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THE COULTER PRINCIPLE: FOR THE GOOD OF HUMANKIND

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THE COULTER PRINCIPLE:
FOR THE GOOD OF HUMANKIND

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Arts in the
College of Arts and Sciences
at the University of Kentucky

By

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Nicholasville, Kentucky

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2020

ABSTRACT OF THESIS

THE COULTER PRINCIPLE:
FOR THE GOOD OF HUMANKIND

The atomic bombings of Hiroshima and Nagasaki in August 1945 made Wallace H. Coulter abruptly comprehend the critical need for rapid and accurate blood-cell counts in providing care for victims of radiation exposure. This thesis documents the unwritten story of his journey from that comprehension through his invention and implementation of the Coulter Principle, its commercialization in the first widely available automated blood-cell counter, and elaboration of that ground-breaking counter into increasingly sophisticated instrumentation for analysis not only of blood cells, but of particles involved in many other scientific disciplines. International cold-war politics and the burgeoning of increasingly powerful nuclear weapons were important motivations for him throughout the period here considered; these are summarized as context for his developmental activities.

The Coulter Principle states that if a suspension of blood cells is passed through a small restriction simultaneously with an electric current, the cells will modulate the current, so enabling them to be counted and sized. Today, hematology analyzers based on the Coulter Principle daily process blood samples from many more patients than the number of casualties from the Hiroshima and Nagasaki bombings.

In closing, significant recognitions of Coulter's contributions are summarized.

KEYWORDS: Wallace Coulter, Coulter Principle, Coulter Counter, blood-cell counting, hematology analyzers
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Marshall D. Graham

November 18, 2020
Date

THE COULTER PRINCIPLE:
FOR THE GOOD OF HUMANKIND

By

Marshall D. Graham

Scott K. Taylor
Director of Thesis

Amy Murrell Taylor
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November 18, 2020
Date

DEDICATION

Wallace Henry Coulter

February 17, 1913 - August 7, 1998

“People who don’t try, don’t make mistakes.”

Joseph Richard Coulter, Jr.

August 18, 1924 - November 27, 1995

“Some things are more important than money.”

ACKNOWLEDGEMENTS

This thesis originated in the University of Kentucky's recruitment day, May 7, 1959, which I attended as a graduating high-school senior. There I saw a Coulter Counter® Model A exhibited by the Biology Department and was allowed to operate it. Its sensing aperture puzzled me, and I began searching library stacks for explanations. My findings led to purchase of a Coulter Counter® Model ZB during my Ph.D. research at Duke University, which prompted a call from Wallace H. Coulter wanting to know why I required its circuit diagrams. That discussion led him to have me recruited into the Coulter organization in 1976, where in June 1978 I became his technical advisor, a position I held until Coulter Corporation was sold to Beckman Instruments in late 1997. My work required detailed knowledge of sensing apertures, which furthered my continuing interest. To the many people who lent a hand during my 60-year journey, I can only say, "Thank you."

I thank Ms. Doris Zagon, Wallace Coulter's only administrative aide, who checked my transcriptions of his handwritten materials; Ms. Laura Coulter Jones, who provided scans and copies of material in her grandfather's files; and Dr. Garry Wheeler Stone, whose review of my text improved its lucidity.

Special thanks are due to my graduate committee for their willingness to serve and helpful comments. Dr. Scott K. Taylor, who took time to understand my interest in how historians think, and Dr. Amy Murrell Taylor, who encouraged me to undertake this thesis topic, have earned my sincere appreciation. The staff of the University's Interlibrary Loan program has obtained many helpful articles I had failed to locate in years of searching. And I deeply appreciate being able to pursue a study of history as a Donovan Scholar.

Without understanding from my wife Moon, I would not have gotten to this stage, and our daughter Dawn spent four of her early summers helping me archive old articles. Finally, my brother William Gerry Graham has enabled many improvements in the Coulter sensing apertures used in current implementations of the Coulter Principle.

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CHAPTER 1. INSPIRATION

In early August, 1945, people by the tens of thousands died instantly in two blinding flashes of light, but death did not always come so quickly for other victims of the Hiroshima (Figure 1.1) and Nagasaki (Figure 1.2) bombings: Burns and other injuries had yet to kill other tens of thousands, while lethal radiation effects had only begun to claim thousands more.[1] Japanese doctors initially were puzzled by symptoms exhibited by the latter victims, and to minimize negative publicity, U.S. officials discredited their reports.[2] However, a Dutch physician who survived the Nagasaki bombing confirmed the Japanese findings while reporting decreased counts of both red and white blood cells in such victims.[3] Later, after visiting Nagasaki, an officer of the U.S. Navy Medical Corps stated that "the 'radiation sickness' produced a form of anemia, due directly to the fact that rays from the bomb interfered with the functions of the bone marrow – one of the principal sites of manufacture of red blood cells."[4] As a result, the normal concentration of red blood cells (erythrocytes) circulating in a victim's veins decreased according to the person's radiation exposure, causing for many heavy fatigue and a sense of weakness before a slow death. For others, impaired production of white blood cells (leukocytes) and platelets resulted in fatal infections or bleeding. Those two bombings helped to end WWII without an invasion of the Japanese homeland, but they inflicted tremendous costs on the Japanese people.[5]

"We have spent $2,000,000,000 on the greatest scientific gamble in history and won." So it was that U.S. President Harry S. Truman characterized the Manhattan Project

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[1] Actual fatality figures, either total or according to time or cause of death, are unknown; David Richardson, "Lessons from Hiroshima and Nagasaki: The most exposed and the most vulnerable," *Bulletin of the Atomic Scientists* 68 (May 2012): 29-35.

[2] James F. McGlincy, "Writers tell of utter ruin in Hiroshima, *Detroit Times*, Detroit, MI, September 5,1945, 1; "Japanese radio proved lying about atomic bomb effects," *The Greensboro Record*, Greensboro, NC, September 12, 1945, 1; "Little radioactivity found in bomb-blasted Hiroshima," *Durham Morning Herald*, Durham, NC, September 13, 1945, 1.

[3] "Atom bomb's horror told," *Detroit Times*, Detroit, MI, September 11, 1945, 2.

[4] Joseph J. Timmes, "Radiation sickness in Nagasaki: preliminary report," *U.S. Naval Medical Bulletin* 46 (1946): 221-23.

[5] Eisei Ishikawa, *Hiroshima and Nagasaki: The Physical, Medical, and Social Effects of the Atomic Bombings*, trans. David L. Swain (New York: Basic Books, Inc., 1981.

Figure 1.1. An American view of the Hiroshima bombing, August 6, 1945.[6] The bombing plane, the U.S. B-29 *Enola Gay*, is shown some eleven miles from the explosion, at an altitude of 1,900 feet, of the uranium bomb Little Boy. The explosive yield was equivalent to that produced by some 15 kilotons (KT) of TNT, and the mushroom cloud rose to an altitude of more than eight miles. The photograph was made from a second B-29, *Necessary Evil*, which carried the mission photographers.[7]

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[6] "America conducted worlds' first nuclear attack in Hiroshima seventy-two years ago today," *Pakistan Today*, Foreign News, Selection 4, August 6, 2017, website accessed April 30, 2018; Wilfred G. Burchett, "The atomic plague," *Daily Express*, London, UK, September 5, 1945, 1, website accessed September 27, 2020.

[7] Paul Ham, *Hiroshima Nagasaki: The Real Story of the Atomic Bombings and Their Aftermath* (New York: Thomas Dunne Books, St. Martin's Press, 2011), 315-38. An instructive overview is available: Cynthia C. Kelly, ed., *The Manhattan Project: The Birth of the Atomic Bomb in the Words of its Creators, Eyewitnesses and Historians* (New York: Black Dog & Leventhal Publishers, 2007), 329-45.

Figure 1.2. A Japanese view of the Nagasaki bombing, August 9, 1945. The bombing plane was the U.S. B-29, *Bockscar*, and the plutonium bomb Fat Man detonated at an altitude of 1,640 feet. The explosive yield was some 21 KT, and the mushroom cloud reached an altitude of about eight miles.[8] Hiromichi Matsuda made this photograph from a distance of some six miles about 15 minutes after the explosion.[9] By then the mushroom cloud, of which only the lower portion is apparent, had been reshaped by wind patterns.

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[8] Ibid. 357-79.

[9] "The first use of the atomic bomb," *CNN World*, August 4, 2015, image 14 and its attribution, website accessed April 30, 2018. An eyewitness account is available: Kelly, *The Manhattan Project*, 345-51.

via which the bombs were developed that had killed women and children by the tens of thousands in Hiroshima and Nagasaki.[10] Those bombings did precede a quick surrender by Japanese militarists.[11] However, the opinion occasionally voiced by Wallace Henry Coulter was, "It's the worst mistake this country ever made." [12]

Born in Little Rock, Arkansas, in 1913, Wallace had gone to the Far East in December 1939 as a sales and service engineer for Chicago's General Electric X-Ray Corporation. While returning to Singapore City on December 8, 1941, from hospital visits in Java, he had watched the first Japanese bombing of that city within a few hours of the Japanese attacks on Hong Kong, Manila, and Pearl Harbor. On February 8, 1942, the Japanese army had crossed the Strait of Jahor, prompting him to start homeward. He gained passage on one of the last boats to Java from which, when the Japanese navy approached, on February 24 he continued to Mumbai, India, where he arrived two weeks later. Before leaving Mumbai on April 8 for Dar es Salaam, Tanganyika, he wrote to his parents: "It is nice to be away from Singapore. Those bombing raids weren't so bad individually, but when they came at all hours and for from 30 minutes to three hours at a time it got damn annoying. I had some near misses but the only damage done was getting my knees and hands skinned diving into a ditch once!" [13] As opportunities arose, he persevered westward to South Africa, onward to Argentina, and finally from Buenos Aires to New York on Christmas Day, 1942. There, he would supervise radio transmitter design at Press Wireless, Inc., in Hicksville, Long Island, for the remainder of the war.[14] By mid-

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[10] J. A. Fox, "Atomic bomb, world's greatest, hits Japs," *The Evening Star*, Washington, D.C., August 6, 1945, A1; Harry S. Truman, "The Report of President Truman on the atomic bomb," *Science* 102(August 17, 1945): 164.

[11] "Japs report peace offer accepted," *Greensboro Daily News*, Greensboro, NC, August 14,1945, 1; Garnett D. Homer, "Surrender signed on board Missouri; Japs stripped of all their conquests," *The Evening Star*, Washington, D.C., September 2,1945, 1.

[12] Wallace's commentary in subsequent conversations with the author.

[13] Wallace Coulter, letter to Joseph Coulter, dated April 8, 1942; Joseph R. Coulter Files (hereinafter the JRC Files), privately held in the Coulter Family Collection, Coral Gables, Florida. Ms. Laura Coulter Jones gave access to her grandfather's files regarding her father Joseph R. Coulter, Jr., and her Uncle Wallace and provided photocopies or scans of those items cited herein.

[14] Wallace's family background, details of his youth and homeward journey from the Far East, and his experience in radio, as well as information about his only sibling, Joseph R. Coulter, Jr., are outlined in Marshall Don. Graham, "The Coulter Principle: The Arkansas background," *The Arkansas Historical Quarterly* 73 (Summer 2014): 166-75.

1945, he and a business friend, David A. Garrick, would propose establishing an electro-medical group in Chicago for Raytheon Manufacturing Company; Wallace was to develop low-noise amplifiers for electrocardiographic equipment and pulse circuits for muscle stimulators, while Garrick was to handle business responsibilities.[15]

Then came those bombings of Hiroshima and Nagasaki.

During his hospital visits in the Far East, Wallace had seen blood-cell counts being done by hematology technologists using hemocytometers (specialized microscope slides in which the cover slip was held at a particular distance above a grid to form a counting chamber of specified volume). Library research had taught him that normal human blood contained about 5,000,000 erythrocytes and 7,000 leukocytes per microliter (µl), with only a few hundred cells in the volume of diluted blood pipetted between the hemocytometer's coverslip and grid being counted by a technologist using a microscope. For normal blood samples and commercial hemocytometers the cellular concentration from an accurate count was significant within only ±16% for erythrocytes but within only ±21% for leukocytes, uncertainties which incompetency or inattention might double or triple. Moreover, because the sample's cellular concentration was estimated by dividing the technologist's count by the sample's dilution ratio, any uncertainty in the latter further magnified errors in the estimated concentration. For a normal blood sample the counting process would require 15 to 30 minutes for a competent technologist to complete, while some abnormal samples could double this time.[16] Although a typical patient might need only a single blood-cell count in several years, the inaccuracy and time requirement of manual counts had caused Wallace to reflect on the possibility of automating them. But reports about radiation effects of the Hiroshima and Nagasaki bombings now made brutally obvious to him the critical need for blood-cell counts of greatly improved accuracy, not just occasionally for individuals but repeatedly and at close intervals for whole populations, to monitor recovery of the many victims' bone-marrow from radiation

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This article augments items from the JRC Files with information drawn from a number of other primary sources.

[15] Joseph R. Coulter, Jr., letters to Joseph R. Coulter, Sr., dated August 26 and 28, 1945; JRC Files.

[16] Preben Plum, "Accuracy of haematological counting methods," *Acta Medica Scandinavica* 90 (1936): 342-64; Joseph Berkson, Thomas B. McGath, and Margaret Hurn, "The error of estimate of the blood cell count as made with the hemocytometer," *American Journal of Physiology* 128 (1939): 309-23; M. L. Verso, "The evolution of blood-counting techniques," *Medical History* 8 (April 1964): 149-58.

exposure.[17] The accuracy, repeatability, and rapidity necessary for blood-cell counts meeting this need clearly demanded an automated counting process. These realizations, made when he was 32 years old, changed the course that his life would thereafter follow.

Wallace's library searches had located a brief description of an attempt to adapt a phototube to sense greatly magnified blood cells in a suspension flowing through a capillary tube mounted on a microscope stage.[18] His early experiments would confirm technical difficulties with this approach, and he would then consider replacing the capillary tube with an aperture through which both the cellular suspension and the illuminating light passed. Unable to obtain an acceptable signal from individual cells, he would remember a method he had encountered as a student of electrical engineering in the early 1930s, one that enabled calculation of the electrical resistance of particle suspensions when the electrical conductivity of the particles differed from that of the suspending liquid.[19] This would lead him to theorize that apertures comparable in size to a blood cell, through which a flowing cellular suspension formed a path not for light but for an electrical current, could satisfy the method's assumptions and so might enable counting of the suspended cells.[20]

But could passing blood through a small aperture, a little bit of nothing in a short bore of length L between two orifices of diameter D, and measuring changes in an electrical current through it really provide help for survivors of a nuclear event such as those illustrated above? This thesis will detail the unwritten history of how Wallace developed his theory, first into the Coulter Principle and then into the revolutionary Coulter Counter® Model A, the descendants of which daily affect the lives of millions of people worldwide.[21] By 1988, Coulter companies had produced the 80,000th instrument

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[17] "Atom bomb's horror told," *Detroit Times*, Detroit, MI, September 11, 1945, 2; Thomas R. Henry, "Navy issues report on Nagasaki victims of atomic radiation," *The Evening Star*, Washington, D.C., January 31, 1946, A8; James S. P. Beck and William A. Meissner, "Radiation effects of the atomic bomb among the natives of Nagasaki, Kyushu," *American Journal of Clinical Pathology* 16 (September 1946): 586-92.

[18] Andrew Moldavan, "Photo-electric technique for the counting of microscopical cells," *Science* 80 (August 24, 1934): 188-89.

[19] James Clerk Maxwell, *Treatise on Electricity & Magnetism*, 3rd ed., vol.1 (Oxford, UK: Clarendon Press, 1891; reprint, New York: Dover Publications, Inc., 1954), 440-41.

[20] For a discussion of "aperture" as herein used, see Appendix A, fourth paragraph.

[21] "679,591, Coulter Counter," *Official Gazette of the United States Patent Office* (hereinafter, *Official Gazette*) 743 (Jun. 2, 1959): TM 37.

incorporating the Coulter Principle.[22] By 2018, at least 6,500 DxH 800 hematology analyzers were installed, each one fully automated to process 100 blood samples per hour through Coulter apertures; if operated only 12 hours per day at 70 samples per hour, these could process more than 5.4 million samples daily. Of those samples, perhaps some 220,000 would have an abnormality affecting a patient's diagnostics, and samples run on many older models still in use would greatly increase this number. Furthermore, particle-analyzing models are used in manufacturing processes for hundreds of commercial products for which the number or size of constituent particles affect function or acceptability, for example, chocolate, wines, medicines, cosmetics, building materials and supplies – the list goes on. The undeniable importance of these instruments has attracted envious attention, and on expiration of its patent protection, competitive instruments have incorporated the Coulter Principle. These have also helped improve the health and quality of life for millions, and Wallace took reluctant pride in having made such rivalry possible.

Wallace was a modest and very private person who never married and who had no immediate survivors at his death in 1998. An experienced engineer, he published but a single paper publicizing what became his life's work, while to protect his progress he contributed as inventor to 85 U.S. patents. These, and details spread through his personal papers, are the only record of his achievements he himself left. Unfortunately many of his papers were apparently discarded or lost during the 1992 relocation of the corporation he and his brother, Joseph R. Coulter, Jr., had founded. Hence, the origin and development of the Coulter Principle are not well understood.[23] But among those personal papers were ones that support Wallace's motivation being accurate and rapid blood-cell counts, a motivation inspired by the need to effectively monitor bone-marrow recovery from radiation exposure such as endured by survivors of the Hiroshima and Nagasaki bombings.

This thesis has its direct origins in my service as Wallace's technical advisor from mid-1978 until Beckman Instruments bought and merged with Coulter Corporation in late 1997; it draws extensively from the personal papers he provided while I served in this role. It will document, insofar as now possible, Wallace's journey from abrupt comprehension of the critical need for accurate and rapid blood-cell counts through his invention, implementation, commercialization, and elaboration of the first commercially available

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[22] "Back to the future with Coulter," *Coulter Viewpoint* 1 (1988): 4.

[23] Marshall Don. Graham, "The Coulter Principle: Imaginary origins," *Cytometry A* 83A (2013): 1057-61.

automated blood-cell counter, which gained worldwide renown as the Coulter Counter® Model A. Then, a brief contemplation of the significant recognitions his efforts brought him will bring this thesis to its close.

And yes, it will show that a small aperture, a little bit of nothing, could indeed help victims of a catastrophic nuclear event. But first, Wallace had to vacate Hicksville.

CHAPTER 2. PREPARATION

The Japanese surrender brought a vast reorganization for the U.S. economy and a rapid demobilization of U.S. service personnel. As federal work at Press Wireless, Inc., declined, Wallace Coulter completed work on an ultra-stable transmitter oscillator and, desiring more freedom in his work projects, proposed and briefly co-managed an electro-medical development group at Raytheon Manufacturing Company while returning to Chicago from Hicksville. As a personal project, he began the design of a noise-cancelling amplifier for electrocardiography. However, because of shifting economic conditions the Raytheon proposal was never formalized. Wallace continued his library search for an instrument design that might be adapted to provide blood-cell counts equal to the needs of radiation victims, but found little of relevance. He recruited his brother to help.[24]

Joseph Richard Coulter, Jr., younger by some eleven years than Wallace, had joined the U.S. Army in October of 1942 and had spent sixteen months in the Army Specialized Training Program (ASTP) at Ohio State University studying toward a degree in electrical engineering. He had then worked as a radio operator in the Army Signal Corp at Camp Crowder, Missouri, and was anticipating discharge.[25] In August 1945, Joseph informed their parents that Wallace was "busy with his blood-cell counter," had bought an electronics reference book covering the period from 1925 to 1945, had sent him a list of thirty references to read and summarize, and had asked him to go to the Alien Property Office to obtain information on a U.S. patent, granted to the Norwegian Jan Kielland, for a blood-cell counter.[26] So that Joseph could complete his degree, Wallace registered him at the Illinois Institute of Technology; in response, Joseph reported his activities and

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[24] "New Fellows in the Industry Applications Society, Wallace H. Coulter," *IEEE Transactions on Industry Applications* IA-20 (January/February 1984): 4; Joseph R. Coulter, Jr., letters to Joseph R. Coulter, Sr., dated August 26 and 28, 2945; JRC Files. In his letter of March 20, 1946, to Wallace Coulter, Leslie Norde described management changes at Press Wireless, remarked that tests on Wallace's oscillator would start March 21, stated that he had returned all Raytheon equipment, and asked what to do with Wallace's equipment still in Hicksville; JRC Files.

[25] Ohio State University transcript, dated February 15, 1946, and U.S. Army Separation Qualification Record, dated February 19, 1946, both for Joseph R. Coulter, Jr.; JRC Files.

[26] Joseph R. Coulter, Jr., letters to Joseph R. Coulter, Sr., dated August 26 and 28, 1945; JRC Files.

observed, “Among other things I think that when I come to Chicago to go to school that we should live together. It would be cheaper and I could absorb some of your experience.” Restive under the top-down orders of his Army service, he finished his thought by adding, “I’ve really got my heart set on us being in business someday and the sooner the better. It would be very nice to be in a position to run something like you wanted it.” [27] Discharged on February 19, 1946, Joseph joined Wallace in Chicago, and Wallace began working as a sales engineer with Illinois Tool Works around that time. In March their father wrote the brothers that he had just expressed them a box containing technical books, electronics magazines, and “the Indices, etc., that had come in the last few days.” [28]

The Chicago in which the Coulter brothers began their quest for autonomy in 1946 was not the Chicago that Wallace had left in 1939.[29] Events of 1939 had allowed actions of Robert M. Hutchins to non-obviously, but significantly, reshape the city. Hutchins, installed as President of the University of Chicago in 1929, was a young idealist who saw the function of a college as teaching students how to think and understand, rather than how to make a living.[30] In his view, collegiate specialization, especially any tending toward a vocation, should be discouraged, while trade skills should be learned in industry; discussions amongst students and with their professors were essential, and activities that interfered with these, such as time-consuming varsity football practices, were also undesirable.[31] Under his supervision the University’s many departments were reorganized into four graduate divisions – Physical Sciences, Biological Sciences, Social Sciences, and Humanities – to which was added the office of university examiner. Each division provided a survey course which students could navigate and augment with divisional electives as they chose before passing a comprehensive divisional examination for their

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[27] Joseph R. Coulter, Jr., letter to Wallace Coulter, dated January 31, 1946; JRC Files.

[28] Joseph R. Coulter, Sr., letter to Wallace and Joseph R. Coulter, Jr., dated March 12, 1946; JRC Files.

[29] Richard G. Hewlett and Oscar E. Anderson, Jr., *The New World, 1939-1946: A History of the United States Atomic Energy Commission, Vol. 1* (University Park, PA: Pennsylvania State University Press, 1962).

[30] William H. McNeill, *Hutchins’ University: A Memoir of the University of Chicago, 1929-1950* (Chicago, IL: University of Chicago Press, 1991).

[31] Robert M. Hutchins, “The idea of a college,” *Measure* 1 (Fall 1950): 363-71, website accessed October 10, 2020.

degree.[32] Before Hutchins' presidency the University's varsity football team, the Maroons, had won seven Big Ten championships, but the Depression brought a drop in both enrollments and the number of men wanting to play football, and under his reorganization, the Maroons had to pass the same divisional examinations as other students. This requirement limited their spring practice to about ten days compared to two or three times that for their competitors, and in their 1939 season, the Maroons scored only 37 points while their eight opponents scored a total of 308 points.[33] That December, to the disappointment of alumni, Hutchins ended the University's varsity football program and left the grandstands at the University's Stagg Field, with their seating capacity of 56,000 fans, to quietly ruin.[34]

In March 1939, Walter P. Murphy, President of Chicago's Standard Railway Equipment Manufacturing Company, had donated $6,735,000 to Northwestern University to initiate its Institute of Technology in Evanston.[35] Murphy was an elderly realist who had obtained more than a hundred U.S. patents; although unable to complete college, he was persuaded that closely integrating academic courses with practical application in industrial settings would provide the best engineering education. To provide such a cooperative program, Northwestern had accepted Murphy's donation, and his foundation had also offered to establish a similar School of Engineering at the University of Chicago. However, Hutchins preferred a program of research and graduate education similar to that at the Massachusetts Institute of Technology. During a dinner at Murphy's Lake Bluff home, he expressed pleasure that prospects were bright for Northwestern's Institute, but instead of accepting the offer of an engineering school, he solicited a donation in support of the

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[32] McNeill, Hutchins' University, 31.

[33] "Stagg retired as Chicago athletic director," *The Grand Rapids Press*, Grand Rapids, MI, October 14, 1932, 27; Carl Larsen, Opinion article, *Sunday Tim*es, Chicago, IL, November 19, 1939, Magazine, 3; "An old story: Chicago loses," *The Milwaukee Journal*, Milwaukee, WI, November 26, 1939,Sports 2.

[34] "New Stagg Field to seat 56,000," *Aberdeen American-News*, Aberdeen, SD, July 24, 1927, 6; "They solve problem – Maroons give up football" and "Frosh star going back; alumni meet," *Daily Times*, Chicago, IL, December 22, 1938, 43; Charles Dunkley, "Chicago alumni favor football," *The News and Courier*, Charleston, SC, December 23, 1939, 6; McNeill, *Hutchins' University*, 95-97.

[35] "Evolves new way to use money for poor," *Daily News*, Chicago, IL, January 15, 1926, 3; "$6,735,000 for N. U. School," *Daily Times*, Chicago, IL, March 22, 1939, 49.

University of Chicago's Medical School. No agreement was reached.[36] After the German invasion of Poland on September 1, 1939, concerns grew that the U.S. would be drawn into the European conflict, and by late 1941, both universities would be operating programs for governmental research and to familiarize officer trainees with the sciences. However, at the University of Chicago Hutchins' decision inhibited the interactions between engineering and the sciences that elsewhere produced so many developments in technology.[37] Dedicated in June 1942, Northwestern's Institute would attract excellent faculty and students, and in his will Murphy provided an additional $20,000,000 to develop, operate, and maintain it.[38] During the war, Northwestern University would accept responsibility for some 28 government research projects at its Evanston campus and 12 more at its medical school in Chicago. In addition to supporting governmental research in engineering and physical sciences, the Institute's laboratories with their new equipment would draw federal funds for intensive training of over 50,000 military personnel.[39]

The European conflict brought another concern that would reshape not only the Chicago of 1939, but the world itself. In his letter to U.S. President Franklin D. Roosevelt of August 2, 1939, Albert Einstein warned that "it may become possible to set up a nuclear chain reaction in a large mass of uranium," thereby generating immense power and conceivably leading to construction of extremely powerful bombs; he noted that Germany had stopped sales of uranium from the Czechoslovakian mines it had recently annexed.[40]

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[36] Robert C. Michaelson, "Opportunity lost and gained: A sidelight on the Walter P. Murphy gift," in *Tech, The Early Years: An Anthology of the History of the Technological Institute of Northwestern University from 1939 to 1969*, ed. Morris E. Fine, 5-10 (Evanston, IL: Northwestern University, 1995).

[37] The University of Chicago first offered a B.S. in engineering and formed its first engineering school, the Pritzker School of Molecular Engineering, in 2019: Dawn Rhodes, "University of Chicago receives $75M to launch campus' first engineering school," *Chicago Tribune*, May 28, 2019, website accessed October 10, 2020.

[38] "Rail leaders left funds in Murphy will," *Rockford Morning Star*, Rockford, IL, January 1, 1943, 3; Mel Hodell ,"Murphy will leaves NU $20,000,000," *Daily Northwestern*, Evanston, IL, January 5, 1943, 1; "Murphy bequest raises NU to fifth wealthiest university," ibid. January 6, 1943, 3.

[39] "N. U. to dedicate campus dream – Tech Institute," *The Chicago Daily News*, Chicago, IL, June 13, 1942, 1, 5, and Photogravure 3; Jeanette Lowrey et al., "Science in Chicago," *The Scientific Monthly* 65 (December 1947): 470.

[40] Albert Einstein, letter dated August 2, 1939, to F. D. Roosevelt and Roosevelt's reply are transcribed in Kelly, *The Manhattan Project*, 42-44. Einstein's original letter is available online, website accessed October 20, 2020.

Little was known about Germany's research into atomic fission, but its repeated purchases of Norwegian heavy water (deuterium oxide) stimulated concern that it might be progressing toward a plutonium bomb via a uranium reactor moderated with heavy water.[41] Roosevelt was convinced that the U.S. could not risk Germany unilaterally developing such bombs and replied on October 19 that he had convened a board to investigate Einstein's suggestion regarding uranium; the Manhattan Project, created on August 13, 1942, would result.[42] Only a few details regarding this complex program are needed here; both summary and detailed histories are available.[43]

In May 1940, Hutchins had named a 1927 Nobel laureate in physics, Arthur H. Compton, as Dean of Physical Sciences at the University of Chicago.[44] The unexpected Japanese attack on the U.S. naval base at Pearl Harbor on December 7, 1941, provoked the U.S. into declaring war, and on December 10th both Germany and Italy declared war on the U.S. After accepting responsibility on December 18th for the theoretical and experimental work to build an atomic reactor and produce plutonium for a fission bomb as quickly as possible, Compton organized the nationwide Metallurgical Laboratory (Met Lab) in January and authorized construction of a small reactor pile in the racquets court under the unused west grandstands of Stagg Field.[45] Experiments with it showed that the probability *k* of a neutron released in a nuclear fission causing a subsequent fission was 0.94±0.02, whereas a *k* of 1.00 was needed for a self-sustaining reaction and a *k* greater than 1.00 was necessary for the runaway chain reaction required for a fission explosion. Using the same neutron sources and better experimental methods with his graphite-moderated pile at Columbia University, Enrico Fermi obtained a *k* of 0.995 in early May.[46] On May 23rd, $25,000,000 was allotted to provide one or more plutonium-producing piles

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[41] Hewlett and Anderson, *The New World, 1939-1946*, 29 and 119.

[42] Franklin D. Roosevelt, letter dated October 19, 1939, to Dr. Albert Einstein; Atomic Heritage Foundation, "The Manhattan Project," websites accessed October 20, 2020.

[43] F. G. Gosling, *The Manhattan Project: Making the Atomic Bomb*, DOE/MA-0002 (Washington, D.C.: United States Department of Energy, 2010); H. D. Smyth, *Atomic Energy for Military Purposes* (York, PA: Maple Press, 1945); Hewlett and Anderson, *The New World, 1939-1946*.

[44] "Scientist named dean," *San Francisco Chronicle*, San Francisco, CA, May 4, 1940, 9; McNeill, *Hutchins' University*, 104.

[45] Ibid. 104-106; Hewlett and Anderson, *The New World, 1939-1946*, 29, 36-38, and 46-52.

[46] Ibid. 68, 70-71.

by January 1944, and Compton began gathering researchers from the Berkeley, Columbia, and Princeton programs in Chicago. Using ultra-pure uranium oxide in a replica of the earlier Stagg Field pile, Fermi obtained an estimated *k* of 1.014 in August, and without seeking approval, Compton authorized him to build a larger self-sustaining pile under the west grandstands at Stagg Field. In a review by the S-1 Executive Committee on November 14, concerns about those stands being in one of the most densely populated areas in the U.S., Fermi's last *k* value, and the practicality of plutonium as a bomb material combined to trigger a reappraisal of Compton's approach. However, the new pile was completed the night of December 1, and the next day Fermi brought it into self-sustaining operation while Compton and a member of the reappraisal committee watched.[47] They saw the theories that would make fission bombs possible validated beneath the empty grandstands that Hutchins had abandoned to ruin.

Producing sufficient fissionable uranium or plutonium to make a practical bomb still faced many difficult problems. In March 1943 Fermi's reactor was rebuilt for experimental use in the Argonne Forest Preserve in Lemont, IL, and much of the development was done under the U.S. Army Corps of Engineers at other sites.[48] In the Trinity Test on July 16, 1945, the first plutonium bomb was detonated in present-day White Sands Missile Range; on August 6 the first uranium bomb was dropped on Hiroshima (Figure 1.1); and on August 9 the second plutonium bomb was dropped on Nagasaki (Figure 1.2). After the war's end in September, the G.I. Bill would bring veterans in their tens of thousands into the science and engineering programs at Chicago's universities, and in July 1946, the Met Lab would become Argonne National Laboratory.[49] Basic research was vital to national security, but the secrecy typical of military/industrial research was a problem for academic researchers; to encourage non-governmental research, the Office of Naval Research was organized that August. Through it, federally funded contracts would be let without undue restrictions on the contractor's freedom to publish, and within a year a regional office was operational

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[47] Ibid. 88-89, 108-13; Sid Moody, "When atomic era opened: Flashback to Dec. 2, 1942," *Sunday Star*, Washington, D.C., December 2, 1962, B3; *The First Reactor*, DOE/NE-0046 (Washington, D.C.: United States Department of Energy, December 1982).

[48] Hewlett and Anderson, *The New World, 1939-1946*, 116-73 for uranium, 174-226 for plutonium, and 227-54 and 289-321 for the fission bomb effort.

[49] Leonard Greenbaum, *A Special Interest: The Atomic Energy Commission, Argonne National Laboratory, and the Midwestern Universities* (Ann Arbor: University of Michigan Press, 1971).

in Chicago.[50] These federally sponsored programs would continue to attract skilled personnel and would bring significant additional service and industrial activity to Chicago.

Meanwhile, plans had matured for the world's fourth and fifth fission explosions. On July 1 and 25, 1946, the U.S. made the second and third tests of the Nagasaki bomb design at Bikini Atoll in the Marshall Islands.[51] Unlike the Trinity Test in July 1945, these tests were intended to study effects of nuclear explosions on naval ships and planes, as well as animals; the first bomb was dropped over 95 unmanned ships, while the second was detonated 25 feet underwater beneath survivors of that fleet.[52] In the first test 176 goats, 146 pigs, 57 guinea pigs, 3,030 white rats, and 109 mice were distributed on 22 of the ships; in the second, 20 pigs and 200 white rats were dispersed onto four of the ships.[53] To follow the animals' bone-marrow recovery, 100 technologists from the Naval Medical Research Section did blood-cell counts on each one before the explosions and repeatedly on the survivors; although there were specie differences, about 15% of all animals died due to radiation effects.[54] A large press contingent attended the tests, and news reports mentioning the many labor-intensive blood-cell counts would remind the Coulter brothers of the need for an automated method.[55] Only later was it apparent that mist from the second test had spread lingering radioactive contamination that caused serious health problems for servicemen assigned to the prolonged cleanup.[56]

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[50] Alan T. Waterman and Robert D. Conrad, "The Office of Naval Research," *The American Scholar* 16 (Summer 1947): 354-56; Rayy Mitten, "Office of Naval Research not yet sure it can control its rain-making device," *The Corpus Christi Times*, Corpus Christi, TX, August 26, 1947, editorial page.

[51] Lloyd J. Graybar, "The 1946 atomic bomb tests: Atomic diplomacy or bureaucratic infighting?" *Journal of American History* 72 (March 1986): 888-907.

[52] "Blast toll mounting at Bikini" and "Scenes as fourth atomic bomb dropped," *Evening World-Herald*, Omaha, NE, July 1, 1946, 1; W. A. Shurcliff, *Bombs at Bikini: The Official Report of Operation Crossroads* (New York: Wm. H. Wise & Co., 1947).

[53] Joint Task Force One Historian, *Operation Crossroads: The Official Pictorial Record* (New York: Wm. H. Wise & Co., 1947), 108-10, 220.

[54] Clark Lee, "Quick bomb news," *The Kansas City Star*, Kansas City, MO, June 24, 1946, 7; Graybar, "The 1946 atomic bomb tests," 901-02.

[55] "Bikini," *Life* 23 (August 11, 1947): 74-88, website accessed June 14, 2018.

[56] Ibid. 84-85; Phillip W. Smith, "Sailors kept on radioactive ships in test," *The Times-Picayune*, New Orleans, LA, November 7, Section 1, 20; Tim Ahern, "Radiation seen more extensive," *Mobile Register*, Mobile, AL, December 5, 1985, 5A.

In April 1947 Wallace and Joseph purchased the property at 3023 W. Fulton Boulevard, Chicago, and the unfinished partial basement in the brothers' new home offered space for undisturbed experiments.[57] When his duties at Illinois Tool Works permitted, Wallace continued his research on amplifier designs and cell-counting (Figure 2.1). Joseph received his degree in electrical engineering the following June, accepted a position as project engineer in the Communications Division of Motorola Corporation, and continued the brothers' partnership as Coulter Electronics.[58]

Despite the crucial role that blood plays in physical health, the Coulters' research had located little information related to instrumented analysis of its cellular components. Considerable work had been done on light transmission through blood diluted with various solutions, but estimation of cell number by this approach required information regarding cell size and shape; even when this was provided, the estimated cell number was less reliable than counts obtained via the hemocytometer method Wallace had observed in the Far East.[59] Frank Twyman and David Follett had patented a concept based on the cellular diffraction patterns from thin static films of blood. This assumed that the intensity of the light diffracted from a film area would be proportional to the total area of diffracting blood cells and dividing that intensity by the average blood-cell area would provide an estimate of the cellular number. However, this approach also required information about cellular size and shape.[60] In a normal blood sample the native cells range in both parameters, a situation worsened in anemias, and both cellular parameters depended on the preparation of the film.[61] Moreover, both the apparatus required to form the diffraction patterns and interpretation of those patterns seemed difficult to automate.

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[57] Document 14053001 recorded May 9, 1947, in Cook County, IL, Book 42164, 123; WHC Papers.

[58] 1947 commencement program for Illinois Institute of Technology; JRC Files. Regarding the high-fidelity amplifier designs, see W. H. and J. R. Coulter, "O-T-L amplifiers," *Audio Engineering* 36 (Sept. 1952): 10 and Richard H. Dorf, "Audio patents," *Audio* 38 (July 1954): 2, 6.

[59] Eric Ponder, The relation of red cell diameter and number to the light transmission of suspensions," *American Journal of Physiology* 111 (1935): 99-106.

[60] Frank Twyman and David Henry Follett, "Counting of microscopical bodies, such as blood corpuscles," U.S. Patent 1,974,522, filed Feb. 7, 1933, and issued Sep. 25, 1934.

[61] Adrainus Pijper, "An improved diffraction method of diagnosing and following the course of pernicious and other anemias," *British Medical Journal* 1 (1929): 635-38.

Figure 2.1. 3023 W. Fulton Boulevard, Chicago. The Coulter brothers owned the property between April 1947 and June 1956. The two-story building contained 2,192 square feet of floor space and in 2015 still had the unfinished partial basement that served the brothers as a makeshift workshop and laboratory.[62] In addition to experimental research resulting in Wallace's invention of the Coulter Principle and the brothers' implementation of it, other efforts there would lead to patents for both noise-cancelling and high-fidelity amplifiers.[63]

Wallace formed Coultamp Company to commercialize the latter, but development of the Coulter Principle into the Coulter Counter® Model A delayed this venture, and introduction of silicon power transistors in 1957 made vacuum-tube high-fidelity amplifiers unpopular for the next several decades. Coultamp Company became a forlorn aspiration.

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62 Redfin.com, "3023 W. Fulton Street, Chicago, IL," website accessed December 11, 2018; Glenn Law, "From basement to boardroom: Inside Coulter Electronics," *New Miami* 1 (April 1989): 31-5, 52-3; Gregg Fields, "A Life of Discovery," *The Miami Herald Business Monday*, Miami FL, April 7, 1992; Bruce Upbin, "What have you invented for me lately?" *Forbes* 158 (Dec. 16, 1996): 330-34.

63 Wallace H. Coulter and Joseph R. Coulter, Jr., "Interference eliminating device for measuring instruments," U.S. Patent 2,622,150, filed Jan. 13, 1949, and issued Dec. 16, 1952. For the high-fidelity amplifiers, see Wallace H. Coulter, "Amplifier circuit having series-connected tubes," U.S. Patent 2,659,775, filed Mar. 21, 1949, and issued Nov. 17, 1953; "Amplifier having series-connected output tubes," U.S. Patent 2,743,321, filed Mar. 21, 1952, and issued Apr. 24, 1956; and ; "Amplifier having series-connected output tubes," U.S. Patent 2,763,733, division of U.S. Patent 2,743,321, issued Sep. 18, 1956.

In contrast, Andrew Moldavan had outlined a simpler approach requiring no interpretation.[64] A diluted blood sample would be made to flow through a capillary tube mounted on the specimen stage of a laboratory microscope, with the microscope objective focused on the flowing cellular suspension. A phototube mounted so as to intercept part of the image formed by the objective would generate a transient change in the voltage applied to the phototube as each cell passed through its field of view. The changes in voltage (the cellular signals) would be indicated by an appropriate meter and could be amplified for recording by unspecified means. Remarking both the difficulty of optically matching the objective to the circular cross-sections of non-standardized capillary tubes and the inadequate phototube response to the magnified cells within such capillaries, Moldavan's brief note only established his priority regarding an insightful concept. The Coulter brothers found the concept's simplicity appealing, and their research had suggested that its acknowledged problems might be resolvable. Jan Kielland, the Norwegian for whose patent Wallace had sent Joseph to the Alien Property Office, had proposed avoiding the optical difficulties by using a capillary tube having rectangular inner and outer cross-sections so that cells could be imaged much as if they were in a hemocytometer.[65] And sensitive photomultiplier phototubes, intensively developed during the war, promised useful responses to cells passing through the bore of such tubes.[66] If these improvements acceptably mitigated Moldavan's technical difficulties, cellular signals could be amplified to trigger a pulse counter, the cellular concentration in undiluted blood samples then being the indicated cell count after its appropriate modification by both the volume of diluted sample from which it was taken and the sample dilution ratio.

The Coulters and Carl Lagercrantz, a professor at the Institute of Medical Chemistry, University of Uppsala, Sweden, undertook independent experiments with Moldavan's photo-electric concept. However, the optical difficulties posed by cylindrical capillaries proved insurmountable, and practicable versions of Kielland's capillary tubes

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[64] Moldavan, "Photo-electric technique," 188-89.

[65] Jan Kielland, "Method and apparatus for counting blood corpuscles," U.S. Patent 2,369,577, filed May 12, 1941, and issued Feb. 13, 1945.

[66] V. K. Zworykin, G. A. Morton, and L. Malter, "The secondary emission multiplier – a new electronic device*," Proceedings, Institute of Radio Engineers* 24 (1936): 351-75.

with noncircular cross-sections were still many years in the future.[67] Lagercrantz resorted to mechanically moving a conventional hemocytometer across a microscope stage and so though the field of view of a photomultiplier phototube fed by one port of a double eyepiece.[68] By contrast, the Coulters focused the microscope along the capillary bore into the suspension flow rather than through the capillary wall and across the flow as Moldavan had proposed. By late 1947 Wallace had shortened the capillary as much as he could; his experimental description begins, “A fluid bearing the particles is made to flow thru a small aperture thru which light is also directed” (Appendix 2). While the Coulters’ axial-sensing approach minimized difficulties caused by optical properties of the capillary wall, non-uniform illumination within the aperture caused the phototube’s response to vary unacceptably with a cell’s path through the aperture, and multiple cells often simultaneously appeared in its field of view. A contemporary particle counter had been developed during the war for counting smoke particles used in evaluating filters for gas masks; it avoided the sensing difficulties encountered by Lagercrantz and the Coulter brothers by eliminating the capillary tube and sensing the particles as aerosols, but this approach was not feasible with blood cells.[69] In brief, photo-electric counters seemed unlikely to provide significant help to victims of radiation exposure. Wallace’s thoughts turned to other approaches.

Meanwhile, survivors of the Hiroshima and Nagasaki bombings continued to suffer the long-term effects of their radiation exposure, as did some of those who came to help them. Such consequences would be monitored for the next several decades by a joint U.S. and Japanese commission.[70] And some U.S. servicemen who had participated in the

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[67] Carl Lagercrantz, “Photoelectric counting of individual microscopic cells,” *Upsala Läkareförenings Förhandlingar* 52 (1947): 287-303; Francois Maystre and Alfredo E. Bruno, “Laser beam probing in capillary tubes,” *Analytical Chemistry* 64 (1992): 2885-87; Marshall Donnie Graham et al., “Monolithic optical flow cells and method of manufacture,” U.S. Patent 8,189,187, filed Nov. 13, 2009, and issued May 29, 2012.

[68] Carl Lagercrantz, “Photoelectric counting of individual microscopic plant and animal cells,” *Nature* 161 (January 3, 1948): 25-6; “On the theory of counting microscopic cells by photoelectric scanning: An improved counting apparatus,” *Acta Physiological Scandinavica* 26 (1952), Supplementum 93.

[69] John Urbanek, “NU lab develops particle counter,” *Daily Northwestern*, Evanston, IL, April 22, 1947, 2; Frank T. Gucker, Jr., et al., “A photoelectronic counter for colloidal particles,” *Journal of the American Chemical Society* 69 (1947): 2422-31.

[70] John E. Dowling, “An introduction to ‘a song among the ruins’,” *Proceedings of the National Academy of Sciences* 95 (May 12, 1998): 5423. The prefaces four papers on

Operation Crossroads tests of July 1946 suffered similar effects, but were unfortunately allowed to scatter with their units without monitoring.[71] Although still generally underappreciated, the need for rapid accurate blood-cell counts was increasing.

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radiation effects of those bombings: Itsuzo Shigematsu, “Greetings: 50 years of Atomic Bomb Casualty Commission – Radiation Effects Research Foundation research,” 5424-25; Frank W. Putnam, “The atomic Bomb Casualty Commission in retrospect,” 5426-31; James V. Neel, “Genetic studies at the Atomic Bomb Casualty Commission – Radiation Effects Research Foundation: 1946-1997,” 5432-36; and William J. Schull, “The somatic effects of exposure to atomic radiation, 1947-1997,” 5437-41.

[71] Shurcliff, *Bombs at Bikini: The Official Report of Operation Crossroads*; Philip W. Smith, “Sailors kept on radioactive ships in test,” *The Times Picayune*, New Orleans, LA, November 7, 1982, Section 1, 20; Ahern, “Radiation seen more extensive,” *Mobile Register*, Mobil, AL, December 5, 1985, 5A.

CHAPTER 3. INVENTION

A year of library research and experimentation had stifled Wallace Coulter's hope that photo-electric methods might provide a blood-cell counter useful in monitoring bone-marrow recovery from radiation exposure. His search for an alternative approach continued, but finding nothing of promise, he began speculating about possibilities.

During his study of electrical engineering at Atlanta's Georgia School of Technology, Wallace had read about a method for calculating the electrical resistance of particle suspensions when the electrical conductivity of the particles differed from that of the suspending liquid.[72] He knew that the resistance of the suspension differed from that of both particles and suspending liquid, but he did not know whether the conductivity of blood cells differed sufficiently from that of a compatible suspending solution that they could be sensed. Around Christmas in 1947, he was thinking how any such difference might be maximized and realized that the suspension volume used in such determinations should not greatly exceed the aggregate volume of the blood cells. Furthermore, if sufficient difference existed between the conductivity of cells and suspending liquid, an aperture comparable in size to a single blood cell might provide this condition for individual cells while also allowing throughflow of a cellular suspension. If then the flowing cellular suspension formed a path for an electrical current rather than light as in the Coulters' photo-electric experiments, changes in the current caused by passing cells might allow them to be individually detected. A theory that would become the Coulter Principle was beginning to take form. To shape it, Wallace needed to know the electrical conductivity of blood cells and compatible suspending media. And given a suitable difference in these, he needed to know appropriate values of the length L and the diameter D of that little bit of nothing between the two orifices of that small aperture.

In early 1948, news articles reinforced Wallace's urgency in finding information regarding the conductivity of blood cells and a source for very small apertures: The U.S. initiated the world's sixth, seventh, and eighth nuclear detonations on Enewetak Atoll in the Marshall Islands. These tests of improved fission bombs were cloaked in high secrecy, but their delayed description verified that both the sixth and seventh explosions were

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[72] Maxwell, *Treatise on Electricity & Magnetism*, 440-41. In 1948, the Georgia School of Technology became the Georgia Institute of Technology.

significantly more powerful than any of the previous five.[73] The press conference on May 19, 1948, only produced a flurry of short news articles devoid of informative details.[74]

Blood was then widely thought to be a homogeneous mixture of blood cells and plasma in which the predominant erythrocytes were uniform in size and constituency, and the following July Wallace obtained a reprint of a brief note based on this concept (Figure 3.1). It described use of a conductivity cell to demonstrate erythrocytes being poor conductors of electrical current, confirmed blood cells to be relatively non-conductive compared to physiologic saline solution, and gave a simple equation for estimating the concentration of such cells based on the conductivities of plasma and whole blood. In their last paragraph the authors stated their intention to develop an electronic circuit providing accurate erythrocyte counts.[75] Wallace now had part of the information he needed, and the authors' intent redirected his research toward conductivity cells. His reading led him to imagine a small J-shaped tube inverted into fluid in two metal cups; a wire attached to both cups allowed them to connect an electrical current to the liquid in the tube bore, which he supposed would siphon from the upper cup through the tube's short leg and down the longer leg into the lower cup (Appendix 3). Then, after mentally miniaturizing the conductivity cell by substituting his very small (theoretical) aperture for its small tube, on July 26, 1948, he wrote the first statement of the Coulter Principle (Appendix 4). Over the next few days he then added details, as well as thoughts on both what the dimensions of a practical aperture might be and how one might be made. Because the authors of the note in Figure 3.1 did not publish full details of their experiments until 1950, Wallace's expanded description offers interesting insights into his evolving thought processes.[76]

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[73] L. H. Berkhouse et al., *Operation Sandstone 1948*, Report No. DNA 6033F (Washington, D.C.: Nuclear Defense Agency, December 19, 1983), 1. The three bombs were detonated on April 15, April 30, and May 15, 1948.

[74] Examples: "Gain in atomic bomb efficiency seen result of Eniwetok test," *The Boston Herald*, Boston, MA, May 19, 1948, 7; "Eniwetok atom tests show great progress in developing bomb," *The Evening Star*, Washington, D.C., May 19, 1948, A3; "Latest atom weapons vastly better than first types," *The Columbus Dispatch*, Columbus, OH, May 19, 1948, A3.

[75] Fred G. Hirsch et al., "The relationship between the erythrocyte concentration and specific electro conductivity of blood," *Bulletin of the New York Academy of Medicine*, 2nd Series, 24 (June 1948): 393-94.

[76] Frederic G. Hirsch et al., "The electrical conductivity of blood: I. Relationship to erythrocyte concentration," *Blood* 5 (1950): 1017-35; E. Clinton Texter, Jr., et al., "The electrical conductivity of blood: II. Relation to red cell count," *Blood* 5 (1950): 1036-48.

*The Relationship Between the Erythrocyte Concentration and the Specific Electro Conductivity of Blood**

Fred G. Hirsch, Lloyd A. Wood, Ph.D., William C. Ballard, Ph.D., Constance Frey, B.A., and Irving S. Wright

It has long been known that the erythrocyte constitutes an almost perfect non-conductor of electrical current in which characteristic it is almost unique among tissues. Stewart, Oker-Blom, Wilson and others have utilized this characteristic to make possible the determination of the hematocrit and mean cell volume by electrical techniques.

This study is an attempt to utilize the non-conducting property of the red blood cell for the determination by electrical methods of the numbers of erythrocytes per unit volume of whole blood.

The specific conductivity of physiologic saline solutions containing various concentrations of washed red blood cells has been measured on an alternating current bridge and the results plotted against actual red blood cell counts of the samples using the standard technique.

The electrical circuit used employed a variable frequency oscillator which supplied an alternating current to a modified wheatstone bridge with an incorporated Wagner ground. A cathode ray tube was employed as the null indicator. Provision was also incorporated for the use of a cathode ray oscilloscope as a null indicator. The experiments were run at a frequency of 5000 cycles per minute since it was determined experimentally that minimal polarization effects were present under these circumstances. The conductivity cell was of a standard U type employing platinum electrodes.

The empirical results of these experiments revealed the probable existence of a mathematical relationship. This was evolved and appears to be:

$$g = C \times \frac{K_0 - K}{K_0 + K}$$

when

g = concentration of r.b.c. in millions per cu. millimeter.

C = a constant which has a value of 9.62×10^6.

K_0 = the specific conductance of plasma.

K = the specific conductance of whole blood.

The accumulation of experimental data indicates that the formula is valid. By using it we have been able to make satisfactory red cell counts on both saline cell suspensions and heparinized whole blood samples with reasonable accuracy.

It has been found that patients with normocytic anemias have bloods of a higher specific conductivity than do normal people; it also has been shown that patients with polycythemia have blood of a lower specific conductivity than do normals. It has been possible to satisfactorily calculate the red blood cell count on these patients using the specific conductivity as the basis for calculation.

Work is at present in progress to design a satisfactory electronic circuit which will make possible red blood cell counts of a greater accuracy, and with less tedious work than is now possible using present techniques.*

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* The opinions expressed are the personal ones of the authors and may not be construed as official, or as representing the views of the Navy Department as a whole.

This work has been aided by grants from the Eli Lilly Co., Sharp and Dohme Inc., the Albert and Mary Lasker Foundation and the Hyde Foundation through the New York Heart Association, and the U. S. Navy.

Reprinted from BULLETIN OF THE NEW YORK ACADEMY OF MEDICINE
June, 1948, second series, vol. 24, no. 6, pp. 393-394

Figure 3.1. Wallace Coulter's reprint of a crucial note; WHC Papers. This convinced him that his theory about a method for blood-cell counting might be practicable.

This description (Appendix 5) modified both the particle suspension and conductivity cell of standard electro-chemical practice. The particles were required to be ungrouped, that is, individual; of different electrical conductivity than their suspending liquid; and diluted sufficiently that “the particle concentration would be only one particle to 5, 50 or perhaps more equivalent aperture volumes.” These three suspension requirements of the Coulter Principle would enable practical cell and particle counters.

In contrast to traditional conductivity cells, Wallace described a dual-chambered insulative structure with a large electrode in each chamber; the only fluidic and electrical connection between the two chambers was a submerged small aperture, as short as possible, in the dividing wall. These modifications would enable a transient change in electrical resistance between the electrodes as a greater liquid level in one chamber caused individual particles to be carried through the aperture into the other chamber, thus activating a suitable counter in response to the passage of each particle. He proposed using electrical arcs to make apertures in thin mica sheets and provided reasonable estimates for both the resistance of an aperture, scaled to erythrocytes with a diameter D of 20 μm in a substrate of the same thickness L, and the resistance change occurring when a particle of known volume passed through such an aperture.[77] These estimates were purely theoretical.

Meanwhile, the Coulters had received significant reinforcement. While in the ASTP at Ohio State University, Joseph had served with Walter R. Hogg, whose Army discharge occurred on March 10, 1946, some three weeks after his. The two native Missourians were classmates as they earned degrees in electrical engineering from the Illinois Institute of Technology. Hogg soon began volunteering in the Coulters’ basement and helped with the brothers’ amplifier development and their experiments toward blood-cell counting.[78] On August 2, 1948, he witnessed Wallace’s expanded description (Appendix 5), which would become the core of the future patent application on the Coulter Principle.

After reflection Wallace outlined some possible aperture geometries in a single handwritten page (Appendix 6). He had now conceptualized a method he was convinced would allow electrical detection and counting of blood cells or other microscopic particles.

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[77] The metric μm, pronounced and sometimes written “micron” as in Wallace’s notes, is one millionth of a meter or 0.00003937 inch. The plural is either “microns” or “micra.”

[78] “In memory of Walter R. Hogg,” *The Coulter Countdown* 12 (Summer 1982): 3-4. Hogg became the first full-time employee of Coulter Electronics, Inc., in 1958 and the only employee named inventor or co-inventor on more U.S. patents (95) than Wallace (85).

But suitable small apertures remained elusive. While electrical arcs though thin mica sheets did indeed produce small apertures, he found these were both unpredictable in size and erratic in quality. Increasingly frustrated, on October 16 he heated the tip of a carefully sharpened needle and burned a hole in a cellophane wrapper from a pack of Joseph's cigarettes, bound the wrapper to one end of a glass tube with rubber bands, and showed that individual cells in his diluted blood flowing out of the tube through the hole produced a detectable change in an electrical current also flowing through the hole. He later remarked of the cellophane, "It didn't hold up long, but we were able to count some cells."[79] Wallace resigned from Illinois Tool Works to devote more time to experiments and requested samples of commercial films thought to have better water resistance. Of samples from several suppliers, Eastman Kodak's seemed to withstand water exposure best. On October 30, he and Hogg set up a second experiment with a needle-made aperture, this one 3 mils (76 μm) in diameter through the 0.88 mil (22 μm) thickness of a cellulose acetate film from Eastman Kodak (Figure 3.2a). A microscope focused on the aperture allowed visual correlation of the changes in electrical current, displayed on an oscilloscope, that resulted from the passage of blood cells through the aperture under the pressure (or head) of a column of diluted blood a few cm in height above the aperture.[80] Wallace noted (Figure 3.2b), "The cells flowing throu the aperture could be readily seen in the microscope. The electrical pulses which they produced were very distinct on the oscilloscope. The pulse duration was of the order of 1 millisecond. No effort was made to obtain a particular rate of flow or pulses. A dilution of several thousand times was used for the solution." Despite this dilution ratio, multiple cells were seen as they coincidently passed through the improvised aperture.

Those cellular signals, obtained from an aperture nearly four times the 20-μm diameter assumed in his theoretical description, caused Wallace to reconsider aperture dimensions. He sketched a sharp-edged cylindrical aperture having a diameter D of 50 μm and length L of 25 μm and calculated that a dilution of 4,000 to 10,000 times was needed to reduce simultaneous passage of multiple erythrocytes to an acceptable level.[81]

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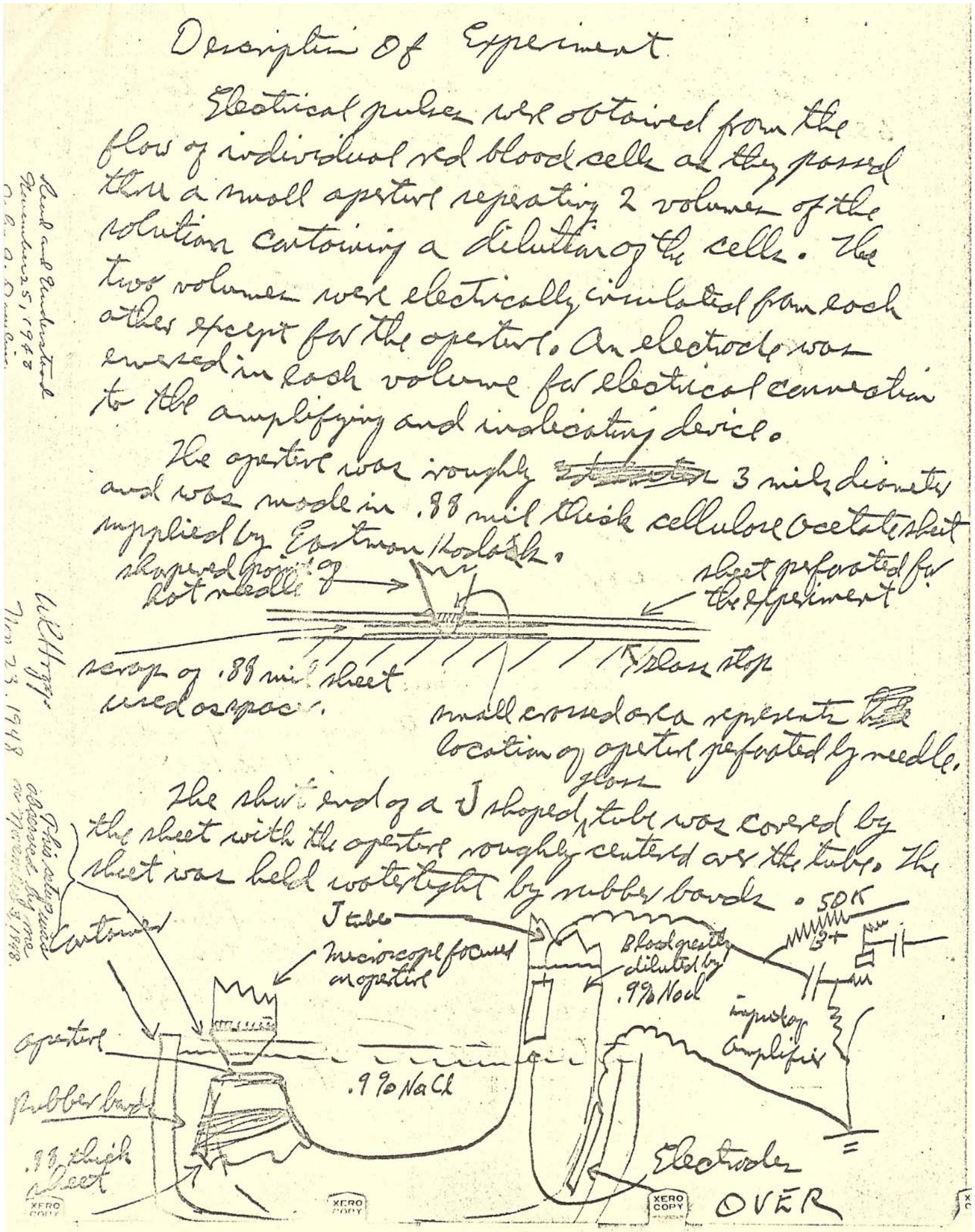
[79] Marshall Don. Graham, "The Coulter Principle: Foundation of an industry," *Journal of the Association for Laboratory Automation* 8 (December 2003): 72.

[80] An oscilloscope is an electronic instrument which displays a changing electrical quantity, such as the cellular pulses, as a visible graph on a display screen.

[81] Wallace Coulter, untitled and undated sketch of aperture with dilution calculations; WHC Papers.



Description Of Experiment

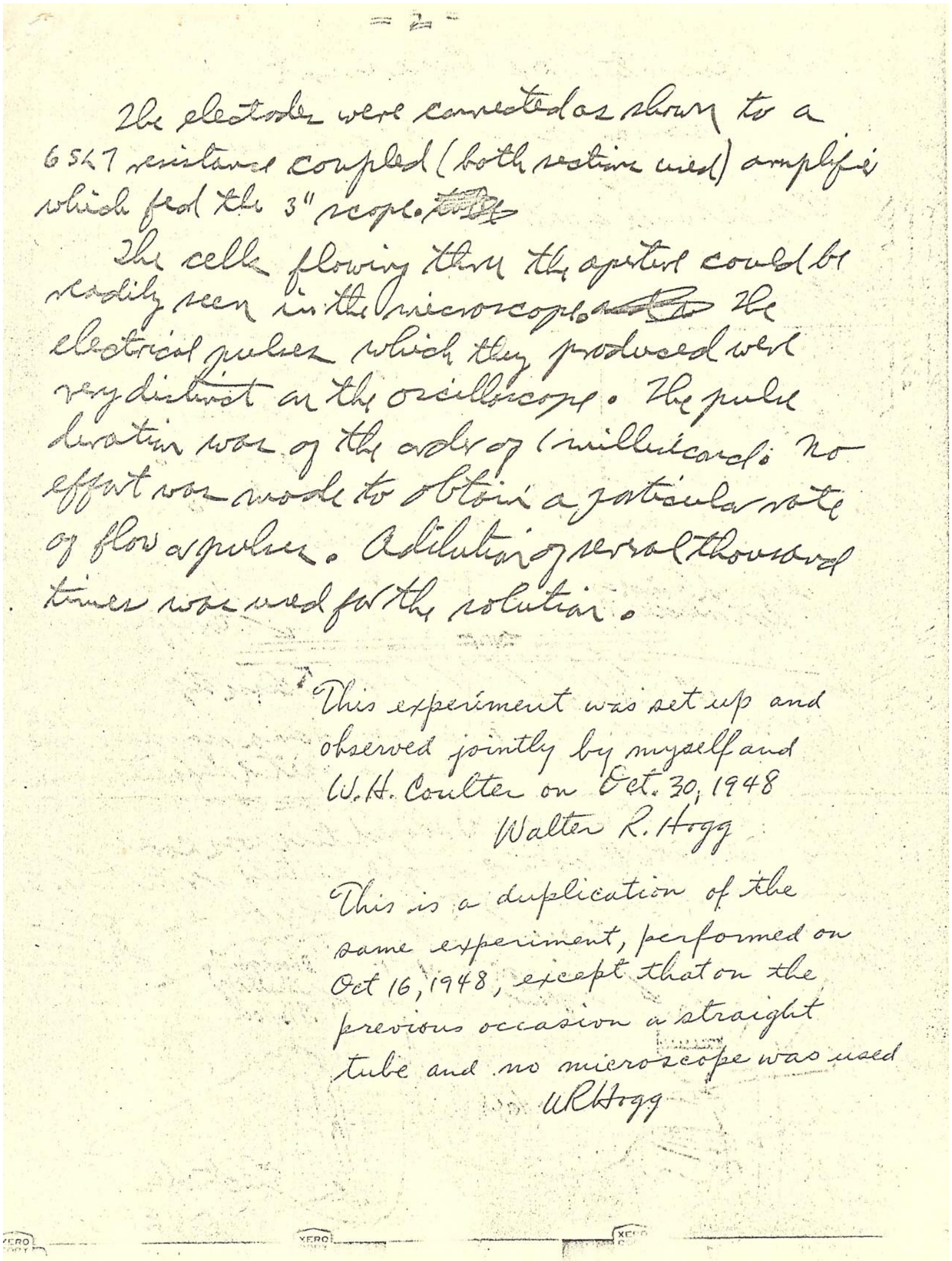
Electrical pulses were obtained from the flow of individual red blood cells as they passed thru a small aperture separating 2 volumes of the solution containing a dilution of the cells. The two volumes were electrically insulated from each other except for the aperture. An electrode was immersed in each volume for electrical connection to the amplifying and indicating device.

The aperture was roughly 3 mils diameter and was made in .88 mil thick cellulose acetate sheet supplied by Eastman Kodak.

The short end of a J shaped glass tube was covered by the sheet with the aperture roughly centered over the tube. The sheet was held watertight by rubber bands.

Figure 3.2a. Photocopy of the obverse of Wallace Coulter's experimental description.[82] This illustrates the second demonstration of the Coulter Principle, done on October 30, 1948. The aperture is indicated in the lower left corner. Please see text for an explanation and Figure 3.2b for the reverse; a transcription of both sides is provided in Appendix 7.

[82] Experimental notes; WHC Papers.



\- 2 -

The electrodes were connected as shown to a 6SL7 resistance coupled (both sections used) amplifier which fed the 3" scope.

The cells flowing thru the aperture could be readily seen in the microscope & the electrical pulses which they produced were very distinct on the oscilloscope. The pulse duration was of the order of 1 millisecond. No effort was made to obtain a particular rate of flow or pulses. A dilution of several thousand times was used for the solution.

This experiment was set up and observed jointly by myself and W.H. Coulter on Oct. 30, 1948
Walter R. Hogg

This is a duplication of the same experiment, performed on Oct 16, 1948, except that on the previous occasion a straight tube and no microscope was used
WRHogg

Figure 3.2b. Photocopy of the reverse of Figure 3.2a. Walter R. Hogg's second addenda is the only record of the first demonstration of the Coulter Principle, done on October 16, 1948. Dimensions of that sensing aperture are not known, but the same electrical arrangement and oscilloscope were used in both October demonstrations.

To do erythrocyte counts, hematology technologists utilized a 100-times diluting pipette to fill a hemocytometer counting chamber, and Wallace realized that two consecutive dilutions with such a pipette would produce a volume of diluted blood much too small to cause it to flow out of a sample tube through the tube's aperture as in the two October experiments. And while those experiments had proven the potential of his evolving Principle, they had also demonstrated the need for better apertures.

Enquiries led Wallace to Sam Gutilla, a glassworker at the University of Chicago (Figure 3.3). Gutilla soon demonstrated that, by raising a pimple on the wall of a heated test tube from inside and then carefully polishing off the external tip of the pimple after cooling the tube, he could create apertures approximately 100 µm in diameter near the tube's closed end. When such tubes were substituted in the setup used in Wallace's October experiments, some gave cellular signals with ten times the amplitude of those the Coulters had obtained with their axial method of photo-electric cell sensing (Appendix 2).[83] The improved signals suggested to Wallace that cellular volumes might be estimated, and he began searching for an attorney to prepare a patent application on his electrical counting method. But the attorneys to whom he spoke failed to grasp the method's underlying principle, and to his disappointment, he was repeatedly told, "You can't patent a hole." [84]

A former co-worker came to his rescue. Eugene Mittelmann had been the director of electronic research and development at Illinois Tool Works while Wallace was employed there. He had accumulated some 20 U.S. patents as sole inventor and, in the process, become acquainted with a number of Chicago's patent attorneys.[85] In late 1948 he introduced Wallace to I. Irving Silverman, who had earned both a B.S. in electrical engineering and a Juris Doctorate law degree and who as an Air Force Captain had during the war participated in the Army Electronics Training Center at Harvard University and Massachusetts Institute of Technology.[86] Silverman immediately recognized the significance of Wallace's unusual method, outlined the legal requirements for a patent

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[83] Graham, "The Coulter Principle: The Arkansas background," 177-178.

[84] Ibid. 177-178.

[85] "I.R.E. People: Eugene Mittelmann," *Proceedings, Institute of Radio Engineers* 35 (1947): 709. A listing of 32 U.S. patents issued to him is included in the WHC Papers. Wallace would later work as Mittelmann's sales manager at Century Steel.

[86] "In memoriam: Patent counsel I. Irving Silverman," *The Coulter Countdown* 14 (Spring 1984): 2.

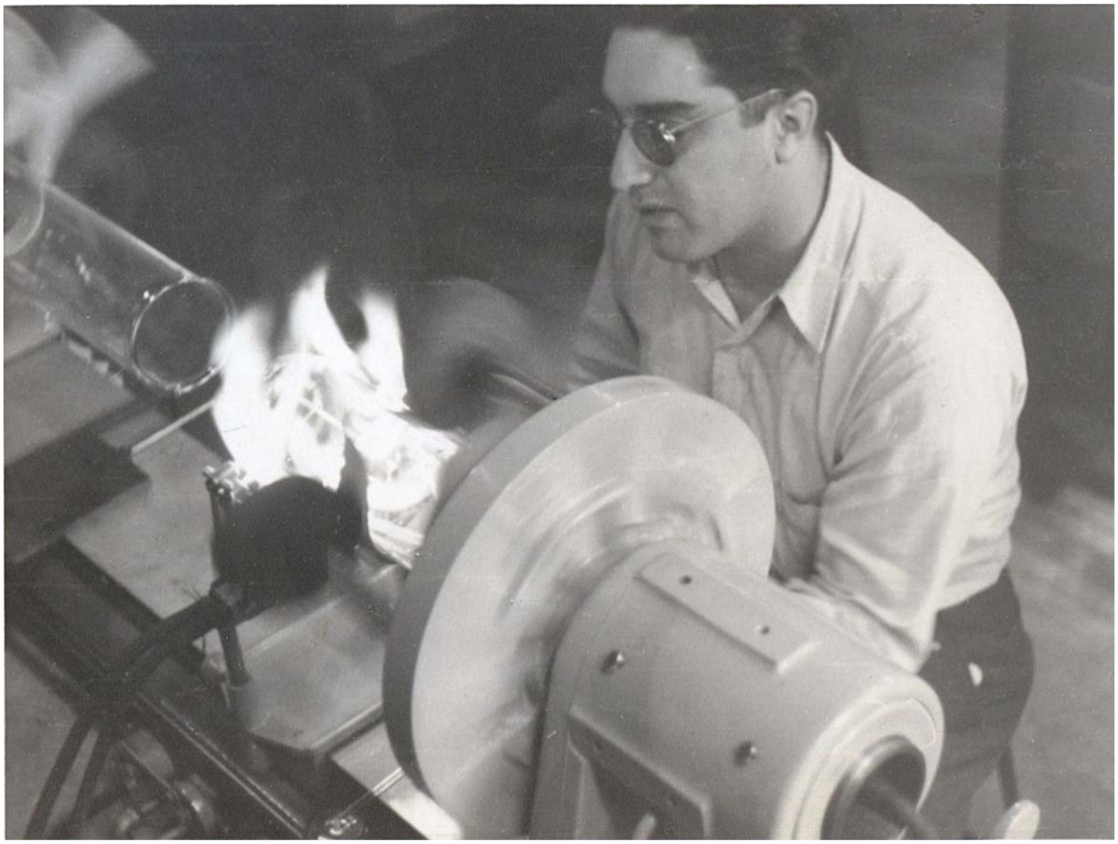

Figure 3.3. Sam Gutilla lathe-forming a glass component in the early 1950s. Gutilla had learned his craft as a young teenager in his uncle's company, had been drafted in 1943 into the U.S. Marine Corp, and because of his exempt skills, been immediately assigned to the Manhattan Project at the University of Chicago's Stagg Field. When the reactor pile was moved to Argonne National Laboratory in Lemont, Gutilla remained with the University. He later founded Delmar Scientific Laboratories, then Fusion Scientific Glass Company, through which he supplied glass components to Coulter Electronics throughout its several reincarnations until 2009.[87] *Photograph courtesy of Sam Gutilla*.

[87] Sam Gutilla, emails to the author, dated June 10, 2005, and September 10, 2008, with the photograph above among those he provided of himself; WHC Papers.

filing, and agreed to prepare and file a patent application with the U.S. Patent Office when Wallace could provide necessary details.

The Coulter brothers had continued development of Wallace's noise-cancelling electrocardiographic amplifiers, and thinking that one of their designs might be patentable, Silverman filed an application on it while Wallace worked on a description of his nascent cell counter (Appendix 5).[88] A patent examiner with whom Silverman interacted also doubted that a hole could be patented, but had the grace to surmise that if examples other than an axial flow of electrical current through an aperture were included, a patent might be allowable on the principle of sensing particles in a constricted current path.[89] Wallace's application, filed on August 27, 1949, included alternative current paths transverse to the suspension flow and apertures of non-cylindrical cross-section, as well as a device in which an insulated needle was mechanically swept past particles in a stationary suspension, a particle's presence being signaled by a pulse in the current between the moving needle and a stationary electrode in contact with the conductive suspending liquid. He expected to solicit developmental support from the U.S. Navy, and patent claims were designed so that his patent rights would not be jeopardized if he accepted Navy monies.

Thus defined, Wallace's germinal patent on the Coulter Principle would issue on October 20, 1953; it is freely accessible.[90] The patent's Figure 1 is a conductivity cell such as prompted Wallace's experiment with a needle and a cigarette wrapper. Gutilla's pinhole aperture was the preferred embodiment of a constricted current path (Figure 6), and sample flows through the aperture were due to suspension heads of a few cm (Figures 1, 6, and 7). No range of particle concentrations or method of measuring the count volume was specified; instead, the indicating method was shown schematically as a rate-meter that would indicate the number of cells passing through the aperture each second (Figure 7). Non-axial constricted current paths (Figures 5 and 10) and apertures of non-cylindrical cross-section (Figures 10 and 13) were illustrated, as well as the mechanical device for sweeping a needle past particles in a stationary suspension (Figure 8).

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[88] The application yielded U.S. Patent 2,622,150 to the Coulter brothers on Dec. 16, 1952. This was the first of several amplifier patents that would result from work done in the Coulters' West Fulton basement (Figure 2.1).

[89] Graham, "The Coulter Principle: Foundation of an industry," 73.

[90] Wallace H. Coulter, "Means for counting particles suspended in a fluid," U.S. Patent 2,656,508, filed Aug. 27, 1949, and issued Oct. 20, 1953. The References have a link.

Wallace's theory had become a principle that would receive U.S. patent protection, but while many of Gutilla's pinhole apertures individually gave good cellular signals, variability in aperture geometry frequently caused excessive variation between signals from different apertures. Moreover, Wallace had seen cells transiting the needle-made aperture of his October 30 experiment (Figure 3.2a), so he knew that useful cell counts required much higher flow rates through an aperture than provided by sample heads of a few cm. And he had yet to determine the practical dilution ratio and volume of diluted blood from which the cell count was taken, both required with high accuracy and repeatability.

Wallace had gained an essential victory, but major technical challenges still lay ahead. Then, two days after his patent application on the Coulter Principle was filed, on August 29, 1949, the Soviet Union exploded its first fission bomb.[91] This surprisingly early potential for atomic attack on the U.S. homeland rippled throughout news coverage and would result some two years later in the widely publicized "Duck and Cover" program in public schools (Figure 3.4).[92] Nor was this the only reminder of the need for urgency.

The U.S. Army was meanwhile developing artillery projectiles based on the Hiroshima bomb and a mobile 280-mm cannon to fire them.[93] On June 25, 1950 the Korean War would begin, and the prospect of nuclear weapons being used in war arose once more.[94] In late November 1950, Chinese troops crossed the Yalu and halted the United Nations' advance into North Korea. On November 30, President Harry S. Truman told reporters that he would take all necessary actions to win in Korea, including using nuclear weapons.[95] Korea's President Syngman Rhee supported bombing North Korea with bombs like those dropped on Hiroshima or Nagasaki, but there was congressional

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[91] Ernest B. Vaccaro, "Atomic explosion inside Russia detected by U.S., says Truman," *The Seattle Daily Times*, Seattle, WA, September 23, 1949, 1.

[92] Felix Cotton, "Bert the Turtle stars in raid defense," *Daily Record*, Boston, MA, December 17, 1951, 42.

[93] Andrew J. Bacevich, *The Pentomic Era: The U.S. Army between Korea and Vietnam* (Washington, D.C.: National Defense University Press Publications, 1986), 81-87; C. G. Sweeting, "Doomsday on Wheels?" *MHQ* 26 (Winter 2014): 98-104.

[94] Roger Dingman, "Atomic diplomacy during the Korean War," *International Security* 13, (Winter 1988-1989): 50-91.

[95] "'Consider' atomic bomb use," *The Kansas City Star*, Kansas City, MO, November 30, 1950, 1; "U.S. forces ready to use A-bomb," *San Diego Union*, San Diego, CA, December 1, 1950, 3, col. 7.

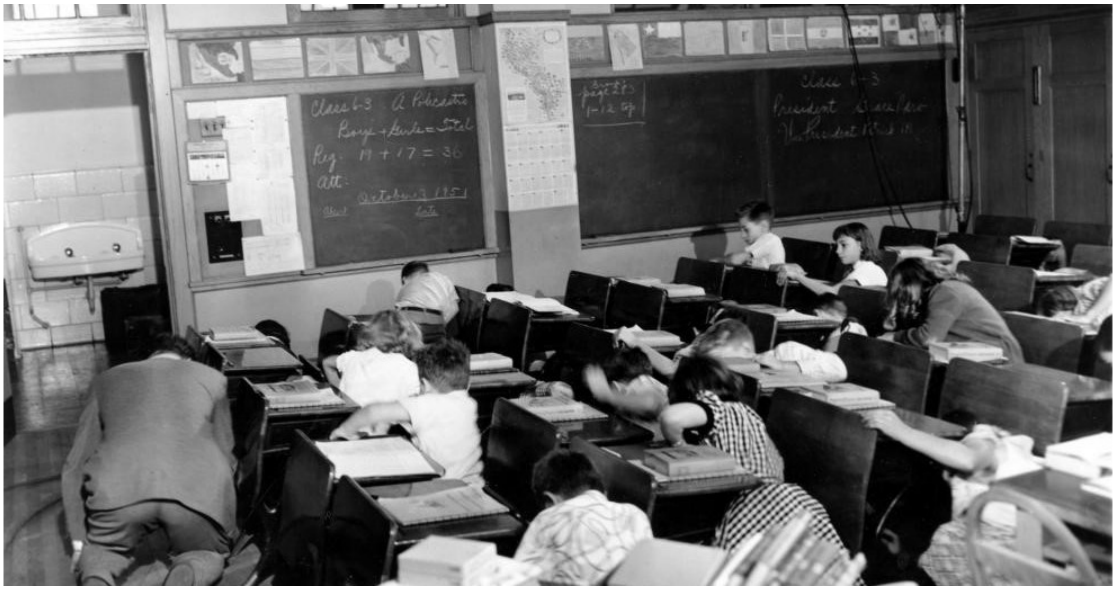

Figure 3.4. “Duck and Cover” illustration.[96] Sixth-grade students at Public School 152, Queens, New York, act out a scene for the film “Duck and Cover” by ducking under their desks as instructed by their teacher. Such exercises were initiated well into the 1960s in many public-school classrooms by the teacher suddenly saying, “Drop.” The image here is adapted from one included on a photo-blog website.[97]

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[96] “Pupils star in Civil Defense movie,” *Sunday Star Pictorial Magazine*, Washington, D.C., December 23, 1951, 10.

[97] Kelly Gonsalves, “America’s era of duck-and-cover: Images from a bygone era of nuclear panic,” image 8, *The Week; Captured: A photo blog*, website accessed October 22, 2018.

disagreement about such use.[98] Then, on 9 December, General Douglas MacArthur, Commander-in-Chief of the United Nations Command, requested commander's discretion to use atomic weapons in the Korean theatre and on 24 December submitted "a list of retardation targets" for which he required 26 fission bombs. Truman stalled a decision and, by mid-April 1951, relieved MacArthur of his command.[99] In interviews published posthumously, MacArthur would say that he would have won the war in ten days: "I would have dropped 30 or so atomic bombs . . . strung across the neck of Manchuria." [100]

To Wallace, and to the researchers pursuing photo-electric cell counting, it seemed as if the unsatisfied need for automated blood-cell counters might all too soon become life-threatening to large civilian populations.

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[98] "Rhee would use 'old style' atomic bombs," *The Seattle Times*, Seattle, WA, December 1, 1950, 8, col. 7; "U.S. lawmakers split on use of dreaded weapon against foe," *San Diego Union*, San Diego, CA, December 1, 1950, 3, col. 4.

[99] Dingman, "Atomic diplomacy during the Korean War," 71-75.

[100] Bruce Cummings, "Korea: Forgotten nuclear threats," *Le Monde Diplomatique*, December 2004; website accessed September 11, 2018.

CHAPTER 4. IMPLEMENTATION

Wallace Coulter had learned from his library research that determination of the cellular concentration in a blood sample required knowing three things: an accurate cell count from a diluted sample, the volume of diluted sample from which the count was obtained, and the dilution ratio by which the count volume was obtained from the original blood sample. The first two requirements determined the design of a practical cell counter, while the third determined the accuracy of the counter's final result. Furthermore, the volume of diluted sample had to satisfy the counter's operational requirements, and its volume flow rate through the counter had to provide acceptable sample processing rates. Now, he needed ways both to determine the suspension volume from which a cell count was made and to increase the flow rate of that volume through the sensing aperture. He also needed apertures that gave consistent cellular signals and a reliable means of providing sufficient diluted blood at accurate dilution ratios. As he later summarized his quandary, "Challenges are good, and we sure had our share of good." [101]

In Wallace's patent application the concentration of particles in any suspension volume was the ratio of a rate-meter's time-averaged count of particles in the volume to that volume's flow rate during the time the count was obtained.[102] However, he had realized that the design of a counter would be simplified if the count volume, rather than the count time, controlled the counter: The concentration of particles in the suspension would then be simply the ratio of the accumulated count to the count volume. He outlined first thoughts in a page of undated handwritten notes, "For Speed Count in a Volume" (Appendix 8). To a sketch of his October 16, 1948, experiment he added two insulated 'needle points' mounted close together on a support the position of which could be adjusted above the liquid in the vessel into which the suspension flowed through the aperture (Figure A8.1). These needle points were separated vertically by a fixed distance determined by the rise of the liquid level in the vessel that corresponded to the desired count volume. As in Figure 3.2a, the diluted sample flowed from inside a vertical sample tube containing a metal electrode through a sensing aperture into a beaker in which a second metal electrode was located. Wallace intended a counter to begin accumulating

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[101] Graham, "The Coulter Principle: Foundation of an industry," 76, col. 1.
[102] Coulter, U.S. Patent 2,656,508, Figure 7 and discussion, col. 10, lines 7-53.

cellular pulses when the liquid contacted the lower needle point and stop when it made a similar contact to the upper needle point. However, in practice the resulting count volumes lacked the repeatability needed for accurate calculation of cellular concentrations.

Providing sample flow rates sufficient to yield acceptable cellular pulse rates was another worrisome challenge. In Wallace's experiments of October 1948, in his patent application of 1949, and in his undated "For Speed Count in a Volume," cellular suspensions flowed through a sample tube's sensing aperture due to the hydrostatic head of the sample within the tube (for example, Figures 3.2a and A8.1). During the October 30 experiment he had watched erythrocytes transit the aperture under a sample head of a few cm and correlated such passages with the pulses seen on an oscilloscope; the cellular pulse rate could have been only a few pulses per second. He used another version of the October 16 experiment to better define the relation between sample head and erythrocyte pulse rate. Volume flow rates through a cylindrical aperture, one mm in diameter in a membrane 23 μm thick and attached to the bottom of a sample tube containing a water column 71 cm in height, were scaled to estimate a cellular pulse rate of 110 per second for a blood dilution giving one cell per ten equivalent aperture lengths and flowing through a similar aperture 25 μm in diameter. This suggested that a hydrostatic head of some 1.78 meters (5 feet, 10 inches), and corresponding volumes of diluted blood, might be needed to generate a cellular pulse rate of a few thousand per second.[103] Such unrealistic suspension column heights and volumes made it impractical to use a sample's hydrostatic head to flow it through an aperture.

To improve suspension flow rates, the Coulter brothers tried motor-driven pumps modeled on medical syringes. While prototype pumps provided useful sample flow rates, the sample volumes required were excessive, and available methods to determine sample count volumes lacked sufficient accuracy and repeatability.[104] Determining count volumes with acceptable accuracy and providing practical suspension flow rates both persisted as serious technical challenges.

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[103] Wallace Coulter, untitled, unsigned, and undated handwritten notes; WHC Papers.

[104] The approach was later patented against future utility: Wallace H. Coulter and Joseph R. Coulter, Jr., "Fluid Metering System and Apparatus," U.S. Patent 3,015,775, filed Jan. 9, 1959, and issued Jan. 2, 1962. It was successfully implemented as a replacement for the mercury volume-control manometer once precision stepper motors became available.

Meanwhile, researchers who were pursuing photo-electric particle detection presented a competitive challenge. Glenn C. Wolf had adapted a photodetector and microscope to view a rectangular capillary channel similar to Kielland's; the cellular suspension was static and a mechanical stage provided a unidirectional scan along the channel.[105] James Hillier had eliminated all mechanical scanning of blood-smear slides by adapting the two-dimensional scanning pattern from a cathode-ray tube as the illumination source.[106] To scan such slides, Sandorff and Foster substituted a mechanical stage that combined simultaneous unidirectional and offsetting motions; the resulting scan path was spiral, the view of the photodetector being limited by an aperture to a cell-sized area.[107] Wolff used a mechanical stage having a unidirectional motion along a hemocytometer, with an offset done after each length scan; the photodetector viewed the chamber through a rectangular aperture about the width of a cell and twice as long.[108] Two prototypes of Wolff's instrument, one scanning a blood-smear slide and the other a hemocytometer, were exhibited at a congress in England during August, 1950.[109] Someone sent Wallace a brief unattributed news item describing those counters.[110] Since his resignation from Illinois Tool Works in 1948, the brothers' living expenses, mortgage payments, costs of parts and materials for experimental work, and patenting costs altogether exceeded Joseph's salary and the occasional income from Coulter Electronics' amplifier contracts. The brothers' funding shortage was becoming a serious challenge to their cell-counting project, and other researchers' progress toward competitive counting instruments focused Wallace's thoughts on finding developmental support.

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[105] Glenn C. Wolf, "Apparatus for the observation and counting of microscopic bodies," U.S. Patent 2,480,312, filed Feb. 20, 1947, and issued Aug. 30, 1949.

[106] James Hillier, "Method and apparatus for electronically determining particle size distribution," U.S. Patent 2,494,441, filed Jul. 28, 1948, and issued Jan. 10, 1950.

[107] P. E. Sandorff and H. W. Foster, "Apparatus for counting blood corpuscles," U.S. Patent 2,584,052, filed Aug. 30, 1949, and issued Jan. 29, 1952.

[108] H. S. Wolff, "An apparatus for counting small particles in random distribution, with special reference to red blood corpuscles," *Nature* 165 (June 17, 1950): 967; Heinz Siegfried Wolff, Marjorie Grace Story, and Derick Jacob Behrens, "Apparatus for counting microscopic particles," U.S. Patent 2,661,902, filed Jan. 10, 1951, and issued Dec. 8, 1953.

[109] C. V. Moore, ed., *Proceedings of the Third International Congress of the International Society of Hematology, Cambridge, England, August 21-25, 1950* (New York: Grune and Stratton, 1951).

[110] "Electronic red blood cell counters," 238-39 of unknown source; WHC Papers.

Because the U.S. Navy had supported the research that redirected his attention to conductivity cells (Figure 3.1), Wallace first described his embryonic blood-cell counter to Lloyd White of the Chicago office of the Office of Naval Research (ONR). Then, on September 13, 1950, he demonstrated an experimental setup for White and Dr. Morris Jones, an ONR microbiologist, and they stated their intent of sending a favorable report to ONR's headquarters in Washington, D.C.[111] On the 14th Wallace contacted the Chicago office of the Atomic Energy Commission and ultimately spoke with William Bigler, assistant to Dr. Walter H. Zinn, director of Argonne National Laboratory (ANL).[112] Bigler suggested contacting Dr. Austin M. Brues, Director of ANL's Division of Biological and Medical Research.[113] Brues was in conference when Wallace called September 15, so he spoke with Brues' administrative assistant, Ms. Jean Gilbert, who requested further information and descriptive literature. Wallace finally awakened traces of interest by describing his cell counter and arguing the value of improved cell-counting instrumentation. After this exasperating interchange, he contacted several other institutions in the Chicago area, but while he received expressions of interest, of encouragement, and of ideas for other potential counter applications, he received none regarding a potential funding source.[114]

During the next few months he prepared a four-page proposal for developmental support (Appendix 9); its third paragraph began (Figure A9.1), "In the event of atomic attack against either the military or the civilian population an accurate, simple, and rapid means of obtaining red blood-cell counts would greatly facilitate the work of the inevitably over-burdened medical personnel in their task of assessing radiation damage to large numbers of casualties." This sentence crystallized Wallace's understanding of both what was needed and its critical importance. A drawing on the proposal's third page accurately reflected his patent application (Figure 4.1). Page 4 of the proposal requested a budget of $17,769.42 for a 34-week developmental effort by the Coulter brothers (Figure A9.3), and

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[111] Wallace Coulter, untitled handwritten notes regarding contacts, September 1950; WHC Papers. These were stapled to the aforesaid news item regarding Wolff's counters.

[112] Greenbaum, *A Special Interest*, prologue and 5-22.

[113] Austin M. Brues, ed., *Quarterly Report, November, December, 1950 and January 1951*, Report ANL-4571, (Division of Biological and Medical Research, Argonne National Laboratory, Chicago, 1951), 7-8.

[114] Wallace Coulter, untitled handwritten notes regarding contacts, September 1950; WHC Papers. Wallace's concerns were increased by U.S. President Truman's suggestion that atomic bombs might be used in Korea; see "Truman A-bomb threat in Korea jolts world," *San Diego Union*, San Diego, CA, December 1, 1950, 1, col. 1.

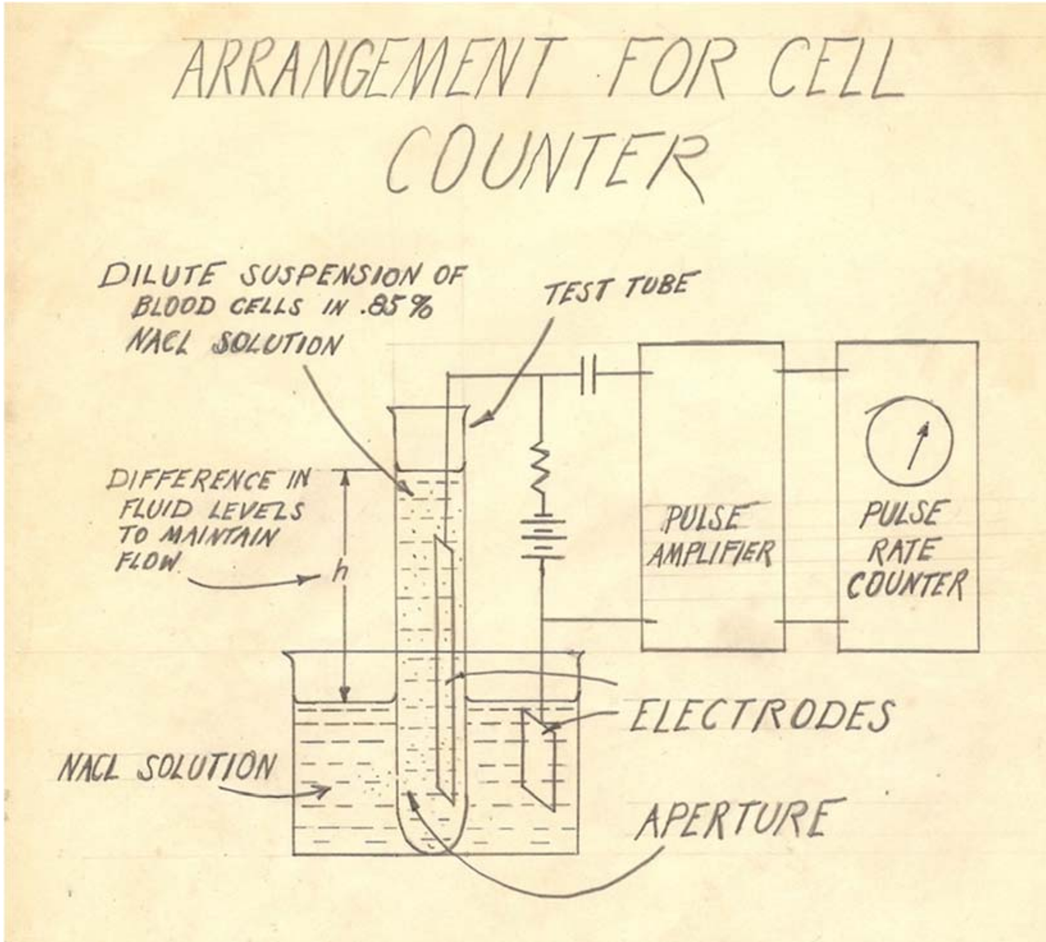

Figure 4.1. Third page of the ANL Proposal.[115] This sketch is an accurate summary of the preferred embodiment (Figure 7) in Wallace's U.S. Patent 2,656,508 on the Coulter Principle. A blood sample was diluted in 0.85% saline (NaCl) solution, and the cellular suspension was poured into a test tube in which Gutilla had made a pinpoint aperture near its lower end. The filled tube was then stood upright in a container of saline solution enclosing an electrode, and a second electrode was placed in the tube. As the difference in liquid heights (the head) caused the cells, indicated by dots, to be carried out of the tube through the aperture, their relative non-conductivity caused a transient decrease in the electrical current flowing between the electrodes from the voltage source, indicated by the connected electrical symbols for a resistance (zig-zag line) and battery.

A capacitor, indicated by the two parallel lines beneath "TEST TUBE," coupled the transient resistance change due to a cell's transiting the aperture to a pulse amplifier while blocking direct current from the battery. The amplified pulses triggered a pulse rate counter, which determined the number per second of cells transiting the aperture.

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[115] ANL proposal draft; WHC Papers. The proposal text appears in Appendix 9, Figures A9.1 through A9.3.

in his transmittal letter of January 26, 1951, to ANL's Ms. Jean Gilbert he offered to discuss the cell counter with anyone who was interested.[116] Gilbert responded February 19, 1951, that while there was interest among the personnel of Brues' Division, there was no present need for such an instrument in its research program. She suggested that he might contact Major Lenox Lohr, the civil defense director for Illinois (Figure A9.4).[117] Lohr seems to have suggested that he contact Dr. Freeman H. Quimby of ONR's physiology branch in Washington, D.C.

On March 6, 1951, Wallace met with Quimby in his Washington office and discussed the content of his ANL proposal. He had similar discussions the next day with Dr. Carl F. T. Mattern and other scientists from the National Institutes of Health (NIH) in Bethesda, MD. Although these discussions raised difficult questions about achievable dilution accuracies and led to changes in the ANL proposal, they were generally encouraging. On returning to Chicago, Wallace retained the emphasis of the ANL proposal as he redrafted it to incorporate some of the suggestions he had received.

He also began a search for a diluting apparatus capable of providing acceptable accuracy at the high dilution ratios his calculations had suggested would be required. In a letter to Gamma Scientific Company, Wallace indicated why he needed blood dilutions of the order of 1:100,000: "For the present a method is required for our own laboratory use. Another and more difficult problem is to find a means that would be suitable for field use as in the armed forces and civilian defense work." [118] No trace of a reply has been found.

On April 30, 1951, Wallace sent to Lloyd White and Morris Jones of the ONR's Chicago office his revised proposal, noting in his letter of transmittal the March meetings in Washington and Bethesda.[119] The proposal (Appendix 10) comprised a single-page overview of Coulter Electronics, a three-page summary of his intended approach to design

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[116] Wallace H. Coulter, carbon copies of letter dated January 26, 1951 and of the accompanying proposal, to Mrs. Jean Gilbert, ANL; WHC Papers.

[117] "City officials panel leaders," *Rockford Register-Republic*, Rockford, IL, October 5, 1950, 8.

[118] Wallace H. Coulter, carbon copy of letter to Gamma Scientific Company, dated April 23, 1951; WHC Papers. According to his first handwritten annotation, he had found the company via a reference to it in B. B. Cunningham, "Microchemical methods," *Nucleonics* 5 (Nov. 1949): 62-85. His second annotation quotes from the first footnote on 70, col. 1.

[119] Wallace H. Coulter, letter to Lloyd White and Morris Jones, ONR, dated April 30, 1951, with accompanying proposal; WHC Papers. Both are transcribed in Appendix 10.

of an erythrocyte counter, and the same single-page budget request he had sent to the ANL (Figure A9.3). Although the summary's description of the operative principle followed that of the ANL proposal (Figure 4.1), it was not illustrated; the text began:

> "The purpose of this proposal is to supply a laboratory model employing the principle as adapted specifically to the counting of red blood cells.
>
> The application to red blood cell counts is proposed because of the need of rapid, more accurate and less tedious means than the present method which requires the skills of highly trained laboratory technicians. As the red blood count is of great significance in detecting and following radiation damage and treatment and as the possibility exists of having an enormous number of radiation casualties in atomic attacks the need of a better method is of critical concern."

White and Jones forwarded Wallace's documents to ONR headquarters in Washington, D.C., and D. E. Gruber of that office acknowledged receipt of the proposal, "To Supply Blood Cell Counter," on May 16, 1951 (Figure A10.1). To help meet expenses, Wallace then began work as a sales manager at the Mittelmann Electronics Division of Century Steel.[120] Late that summer he learned that his ONR proposal had gained favorable reviews from several groups in ONR's technical staff, but had caused concerns in ONR's budget office. Wallace had tried to avoid the latter reaction by having the ONR retain ownership of the single item of capital equipment, a specific digital counter (last item in Figure A9.3), but ONR also had concerns about other aspects of his budget. In a letter drafted September 29 to Dr. Byron Olson, one of the NIH scientists he had met in March, Wallace indicated that it was fairly certain that ONR would be unable to act on his proposal. He noted another researcher's effort to devise instrumentation for aerosol determinations, observed that his aperture method should be more direct and accurate, and wondered whether this might be an application.[121] However, before he sent the letter, one of the NIH scientists phoned to inquire about his proposal's status, and he indicated its apparent failure. As Wallace would later tell it, the NIH scientists then helped him convince the ONR to approve his proposal, but initially only partially fund its budget until he demonstrated his

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[120] Headed by Eugene Mittelmann, who had recommended I. Irving Silverman to file Wallace's patent application on the Coulter Principle. Wallace's work in Mittelmann's area of interest produced two more patent applications; these resulted in his U.S. Patents 2,712,504 and 2,799,216 on rapid liquid pasteurization and U.S. Patent 2,733,604 on an electromagnetic flowmeter.

[121] Wallace H. Coulter, carbon copy of letter to Dr. Byron Olson, drafted September 29, 1951, but unsigned and annotated in Wallace's handwriting, "Not sent." WHC Papers.

counter's feasibility. Partial funding under ONR Contract NONR-1054 (00), "To Supply Blood Cell Counter," would enable the Coulters' to continue their cell-counting efforts.

In the interim, Joseph had uncovered a paper long buried in library stacks, one Wallace felt lucky to acquire. To measure the flight time of cannon projectiles, a Belgian artilleryman had used two electromagnets to start and stop a constant flow of mercury through a small aperture; one electromagnet held a small valve closed until wires across the cannon muzzle were broken by the exiting projectile and the other electromagnet reclosed the valve when the projectile broke wires in the target at the desired range. The calibrated aperture flow rate and the temperature-compensated weight of the mercury that had flowed through the aperture enabled calculation of the projectile's flight time to within a microsecond.[122] With Gutilla's help, Wallace substituted his two level-sensing needles (Appendix 8) for the artilleryman's sensing wires by sealing start, stop, and common electrodes through the glass wall of a mercury manometer designed so that a horizontal mercury flow connected first the count start, then the count stop, electrode to the common electrode.[123] Unbalanced by a vacuum source, the manometer's mercury column, only 0.127 meter high but equivalent to a water column 1.74 meters high, gently drew a controlled count volume of cell suspension from a diluted blood sample through the sensing aperture at practical flow rates as it resumed its equilibrium position. When fully developed (Figure 4.2), such volume-control manometers could consistently provide count volumes of 0.25% accuracy. As Joseph later commented, "It was the manometer that made the counter work. It was simple, it was easy to control, and it kept working." [124]

The Coulters had now resolved two of their technical challenges: how to accurately determine the suspension volume from which a cell count was made, and how to acceptably increase the flow rate of that volume through a sensing aperture. They combined one of Gutilla's manometers, without volume-control electrodes, and one of his pinhole aperture tubes to form a rudimentary sample stand, and Wallace took it and the

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[122] P. Le Boulengé, *The Electric Clepsydra*, trans. J. D. Marvin (Washington, D.C.: Government Printing Office, 1873). The title comes from the Athenian court timer, called a κλεψύδρα (klepsydra) by Aristophanes in his The Acharnians of 424 BCE and The Wasps of 422 BCE; Benjamin Bickley Rogers, *Aristophanes*, vol. I (London: William Heinemann Ltd, 1924), "The Acharnians," line 693 and "The Wasps," line 93.

[123] Wallace Coulter, "Top view of Calibrated Length," unsigned and undated handwritten description of the volume-control portion of a manometer tube; WHC Papers.

[124] Graham, "The Coulter Principle: Foundation of an industry," 74.

Figure 4.2. A volume-control manometer, reduced view at an angle from below.[125] The manometer was first unbalanced by allowing a vacuum to pull mercury upward into the reservoir until the holding bulb was about half empty.[126] When the reservoir port was opened to atmosphere, the mercury resumed its equilibrium position by flowing out of the reservoir and through the holding bulb, first making an electrical connection between the common electrode and the start electrode to activate the counting circuitry and then with the stop electrode to end the count.[127] For blood-cell counting with apertures with diameters D of 50 µm or 100 µm, the control volume between the two electrodes was 500 µl. To accommodate interchangeable sample tubes with apertures of different diameters, some manometers had multiple horizontal U-shaped bends, with additional stop electrode(s) located to allow one of two or three count volumes to be selected from 50, 500 or 2,000 µl control volumes.

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[125] Marketing photograph, WHC Papers.

[126] In much of the Coulters' experimentation, as well as for a number of early counter installations, the vacuum was provided by a low-volume water aspirator connected to the laboratory water supply via a flow regulator and filter. Fluctuating water pressures often led to the aspirator unit being replaced with a small electric vacuum pump.

[127] Wallace H. Coulter and Joseph R. Coulter, Jr., "Fluid Metering Apparatus," U.S. Patent 2,869,078, filed May 9, 1956, and issued Jan. 13, 1959.

Coulters' electronics module (Figure 4.3) to ONR headquarters and demonstrated cellular pulses at practical count rates on an oscilloscope (Appendix 11). Gaining a commitment for his proposed funding, he soon acquired his predetermined counter, a Berkeley Scientific Model 410 (Figure 4.4). The counter facilitated integration of its decade counting modules with the electronics module, and by late 1952 the Coulters had developed a complete preliminary electronics design that also included the necessary elements of an oscilloscope.

Meanwhile, challenges arising in Cold War politics had intensified. Production of the Army's mobile 280-mm atomic cannon began in 1952.[128] On October 3, 1952, Great Britain exploded its first fission bomb, and on November 1 the U.S. would detonate its first hydrogen device.[129] President-elect Dwight D. Eisenhower saw such weaponry as a limit to Communist aims and would have the Army's atomic cannon publicized before including one in his inaugural parade in January 1953.[130] Atomic cannon would be displayed in New York and Philadelphia during Armed Forces Week, this followed by announcements of the test-firing of a fission projectile on May 25, 1953.[131] The unrestricted Grable shot received broad news coverage, and the 15-KT explosion seven miles from the cannon would be followed by execution of the Korean War Armistice on July 27, 1953.[132] In August, the Soviet Union would explode its first hydrogen device, with speculation about its acquiring a knockout capability against the U.S., but President Eisenhower's advisors viewed taxes

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[128] "U.S. producing atomic cannon, magazine says," *The Augusta Chronicle*, Augusta, GA, April 11, 1952, 6D; Elton C. Fay, "Army unveils 12-inch atomic gun," *The Seattle Daily Times*, Seattle, WA, September 30, 1952, 3.

[129] "British make secret atom test," *San Diego Union*, home ed., San Diego, CA, October 3, 1952, 1; Richard Gott, "The evolution of the independent British deterrent," *International Affairs* 39 (April 1963): 238-52; "U.S. admits H-bomb research was included in recent tests," *Richmond Times-Dispatch*, Richmond, VA, November 17, 1952, 1.

[130] "750,000 at Washington see marathon parade," *Springfield Union*, Springfield, MA, January 21, 1953, 1; Sweeting, "Doomsday on Wheels?" 104.

[131] Herbert O. Johansen, "New guns you should know about," *Popular Science* 162 (May 1953): 92-95, 228; "Atomic cannon goes to proving grounds," *Springfield Union*, Springfield, MA, May 19, 1953, 35.

[132] "First atomic cannon blast set for today," *Springfield Union*, Springfield, MA, May 25, 1953, 10; Bill Becker, "Atomic cannon is fired in test," *The Times-Picayune*, New Orleans, LA, May 26, 1953, 1; "Atomic cannon in action," *Cleveland Plain Dealer*, Cleveland, OH, May 26, 1953, 48; Rosemary J. Foot, "Nuclear coercion and the ending of the Korean Conflict," *International Security* 13 (Winter 1988-1989): 92-112.

Figure 4.3. The electronics module from the initial ONR work. It integrated the voltage supply for aperture current and the amplifier for cellular pulses resulting when cells modulated the current as they transited the aperture. The first switch from the right controlled the current to the aperture, and the second one set the pulse amplification. The large center knob controlled the threshold, or level, above which the amplitude of a pulse had to be for it to be counted. Connectors for the aperture current, aperture signal, and an oscilloscope are on the module's rear panel. *Photograph courtesy of William G. Graham.*

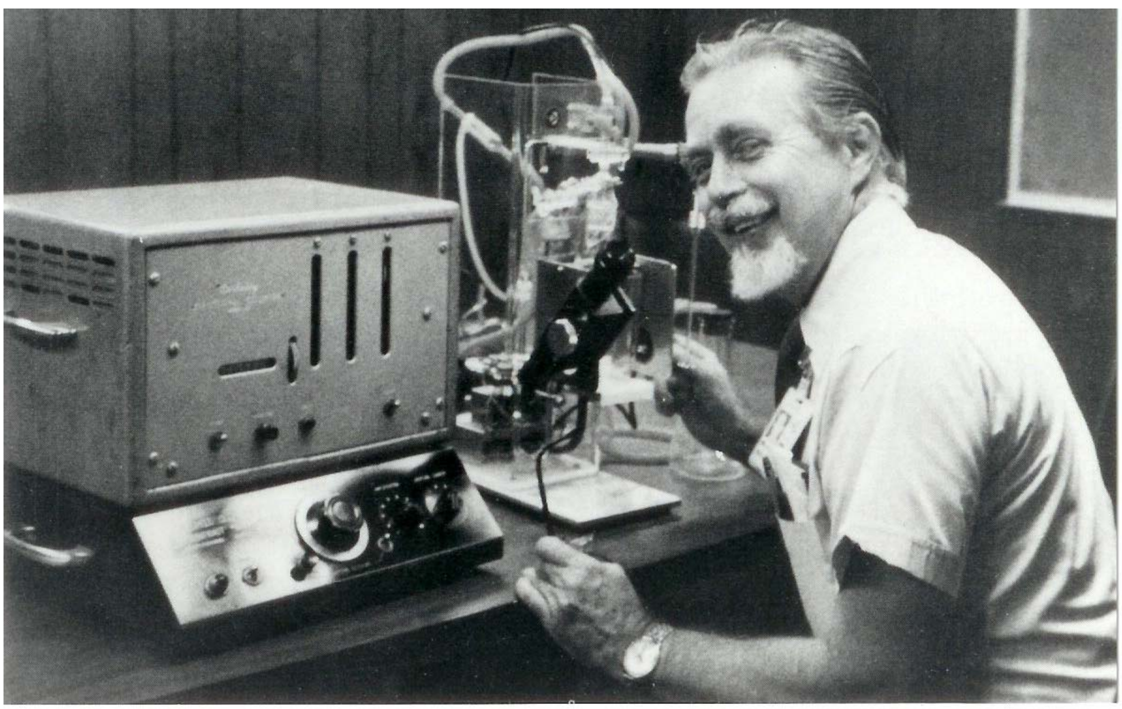

Figure 4.4. Walter Hogg with a reconstruction of the ONR feasibility demonstration.[133] The Berkeley Model 410 counter rests in its intended position on the electronics module of Figure 4.3. Both units were found in a forgotten company closet in the late 1970s, and to illustrate the original setup, Hogg combined them with a 1970s industrial sample stand. The only item missing was the oscilloscope used to display cellular pulses (Figure A11.1).

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[133] "In memory of Walter R. Hogg," *The Coulter Countdown* 12 (Summer 1982): 3.

as “more dangerous than hydrogen bombs.” [134]That October the U.S. would deploy six of the 280-mm atomic cannon in West Germany.[135]

For Wallace, the drumbeat of nuclear news reaffirmed the critical need for automated erythrocyte counting. But in early 1953 Joseph had helped Hogg find a position at Motorola Corporation, and this had slowed instrument development. The Grable shot caused Wallace to resign from Mittelmann Electronics in order to spend more time incorporating their electronics and volume-control manometer into the first integrated instrument (Figure A11.3), which he took to ONR. The demonstration again went well, and to provide the contract’s laboratory model, an experimental counter was carefully assembled (Figure 4.5) and left at ONR for functional testing. On October 20, 1953, Wallace’s patent on the Coulter Principle issued. Now comfortable including all known improvements in a prototype instrument, the Coulters began constructing a cell counter that would be sufficiently robust for evaluation of its clinical performance (Figure 4.6).

But another of the Coulters’ technical challenges remained a significant concern. Although Gutilla’s pinhole apertures in the wall of the interchangeable sample tubes gave useful cellular signals and were durable, for the same blood sample geometric variations in the apertures frequently caused unacceptable disparities in signals from different tubes. To improve aperture geometry, Wallace tried cementing thin glass wafers, cut and polished from small-bore capillary tubing, over larger holes made by polishing away the entire raised area surrounding the pinpoint aperture of Gutilla’s sample tubes, but existing cements often failed and Gutilla’s attempts to flame-fuse such wafers to the sample tubes typically ruined the apertures. Wallace then replaced the glass wafers with ring jewels, made as shaft bearings for gears in Swiss mechanical watches. The shaft holes in such jewels were cylindrical bores polished to precise diameters through a disk of synthetic ruby or sapphire on which the parallel flats were then polished. Available in in a range of

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[134] “Russia explodes hydrogen bomb,” *Springfield Union*, Springfield, MA, August 20, 1953, 1; Joseph Alsop, “Matter of fact: Taxes and H-bombs,” *Lexington Leader*, Lexington, KY, August 25, 1953, 4.

[135] Tom Stone, “30 U.S. A-cannon now in Germany,” *The Times-Picayune*, New Orleans, LA, April 30, 1954, 1. Stone’s title probably exaggerated by 12 the number of atomic cannon actually sent; the Army officially announced that three battalions, each with six of the cannon, had gone or were going to Europe (“A-gun’s shell power can rival Hiroshima bomb, Ridgeway says,” *The Evening Star*, Washington, D.C., May 2, 1954, A41). Furthermore, only 20 of the cannon were produced [R. J. Ritter, “Mk-65 (280mm) atomic cannon – 1953,” *NAAV*, Houston, TX, April, 2007, 10].

Figure 4.5. ONR laboratory model cell counter. This experimental instrument included refinements gained via work with the integrated instrument (Figure A11.3); WHC Papers.

Figure 4.6. Electronics unit of a prototype Coulter Counter® Model A. The appearance differed from that of the first integrated and laboratory units via the panel label and the frame around the oscilloscope display being taller than it was wide. Wallace Coulter works behind the unfinished unit in the Coulters' W. Fulton basement; WHC Papers.

bore dimensions from several Swiss companies, ring jewels had orifices and bores of a given diameter that were virtually identical; if the sharp-edged orifices were acceptably free of chips and cracks as shown in Figure 4.7, such apertures provided cellular pulses consistent in both quality and uniformity when attached to Gutilla's modified sample tubes with a proprietary cement. Sample tubes having apertures of diameter D about 100 μm were used during Wallace's final development of the prototype cell counter.

Accurately providing sufficient blood at an appropriate dilution still remained a serious challenge. In his letter to Gamma Scientific Company, Wallace had indicated its severity: "The bottleneck of the whole operation, insofar as time is concerned, is in taking the sample and making the dilution." [136] And dilutions made with commercial pipettes also varied from pipette to pipette, so yielding inaccurate cellular concentrations. Assigned the volume calculated from the weight of mercury they delivered, individual pipettes could with care dispense volumes accurate to 0.2%.[137] However, the time requirement and technique for making manual dilutions with them continued to be bottlenecks.

A prototype Coulter Counter® Model A (Figure 4.8) went to ONR near the end of 1953. By then the inaccuracy of manual erythrocyte counts had caused their value as a routine clinical tool to be questioned, but interest in leukocyte counts was increasing.[138]

Wallace began considering how the counter might be used for other blood constituents. In addition to low erythrocyte counts, many radiation victims of the Hiroshima and Nagasaki bombings had reduced leukocyte and platelet counts, with degraded capability to fight infections and to form blood clots, respectively.[139] Like the erythrocyte count, both leukocyte and platelet counts typically increased as the bone marrow recovered, but monitoring either recovery would require a new sample dilution and different instrument characteristics. With reference to Table 4.1, normal blood samples contain some 715 times more erythrocytes than leukocytes, and with a threshold setting that eliminated platelet pulses, the counter could provide an acceptable erythrocyte count

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[136] Wallace H. Coulter, carbon copy of letter to Gamma Scientific Company, dated April 23, 1951; WHC Papers.

[137] Cunningham, "Microchemical methods," first footnote under Section B.1. "Apparatus and General Methods."

[138] George Discombe, "The normal blood count," *British Medical Journal* 1 (February 6, 1954): 326-28. This is a citation in undated notes; WHC Papers. See also George Discombe, "Blood-counting by machine," *The Lancet* 270 (August 3, 1957): 240.

[139] Timmes, "Radiation sickness in Nagasaki: preliminary report," 221-23.

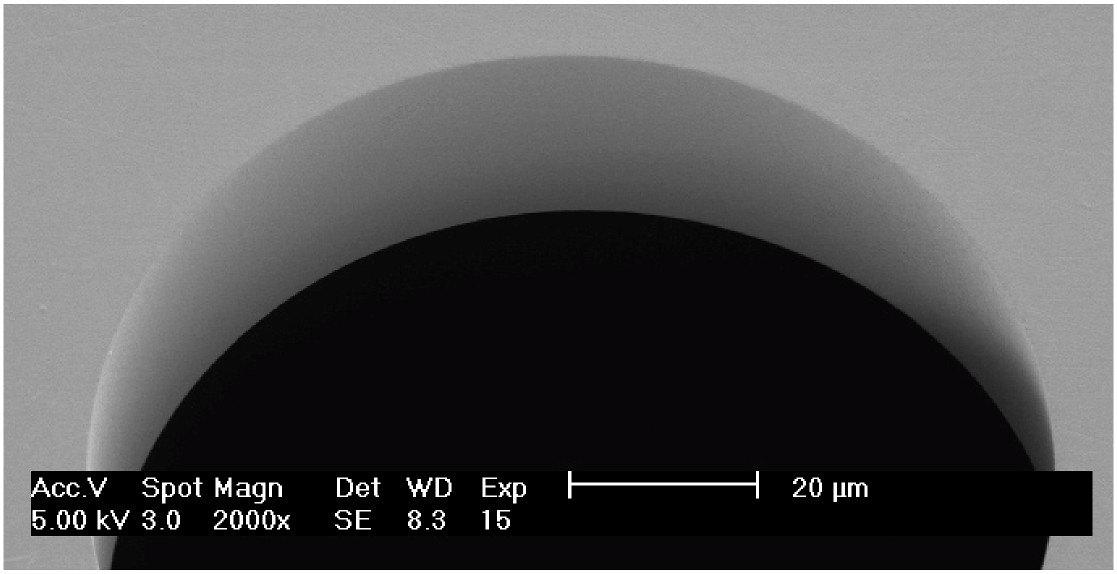

Figure 4.7. The little bit of nothing in a defect-free ruby ring jewel. The diameter D of the aperture's cylindrical bore is 100 µm and its length L is 75 µm; a typical human scalp hair will measure between those dimensions. This scanning-electron micrograph at 2,000x magnification illustrates the quality necessary in ring jewels for their acceptable use as a Coulter sensing aperture.[140] To better show the sharp-edged orifice formed by the square intersection of the bore with the jewel flat, the jewel is tilted at 15 degrees so that the view is down the bore into the mounting material blocking the second orifice. The sharp orifice periphery, aperture bore, and jewel flat are defect-free well below the sub-µm level.

Ruby ring jewels provided a visual contrast with typical samples that facilitated visual monitoring of the apertures for clogs during a sample run. Much of Wallace's developmental work was done with apertures of bore length L approximately equal to their diameter D; cells or particles in samples of appropriate dilution yielded acceptable signals if their equivalent diameters were between about 2% and perhaps 40% of D. Cells or particles smaller than this size range gave signals that were buried in instrument noise, while those larger often gave atypical signal pulses and frequently caused aperture clogs. Signal consistency could sometimes be improved by using apertures having L greater than D; see the following discussion of Figure 4.9. For a given sample, smaller aperture diameters D reduced the probability of two or more cells or particles simultaneously being in the aperture's sensitive volume, but increased the importance of having particle-free electrolyte as the diluent.

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[140] Marshall D. Graham, "Volumetric flow in 100-micra Coulter sensing conduits at 150 mmHg differential pressure," poster manuscript, Figure 1, XXI International Congress, International Society for Advancement of Cytometry, May 4-9, 2002, San Diego, CA. The poster abstract, sans the figure above, appears in *Beckman Coulter Bulletin 9283; Abstracts from ISAC XXI International Congress, May 4-9, 2002, San Diego, CA* (Brea, CA: Beckman Coulter, Inc., 2002), 12-13.

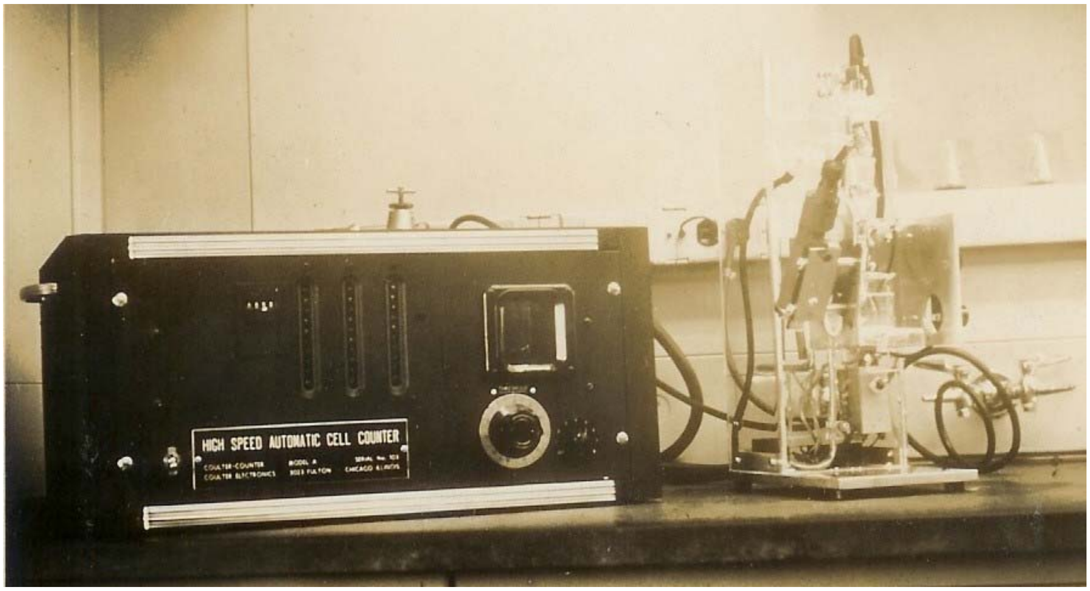

Figure 4.8. A prototype Coulter Counter® Model A.[141] The panel label identified the electronics unit as "Coulter Counter…Model A…Serial No. 101" and gave its origin as "Coulter Electronics…3023 Fulton…Chicago, Illinois." In contrast to the experimental instrument (Figure 4.5), the volume-control manometer, visible as the U-shaped tube inside the sample stand, had two count-control volumes selectable by the shielded switch beneath the stand's sample platform. The microscope for monitoring the condition of the sensing aperture is mounted on the left side of the sample shield around the sample platform.[142] Unfortunately, the two stopcocks near the top of the stand that controlled vacuum application and rinsing electrolyte are just barely visible (see Figure 5.2).

The electronics unit included 33 vacuum tubes plus an oscilloscope tube to process, count, and display pulses generated by cells (or particles) drawn through the sample tube's aperture as mercury flowed between the manometer's volume-control electrodes. Glassware for the electrolyte supply and waste collection is not shown. This photograph was taken in a government laboratory and is from the JRC Files. *Photograph courtesy of Ms. Laura Coulter Jones.*

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[141] To simplify the following text, Coulter Counter® will refer to a counter based on the Coulter Principle, while specific Coulter Counter® models will be indicated as the Model x counter, where x is a designator such as A, B, C, and so on.

[142] The microscope was a modified rifleman's spotting telescope bought as military surplus. Wallace had an optician design a housing carrying a collimating lens of short focal-length and containing a prism to bend the line-of-sight by 90 degrees. The housing was mounted in front of the telescope objective so that light from the illuminated Coulter sensing aperture could travel through the collimating lens and prism into the telescope, which formed a magnified image of the aperture.

Table 4.1. Constituents of normal human blood.[143] Values appearing below are illustrative; actual values may depend on the age and sex of the donor, as well as details of practice at the facility doing the analysis.[144] In whole blood, erythrocytes are biconcave discoids about 8 μm in diameter, whereas the equivalent spherical diameters of leukocytes typically range between 7.6 and 10.4 μm (bolded entries); volumes of the rare eosinophils and basophils overlap those of neutrophils and monocytes. The equivalent spherical diameters of platelets usually range between 2.6 and 2.9 μm.

Blood Constituent	Count, per μl		Volume, μm^3	
	Mode	Range	Mode	Range
Erythrocytes	5,000,000	3,500,000 – 5,900,000	90	80 - 100
Leukocytes	7,000	4,500 - 11,000		
neutrophils	3,500	1,800 - 7,700	468	444 - 492
lymphocytes	2,850	1,000 - 4,800	247	**229** - 265
monocytes	400	0 - 800	534	487 - **579**
eosinophils	250	0 - 450		
basophils	100	0 - 200		
Platelets	300,000	150,000 - 450,000	11	9.5 - 12.5

[143] Errett C. Albritton, ed., *Standard Values in Blood*, 5, 22, and 37. The volume data for leukocytes is from Zipursky et al., “Leukocyte density and volume in normal subjects and in patients with acute lymphoblastic leukemia.”

[144] Plum, “Accuracy of hæmatological counting methods,” 342-64; Berkson et al., “The error of estimate of the blood cell count as made with the hemocytometer,” 309-23.

from a 1:50,000 dilution made without first removing the leukocytes or platelets. While erythrocytes have a modal cellular volume of about 90 cubic µm (µm^3), the five subpopulations of normal leukocytes vary in cellular volume from about 230 µm^3 for lymphocytes to about 580 µm^3 for monocytes. The counter's pulse amplification could be adjusted downward by some 80% to accommodate the greater pulse amplitudes produced by the larger leukocytes, but for a count accuracy equivalent to that of an erythrocyte count, the sample's erythrocyte content would first need to be reduced to about ten cells per µl before making a 1:70 dilution. Wallace thought that differences in the specific gravities of cellular types might enable erythrocyte depletion by centrifugation and combed through hematology reference works.[145] He found promising data for erythrocytes, but nothing comparable leukocytes.[146] Persistent as always, he directed his research toward the possibility of counting platelets.

As indicated in Table 4.1, normal blood samples contain some 17 times more erythrocytes than platelets, with an individual volume about eight times greater. A dilution of 1:3,000 to provide a platelet concentration equal to that of erythrocytes in a 1:50,000 dilution would leave some 1,670 erythrocytes in the diluted sample. Wallace knew that pulse amplitudes from platelets comparable to those produced by erythrocytes would require some combination of significantly increased pulse amplification and significantly reduced diameter D of the sensing aperture. However, electronic noise limited useful pulse amplification, and the increased likelihood of erythrocytes clogging suitable smaller apertures made it preferable to remove them before dilution. Wallace located a centrifugation method that seemed promising for separating platelets from normal blood samples, but he lacked access to a centrifuge to test whether it was practicable.[147] Instead of the answers he had hoped to find, he had only found more challenges.

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[145] Wallace Coulter, reference list entitled "Jan 14 54 Ill Med School Library"; WHC Papers.

[146] Albritton, ed., *Standard Values in Blood* (Philadelphia, PA: W. B. Saunders & Co., 1952), 5 and 37. In 1954 separation methods could not reliably provide comparable data for leukocytes; see, for example, Alvin Zipursky et al., "Leukocyte density and volume in normal subjects and in patients with acute lymphoblastic leukemia," *Blood* 48 (September 1976): 361-71.

[147] Wallace Coulter, untitled and undated reference list regarding platelets; WHC Papers. Allen H. Minor and Lee Burnett, "A method for separation and concentrating platelets from normal human blood," *Blood* 7 (July 1952): 693-99; Mario Stefanini and William Dameshek, "Collection, preservation, and transfusion of platelets," *New England Journal of Medicine* 248 (May 7, 1953): 797-802.

On March 1, 1954, the drumbeat of nuclear news began to crescendo. By then the Soviets had exploded at least seven fission bombs and one hydrogen device, while Great Britain had tested three fission bombs.[148] On that date, the U.S. Army announced that a third atomic cannon battalion and its six 280-mm cannon would soon be sent to Europe, and the Atomic Energy Commission (AEC) announced the Operation Castle nuclear tests at Bikini Atoll in the Marshall Islands.[149] The first of these, Castle Bravo, was the 48th nuclear detonation by the U.S., but it exposed the poor preparation of those heading U.S. development of thermonuclear weapons.[150] Fusion of the bomb's dry thermonuclear fuel, the lithium-6 isotope, was intended to cause fission of its uranium-238 jacket, with the total explosive yield expected to range between four and eight million tons (MT) of TNT. However, about 60% of the bomb's lithium content was the lithium-7 isotope, which the bomb's designers mistakenly assumed would be inert, and the lithium yield alone was later estimated at 5 MT. Uranium-238 fission brought the total yield to 15 MT while creating strongly radioactive atomic fragments.[151] The explosive yield was about 1,000 times greater than for either of the Hiroshima or Nagasaki fission bombs, with significantly worse fallout, and effects of the latter were made worse by failures in weather forecasting, the failure to postpone the test following unfavorable changes in wind direction, and the failure to conduct precautionary pre-test evacuations. Fallout from the unexpectedly high yield blanketed 236 Marshall Islanders on three other atolls and 28 U.S. servicemen manning a weather station on a fourth. In addition, the crew of the Japanese trawler *Lucky Dragon*, some 95 statute miles from the explosion and well outside the official danger area, received fallout burns that hospitalized all 23 members, one of whom died.[152] At least two other Japanese fishing boats were contaminated, and tons of fish later caught in waters

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[148] Nils-Olov Bergkvist and Ragnhild Ferm, *Nuclear Explosions, 1945-1998*, FOA-R-00-01572-180-SE (Stockholm: Defence Research Establishment, July 2000), 8, 10, 14.

[149] "More atomic cannon to go to Europe," *San Diego Union*, San Diego, CA, March 2, 1954, 3, col. 2; "New atomic blasts start in Pacific; details kept secret," ibid. 1.

[150] Arlana Rowberry, "Castle Bravo: The largest U.S. nuclear explosion," website accessed June 14, 2018.

[151] "Operation Castle; Castle Bravo," Nuclear Weapon Archive, website accessed June 14, 2018.

[152] "Atomic explosion test danger area extended by AEC," *San Diego Union*, San Diego, CA, March 25, 1954, A3, col. 1; "Fisherman describes atom blast," ibid. A3, col. 8; "Japanese fisherman dusted by U.S. hydrogen bomb dies," *Fort Worth Star-Telegram*, Fort Worth, TX, September 24, 1954, 1.

contaminated by fallout proved radioactive and required safe disposal.[153] Nor did distance guarantee avoidance of fallout: 92 crewmembers of the *USS Patapsco* received significant radiation exposure from fallout although the tanker was some 650 statute miles from Bikini. Only later did the fact emerge that the death zone from radioactive fallout "covered a cigar-shaped area up to 7,000 square miles." [154] News coverage of Operation Castle and its consequences would continue for years; as of 2016 Bikini Atoll still had areas with radiation levels greater than thought safe for human habitation.[155]

Meanwhile, Wallace had found a note on spherical latex particles of uniform 0.259-µm diameter.[156] Curious if similar particles might provide a calibration method for cellular volumes, he obtained some of the largest polystyrene latex particles that Dow Chemical Company had yet made, 1.1 µm in diameter and later used as calibration standards in microscopy, and began experimenting with them.[157] At first, noise in the counter signal obscured the particle pulses, and he sought larger particles while working to reduce the noise.[158] He substituted a special transformer in another prototype Model A counter that, prior to July 3, 1954, went to Lt. Col. Joseph H. Akeroyd at the Walter Reed Medical

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[153] Richard C. Cuddihy and George J. Newton, *Human Radiation Exposures Related to Nuclear Weapons Industries*, LMF-112/UC-48 (Albuquerque, NM: Inhalation Toxicology Research Institute, 1985), 107-18; "Japan undergoes another scare," *The Evansville Sunday Courier and Press*, Evansville, IN, October 10, 1954, 6.

[154] Edwin Diamond, "1954 tests show U-bomb surpassing estimates," *The Oregonian*, Portland, OR, March 6, 1955, 12.

[155] Thomas Kunkle and Byron Ristvet, *Castle Bravo: 50 Years of Legend and Lore*, DTRIAC SR-12-001 (Kirtland Air Force Base, NM: Defense Threat Reduction Information Analysis Center, 2013); Autumn S. Bordner et al., "Measurement of background gamma radiation in the northern Marshall Islands," *Proceedings of the National Academy of Sciences* 113 (2016): 6833-38.

[156] Wallace Coulter, undated note on a scrap of a planning form; WHC Papers. The first of two references cited Robert C. Backus and Robley C. Williams, "Small spherical particles of exceptionally uniform size," *Journal of Applied Physics* 20 (1949): 224-25.

[157] Wallace H. Coulter to Carl T. F. Mattern, letter dated February 5, 1955 (Figure 4.9); WHC Papers. Dow Chemical Company announced a range of larger latex particles at the 12th Annual Meeting of the Electron Microscope Society of America, Highland Park, IL, October 14-16, 1954; see E. B. Bradford and J. W. Vanderhoff, "Electron microscopy of monodisperse latexes," *Journal of Applied Physics* 26 (July 1955): 864, *fn. Wallace's 1.1-µm particles came from Lot LS-067-A, Tables VIII and IX, page 870.

[158] Wallace Coulter, notes on first page of U.S. Patent 2,609,256, "Describes method of producing uniform spheres down to 5 microns, refers to copending application covering same"; WHC Papers. This patent, entitled, "Ball Bearing," was filed by William O. Baker and Field H. Winslow on April 28, 1951, with issuance on September 2, 1952.

Center, then the U.S. Army's flagship medical facility, for appraisal as a leukocyte counter.[159] However, the transformer provided little improvement, and Wallace considered other approaches. Uncertain whether some noise arose in electrochemical effects due to the aperture excitation current interacting with the electrodes in the sample vessel and aperture tube, he imagined adding one or two voltage-sensing electrodes either in or near the aperture through which negligible current passed while the excitation current passed through the usual electrodes. The concept was valid, but difficult to implement.[160]

While Wallace was working to reduce noise in counter signals, ONR functional testing of the experimental and prototype counters had progressed favorably, and for his first three production instruments, in late 1954 he ordered 12 sample tubes and three volume-control manometers from Gutilla.[161] When functional testing of the prototype counter (Figure 4.8) was completed, ONR requested Dr. Carl F. T. Mattern, of NIH's National Institute of Allergy and Infectious Diseases, to evaluate its clinical performance, work for which Dr. Freeman H. Quimby arranged ONR's partial support.[162] Mattern had begun working with the ONR experimental instrument (Figure 4.5), but finding some performance differences, he seems to have used the prototype instrument for much of his clinical evaluation. Dr. George Brecher, of NIH's Clinical Center and National Cancer Institute, and Lt. Col. Joseph H. Akeroyd, of Walter Reed's Clinical Hematology Department, were among those whose help would be acknowledged." [163] Mattern would later lend Brecher one of the two ONR counters for a second evaluation, and as noted above, Akeroyd had received another prototype Model A counter in early 1954.

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[159] Wallace Coulter, two pages of handwritten notes regarding a low-capacity filament transformer, "used only on unit at Walter Reed," dated July 3, 1954; WHC Papers.

[160] Graham et al., "Potential-sensing method and apparatus for sensing and characterizing particles by the Coulter Principle," U.S. Patent 6,175,227, issued Jan. 16, 2001.

[161] Wallace Coulter, undated pencil sketches of an aperture tube and the calibrated length of a volume-control manometer, the first with the heading, "12 ordered late '54 for 1st lot of 3 units," and the second, "For 1st lot of 3, late '54"; WHC Papers.

[162] Carl F. T. Mattern, Frederick S. Brackett, and Byron J. Olson, The determination of number and size of particles by electrical gating: Blood cells," *Journal of Applied Physiology* 10 (January 1957): 56, fn 4, and 70, col. 1.

[163] For experimental model, ibid. 56, fn 4; 63, col. 1, first complete paragraph; and 67, col. 1, first complete paragraph; for acknowledgements, 70, col. 1.

As Mattern progressed through his evaluation, Wallace replied to some of his notes and comments (Figure 4.9).[164] This seems to follow points in one of Mattern's letters, now unavailable, and might be confusing. However, it is a rare example of Wallace personally documenting such technical details in his own individualistic style and for this reason alone deserves a careful reading. The following overview is intended to aid understanding.

The spatial distribution of a sensing aperture's electrical excitation current and suspension throughflow depends in a complex manner on the diameter D and length L of the aperture bore, which together provide sufficient information that the spatial distribution of electrical current can be defined analytically. However, these geometric parameters are insufficient to allow accounting for the inertia and volume continuity of suspension passing through the aperture, and analytic methods addressing such liquid aperture throughflows require that multiple assumptions be made (Appendix 12). In the first sentence of his second paragraph, Wallace recognized that a cell's presence might change the excitation current in a suspension volume which extended outside both ends of the geometric volume defined by the aperture's bore diameter D and bore length L; he wondered if the two external sensitive regions were not each approximately a hemisphere centered on the aperture axis and extending outward on the surface surrounding the aperture, as sketched in the left margin of the paragraph. If so, the radius of the hemispherical surface should scale with the aperture diameter D, and so far, this was an acceptable description. But contrary to his second sentence, the surface of such hemispheres is the locus of points having an equal voltage, not an equal density of electrical current. His third sentence, completed with the phrase handwritten above the first paragraph, has an analogous misunderstanding of suspension flows: The hemispherical surface on the entry side of the aperture is the locus of points of equal pressure, not of equal flow velocity. Otherwise, his impressions of the aperture's sensitive volume and its entering flow were accurate.

Wallace's third paragraph notes Mattern's belief that the sensitive volume was different for the large and small apertures in the Swiss ring jewels cemented on the ONR sample tubes. Because he would have expected the sensitive volume to scale with aperture diameter D, Wallace interpreted this to mean something more underlay Mattern's observations than a difference in designated aperture diameter. Moreover, as indicated in Wallace's sixth paragraph, Mattern had used flow measurements to evaluate whether

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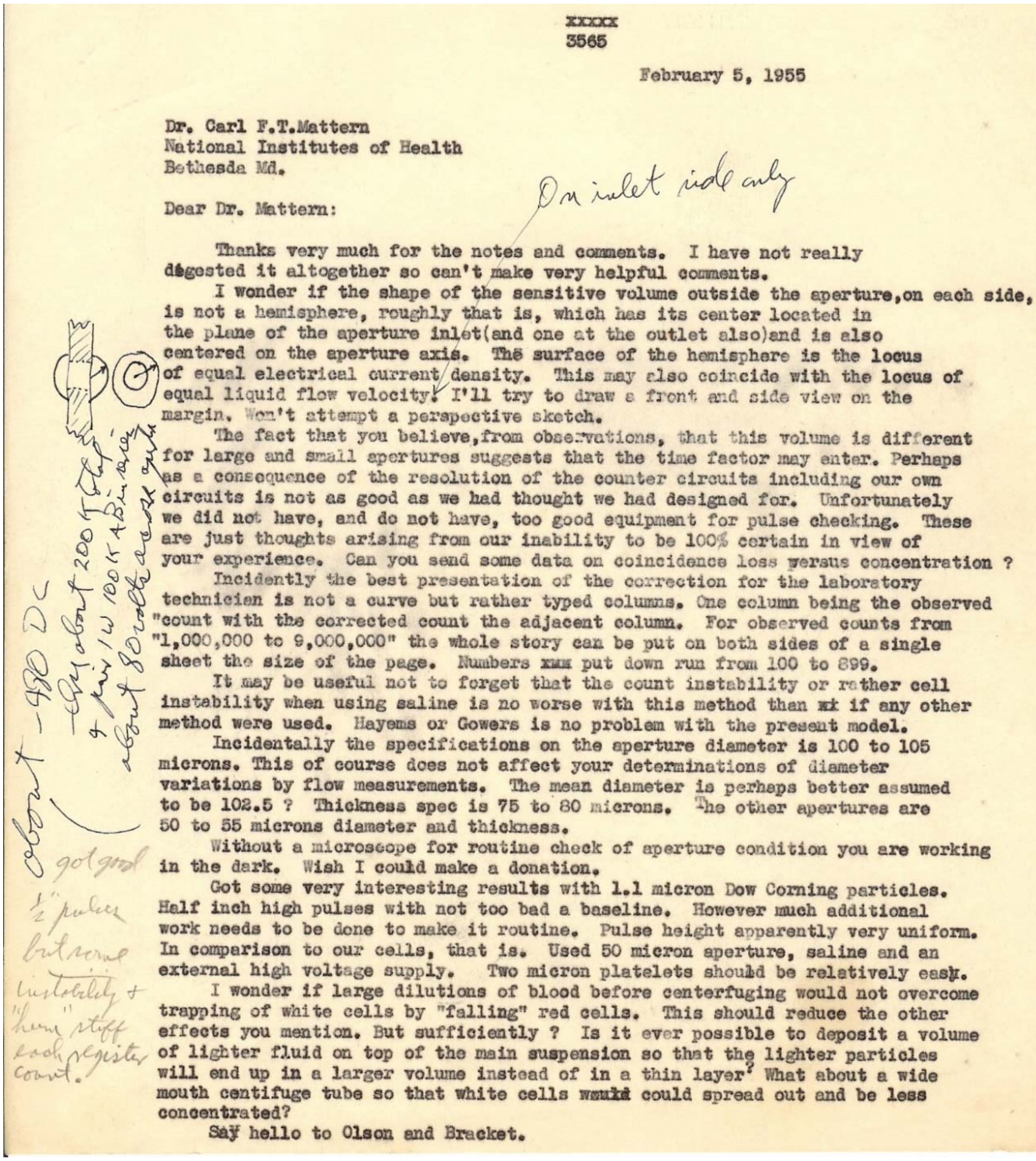
[164] Wallace Coulter, carbon copy of typed letter to Dr. Carl F. T. Mattern, dated February 5, 1955; WHC Papers.



xxxxx
3565

February 5, 1955

Dr. Carl F.T.Mattern
National Institutes of Health
Bethesda Md.

Dear Dr. Mattern:

Thanks very much for the notes and comments. I have not really dégested it altogether so can't make very helpful comments.

I wonder if the shape of the sensitive volume outside the aperture,on each side, is not a hemisphere, roughly that is, which has its center located in the plane of the aperture inlet(and one at the outlet also)and is also centered on the aperture axis. The surface of the hemisphere is the locus of equal electrical current density. This may also coincide with the locus of equal liquid flow velocity. I'll try to draw a front and side view on the margin. Won't attempt a perspective sketch.

The fact that you believe,from observations, that this volume is different for large and small apertures suggests that the time factor may enter. Perhaps as a consequence of the resolution of the counter circuits including our own circuits is not as good as we had thought we had designed for. Unfortunately we did not have, and do not have, too good equipment for pulse checking. These are just thoughts arising from our inability to be 100% certain in view of your experience. Can you send some data on coincidence loss versus concentration ?

Incidently the best presentation of the correction for the laboratory technicien is not a curve but rather typed columns. One column being the observed "count with the corrected count the adjacent column. For observed counts from "1,000,000 to 9,000,000" the whole story can be put on both sides of a single sheet the size of the page. Numbers xxx put down run from 100 to 899.

It may be useful not to forget that the count instability or rather cell instability when using saline is no worse with this method than xx if any other method were used. Hayems or Gowers is no problem with the present model.

Incidentally the specifications on the aperture diameter is 100 to 105 microns. This of course does not affect your determinations of diameter variations by flow measurements. The mean diameter is perhaps better assumed to be 102.5 ? Thickness spec is 75 to 80 microns. The other apertures are 50 to 55 microns diameter and thickness.

Without a microscope for routine check of aperture condition you are working in the dark. Wish I could make a donation.

Got some very interesting results with 1.1 micron Dow Corning particles. Half inch high pulses with not too bad a baseline. However much additional work needs to be done to make it routine. Pulse height apparently very uniform. In comparison to our cells, that is. Used 50 micron aperture, saline and an external high voltage supply. Two micron platelets should be relatively easy.

I wonder if large dilutions of blood before centerfuging would not overcome trapping of white cells by "falling" red cells. This should reduce the other effects you mention. But sufficiently ? Is it ever possible to deposit a volume of lighter fluid on top of the main suspension so that the lighter particles will end up in a larger volume instead of in a thin layer? What about a wide mouth centifuge tube so that white cells would could spread out and be less concentrated?

Say hello to Olson and Bracket.

Figure 4.9. Wallace Coulter's response to Mattern's communication.[165] This contains the first description of an aperture's sensitive volume and suggests the important role of aperture tolerancing in counter operation; an explanation of the marginal entries is provided in the text.

[165] Carbon copy; WHC Papers. Mattern replicated Wallace's sensitive-volume sketch in Fig. 4 of "The determination of number and size of particles," 58. He defined this volume as that in which presence of multiple cells would cause a cellular signal differing in amplitude or shape from one caused by a single cell; for 100-µm apertures, it appeared to be about three times the aperture's geometric volume (58, col. 2).

variations in aperture diameters within a nominal diameter designation might be the cause. Wallace supported Mattern's belief and provided a partial explanation by stating that the larger apertures had a specified bore diameter D of 100 to 105 µm, with a specified bore length L of 75 to 80 µm, while both the diameter D and bore length L of the smaller ones were specified to be between 50 and 55 µm. Such diametrical variation within both the larger and smaller apertures would affect the sensitive volumes and could be detected by appropriate flow measurements. However, another aspect of aperture geometry could also influence apparent sensitive volumes: The different L/D ratios for the two aperture sizes (0.71 to 0.80 for the 100-µm apertures and 0.91 to 1.10 for the 50-µm ones) would cause different distributions of the excitation current and sample flow within the aperture sensitive volumes, potentially causing cellular pulses to differ significantly in amplitude and shape as a result. Wallace also acknowledged that the time resolution of the counter's circuit design may have caused different responses for such pulses from the two aperture sizes, and to compare the coincidence corrections he was developing (Appendices 5 and 12), he requested Mattern to send him data on count loss due to multiple cells simultaneously being in the aperture's sensitive volume.

The eighth paragraph of the letter summarizes results of Wallace's experiments with 1.1-µm latex particles and indicates his progress toward noise reduction in counter signals. Whereas in his first such experiments the particle pulses were obscured by the counter noise, he now reported seeing, "Half inch high pulses with not too bad a baseline," on the counter's oscilloscope display. The faint note in the lower margin elaborates this to, 'got good ½" pulses but some instability & "hum" stuff each register count.' This result was obtained using saline as the particle suspending medium, a 50-µm aperture, and a high-voltage aperture supply external to the counter. The upper marginal note describes the aperture excitation: an external supply providing about 480 volts direct current (DC) was connected through a pair of 100,000-ohm, one-watt, **A**llen-**B**radley resistors in series with the excitation electrodes on either side of the 50-µm aperture. About 80 volts was applied between the two electrodes, with an excitation current through the aperture of about 2 milliamperes and an average current density of about one microampere per square µm. Wallace now thought that, if erythrocytes and leukocytes could be eliminated from a sample, platelet counting "should be relatively easy." Appropriate modifications to the design of the ONR counters would be incorporated in the lot of three counters Wallace had begun to assemble, and he would continue experiments toward noise reduction and use of latex particles as calibration aids.

In his ninth paragraph Wallace responded to Mattern's comments regarding his attempts to reduce the red cell (erythrocyte) concentration in a blood sample sufficiently that a white cell (leukocyte) count might be made. Mattern had tried centrifuging the samples and counting the supernatant, but found that leukocytes were entrapped and dragged down by the "falling" erythrocytes. Wallace wondered if, in addition to a large dilution, overlaying the sample with a liquid of lesser density prior to centrifuging might produce a suspension rich in leukocytes in the lighter liquid. Some two weeks later in another letter to Mattern (Appendix 13) he proposed the use of a rotor with angled bores in which to centrifuge the sample tubes and questioned whether treating the samples with a surface-active nonhemolytic reagent such as Triton WR-1339 might help. He also informed Mattern of a blood-cell counter, based on Wolff's photo-electric approach, that Jarrell-Ash Company was introducing and summarized his own experiments toward defining count loss due to simultaneous passage of multiple cells through a sensing aperture.[166] Wallace would continue his research on physical preparation of leukocyte samples for some months, but found no method that provided significant improvements; his major advance was a redesign of the volume-control section of the Model A manometer that improved repeatability of its count volume.[167]

Then at a technical conference in mid-1955, Wallace reconnected with Joseph Gardberg, who had been a classmate at Atlanta's Georgia School of Technology during the early 1930s and who now lived in an apartment building at 5227 North Kenmore Avenue, Chicago. Both men were inventors, and a working relationship soon evolved.[168] On October 26, 1955, Wallace outlined a way to increase a radio's selectivity, and both he and Gardberg signed and dated it that day.[169] Meanwhile, both the NIH counter evaluations continued to progress favorably, and by year's end Wallace had agreed to

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[166] Wolff, "An apparatus for counting small particles in random distribution," 967; Alan Richardson Jones and Frederick Brech, "Apparatus for counting discrete microscopic particles," U.S. Patent 3,076,600, filed Sep. 3, 1954, and issued Feb. 5, 1963.

[167] Wallace Coulter, sketch of volume-control manometer dated July 2 '55; WHC Papers.

[168] "Joseph Gardberg, administrative assistant," *The Coulter Countdown* 2 (June 1972): 2. In late 1955, Gardberg filed three applications that issued as U.S. Patents 2,865,997, 2,886,797, and 2,900,448. These were the first of seventeen such that he received.

[169] Wallace Coulter, untitled sheet with three radio input circuits, signed at bottom: W. H. Coulter Oct 26 55, J. Gardberg 10-26-55, and Walter R. Hogg 10-29-55; WHC Papers.

provide a cell counter to Baylor Hospital in Dallas, Texas.[170] But he was increasingly handicapped by the partial basement at 3023 W. Fulton Boulevard, and Joseph was anticipating marriage; a change in work and living arrangements seemed justified. Gardberg offered to rent Wallace basement space at 5227 North Kenmore, and the Coulters put their property on sale. On January 15, 1956, Joseph and Laura Belle May were married.[171] Wallace began transferring Coulter Electronics to Gardberg's basement, and on April 15 the Coulters accepted an offer on the W. Fulton property that was finalized on June 14.[172] That day Brecher's evaluation report was first received by the publisher, while Mattern's was accepted for publication on June 25; both listed 5227 North Kenmore Avenue, Chicago, as the address for Coulter Electronics.[173] It was time to make the favorable results described in both reports available to the broader clinical community.

During the nine years that Wallace and Joseph had owned the property at 3023 W. Fulton (Figure 2.1), their intensive library research had shown the limitations of experimental blood-cell counters then under development and suggested to Wallace his innovative Coulter Principle for which his experiments with a needle and cellophane wrapper in the property basement had confirmed technical feasibility. Additional work there had supported filing for two crucial U.S. patents and identified several technical challenges in need of solutions, this even as Cold War politics and personal finances

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[170] "Grant given for medical study tools," *The Dallas Morning News*, Dallas, TX, December 28, 1955, Part 3, 1, col. 6; Helen Bullock, "Hospital acquires blood-cell unit," ibid. August 23, 1956, Part 3, 1, col. 3; "Medical benefactor to receive award," ibid. March 10, 1957, Part 3, 1, col. 4. The connection may have been made via Wallace's paternal aunt, Ms. Sybil Coulter Holiman, who retired from Baylor Medical Center.

[171] Laura Coulter Jones, email to Don. Graham, March 6, 2012; WHC Papers.

[172] State Management Corporation, disbursement sheet dated June 14, 1956, $3,034.26 to Wallace H. Coulter, Joseph R. Coulter, Jr., and Laura Belle Coulter; WHC Papers.

[173] George Brecher, Marvin Schneiderman, and George Z. Williams, "Evaluation of electronic red blood cell counter," *American Journal of Clinical Pathology* 26 (December 1956): 1439, footnotes; Mattern, Brackett, and Olson, "The determination of number and size of particles," 56, footnotes. The building at 5227 North Kenmore Avenue has been demolished; the site is presently associated with the modern high-rise residential structure at 5225 North Kenmore. It is unclear where Wallace's living quarters were; they may have been at 5227 North Kenmore, but the advertisement for the Model A counter in the December 1958 issue of *Journal of Clinical Investigation* listed a 5220 North Kenmore address, while the letter of June 21, 1960, notifying Wallace of his John Scott Medal was sent to 5045 North Kenmore; WHC Papers.

added other challenges.[174] However, ONR financial support had enabled the Coulters to develop and construct a superior cell-counting instrument. During this process, most of the known technical challenges had been acceptably resolved, and their Model A counter had successfully undergone feasibility demonstrations and thorough evaluations. It had enabled rapid automated erythrocyte counts in precise sample volumes, and the accuracy and rapidity of such counts seemed sufficient not only for effective monitoring of bone-marrow recovery in victims of radiation exposure but likely to also restore erythrocyte counts as a routine clinical tool. Moreover, if proper sample preparation methods could be developed, these capabilities should also permit counting of leukocytes and platelets. A method to sufficiently improve repeatability of whole-blood dilutions to provide acceptable cellular coincidence levels remained the significant technical challenge; a practical resolution would not only facilitate the erythrocyte count that had originally motivated Wallace's research, but could also increase the reliability of leukocyte and platelet counts. And Wallace's experiments with latex particles had not only provided a calibration method for such cell counts, but suggested the counter's potential for particle analysis of interest to commercial firms.

The Coulters' persistence had overcome significant challenges during their residence at 3023 W. Fulton, but as Wallace settled Coulter Electronics at 5227 North Kenmore Avenue, they knew that the medical community was largely unaware of the Model A counter. As Joseph would later summarize the decade they had invested in their ground-breaking blood-cell counter, "We knew there were problems, but we also knew we had something useful." And as Wallace sometimes observed, "If it's useful, people will buy it." [175] However, they knew that their limited resources made even the attempt to introduce the Model A counter a serious gamble. But the conviction that had brought Wallace this far had not weakened, and he saw the move to 5227 North Kenmore as a first step toward resolving the business challenges of producing and distributing an automated counter with not only the demonstrated capability of blood-cell analysis but also the potential applicability to analysis of industrial particles. And Joseph saw it as a possible realization of his desire "to be in a position to run something like you wanted it." [176]

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[174] Coulter, U.S. Patent 2,656,508; Coulter and Coulter, U.S. Patent 2,869,078.
[175] Graham, "The Coulter Principle: Foundation of an industry," 74, col. 2 and 77, col. 1.
[176] Joseph R. Coulter, Jr., letter to Wallace Coulter, dated January 31, 1946; JRC Files.

CHAPTER 5. PROMOTION

The bombings of Hiroshima and Nagasaki in August 1945 (Figures 1.1 and 1.2) had made clear to Wallace Coulter the need for automated blood-cell counts to assess survivors' bone-marrow recovery from radiation damage, and weapons news originating in Cold War politics had become a constant reminder of the possibility for similar tragedies. It has been estimated that by mid-1956, the U.S. had some 3,692 nuclear devices, the Soviets about 426, and Great Britain about 21.[177] The unending drumbeat of news stories during this proliferation had sustained Wallace's apprehensions as he and his brother Joseph developed their Model A counter.

Artillery capable of firing fission projectiles had been especially worrisome to Wallace. The U.S. Army's mobile 280-mm cannon had been developed to fire a version of the 15-KT Hiroshima bomb to a range of more than 20 miles, and President-elect Dwight D. Eisenhower had the cannon disclosed before including one in his inaugural parade in January 1953.[178] The unrestricted and publicized Grable test shot on May 25 was followed on July 27, 1953, by the Korean War Armistice.[179] By July 1955, 18 of those cannon had been deployed in West Germany, and because the terms of the Armistice prohibited introduction of new weaponry into Korea, others were expected to be staged on Okinawa that August in case Korean deployment seemed necessary.[180] March 1956 brought news that the Soviets had developed two types of fission projectiles, one for a 203-mm gun and another for a 240-mm mortar; there were rumors that one of the guns had been brought to the Russian border with North Korea, where inadequate roads halted its further

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177 Robert S. Norris and Hans M. Kristensen, "Global nuclear weapons inventories, 1945-2010," *Bulletin of the Atomic Scientists* 66 (July-August 2010): 77-83.

178 "750,000 at Washington see marathon parade," *Springfield Union*, Springfield, MA, January 21, 1953, 1.

179 "Atomic cannon goes to proving grounds," *Springfield Union*, Springfield, MA, May 19, 1953, 35; "First atomic cannon blast set for today," ibid. May 25, 1953, 10; Becker, "Atomic cannon is fired in test," *The Times-Picayune*; Foot, "Nuclear coercion and the ending of the Korean Conflict."

180 "Okinawa gets atomic cannon," *Trenton Evening Times*, Trenton, NJ, July 28, 1955, 1; Robert S. Allen, "Calls for atomic cannon follow Red Korea buildup," *The State*, Columbia, SC, August 1, 1955, 4A; "Brucker says Quemoy blow unlikely now," *Springfield Union*, Springfield, MA, December 31, 1955, 21.

progress.[181] When its 280-mm cannon proved impractically cumbersome, the U.S. Army would by May 1957 develop a fission projectile for both its maneuverable 203-mm gun and howitzer.[182] Unintended use of such fearsome artillery seemed probable to Wallace, with dire consequences for civilian populations.

Meanwhile, the two NIH evaluations of the Coulters' prototype Model A counters had demonstrated these might rapidly provide an acceptable assessment of survivors' bone-marrow recovery; both reports are too detailed for more than a concise summary here. Mattern, Brackett, and Olson began theirs by briefly reviewing previous approaches to cell counting, then thoroughly describing the Model A counter and its operational characteristics; this summarized the Poisson statistics of multiple cells occurring in the aperture's sensitive volume.[183] Brecher, Schneiderman, and Williams briefly summarized the counter's description and characteristics before detailing a robust procedure for the counter's use in a clinical setting.[184] Although sample tubes having apertures with diameters D of 50 µm and 75 µm had been provided, to minimize aperture clogging much of both evaluations was done using tubes having apertures for which D and length L were 100 µm and 75 µm, respectively. Both groups used dual dilutions with calibrated pipettes and 0.9% saline solution to prepare the 1:50,000 dilutions needed for erythrocyte counts, but Mattern's group used blood samples from mice, sheep, goats, and humans to check effects of erythrocyte size while Brecher's group processed only clinical samples. Both groups compared the resulting counts with manual ones made using hemocytometers and the usual 1:200 dilutions, and both found unexpected errors in the latter due to differences in the hemocytometer filling rate. For consistent filling rates, both found the counter reduced sample processing times to a third that of manual counts, with a simultaneous three-fold improvement in count accuracy and much less technologist fatigue. Brecher's group checked the reproducibility of the automated counts via counts on dilutions greater than 1:50,000 and found it to be independent of the dilution ratio. The only disadvantage

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[181] "New A-weapons credited to Reds," *San Diego Union*, San Diego, CA, March 30, 1956, A2.

[182] "Army replacing huge A-cannon," *Lexington Sunday Herald Leader*, Lexington, KY, September 11, 1955, 1; US confirms 8-inch guns atomic shells," *The State*, Columbia, SC, May 2, 1957, 2A.

[183] Mattern, Brackett, and Olson, "Determination of number and size of particles," 58-59. Wallace's marginal sketch of the sensitive volume in Figure 4.9 appears in Fig. 4.

[184] Brecher, Schneiderman, and Williams, "Evaluation of electronic red blood cell counter."

of using the Model A counter was the need for large-ratio dilutions and therefore the need for larger volumes of diluent than required with manual hemocytometry, but the improved count accuracy it provided offset this concern. Mattern's group also explored the counter's use for leukocyte counts.

As noted in preceding discussion regarding Figure 4.9, Matten had tried to deplete the greater erythrocyte concentration in blood samples by centrifuging them and counting the supernatant, but many leukocytes were entrapped in the settling erythrocytes. However, he found papers on the hemolytic effect of saponin and reported its use in his preliminary leukocyte counts.[185] The saponin selectively removed cholesterol from the erythrocyte membranes, leaving holes which made the erythrocytes nearly as conductive as the suspending saline and thus uncountable, but added some small debris particles to the suspension.[186] Mattern's group compared Model A leukocyte counts on blood diluted 1:200 in 0.9% saline solution containing a 1:10,000 dilution of a commercial saponin with hemocytometer counts using the same dilution of blood and found that their results showed considerable promise for automated leukocyte counting. This use of saponin avoided the centrifugation step Wallace had been pursuing (Figure 4.9, ninth paragraph), and to conclude their report, Mattern's group wrote, "The instrument's potentialities for counting and 'sizing' a variety of particles makes its continued development and testing highly desirable."

Pleased with the NIH evaluations, Wallace had a U.S. patent application filed in May on the Model A counter's volume-control manometer (Figure 4.2) and delivered the updated unit he had promised to Baylor Hospital.[187] He also submitted a preliminary draft about the updated counter to the National Electronics Conference (NEC), to be held that October in Chicago. As he prepared for his NEC presentation, he worked to start counter production in Gardberg's North Kenmore basement, which contained a small unfinished

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[185] Mattern, Brackett, and Olson, "Determination of number and size of particles," References 19 and 20.

[186] P. Seeman, D. Cheng, and G. H. Iles, "Structure of membrane holes in osmotic and saponin hemolysis," *Journal of Cell Biology* 56 (1973): 519-27.

[187] "Grant given for medical study tools," *The Dallas Morning News*, Dallas, TX, December 28, 1955, Part 3, 1, col. 6; Helen Bullock, "Hospital acquires blood-cell unit," ibid. August 23, 1956, Part 3, 1, col. 3. A second Coulter Counter® was later provided by the same donor: "Counter presented," ibid. December 20, 1961, Section 4, 2.

area near the furnace and a finished area occupied by a ping-pong table. And at some point he purchased the entire stock of saponin held by Mattern's source.

To assemble the counters, a capable technician was needed. One of Joseph's co-workers had interviewed Ernest Kenji Yasaka, a Hawaiian, a former U.S. Navy serviceman, and a recent graduate of Chicago's DeVry Technical Institute. Wallace met with him, was impressed, and offered him $2.00 an hour to build Model A counters. Yasaka became Coulter Electronics' first employee and set up his work space in the unfinished area of Gardberg's basement (Figure 5.1).[188]

While Yasaka developed his assembly technique, Wallace introduced what would become the first commercially successful automated blood-cell counter in his NEC presentation on October 3, 1956.[189] He summarized the problems with manual blood-cell counts and featured an image of a Model A counter as a model "now in use in a number of laboratories (Figure 5.2)." [190] His text reflected the counter's developmental process and the three sample requirements stated in his extended description of the Coulter Principle (Appendix 5): the cells were to be ungrouped, that is, individual; of different electrical conductivity than the suspending liquid; and diluted sufficiently that there would seldom be more than one cell in the aperture's sensitive volume. The counter's sensing aperture, the shaft hole in a watch jewel (Figure 4.7), was reported to have a diameter D of 100 μm and a length L of 67 μm.

Diluted blood samples were pulled through the aperture by mercury flow in a modified manometer; mercury contact first with a "start," then a "stop," electrode through the manometer's glass wall determined the sample volume from which cell counts were taken (Figure 4.2). Under ideal conditions, 90,000 individual cells might be counted in the

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[188] "Meet Ernest Yasaka - Coulter's first employee," *The Coulter Countdown* 2 (February 1972): 3; "He's oldest employee," ibid. 2(12)/3(1), (1972): 4.

[189] Scans of both the preliminary draft and the published paper are available via links in Marshall Don. Graham, "Wallace Coulter's one technical paper," *Purdue Cytometry Disc Series* 10 (2007), website accessed October 12, 2018. For the original publication, see: Wallace H. Coulter, "High speed automatic blood cell counter and cell size analyzer," in *Proceedings of the National Electronics Conference*, vol. 12 (Chicago: National Electronics Conference, Inc., 1957), 1034-40. The paper is reprinted in *Hematology Landmark Papers of the Twentieth Century*, ed. Marshall A. Lichtman et al. (San Diego: Academic Press, 2000), 911-21.

[190] Coulter, "High speed automatic blood cell counter and cell size analyzer," 1042; a second statement regarding usage in a number of laboratories appears on 1037.

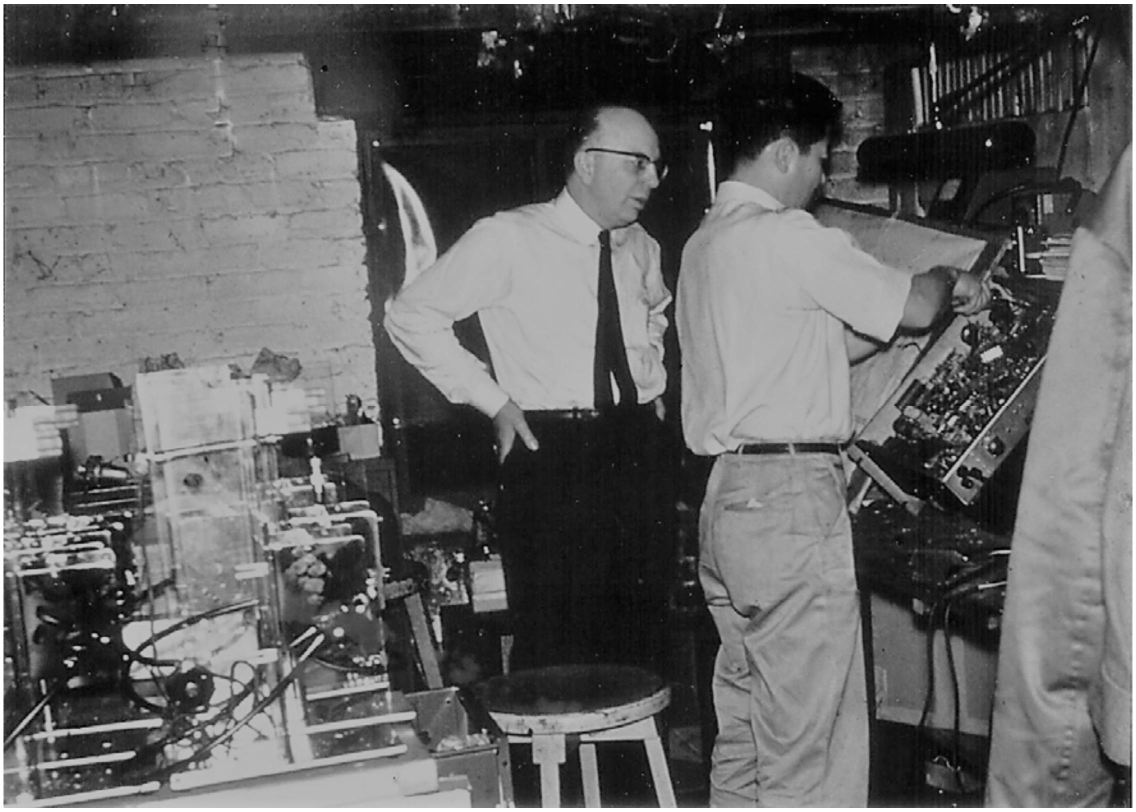

Figure 5.1. The work area in Gardberg's basement.[191] Wallace Coulter watched as Ernest Yasaka assembled the electronics chassis for one of the first production Model A counters; several of the counter's innovative sample stands awaited completion on the bench behind him. Yasaka would construct hundreds of the Model A counters here before returning permanently to Hawaii in 1959. He then became a lab technician at the University of Hawaii and completed two years of coursework as a part-time student.[192] He would also work for Coulter Electronics, Inc., first as a part-time representative on commission, then as a full-time sales and service engineer from 1972 until his retirement at age 65 in June 1992.

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[191] Photographic print, WHC Papers; the image has appeared in Graham, "The Coulter Principle: The Arkansas background," 180. A slightly different view was also published: "Look ahead with expectation, look back with pride," *The Coulter Countdown* 10 (Special 1980): 4.

[192] David H. Crowell, Ernest K. Yasaka, and Doris C. Crowell, "Infant stabilimeter," *Child Development* 35 (1964): 525-32.

Figure 5.2. An early production Coulter Counter® Model A.[193] According to the panel label, the electronics unit was Coulter Counter, "Model A ----- serial 111," from "Coulter Electronics…5227 North Kenmore…Chicago." Production instruments retained the same panel layout as the prototype, but were repackaged to improve presentation (see Figure 4.8), their electronics were less noisy, and their volume-control manometer drew count volumes of better accuracy from the sample beaker on the sample stand's platform.

Briefly, the rotary switch at the lower right of the electronics' panel controlled the electrical current through the sensing aperture, at the lower end of the sample tube extending downward into the sample breaker. Pulses in the aperture current between the electrodes in the sample tube and the sample beaker due to cells transiting the sensing aperture were amplified for display on the oscilloscope tube; those above the level set by the threshold control beneath the display were accumulated on the three low-digit decade counters and high-digit mechanical counter to the left of the display. Here, the two stopcocks near the top of the stand that controlled vacuum application (knob to the right) and rinsing electrolyte (knob to the left) are clearly visible. The vacuum pump used to unbalance the volume-control manometer and the glassware for the electrolyte supply and waste collection are not shown. A circuit schematic is available.[194]

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[193] Marketing photograph, WHC Papers. The image has appeared in Graham, "The Coulter Principle: The Arkansas background," 180, and *The Coulter automatic blood cell counter and cell size analyzer*, Bulletin A-1, Coulter Electronics, 1957. The WHC Papers include a copy both of Bulletin A-1 by Coulter Electronics, Inc., and its Portuguese translation (BR-3060).

[194] Coulter Counter Model A, 6301011D, Dwg 101, Rev. 15, Nov. 1960; WHC Papers.

approximately 15 seconds between those two contacts, but in practice, multiple cells coincidently passing through the aperture's sensitive volume required sample dilutions yielding counts of some 50,000 cells, or a cellular flow of about 3,300 cells per second. By comparison, a manual count of 500 cells required perhaps 20 minutes. The automated cell count reduced count errors to one-tenth of those for a manual count done by an expert technologist, this by a method that not only required less-skillful technologists, but needed only 1.25% of the count time.

Wallace acknowledged construction support for an experimental counter by the Office of Naval Research via Contract NONR 1054 (00) and cited both NIH evaluation reports on the contract's product while including drawings and experimental results from the report by Mattern, Brackett, and Olson.[195] The paper would be published shortly after publication of the NIH evaluations and the first advertisement for the Model A counter.[196] The text of the latter summarized the conference paper, with claimed capabilities being beyond those of electro-optical counters then being described in the literature (Figure 5.3).

A scientist at Fort Detrick's Biological Warfare Laboratories had seen one of the prototype Model A counters at NIH and inquired whether one might be used to count bacteria about one µm in diameter.[197] This was not feasible with the production Model A counter, but with care Wallace had gotten countable pulses from latex particles 1.1 µm in diameter (Figure 4.9) and had sold a unit to Herbert Kubitschek, a Ph.D. physicist working as a radiation microbiologist in ANL's Division of Biological and Medical Research.[198] Kubitschek had tried unsuccessfully to build an optical counter for bacteria.[199] On learning of the Model A counter, he acquired one from Wallace, equipped it with 10-µm sensing apertures made from redrawn capillary tubing, and using his experience with radiation counting instruments, modified its electronics so that it could count bacteria and latex particles as small as one µm in diameter, as well as drive a pulse-height analyzer to record

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[195] Ibid. 1042.

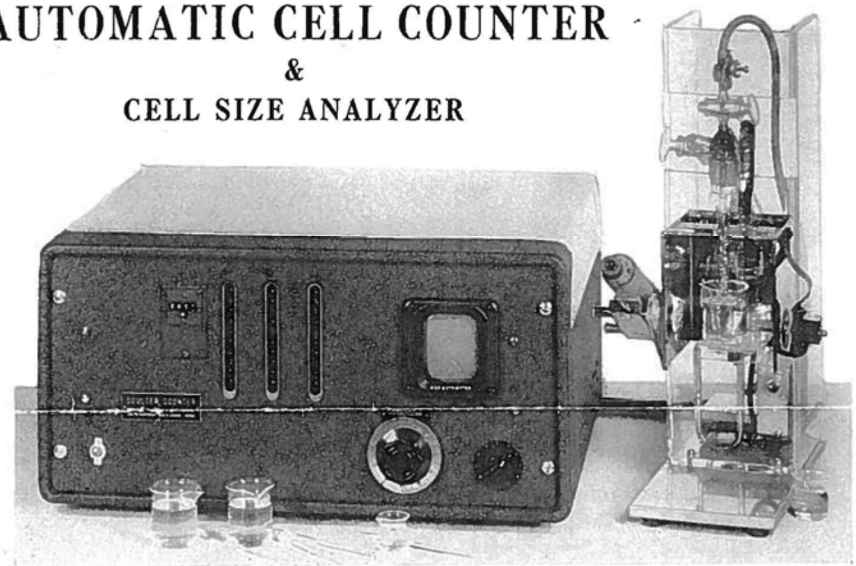
[196] Figure 5.3, from *American Journal of Clinical Pathology* 26, December 1956.

[197] Nelson E. Alexander, letter to Wallace Coulter dated October 29, 1956; WHC Papers.

[198] Austin M. Brues, ed., *Quarterly Report, August, September, October, 1951, Report ANL-4713*, (Division of Biological and Medical Research, Argonne National Laboratory, Chicago, 1951), 5.

[199] H. E. Kubitschek, "Electronic measurement of particle size," *Research* 13 (April 1960): 129, col. 2, for a mention of the optical counter.

AUTOMATIC CELL COUNTER
&
CELL SIZE ANALYZER



The new COULTER COUNTER provides *accuracy, speed and reliability* not approached by any other method.

- Counts in excess of 6,000 individual cells per second.
- Each count is equivalent, in number of cells counted, to the average of 100 chamber counts to reduce the sampling error by a factor of approximately 10 times.
- Unit takes its own precisely metered sample from a sample beaker to eliminate counting chamber errors.
- An oscilloscope display provides immediate information on relative cell size and relative size distribution.
- Threshold level control provides a means of rapidly obtaining complete cell size distribution data.
- Oscilloscope display providing a check of circuit performance coupled with simplicity of mechanical design affords highest reliability.
- Sensitivity extends to particles smaller than 2 microns.
- Sample capacity exceeds 100 counts per hour on a production basis.

Coulter Electronics 5227 N. Kenmore Chicago 40, Illinois

Figure 5.3. The first advertisement for the Model A counter. Features that distinguished it from possible competitors were itemized, with emphasis being given to performance improvements in cell counting (points 1-3, and 8) and the counter's unique cell-sizing capability (points 4 and 5). This image is from the WHC Papers.

a distribution of the resulting pulses.[200] On January 4, 1957, Wallace sent the Fort Detrick scientist a copy of his NEC paper and suggested that he contact Yasaka; the scientist acknowledged the preprint and expressed appreciation for Yasaka's suggestion that a discussion with Kubitschek might be helpful.[201]

Wallace's NEC presentation prompted Robert H. Berg to propose exploring design improvements that would enable the Model A counter to process suspensions of particles used commercially, then developing a market for the upgraded instrument. After receiving the M.S. degree in chemical engineering and instrumentation from the University of Wisconsin, Berg had worked for several years in chemical process control before founding his proprietorship Process Control Services Company (PCSC) in Elmhurst, Illinois.[202] On April 1, 1957, the Coulter brothers signed a Sales Franchise Agreement that required him to develop industrial markets for the Model A counter via Coulter Industrial Sales Company (CISC), of which he was to be the sole officer and controlling stock holder; he was to finance this development and be compensated for his efforts through sales of counters for industrial uses.[203] Soon afterward, Shepard Kinsman proposed to Berg that he form a particle-analysis service using the Model A counter. With Wallace's agreement, Kinsman incorporated Particle Data Laboratories, Inc. (PDLI) in which Berg became a minority shareholder in return for space and use of Berg's PCSC/CISC phone number at his home at 196 Clinton Avenue, Elmhurst, Illinois.[204] (CISC and PDLI would use Elmhurst P. O. Box 22 and P. O. Box 265 as their respective addresses.) In mid-April a Model A counter was first displayed publicly in a Chicago trade-show (Figure 5.4). A promotional item noting CISC's formation was prepared for June publication; it mentioned NIH's successful

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[200] Herbert E. Kubitschek, "A lossless anticoincidence circuit," *Review of Scientific Instruments* 23 (October 1952): 567-68; "Electronic counting and sizing of bacteria," *Nature* 182 (July 26, 1958): 234-35; H. E. Kubitschek, "Apertures for Coulter Counters," *Review of Scientific Instruments* 35 (1964): 1598-99.

[201] Nelson E. Alexander, letter to Wallace Coulter dated January 29, 1957; WHC Papers. Wallace's letter of January 4 is mentioned therein.

[202] Robert H. Berg, "Sensing zone methods in fine particle size analysis," *Materials Research and Standards* 5 (1965): 119, col. 2.

[203] The agreement is Exhibit 1 with Berg's "Answer to Complaint" filed June 21, 1961, in Case 1-61-141, Circuit Court of DuPage County, Wheaton, Illinois. The case title is "Coulter Electronics, Inc. v. Robert H. Berg, individually and doing business as Process Control Services Co., Particle Data Laboratories, Inc., and Coulter Industrial Sales Co."

[204] Robert H. Berg, Process Control Services Company, letter dated September 19, 1960, to Coulter Electronics, Inc., 2525 N. Sheffield Avenue, Chicago, p. 9; WHC papers.

Figure 5.4. The first trade-show exhibition of the Model A counter.[205] According to the *Sun-Times* legend, Ms. "Virginia Mackay of the University of Chicago demonstrates a Coulter automatic blood cell counter and cell-size analyzer at Conrad Hilton Hotel."

[205] Photographic print, WHC Papers. A clipping of the legend, date-stamped April 16, 1957, and typed notes are glued to the reverse of the print. According to these, the photograph was made for the *Chicago Sun-Times* by Larry Nocerkno on April 15, 1957; cataloged as "B-623-Blood," it later found its way to an Ebay site, from which it was acquired by the author.

evaluations of the Model A counter for blood-cell counting, stated that 20 counters were at work in medical or biological fields, and indicated that tests with some industrial particles had yielded good preliminary results.[206] This would be followed by similar articles.[207]

During Coulter Electronics' first year in Gardberg's basement, Wallace's sales efforts had placed most of those 20 Model A counters. He still drove his 1949 Kaiser Traveler, and in after-dinner reminisces he would later sometimes tell about Yasaka's "spiffy" 1954 Ford: "He built the counters faster than I could sell them, so he let me use his car, to improve my chances on sales calls. But I always put gas in it before I brought it back." And as he told a reporter in 1976, "We built one machine, got our money back, built two, sold those, and bootstrapped an organization that now employs several thousand people." [208] But by mid-1957 the partnership's progress was increasingly limited by the Coulter brothers' resources and capabilities.

Joseph R. Coulter, Sr., the brothers' father, had worked since age 16 as a railroad telegrapher and train dispatcher; when train schedules permitted him to maintain his own work schedule, he had used his rail pass to make weekend trips from Monroe, Louisiana, to help his sons with their growing correspondence and bookkeeping duties.[209] One side of Gardberg's ping-pong table had become his desk, while vacant areas were used by his sons for their engineering work (Figure 5.5). On August 7, 1957, at age 67, he joined Coulter Electronics as a partner, secretary, and treasurer, then resigned the next day from service with Missouri Pacific Railway Company.[210] Necessity had taught him discipline and frugality; it was time to instill more of both into the family partnership.

That August 27th, newspapers across the U.S. reported that a broadcast by Moscow radio claimed the Soviet Union had unexpectedly tested the first intercontinental

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[206] "Solving a tiny problem," *Chemical and Engineering News* 35 (June 17, 1957): 92.

[207] "Blood cell counter may have versatile applications," *Analytical Chemistry* 29 (June 21, 1957): 76A; "R-D team gets new tool for particle count," *Industrial Laboratories*, June 1957; "Counts, sizes 100,000 fine particles in 15 seconds," *Food Processing* (August 1957): 48; Robert H. Berg, "Rapid volumetric particle size analysis via electronics," *IRE Transactions on Industrial Electronics* PGIE-6 (May 1958): 46-52.

[208] Dave Canfield and Donald Zochert, "Blood cells," in "Coffee-stirrer is among amateurs' many inventions," *Arkansas Gazette*, Little Rock, AR, June 11, 1976, 16A, col. 6-7.

[209] Graham, "The Coulter Principle: The Arkansas background," 165-69, 182-83.

[210] Joseph R. Coulter, Sr., letter dated August 8, 1957, to Missouri Pacific Railway Company; JRC Files.

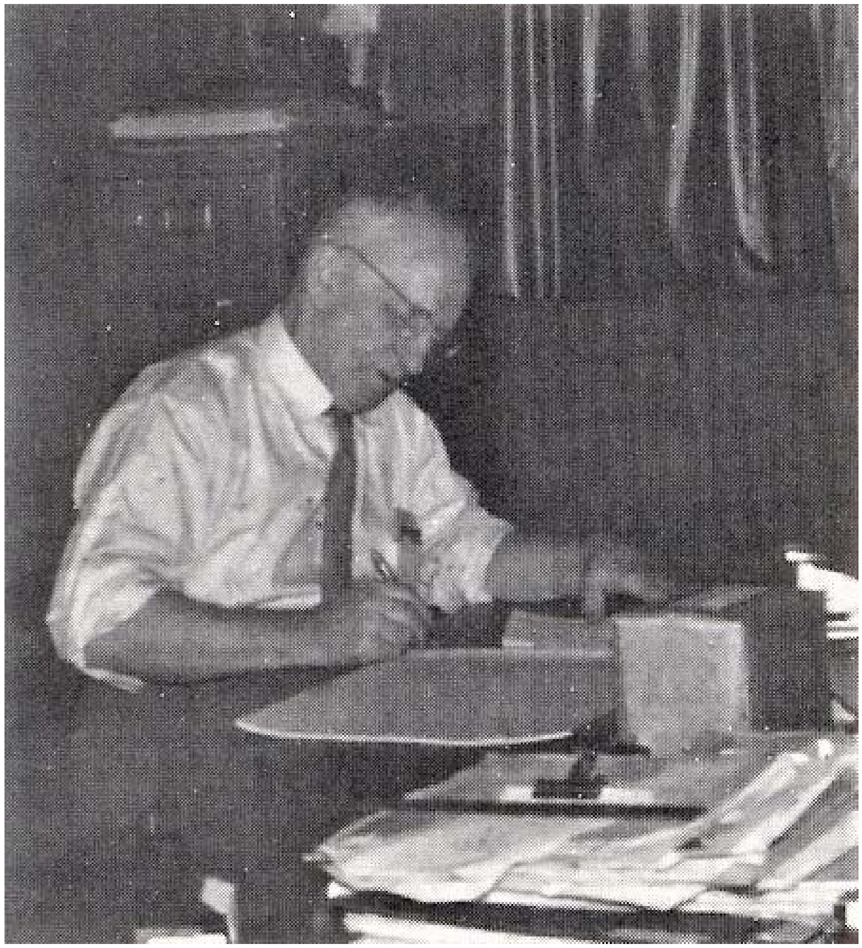

Figure 5.5. Joseph R. Coulter, Sr., working at Gardberg's ping-pong table.[211] Here, he took charge of bookkeeping and correspondence for Coulter Electronics and, after April 14, 1958, Coulter Electronics, Inc., until the latter moved in mid-1960 to 2525 N. Sheffield Avenue, Chicago. Joseph, Sr., continued his secretarial and bookkeeping duties through the corporation's move to Hialeah, Florida, in December 1961 and only semi-retired at age 81 in 1971.

[211] The image appeared in "In Memoriam – Joseph R. Coulter, Sr. – A beloved friend," *The Coulter Countdown* 9 (No. 2, 1979): 2-3. For further details, see also Stewart D. Allen, "'Mr. Senior' 1890-1979," ibid. 1.

ballistic missile (ICBM), one it claimed capable of "hitting any spot on the globe." [212] Two days later, a Soviet military engineer was quoted as saying that the new Soviet missile could carry a hydrogen warhead to an altitude of 600 miles before crashing it within 6 to 12 miles of any target on earth at speeds up to 16,000 miles per hour. [213] Just 15 weeks earlier Britain's Short Granite test, with a reported explosive yield "in the megaton range," had allowed it to join the U.S. and the Soviet Union in the world's nuclear club.[214] Coverage of the several fusion detonations had made the public aware that the tremendous power of thermonuclear weapons greatly increased the area exposed to blast and radiation damage, so reducing their required targeting accuracy. If the Soviet claims were true, the consequences of the 280-mm atomic cannon projectiles that had kept Wallace motivated would seem trivial.

And the Soviet claims were not the only worrisome news. That September Wallace received a foreboding letter from George Brecher, first author of one of the NIH evaluations of the Coulter Counter®.[215] At a German trade show Brecher had examined a Celloscope counter he thought to be a close functional copy; his letter was accompanied by a descriptive brochure from the manufacturer, Lars Ljungberg & Co. Brecher reported that the instrument's developer had been working toward an electro-optical method for counting particles when he became aware of the Model A counter. Integration of simpler electronics and sample stand into a single unit had created a counter both smaller and less costly than the Coulter instrument; rather than a vacuum pump, it used a rubber

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[212] "Russia claims she has tested world missile," *The Evening Star*, Washington, D.C., August 27, 1957, 1; "Intercontinental missile tested, Russia claims," *Rockford Morning Star*, Rockford, IL, August 27, 1957, 1; "Soviet Russia declares it has successful ICBM," *Illinois State Journal*, Springfield, IL, August 27, 1957, 1; "Russ claim rocket with super range; first nation to report an ICBM," *San Francisco Chronicle*, San Francisco, CA, August 27, 1957, 1; "Rocket claims interest Pentagon," *Aberdeen American-News*, Aberdeen, SD, August 27, 1957, 1; "U.S. faces big threat, Dulles says," ibid.

[213] "Russian claims missile could carry H-warhead," *The Dallas Morning News*, Dallas, TX, August 29, 1957, 14; "Missile has pinpoint aim," *San Diego Union*, San Diego, CA, August 29, 1957, 3.

[214] "Britain fires an H-bomb; little fallout," *Milwaukee Journal, Milwaukee*, WI, May 16, 1957, 1; "British hail H-test," *Riverside Daily Press*, Riverside, CA, May 16, 1957, 1; "British test in megatons," *World-Herald*, Omaha, NE, May 16, 1957, 3. But see Norman Dombey and Eric Grove, "Britain's thermonuclear bluff," *London Review of Books* 14 (October 22, 1992): 8-9.

[215] Brecher, Schneiderman, and Williams, "Evaluation of electronic red blood cell counter." For a brief Brecher biography, see: E. Nečas, "Tributes to George Brecher, MD., (1913-2004)," *Prague Medical Report* 106, No. 2 (2005): 217-20.

suction bulb to somehow cause a fixed volume of diluted blood to flow through a 30-μm sensing aperture.[216] Brecher had been allowed to make trial sample runs. Except for the small aperture's tendency to clog and an unstable baseline on the oscilloscope display, he found that the Celloscope worked "quite well" for erythrocytes, but based on his experience with the Model A counter, he doubted the exhibitor's claim that it could count white cells. He thought that Wallace's U.S. Patent 2,656,508 on the Coulter Principle would preclude U.S. sales of the Celloscope, but he wondered whether sales in England might be a problem. Although patents on the Coulter Principle had by then issued in Great Britain, France, and Germany, Wallace had not had applications filed in the Scandinavian and smaller European countries; his study of the descriptive brochure convinced him that his lack of Scandinavian patents had become a serious vulnerability.

Then on October 4 the Soviets orbited Earth's first satellite, Sputnik 1; at 184 lb its weight was roughly half that of the fission projectiles for the 280-mm cannon that had so worried Wallace.[217] Although high-level U.S. officials had known of the Soviet satellite program, most U.S. citizens did not; however, many had learned that research underlying the thermonuclear bombs tested by the U.S., the Soviet Union, and Great Britain had shown that smaller and lighter hydrogen warheads were possible. Panicked speculation began not only about whether those devices could be mated to a ballistic missile such as had orbited Sputnik 1, but whether the U.S. now lagged the Soviet Union in technical capabilities.[218] Both concerns increased that November when the Soviets orbited a dog in Sputnik 2.[219] Weighing 1,120 lb, this satellite was more than three times as heavy as the fission projectiles for the 280-mm cannon. Those concerns grew further that December when the U.S. Navy failed in its first attempt to launch its 3-lb satellite Vanguard 1A, but would be moderated slightly when the U.S. Army's Jupiter-C rocket placed the 30-lb Explorer 1 into orbit January 31, 1958.[220]

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[216] G. Brecher, letter to Wallace Coulter dated Sept 10, 57 and *The Celloscope: High Speed Automatic Particle Counter* (Stockholm: A. B. Lars Ljungberg & Co., 1957); WHC Papers.

[217] "Russian 'moon' now circling the globe every 95 minutes," *Evening World-Herald*, Omaha, NE, October 5, 1957, 1.

[218] L.A. DuBridge, "The Challenge of Sputnik," *Science and Engineering* 21 (1958): 13-18.

[219] "Dog in satellite living normally, Russia says," *Richmond Times-Dispatch*, Richmond, VA, November 5, 1957, 1.

[220] "Vanguard wrecked in test; rocket falls after rise of 3 feet," *San Diego Union*, San Diego, CA, December 7, 1957, 1; L. Edgar Prina, "First U.S. satellite put into orbit, races

Meanwhile, having grown increasingly concerned about the relationship with Berg and CISC, Joseph, Sr., had prodded Berg into providing a statement of account for CISC activities through November 30, 1957. Berg asserted that the promotional work summarized above had occupied him from execution of the CISC contract on April 1 into August, when he had rented two Model A counters for evaluation; for reasons unstated, one was returned within its first rental month. Thereafter, he had rented six more counters and sold one for which he received $3,840 and would pay Coulter Electronics $2,304. In addition to his promotional work Berg had used experience gained with the counters to help produce two brochures, *Theory of the Coulter Counter*, which quoted and expanded the statistical treatment of cellular coincidence Mattern, Brackett, and Olson had outlined in their evaluation report, and an operator's *Instruction Manual, Model A*.[221] From his experimentation with electro-optical counters Wallace had learned to minimize multiple cells simultaneously occupying a counter's sensitive volume, and the blood sample used in his October 30, 1948, experiment was "greatly diluted by 0.9% NaCl" (Figure 3.2). He had suggested to Mattern that technologists correct for coincidence loss via tables he was preparing by counting serial dilutions of samples; these he later reduced to a plot of coincidence loss against the counter result. *Theory of the Coulter Counter* described use of his method to correct the counter's background count for cell-free diluent (if necessary) and for the diluted blood sample by appropriately incrementing the counter result, then correcting the latter for particles in the diluent by subtracting the former.[222] A chart enabling such corrections for apertures having diameters D of 50, 70, 100, 140, or 200 μm and used with the 500 μl volume-control manometer was included in *Instruction Manual, Model A*. The two brochures would prove to be significant sales aids for the Model A counter. Berg billed Coulter Electronics via PCSC $420 for engineering services and $43.55 for

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around Earth 6 times," *The Evening Star*, Washington, D.C., February 1, 1958, 1; "Von Braun team gets its chance and delivers," ibid. 7.

[221] Mattern, Brackett, and Olson, "The determination of number and size of particles," 58-59; *Theory of the Coulter Counter*, Bulletin T-1, Coulter Industrial Sales, Inc., 1957; *Instruction Manual, Model A*, Coulter Electronics, 1957. A reprint of the first by Coulter Electronics, Inc., and an early copy of the second are in the WHC Papers.

[222] See discussion of Figure 3.2, the fourth paragraph in Figure 4.9, and the last paragraph in Appendix 13.

printing instruction manuals.[223] Wallace approved and Joseph, Sr., processed Berg's statement of account.

Then, on January 31, 1958, the U.S. deployed its 280-mm atomic cannon in Korea, as it would its 203-mm atomic howitzer by the following October.[224] Unintended use of such weaponry seemed more likely to Wallace than an ICBM attack: That millions could die in any hostile thermonuclear explosion, with millions more suffering radiation damage, made him hopeful that their sheer atrocity would inhibit such attacks.

But if not, millions of rapid, accurate, and repeatable blood-cell counts would be needed to monitor survivors' bone-marrow recovery, and Lars Ljungberg's Celloscope counter had taken on new significance. It was time to make the Model A counter available in numbers beyond the capabilities of the Coulter family partnership. While Wallace pondered possible ways to increase counter production, he continued to support Berg's application of the Model A counter for particles used in industrial products.

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[223] Robert H. Berg, Coulter Industrial Sales Co., statement of account dated December 2, 1957, to Wallace H. Coulter, Coulter Electronics; JRC Files. The charges originated in the two-page letter agreement of March 28, 1957, executed by Wallace Coulter on April 1, 1957, and attached as Exhibit 2 to Berg's "Answer to Complaint" filed June 21, 1961, in Case 1-61-141, Circuit Court of DuPage County, Wheaton, Illinois.

[224] "U.N. ducks A-arms tale," *The Oregonian*, Portland, OR, January 31, 1958, 2; "South Korea ready if Reds attack," *Illinois State Journal-Register*, Springfield, IL, October 12, 1958, 29.

CHAPTER 6. COMMERCIALIZATION

Early industrial users of the Model A counters had quickly demonstrated serious challenges in counting and sizing industrial particles that were unlikely when analyzing blood diluted with physiologic saline. Some common particles were much larger than blood cells and required both sensing apertures of larger diameter and volume-control manometers having greater count volumes. Wallace Coulter worked with Sam Gutilla (Figure 3.3) to provide sample tubes to which watch jewels having a range of aperture diameters larger than 100 µm were cemented and manometers having another "stop" electrode located to give a second count volume of 2,000 µl (Figure 4.2). Furthermore, dense particles often settled out of suspension before they could be counted. For these, Gutilla developed a glass stirring rod with one end formed like a propeller; it was to be spun by a small variable-speed electric motor. On October 28, 1957, Gutilla had provided Robert Berg with six stirring rods and four of the new dual-volume control manometers.[225]

Wallace also worked toward incorporating Coulter Electronics. To begin, in March 1958 he recruited Walter Hogg, the long-time volunteer in the W. Fulton basement (Figure 4.4), as Coulter Electronics' first full-time employee.[226] He and Hogg focused their attention on two technical challenges caused by the chemical mixtures Berg was recommending as suspending electrolytes for industrial particles. Some of these dissolved the cement bonding the aperture ring jewels to the glass sample tubes. Wallace had worked with Gutilla to eliminate the cement by heat-fusing the ring jewels to the sample tubes, but the large difference between the coefficients of thermal expansion for sapphire or ruby and typical glasses had made reliable joins difficult, and even successful joins frequently separated if the sample tubes were cleaned in hot water. Hogg found that careful annealing of the fused tubes could reduce the frequency of such failures.

Berg's second electrolyte challenge was more demanding. To reduce electro-chemical polarization at the electrodes, the Model A counter reversed the polarity of the electrical current supplied to the sensing aperture after each particle count. This approach

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225 Delmar Scientific Laboratories, Invoice No. 2795 dated October 25, 1957, to Coultier (sic) Electronics; R. H. Berg, receipt dated October 28, 1957, for "4 manometers as per sketch, 6 stirring Rods, addressed to W. Coulter, from Delmar Sci Labs." WHC Papers.

226 "Walt Hogg marks twenty years in service," *The Coulter Countdown* 8 (February 1978): 2; "In memory of Walter R. Hogg," ibid. 12 (Summer 1982): 3-4.

worked well with simple electrolytes, but some of Berg's chemical mixtures interacted with certain particle materials to create lingering polarity-sensitive electrode polarizations, so causing unequal aperture resistances for aperture currents of opposite polarity. The electronic circuitry of the Model A counter could not compensate for this artifact, but gave different particle counts and size indications in successive counts for the same particle suspension. This was an unintended result of the high-resistance voltage source Wallace had used to supply the aperture current, but he knew replacing it with a constant-current source would resolve the problem. He and Hogg began developing the complex current source while he continued to sell Model A counters for clinical and biological applications.

Meanwhile, Berg's promotional articles had become increasingly ambiguous about whether technical contributions originated with PCSC, PDLI, CISC, or Coulter Electronics. On March 31, 1958, the Coulters had the first CISC franchise agreement terminated and a clarifying renewal prepared that would be effective that April 1. However, Berg had not provided an overview of recent CISC activities, and while Joseph, Sr., pursued one, the Coulters tabled the renewal agreement. Then, on April 14, the Coulters dissolved their family partnership and incorporated Coulter Electronics, Inc. (CEI); Wallace became Vice President of the new corporation, while his brother Joseph became its President.[227] Hogg and Joseph, Sr., became its first and second full-time employees. Hogg would head CEI's technical activities, and Joseph, Sr., continued his secretarial and bookkeeping duties. His tenacity gained a copy of Berg's update on CISC's activities as of May 1, and Wallace carefully went through its details.

Berg listed 14 counters he had placed for industrial applications, of which seven had been purchased, three were rental units, one had been returned because of particle settlement, and three were awaiting a solution for their different responses to identical particles when the polarity of the aperture current was reversed. With increasing orders to both CEI and CISC (Table 6.1), Ernest Yasaka was no longer able to complete Model A counters quickly enough; the update listed a backlog of 19 unfilled orders for counters, of which one was intended for use in PDLI's particle-counting service.[228]

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[227] Illinois Articles of Incorporation Certificate 32006 filed April 14, 1958, Joseph Rubin, Gilbert Lynn, and I. Irving Silverman, Incorporators; amended in Illinois Articles of Amendment Certificate 13507 filed December 20, 1960, Joseph R. Coulter, Jr., President; Joseph R. Coulter, Sr., Secretary; WHC Papers.

[228] Coulter Industrial Sales Co., memo "Applications to Date," dated May 1, 1958, to "All field offices"; JRC Files.

Table 6.1. Cumulative placements of the Model A counter. These counters were rapidly accepted by researchers working in many disciplines. Until late 1960, all instruments sold by both Coulter Electronics and Coulter Electronics, Inc. (CEI) were to users in biological, medical, or clinical institutions, but due to the Coulters' deactivation of Robert Berg's Coulter Industrial Sales Company (CISC) on September 8, 1960, the entries for November 1960 and February 1961 may include units CEI sold through that January to fill orders Berg had already accepted from industrial users.[229] The first instruments sold by Berg through CISC were also the biological version shown in Figure 5.2, but those sold later may have included some or all of the industrial adaptations noted in Figure 6.1.

Timeline	Coulter Electronics or CEI.	Berg (CISC).[230]
June, 1957	20 [231]	0
December, 1957	Unknown	8
December, 1958	More than 150 [232]	40
April, 1959	More than 200 [233]	49
September, 1959	More than 450 [234]	61
January, 1960	More than 700 [235]	89
April, 1960	More than 750 [236]	101
November, 1960	More than 950 [237]	149
February, 1961	More than 1,500 [238]	161

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229 See Chapter 7 herein for discussion of CEI's termination of the CISC relationship.

230 Joseph R. Coulter, Sr., listing of "Industrial Purchases – Coulter Counter," August 16, 1957, through January 1961; JRC Files.

231 "Solving a tiny problem," *Chemical and Engineering News* 35 (June 17, 1957): 92.

232 "Substantiated success ... important to you!" *Journal of Clinical Investigation* 37 (December 1958): ad page 1. This journal title is abbreviated below as *JCI*.

233 "Solving a tiny problem – great industrial strides are made by the Coulter Counter," *Analytical Chemistry* 31 (April 1959): 10A.

234 "Proved! Coulter Counter®," *JCI* 38 (September 1959): ad page 1.

235 "Proved! Coulter Counter®," *JCI* 39 (January 1960): ad page 13.

236 "Over 750 installations," *JCI* 39 (April 1960): ad page 18. According to other advertisements therein, the exhibition was part of a conference organized by the American Society for Clinical Investigation.

237 "Over 950 installations," *JCI* 39 (November 1960): ad page 24.

238 "Breakthrough in fine particle analysis," *Analytical Chemistry* 33 (February 1961): 166A.

Berg's update also noted work toward the stirring rod to reduce particle settlement and indicated that both a new volume-control manometer providing three count volumes and sample tubes having the ring-jewel aperture discs fused to them would be shippable in a few weeks. CISC's Bulletin A-2 featured a photograph of a Model A counter the sample stand of which included Gutilla's stirring rod and its drive motor (Figure 6.1); the accompanying price list included the sample agitator and speed control, with spare stirring rod, as part of the basic counter offered by CISC. The list also included a choice of sample tubes having ring jewels with a range of aperture diameters either cemented or fused onto them and of volume-control manometers providing single, dual, or triple count volumes.[239] These options improved the versatility of the Model A counter and for some commercial customers would enable reliable particle counts important to their business.

Wallace's concern persisted regarding Berg's ambiguity as to which company originated technical contributions, but after anxious discussion the Coulters forwarded CEI's renewal Sales Franchise Agreement to Berg, who received it June 18, 1958, and met with Joseph R. Coulter, Jr., to sign it on June 24th.[240] While between agreements he had drafted a paper, obtained Wallace's oral approval of it, and had it accepted for presentation at the annual meeting of the American Society for Testing of Materials (ASTM), June 26-27, 1958.[241] Then, as though he were free to ignore his obligations under the original franchise agreement, he also had a supply printed of what he posited to be an authorized reprint (Appendix 14), while the paper as approved by Wallace would appear in the ASTM symposium proceedings published in August 1959.[242] A comparison of the two shows that Berg's 1958 "authorized reprint" was not an actual reprint, but a modified and reformatted version of the ASTM content (Table 6.2). In both versions Berg had indicated his affiliation with his consulting activity PCSC, this with no mention of CISC, Coulter Electronics, or CEI. In addition to the photograph in Figure 6.1, both versions had

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[239] *Coulter Counter for Particle Content and Size Distribution*, Bulletin A-2, Coulter Industrial Sales Co., 1958; "Price List" dated June 1, 1958; WHC Papers.

[240] The renewal Sales Franchise Agreement, effective on April 1, 1958, but not executed until June 24, 1958, is Exhibit 1 with CEI's "Complaint" filed January 20, 1961, in Case 1-61-141, Circuit Court of DuPage County, Wheaton, Illinois.

[241] Robert H. Berg, letter to Coulter Electronics, Inc., Sept. 19, 1960, p. 11; WHC papers.

[242] Robert H. Berg, "Electronic size analysis of subsieve particles by flowing through a small liquid resistor," in *Symposium on Particle Size measurement, ASTM Special Technical Publication No. 234* (Philadelphia: American Society for Testing Materials, August 1959), 245-58.

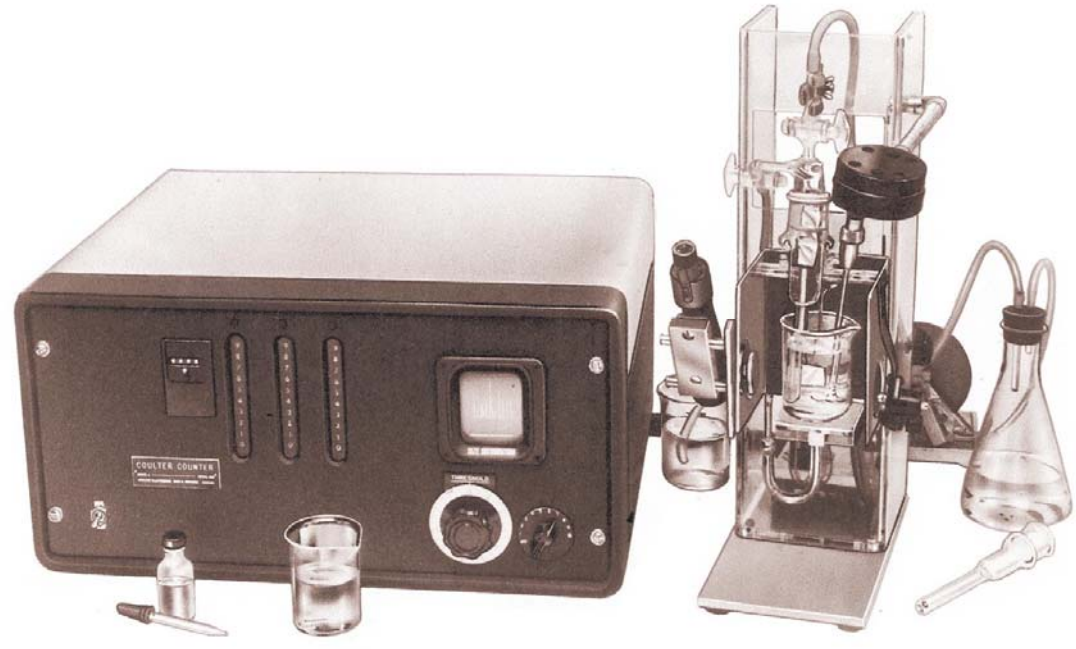

Figure 6.1. An industrial version of the Model A counter.[243] There were few visible or operational differences between it and the early production counter in Figure 5.2. The most obvious difference was the round black object at the upper right of the sample stand; it was a variable-speed electric motor which spun the glass stirring rod extending down into the sample beaker. The stirring rod ended in a small two-bladed propeller the rotation of which helped keep dense particles from settling; its rotational speed was controlled by the black knob just barely visible against the upper part of the vacuum pump's disk behind the sample stand.

Other versions of the sample stand employed a dual or triple volume manometer from which the desired count volume was selected by a switch beneath the stand's sample platform, as shown in Figure 4.8. The electronics unit provided lower noise levels over a wider frequency range, but otherwise was unmodified from that in Figure 5.2. Although seldom referred to as the Coulter Counter® Model K, that was the official name for the industrial versions. A circuit schematic is available.[244]

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[243] Marketing photograph, WHC Papers. The image appeared in *Coulter Counter for Particle Content and Size Distribution*, Bulletin A-2, Coulter Industrial Sales Co., 1958, a copy of which is in the WHC Papers. The image has also appeared in Graham, "The Coulter Principle: Foundation of an industry," 75.

[244] Coulter Counter Model K, 6301010E, DWG. 102, Rev. 4, April 1963; WHC Papers.

Table 6.2. Berg's ASTM presentation, June 1958. This exists in two versions, the official 1959 one in ASTM Special Technical Publication No. 234 and Berg's 1958 "authorized reprint" in Appendix 14. In both, Berg indicated his affiliation with Process Control Services Company (PCSC). The 1958 version is not a reprint of the 1959 one, but is an earlier printing of its reformatted content, with all mentions of "Coulter," Coulter Principle," "Coulter Method," or "Coulter Counter" replaced by non-eponymous wording. Berg would later cite it in support of these exaggerated claims: "The response theory and size analysis capability of the electrolytic sensing zone were originally reported by Berg." [245] and "This well-established method was first detailed extensively by Berg." [246]

Detail	ASTM STP No. 234	Berg's "Authorized Reprint"
Berg's affiliation	PCSC	PCSC
Print information	Baltimore, August 1959.	Unknown, 1958.
Print format	Dual column.	Triple column.
Pagination	245-58.	Cover; 1-5.
Figures	Eight.	Same eight, but two altered.
Coulter"	p. 245, col. 2, RP, line 3.	p. 1, col. 1, "a resistance."
"Coulter"	p. 246, col. 1, Fig. 1 legend.	p. 1, col. 2, deleted.
"Coulter Principle"	p. 247, Fig. 2, top line in figure.	p. 2, "Electric Principle."
"Coulter Method"	p. 247, Fig. 2, legend.	p. 2, "Electric Method."
"Coulter Principle"	p. 248, col. 1, A&O, lines 1-2.	p. 2, col. 1, "electric principle."
"Coulter Counter"	p. 251, Fig. 5, in lower chart.	p. 4, "Particle Counter."
"Coulter Counter"	p. 253, Fig. 7, in chart.	p. 4, "Particle Counter."
References	Four; 1) is WHC's NEC Paper.	Same four.
Discussion	pp. 256-58.	None.
Compliments of	No donor specified.	p. 5, "Particle Data, Inc."

[245] Richard F. Karuhn and Robert H. Berg, "Particle shape analysis by electrolytic sensing zone," in *International Powder and Bulk Solids Handling and Processing: Proceedings of the Technical Program, Rosemont, Illinois, May 16-18, 1978*, 255 (Chicago: Industrial and Scientific Conference Management, Inc., 1978).

[246] Richard F. Karuhn and Robert H. Berg, "Practical aspects of Electrozone size analysis," in *Particle Characterization in Technology, Vol. 1. Applications and Microanalysis*, ed. John Keith Beddow, 158 (Boca Raton, FL: CRC Press, Inc., 1984).

figures and information from CISC's Bulletin A-2; the final introductory paragraph of both stated, "During the past ten years a basically new principle has been developed for particle size analysis. It was first applied to blood cell counting about four years ago." While in both versions this last sentence cited Wallace's 1956 NEC paper, it did so without mentioning his role in originating and developing that new principle during those ten years.

But the comparison also reveals devious differences: In Berg's 1958 "authorized reprint," all mentions in the ASTM paper of Coulter, the Coulter Principle, and the Coulter Counter® were replaced by non-eponymous wording (Table 6.2). Moreover, the post-talk Discussion was omitted; this contained references to "Coulter data," "the Coulter method," and (twice) "the Coulter Principle." The "authorized reprint" seems intended to convince prospective customers that the Model A industrial counter was the result of Berg's efforts rather than a Model A counter improved and produced by the Coulters; be that as it may, numerous copies were retrieved from old CEI customer-service files.

Wallace only became aware of Berg's pretense when a customer gave him a copy of the "authorized reprint." Now deeply concerned by Berg's actions but unable to support his field and service activities, CEI cancelled the renewal Sales Franchise Agreement on October 1, 1958. A supplement, effective that date and executed by both parties that November 1, reduced CISC's commissions and required CEI to provide CISC an advertising allowance, a fifth of which Berg was to use demonstrating the Model A counter at conferences and trade shows; it also defined industrial uses and allowed Berg to devote a tenth of his productive time to interests other than CISC, provided that such interests were not competitive to either CEI or CISC. But the supplement did not alter the agreement's prohibition during its term of CISC or Berg promoting or selling any apparatus that was competitive with the Coulter Counter® and of CISC or Berg using variants of "Coulter," "Coulter Counter®," or "Coulter Industrial Sale Corporation" for one year thereafter. Goodwill resulting from CISC's activity was to remain the sole property of CEI, and any inventions related to the Coulter Counter that were dominated by CEI patent claims were to be assigned to CEI; any others would be the property of CISC, with CEI retaining an unequivocal license to use such inventions as it might choose.[247] If honored,

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[247] The Renewal Sales Franchise Agreement and its Supplement are Exhibits 1 and 2, respectively, with CEI's "Complaint" filed January 20, 1961, in Case 1-61-141, Circuit Court of DuPage County, Wheaton, Illinois.

the supplement would address many of the Coulters' concerns, but experience would show that to be an unrealistic expectation.

It was during this period that Berg hired Shepard Kinsman, the principal of PDLI, as CISC's sole employee, and on August 20th Kinsman forwarded Berg's update of May 1 to "All Agents." He noted that delivery time for Model A counters was decreasing and that the enthusiasm shown by customers who had made a "serious study of the Coulter Counter is almost unbelievable." He also stressed the need to reduce the number of free samples being run at the office, suggesting instead that existing data from runs on similar samples be offered, and proposed guidelines for successful demonstrations, one of which was to avoid giving a customer demonstration results because the hurried circumstances could produce invalid data that might later prove detrimental.[248]

Kinsman's professional approach helped ease the concerns about CISC held by all the Coulters. However, Berg's ASTM ruse had taught Wallace that better control was needed over information made available to potential customers, and he negotiated a distribution agreement with Scientific Products, a division of American Hospital Supply Corporation. The first advertisement appeared in September; it summarized the operational principle and advantages of the Model A counter before offering electronic counts of both leukocytes and erythrocytes, with accurate plots of cell-size distributions via single-threshold control, from an instrument priced at $3,350.[249]

Cell-size distributions were obtained by the operator repeatedly counting a sample at progressively incremented settings of the counter's single threshold control and recording the corrected individual counts on a chart. This required a substantial volume of diluted sample and perhaps two minutes to step through the incrementation.[250] Although accurate, the resulting cumulative distribution was not as desirable as a correct differential one, and experience showed that the manual arithmetical steps necessary to reduce a cumulative distribution to a differential one were both time-consuming and error-prone.

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[248] Shepard Kinsman, inter-office memorandum "Sample List" dated August 20, 1958, to All Agents; Coulter Industrial Sales Co., memo "Applications to Date," dated May 1, 1958, to "All field offices"; JRC Files.

[249] "Coulter Blood Cell Counter," *Journal of Clinical Investigation* 37 (September 1958): ad page 5.

[250] *The Coulter Automatic Blood Cell Counter and Cell Size Analyzer*, Bulletin A-1, Coulter Electronics, 1957; a later reprint by Coulter Electronics Sales Company, Inc., is in the WHC Papers.

After discussions with Kubitschek, Wallace began adapting circuitry used in pulse-height analyzers to form a dual-threshold pulse amplifier that could define a differential distribution bin. The bin would still require manual incrementation through repeated counts and count recording, but the approach would eliminate arithmetical errors.

The first reports by users of the Model A counter also appeared in 1958. Kubitschek's paper on counting and sizing bacteria with his modified Model A counter, one of his 10-μm apertures, and a pulse-height analyzer was published in July. It included an equation estimating the resistance change induced by a particle passing through the aperture and contained the first differential distributions for both suspensions of single-specie bacteria and mixed polystyrene latex spheres.[251] In November, the first paper from a hospital using a Model A counter was published. The decision to have all blood-cell counts done by medical technologists, rather than by medical students, and lack of qualified technologists had motivated a trial of the counter. Although erythrocyte counts were done without removing leukocytes from the samples, experience over four months showed the counter "to be reliable, accurate, and efficient, as well as economical." [252] Such counts by a Model A counter also enabled better understanding of human erythrocyte geometry.[253] And a comparison of hemocytometer counts of cultured fibroblasts with those made by a Model A counter concluded "that the electronic counter is superior in deriving the true average cell number at points on the fibroblast growth curve." [254] In December a favorable news story featured a Model A counter in clinical use, and Wallace sponsored a new independent advertisement.[255] Claiming 150 counter placements (Table 6.1), this detailed the counter's operational abilities and emphasized that its success was due to its innovation technologically, psychologically, and economically. For direct counting and sizing of bacteria and other small particles, a final

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[251] Kubitschek, "Electronic counting and sizing of bacteria."

[252] Irene E. Roeckel, "A new method for blood cell counting," *Bulletin, Georgetown University Medical Center* 12 (November 1958): 60-61.

[253] Donald N. Houchin, John I. Munn, and Benjamin L. Parnell, "A method for the measurement of red cell dimensions and calculation of mean corpuscular volume and surface area," *Blood* 13 (1958): 1185-91.

[254] Robert J. Kuchler and Donald J. Merchant, "Growth of tissue cells in culture," *University of Michigan Medical Center Journal* 24 (1958): 209-10.

[255] "Hospital's new device speeds blood analysis, San Diego Union, San Diego, CA, December 5, 1958, a24; Substantiated success ... important to you!," *Journal of Clinical Investigation* 37 (December 1958): ad page 1.

note indicated availability of specially designed research models. During a dinner conversation in the mid-1980s, Wallace remarked that this was his best advertisement.

The 1958 event likely of most interest to Wallace was the filing of a U.S. Patent application on an improved sample tube for the Model A counter.[256] He had worked with Gutilla for months to develop a viable method for heat-fusing ring-jewel aperture wafers to glass sample tubes. During prosecution of the original U.S. patent filing on the tube structure the manufacturing method assumed greater significance, and additional details Gutilla provided led to a divisional prosecution on that method.[257] As a co-inventor on both resulting U.S. Patents, Berg was required to assign them to CEI.

Lars Ljungberg was still producing the Celloscope counter and distributing it throughout Europe.[258] In late 1958 or early 1959 Wallace took a Model A counter to Europe and began warning Celloscope users about their possible legal liabilities and authorizing infringement lawsuits.[259] Wallace's efforts upset some academicians who, whether from ignorance or arrogance, had ignored patent restrictions on their interests. He visited the Max Planck Institut für Biochemie in Martinsried, West Germany, where he met Gerhard Ruhenstroth-Bauer, who purchased a Model A counter and began studying the cell-size distributions produced from its cell counts.[260] Wallace returned to the U.S. with a deeper appreciation of Europe's potential as a market for cell counters. In late 1958 Joseph had

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[256] Wallace H. Coulter, Robert H. Berg, and Fred L. Heuschkel, "Scanner element for particle analyzers," U.S. Patent 2,985,830, filed Dec. 29, 1958, and issued May 23, 1961. Berg's assignments were required by his letter agreement with Coulter Electronics of March 28, 1957, executed by Wallace Coulter on April 1, 1957.

[257] Silverman, Mullin, and Cass, "Memorandum in Re: Coulter Scanner Element – Method of Manufacture," October 28, 1960, 14 typed pages plus two pages of sketches produced from Gutilla's handwritten original, WHC Papers; Wallace H. Coulter, Fred L. Heuschkel, and Robert H. Berg, "Method of making a scanner element for particle analyzers," U.S. Patent 3,122,431, filed Dec. 29, 1958; and issued Feb. 25, 1964.

[258] Erik Öhlin, "Automatisk cellräknings-method – speciellt för blodkröppar," *Nordisk Medicin* 59 (1958): 577-78. Öhlin would later be acknowledged as the originator of the Celloscope; see "Swelab" and "Boule Medical AB," websites accessed August 25, 2019.

[259] Wallace had by then patented his Coulter Principle in England (Patent 722,418A, granted January 16, 1955), France (Patent 1,080,716A, granted December 13, 1954) and Germany (Patent 964,810C, granted May 29, 1957) and had also made a similar filing July 18, 1953, in Holland; "Foreign Filings" file, WHC Papers.

[260] Günter Valet, "History & concepts of Martinsried flow cytometry group," *Purdue Cytometry Disc Series* 10 (2007), website accessed April 3, 2019.

established Coulter Electronics, Ltd., in a basement on London's Edith Road.[261] Initially manufacturing and selling Model A counters, the subsidiary's first developmental project would be a simplified counter allowing the technologist to select either an erythrocyte count or a leukocyte count by flipping a switch. More competitive with the Celloscope counter than was the Model A, when brought into production by CEI this counter would be welcomed by smaller hematology laboratories.

In February 1959, a group headed by Lt. Col. Joseph H. Akeroyd of the Walter Reed Medical Center published a study, begun in mid-1954, on the practicality of using the Model A counter to do routine leukocyte counts in clinical laboratories. It was reported that lot-to-lot variability in available saponin made it unacceptable for preparation of clinical leukocyte samples, whereas Triton X-100, a poly-ether alcohol, was effective in preferentially removing erythrocytes from the cell count if one part of the blood sample were diluted into 200 parts of a 1:2000 dilution of Triton X-100 in physiologic saline and cell counts were made within five minutes of the sample dilution. Use of the Model A counter with such dilutions was practical in clinical laboratories and gave a standard error in the leukocyte count of 2.8%.[262] However, a study at Stamford Hospital, Stanford, CT, found that action of Triton X-100 solution on leukocytes was too rapid for use in that laboratory's routine procedure and recommended use of a 0.5% solution of saponin, acquired from CEI, in physiologic saline to remove erythrocytes from blood samples at a final saponin dilution of 1:500. After a year's experience with this method, the Model A counter was placed in routine clinical use for both erythrocyte and leukocyte counting, with increased accuracy in results, reduced fatigue among the laboratory technologists, and substantial savings in time.[263] A second study at Mayo Clinic, Rochester, MN, also found the Triton X-100 solution used by Akeroyd's group was too aggressive on leukocytes and used a saponin solution, the authors noting that CEI owned the entire supply of a suitable

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[261] Wallace H. Coulter and Joseph R. Coulter, Jr., letter dated March 26, 1960, to Mike I. Henderson, Coulter Electronics, Ltd.; carbon copy in the WHC Papers. The subsidiary's early advertisement for the Model A counter includes the complete address: "Now manufactured in Britain!" *Chemical Age* 84 (Sept. 24, 1960): 476.

[262] Joseph H. Akeroyd et al., "On counting leukocytes by electronic means," *American Journal of Clinical Pathology* 31 (February 1959): 188-92.

[263] Walter J. Richar and Edward S. Breakell. "Evaluation of an electronic particle counter for the counting of white blood cells," *American Journal of Clinical Pathology* 31 (May 1959): 384-93. See 386, col. 1, for remarks regarding saponin and the authors' source for it and Addendum, 393, col. 1, for comments on Akeroyd's use of Triton X-100.

saponin.[264] CEI used its saponin to lyse erythrocytes during developmental work and, finding customer interest, would begin selling reagents incorporating it before filing for the trademark "ZAPonin" on the first of these in July 1966.[265] Coulter Diagnostics, Inc., would be formed in 1967 to produce and market a growing line of Coulter reagents.

These reports in early 1959 persuaded Wallace that not only was his non-optical method for blood-cell counting capable of effectively assessing survivors' bone-marrow recovery from radiation exposure, but that additional staffing and a national sales organization were needed. Hogg began increasing his technical staff, then moved it from Gardberg's basement into an apartment on Broadway, some three blocks from 5227 North Kenmore Avenue. To build and manage a sales group, Wallace hired a fellow Arkansan, Floyd E. Henderson.[266] The timing was fortuitous: That summer the U.S. Department of Commerce selected the Model A counter for exhibition in Munich, Germany, and the favorable publicity it produced would considerably ease Henderson's tasks.[267] Meanwhile, Wallace and Hogg had made significant improvements to the counter's electronic circuitry.

It was noted above that several Model A counters Berg had rented on trial had been returned because some of his complex electrolytes caused different count and size data for the same particle suspension when the polarity of the electric current through the sensing aperture was alternated. To solve this problem, Wallace and Hogg developed a low-resistance constant-current source to replace the high-resistance constant-voltage source Wallace had designed into the Model A counter. Moreover, the new aperture current source also eliminated the need to recalibrate the counter while processing a sample if room temperature changed several degrees or if there were an interchange of either electrolytes having different electrical resistivity or sample tubes having apertures of different dimensions. They had likewise demonstrated that dual threshold controls set to define lower and upper bin limits could, when progressively incremented on sequential

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[264] Thomas B. Magath and Joseph Berkson, "Electronic blood-cell counting," *American Journal of Clinical Pathology* 34 (September 1960): 203-13; the comments regarding the saponin source and Triton X-100 appear on 203, col. 2,*fn.

[265] "SN 249,506, ZAPonin," *Official Gazette* 836 (Mar. 7, 1967): TM 37 and "828,977, ZAPONIN," Official Gazette 838 (May 23, 1967): TM 180. CEI's first advertisement for this new reagent appeared in *Journal of Clinical Investigation* 45 (April 1966): ad page 12. ZAPonin would also be used to prepare spermatozoa samples for counting.

[266] "Coulter people around the world mourn death of Floyd Henderson," *The Coulter Countdown* 7 (11-12), 1977): 1.

[267] "Coulter Electronics, Inc.," *The Coulter Countdown* 1 (September 1970): 1.

counts on the same sample, allow an operator to manually record the resultant bin counts as a differential size distribution. In August, 1959, Wallace had an application on these improvements filed with the U.S. Patent Office, which would allow two patents on what would become the Coulter Counter® Model B. Wallace had also begun work on circuitry that could accept the manually incremented bin counts and interface them to a modified strip-chart recorder so that a differential size distribution could be automatically plotted.[268]

While the Coulters developed CEI and Wallace enhanced counter capabilities, Berg had resumed his promotional activities in the industrial arena. In April 1959, under the CISC byline he had published another item in which, beneath an illustration of the industrial version of the Model A counter (Figure 6.1), he stated that "Fine particle measurement has been greatly advanced by the Coulter Counter in over 50 leading industrial laboratories since it was first announced," and claimed that 200 counters were being used in the biological or clinical fields. He listed 27 classes of industrial materials to which the counter had been applied and indicated the number of users in each class.[269] Then in May, Berg ran an advertisement suggesting the counter's culinary uses, for example, to control particle size in catsup, and in June in New York City he described application of the Model A counter to quantification of contaminating particles in hydraulic fluids for the Society of Automotive Engineers.[270]

In September 1959 Wallace placed an advertisement headed, "PROVED! COULTER COUNTER® accuracy and speed for counting red cells, white cells, tissue cultures, bacteria." It noted more than 450 Model A counter installations for non-industrial applications, and Berg had placed 61 Model A industrial counters across a broad spectrum of commercial organizations, with orders pending that would require another 28 counters by January 1960. However, Yasaka was returning to Hawaii, and Hogg's technical group was struggling to fill orders that by then would require 250 more biological Model A

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[268] Wallace H. Coulter et al., "Particle analyzing device," U.S. Patent 3,259,842, filed Aug. 19, 1959, and issued Jul. 5, 1966; "Particle studying device pulse analyzer," U.S. Patent 3,295,059, filed Aug. 19, 1959, and issued Dec. 27, 1966.

[269] "Solving a tiny problem – great industrial strides are made by the Coulter Counter," *Analytical Chemistry* 31 (April 1959): 10A.

[270] "At last! Fast and accurate fine particle measurement for foods of all kinds," *Food Technology* (May 1959); Robert H. Berg, memorandum "A discussion of the Coulter Counter and its application to the measurement of contamination in oils, etc." to the Contamination Control Panel, Committee A-6, Society of Automotive Engineers, dated June 19, 1959; WHC Papers.

counters (Table 6.1). Furthermore, Hogg needed technicians to integrate the new dual-threshold pulse amplifiers into a prototype Model B counter and to help move Wallace's experimental distribution recorder into producible form as the Model H Distribution Plotter.[271] Wallace and Hogg had also continued to improve the dual-threshold pulse amplifiers, but now needed additional technical support to combine them into a multi-bin pulse-height analyzer that could eliminate the manual incrementation of counts on repeated sample runs required by the paired Model B and Model H. If successful, the result would automatically provide a differential size distribution from simultaneous bin counts acquired in roughly one-fifth of the time required by the Models B and H.

The hundreds of Model A counters by then being applied in a variety of disciplines could rapidly and accurately count the cells or particles in a precise and repeatable volume of diluted sample, but an accurate estimate of the concentration in the original sample still required accurate knowledge of the dilution ratio for that volume. The Coulter brothers had experimented with several dilution methods, but had found none capable of reliably yielding dilution accuracies approaching those obtained by manually dispensing the blood sample into the suspending electrolyte from pipettes calibrated by weighing the mercury they delivered.[272] By early 1959 the Coulters had designed an automated diluting apparatus and, with Gutilla's help, provided a prototype to researchers at Mayo Clinic. Although sound in principle, this instrument was so cumbersome in use that it prompted the researchers to design a simpler version and have it constructed.[273] Nonetheless, the Coulters would apply for a U.S. patent for their prototype, and interactions with the patent examiner during its lengthy prosecution would enable Wallace to simplify its design and operation.[274] The Dual Diluter (Figure 6.2) would resolve the last fundamental challenge Wallace had faced in converting his Coulter Principle from a concept into an accurate method of automatically determining blood-cell counts for clinical blood samples.

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[271] Wallace H. Coulter and Abraham Siegelman, "Particle distribution plotting apparatus," U.S. Patent 3,331,950, filed Feb. 27, 1961, and issued Jul. 18, 1967.

[272] Joseph L. Grant, Melvin C. Britton, Jr., and Thomas E. Kurtz, "Measurement of red cell volume with the electronic cell counter," *American Journal of Clinical Pathology* 33 (February 1960): 138-43.

[273] Magath and Berkson, "Electronic blood-cell counting," 204 and 205, Fig. 1.

[274] For a state-of-art summary, see Thomas V. Feichtmeir et al., "Electronic counting of erythrocytes and leukocytes" and "A device to pipet and dilute fluid semi-automatically," *American Journal of Clinical Pathology* 35 (April 1961): 373-77 and 378-89.

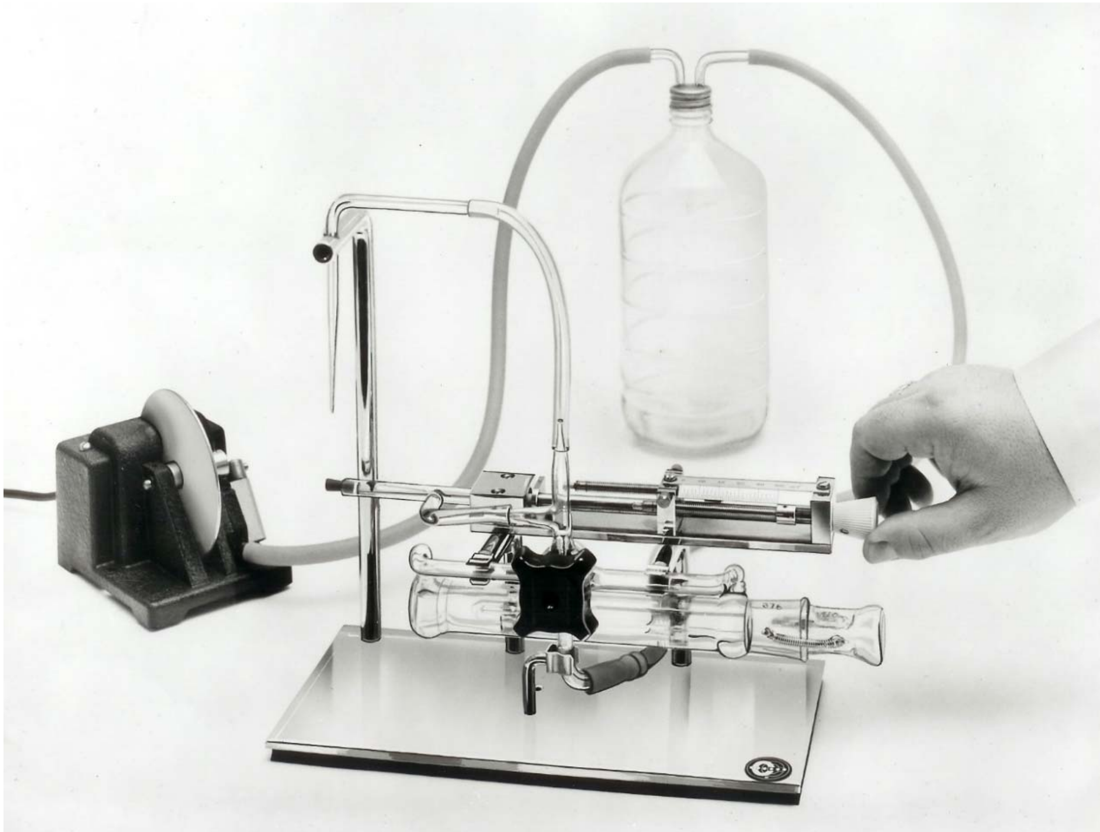

Figure 6.2. The Coulter Dual Diluter.[275] Stops for the syringe piston could be selected by the operator to provide dilutions giving acceptable cellular coincidence rates for either an erythrocyte or a leukocyte count. The black knob in the center of the apparatus controlled a two-way valve; in its first position the valve allowed vacuum from the vacuum pump at the left to draw the appropriate volume of whole blood from a sample vial held under the tapered tube at the left of the apparatus into the predetermined volume of diluent in the cylinder beneath the control knob. The cylinder contained a movable plug piston. When the control knob was then rotated to its second position, pressure of the air compressed on the other side of piston's movement caused the diluted sample to flow into a second vial then held under the tapered tube.

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[275] Marketing photograph; WHC Papers. The image appeared in company publications as well as in advertisements, for example, "Dilutions?" *Journal of Clinical Investigation* 43 (May 1964): ad page 15. Details are included in two brochures by Coulter Electronics, Ltd.: "Here are 2 ways to Coulter Coefficiency in the Haematology Department" and "Operating and Assembly Instructions for the Coulter Dual Diluter - Model "R";" WHC Papers. For further diluter developments, see Wallace Henry Coulter, Joseph Richard Coulter, Jr., and William Anthony Claps, "Automatic diluting apparatus," U.S. Patent 3,138,294, filed Nov. 17, 1960, and issued Jun. 23, 1964; and Wallace H. Coulter, "Automatic diluting apparatus," U.S. Patent 3,138,290, filed Aug. 31, 1962, and issued Jun. 23, 1964.

The Model A counter had begun to restore erythrocyte counts as a routine clinical tool, and the Dual Diluter would facilitate this recovery. Wallace arranged to have a Model A Counter exhibited at the 1959 meeting of the American Association for the Advancement of Science (AAAS).[276] By then practical alternatives to the counter's voltage excitation for the aperture and its voltage-sensitive amplifier for cellular signals had been demonstrated, and prototypes of the Model B counter and its 25-bin Model H Distribution Plotter were in final development. Wallace provided a description of these new Coulter instruments to Scientific Products and organized their exhibition during the following October at the NIH Instrument Symposium in Bethesda.[277] Meanwhile, he and Hogg were adapting the dual-threshold single-bin circuitry used in the Model B counter to multi-bin usage by causing the upper threshold control of one distribution bin to also function as the lower threshold control of the next-higher bin; this technique could enable automatic acquisition of a differential size distribution from a single sample run lasting only some 15 or 20 seconds. Insights gained from the exhibit interactions would help shape their implementation of the multi-bin design into an experimental Coulter Counter® Model C that, like the Model A and Model B counters, would be based on vacuum-tube technology.

The year 1959 brought another event with significant implications for Coulter endeavors: That June a report was published on erythrocyte counting with Ljungberg's Celloscope counter; it confirmed not only that the Celloscope was based on Wallace's patented Coulter Principle, but that the sample flow velocity and count volume through its 30-μm sensing aperture were controlled by a mercury manometer closely resembling the one the Coulter brothers had patented and used in the Model A counter (Figure 4.2).[278] Facing infringement lawsuits in several European countries, Ljungberg thought that he could have the Coulters' patents invalidated.[279] Then, not content with infringements of

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[276] Raymond A. Taylor, "AAAS Chicago Meeting," *Science* NS 130 (Dec. 4, 1959): 1555.

[277] "Coulter Counter," *Bulletin*, Scientific Products, April 1960, 7; WHC Papers, and "NIH Instrument Symposium and Exhibit Program listed," *Analytical Chemistry* 32 (September 1960): 44A and 54A.

[278] Cato Oulie, "Telling av de röde blodlegemer ved deres elektriske motstand," *Nordisk Medicin* 62 (1959): 1421-25.

[279] Lars Ljungberg, AB Lars Ljungberg & Co., letter dated January 25, 1962, to Wallace H. Coulter, 2525 North Sheffield Avenue, Chicago; photocopy in WHC Papers. He suggested that CEI's pending lawsuit regarding his infringement of French Patent 1,080,716A should proceed, but to his surprise the courts would decide not only that he had infringed, but that his appeal of that decision was without merit.

their European patents, he began recruiting agents to distribute Celloscope counters in the U.S., and Wallace filed an infringement lawsuit against one of these, Schueler & Co.[280] This discouraged formation of other such alliances, but because CEI held no Swedish patents, it did little to deter Ljungberg himself, and his unremitting promotion of the Celloscope counter would cause increasingly significant challenges for CEI.

On February 13, 1960, France's detonation of a fission bomb made it the fourth member of the world's nuclear club, and soon news reports began appearing about new 'baby' nuclear weapons that, like atomic artillery, seemed liable to unintended use.[281] As he considered this new menace, Wallace took satisfaction in knowing that a blood-cell counter capable of meaningfully assessing bone-marrow recovery from radiation damage was in commercial production and that proven refinements would even better suit its successors to a task he ardently hoped would never materialize. The CEI advertisement he ran that April claimed more than 750 installations of the Model A counter and invited readers to "See us in Booth #1, Steel Pier – Atlantic City, May 1-3." [282] And in May, over his CISC byline, Berg discussed the counter's many applications in food processing.[283]

It was now some 15 years since the need to adequately assess bone-marrow recovery from radiation damage had made Wallace understand that an automated method of counting blood cells must be developed. In mid-1956 he had moved Coulter Electronics into Gardberg's North Kenmore basement, recruited an electronics technician, and described the Model A counter in a conference presentation. As the technician built instruments one by one, Wallace had sold them, and he and his brother Joseph, Jr., had hired Walter Hogg to recruit and oversee a technical staff, had established Coulter Electronics, Inc., and had self-funded entry of the Model A counter into the clinical and biological markets. Further extension of a marketing presence then exceeded their financial resources, but Wallace's conference paper had attracted Robert Berg, who had

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[280] Case 60/540 filed February 9, 1960, in District Court, Second District of New York; see "2,656,508," *Official Gazette* 752 (Mar. 29, 1960): 1085.

[281] David Mason, "France plans nuclear arms," *Boston Sunday Herald*, Boston, MA, February 14, 1960, 1; Ray Cromley, "Mysterious series of 'baby' nuclear weapons may revolutionize warfare on land," *Lexington Leader*, Lexington. KY, March 31, 1960, 37.

[282] "Over 750 installations," *Journal of Clinical Investigation* 39 (April 1960): ad page 18.

[283] Robert H. Berg and George M. Kovac, "Here's how to clinch vital particle-size control," *Food Engineering* 32 (May 1960): 40-43. For a review of this art, see Charles H. Coleman and John E. Despaul, "Measuring the size compliance of foods," *Food Technology* 9 (1955): 94-102.

proposed that he develop markets for the Model A counter in industrial fields. He had agreed to do so via a dedicated company which, as the sole officer and controlling stock holder, he was to finance with compensation for his sales of counters for purely industrial usages. On April 1, 1957, the Coulters had accepted his proposal via a sales franchise agreement, and although this alliance of convenience had at times proven worrisome, by April 1960 the Coulters' dedicated bootstrapping had placed more than 750 Model A counters for clinical and biological applications and provided Berg another 101 counters for a variety of industrial applications (Table 6.1).

Increasing sales of Model A counters for both biological and industrial applications, plus the prospects represented by the new counting instruments under development, emphasized the limitations imposed on CEI's operations by Gardberg's basement and the Broadway apartment some three blocks from it. The Model B counter with its Model H Distribution Plotter and the experimental Model C counter would require more space as they advanced through development and into production.

The Coulters began looking for facilities better suited to their growing company.

CHAPTER 7. TRANSITION

In June 1960 Wallace Coulter received a letter from the City of Philadelphia informing him that he had been selected to receive a John Scott Medal, presented to those who, by their inventions, "have contributed in some outstanding way to the comfort, welfare, and happiness of mankind." [284] Greatly encouraged by his selection, Wallace leased space in what seemed to be a suitable building at 2525 North Sheffield Avenue, Chicago.[285] To minimize disruption to Model A counter production, the Coulters began gradually consolidating CEI activities into its new space, and by late August Joseph R. Coulter, Sr., would be issuing invoices bearing the new address.[286] In November the first updated advertisement would be published, and when Wallace received his John Scott Medal that December, CEI would be operating from 2525 North Sheffield Avenue.[287]

The focus hereto has been on Wallace's lengthy journey from the fatal flashes over Japan in August of 1945 (Figures 1.1 and 1.2) and his abrupt comprehension of the critical need for accurate and rapid blood-cell counts; through his invention of the Coulter Principle (Figures 3.2a and b), its implementation in his Chicago basement via an ONR contract (Figure 4.6; Appendix 11), and his many commercialization efforts from a second Chicago basement (Figure 5.1); to the public recognition in December 1960 of his efforts for the good of humankind. My goal has been to detail that untold journey with sufficient context to make its telling as complete as now possible. The extensive literature concerning research on, and applications of, the Coulter Principle and its many implementations over the past six decades will not be addressed; its adequate treatment

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[284] G. Curtis Pritchard, Secretary, City of Philadelphia Board of Directors of City Trusts, to Wallace H. Coulter, letter dated June 21, 1960, carbon copy in WHC Papers; "Ex-Monroyan wins coveted science award," unattributed newspaper clipping, JRC Files; Robert Fox, "The John Scott Medal," *Proceedings of the American Philosophical Society* 112 (December 9, 1968): 416-30, page 430 of which confirms Wallace's award.

[285] The building is located just south of West Lill Avenue, on the east side of North Sheffield, and is bounded on its east by elevated tracks for the "L" trains. Now remodeled, it contains 28 condominiums (Apartments.com, "2525 N. Sheffield Avenue, Chicago, IL," website accessed December 11, 2018).

[286] Coulter Electronics, Inc., Invoice No. 4773 dated August 29, 1960, to Coulter Industrial Sales Co., for "400 micron fused aperture tube #1009," JRC Files.

[287] "Over 950 installations," *Journal of Clinical Investigation* 39 (November 1960): ad page 24; "Chicago inventor wins Scott honor," *World-Herald*, Omaha, NE, December 13, 1960, 13, col. 1.

would require several volumes. Perhaps a historian of technology will be intrigued into exploring this extensive knowledge base, to which my earlier paper may provide an introduction.[288] Here, supplementary context regarding events Wallace endured will be more appropriate than such detailed technical exposition.

In his letter of February 5, 1955, to Mattern (Figure 4.9), Wallace speculated that the time resolution of the Model A counter's circuits may have caused different responses for pulses from the two aperture types Mattern was provided and noted the diameters D and lengths L for both types. The different L/D ratios for the two aperture sizes would cause the excitation current to be distributed differently within the apertures' sensitive volumes, which suggested cellular pulses would differ in amplitude and shape. Throughout development of the Model A counter, cell-counting speed had been a high priority, and Wallace had minimized aperture length L to decrease the apertures' sensitive volume and thereby cellular coincidence rates, then optimized the counter electronics for the small aperture L/D ratios. As previously noted, Herbert Kubitschek made aperture tubes for his microbiological research by cutting an appropriate section from redrawn capillary tubing and polishing its ends to obtain the desired aperture length L in a wafer he cemented onto the sample tube.[289] Using such wafers in a variety of aperture L/D ratios, he confirmed Wallace's speculation about the time resolution of Model A counters by showing that, for aperture L/D ratios too small and counter electronics unmatched to the aperture, particle pulses failed to attain maximum amplitude, with consequent poor sensitivity and size resolution. Furthermore, unless such small apertures had no orifice defects, the particle signals also demonstrated excessive noise. Wallace had made similar sample tubes using aperture wafers made from standard capillary tubes having larger internal diameters, but found that orifice defects caused noisy particle signals for many of these, as well as for Swiss watch jewels made with non-standard smaller aperture diameters D.

A persistent limitation to industrial sales had been that analysis of suspensions such as clay particles required smaller sensing apertures than CEI could dependably provide. Robert Berg interacted with Kubitschek, then had a glassblower make sample tubes having small sensing apertures formed by Kubitschek's method.[290] Wallace

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[288] Graham, "The Coulter Principle: Foundation of an industry."

[289] Kubitschek, "Electronic counting and sizing of bacteria."

[290] Kubitschek, "Electronic measurement of particle size." See page 31, Figure 3 for acknowledgement of Berg's Particle Data Laboratories, Inc.; 134, Table 1, and 135,

evaluated some of Berg's sample tubes but found that they gave unreliable data because of orifice defects, thus making them likely to damage CEI's reputation. However, to reduce his risk of having counters returned to CISC due to lack of small sensing apertures, Berg seems not only to have sent doubtful apertures to customers, but to have done so without appropriately optimizing the counters' electronic circuitry.

Berg's treatment of customers desiring small apertures troubled Wallace, and the ASTM "reprint" (Appendix 14) still rankled. He had CEI attorney I. Irving Silverman define obligations between CEI and Berg's organizations, reach agreement with Berg to terminate the CISC franchise relationship, and dissolve CISC as of midnight September 8, 1960. Silverman informed Berg of CEI's expectations by letter dated August 26, 1960. On September 9 he collected seven cartons of CISC documents and in his acknowledgement thereof committed CEI to properly care for them, provide Berg access to them, and complete any unfinished CISC business documented in them.[291] Berg responded as an injured party by letter on September 19th, but committed himself and his employees to avoid, until September 9, 1961, promoting or selling any apparatus that was competitive with the Coulter Counter® and thereafter to avoid using any variation of the word Coulter, the term Coulter Counter, or the phrase Coulter Industrial Sales Corporation. He also proposed how CEI might acquire partial or total control of both PDLI and PCSC.[292] However, those proposals proved too unacceptable to be formalized, and Wallace suspected that Berg continued using information acquired by CISC, to which the franchise agreement assigned CEI sole ownership.

Still impressed with Shepard Kinsman, the Coulters hired him as the first employee of their newly-organized Coulter Electronics Sales Company (CESC). On October 1, 1960, Kinsman issued CESC's first product listing; in addition to the Model A counter, it included

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col. 2, for acknowledgement of help from Berg's CISC. Berg's interaction with the glassblower is outlined in his memorandum, "11 & 19 micron tubes," dated July 19, 1960, to W. H. Coulter, with copy to J. R. Coulter, Sr.; JRC Files.

[291] I. Irving Silverman, letters to CISC dated August 26, 1960, and September 9, 1960. The first is attached to Berg's "Second Amended Counter-Claim" and the second is Exhibit A to CEI's "Reply to Deny Motion to Inspect Certain Documents" filed January 5, 1962, and December 17, 1962, respectively, in Case 1-61-141, Circuit Court of DuPage County, Wheaton, Illinois.

[292] Robert H. Berg, Process Control Services Company, letter dated September 19, 1960, to Coulter Electronics, Inc., 2525 N. Sheffield Avenue, Chicago; WHC Papers. Item B on page 8 is the non-compete commitment, and pages 10 and 10A concern PDLI.

both the Model B counter and the Model H Distribution Plotter.[293] While he developed CESC to replace CISC, Kinsman implemented Silverman's commitment to complete Berg's unfilled orders and to fulfil his exhibition commitments (Figure 7.1).[294] He would also co-author a paper with Joseph Coulter, Jr., for presentation at the 1961 meeting of meeting of the American Ceramic Society.[295]

Meanwhile, at the invitation of the U.S. State Department, a Model A counter was included in the first exhibition of U.S. medical instruments held behind the Iron Curtain, this at the 19th International Fair in Plovdiv, Bulgaria. Visited daily by some 50,000 people, the exhibit introduced the innovative instrument to many receptive clinicians.[296] The Model B counter and the Model H Distribution Plotter would be exhibited October 4-7 by both CEI and Scientific Products at the 10th NIH Instrument Symposium and Exhibit in Bethesda.[297] Furthermore, CEI would occupy two display booths at the AAAS meeting and exhibition that December in New York, and the experimental Model C counter was being readied for another exhibition, this at the 12th Annual Pittsburgh Conference, February 27 to March 3, 1961.[298] Wallace's lawsuit against Schueler & Co. was proceeding favorably and would result that July in a consent judgement that the company's distribution of the Celloscope and its use in the U.S. infringed both his U.S. patent on the Coulter Principle and the Coulter brothers' U.S. patent (2, 869,078) on the volume-control manometer.[299] As if to emphasize these encouraging developments, CEI's February advertisement would claim over 1,500 Model A counter installations (Table 6.1).

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[293] Coulter Electronics Sales Co., "Coulter Counter® -- Prices and Parts List," October 1, 1960; JRC Files; *The Coulter Counter® Model B Research Counter*, four-page sales brochure, Coulter Electronics, no date; WHC Papers.

[294] "Coulter Industrial Sales Co." *Analytical Chemistry* 33 (February 1961): 92A, col. 2; "Coulter Industrial Sales Co." *Analytical Chemistry* 34 (February 1962): 72A, col. 2.

[295] Shepard Kinsman and Joseph R. Coulter, Jr., "Particle size measurements using the resistance change method," incomplete carbon copy; WHC Papers.

[296] "Coulter Electronics, Inc.," *The Coulter Countdown* 1 (September 1970): 1; "U.S. show big Bulgaria hit, White says," *Boston Evening American*, Boston, MA, September 22, 1960, 6.

[297] "NIH Instrument Symposium and Exhibit Program listed," *Analytical Chemistry* 32 (September 1960): 44A and 54A respectively.

[298] Raymond A. Taylor, "AAAS New York Meeting," *Science* NS 132 (Dec. 2, 1960): 1637; "Coulter Industrial Sales Co." *Analytical Chemistry* 33 (February 1961): 92A, col. 2.

[299] Case 60/540 filed February 9, 1960, in District Court, Second District of New York; see "2,656,508," *Official Gazette* 780 (Jul. 17, 1961): 728.

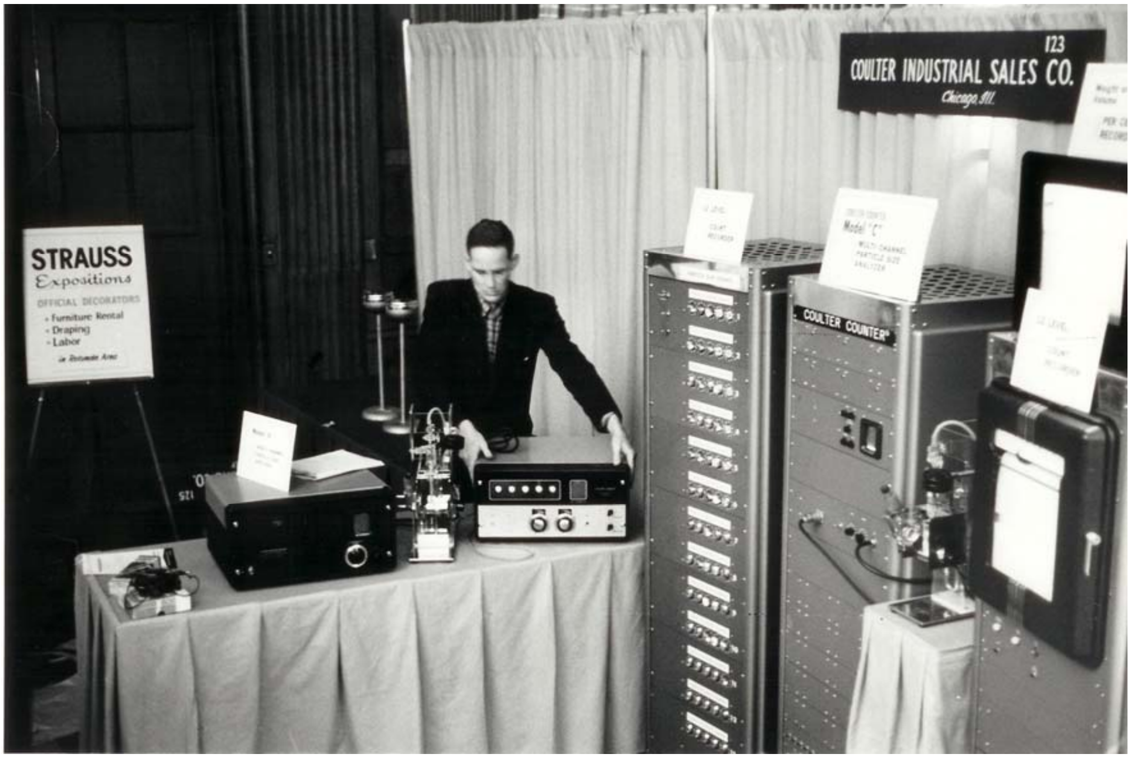

Figure 7.1. Trade show booth by Coulter Industrial Sales Co.[300] Shepard Kinsman is shown preparing the display. The electronics unit for a single-threshold industrial Model A counter is to his right, and he is positioning the one for a dual-threshold industrial Model B counter; the three electronics racks for the experimental Model C counter to his left include, respectively, the 12-bin pulse-height analyzer, the pulse amplifiers and power supply, and this side of the sample stand, the plotter for size distributions. All three counter versions used the industrial sample-stand (Figure 6.1), shown here between the Model A and B electronics units and to the left of the Model C plotter. Kinsman's proficiency with the various Coulter instruments gained him wide recognition.[301]

The experimental Model C counter demonstrated the practicality of twelve-bin pulse-height analysis for both clinical and industrial applications, but it had to be disassembled to be transported, and its 350 vacuum tubes required more electrical power than was usually provided in clinical laboratories. While it demonstrated the practicality of twelve-bin pulse-height analysis, neither its size nor power requirement was practical; to reduce these, development of a transistorized Model C counter was begun soon after this photograph was made.[302]

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[300] Marketing photograph, WHC Papers. The image was published in "A page from history," *The Coulter Countdown* 23 (Spring 1992): 10-12.

[301] Shepard Kinsman, "Electrical resistance method for automated counting of particles," *Annals of the New York Academy of Sciences* 158 (June 1969): 703-09; S. Kinsman and E. V. Hoff, "A new Coulter Counter® for particle size analysis in the sieve range," *Powder Technology* 24 (1979): 155-58; "Retiree Shep Kinsman receives ASTM Award of Merit," *The Coulter Countdown* 12 (Spring 1984): 6.

[302] "A page from history," *The Coulter Countdown* 23 (Spring 1992): 10-12.

That last paragraph could suggest that CEI's relocation to 2525 North Sheffield Avenue had been a resounding success. However, vibrations coupled into the building from "L" trains rumbling along the elevated tracks immediately behind it frequently caused unreliable test results and limited the time available for the testing necessary for both production Model A counters and the developmental Coulter instruments. And at age 48 Wallace was finding "winters were just too damn cold" in Chicago.[303] He began looking for a more congenial location for CEI, a search which eventually led to his interacting with the Dade County (Florida) Development Department. It would not be until that autumn that an acceptable facility was found and agreement reached for its occupation.[304]

A quivering building and cold winters were not Wallace's only worries: 1960 had brought reports of Lars Ljungberg's Celloscope being used to count both white blood cells (leukocytes) and platelets.[305] Taro Nakatani, Vice President of Japan's TOA Electric Co., Ltd., had traveled extensively in the U.S. in quest of a new business opportunity in medical electronics.[306] In Munich, Gerhard Ruhenstroth-Bauer's group confirmed the finding by Mattern, Brackett, and Olson that data from the Model A counter yielded volume distributions for normal human erythrocytes that contained excessive numbers of larger cells, whereas those for normal leukocyte subtypes closely approached a Gaussian distribution.[307] And in Chicago, comments by prospective customers suggested that Robert Berg was continuing to use information acquired during his CISC endeavors; considering this to be both unfair competition and a breach of the several franchise documents Berg had executed, on January 20, 1961, Wallace filed Case 1-61-141 in the Circuit Court of DuPage County, Illinois, regarding Berg's problematic activities.[308]

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[303] "Contributions to Laboratory Medicine 1961-1980," commemorative wall panel at Coulter Clinical Pathology Laboratory, Jackson Memorial Hospital, Miami, Florida.

[304] "New industry growing with Metropolitan Dade County Development," *Metropolitan Miami Memo* 4 (May 1963): 1.

[305] B. Rosenlund and Olav Per Foss, "Automatisk telling av hvite blodlegemer," *Nordisk Medicin* 63 (1960): 556-58; Olav Per Foss, Bent Rosenlund, and Olina Vik, "Automatisk telling av blodplater," *Nordisk Medicin* 64 (1960): 1350-53.

[306] Sysmex Corporation, "50 years of Sysmex," website accessed October 14, 2019.

[307] Mattern, Brackett, and Olson, "Determination of number and size of particles"; G. Ruhenstroth-Bauer and D. Zang, "Automatische Zählmethoden: das Coulter'sche Partikelzählgerät," *Blut* 6 (1960): 446-62.

[308] "Coulter Electronics, Inc. v. Robert H. Berg, individually and doing business as Process Control Services Co., Particle Data Laboratories, Inc., and Coulter Industrial Sales Co.," Case 1-61-141, Circuit Court of DuPage County, Wheaton, Illinois. In December 2019 a photocopy of the approximately 350-page case file was obtained by the author

Meanwhile, international politics would fuel broader concerns. On October 8, 1960, Senator John F. Kennedy came to the University of Kentucky and gave a short speech from a portable podium in front of the Spanish cannon on the drill field. In 1955 Kentucky had lowered its minimum voting age to 18, and the 1960 presidential election was the first in which I would be eligible to vote.[309] Then a sophomore in the Department of Electrical Engineering, I was among the many University students who crowded onto the drill field to listen. Kennedy said that we would be living in the most hazardous time of the country, that students were "not developed to advance the purpose of college – they have a higher purpose – they must pursue the welfare of the nation." [310] The Soviet satellite launches of 1957 had made Soviet superiority in military technology a topic of frequent campus discussions, and Kennedy's words registered forcefully. Events following his inauguration on January 20, 1961, would prove the accuracy of his prediction regarding our future.

During 1960 U.S. President Dwight D. Eisenhower had approved development of a plan for a paramilitary operation to neutralize Cuba's Prime Minister, Fidel Castro, but left a decision on its implementation to his successor. In March of 1961 President Kennedy approved an invasion of Cuba by some 1,400 Cuban exiles.[311] Then, perhaps distracted by the Soviets placing Yuri Gargarin in earth orbit that April 12, on April 16 he cancelled air support crucial to the exiles' success at Cuba's Bay of Pigs, and the next day more than 1,100 of the invaders were captured by Castro's troops, the rest being killed or scattered.[312] Kennedy wished to appear a vigorous president, and one of his highest

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from the Civil Department, DuPage County Judicial Center, Wheaton, Illinois, and placed in the WHC Papers.

[309] "Tax exemption, lower voting age amendments now in effect," *Lexington Leader*, Lexington, KY, December 1, 1955, 2.

[310] "Kennedy arrives in city for major address today," *Lexington Herald*, Lexington, KY, October 8, 1960, 1; Sen. Kennedy tells audience at UK that 1960s will be 'most hazardous time' for United States," *Lexington Sunday Herald-Leader*, Lexington, KY, October 9, 1960, 1.

[311] Rebecca R. Friedman, "Crisis management at the dead center: The 1960-1961 presidential transition and the Bay of Pigs fiasco," *Presidential Studies Quarterly* 41 (June 2011): 309, 313-14, 325-28; Jack Hawkins, *Record of Paramilitary Action against the Castro Government of Cuba: 17 March 1960 – May 1961*, Clandestine Services Historical Paper No. 105 (Washington, D.C.: Central Intelligence Agency, 1961).

[312] "Man enters space," *The Huntsville Times*, Huntsville, AL, April 12, 1961, 1; "Foes of Castro invade Cuba, say Fidel's militia deserting" and "Invasion help is ruled out," *World-Herald*, Omaha, NE, April 18, 1961, 1; Michael Dunne, "Perfect failure: The USA, Cuba and the Bay of Pigs, 1961," *The Political Quarterly* 82 (July-September 2011):

priorities was to meet with Soviet Premier Nikita Khrushchev, who he hoped could be convinced to cooperate toward easing Cold War tensions. A meeting was arranged for June 3-4 in Vienna; but there Kennedy would find that his fumbling of the Bay of Pigs Invasion gave Khrushchev an uncomfortable advantage.[313]

On July 25th Kennedy gave a summary of his Vienna meeting with Khrushchev and his response to that discussion in a nationally televised speech.[314] Khrushchev chose to see this as an ultimatum triggered by his proposal to unilaterally enter into a peace agreement with East Germany and used the safe return of Gherman Titov from seventeen Earth orbits to mention Soviet ICBMs and warn of nuclear war.[315] Then, as August 13 dawned, the East Germans brought Soviet tanks to the border between East and West Berlin and began erecting barbed-wire barriers along it.[316] As a further complication, around that time, the U.S. relocated the technicians installing its Jupiter missiles from completed sites in Italy to proposed sites in Turkey; these missiles carried a 1.44-MT thermonuclear warhead weighing some 1,650 lb. The fact that Turkey shared a national border with the Soviet Union would place considerable Soviet territory within their 1,500-mile range.[317] The first five Turkish launch positions for the 15-missile NATO II Squadron would become operational that November 6, and the final such installation would become

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456, col. 1; Dániel Laykó, "Causes of the Bay of Pigs Invasion's failure," *Corvinus Journal of International Affairs* 2 (2017): 45.

[313] "Text of Kennedy's address on visit to Europe," *Milwaukee Journal*, Milwaukee, WI, June 7, 1961, Part 1, 14; Alexander Akalovsky, "33. Memorandum of Conversation," website accessed February 2, 2019; Günter Bischof, Stefan Karner, and Barbara Stelzl-Marx, eds., *The Vienna Summit and Its Importance in International History* (Lanham: Lexington Books, 2014).

[314] "Here is text of President Kennedy's address on Berlin Crisis," *The Knoxville News-Sentinel*, Knoxville, TN, July 26, 1961, 4; "Kennedy replies to Khrush" and "4000 E. German refugees in record trek to W. Berlin," *Boston American*, Boston, MA, July 17, 1961, 2.

[315] "Khrushchev calls for talks on Berlin, warns of A-war" and "A call from the Kremlin," *San Francisco Chronicle*, San Francisco, CA, August 8, 1961, 1.

[316] "Violence swells Berlin tension as Reds halt flight of refugees; barb wire, tanks seal off escape routes," *Cleveland Plain Dealer*, Cleveland, OH, August 14, 1961, 1; "West Berliners, Communist police clash after refugee routes closed" and "Clatter of pneumatic drills heralds Communist order closing border," *Lexington Herald*, Lexington, KY, August 14, 1961, 1.

[317] "Threat answered by 30 Jupiters," *The Virginian Pilot*, Norfolk-Portsmouth, VA, October 28, 1962, A11; "Second Polaris sub assigned to Mediterranean area," *Arkansas Gazette*, Little Rock, AR, April 13, 1963.

so on March 5, 1962. All Jupiter missiles would be removed from service in April 1963, with Polaris-armed submarines assuming their role.[318]

On August 31, 1961, Khrushchev used France's four Reggane tests of its fission devices as justification for resumption of Soviet testing in order to develop thermonuclear bombs of 20, 30, 50, and 100 MT explosive yield, bombs that could be delivered anywhere on earth by rockets like those that had put Yuri Gargarin and Gherman Titov into earth orbit.[319] This declaration confounded western leaders: On October 31, 1958, the U.S. and Great Britain had entered into a voluntary moratorium on nuclear testing and had been joined in January 1959 by the Soviet Union; neither western country had resumed testing. Furthermore, although Khrushchev had sometimes suggested that the Soviets might develop a 100-MT superbomb, U.S. experts considered such a weapon to be impractical.[320] Regardless of such opinions, Khrushchev was determined to resume testing, and on September 1, 1961, the Soviets detonated the first of the 57 nuclear devices they would explode by that November 4.[321]

In the interim, Cold War politics had led many academicians to realize that hematological methods required standardization, and the first international conference to address this need convened in Vienna that September.[322] Wallace was among the 19 conferees, many of whom were influential members of national health-care organizations (Figure 7.2). As if to stimulate discussion, during that month the Soviets conducted 26 above-ground nuclear detonations with significant radioactive fallout; of these, five were

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[318] "Jacob Neufeld, *The Development of Ballistic Missiles in the United States Air Force 1945-1960* (Washington, D.C.: Office of Air Force History, 1990), 227.

[319] "French set off 4th A-bomb," *The Evening Star*, Washington, D.C., April 25, 1961, 6; "French tests again hit by Soviet," *Richmond Times-Dispatch*, Richmond, VA, May 19, 1961, 18; Joseph L. Myler, "Superbomb goal raises world fears – rocket threat taken seriously," *The Evansville Press*, Evansville, IN, August 31, 1961, 1; Bob Considine, "On the line: Measure of Khrush H-bomb," *Boston Daily Record*, Boston, MA, September 6, 1961, 16.

[320] "Speculation equals size of K's 100-megaton boast," *The Biloxi Daily Herald*, Biloxi, MS, September 7, 1961, 4; "Soviet superbomb termed foolish; U.S. experts call it too potent," *Lexington Herald*, Lexington, KY, August 11, 1961, 20.

[321] Tom Wicker, "Reds explode nuclear bomb," *The Boston Herald*, Boston, MA, September 2, 1961, 1; Tom Szulc, "Reds fire 2nd nuclear blast," *Cleveland Plain Dealer*, Cleveland, OH, September 5, 1961, 1; "1961 Soviet nuclear tests," website accessed June 7, 2019.

[322] S. M. Lewis, "International Council for Standardization in Haematology – the first forty years," *International Journal for Laboratory Haematology* 31 (June 2009): 254.

Figure 7.2. Fireside Conference, Vienna, September 1961.[323] The conference theme was "Experiences with blood-cell counting apparatus;" chaired by Ch. G. v. Boroviczény of the Medical University Clinic, Freiburg, Germany, it was the first international conference to consider standardization in clinical hematology. Wallace Coulter is the third seated man on the right side of the table. George Brecher seems to have been the photographer; his distinctive handwriting on the photo's reverse provides the preceding information.

Boroviczény was well connected within the hematological research community and had just completed an extensive survey of the cell-counting art in which he included a discussion of the dual-function Model D counter then being developed by Coulter Electronics, Ltd.[324]

The proceedings editor mislaid the manuscripts from this conference; what little is known about it appears in Boroviczény's later footnote.[325] Other conferees included J. F. Coster, P. J. Crosland-Taylor, G. Discombe, T. Gecsö, D. Goodchild, S. A. Killmann, N. Kleine, J Larsson, K. Lennert, J. Libánski, E. W. Meyer, O. J. Nash, L. Poller, H. Reiser, G. Ruhenstroth-Bauer, and I. Wessely. As time permitted, Wallace would continue to interact with standardization committees into the 1980s.[326]

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[323] Annotated photograph; WHC Papers.

[324] K. G. v. Boroviczény, "Erhfahrungen mit Blutkörperchenzählapparate," *Das Ärzltliche Laboratorium* 8 (1961): 168-71.

[325] Ch. G. v. Boroviczény, "On the standardization of blood cell counts," *Bibliotheca Haematologica* 24 (1966): 24, fn.

[326] International Committee for Standardization in Haematology, "Protocol for evaluation of automated cell counters," *Clinical and Laboratory Haematology* 6 (1984): 69-84.

of at least one MT in explosive yield.[327] Then, on September 15, the U.S. also resumed testing with the first of 44 low-yield underground detonations.[328]

When Wallace returned to Chicago from Vienna, he found that prototype Model B counters and Model H plotters were progressing acceptably through evaluations at two institutions as the first advertisement appeared for them.[329] Furthermore, a prototype six-bin transistorized Model C counter would be functionally complete by that December, and arrangements were progressing toward CEI's relocation to Hialeah, Florida.

By then, the barrier between East and West Berlin included sections of concrete wall with armed guards in watch towers; the checkpoints through which people might pass had been reduced to a stifling few. Disagreement over whether East German or Soviet guards could examine documents authorizing travel of U.S. diplomats between the two Berlins prompted the U.S. to position tanks at Checkpoint Charlie, their cannons aimed toward the East German troops positioned behind the wall. On October 27, the Soviets positioned their tanks in East Berlin with their cannons aimed at the U.S. tanks 200 yards away.[330] To his credit, Kennedy was able to convince Khrushchev that if the Soviet tanks were withdrawn, he would have the U.S. tanks withdrawn, and after 16 hours the dangerous standoff ended peacefully.[331]

Meanwhile, nuclear testing had continued. All 21 Soviet tests in October were above ground, most with significant fallout, and six were 1.5 MT or greater in explosive yield. The detonation on October 23 was first reported to have generated an explosive yield of 30 to 50 MT, but this was denied by a Soviet diplomat, who indicated that a 50-MT bomb would be tested on October 30.[332] (The air-drop test on the 23rd was later officially listed at an explosive yield of 12.5 MT.[333]) And on October 30, Earth shook when

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[327] "1961 Soviet nuclear tests," website accessed June 7, 2019.

[328] "Operation Nougat," website accessed June 9, 2019.

[329] "The Coulter Counter®," *Scientific American* 205 (September 1961): 252 and *Science* NS 134 (October 20, 1961): 1270.

[330] "Soviet tanks roll into Berlin," *Lexington Herald*, Lexington, KY, October 27, 1961, 1.

[331] "U.S. demands Russia restore Berlin access" and "U.S., Red tanks face each other at Berlin line," *San Diego Union*, San Diego, CA, October 28, 1961, 1; 1961, 1; Ingo Wolfgang Trauschweizer, "Tanks at Checkpoint Charlie: Lucius Clay and the Berlin Crisis, 1961-62," *Cold War History* 6 (May 2006): 205-28.

[332] "Shocked by huge bomb" and "Biggest atom blast set for October 30," *The Kansas City Star*, Kansas City, MO, October 24, 1961, 1.

[333] "1961 Soviet nuclear tests," website accessed June 7, 2019.

the Soviets exploded the most powerful thermonuclear bomb ever detonated; at 50 MT explosive yield the parachuted bomb was some 3,300 times more powerful than the 1945 Hiroshima bomb.[334] In fact, its yield was ten times the total power of all explosives used during WWII, including the fission bombs dropped on Hiroshima (Figure 1.1) and Nagasaki (Figure 1.2). According to one of the test's participants, as Khrushchev saw it, the test was not of a weapon but of a trigger for a 100-MT bomb done with less than the full 100-MT load of fusion fuel, while according to Andrei Sakharov, Khrushchev saw the test as hanging the terror sword of Damocles over the heads of capitalists.[335] Of the ten remaining Soviet tests, nine were 0.4 MT yield or less, that on November 4 was 1.5 MT.[336]

However, radioactive fallout was not the only cloud on the Coulters' horizon. In Sweden, Lars Ljungberg was improving the Celloscope counter, and in Japan, Taro Nakatani had initiated development of a blood-cell counter at TOA Electric Co., Ltd.[337] Furthermore, competitive interests at several German academic institutions were gaining momentum.[338] And in DuPage County, Robert Berg's responses to CEI's complaint in Case 1-61-141 were both dilatory and evasive while he continued using information acquired in his CISC activities, a situation he escalated on July 26, 1961, by applying to register "ElectroZone" as a PDLI trademark for the Coulter sensing aperture.[339] It was then

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[334] "French report Soviet A-blast; Swedes and British call it earthquake," *Lexington Herald*, Lexington, KY, October 30, 1961, 1; "Russians detonate device reported at 50 megatons," *Arkansas Gazette*, Little Rock, AR, October 31, 1961, 1.

[335] Yu. N. Smirnov, "Three interesting episodes in the Soviet nuclear program," in *Monitoring a Comprehensive Test Ban Treaty*, ed. E. S. Husebye and A. M. Dainty, 11-15 (Dordrecht: Kluwer Academic Publishers, 1996).

[336] "1961 Soviet nuclear tests," website accessed June 7, 2019.

[337] Erich Treiber, "Gegenwartsprobleme der Viskosechemie," *Lenzinger Berichte* 12 (September 1962): 15; Sysmex Corporation, "50 years of Sysmex," website accessed October 14, 2019.

[338] Boroviczény, "Erhfahrungen mit Blutkörperchenzählapparate"; H. P. Schudt, "Elektronische Verfahren zur automatischen Zählung von Blutkörperchen," *Wissenschaftliche Zeitschrift der Hochschule für Elektrotechnik, Ilmenau* 7 (1961): 269-78; K. G. v. Boroviczény, R. Giencke, and E. Baumgarten, "Erythrozytenzählung mit einem elektronischen apparat," *Wiener Zeitschrift für innere Medizin* 6 (June 1961): 267-78. G. Pfeiffer, "Methoden und Geräte zur elektronische Zählung von Blutkörperchen und zur Bestimmung ihrer Größenverteilung," *Nachrichtentechnik* 12 (1962): 47-50; "Elektronische Blutkörperchen-zählung und Grössenverteilungs-bestimmung," *Zeitschrift fur Medizinische Labortechnik* 3 (1962): 57-87.

[339] "SN 124,750, Electrozone," *Official Gazette* 779 (Jun. 19, 1962): TM 121. For a summary of the background for Case 1-61-14, see I. Irving Silverman, "In the matter of

still six weeks until the expiry of his non-compete commitment.[340] These increasing competitive pressures strongly emphasized the Coulters' need to relocate CEI into a facility suited to efficient development and stable production of their cell and particle counters.

As atmospheric disturbances from Khrushchev's inhuman blast died away, the Coulters began preparations to remove CEI from 2525 N. Sheffield Avenue and locate it in the warehouse building at 590 West 20th Street in Hialeah, Florida. In early December 1961, a truck convoy moved equipment and fifteen key personnel, including the company's production employees and sales manager, Floyd Henderson, into CEI's new facility.[341] Florida Power and Light Company welcomed CEI with a display of a Model A counter under a placard bearing the corporation's new address and phone number.[342]

Application Serial No. 124,750 published in the *Official Gazette* on June 19, 1962," Opposition No. 42,198, September 18, 1962; "Electrozone" file, WHC Papers.

[340] Robert H. Berg, Process Control Services Company, letter dated September 19, 1960, to Coulter Electronics, Inc., 2525 N. Sheffield Avenue, Chicago; WHC Papers. The non-compete commitment is on page 8.

[341] "Coulter Electronics, Inc.," *The Coulter Countdown* 1 (September 1970): 1; "New industry growing with Metropolitan Dade County Development," *Metropolitan Miami Memo* 4 (May 1963): 1. After CEI completed its move to Kendall in the early 1990s, the building was replaced with a parking lot.

[342] Photograph of the display, "Builders of South Florida," WHC Papers.

CHAPTER 8. ELABORATION

The Coulter brothers, Wallace and Joseph, Jr., accompanied the group of CEI employees who arrived in December 1961 at the empty warehouse at 590 West 20th Street, Hialeah; they placed a door on two sawhorses as a shared desk and resumed their duties. Meanwhile, in addition to the Model B counter, the Model H Distribution Plotter, and the transistorized Model C counter, other products were moving through development in Chicago; among these were timers for monitoring multiple laboratory processes and an experimental blood coagulation timer. Wallace continued supporting CEI's actions in DuPage County Case 1-61-141 and Walter Hogg and the technicians as they readied prototype instruments for removal to Hialeah. While Joseph, Jr., organized and restarted production of Model A counters, Floyd Henderson reconstituted his sales organization; one of his early hires, Ms. Doris Zagon (Figure 8.1), would become an exceptional asset for Wallace. When Hogg and the technicians arrived in Hialeah in February 1962; one of their first tasks was preparing an exhibition of the growing CEI product line (Figure 8.2). A comparison of this figure with Figure 5.4 will suggest the progress of the Coulters' company during their last five years in Chicago.

While Wallace juggled legal and technical duties, Joseph, Jr., found the Hialeah location advantageous as he rebuilt CEI's production capability. After Castro's ousting of Cuba's Batista regime on December 31, 1958, between 1,600 and 1,700 Cubans per week had begun exiling themselves to the U.S. via commercial flights, usually to Miami where many would settle in Hialeah. These exiles were typically well-educated, many having held upper government or business positions. Kennedy's Bay of Pigs fiasco in April 1961 had increased both immigration rates and the number of true refugees, some of whom were mid-level professionals or merchants with relatives among earlier arrivals. Appreciative of any employment opportunity, these new arrivals brought an intelligent work ethic and stability to the positions Joseph needed to fill. This phase of Cuban immigration would end in November 1962, when Castro stopped all commercial flights from Cuba in retaliation for the U.S. quarantine of Cuba during October's missile crisis.[343]

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[343] John F. Thomas, "Cuban refugees in the United States," *The International Migration Review* 1 (Spring 1967): 47-52; Silvia Pedraza-Bailey, "Cuba's exiles: Portrait of a refugee migration," ibid. 19 (Spring 1985): 9-14; "Castro grounds airline flights," *Chicago Daily News*, Chicago, IL, November 19, 1962, 13.

Figure 8.1. Ms. Doris Zagon, Wallace Coulter's only administrative aide.[344] Doris joined CEI January 10, 1962, shortly after the company's initial move from Chicago to Hialeah. She was recruited as a secretary for the company's first sales manager, Floyd Henderson, but Wallace began asking her to type his letters and notarize CEI legal documents, so Henderson recruited himself another secretary.[345] Doris soon came to understand the Coulter brothers and their approach to their growing company and its employees. Wallace valued her effectivity and would often comment about something needing attention, "Get Doris to get it done." She served as his aide until several months after Beckman Instruments assumed control of Coulter Corporation in late 1997. Here, she is shown in early 1963 as a technologist operating one of the bin-threshold controls of a Coulter Counter® Model B with the biological sample stand.

Incrementing the interlocked bin-threshold controls enabled the accessory Model H Distribution Plotter above the Model B counter to record a 25-bin differential size distribution from a continuous sample run lasting 100 seconds. The count for each bin was displayed on the five dekatron counter tubes above the counter's threshold controls, and the cell pulses having amplitudes between the bin thresholds were displayed on the oscilloscope tube to the right of the dekatron tubes.[346] *Photograph courtesy of Ms. Zagon.*

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[344] The image appeared in "New industry growing with metropolitan Dade County development," *Metropolitan Miami Memo* 7 (May 1963): 1. Ms. Zagon provided a photocopy of the article with her notes on May 6, 2018; WHC Papers.

[345] Ms. Zagon's husband Harold would work in Henderson's organization until retirement.

[346] *The Coulter Counter® Model B Research Counter*, four-page sales brochure, Coulter Electronics, no date; WHC Papers.

Figure 8.2. CEI's first trade-show exhibition after its move to Hialeah.[347] The display was an accurate representation of the company's product offering by mid-1962. Two prototype Model E timers for monitoring multiple laboratory processes appear on the left arm of the display.[348] Behind them is an electronics unit for the Model B counter, to the right of which are the counter's biological sample stand and Model H Distribution Plotter (see Figure 8.1). A Model A counter with its biological sample stand (Figure 5.2) occupy the center of the display; to its right is a second Model B counter and Model H Distribution Plotter.

The thresholding circuitry for a prototype six-bin transistorized Model C counter is exhibited in the display corner; the industrial sample stand sits to the right of it while the counting, count display, and power electronics are in the two modules on the floor at the end of the right arm of the display.[349] The instrument between the Model C circuitry cabinets is a prototype apparatus for determining the coagulation time of blood samples; after repackaging, it joined the CEI product line as the Coulter Coagu-Chron.[350]

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[347] Marketing photograph, 54th Annual Meeting of the American Society for Clinical Investigation, Atlantic City, NJ, April 1962; [*Journal of Clinical Investigation* 41 (April 1962): i-ii]; WHC Papers.

[348] Jack F. Crawford, "Time interval indicator having a rotatable transparent plate concentric with a fixed calibrated plate," U.S. Patent 3,187,319, filed Sept. 4, 1962, and issued Jun. 1, 1965. This timer was used to control the 100-second sample runs required by the paired Model B counter and Model H plotter.

[349] An image of a six-bin Model C counter appeared in introductory advertisements; see, for example, "'The' specialists in particle size analysis," *Analytical Chemistry* 36 (February 1964): 181A, col. 1.

[350] Standford L. Adler and Wallace H. Coulter, "Automatic coagulometer apparatus," U.S. Patent 3,216,240, filed Aug. 30, 1962, and issued Nov. 9, 1965.

In July 1962 Castro had agreed that the Soviet Union could place nuclear missiles in Cuba, and the Soviets had significantly increased Cuban shipments of personnel and supplies, seemingly intending to present the U.S. with the fait accompli of a nuclear threat analogous to that it felt from NATO Jupiter missiles the U.S. had installed in Turkey.[351] On October 16 President Kennedy was informed of new construction activity apparent in reconnaissance photographs made during Cuban overflights the previous day, and subsequent images showed camouflaged launch sites for nuclear ballistic missiles. In a nationally televised message on October 22, Kennedy announced that the U.S. would stop Soviet shipments of offensive arms by imposing a naval quarantine on Cuba, which prompted the Soviet response that this was piracy and a step toward thermonuclear war. Dr. Thomas C. Clark, then chairman of the Department of History at the University of Kentucky, called the decision “exceedingly grim,” but added that he did not “favor doing nothing and letting them build up arms right in our front door.” [352] On October 25, twelve Soviet ships were turned back from Cuba; on the 27th, Cubans shot down a U.S. reconnaissance plane, and in a broadcast to Kennedy, Soviet Premier Khrushchev offered to “remove from Cuba those means which you regard as offensive means” if U.S. representatives would declare that the U.S. would “remove its similar means from Turkey.” Kennedy ignored this message, instead responding agreeably to Khrushchev’s message of the previous day, which did not link the Cuban and Turkish offensive weapons. Kennedy required that first, under United Nations supervision, work stop on Cuban offensive missile

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[351] Raymond L. Garthoff, “Cuban Missile Crisis: The Soviet story,” *Foreign Policy* 72 (Autumn 1988): 61-80; Aiyaz Husain, “Covert actions and US Cold War strategy in Cuba, 1961-62,” *Cold War History* 5 (February 2005): 23-53; “Soviet Union increases its deliveries to Cuba” and “Senate warns Kremlin about buildup in Cuba,” *Lexington Herald*, Lexington, KY, September 21, 1962, 1; “Soviet ship in Havana” and “Resolution on Cuba is carefully worded,” *Lexington Leader*, Lexington, KY, September 27, 1962, 1; John M. Hightower, “U.S. Naval blockade of Cuba could bring long repercussions,” *Lexington Herald*, Lexington, KY, October 14, 1962, 46; Elton C. Fay, “Soviet-bloc shipments for Castro of late far more than trickle,” *Lexington Leader*, Lexington, KY, October 19, 1962, 1.

[352] John M. Hightower, “U.S., Soviet on collision course; blockade ordered to halt flow of arms to Cuba,” *Lexington Leader*, Lexington, KY, October 23, 1962, 1; George Syvertsen, ”Soviet in serious warning to U.S.” ibid.; John Alexander, “Lexingtonians feel that possibility of war is real; they back Kennedy,” ibid.; “Kennedy-K showdown shapes up; blockade only hours away,” *The Knoxville News-Sentinel*, Knoxville, TN, October 23, 1962, 1. For a timeline, see: Marcus D. Pohlmann, “Constraining presidents at the brink: The Cuban Missile Crisis,” *Presidential Studies Quarterly* 19 (Spring 1989): 337-46.

bases and all offensive weapons in Cuba be rendered inoperable.[353] He did not agree to removal of the NATO Jupiter missiles from Turkey but, given no option, may have had a proposal in readiness to do so on removal of the Cuban offensive missiles.[354] To the surprise of many, Khrushchev accepted Kennedy's requirements, this without linking dismantling of the missile bases to a Berlin settlement, and Wallace (and people of the western hemisphere) began to breathe more easily.[355] So ended a confrontation that, for a second time in the first two years of Kennedy's presidency, had brought the distinct possibility of hot war between the U.S. and the Soviet Union.

But lest it seem that national rationality had suddenly flourished, in the thirteen days from October 16th to the 29th, 1962, both world powers tested seven nuclear devices, bringing the total tests for each to some 100 since September 1, 1961, when the Soviets had abrogated the voluntary nuclear-test moratorium.[356] Meanwhile, school children continued to practice "Duck and Cover" (Figure 3.4).[357]

By mid-1962 Joseph, Jr., had established a production capability for the Model A and Model B counters and Model H Distribution Plotter (Figure 8.3) and begun planning production of the Model C counter (Figure 8.4). Wallace had been supporting CEI's actions in DuPage County Case 1-61-141 and two groups evaluating paired Model B and Model H prototypes: NIH's George Brecher and coworkers were assessing instrument capabilities, and Sipe and Cronkite were exploring their utility for platelet counting. The

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[353] "U.S. intercepts Soviet ship, 12 others turn back," *The Knoxville News-Sentinel*, Knoxville, TN, October 25, 1962, 1; "U.S. recon plane reported missing" and "Peace in sight if Cuba bases go, U.S. would then recall ships, JFK tells K," ibid. October 28, 1962, A1; Khrushchev's broadcast message of October 27, Kennedy's reaction to it, and his response to that of October 26 are all printed on A4. For the actual documents, see: "John F. Kennedy and the Cuban Missile Crisis; Thirteen days in October 1962," John F. Kennedy Presidential Library and Museum, website accessed October 19, 2019.

[354] James G. Blight, Joseph S. Nye, Jr., and David A. Welch, "The Cuban Missile Crisis revisited," *Foreign Affairs* 66 (Fall 1987): 178-79; Süleyman Seydi, "Turkish-American relations and the Cuban Missile Crisis, 1957-63," *Middle Eastern Studies* 46 (May 2010): 433-55.

[355] "Khrushchev orders missile bases dismantled, Kennedy lauds Premier's action, underscores need for verification," *Lexington Herald*, Lexington, KY, October 29, 1962, 1; "Thant says Red missile bases being dismantled," ibid. November 1, 1962, 1.

[356] "Nuclear tests – databases and other material," website accessed November 10, 2019.

[357] "In case of brief warning," *The Knoxville News-Sentinel*, Knoxville, TN, October 28, 1962, F8.

Figure 8.3. CEI's production area, 590 West 20th Street, Hialeah.[358] Here, Joseph R. Coulter, Jr., is shown in early 1963 as he watched construction of an electronics unit for a Model A counter.[359] The Model B counter was then entering production.[360] Behind Joseph on his right several Coulter Dual Diluters (Figure 6.2) were being assembled. As part of his CEI duties while in Chicago, in late 1958 Joseph had established Coulter Electronics, Ltd., in a London basement, and then in 1961 he had organized Coultronics France S. A. in Margency, France.[361] He would continue expanding CEI's commercial operations throughout the company's Hialeah residency, its merger with Coulter Corporation in 1991, and its move to Kendall, Florida, in 1992 through 1995.[362]

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[358] The central part of the image was featured in "New industry growing with metropolitan Dade County development," *Metropolitan Miami Memo* 7 (May 1963): 1, while the complete image was published in "Look ahead with expectation, look back with pride," *The Coulter Countdown* 10 (Special 1980): 4.

[359] "This 1 technician counts and sizes blood cells using a Coulter Counter®," *Journal of Clinical Investigation* 41 (February 1962): ad page 3.

[360] "Coulter biological particle counter," *Science* NS 135 (February 16, 1962): 470; "Automation for biological sizing and counting," *AIBS Bulletin* 12 (August 1962): 45.

[361] Coulter Electronics, Ltd., was located at 4 Auriol Mansions, Edith Road, London W14 ["Now manufactured in Britain!" *Chemical Age* 84 (Sept. 24, 1960): 476], while Coultronics France S. A. was at 14 Rue Eugène Legendre, 95 Margency ["Le Coulter Counter Modele S," *Medicaments*, 1971, ref. 69822].

[362] "Joseph R. Coulter, Jr., 1924-1995," *Coulter Viewpoint* 18 (1996): 4-5; "Joseph Coulter, Jr., built worldwide enterprise," *The Miami Herald*, state ed., Miami, FL, November 29, 1995, 4B.

Figure 8.4. A prototype twelve-bin Coulter Counter® Model C.[363] The thirteen transistorized threshold controls filling the second panel of the electronics cabinet on the left defined the bin limits as the twelve bins were simultaneously acquired; the bin display tubes occupy the second and third panels of the cabinet on the right. The power unit, shown beneath the display cabinet of the six-bin display cabinet in Figure 8.2, is beneath the bench. A comparison with the experimental Model C counter in Figure 7.1 may be of interest.

Intended for industrial applications, the complex Model C counter did not require the long sample runs needed by the Model H plotter, but it was expensive; to attract buyers it was introduced in six-bin, nine-bin, and twelve-bin versions with optional numbers of dekatron display tubes for the bin counts. The full complement of display tubes was not installed in this twelve-bin instrument; only the bottom row of display tubes contains six tubes for each of those two bins, whereas the top three double rows contain only four display tubes for each of those six bins. A summary and an image of a twelve-bin instrument with the full complement of display tubes have been published.[364]

Only a few Model C counters were sold, but Xerox Corporation bought three or four for use with its toners.[365] Experience gained in designing the Model C enabled design of the transistorized replacement for the Model A, the Model F counter, and the smaller and more versatile Model T counter after integrated circuits replaced discrete transistors.

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[363] Marketing photograph, WHC Papers. This image appeared in the historical summary, "A page from history," *The Coulter Countdown* 23 (Spring 1992): 10-12.

[364] R. W. Lines and W. M. Wood, "Automatic counting and sizing of fine particles," *Ceramics* 16 (May 1965): 29.

[365] H. Walser, "Precision of the Model C Coulter Counter," Xerox Corporation, Webster, NY; presented at the Coulter Counter Users' Conference, Chicago, January 23, 1968.

first evaluation emphasized differences between the Model A and Model B counters, and both would be favorable to promising features of the Model B and Model H instruments.[366] Constant-current excitation for the sensing aperture of the Model B counter made it insensitive to tolerancing differences in sensing apertures of a given geometry and to variations in the resistivity of the suspending electrolyte and the temperature of the aperture's environment. Dual threshold controls of the Model B counter's current-sensitive amplifier were interlockable to form a movable bin controlled by a sequencing four-second timer, thereby enabling the Model H plotter to automatically accumulate 25-bin differential volume distributions from constant-flow sample runs lasting some 100 seconds.[367]

Although the circuitry improvements in the Model B counter and the innovative capability of the Model H Distribution Plotter could improve analytic efficiency, they would also focus attention on functional characteristics of Coulter sensing apertures (Figure 4.7). Sipe and Cronkite concluded that both the Model A and Model B counters could rapidly give accurate platelet counts on known dilutions of separated normal platelets but that making platelet counts was impractical on either whole blood or blood from patients with a variety of diseases. Moreover, when visual platelet counts by phase microscopy fell below 100,000 per mm^3, instrument counts were consistently higher than the visual counts, and the authors speculated that small particles invisible by phase microscopy were responsible.[368] Analysis of the cellular signals would allow Hogg to demonstrate that the phantom platelets were cells that recirculated in the aperture's toroidal exit flow into its sensitive volume to be counted a second time, a preventable artefact if a cell-free flow of electrolyte were provided at the aperture's exit orifice to sweep exiting cells away from the aperture.[369] However, the paired Model B counter and the Model H plotter did not

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[366] George Brecher et al., "Size distribution of erythrocytes," *Annals of the New York Academy of Sciences* 99 (June 1962): 242-61; Clyde R. Sipe and Eugene P. Cronkite, "Studies on the application of the Coulter electronic counter in enumeration of platelets," ibid. 262-70. Brecher's technologists found the count display of the Model B counter easier to read if the counter were positioned to place the dekatron display tubes closer to eye level (see Figure 8.1); George Brecher, letter dated July 6, 1964 to Wallace H. Coulter, WHC Papers.

[367] *The Coulter Counter® Model B Research Counter*, four-page sales brochure, Coulter Electronics, no date; WHC Papers.

[368] Sipe and Cronkite, "Studies on the application of the Coulter electronic counter in enumeration of platelets," 268-69. See Table 4.1 for the range of normal platelet counts.

[369] Walter R. Hogg, "Aperture tube structure for particle study apparatus," U.S. Patent 3,299,354, filed Jul. 5, 1962, and issued Jan. 17, 1967.

sufficiently separate platelet volume distributions from background noise, which would require better orifice quality in sensing apertures and reduced electronic noise in the Model B counter.[370] Such improvements would soon result in the industrial Model B counter.

In both evaluations of the paired Model B and Model H instruments the long sample runs were often interrupted by debris adhering around the aperture's entry orifice or clogging its bore. Although such count interruptions had been common throughout Wallace's development of his Coulter Principle, they were detectable via the microscopes fitted to the sample stands used with the Model A (Figure 5.2), Model B (Figure 8.1), and Model C (Figure 8.4) counters, and repeated sample runs neither onerously increased technologist time nor risked sample depletion as did those encountered in the present evaluations. These emphasized the value of simultaneous counts by multi-bin pulse-height analyzers such as used in the Model C counter, but even with such advanced counter circuitry, fewer interrupted sample runs would be advantageous. Analysis of the counter pulse stream allowed Wallace and Hogg to develop ways to warn the operator of debris adhering around the aperture entry orifice or clogging its bore.[371] Although these methods were helpful, they were only partial solutions, and Wallace would later extend them to provide automatic removal of the interfering material.[372]

Brecher's group found that the differential volume distributions of normal human erythrocytes created by the Model B counter and Model H plotter contained excessive numbers of larger cells suggestive of bimodal populations. In their evaluation of the Model A counter, Mattern, Brackett, and Olson had found similar skewness in differential volume distributions calculated manually from the counter data, a finding subsequently confirmed by Ruhenstroth-Bauer and Zang.[373] Observing that counter pulse heights resulting from simultaneous presence of two cells in the aperture's sensitive volume depended on the axial distance between them, Mattern's group had given a statistical model for such

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[370] M. J. Eggleton and A. A. Sharp, "Platelet counting using the Coulter electronic counter," *Journal of Clinical Pathology* 16 (1963): 164-67.

[371] Wallace H. Coulter and Walter R. Hogg, "Debris alarm," U.S. Patent 3,259,891, filed May 1, 1964, and issued Jul. 5, 1966.

[372] Wallace H. Coulter, "Method and system for cleaning an aperture in a particle study device," U.S. Patent 3,963,984, filed Nov. 4, 1974, and issued Jun. 15, 1976.

[373] Mattern, Brackett, and Olson, "Determination of number and size of particles," 58-59 for the coincidence model and 67 for the skewness discussion; Ruhenstroth-Bauer and Zang, "Automatische Zählmethoden: das Coulter'sche Partikelzählgerät."

occurrences in mid-aperture and attributed the large-volume distribution skewness to cellular coincidence. The Model H plotter eliminated manual arithmetic errors in distribution generation, and while the degree of skewness did not follow coincidence rates, for want of a better reason Brecher's group reported that "increased coincidence produces a slight broadening of the skewed portion of the standard graph, but the peaks remain unchanged." [374] After substituting a 100-bin pulse-height analyzer for the Model H plotter, Lushbaugh, Basmann, and Glascock found that the skewness was not due to leukocytes in the sample, but seemed to indicate a phantom population of erythrocytes invisible in microscopy of the blood films.[375] Lushbaugh's continued research would later suggest that the invisible erythrocytes might be normal erythrocytes modified by the aperture excitation current.[376] However, similar distribution skewness was also reported for monodisperse latex particles, which suggested that it was a measurement artefact rather than of cellular origin.[377] As will be discussed, over the next decade much effort would be expended by several research groups before the skewed volume distributions would be demonstrated to originate in aperture hydrodynamics.

Analysis of the cellular signal stream from an aperture's sample throughflow had enabled Wallace and Hogg to accommodate two functional characteristics of sensing apertures, and CEI would build on that experience to correct counts and volume distributions for both cell-free electrolyte counts and the two types of coincidental signals resulting from multiple cells being within the aperture's sensitive volume.[378] While such

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[374] Brecher et al., "Size distribution of erythrocytes," 250-51.

[375] C. C. Lushbaugh, N. J. Basmann, and B. Glascock, "Electronic measurement of cellular volumes: II. Frequency distribution of erythrocyte volumes," *Blood* 20 (1962): 242; "Correspondence," *Blood* 23 (1964): 403-05.

[376] Amiram Ur and C. C. Lushbaugh, "Some effects of electrical fields on red blood cells …," *British Journal of Haematology* 15 (1968), 527-38.

[377] M. Wales and J. N. Wilson, "Theory of coincidence in Coulter particle counters," *Review of Scientific Instruments* 32 (1961): 1132-36; "Coincidence in Coulter Counters," ibid. 33 (1962): 575-76; Herbert E. Kubitschek, "Loss of resolution in Coulter Counters," ibid. 33 (1962): 576-77.

[378] J. R. Didry, A. German, and J. Maccario, "Etude du phenomene de coincidence dans un compteur de type Coulter," *Biometrics* 29 (June 1973): 281-92; Graham, "The Coulter Principle: Foundation of an industry," refs. 54 through 61 and 97 through 107. Other examples include Jacques A. Pontigny and Claude J. Collineau, "Digitized coincidence correction method and circuitry for particle analysis apparatus," U.S. Patent 3,626,164, filed Jun. 16, 1969, and issued Dec. 7, 1971, and Wallace H. Coulter and Walter R. Hogg, "Methods and apparatuses for correcting coincidence count

editing methods initially required somewhat longer count times, these were not onerous, and instruments incorporating them would prove to be both producible on a commercial scale and maintainable in routine use. Accruing experience would enable development of editing circuitry and techniques that could correct the count for artefactual pulses, as well as reduce the skewness in volume distributions of both cells and particles.

Although international competition in weapons development and cold-war politics had inspired Wallace's invention of the Coulter Principle and motivated its implementation, increasing commercial competition had now become his most urgent concern.

In the U.S., Robert Berg had continued using information acquired during the tenure of the CISC franchise agreements and at some point began purchasing used Model A counters, rebuilding them, and selling them via PDLI while still bearing their Coulter trademarks. On July 26, 1961, Berg escalated these trademark infringements by applying for PDLI's registration of the trade-mark "ElectroZone" for the electrolytic sensing zone formed by an aperture through which an electrical current and a suspension flow were simultaneously passing, that is, the preferred Coulter sensing aperture of Figure 4.1. He claimed a first-use date for "ElectroZone" of March 1, 1960, which preceded CEI's termination of the franchise relationship and so under the terms of those agreements made the trademark assignable to CEI. On June 19, 1962, the U.S. Patent Office published notice of Berg's "ElectroZone" application.[379] Three months later CEI's attorney, I. Irvin Silverman, filed an opposition to PDLI's registration in which he summarized background and concerns for which CEI had sought recourse via DuPage County Case 1-61-141.[380] In late 1962 CEI began notifying PDLI's suppliers, sales representatives, and customers of their probable infringement of U.S. Patent 2,656,508 on the Coulter Principle. Then in February 1963 the U.S. Patent Office suspended actions on the "ElectroZone" trademark pending resolution of Wallace's DuPage County Case 1-61-14, and this decision carried important ramifications: As indicated in Appendix 15, it began an interval during which Berg would continue infringing activities; it would enable him to prolong that interval via evasive and dilatory responses to court procedures until Wallace's U.S. patent

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inaccuracies in a Coulter type of particle analyzer," U.S. Patent 3,949,198, filed Mar. 26, 1974, and issued Apr. 6, 1976.

379 "SN 124,750, ElectroZone," *Official Gazette* 779 (Jun. 19, 1962): TM 121.

380 I. Irving Silverman, "In the matter of Application Serial No. 124,750 published in the *Official Gazette* on June 19, 1962," Opposition No. 42,198, September 18, 1962; "ElectroZone" file, WHC Papers.

on the Coulter Principle was nearing expiry; and it presaged similar decisions by other courts hearing future CEI infringement lawsuits originating in his activities.

In Sweden, Lars Ljungberg had continued promoting Celloscope counters, but as a countermeasure CEI's English subsidiary, Coulter Electronics, Ltd., had developed the Model D counter (Figure 8.5). Introduced in 1963, it promised significant competition for Ljungberg's counters. And by then, 77 employees were working in CEI's Hialeah facility, where the technical staff was developing the transistorized Model F counter and Model J plotter to replace the aging Model A counter and the unreliable Model H plotter.

Meanwhile in Japan, TOA Electric Co., Ltd., (TOA) had patented an analog of the Coulter Principle and in 1963 began selling its CC-1001 blood-cell counter.[381] This counter avoided direct infringement of Wallace's Japanese Patent 217,947 on the Coulter Principle by diluting the blood sample with a liquid differing in dielectric constant (rather than electrical conductivity) from that of blood cells and flowing the diluted sample through a narrow tube (rather than an aperture) the wall of which contained diametrically opposed electrodes that were <u>preferably</u> insulated from the <u>preferably</u> non-conductive diluting liquid. An alternating current (rather than a direct current) applied to the electrodes enabled detection of blood cells passing between them via the change in capacitance due to the cells' dielectric constant.[382] But this method had limitations the Coulter Principle did not, and the ambiguous "preferably" in the patent description allowed use of exposed electrodes and conductive diluting liquids. The successor company's website entry about the CC-1001 counter admits, "Later, capacitance method was switched to an electrical resistance method using the same electrical principles. Since then, measurement accuracy has been dramatically improved by this technology." [383] That is, the patented capacitance sensing method was advantageously replaced with the resistance sensing

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[381] Sysmex Corporation, "Sysmex Technopark, R&D Center," website accessed May 1, 2019; "50 years of Sysmex," website accessed October 14, 2019.

[382] H. Imadate, "Particle counting device including fluid conducting means breaking up particle clusters," U.S. Patent 3,390,326, filed Nov. 14, 1962, and issued Jun. 25, 1968; Kyuhei Tashiro, Tashiro Patent Bureau, letter dated June 6, 1964, to Silverman & Cass; WHC Papers. The letter notes Ljungberg's secret, agentless sales of Celloscopes.

[383] Sysmex Corporation, "Sysmex Technopark, R&D Center," website accessed May 1, 2019; see text following the image of TOA's CC-1001 cell counter. For an assessment of the TOA CC-1002 counter, see P. W. Helleman and C. J. Benjamin, "The TOA Micro Cell Counter: I. A study of the correlation between the volume of erythrocytes and their frequency distribution curve," *Scandinavian Journal of Haematology* 6 (February 1969): 69-76 and "The TOA Micro Cell Counter: II. A study of the 'coincidence loss'," ibid. (May

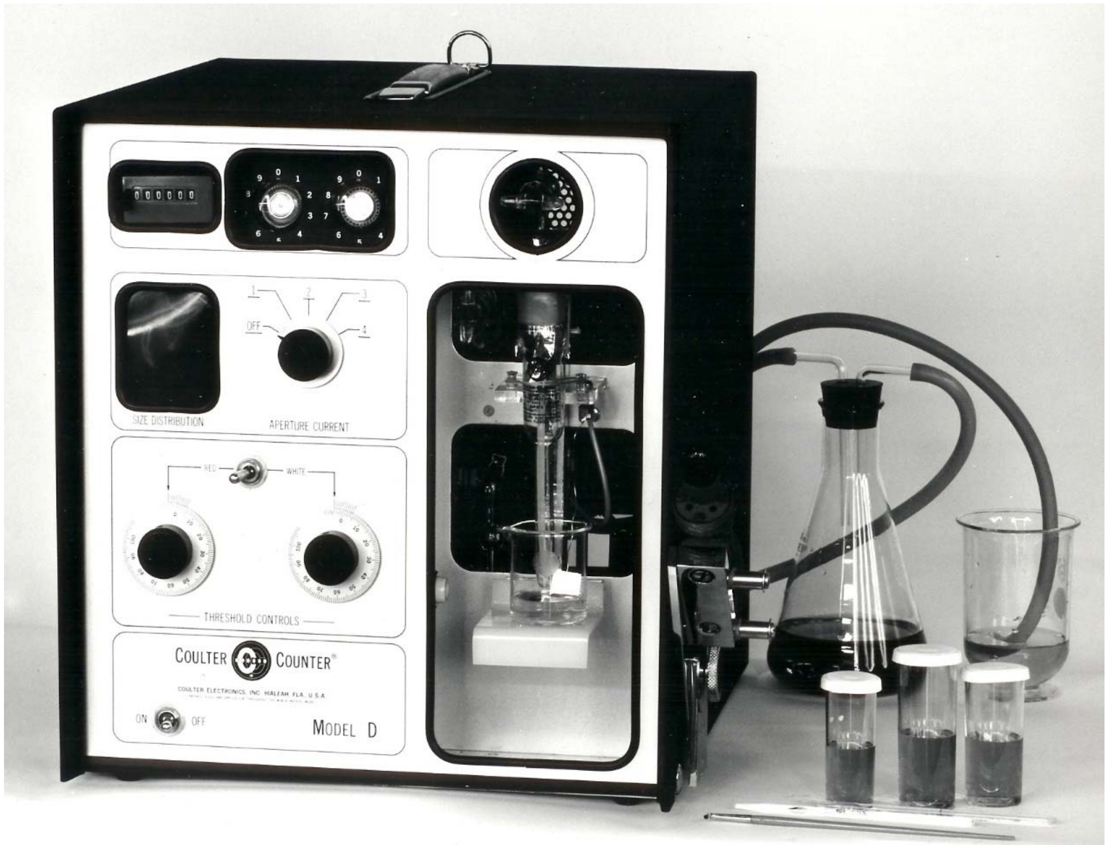

Figure 8.5. A Coulter Counter® Model D, developed by Coulter Electronics, Ltd.[384] This, the fourth implementation of the Coulter Principle, was a simplified dual-function counter adapted for erythrocyte or leukocyte counts as selected by flipping the switch above the two independent threshold controls. The Model D counter was the first Coulter counting instrument to integrate the sample stand into the electronics cabinet, thereby providing a self-contained instrument that, like the Celloscope counter, was readily portable; placement of the mechanical and dekatron count indicators near the top of the panel made them easier to read than the dekatron indicators in the Model B counter had been (Figure 8.1). Although some technologists found it uncomfortable to use the microscope on the lower right of the cabinet, the Model D would be welcomed by smaller hematology laboratories worldwide. Discussion of a prototype, an early advertisement, and a user's report are available.[385] Initially produced with vacuum-tube technology, later versions of the Model D counter incorporated transistors and then integrated circuits; some of the latter versions permitted broader application than the original Model D counter.

1969), 128-32; P. W. Helleman, "The TOA Micro Cell Counter: III. A comparative study of the results of erythrocyte and leukocyte counts with the TOA Micro Cell Counter and with the Coulter Counter," ibid. (July 1969), 160-65.

[384] Marketing photograph; WHC Papers.

[385] Boroviczény, "Erhfahrungen mit Blutkörperchenzählapparate," 168-71 (the Celloscope Counter is illustrated on 171); "50000 cells counted – one by one – in 15 seconds," *Journal of Clinical Pathology* 16 (1963): xxviii; A. N. Blades and H. C. G. Flavell,

one fundamental to the Coulter Principle. In Wallace's view the lawyerly wording of Imadate's patent was formulated in anticipation of this eventuality, which enabled descendents of TOA's CC-1001 counter to proliferate into present-day competition for CEI's successor company.[386]

In the U.S., Robert Berg had meanwhile instigated a process that would result in successors to Ljungberg's Celloscopes becoming similar competition. That story has long hidden in unfamiliar legal sources, but for anyone interested, it is outlined in Appendix 15. Although Berg could not invalidate Wallace's patent on the Coulter Principle, he was able to patent variations of key counter components that the Coulters had already patented. His exaggerated claims about his role during the early commercialization of the Coulter Counter® were cited in the legend for Table 6.2. Those claims and his "authorized reprint" (Appendix 14), aided by the long pendency of DuPage County Case 1-61-141 resulting from CEI's opposition to his registering the "ElectroZone" trademark, assisted him in his later activities, as would research on the Coulter Counter® at the Illinois Institute of Technology. The latter yielded useful reviews of the Coulter sensing art, and one of the researchers, Richard Karuhn, later joined Berg's Particle Data, Inc. (Table 6.2).[387] In 1997, Berg sold both his business interests and the elaborated Celloscope counter, an updated version of which is presently available under the trademark "Elzone." [388]

Moreover, during the lengthy pendency of DuPage County Case 1-61-141, significant competitive ventures had arisen in Germany. Researchers at the present-day Ilmenau University of Technology in Thuringia, from the Free University of Berlin in West Berlin, and in Ruthenstroth-Bauer's research consortium at Max Planck Institut für Biochemistry in Martinsried had developed cell counters based on the Coulter Principle. Given present space constraints, these ventures can only be summarized here, but representative sources will be cited.

"Observations on the use of the Coulter Model D electronic cell counter in clinical haematology," ibid. 158-63 with corrections on 292.

[386] Sysmex Corporation, "50 years of Sysmex," website accessed October 14, 2019.

[387] R. Davies, R. Karuhn, and J. Graf, "Studies on the Coulter Counter, Part II. Investigations into the effect of flow direction and angle of entry of a particle on both particle volume and pulse shape," *Powder Technology* 12 (1975): 157-66.

[388] "ELZONE," Registration No. 3741856, renewed March 2, 2019, website accessed August 12, 2020; "Elzone II 5390 Particle Size Analyzer," Micromeritics Instrument Corp., Norcross, GA, website accessed August 12, 2020.

The Ilmenau “TUR” ZG1 cell counter was similar to the Model A counter in both functionality and capability.[389] Descriptions of its evolving design were published, and a prototype was developed.[390] However, Wallace’s German patent on the Coulter Principle was still in force, and although a few application papers were published, the “TUR” ZG1 counter does not seem to have gained wide distribution.[391]

The cell counters originated by the other two German research groups, between which there was some interaction, would prove more competitive. As recounted regarding their evaluation of the Model B counter and Model H plotter, Brecher’s group had confirmed the finding by Ruhenstroth-Bauer and Zang that differential volume distributions of normal human erythrocytes seemed to contain excessive numbers of larger cells suggestive of bimodal populations.[392] Bull subsequently confirmed this finding and reported that with the industrial Model B counter such skewness could be reduced either by increasing the aperture L/D ratio or by sampling the cellular pulse heights when cells were midway through the aperture; he suggested that the skewness might originate in cells passing through the aperture on non-axial trajectories, an interpretation that Shank

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[389] H. P. Schudt and H.-Chr. Reissmann, "Die elektronische Zählung von Blutkörperchen und anderen Partikelarten nach dem Leitfähigkeitsprinzip," *Wissenschaftliche Zeitschrift der Hochschule für Elektrotechnik, Ilmenau* 8 (1962): 247-56; H. Baufeld, “Erfahrungen mit der elektronischen,” *Zeitschrift fur die gesamte innere Medizin* 19 (1964): 501-04.

[390] G. K. Hinkel, W. Rose, and P. Wunderlich, “Über die elektronische Blutzellzählung mit dem “TuR” ZG1: 1. Das Prinzip des Gerätes und allgemeine Richtlinien für seinen Einsatz,” *Das Deutsche Gesundheitswesen* 21 (1966): 371-77; 2. “Über die elektronische Blutzellzählung mit dem “TuR” ZG1: 2 Zur Erythrozytenzählung,” ibid. 21 (1966): 732-36; 3. “Zur Volumenbestimmung von Erythrozyten und der Aufstellung von Verteilungskurven,” ibid. 21 (1966): 1909-13; W. Rose and I. Bachman, “Über die elektronische Blutzellzählung mit dem “TuR” ZG1: 4. Zur Leukozytenzählung,” ibid. 23 (1968): 2471-76.

[391] P. Wunderlich and G. Hinkel, “Electronic Counting of Erythrocytes and Measurement of Erythrocyte Volumes with the “TuR” ZG 1,” *Bibliotheca Haematologica* 24 (1966): 63-66.

[392] Ruhenstroth-Bauer and Zang, “Automatische Zählmethoden: das Coulter’sche Partikelzählgerät;” K. D. Zang, “Automatische Zählung und Volumenbestimmung von Zellen auf elektronischem Wege,” *Deutsche Medizinische Wochenschrift* 89 (1964): 1710-16; G. Ruhenstroth-Bauer et al., “Zur Volumenverteilung von Erythrozytenpopulationen,” *Folia Haematologica* 83 (1965): 158-63; J. Gutmann, G. Hofmann, and G. Ruhenstroth-Bauer, “Exact methods for measuring the volume distribution of erythrocytes on the basis of the Coulter Principle,” *Bibliotheca Haematologica* 24 (1966): 42-53.

and co-authors soon supported with experimental data.[393] Hypothetically then, if the cell stream were sheathed in a cell-free isotonic electrolyte so that it went through the center of the aperture, the resulting volume distribution should be an unskewed Gaussian. An electro-optical cell counter had incorporated such hydrodynamically focused sample flows, and Spielman and Goren had demonstrated their use with a Coulter sensing aperture.[394] By drawing model particles through an oversized sensing aperture in an electrolytic tank, researchers at the Free University of Berlin showed that those on curving trajectories through the greater excitation current densities near the peripheries of the aperture orifices would produce M-shaped signals the peaks of which were greater than the peakless pulses from identical particles on straighter trajectories along or near the axis of the aperture's sensitive volume.[395] These researchers demonstrated that such peaks could result in large-volume distribution skewness, an artefactual consequence of the spatial distribution of an aperture's electric and hydrodynamic fields that could indeed be avoided by hydrodynamically focusing the sample stream through the aperture near its axis. Thom patented this approach in the U.S. and a prototype counter embodying it was developed by AEG-Telefunken.[396] In 1973, CEI purchased the U.S. manufacturing rights

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[393] Brian S. Bull, "On the distribution of red cell volumes," *Blood* 31 (1968): 503-13; Brenda Buckhold Shank et al., "A physical explanation of the bimodal distribution obtained by electronic sizing of erythrocytes," *Journal of Laboratory and Clinical Medicine* 74 (1969): 630-41.

[394] P. J. Crosland-Taylor, "A device for counting small particles suspended in a fluid in a tube," *Nature* 171 (1953): 37-38; P. Crosland-Taylor, J. W. Stewart, and G. Haggis, "An electronic blood-cell-counting machine," *Blood* 13 (1958): 398-409; Lloyd Spielman and Simon L. Goren, "Improving resolution in Coulter counting by hydrodynamic focusing," *Journal of Colloid and Interface Science* 26 (1968): 175-82.

[395] R. Thom, "Anordnung zur Gewinnung von Größen, die den Mengen von in der Untersuchungsflüssigkeit enthaltenen Teilchen verschiedenen Volumens entsprechen," German Patent DE1,806,512, priority Nov. 2, 1968; R. Thom, A. Hampe, and G. Sauerbrey, "Die elektronische Volumenbestimmung von Blutkörperchen und ihre Fehlerquellen," *Zeitschrift für die gesamte experimentelle Medizin* 151 (1969): 331-49.

[396] R. Thom and V. Kachel, "Fortschritte für die elektronische Größenbestimmung von Blutkörperchen," *Blut* 21 (1970): 48-50; R. Thom, "Method and results by improved electronic blood-cell sizing," in *Modern Concepts in Hematology*, eds. G. Izak and S. M. Lewis, 191-200 (New York: Academic Press, 1972); Reinhard Thom and Jurgen Schulz, "Particle volume and cross-section measurement," U.S. Patent 3,793,587, filed Mar. 8.1972, and issued Feb.19, 1974; Reinhard Thom, "Particle analysis method and apparatus wherein liquid containing particles is sucked into a constricted flow path," U.S. Patent 3,810,010, filed Nov. 27, 1972, and issued May 7, 1974; R. Thom, ed.,

and introduced the counter as the Coulter TF.[397] Individual TF counters could yield excellent results, but unit-to-unit variability in production units and their expensive maintenance would prompt CEI to discontinue them.

In his doctoral research Volker Kachel, one of Ruthenstroth-Bauer's researchers at Max Planck Institut für Biochemistry, used ultra-short light flashes to photograph compound aperture throughflows and demonstrate that they eliminated skewness in volume distributions of normal erythrocytes.[398] Kachel would then introduce the Metricell, another cell counter based on the Coulter Principle and using hydrodynamic focusing of the cell stream through the aperture's sensitive volume.[399] An accessible discussion of this evolution, with an alternative solution via analysis of the counter pulse stream, is available.[400] Analyzing the stream of cellular signals from an aperture's unfocused throughflow and deleting those pulses from cells on non-axial cellular trajectories avoided the complex sensing structure and fluidic system required for hydrodynamically focusing the sample stream along the aperture axis. In a decision reinforced by its experience with the AEG-Telefunken counter, CEI would patent a number of increasingly sophisticated analytic methods to mitigate the skewed volume distributions.[401]

Vergleichende Untersuchung zur elektronischen Zellvolumen-Analyse, Publication N1/EP/V 1689 (Ulm: AEG-Telefunken, 1972).

[397] J. Schulz and R. Thom, "Electrical sizing and counting platelets in whole blood," *Medical and Biological Engineering* 11 (1973): 447-54; Brian H. Kaye, *Characterization of Powders and Aerosols* (New York: Wiley-VCH, 1999), 174-75; Günter Valet, "Concept Developments in Flow Cytometry," in *Cellular Diagnostics: Basic Principles, Methods and Clinical Applications of Flow Cytometry*, eds. U. Sack, A. Tárnok, and G. Rothe, 34-35 (Basel: Karger, 2009).

[398] V. Kachel, H. Metzger, and G. Ruhenstroth-Bauer, "Der Einfluβ der Partikeldurchtrittsbahn auf die Volumenverteilungskurven nach dem Coulter-Verfahren, *Zeitschrift fur die gesamte experimentelle Medizin* 153 (1970): 331-47; H. Metzger, V. Kachel, and G. Ruhenstroth-Bauer, "Einfluβ der Partikelgröβe, -form und –konsistenz auf rechtsschiefe Coulter Volumenverteilungskurven," *Blut* 23 (1971): 143-54; Volker Kachel, *Methoden zur Analyse und Korrektur apparativ dedingter Meßfehler beim elektronischen Verfahren zur Teilchengroßenbestimmung nach Coulter* (PhD diss., Technische Universitat Berlin, 1972).

[399] V. Kachel, "Basic principles of electrical sizing of cells and particles and their realization in the new instrument "Metricell"," *Journal of Histochemistry and Cytochemistry* 24 (1976): 211-30.

[400] Cleveland S. Waterman et al., "Improved measurement of erythrocyte volume distribution by aperture-counter signal analysis," *Clinical Chemistry* 21 (1975):1201-11.

[401] Examples: Walter R. Hogg, "Axial trajectory sensor for electronic particle study apparatus and method," U.S. Patent 3,700,867, filed Dec. 24, 1970, and issued Oct.

Despite the diversion of resources due to Berg's activities, the optimism on display in Figure 8.2 allowed CEI to improve its product offering during the lengthy pendency of DuPage County Case 1-61-141. The transistorized Model F counter (Figure 8.6) replaced the Model A counter in many hematology laboratories. Findings by Sipe and Cronkite regarding the Model B counter in their platelet study led to an improved industrial version, and the solid-state Model J plotter replaced the unreliable Model H plotter.[402] Bull, Schneiderman, and Brecher found both the Model A and industrial Model B counters capable of acceptable platelet counts on diluted blood plasma and described a method for doing so for which CEI began offering disposable kits.[403] Mattern, Brackett, and Olson had used saponin to lyse erythrocytes in samples for leukocyte counts, and CEI's ZAPonin reagent made the method widely available for both leukocyte and spermatozoa counts.[404] Accessory modules for the Models A, B, and F counters enabled rapid computation of the mean cell volume (MCV) and hematocrit (Hct) of a blood sample (Figure 8.6); the Model M Volume Converter calculated the total cell or particle volume in a sample.[405] The introduction of CEI's isotonic diluent, Isoton, normalized cell counting and analysis across its growing counter offerings.[406]

24, 1972; "Particle study apparatus including an axial trajectory sensor," U.S. Patent 3,801,901, filed Sept. 11, 1972, and issued Apr. 2, 1974; Edward Neal Doty and Walter R. Hogg, "Particle study apparatus including an axial trajectory sensor," U.S. Patent 3,820,019, filed Jan. 5, 1973, and issued Jun. 25, 1974; "Particle study apparatus including an axial trajectory sensor," U.S. Patent 3,820,020, filed Jan. 8, 1973, and issued Jun. 25, 1974.

[402] T. R. Parsons, "An automated technique for determining the growth rate of chain-forming phytoplankton," *Limnology & Oceanography* 10 (October 1965): 598-602.

[403] B. S. Bull, M.A. Schneiderman, and George Brecher, "Platelet counts with the Coulter Counter," *American Journal of Clinical Pathology* 44 (1965): 678-88; "Disposable, accurate, ready to use platelet counts!" *Journal of Clinical Investigation* 45 (June 1966): ad page 3.

[404] "ZAPonin," *Journal of Clinical Investigation* 45 (April 1966): ad page 12; Janet Brotherton, "The interconversion of machine settings and size determinations between seven models of Coulter Counter as illustrated by values for human spermatozoa," *Physics in Medicine & Biology* 20 (1975): 816-24.

[405] R. W. Sheldon and T. R. Parsons, *A Practical Manual on the Use of the Coulter Counter in Marine Science*, 26-27 (Toronto: Coulter Electronics Sales Co., Canada, 1967).

[406] "new particle-free diluent, ISOTON," *Journal of Clinical Investigation* 46 (February 1967): ad page 3. Images were included of the Dual Diluter, the Model F and accessory modules, and the Model J plotter.

Figure 8.6. A Coulter Counter® Model F and accessory modules.[407] The first self-contained counter CEI developed, the transistorized Model F counter located the count display tubes at the technologist's eye level, with screens for monitoring the signal stream and the Coulter sensing aperture just beneath them. The latter received an image projected by an internal optical system focused on the sensing aperture, so eliminating the individual microscope used on the four earlier Coulter counters.[408] The digit counts were indicated by the position of a bright dot on the five dekatron tubes at the top of the panel. In addition to cell counts, the lower accessory module computed the mean cell volume (MCV) and from that result, the upper module computed the sample hematocrit (Hct).[409] Cell-volume distributions could be automatically plotted by the Model J plotter.

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[407] Marketing photograph; WHC Papers.

[408] "Introducing the new Model F Coulter Counter®," *BioScience* 14 (November 1964): 13; "14 major innovations in the NEW Model F Coulter Counter®," ibid. 15 (August 1965): 505; Janet Brotherton, "Calibration of a Coulter Counter Model F for size determination of cells," *Cytobios* 1 (April-June 1969): 95-106.

[409] "fast and accurate, with two new automatics," *BioScience* 16 (February 1966): front matter; "Every second counts," *Journal of Clinical Investigation* 45 (June 1966): ad page 2; "New Coulter Counter® Accessories," ibid. 46 (August 1967): ad page 9.

The dekatron display tubes used in the counter Models B through F indicated the bin counts by the position of a bright dot next to a small numeral, and Brecher's technologists had found that reading the bin counts was eased by locating the Model B counter so its display tubes were at the operator's eye level.[410] Consequently, in the Model D counter (Figure 8.5) and Model F counter (Figure 8.6) the bin-count indicators were located near the top of taller instrument enclosures. Enhancements of operator friendliness continued with the introduction of the Model F_n, which among other improvements substituted nixie display tubes for the dekatron display tubes; in these an illuminated numeral displayed each bin count rather than it being indicated by the position of a bright dot.[411] And in response to other laboratory feedback, CEI's technical staff were developing both a counter specifically for platelets, the Thrombocounter, and automating in a bench-top meter the classic method for measuring a sample's hemoglobin content.[412]

Meanwhile, international political competition had not diminished. China tested its first fission and hydrogen bombs on October 16, 1964 and June 17, 1967, respectively.[413] France exploded its first hydrogen bomb on August 24, 1968.[414] Not only were these initial tests reported in the news media, many of the subsequent developmental tests also received coverage. In 1968 the U.S. is thought to have had some 29,000 nuclear weapons, the Soviet Union some 9,000, Great Britain about 400, and France and China perhaps 35 each.[415] Mutually assured destruction was gaining momentum.

Once Wallace realized the critical need for rapid and accurate blood-cell counts following a nuclear event, he had wanted to provide a cell counter that would accept a blood sample and automatically process it to rapidly provide definitive clinical results. Unlike those engineers who try to perfect an item before marketing it, Wallace understood

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410 George Brecher, letter dated July 6, 1964 to Wallace H. Coulter; WHC Papers.

411 "Now platelet counts too …," *Journal of Clinical Investigation* 47 (March 1968): ad page 5; J. K. L. Pearson, C. L. Wright, and D. O. Greer, "A study of methods for estimating the cell content of bulk milk," *Journal of Diary Research* 37 (1970): 467-80.

412 "Problem: To provide an automated method," *Journal of Clinical Investigation* 50 (Jan. 1971): ad pages 5-7.

413 Daniel Southerland, "Red China explodes atomic bomb," *The Seattle Daily Times*, Seattle, WA, October 16, 1964, 1; Kay Tateishi, "H-bomb is set off, Chinese announce," *The Times-Picayune*, New Orleans, LA, June 18, 1967, 1.

414 "France joins H-bomb club; explodes unit in Polynesia," *Times Advertiser*, Trenton, NJ, August 25, 1968, Part 1, 1.

415 Norris and Kristensen, "Global nuclear weapons inventories."

that it only had to be good enough ("If it's useful, people will buy it."), and he persevered in accumulating the knowledge and personnel to develop successive implementations of his Coulter Principle toward achieving that desire. By late 1968 CEI had added an entire second floor in its Hialeah facility, with nearly 500 employees working there and in its off-site sales and service activities, and Wallace had integrated several new developments into the first automated hematology analyzer, the Coulter Counter® Model S.

To use this ground-breaking instrument (Figure 8.7), the technologist had only to present either a venous or capillary blood sample to the proper sample probe and initiate the automated process. A precise volume of venous blood samples was diluted appropriately for a leukocyte count, then split, one part being lysed and sent to the leukocyte and hemoglobin bath while the other part was diluted appropriately a second time for an erythrocyte count and sent to the erythrocyte bath; capillary samples bypassed the first dilution. The dilution in each bath was drawn through three Coulter apertures, of D of 100 μm and L/D of 0.75 for counting leukocytes and of D of 70 μm and L/D of 1.4 for counting and sizing erythrocytes.[416] The cellular signal streams from the six apertures were analyzed and corrected for coincidence; if there were no inconsistencies in any aperture signal stream, those from the three like apertures were forwarded to independent counting circuits, the results from which were then averaged to provide rapid and accurate results with excellent statistical repeatability. However, if the cellular signal stream from one aperture differed significantly from those from the other two like apertures, for example, due to a partial blockage, that aperture's results were voted out of the data to be averaged, and the technologist was warned of the discordant signal stream. Although the reported counts then depended on only two cellular signal streams, their accuracy was still significantly better than provided by manual methods.

Corrected for cellular coincidence and verified to agree within specified limits, the averaged cell counts provided the sample's leukocyte count (WBC) and erythrocyte count (RBC). The latter was used to calculate the average volume of the erythrocytes (MCV) and combined with a hemoglobin measurement (Hb) from the leukocyte bath to yield the

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[416] Wallace H. Coulter and Walter R. Hogg, "Particle analyzing apparatus and method utilizing multiple apertures," U.S. Patent 3,444,463, filed Feb. 14, 1966, and issued May 13, 1969; "Multiple aperture fittings for particle analyzing apparatus," U.S. Patent 3,444,464, filed Nov. 26, 1965, and issued May 13, 1969; Takashi Okuno, "Red cell size as measured by the Coulter model S," *Journal of Clinical Pathology* 25 (1972): 599-602.

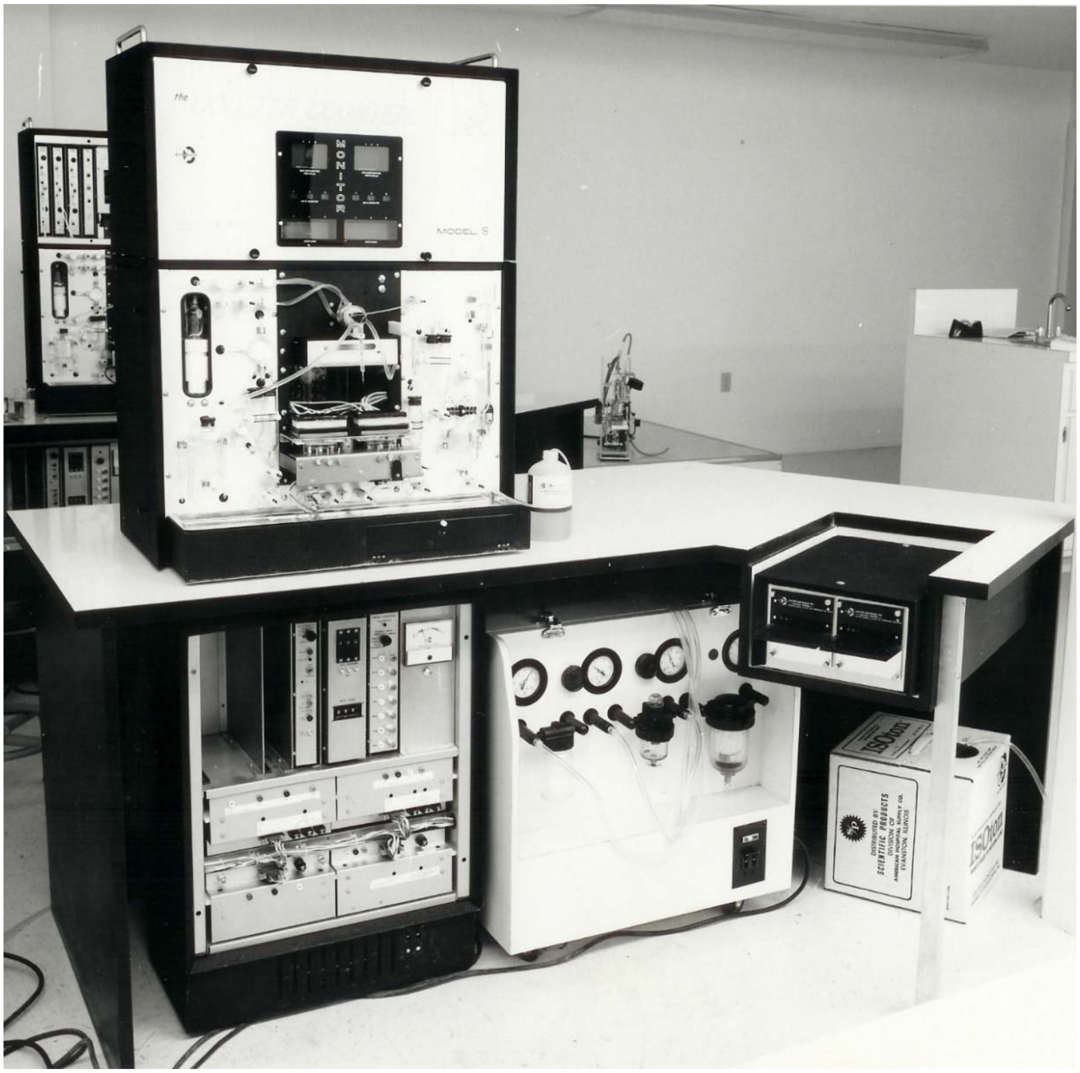

Figure 8.7. A Coulter Counter® Model S hematology analyzer.[417] The Model S comprised four units, the analysis module on the bench; a dual printer module, in the bench cutout; and the electrical power and the vacuum and air supplies, beneath the bench. The carton beneath the printer contains Isoton, the proprietary isotonic NaCl electrolyte used to dilute the blood sample. Interior images of the analysis module and a flow schematic of its sample processing are available.[418]

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[417] Marketing photograph, WHC Papers; William F. Rothermel and Robert I. Klein, "Automatic method and apparatus for obtaining different dilutions from blood or the like samples and processing the same by fluid handling and electronics to obtain certain nonelectric parameters," U.S. Patent 3,549,994, filed Apr. 17, 1967, and issued Dec. 22, 1970; Nell Vaughn, "CBH automates lab procedures," *Lexington Leader*, Lexington, KY, November 21, 1968, 40.

[418] D. F. Barnard et al., "An evaluation of the Coulter Model S," *Journal of Clinical Pathology* 22, Supplement 3 (1969): 26-27; Janny A. de Lange et al., "Automation in the hematology laboratory," *Journal of Clinical Chemistry and Clinical Biochemistry* 14 (1976): 486.

volume percentage of erythrocytes (hematocrit, Hct, or packed cell volume, PCV), the average mass of hemoglobin per erythrocyte (MCH), and the ratio of Hb to Hct (MCHC). For the first time it was possible to efficiently assess a patient's bone marrow recovery from radiation damage via rapid, accurate, and repeatable automated determinations of these seven blood parameters, which were determined and printed within less than a minute of the technologist presenting the blood sample.[419] The first advertisement for the Model S noted, "One operator in one hour can make more seven-parameter blood analyses more accurately than three overworked technologists can in eight." [420] Five months later a second advertisement claimed 1,000 Model S installations in two years.[421]

Although the Model S did not automate sample presentation to its aspiration probe, differentiate the five subtypes of normal leukocytes (Table 4.1), or automate the platelet count, the laboratory efficiency that it enabled revolutionized the practice of clinical hematology and earned it widespread acceptance. Unprecedented demand for the Model S provided a stable foundation upon which CEI expanded its employee base to 800 people by mid-1970 and its facilities to include two nearby buildings at 601 and 701 West 20th Street.[422] To supply the Model S with suitable reagents, Coulter Diagnostics, Inc., began operations in two additional buildings at 740 and 780 West 83rd Street, Hialeah.[423] There were soon some 200 employees in CEI sales and service offices in ten states.

The success of the Model S allowed Wallace to implement the Coulter Principle in a series of increasingly sophisticated hematology analyzers, as well as in smaller particle counters useful not only in hematology, but in a great variety of other disciplines.[424] By 1988, Coulter companies had produced the 80,000th instrument incorporating the Coulter

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[419] Geoffrey M. Brittin, George Brecher, and Carole A. Johnson, "Evaluation of the Coulter Counter Model S," *American Journal of Clinical Pathology* 52 (Dec. 1, 1969): 679-89; P. H. Pinkerton et al., "An assessment of the Coulter Counter model S," *Journal of Clinical Pathology* 23 (March 1970): 68-76; Barnard et al., "An evaluation of the Coulter Model S."

[420] "In one hour with one operator with the Coulter Counter® Model S," *Journal of Clinical Investigation* 49 (May 1970): ad pages 6 and 7.

[421] "SAVINGS BOND," ibid. 49 (October 1970): ad pages 7-11, specifically ad page 10.

[422] "Look ahead with expectation, look back with pride," *The Coulter Countdown* 10 (Special 1980): 2-5.

[423] "What reagents are specifically designed for the Coulter Counter?" *Journal of Clinical Investigation* 49 (November 1970): ad page 11.

[424] "Bibliographic References" (Brea, CA; Beckman Coulter, Inc., 2003).

Principle.[425] By the early 1990s the Coulters' Hialeah operations occupied some thirty-five buildings. In 1991 the brothers merged CEI into Coulter Corporation, then bid $13 million for the AmeriFirst Bank campus in Kendall, Florida, and received a 10% discount for paying cash. The Corporation's relocation was largely complete when Joseph, Jr., died November 27, 1995. Wallace's declining health then led to the Corporation being sold to Beckman Instruments, Inc., with change of control on November 1, 1997. Wallace died August 7, 1998, after arranging for distribution of $100 million of his share of the proceeds to the Corporation's some 5,500 employees according to their position and length of service. To continue improving health care through medical research and engineering, the remainder of his share funded the Wallace H. Coulter Foundation.[426]

Meanwhile, international cold-war politics had continued, with India testing its first fission device on May 18, 1974.[427] But when a nuclear disaster requiring blood-cell counts came, it was due to ill-advised cost cutting, not war. On April 26, 1986, Reactor Unit 4 at the Chernobyl Nuclear Power Plant in Ukraine was destroyed by an explosion twice as powerful, with more than one hundred times the release of contaminating radiation, as that of the fission bombings of both Hiroshima and Nagasaki.[428] On May 5 Wallace authorized donation and expedited airlift of a Coulter S-Plus IV hematology analyzer and reagents for 5,000 complete blood-cell counts from France into Moscow where radiation victims were being treated.[429] Other Coulter instruments, including a T660 hematology analyzer, and reagents would follow later, all gratefully received and effective.[430] Small holes, a little bit of nothing in a short bore of length L between two orifices of diameter D, finally delivered badly needed help to survivors of a nuclear event. Wallace would

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425 "Back to the future with Coulter," *Coulter Viewpoint* 1 (1988): 4.

426 Graham, "The Coulter Principle: The Arkansas background," 186-89.

427 Bernard Weinraub, "India reveals explosion of atomic device," *The Boston Herald*, Boston, MA, May 19, 1974, 1; "Test blast propels India into nuclear power elite," *Washington Star-News*, Washington, D.C., May 19, 1974, 1.

428 V. Poyarkov, et al., *The Chornobyl Accident: A Comprehensive Risk Assessment*, ed. G. J. Vargo (Columbus: Battelle Press, 2000), 1-5; A. V. Yablokov, V. B. Nesterenko, and A. V. Nesterenko, "Chernobyl: Consequences of the Catastrophe for People and the Environment," *Annals of the New York Academy of Science* 1181 (2009): 1-3.

429 "Model S-Plus IV goes on humanitarian mission," *The Coulter Countdown* 17 (Winter/Spring 1987): 16-17; Robert Peter Gale*, Final Warning: The Legacy of Chernobyl* (New York: Warner Books, 1988), 63-64.

430 ""Unflappable" T660 brings help to Chernobyl victims," *The Coulter Countdown* 23 (Spring 1992): 20-21.

sometimes speak with quiet pride of having practical implementations of his needle-made aperture in cellophane applied toward the need he had perceived some forty years before.

CHAPTER 9. CONTEMPLATION

Those killing flashes of light over Hiroshima and Nagasaki in August 1945 (Figures 1.1 and 1.2) brought abrupt comprehension to Wallace Coulter of the critical need for accurate and rapid blood-cell counts. This thesis has described his journey from that comprehension through his invention and implementation of the Coulter Principle, its commercialization in the first widely available automated blood-cell counter, and elaboration of that ground-breaking Model A counter into increasingly sophisticated instrumentation for analysis not only of blood cells, but of particles involved in many other scientific disciplines. As noted in my introductory chapter, by 2018 at least 6,500 DxH 800 hematology analyzers based on the Coulter Principle were installed, each one fully automated to process 100 blood samples per hour.[431] If operated only 12 hours per day at 70 samples per hour, these could process more than 5.4 million samples daily, and perhaps some 220,000 of those samples would have an abnormality affecting a patient's diagnostics. This is approximately the number of people often estimated to have been reduced to nothing in those killing flashes of light, and samples run on the many older models still in use would significantly increase that number.

The Hiroshima and Nagasaki bombs were products of the U.S. response to fears of émigré German scientists that Germany might be working toward a fission bomb; however, in 1945 the Alsos Mission would find that the Germans had yet to devise a self-sustaining reactor pile.[432] To some it may seem ironic that those bombs were used against Japan, but in his announcement of the Hiroshima bombing, U.S. President Harry S. Truman strongly implied that it was retribution for the Japanese attack on Pearl Harbor in December 1941, and he would say of the originating Manhattan Project, "We have spent $2,000,000,000 on the greatest scientific gamble in history and won." [433] The theories on which those two bombs were based were first validated under the direction of Arthur H. Compton in December 1942 under the University of Chicago's Stagg Field grandstands,

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[431] Beckman Coulter, Inc., "DxH 800 Hematology Analyzer," website accessed October 23, 2020.

[432] Kelley, *The Manhattan Project*, 269-73; Atomic Heritage Foundation, "Alsos Mission," website accessed October 20, 2020.

[433] Kelly, *The Manhattan Project*, 339-42, specifically 340; Truman, "The report of President Truman on the atomic bomb," 164.

which the University's idealistic President Robert M. Hutchins had abandoned to ruin a few months before naming Compton as Dean of Physical Sciences. Much of Wallace's early journey benefitted from other unintentional consequences of Hutchins' actions. After the war's end in September 1945, the G.I. Bill brought thousands of veterans, including his brother Joseph, Jr., into the science and engineering programs at Chicago's universities, and in July 1946, Compton's Met Lab became Argonne National Laboratory (ANL).[434] In recognition of the need for less secrecy in sponsored research, the Office of Naval Research (ONR) was organized that August and within a year a regional office was operational in Chicago.[435] These federally sponsored programs continued to attract skilled personnel and bring service and industrial activity to Chicago.

Wallace returned to Chicago in early 1946 after working during the war in a broadcasting project that was largely sponsored by the U.S. government, and he was joined by his brother Joseph following the latter's separation from the U.S. Army. Both wanted greater independence than their recent experiences had provided. Although he was already doing library research toward an automated blood-cell counter, Wallace proposed and briefly co-managed an electro-medical development group at Raytheon Manufacturing Company during his return to Chicago, but the proposal was never formalized. Both brothers found employment with other Chicago companies and together, as personal time allowed, designed amplifier circuits and experimented with approaches to blood-cell counting; in their first effort at self-sufficiency, they built and sold high-fidelity amplifiers as Coultamp Company (legend for Figure 2.1).

After Wallace first demonstrated the Coulter Principle by flowing his diluted blood through a needle-made hole in a cellophane wrapper from a cigarette package (Figure 3.2a and b; Appendix 7), Sam Gutilla helped develop durable aperture tubes for blood-cell counting; he had served the Manhattan Project as a glassworker under the Stagg Field grandstands (Figure 3.3). When Wallace was ready to seek patent protection for his Principle, the lawyer I. Irving Silverman was recommended; Silverman, a former Air Force Captain who had participated in the Army Electronics Training Center at Harvard University and Massachusetts Institute of Technology, had relocated to Chicago because of the innovative activities of its academic and industrial communities. Once a U.S. patent

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434 Greenbaum, *A Special Interest*, 13-22.

435 Rayy Mitten, "Office of Naval Research not yet sure it can control its rain-making device," *The Corpus Christi Times*, Corpus Christi, TX, August 26, 1947, editorial page.

application was filed, Wallace described and demonstrated a basic cell counter to personnel of ONR's Chicago office, who were favorable, and then sent a proposal for developmental support to ANL (Appendix 9) while he awaited an ONR response. ANL saw no need for his proposed counter in its research program (Figure A9.4), and frustrated by its bureaucracy, Wallace submitted a revised proposal to the ONR (Appendix 10); this gained him contract NONR-1054 (00) that enabled implementation of an integrated instrument under the ONR's low-oversight policies (Appendix 11).

There were four people deserving mention who, like Silverman, were in Chicago because of its innovation boom. Joseph Gardberg, the inventor who provided basement space for the initial production of the Model A counter, had relocated from Mobil, AL; Ernest Yasaka, the Hawaiian who built hundreds of Model A counters in Gardberg's basement, had attended Chicago's DeVry Technical Institute after his service in the U.S. Navy; and Robert H. Berg, the Wisconsinite who helped introduce the Model A counter to industrial users, had worked for several years in chemical process control. Finally, there was Herbert E. Kubitschek, a student of Enrico Fermi and attendee of the December 1942 demonstration of the self-sustaining Stagg Field pile, who first demonstrated use of the Model A counter with pulse-height analyzers to analyze bacteria and was helpful in adapting the counter to industrial particles.[436]

Convenient access to needed skills in Chicago facilitated the Coulters' determination to remain independent, and they were able to develop the Model A counter with only the $17,769.42 of federal money received through their one contract with ONR (Figure A9.3). Under ONR guidelines Wallace retained ownership of the early U.S. patents related to the Coulter Principle and was free to publish his NEC paper describing the Model A counter. Devoted work by the Coulters enabled them to self-fund commercialization of the counter and its incremental elaboration into complex hematology analyzers via instrument sales, so retaining their autonomy to "run something like you wanted it."

It is instructive to glance back over the preceding text and realize that $17,769.42 of ONR federal funds allowed the independent Coulters to develop a principle that now daily aids the diagnostics of more people than the total number killed by the first uses of the bombs that $2,000,000,000 in federal funds produced via the military/industrial efforts

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[436] Atomic Heritage Foundation, "Herbert E. Kubitschek," website accessed September 25, 2020; Kubitschek, "Electronic counting and sizing of bacteria" and "Electronic measurement of particle size."

of the Manhattan Project. Of course, a detailed comparison would disclose many relevant qualifiers, but still the general contrast provokes interesting thoughts.

Wallace was modest and often self-effacing; as an engineer focused on building a company, he emphasized documenting his progress and largely left its promotion to others. He authored or co-authored several descriptive brochures and became inventor or co-inventor on 85 U.S. patents, on five of which Joseph was a co-inventor; his last three patents were issued posthumously.[437] His patents reflect decades spent understanding the fundamental interacting minutiae that limited the performance of the then-current implementation of his Coulter Principle. To anyone willing to listen, including competitors met at their exhibits, he would spend minutes explaining some technical detail very few even recognized. Unfortunately, much of what he came to understand was only documented in his notes, now mostly lost.

Wallace's many contributions brought him impressive honorary recognitions, usually because someone who knew of his accomplishments nominated him. In addition to the John Scott Metal he was awarded in 1960, he received the following honors originally itemized elsewhere, which see for sources.[438] These included the Florida Industrialist of the Year Award in 1988 from the Museum of Science and Industry and in 1989, the Certificate for Distinguished Achievement from the American Society of Hematology, the Gold-Headed Cane Award from the Association of Clinical Scientists, and the Lifetime Achievement Award for significant achievement in medical electronics from M. D. Buyline. In 1993, he received one of the first two Distinguished Service Awards given by the International Society of Analytic Cytology, of which he was a charter member. In addition he received honorary doctorates: of science from Westminster College in 1975; of engineering from the University of Miami in 1979; of science from Clarkson University in 1979; of laws from Barry University in 1991; and posthumously, of philosophy from the Georgia Institute of Technology in 2005. And in 2000, in what would have been of especial importance to Wallace, his only published paper, the NEC one describing the first commercially available automated cell counter, was selected as one of the eighty-six most

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[437] U.S. Patent 6,159,740, filed May 17, 1990, and issued December 12, 2000; U.S. Patent 6,579,685, filed September 9, 1994, and issued June 17, 2003; U.S. Patent 6,900,029, filed November 13, 1995, and issued May 31, 2005.

[438] Graham, "The Coulter Principle: The Arkansas background, 189-90.

influential hematology papers of the twentieth century.[439] His invention of the Coulter Principle brought Wallace posthumous induction into the National Inventors Hall of Fame in 2004.[440] And his Principle's first commercial implementation, the Model A counter, was acknowledged in a compendium of significant scientific instruments.[441]

Although Wallace never completed his undergraduate degree in electrical engineering, his contributions were recognized by the Institute of Electrical and Electronics Engineers, and in 2001, he received a memorial tribute from the National Academy of Engineering.[442]

And it was all because of that little bit of nothing in a short bore of length L between orifices of diameter D.

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[439] Wallace H. Coulter, "High speed automatic blood cell counter and cell size analyzer," in *Hematology Landmark Papers of the Twentieth Century*, ed. Marshall A. Lichtman et al. (San Diego: Academic Press, 2000), 911-21.

[440] "Wallace Coulter," *Inductees of the National Inventors Hall of Fame*, 39th ed., 271 (Akron, OH: Invent Now, Inc., 2011), website accessed September 21, 2020.

[441] Wallace H. Coulter, "Counter, Coulter," in *Instruments of Science: An Historical Encyclopedia*, eds. Robert Bud and Deborah Jean Warner, 153-55 (New York: Garland Publishing, Inc., 1998). Although Wallace is listed as the author, the style of the text suggests that it was ghostwritten.

[442] "1980 – Wallace H. Coulter," IEEE Morris E. Leeds Award, website accessed October 21, 2020; "New Fellows in the Industry Applications Society; Wallace H. Coulter." *IEEE Transactions on Industry Applications* IA-20 (January/February 1984): 4; Michael R. Sfat, "Wallace Henry Coulter," in *Memorial Tributes: National Academy of Engineering* 9, 55-57 (Washington, D.C.: National Academy Press, 2001).

APPENDICES

APPENDIX 1. Background

Insights and quotations expressed in this thesis originated in my many conversations with the Coulter brothers as a result of my advisory relationship. Projects undertaken involved both travel and meals with them and resulted in a score each of my publications and U.S. patents, discussions about which frequently ran through dinner to end in Wallace's office.[443] My collection of Wallace's papers began in 1982 when he either gave me or permitted my photocopying items from his personal files while I drafted his nomination as a Fellow of the Institute of Electrical and Electronics Engineers. Our discussions often elicited additional items, since augmented by gifts of similar material from other long-term employees of the Beckman Coulter organization. The resulting collection underlies three of my previous publications, which summarized more of the Coulter story than can be detailed here.[444]

As noted in Chapter 1 herein, Wallace's personal files were apparently discarded during the relocation of Coulter Corporation in 1992. These included reprints, patents, news clippings, handwritten notes, and period Xero photocopies of other notes about his interests and inventive process. As indicated by the last sentence of the note transcribed in Appendix 2, Wallace did not document any experimental work until after he had demonstrated the Coulter Principle in October 1948 and was anticipating the patenting process; the early transcriptions herein are arranged in the chronological order of his experimental work, a sequence he and Joseph independently confirmed during our several discussions regarding their activities during the late 1940s and 1950s.

Wallace's experimental notes were usually written in pencil on letter-size sheets of inexpensive "scratch" paper. His emphasis was on accuracy rather than neatness, and his handwriting was not his strong point. Several of his notes were written on both sides of a single sheet, often with apparent bleed-through; in the following transcriptions, such double-sided notes are indicated by "OVER" in mid-line between text segments. His "Description of Experiment," Figure 3.2 of the thesis text, is representative.

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[443] See the VITA, included herein.

[444] Graham, "The Coulter Principle: Foundation of an industry," "The Coulter Principle: Imaginary origins"; and "The Coulter Principle: The Arkansas background." Typos found therein during the present research have been corrected herein.

Figure 3.2 is Wallace's first illustration of his preferred implementation of the Coulter Principle, elaborated in Appendices 2, 4, 5, 6, 7, 9, and 10. In each of these, and in his U.S. Patent 2,656,508 on that Principle, he used "aperture" to describe his preferred structure for providing a constricted suspension flow. In the hydraulics literature "orifice" is often used instead of "aperture" because the constrictive bore was made through material of a thickness L that was much less than the bore's diameter D, that is, the L/D ratio of the bore was small. "Aperture" as used in both Wallace's documentation and herein indicates that, whatever the substrate thickness, the bore had orifices at both its ends.

Unless otherwise indicated, transcriptions in the following appendices ignore the line sequence of his handwritten text and transparently both include all drafting corrections and correct any typos. The transcriptions have been proofread by other people familiar with Wallace's handwriting and are agreed to be accurate. Signatures are italicized; my descriptive or explanatory comments appear in footnotes.

In addition to notes regarding experimental work, Wallace's files contained informal notes related to a variety of topics, such as publications he wanted to find or had read, sudden insights, feasibility calculations, and phone calls. These tended to be hurriedly written on any paper at hand and were often neither signed nor dated. However, he sometimes provided context for such notes by paper-clipping or stapling them to other items, and several publications cited herein were located via such reminder notes.

Images of Wallace's notes or typescripts included herein have had clear margins electronically cropped or have been electronically reduced, or both, to fit within the required page margins.

APPENDIX 2. Photo-electric Method of Counting Small Particles [445]

A fluid bearing the particles is made to flow thru a small aperture thru which light is also directed. If the particle has a light opacity or transmission different from that of the fluid the particle will modulate the light passing thru the aperture and such modulation may be detected by a suitable photocell.

The light passing through the aperture should come from an "area" and ideally should come from the inside surface of a hemisphere centered on the aperture. The photocell should gather light over an "area" which also would be best if such "area" were a hemisphere centered on the aperture.

Movement of a particle a short distance from the aperture would not greatly affect the total transmission of light from the area source to the area pickup even though the areas not be perfect hemispheres because light will be passing to (or from) the aperture on all sides of the particle. As the particle comes very close to the aperture its angle of interception becomes significant in comparison to the total aperture angle. Also it picks up speed as it moves toward the aperture. This causes a faster change in its effect on the total light flow through the aperture

OVER

and reaches a maximum when it enters the aperture. By suitable arrangements the modulation can be counted.

As with the aperture-electric current method due consideration must be made of the dilution, etc.

W. H. Coulter
Nov. 21, 1948

Witnessed and understood
Nov. 23, 1948 *Walter R. Hogg*

W R Hogg
Nov. 23, 1948

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[445] Transcription from a Xero photocopy of Wallace's handwritten original. Hogg's initialized signature is in the left margin of the obverse.

APPENDIX 3. Conductivity Measurement "Cell" [446]

Usual cells require relatively large fluid volumes. A problem with small cells is the use of smaller electrodes & control of volume.

The fluid to be measured could be contained in a length of capillary tube. The fluid at each end of the tube could be brought in contact with a small quantity of the same fluid at each end which is in contact with electrodes of area much larger than the cross section of the capillary bore. The distance from the each end of capillary to the metal electrodes should be small.

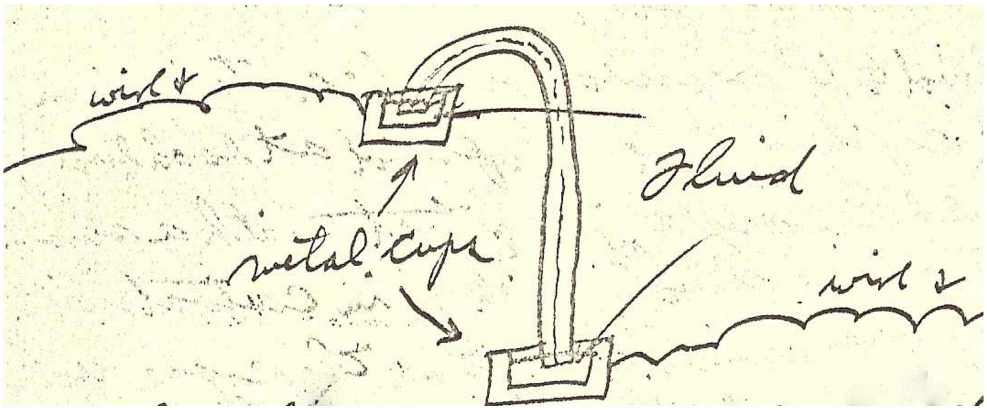
It will be found that the resistance from metal electrode to electrode is almost solely a function of the fluid conductivity and not greatly influenced by moderate contamination of the metal electrodes. The effect of the metal contact resistance & the conduction from the capillary to the electrodes can be reduced indefinitely by increasing the capillary length & decreasing its bore.

The fluid outside of the capillary bore serves as a contact. The bore "conductivity" can be controlled by changing its length to compensate for variations in effective bore cross section as a "production" means.

OVER

[446] Transcription from a Xero photocopy of Wallace's handwritten original. The "Cell" of the title and "cells" of the text refer to the capillary tube(s) and electrodes, not to the blood cells Wallace was contemplating flowing through the capillary bore. This note was prompted by the reprint in Figure 3.1. For the standard U-type conductivity cell mentioned in the fourth paragraph therein, see Hirsch et al., "The electrical conductivity of blood: I," 1020, Figure 3.

The capillary could be formed as shown [447]



This allows convenient contact to the “column” in the bore. Another method would be to simply press gauze-faced electrodes against each end of the capillary. The gauze should be thoroughly prewetted with the sample.

W. H. Coulter Nov. 21, 1948

Witnessed and understood

Nov. 23, 1948 *Walter R. Hogg*

Should be useful for low freq & DC because would minimize polarization effects.[448]

W. H. Coulter, Nov 21, 1948 – [449]

Read and Understood	*WR Hogg*	Witnessed and Understood
November 25, 1948 *John J. Dowling*	Nov. 23, 1948	November 24, 1948 *Allen A. Gault*

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[447] Wallace’s sketch of an inverted J-tube, with both ends immersed in “Fluid” in separate “metal cups”, each connected to a “wire &”, the upper one of which trails off to the left margin and the lower of which does so toward the right margin.

[448] Wallace’s afterthought was jotted above the title, in lighter writing than the main text; “freq” is his abbreviation for “frequency” as of an alternating current and “DC” is the standard abbreviation for “direct current.”

[449] Perhaps because Hirsch et al., stated their intent to develop an electronic circuit giving accurate erythrocyte counts (last text paragraph in Figure 3.1), Wallace placed unusual emphasis on this document. As indicated above, he signed and dated it on the reverse, then as here in the right margin of the obverse; he had Walter R. Hogg endorse it on both sides on Nov. 23, 1948, and the obverse witnessed, from top to bottom in the left margin on the obverse, by Allen A. Gault on November 24, 1948 and by John J. Dowling on November 25, 1948. Both the latter were co-workers at Illinois Tool Works. Wallace paper-clipped the result to his Hirsch et al., reprint (Figure 3.1).

APPENDIX 4. Method of Counting Small Particles (July 26, 1948) [450]

Particles or bodies are suspended in a liquid (in a manner to avoid grouping) if the particles are not already in a liquid. The liquid is made of different electrical conductivity than the particles and is diluted as described below.

The liquid is made to pass thru a small and short aperture from one section of an insulated container to another section. The liquid in both sections of the container are in contact with the aperture and the flow would generally be impelled and controlled by a difference between the levels of the liquids. Ideally but not necessarily the cross section of the aperture should be only large enough to admit and pass the largest particle to be counted. To avoid stoppage the aperture may have to be larger if other larger bodies are present and of course a correction in count will have to be provided if their conductivity effect is very similar to the particles whose count is desired. Extraneous particles may frequently be disposed of by a suitable chemical or other treatment (filtering, etc.) of the liquid. The aperture should also be as "short" as possible consistent with economy, strength, smoothness etc.

It will be observed that the electrical resistance to the flow of electric current between "large" diameter electrodes placed near the aperture and on either side of the aperture will be due, in a very large part, to the small cross section of the constriction even though its length is short. In any event the total electrical resistance from contact to contact thru the liquid will be different when a particle is carried into the constriction than the resistance will be when the liquid only is in the aperture.

This change of resistance "modulates" the electric current and is made to activate a counter as the liquid flow carries the particles thru the aperture.

Wallace H. Coulter July 26, 1948

A means of obtaining short apertures of extremely small cross section is to puncture, perhaps by electrical means, a thin section film or sheet of glass, mica, etc. *WHC* July 26, 1948.[451]

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[450] Transcription from a Xero photocopy of Wallace's handwritten original. Closely written on a single sheet of paper, this is the first statement of the Coulter Principle.

[451] Wallace wrote this bottom to top of the left margin and stapled the photocopy and a circuit diagram of a rate meter to the Xero photocopy transcribed in Appendix 6. His experiments with electrical discharges demonstrated that while short apertures could result, they were unpredictable in size and erratic in quality.

Read and Understood: *J. J. Dowling*, July 28, 1948. [452]

A summary of Wallace's text may be helpful:

A suspension of the particles of interest in a liquid of different electrical conductivity is made to pass through an aperture as small and short as possible between two chambers of an insulative container. The liquid in both chambers of the container is in contact with the aperture, and the suspension flow is controlled by a difference between the liquid levels. The electrical resistance to the flow of electrical current between electrodes placed on both sides of the aperture will be determined by the small cross-section of the aperture and will be different when a particle is carried into the aperture than when only the liquid is in the aperture. This change of resistance modulates the electrical current and is made to activate a counter as the suspension flow carries particles through the aperture.

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[452] Dowling's witnessing signature is written bottom to top in the right margin.

APPENDIX 5. Method of Counting Small Particles (August 1948) [453]

Particles or bodies are suspended in a liquid (in a manner to avoid grouping) if the particles are not already in a liquid. The liquid is made of different electrical conductivity than the conductivity of the particles and is diluted as described below.

The liquid is made to pass thru a small and short aperture or constriction from one section of an insulated container to another insulated section. The fluid in both sections of the container are in contact with the aperture. The liquid flow thru the constriction would generally be impelled and controlled by gravity and determined by a difference in the levels in the two sections. Ideally but not necessarily the cross section of the aperture should be only large enough to admit the largest particle to be counted. The aperture may be larger to pass debris etc. if necessary. Undesired particles may on occasions be eliminated by filtering, chemical treatment, or other means. The aperture should be as short as possible consistent with mechanical strength, smoothness, economy, etc.

It will be observed that the resistance to the flow of electrical current between large diameter metal electrodes placed in each of the two sections is largely concentrated in the aperture between the two sections. The fluid in the aperture serves as an electrical connection between the sections and the fluid in contact with each end of the volume of fluid in the aperture may be considered as electrical contacts to the fluid in the aperture. The electrical resistance of the volume of fluid in the aperture will obviously be affected by any variation in the electrical conductivity of any part of its contents. As the fluid carries one or more particles thru the constriction the change in electrical conductivity can be readily detected in an external electrical circuit. Generally speaking the liquid should be so diluted that the particle concentration would be only one particle to 5, 50 or perhaps more equivalent aperture volumes to reduce the occasions when more than one particle is present in the aperture to a very small percentage of the time when only one is present. The error due to the presence of more than one particle at a time can be reduced by the

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[453] Wallace prepared this description of the Coulter Principle in anticipation of patenting requirements. Unlike the handwritten notes hereto considered, this document is a single-spaced typescript on both sides of a sheet of inexpensive letter-sized paper. The faded text is light blue and may be a carbon copy. As for the previous transcriptions, this one ignores the line sequence of the typed text and silently includes Wallace's handwritten corrections; it has been proofread by others and is agreed to be accurate.

application of probability relations and other means. The change of resistance as individual particles pass thru the aperture can readily be used to actuate a counter.

Considerable information may be obtained regarding particle size by the duration of the change in the electrical resistance of the circuit as the particle passes thru the aperture. As the flow would be relatively constant larger bodies would require a longer time to pass thru. The resolution between different size bodies would naturally be better for the shorter and smaller constrictions. The magnitude of the resistance change would provide valuable information particularly when correlated with the duration and perhaps the “wave shape” of the resistance change. Different size bodies and bodies of different electrical conductivity may selectively activate different counters. The method may be used in conjunction with visual observation to better correlate the various types of data. A microscope may be positioned to view the particles as they pass thru the aperture.

Small apertures may be made by puncturing thin sheets of insulating materials. Sheets or rather flakes of blown glass, split mica, varnish or other films may be obtained as thin as one micron or less. The flakes may be punctured by controlled electric potential and current applied to opposite sides of the flakes by suitable electrodes. By limiting the amount of energy the puncture or hole may be limited to dimensions as small as or smaller than the thickness of the material. Once a small puncture is obtained its size may be increased to a selected dimension and its edges smoothed by flowing a corrosive fluid thru the aperture after it has been suitably mounted. The effect on the edges of the hole will be greater than on the nearby surfaces because of the greater relative rate of fluid flow over the edges. The flow may be continued until a suitable flow rate is obtained. A close inverse relation between fluid flow and electrical resistance will be observed and may be used to measure aperture size by electrical means.

Flake thickness may be determined by optical means or by weight. A flake of glass 20 microns thick and 1/3rd cm. square weighs about 1/50,000 gm. Balances are available to a sensitivity of 1/1,000,000 gm. which should afford 5 to 20 percent accuracy in thickness determinations. Aperture cross section can be determined from the flow rate or by resistance measurement when the flake thickness is known.

Small variations in the aperture flow rates may be compensated for by changes in the amount of pressure or “head” under which they operate in the finished product if it is considered desirable.

To protect the thin flakes from destructive capillary and other forces they may be mounted in a sandwich of two pieces of a thicker material which pieces are provided with apertures perhaps 5 or 20 times larger than the flake aperture. The aperture of the flake is lined up with the sandwich apertures and the three pieces cemented together. If the flake aperture is 20 microns and the sandwich apertures are 20 times larger the resulting dimension will be sufficiently large for fairly “convenient” production and handling.

For an estimation of the magnitude of the resistance change effect for a particular case assume a cylindrical hole and a smaller cubical body as the particle to be “counted”. Assume the aperture length to be 20 microns (1/500 cm) thick (this is about 3 times the diameter of red blood cells) and that the hole is 20 microns in diameter (an area of 1/320,000 sq. cm.). Assume the fluid has a nominal resistance of 50 ohms per cm^3. The resistance of the cylinder of fluid filling the constrictions will be 1/500 divided by 1/320,000 times 50 ohms or approximately 24,000 ohms.

For simplicity assume that the axis of the body is parallel to the axis of the fluid cylinder when the body is inside the cylinder. Assume the body to be a cube of 6 x 6 x 6 microns. The cross section of the cube as an electrical conductor is approximately 1/2,700,000 square cm. This is roughly the 1/9th the cross section area of the cylinder of fluid. The “length” of the cube in the direction of the cylinder axis is roughly 1/3 the length of the cylinder. If it is assumed that the body is a perfect insulator its effect will be to increase the electrical resistance of the cylinder, when it is introduced therein, by an amount of about 1/3 times1/9 or one part in 27 as a very rough approximation. If the resistance of the body differs from the fluid resistance by one part in one thousand then the change in resistance would be roughly one part in 27,000. This change can readily be changed to a voltage change for amplification and actuation of a counter.[454]

Witnessed and understood
August 2, 1948
Walter R. Hogg

Witnessed and Understood
August 7, 1948
John J. Dowling

[454] Uncharacteristically, Wallace neither signed nor dated the typescript, but had Walter R. Hogg and John J. Dowling witness the text as indicated. Dowling also initialed and dated the obverse side in the right margin.

APPENDIX 6. Particle Counter [455]

Further consideration of the use of the small aperture method of counting particles indicates the aperture may be “long” and contain a number of particles at one time as the fluid flows through it provided at least one abrupt change of cross section is provided to give a “sudden” modulation of current as the particle passes through the cross-section. And also providing that the dilution is great enough for distinct modulation to occur for all or most of the particles which pass through the “sudden” change of cross section.

A simple long capillary connecting the input & output fluid bodies would provide a pulse of one priority as it enters the capillary and a pulse of opposite priority as it leaves the capillary. Either or both could of course be counted. The change of conductivity while the body is well within the bore would not present a very distinct current modulation unless the dilution were large enough to keep the number of particles small at any “average” time.

A capillary tube ‘funneled’ at one end would afford marked pulses only away from the funneled end. These more distinct pulses can be satisfactorily counted. “Funneling” avoids an abrupt change of cross section.

Wallace H. Coulter Nov. 21 1948

[455] Transcription from a Xero photocopy of Wallace’s notes handwritten on one side of a sheet of letter-size paper. Along the left margin, beneath, “Witnessed and understood,” Walter R. Hogg signed on November 23; Allen A. Gault on November 24; and John J. Dowling on November 25, 1948. Wallace stapled the photocopy and a circuit diagram of a rate meter to Appendix 4, above, his July 26, 1948, “Method of Counting Small Particles.”

APPENDIX 7. Description of Experiment (October 1948) [456]

Electrical pulses were obtained from the flow of individual red blood cells as they passed through a small aperture separating 2 volumes of the solution containing a dilution of the cells. The two volumes were electrically insulated from each other except for the aperture. An electrode was immersed in each volume for electrical connection to the amplifying and indicating device.

The aperture was roughly 3 mils diameter and was made in .88 mil thick cellulose acetate sheet supplied by Eastman Kodak.[457]

The short end of a J-shaped glass tube was covered by the sheet with the aperture roughly centered over the tube. The sheet was held watertight by rubber bands.[458]

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The electrodes were connected as shown to a 6SL7 resistance coupled (both sections used) amplifier which fed the 3" scope.[459]

The cells flowing through the aperture could be readily seen in the microscope. The electrical pulses which they produced were very distinct on the oscilloscope. The pulse duration was of the order of 1 millisecond. No effort was made to obtain a particular rate of flow or pulses. A dilution of several thousand times was used for the solution.

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[456] Transcription from a Xero photocopy of Wallace's handwritten description of his second demonstration of the Coulter Principle. An image appears as Figure 3.2 of the thesis text.

[457] Here appears a first sketch, of a "sharpened point of hot needle" through two stacked layers of 0.88 mil sheet, the first "sheet perforated for the experiment" and the second, "scrap of .88 mil sheet used as spacer" from the supporting "Glass stop." Hatching indicates the hole made by the needle tip in the top sheet: "small crossed area represents location of aperture perforated by needle."

[458] Here appears a second sketch, of a "container" slightly larger in section than the "J tube" and slightly taller than the short end of the tube, filled slightly above the tube end with ".9% NaCl" solution. The ".88 thick sheet" is draped over the short end of the tube and retained by "Rubber bands," with the "aperture" being the only opening in the portion over the end of the tube. Centered over the aperture is a "Microscope focused on aperture," with the objective inserted into the saline solution. The tube contains "Blood greatly diluted by .9% NaCl," shown a few cm above the level of the solution in the container, and a first "Electrode," which is connected by a 50K-Ohm resistor to the plate supply of the amplifier and to the capacitive "input of Amplifier." To the right of the tube is a second "Electrode," connected to the common ground of the electronics. Wallace's descriptive text is completed on the reverse side of the sheet.

[459] "6SL7" was the designation of a specific type of electron vacuum tube, while "scope" was a short form of "oscilloscope," an instrument for displaying waveforms of electrical signals.

This experiment was set up and
observed jointly by myself and
W. H. Coulter on Oct. 30, 1948
Walter R. Hogg [460]

This is a duplication of the
same experiment, performed on
Oct 16, 1948, except that on the
previous occasion a straight
tube and no microscope was used
W R Hogg

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[460] Hogg's two addenda attest to his participation, as well as his having understood Wallace's description; the second one outlines a simpler test experiment. Wallace neither signed nor dated this document, but had A. A. Gault note in the left margin of the obverse, "This setup was observed by me on November 3, 1948," before also having Walter R. Hogg sign there on November 23, 1948. He then had John J. Dowling sign on November 25, 1948, as having "Read and understood" the description.

APPENDIX 8. For Speed Count in a Volume

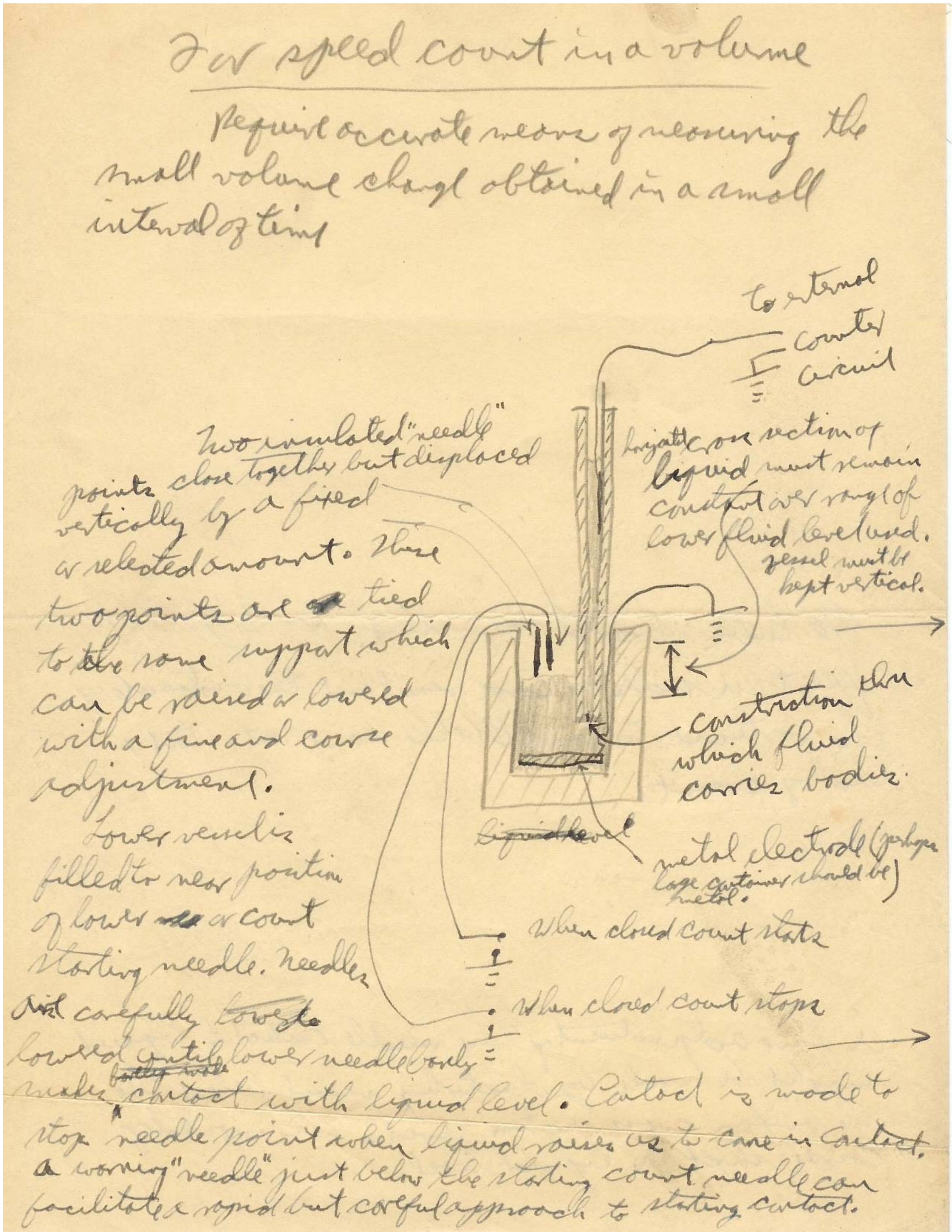
As indicated in the scan of the original (Figure A8.1), Wallace required an "accurate means of measuring the small volume change obtained in a small interval of time."

His plan: Add adjustable level-sensing needles to the setup of the October 16, 1948, experiment (Appendix 7). A "constriction thru which fluid carries bodies" at the bottom of the vertical tube replaces the needle-made aperture. One sensing electrode is in the tube, while the metal common electrode is beneath the constriction in the lower vessel into which the suspension flows from the tube; both these electrodes are connected "to external counter circuit." As noted down the left side and across the bottom of the page, the level-sensing electrodes are: "Two insulated 'needle' points close together but displaced vertically by a fixed or selected amount. These two points are tied to the same support which can be raised or lowered with a fine and coarse adjustment. Lower vessel is filled to near position of lower or count starting needle. Needles are carefully lowered until lower needle only makes contact with liquid level. Contact is made to stop needle point when liquid raises up to come in contact. A warning 'needle' just below the starting count needle can facilitate a rapid but careful approach to starting contact." The counter connections to the two level-sensing electrodes are indicated beneath the lower vessel.

To the right of the vertical tube, two essential requirements are indicated: "Horizontal cross section of liquid must remain constant over range of lower fluid level used. Vessel must be kept vertical."

Although this concept seemed workable in principle, Wallace's experiments found the resultant suspension volumes too nonrepeatable to enable accurate calculation of cellular concentrations from the count data. However, to his amusement the concept was later implemented in a competitive instrument.[461]

[461] D. E. Pegg and A. C. Antcliff, "An evaluation of the Vickers Instruments J12 cell counter," *Journal of Clinical Pathology* 18 (1965): 473, Figure 2.



For speed count in a volume

Require accurate means of measuring the small volume change obtained in a small interval of time

Two insulated "needle" points close together but displaced vertically by a fixed or selected amount. These two points are tied to the same support which can be raised or lowered with a fine and course adjustment.

Lower vessel is filled to near position of lower or count starting needle. Needles are carefully lowered until lower needle barely [illegible] makes contact with liquid level. Contact is made to stop needle point when liquid raises so to come in contact. A warning "needle" just below the starting count needle can facilitate a rapid but careful approach to starting contact.

Figure A8.1. Wallace Coulter's initial approach to measuring the count volume. It elaborates the October 16 setup of Appendix 7. He neither signed nor dated this conceptual description.

APPENDIX 9. Proposal to Argonne National Laboratory (ANL) [462]

By late 1950 Wallace Coulter's decision to spend full time working on experiments toward cell counting, his expenditures for parts and materials, and his expenses for patent applications and filings placed the Coulter brothers in tight financial straits. Figures A9.1-A9.3 below are scans of Wallace's first-draft text of a four-page proposal for developmental support, faintly datelined in Figure A9.1 as 3023 W. Fulton and signed in Figure A9.2 as "Witnessed and understood" by J. J. Dowling on December 21, 1950. The drawing referenced in the final sentence in Figure A9.2 appears as Figure 4.1 in the thesis text, while Figure A9.3 contains the proposed budget. The second draft included the changes indicated in Figures A9.1 and A9.2, while the third draft made only insignificant changes in wording. All three drafts were typed as was this one; the third one Wallace sent to Ms. Jean Gilbert, administrative assistant to Austin M. Brues, Director of ANL's Division of Biological and Medical Research, on January 26, 1951.[463] Her response appears in Figure A9.4.

As noted in the thesis text, here the primary importance of the proposal is its third paragraph (Figure A9.1), which was worded exactly the same in all three drafts.

Similarly noted, a secondary importance is the proposal's drawing (Figure 4.1), wherein the indicating means is a rate-meter (the "pulse rate counter") as illustrated in Figure 7 of Wallace's U.S. Patent 2,656,508 on the Coulter Principle.

The note at the top of the first page and most of the corrections are in the handwriting of Wallace's father, Joseph R. Coulter, Sr., who typed this draft.

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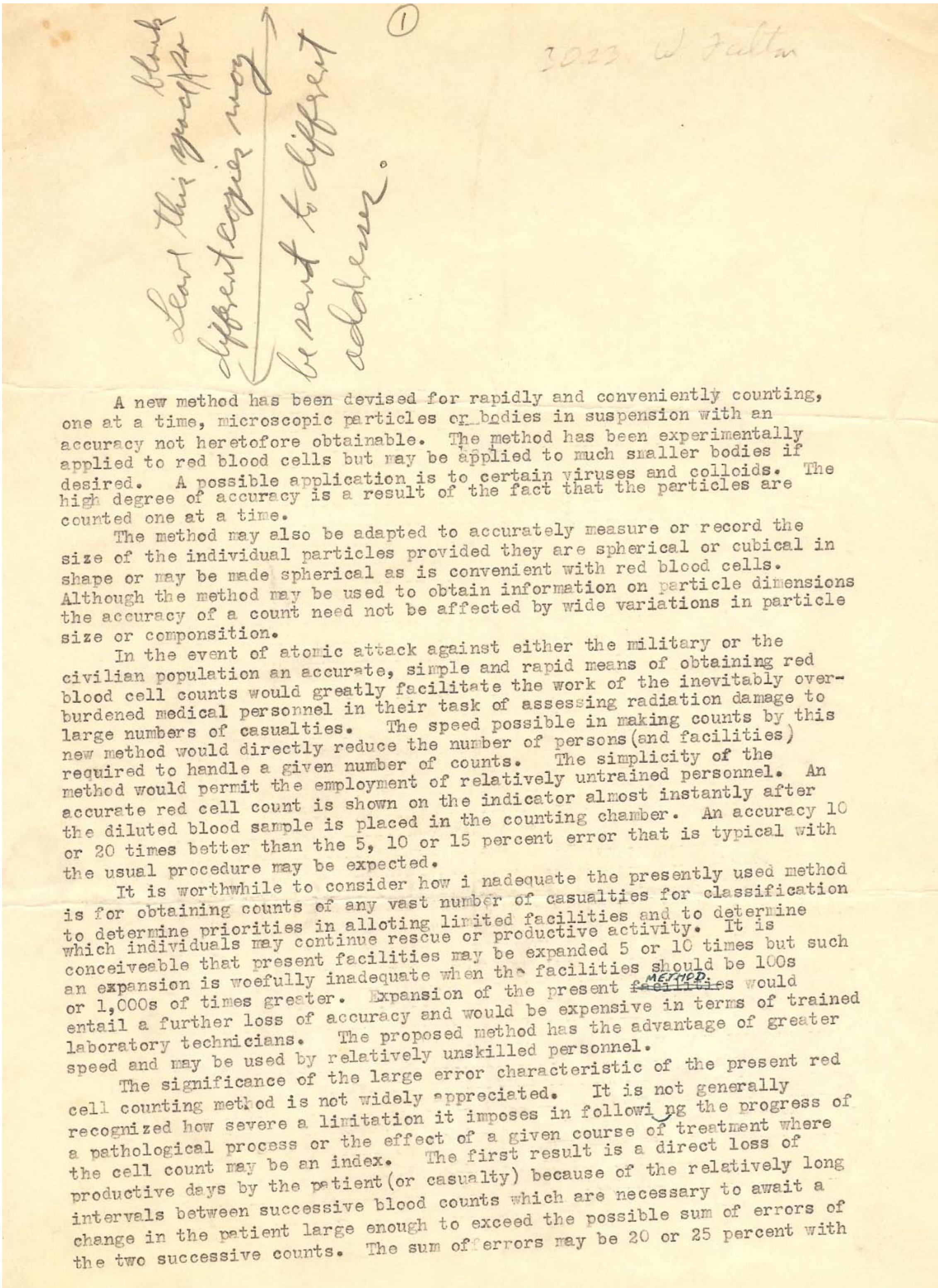
[462] Scans of first proposal draft and letter from Ms. Jean Gilbert; WHC Papers.

[463] Carbon copy of Wallace's letter of transmittal to Ms. Jean Gilbert; WHC Papers. On it he noted the ANL phone number, Butterfield 8-2000, and, "Called her in early Feb. & she said various individuals found it most interesting but not immediately required etc by them. Suggested Major Lenox Lohr etc." This is the call to which Gilbert refers in her letter (Figure A9.4).



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addresses.

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3023 W Fulton

A new method has been devised for rapidly and conveniently counting, one at a time, microscopic particles or bodies in suspension with an accuracy not heretofore obtainable. The method has been experimentally applied to red blood cells but may be applied to much smaller bodies if desired. A possible application is to certain viruses and colloids. The high degree of accuracy is a result of the fact that the particles are counted one at a time.

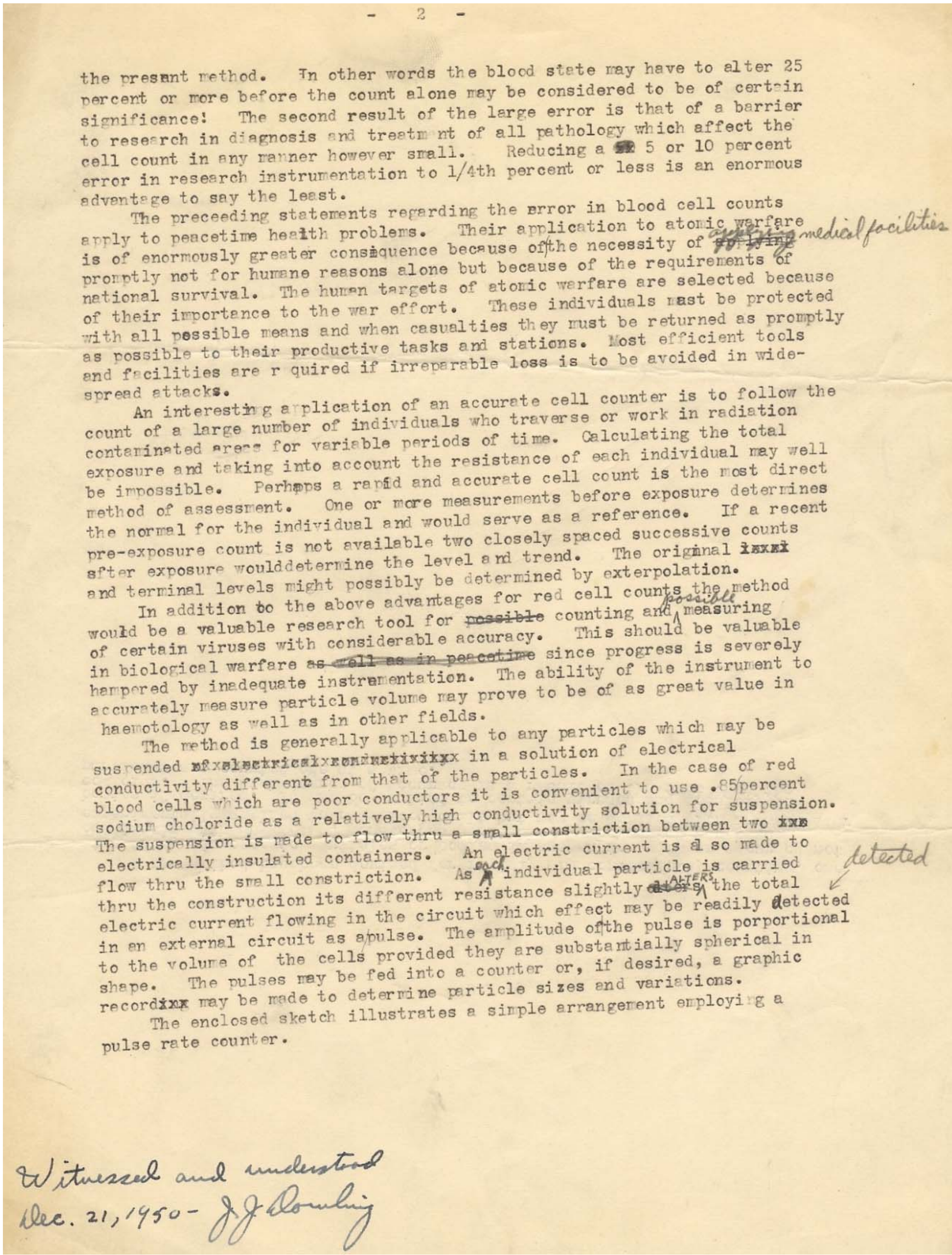
The method may also be adapted to accurately measure or record the size of the individual particles provided they are spherical or cubical in shape or may be made spherical as is convenient with red blood cells. Although the method may be used to obtain information on particle dimensions the accuracy of a count need not be affected by wide variations in particle size or componsition.

In the event of atomic attack against either the military or the civilian population an accurate, simple and rapid means of obtaining red blood cell counts would greatly facilitate the work of the inevitably over-burdened medical personnel in their task of assessing radiation damage to large numbers of casualties. The speed possible in making counts by this new method would directly reduce the number of persons (and facilities) required to handle a given number of counts. The simplicity of the method would permit the employment of relatively untrained personnel. An accurate red cell count is shown on the indicator almost instantly after the diluted blood sample is placed in the counting chamber. An accuracy 10 or 20 times better than the 5, 10 or 15 percent error that is typical with the usual procedure may be expected.

It is worthwhile to consider how inadequate the presently used method is for obtaining counts of any vast number of casualties for classification to determine priorities in alloting limited facilities and to determine which individuals may continue rescue or productive activity. It is conceiveable that present facilities may be expanded 5 or 10 times but such an expansion is woefully inadequate when the facilities should be 100s or 1,000s of times greater. Expansion of the present ~~facilities~~ METHOD would entail a further loss of accuracy and would be expensive in terms of trained laboratory technicians. The proposed method has the advantage of greater speed and may be used by relatively unskilled personnel.

The significance of the large error characteristic of the present red cell counting method is not widely appreciated. It is not generally recognized how severe a limitation it imposes in following the progress of a pathological process or the effect of a given course of treatment where the cell count may be an index. The first result is a direct loss of productive days by the patient (or casualty) because of the relatively long intervals between successive blood counts which are necessary to await a change in the patient large enough to exceed the possible sum of errors of the two successive counts. The sum of errors may be 20 or 25 percent with

Figure A9.1. First page of first ANL proposal draft. The level to which Wallace understood the problem of radiation exposure and requirements for a solution is clearly apparent. A second draft included the indicated changes and the final proposal, only minor rewording.



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the present method. In other words the blood state may have to alter 25 percent or more before the count alone may be considered to be of certain significance! The second result of the large error is that of a barrier to research in diagnosis and treatment of all pathology which affect the cell count in any manner however small. Reducing a 5 or 10 percent error in research instrumentation to 1/4th percent or less is an enormous advantage to say the least.

The preceeding statements regarding the error in blood cell counts apply to peacetime health problems. Their application to atomic warfare is of enormously greater consequence because of the necessity of ~~applying~~ medical facilities promptly not for humane reasons alone but because of the requirements of national survival. The human targets of atomic warfare are selected because of their importance to the war effort. These individuals must be protected with all possible means and when casualties they must be returned as promptly as possible to their productive tasks and stations. Most efficient tools and facilities are required if irreparable loss is to be avoided in widespread attacks.

An interesting application of an accurate cell counter is to follow the count of a large number of individuals who traverse or work in radiation contaminated areas for variable periods of time. Calculating the total exposure and taking into account the resistance of each individual may well be impossible. Perhaps a rapid and accurate cell count is the most direct method of assessment. One or more measurements before exposure determines the normal for the individual and would serve as a reference. If a recent pre-exposure count is not available two closely spaced successive counts after exposure woulddetermine the level and trend. The original ~~ixxxi~~ and terminal levels might possibly be determined by exterpolation.

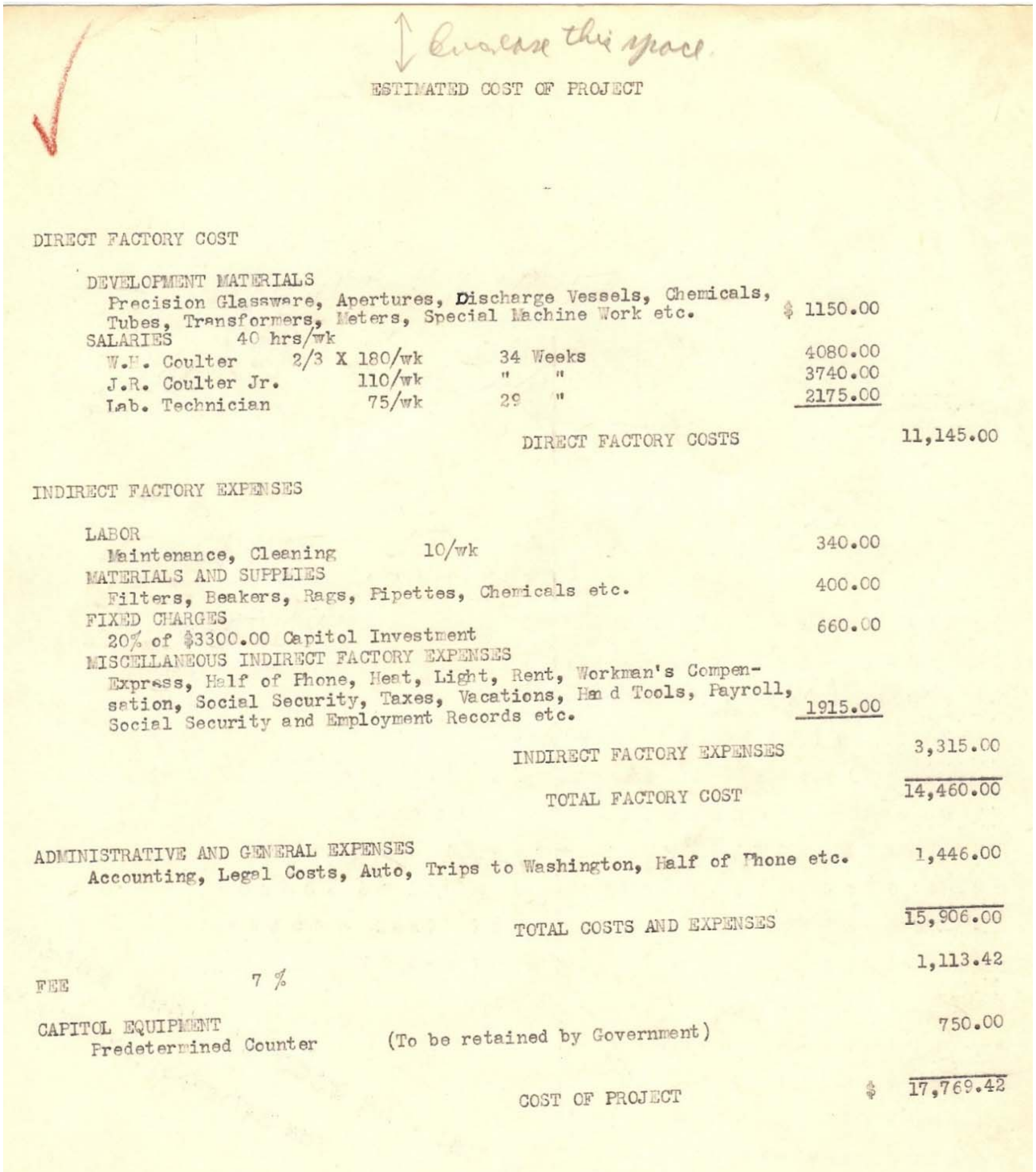
In addition to the above advantages for red cell counts the method would be a valuable research tool for ~~possible~~ counting and possible measuring of certain viruses with considerable accuracy. This should be valuable in biological warfare ~~as well as in peacetime~~ since progress is severely hampered by inadequate instrumentation. The ability of the instrument to accurately measure particle volume may prove to be of as great value in haemotology as well as in other fields.

The method is generally applicable to any particles which may be suspended ~~mfxelectricalxcondnctivityx~~ in a solution of electrical conductivity different from that of the particles. In the case of red blood cells which are poor conductors it is convenient to use .85percent sodium chloride as a relatively high conductivity solution for suspension. The suspension is made to flow thru a small constriction between two ~~ixe~~ electrically insulated containers. An electric current is also made to flow thru the small construction. As each individual particle is carried thru the construction its different resistance slightly alters the total electric current flowing in the circuit which effect may be readily detected in an external circuit as a pulse. The amplitude of the pulse is porportional to the volume of the cells provided they are substantially spherical in shape. The pulses may be fed into a counter or, if desired, a graphic record ~~ixx~~ may be made to determine particle sizes and variations.

The enclosed sketch illustrates a simple arrangement employing a pulse rate counter.

Witnessed and understood
Dec. 21, 1950 - J J Dowling

Figure A9.2. Second page of the ANL proposal draft. The first full paragraph makes a distinction between peacetime health problems and those resulting from atomic warfare. The following paragraph indicates the need to monitor radiation effects on those who travel or work in contaminated areas. The third page of the draft appears in Figure 4.1.



Increase this space

ESTIMATED COST OF PROJECT

DIRECT FACTORY COST

DEVELOPMENT MATERIALS
Precision Glassware, Apertures, Discharge Vessels, Chemicals, Tubes, Transformers, Meters, Special Machine Work etc. — $ 1150.00

SALARIES 40 hrs/wk

W.H. Coulter	2/3 X 180/wk	34 Weeks	4080.00	
J.R. Coulter Jr.	110/wk	" "	3740.00	
Lab. Technician	75/wk	29 "	2175.00	
		DIRECT FACTORY COSTS		11,145.00

INDIRECT FACTORY EXPENSES

LABOR Maintenance, Cleaning 10/wk		340.00	
MATERIALS AND SUPPLIES Filters, Beakers, Rags, Pipettes, Chemicals etc.		400.00	
FIXED CHARGES 20% of $3300.00 Capitol Investment		660.00	
MISCELLANEOUS INDIRECT FACTORY EXPENSES Express, Half of Phone, Heat, Light, Rent, Workman's Compensation, Social Security, Taxes, Vacations, Hand Tools, Payroll, Social Security and Employment Records etc.		1915.00	
	INDIRECT FACTORY EXPENSES		3,315.00
	TOTAL FACTORY COST		14,460.00
ADMINISTRATIVE AND GENERAL EXPENSES Accounting, Legal Costs, Auto, Trips to Washington, Half of Phone etc.			1,446.00
	TOTAL COSTS AND EXPENSES		15,906.00
FEE 7 %			1,113.42
CAPITOL EQUIPMENT Predetermined Counter (To be retained by Government)			750.00
	COST OF PROJECT	$	17,769.42

Figure A9.3. Fourth page of the ANL proposal draft. Funding was requested for 29 weeks of technician effort (Salaries, line 3) and expenses for automobile and trips to Washington (Administrative and General Expenses).[464] This budget request was also submitted to the ONR (Appendix 10).

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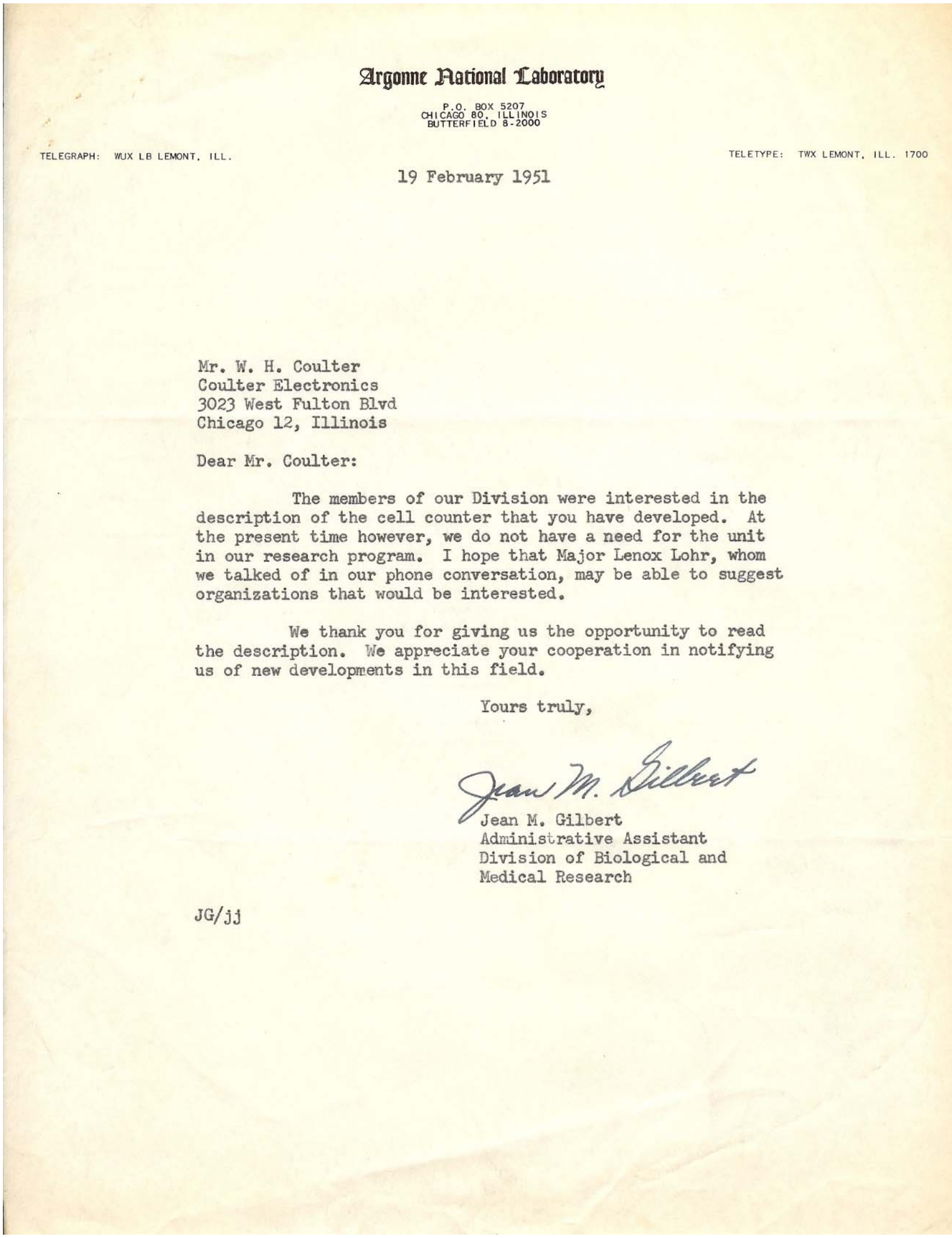
[464] Wallace had purchased a 1949 Kaiser Traveler sedan in November 1949; Stephen L. Kerrigan to Wallace Coulter, letter dated December 3, 1949, with signed insurance transfer effective November 10, 1949, stapled to it; WHC Papers.



Argonne National Laboratory

P.O. BOX 5207
CHICAGO 80, ILLINOIS
BUTTERFIELD 8-2000

TELEGRAPH: WUX LB LEMONT, ILL.

TELETYPE: TWX LEMONT, ILL. 1700

19 February 1951

Mr. W. H. Coulter
Coulter Electronics
3023 West Fulton Blvd
Chicago 12, Illinois

Dear Mr. Coulter:

The members of our Division were interested in the description of the cell counter that you have developed. At the present time however, we do not have a need for the unit in our research program. I hope that Major Lenox Lohr, whom we talked of in our phone conversation, may be able to suggest organizations that would be interested.

We thank you for giving us the opportunity to read the description. We appreciate your cooperation in notifying us of new developments in this field.

Yours truly,

Jean M. Gilbert

Jean M. Gilbert
Administrative Assistant
Division of Biological and
Medical Research

JG/jj

Figure A9.4. ANL's letter rejecting Wallace Coulter's proposal. He did contact Lohr, who apparently suggested that he contact Dr. Freeman H. Quimby of ONR's physiology branch in Washington, D.C. On March 6, 1951, Wallace met with Quimby in his office and discussed the proposal's details.

APPENDIX 10. Proposal to Office of Naval Research (ONR) [465]

April 30, 1951

Messrs Lloyd White, Physicist
Morris Jones, Microbiologist

Office of Naval Research
U. S. Navy
844 N. Rush St.
Chicago 11, Ill.

Subject: Red Blood Cell Counter

Gentlemen:

Herewith is presented a proposal for the construction of a model embodying a new principle of detecting small bodies in suspension as adapted to counting red blood cells. Submission of the proposal has been suggested by Dr. F. H. Quimby, Head of the Physiology Branch of the Office of Naval Research with whom the project was thoroughly discussed at his office on March 6.

In considering this proposal it may be useful to obtain a report of an extended discussion of the method March 7 with a group at the National Institutes of Health in Bethesda. The Institute Scientists present were Dr. Byron J. Olson, Assistant Chief of the Laboratory of Infectious Diseases, Dr. Carl T. F. Mattern, associated with Dr. Olson and Dr. Frederick Brackett, Physicist.

It should be stated that we are most willing to cooperate in the evaluation of the device after completion of the project if such cooperation is requested. In addition Coulter Electronics is anxious to take all necessary measures to put the unit in production should that step be decided upon by the defense agency.

Respectfully yours,
Wallace H. Coulter
Wallace H. Coulter

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[465] This document is a single-spaced typescript on one side of six sheets of letter-sized typing paper; the letter of transmittal was typed on Coulter Electronics' letterhead with the 3023 W. Fulton Blvd. address. As for previous transcriptions, this one ignores the line sequence of the typed text; it has been proofread by others and is agreed to be accurate. Corrections (italicized) have been made of three typos in the proposal body; a) page 1, fourth paragraph, line 8, "desirability" was "desireability"; b) page 2, first full paragraph, line 9, "immersed" was "emersed"; and c) page 2, third full paragraph, line 2, "integration" was "intergration."

COULTER ELECTRONICS

Coulter Electronics is a full partnership wholly owned by Wallace H. Coulter and J. R. Coulter, Jr.

The company was established in 1947 for the purpose of investigating and exploiting unique electronic devices and applications. Several thousand hours have been devoted to the investigation of several projects which incorporate substantial advances over presently known art.

For the purpose of supporting the research objectives a limited amount of production has been undertaken in the electromedical field. Principle items have been special amplifiers incorporating a new interference eliminating method for electrocardiography and a deluxe galvanic generator and muscle stimulator.

W. H. Coulter has a background of 3 years of radio transmitter experience, 5 years of electromedical and high frequency sales engineering and eight years of electronic circuit design and manufacture. J. R. Coulter attended the Ohio State University under the Army Student Training Program and graduated from the Illinois Institute of Technology in 1947 and has been continuously engaged in electronics since that time.

Employees engaged vary with the limited production requirements and material availability. Production may be expanded readily for most instrument requirements.

Net worth exclusive of the value of circuit developments and patent applications but including electronic test equipment, building equity, automobile, cash and accounts receivable: $11,540.00. It is estimated that the value of the circuit developments and patent applications exceeds the above figure several fold.

Location: 3023 W. Fulton Blvd.,
Chicago 12, Illinois.

PROPOSAL: TO SUPPLY MODEL FOR EVALUATING APPLICABILITY OF A NEW PRINCIPLE FOR DETECTING AND COUNTING SMALL PARTICLES WHICH IS SUBSTANTIALLY INDEPENDENT OF PARTICLE SIZE OR COMPOSITION.

The purpose of this proposal is to supply a laboratory model employing the principle as adapted specifically to the counting of red blood cells.

The application to red blood cell counts is proposed because of the need of rapid, more accurate and less tedious means than the present method which requires the skills of highly trained laboratory technicians. As the red blood count is of great significance in detecting and following radiation damage and treatment and as the possibility exists of having an enormous number of radiation casualties in atomic attacks the need of a better method is of critical concern.

The principle to be employed in the proposed laboratory model is extremely simple and holds the promise of ultimately providing, in a small compact instrument, means for making red blood counts in the field in a manner which overcomes the serious limitations of the present method. As the new method detects or counts the particles one at a time, an accuracy 5 or 10 times greater than possible with the conventional means should ultimately be obtained. The new method is faster and should not require the skills of a trained technician. In addition the tedium of the present method is eliminated. A further advantage of the proposed method which is of importance in its evaluation is that an extended period of clinical correlation with conventional counts will not be required.

In order to conserve critical man hours and time the present proposal is to supply a model having the minimum refinements necessary to accomplish the stated objective; namely the evaluation of the method to determine its applicability to the defense effort. Should the results obtained with the proposed model come up to expectations, a second proposal will be made to provide a number of units for trial in the field. It is intended that the original laboratory model also serve to allow closer specifications as to the exact form and performance of subsequent models than is possible to set down at this time. Experience with the proposed laboratory model may indicate the *desirability* of a program for applying the method for: (1) measuring the dimensions of red blood cells, (2) separately or simultaneously obtaining a count of white blood cells or (3) for counting or measuring particles much smaller than red blood cells.

The principle to be incorporated in the proposed apparatus depends upon the fact that particles having an electrical conductivity different from that of the fluid in which they are suspended may be caused to modulate an electric current flowing through the suspension in such a manner that the effect of individual particles can be detected. An electrical path of small dimensions is required and a controlled flow of the particle bearing suspension thru the electrical path varies the electrical resistance of the path as each individual particle is carried in and out of the electrical path. The change in electrical resistance is used to produce a voltage change in an external circuit which is amplified by special circuits for counting or other purposes. Fortunately a structure to provide a sufficiently small electrical path which eliminates the need of correspondingly small and troublesome electrode surfaces has been devised. The metal electrodes that are required may be and are thousands of times the dimensions of the small current path with the result that electrolytic effects at the electrode surfaces are minimized although a very large current concentration in the small electric path is obtained. In addition the structure provides a means for positively

-2-

channeling the flow of the suspension. In the case of red blood cells which are poor electrical conductors it has been found that a .85% sodium chloride solution, which is a relatively good conductor, provides a suitable fluid for suspension.

The above referred to structure which is part of the proposed model consists of the following: A test tube having, near its base, an aperture approximately 1/200th inch diameter and 1/200th inch long thru which a part of the suspension placed in the tube can flow. A second vessel into which the suspension may flow from the aperture and in which the lower end of the test tube is positioned is provided. The second vessel contains enough conductive fluid to at least cover the aperture in the test tube so that a smooth continuous path and flow is provided. The fluid in the test tube is at a higher level than the fluid in the second vessel by a set amount so that a known rate of flow thru the aperture may be established. An electrode is *immersed* in the fluid in the test tube and in the fluid in the second or discharge vessel. It may be seen that an electric current can be made to flow from one electrode to the other thru the constricted fluid and current path between the two otherwise electrically insulated fluid bodies. The volume within the aperture provides the required electric current path of small dimensions where a large concentration of current flow is conveniently obtained. The fluid outside of the aperture at each end of the aperture effectively serves as electrode surfaces of small dimensions.

By connecting the two metal electrodes to a suitable circuit it is possible to produce a signal pulse of several hundred microvolts with the passage of each blood cell thru the aperture. The pulses may be amplified with suitable circuits to any desired level for counting, observation with an oscilloscope, or other purposes. The time required for cell passage thru the aperture which corresponds to the pulse duration is of the order of a millisecond or less depending upon the difference in liquid levels, dilution and other factors. Several hundred cells a second may be detected and counted. By suitable electronic circuit design only those cells exceeding a certain selected minimum size produce a pulse. All pulses are made to produce the same effect on the counting system with the result that the count is made independent of cell size variations.

The possibility exists of obtaining an indication of the count with a simple rate meter. It may be found however that the *integration* interval of which a rate meter is capable is not sufficient for a stable and satisfactorilly accurate indication. Another means of indication would then be provided such as an indication of the total count that occurs during a short fixed interval of time such as 3 or 4 seconds. Other methods of count indications will be considered. Such factors as rate of flow as determined by aperture dimensions and fluid level differences will be explored. Blood sample dilutions much greater than usually employed are required to reduce the frequency with which more than one cell will pass through the aperture at one time. Of course this coincidence effect will be taken into account in whatever method of count indication that is provided. Suitable mixing pipettes and containers will be selected and provided with the model.

A structure to support the test tube “counting” chamber together with convenient means for obtaining the required rate of flow will be provided.

Connections will be provided on the apparatus for feeding the pulse signals to an oscilloscope or high speed graphic recorder to allow visual observations of the pulses produced. Inclusion of a recorder with the model is not proposed for reasons of economy although a recorder would add to the efficiency and speed of the planned work.

It is estimated that completion of the proposed model for evaluation will require 8 months of full time work by two individuals with the assistance and direction of the discoverer of the method, Wallace H. Coulter, devoting 2/3rds of his time to the project. The full time workers will be: (1) J. R. Coulter, Jr., graduate Electronics Engineer, who has been associated with the early work on the method, (2) A laboratory technician who will be recruited for the project. It is intended that the laboratory technician be added to the project approximately 5 weeks after commencement of the project.

Coulter Electronics has on hand the basic apparatus required for electronic development work. Electronic test equipment includes oscilloscopes, vacuum tube voltmeters, volt ohmmeters, Q meter, distortion meter, 20 cycle to 200 kc oscillator, two 20 cycle to 20,000 cycle oscillators, low noise level amplifiers and miscellaneous meters and supplies.

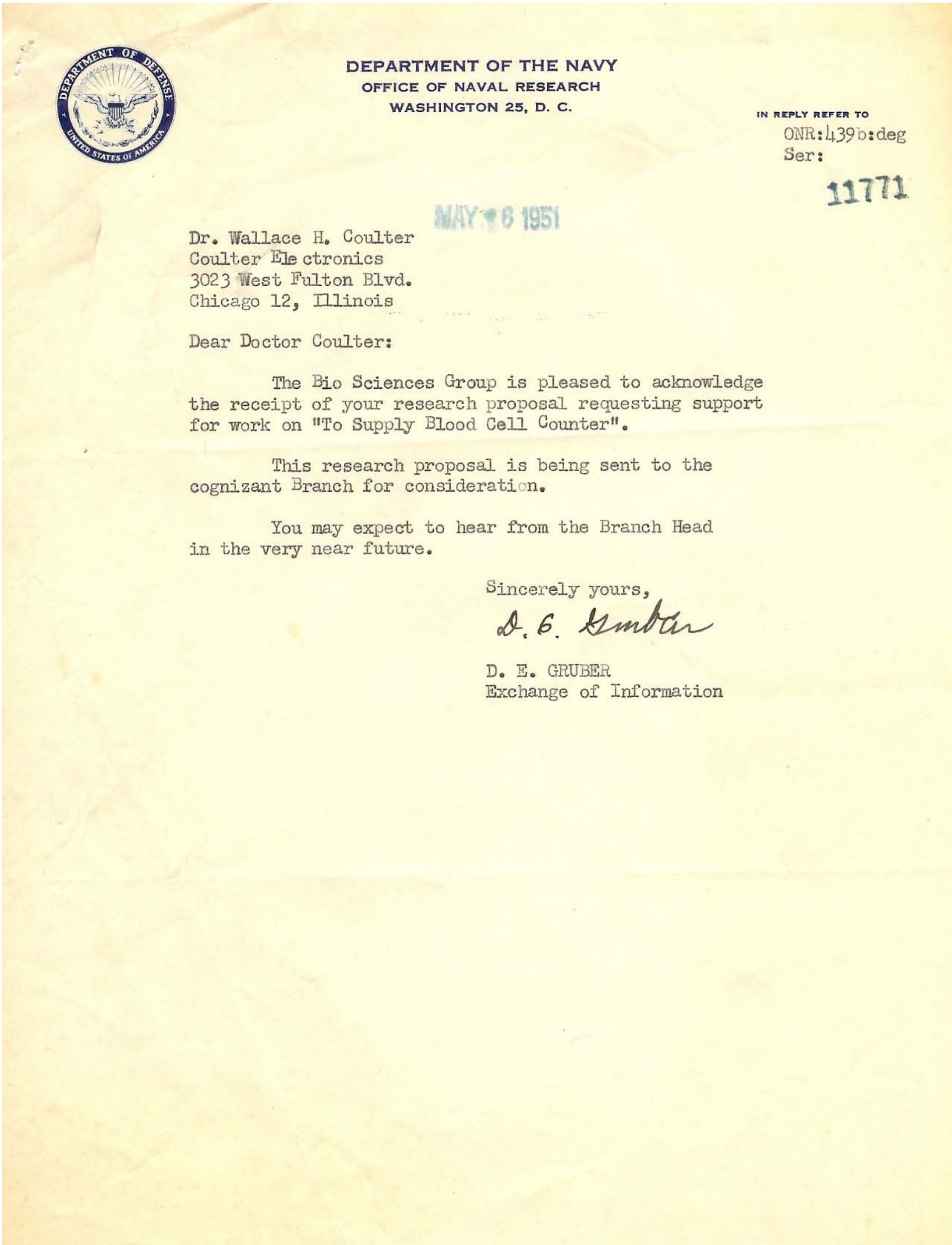
The project will be carried out on the premises of Coulter Electronics. Special machine work and glassware will be obtained from outside sources.

In supplying a model for the Government's evaluation of the principle for defense work, Coulter Electronics does not forfeit any patent rights it may have. It may be well to point out that the investment of Coulter Electronics in the discovery and demonstration of the principle greatly exceeds the estimated cost of the proposed model.

Coulter Electronics certifies that there has not been employed or retained a company or person other than a full time employee to solicit or secure this contract, and agrees to furnish information relating thereto as requested by the Contracting Officer.

NOTES: Page 4 was a typed version of the budgetary page as sent to ANL (Figure A9.3).The ONR's letter acknowledging receipt of this proposal (Figure A10.1) has been previously published.[466]

[466] Graham, "The Coulter Principle: Imaginary origins," Figure 2.



DEPARTMENT OF THE NAVY
OFFICE OF NAVAL RESEARCH
WASHINGTON 25, D. C.

IN REPLY REFER TO
ONR:439b:deg
Ser:
11771

MAY 16 1951

Dr. Wallace H. Coulter
Coulter Electronics
3023 West Fulton Blvd.
Chicago 12, Illinois

Dear Doctor Coulter:

The Bio Sciences Group is pleased to acknowledge the receipt of your research proposal requesting support for work on "To Supply Blood Cell Counter".

This research proposal is being sent to the cognizant Branch for consideration.

You may expect to hear from the Branch Head in the very near future.

Sincerely yours,

D. E. Gruber

D. E. GRUBER
Exchange of Information

Figure A10.1. ONR's letter acknowledging receipt of Wallace Coulter's proposal. The ONR objected to the proposed budget (Figure A9.3), and as of September 29, 1951, Wallace expected no support would be forthcoming. However, his NIH contacts from the March meeting reportedly helped him convince the ONR to approve the proposal, but with only partial funding until he could demonstrate the feasibility of his approach.

APPENDIX 11. Apparatus from ONR Contract NONR-1054 (00) [467]

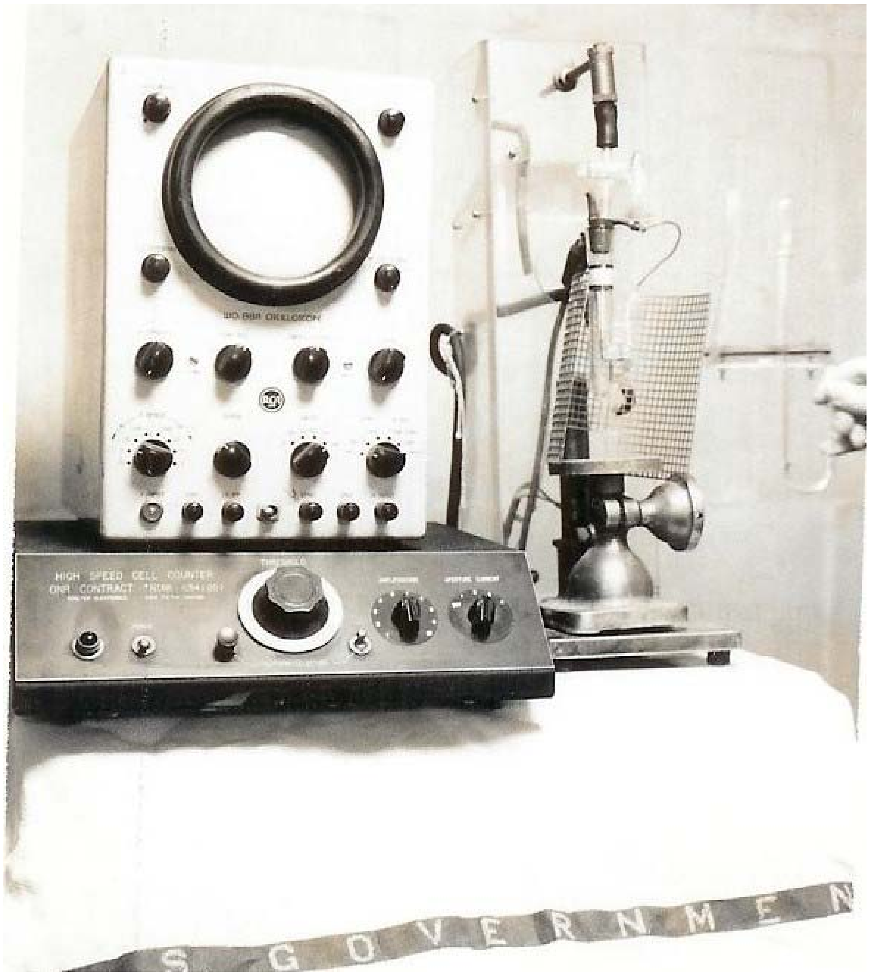


Figure A11.1. Apparatus used in the ONR feasibility demonstration. This consisted of a rudimentary sample stand (Figure A11.2), the electronics module of Figure 4.3 to provide electrical current to the stand's aperture tube and to amplify the pulses generated by blood cells as the vacuum from a manometer drew cellular suspension through the tube's aperture from a vial on the mechanical stage, and a standard oscilloscope to display the amplified cellular pulses from the electronics module. There were no volume-control electrodes on the manometer and no pulse counter; the latter was obtained as a result of the acceptable demonstration of the feasibility of Wallace's proposal. The perforated metal shield around the sample vial and tube reduced electrical interference from the building's power system. Here, Wallace had removed the manometer from the sample stand and held it in his hand at the extreme right of the image.

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[467] These Polaroid photographs were made by Wallace Coulter after retrieving the apparatus from storage during a subsequent ONR visit; WHC Papers.

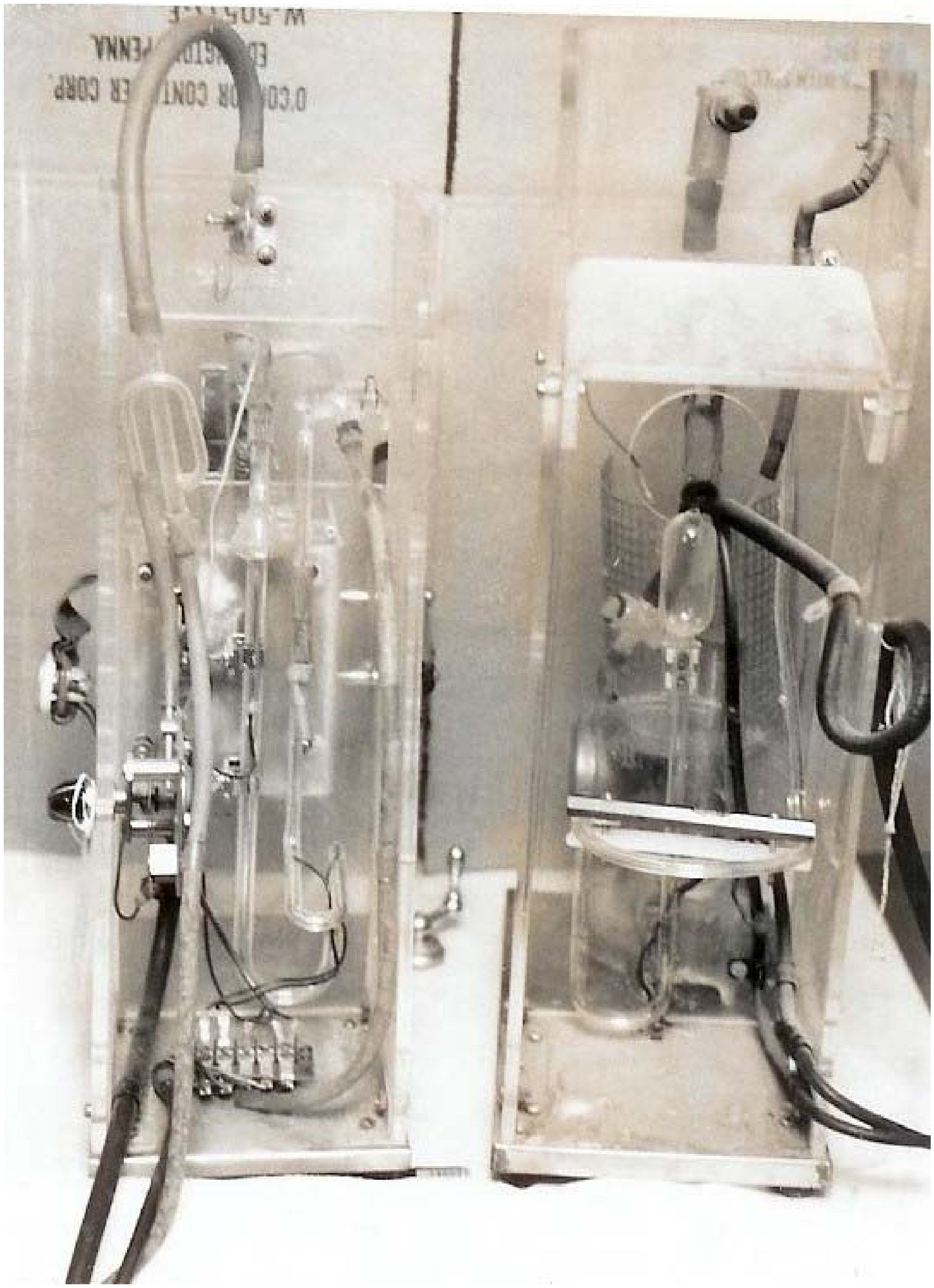

Figure A11.2. Rear view of the ONR sample stands. The rudimentary stand in Figure A11.1 is at the right, while that used in the demonstration of the first integrated counter is at the left (Figure A11.3); the microscope is missing from the latter unit. In both units the glass tubing with the two U-shaped bends is the manometer. The upper U-shaped bend is the segment where mercury flow was horizontal to provide suspension flow at a constant flow velocity. There, in the stand at the left, electrical connections to the volume-control electrodes are visible. Cables for the aperture current and signals exit the stands at the lower corners.

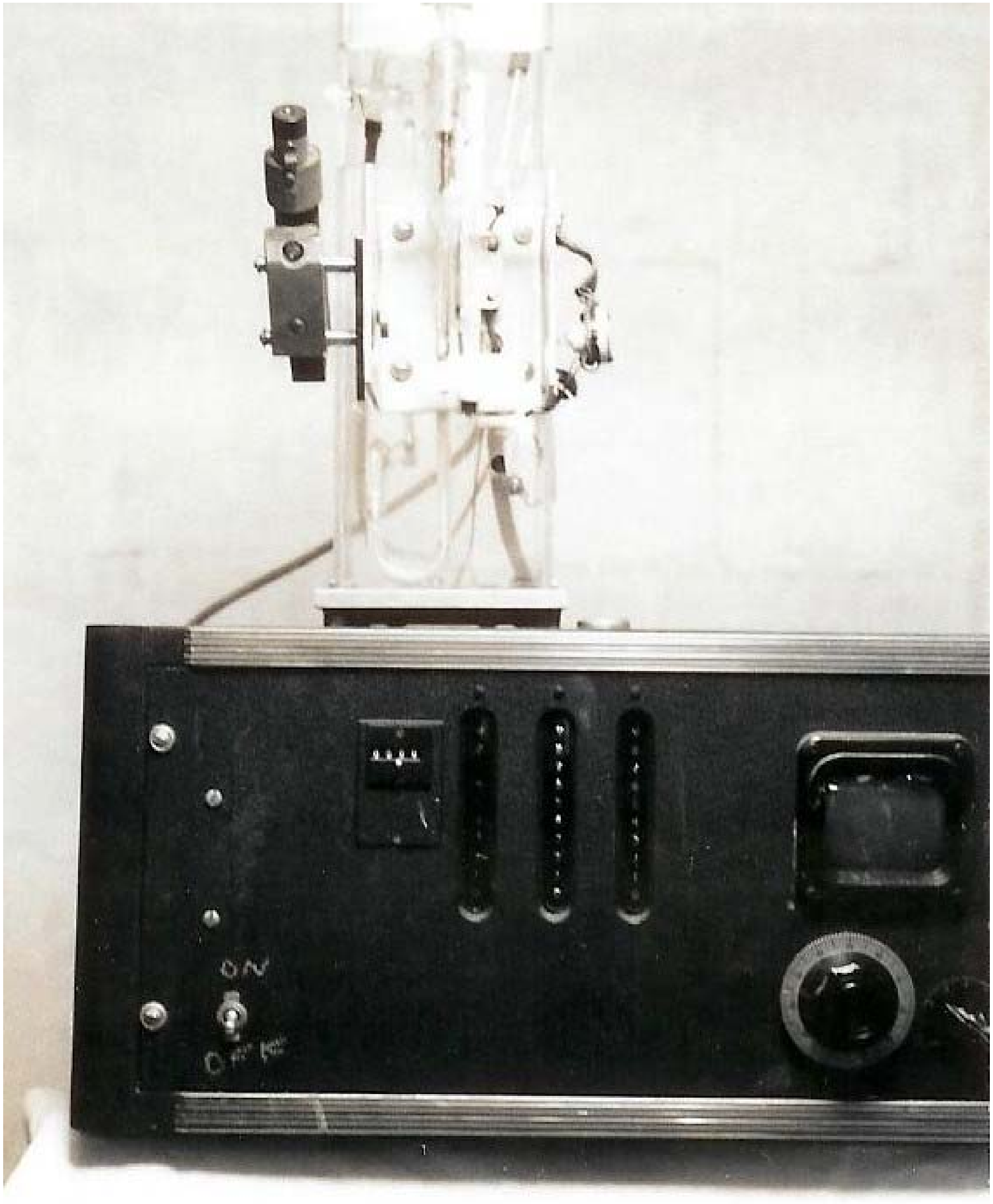

Figure A11.3. First integrated Coulter cell counter. The sample stand at the left in Figure A11.2, now with its microscope in place, rests on the electronics unit that provided all necessary functions of the electronics module and oscilloscope in Figure A11.1, plus three of the decade counting modules used in the predetermined counter of Wallace's proposals, a Berkeley Scientific Model 410. The decade modules registered the rapid low-value digits of a cell count, while the slower high-value digits were registered by the small four-digit mechanical counter to the left of the decade modules.

APPENDIX 12. Characteristics of Coulter Sensing Apertures

As noted regarding Figure 4.9, the spatial distribution of the sensitive volume and suspension throughflow of Coulter sensing apertures depends in a complex manner on the diameter D and length L of the aperture bore, which together provide sufficient information that the spatial distribution of excitation current and sensitive volume can be defined analytically. But inertial effects and surface interactions of suspension passing through the aperture cannot be defined with only these geometric parameters, and understanding liquid aperture throughflows requires carefully defined experiments.

Efforts toward understanding the electric and hydraulic field distributions surrounding sensing apertures were published in 1979 as two chapters in *Flow Cytometry and Sorting*.[468] All authors were with the Max Planck Institut für Biochemie, and their chapters, widely accepted as being definitive and so frequently cited, were republished with minor revisions in a 1990 edition.[469] Except for substituting an “orifice” for the Coulter sensing aperture, Volker Kachel accurately represented Wallace Coulter’s first description of the Coulter Principle (Appendix 4).[470] Kachel’s detailed experimental results closely agree with analytical calculations for the voltages produced by practical aperture currents through ideal apertures and have proven useful in understanding signals, such as the M-shaped pulses responsible for skewed volume distributions, produced by cells or particles in suspensions flowing through defect-free Coulter sensing apertures (Figure 4.7).[471] This chapter is an excellent overview of the spatial distribution of electrical voltage established about and through such apertures by the throughflow of electrical current.

Kachel’s treatment of suspension throughflows about and through Coulter sensing apertures is much less satisfactory. He referred to the second chapter for details and equated the “Coulter orifice” to a short tube in which the flow downstream of the entry orifice was developing tube flow.[472] He assumed that for a sharp-edged entry orifice the

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[468] Myron R. Melamed, Paul F. Mullaney, and Mortimer L. Mendelsohn, eds., *Flow Cytometry and Sorting* (New York: John Wiley & Sons, Inc., 1979).

[469] Myron R. Melamed, Tore Lindmo, and Mortimer L. Mendelsohn, eds., *Flow Cytometry and Sorting*, 2nd ed. (New York: Wiley -Liss, Inc., 1990).

[470] Volker Kachel, “Electrical resistance pulse sizing (Coulter sizing),” ibid. 45-80; for the structure, 46 and Fig. 1. Regarding “orifice,” see Appendix 1, fourth paragraph.

[471] Thom and Kachel, “Fortschritte für die elektronische Größenbestimmung von Blutkörperchen.”

[472] Kachel, “Electrical resistance pulse sizing (Coulter sizing),” 48-49.

flow in the bore downstream was constricted to about 60% of the bore cross-sectional area, with the outer 40% being "a dead zone with vortices." [473] The authors of the second chapter also considered Coulter "orifices" to be short tubes and, among other unsupported assumptions, considered the spatial distributions in suspension flows about and through them as developing tube flows. The authors concluded that sharp-edged entry orifices caused a downstream flow constriction "with a dead water and turbulence zone between the tube wall and the narrowed flow," consequently "geometries with sharp edges must be eliminated or avoided in flow cytometric instrument design." [474]

For cell counts, Wallace's early experiments had led him to initially use ring jewels with a bore D of 100 μm and a length L of 75 μm between the orifices as sensing apertures in production Model A counters; for more than two decades, Coulter organizations had sold blood and particle analyzers incorporating ring jewels selected for sharp orifice edges as sensing apertures (Figure 4.7), and by the late 1980s some 50,000 Coulter instruments had been installed in the U.S. alone. To understand Wallace's experiments, ring jewels having a nominal aperture D of 100 μm were prepared in each of thirteen L/D ratios from 0.059 to 4.883 and inspected to standard quality specifications (Figure 4.7), with D and L measured to within 0.25 μm. The jewels were fused to sample tubes by standard practice, and those undamaged during fusing were tested for their volume throughflow rates.

To determine the volume throughflow rates for the experimental apertures, the 500-μl volume-control manometer of a Coulter Counter® Model ZM controlled both its counting circuits and a precision electronic timer while drawing latex beads suspended in isotonic saline solution through the apertures. As the mercury accelerated from rest in the manometer holding bulb (Figure 4.2), the saline wetted the horizontal aperture bores. Before the mercury reached the manometer's start electrode, the saline's liquid properties and the bore L/D ratios established the suspension's throughflow rates at constant values maintained until the mercury contacted the manometer's stop electrode; the suspension's volume throughflow rate was thus the ratio of 500 μl to the time *t* required for that volume to flow through the apertures. The volume flow coefficients for the apertures of each L/D ratio were obtained by normalizing the observed volume throughflow rates to the

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[473] Ibid. 49, Fig. 7 and col. 2.

[474] Volker Kachel, Hugo Fellner-Feldegg, and Everhard Menke, "Hydrodynamic properties of flow cytometry instruments," in Flow Cytometry and Sorting, 2nd ed., ed. Melamed, Lindmo, and Mendelsohn (New York: Wiley-Liss, 1990), 27-44; for the authors' approach, see 27, col. 1; for their conclusion, see 30, col. 2, continued on 31, col. 1.

theoretical volume throughflow rate for an ideal liquid through an ideal 100-μm aperture (Figure A12.1).

Contrary to Kachel's assumption that a constricted flow filling about 60% of the aperture's bore cross-section occurred behind sharp entry orifices, the volume flow coefficients approached those of 60% bore fills for only the experimental apertures having L/D ratios of 0.059 and 4.883. The largest such constricted fills, with a volume flow coefficient of approximately 75%, occurred for apertures having L/D ratios near 0.586. For those apertures, if wetting forces between the saline and the surfaces defining the sharp orifice withstood liquid inertial forces toward the bore axis all around that sharp orifice, the entry flow could remain attached into the wetted bore. However, if this fragile balance were perturbed by even a sub-μm defect, the entering flow could detach at the orifice defect. Such locally detaching flows could reattach and detach again, or interact with another such flow at another defect, to modify the distribution of aperture excitation current flowing in the suspension throughflow, so causing excessive noise in the bead signals.

For bore L/D ratios less than 0.586, at some point as the mercury moved toward the manometer's start electrode, liquid inertial forces toward the bore axis overcame the wetting forces between the saline and the surfaces defining the sharp entry orifice; flows detached at the orifice and formed constricted throughflows with volume flow coefficients that increased with increasing L/D. However, if effects of the short apertures on the voltage distribution established by their throughflows of electrical current could be accommodated by instrument settings, defect-free sharp entry orifices produced stable detached laminar throughflows that permitted bead counting with reduced volume sensitivity by conduits having L/D ratios somewhat below 0.586.

For bore L/D ratios greater than 0.586, entry flows remained attached on defect-free entry orifices, and viscous losses along the bore wall, rather than flow constrictions downstream of the orifice, produced decreasing volume coefficients with increasing L/D. For Coulter apertures having a D of 100 μm and standard quality under typical conditions of use, liquid wetting and viscous effects ignored in the two chapters enabled stable flow attachment downstream of sharp entry orifices, with repeatable counting and sizing of beads or blood cells at appropriate concentrations.

Equivalent volume flow coefficients on the two slopes of Figure A12.1 indicate similar suspension throughflow volume rates. However, due to the differing influences of liquid inertial and interfacial properties, such throughflows differ in their characteristics: The experimental apertures with bore L/D of 0.059 and 4.883 had Reynolds numbers *Re*

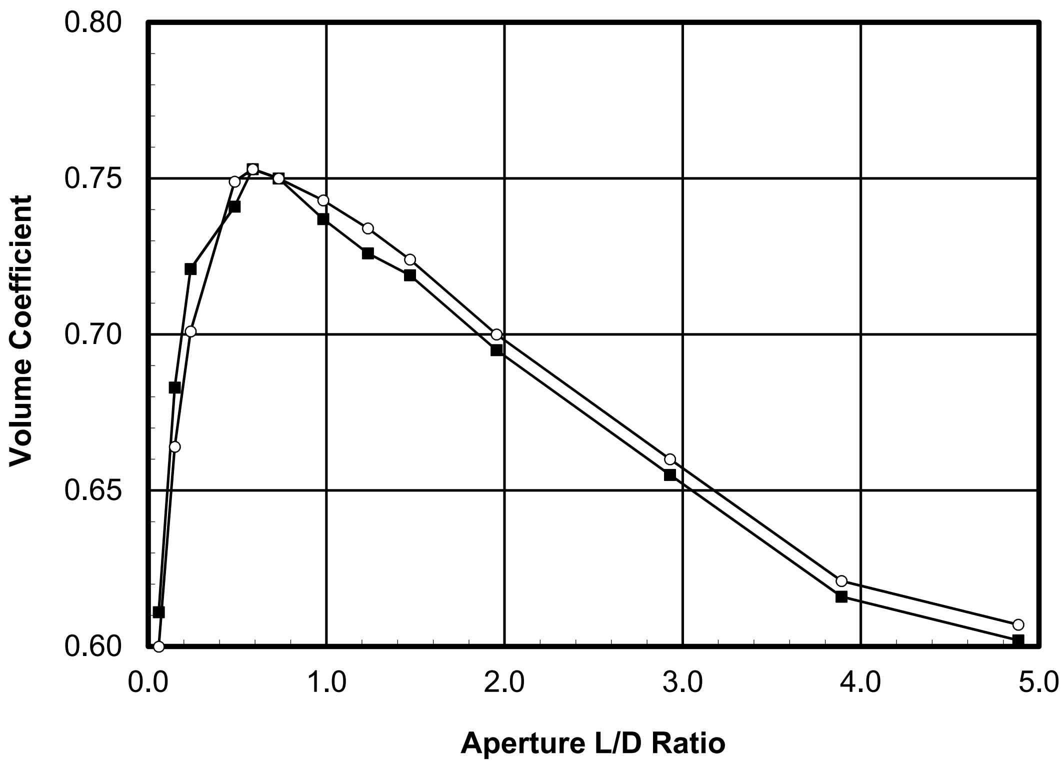

Figure A12.1. Volume flow coefficients for experimental 100-μm apertures.[475] The L/D axis corresponds to throughflow constrictions of 60% of the aperture cross-sectional area. Squares indicate results for an assumed aperture bore diameter of 100 μm, while circles indicate those results corrected for the measured aperture bore diameter D; each data point is the average of ten determinations. The differential in head across the apertures was 150 mm of mercury, with a maximum throughflow Reynolds number *Re* of 480 for L/D approximately 0.586.[476] This figure is based on volumetric flow rates presented elsewhere.[477]

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[475] Actual L/D ratios were 0.059, 0.149, 0.238, 0.485, 0.586, 0.732, 0.982, 1.233, 1.469, 1.955, 2.928, 3.891, and 4.883. The volume coefficients for L/D less than 0.586 are coefficients of constriction, whereas those for L/D greater than 0.586 are more accurately coefficients of discharge.

[476] The Reynolds number *Re* is the dimensionless ratio of the product of the diameter D of the aperture bore and the liquid's average throughflow velocity $\bar{u}$ to the liquid's flow resistance or kinematic viscosity ν.

[477] Marshall D. Graham, "Volumetric flow in 100-micra Coulter sensing conduits at 150 mmHg differential pressure," poster manuscript, Figure 3, XXI International Congress, International Society for Advancement of Cytometry, May 4-9, 2002, San Diego, CA; *Beckman Coulter Bulletin 9283; Abstracts from ISAC XXI International Congress, May 4-9, 2002, San Diego, CA* (Brea, CA: Beckman Coulter, Inc., 2002), 12-13.

of 386 and 385 respectively, yet the first aperture contained Kachel's constricted flow with surrounding vortices while the second contained attached laminar flow. The two aperture throughflows were not even remotely similar, let alone equivalent, in sensing functionality.

Why did members of a widely respected institution so badly miss the nature of liquid flows through sharp-edged orifices in practical sensing apertures? Kachel, Fellner-Feldegg, and Menke ignored not only the proven performance of the many Coulter instruments incorporating apertures with sharp-edged orifices, they also ignored sources describing the basic characteristics of liquid aperture throughflows and provided no experimental data supporting their throughflow assumptions. Moreover, images in those two chapters suggest that some of the non-laminar throughflows apparent therein resulted from edge and wall defects in the test structure, rather than from sharp entry orifices.

Those chapters first appeared while installed Coulter instruments were experiencing serious problems with ring-jewel sensing apertures. In the 1970s electronic watches progressively replaced mechanical ones, and Swiss sources for quality watch ring jewels began reducing production. This trend continued during the 1980s, with an accompanying decrease in the quality of available watch jewels, and repair of installed Coulter instruments and production of new ones became increasingly problematic. My visits to production facilities in 1983 found rising energy costs had combined with decreasing demand to prompt both cost-cutting changes throughout the production process and a growing disinterest in meeting the quality standards required for Coulter sensing apertures. Technical problems that had developed with Swiss watch jewels were resolved, but the problems caused by those two chapters proved less accommodating.

And why do those chapters matter? What seemed to be comprehensive treatments by authors from a respected academic institution made them appear definitive, and they have been repeatedly cited. Graduate students who had recently read those chapters have asked me at conferences whether there might not be better ways to meet hematological screening needs. Moreover, after reading those chapters some clinicians and laboratory technicians have questioned results obtained with Coulter hematology analyzers. How many people may have had unnecessary worries about their diagnosis induced by a doubtful student, clinician, or laboratory technician? Those two chapters matter because their obscure invalidity has made them a persistent source of confusion about clinically significant methods proven by decades of effective performance.

APPENDIX 13. Letter to Dr. Carl Mattern, February 22, 1955 [478]

Feb 22 1955

Dr. Carl Mattern
National Institute of Health

Dear Dr. Mattern:

Since my last notes to you I have had 1 or 2 thoughts about centrifuging to separate white cells. To reduce entrapment by reds, a large dilution and the use of a centrifuge which maintains the tubes at an angle, about 50 degrees I believe, instead of horizontally would reduce the distance and density of cross flow as reds go outward and downward after encountering the sloping wall and the whites come toward the axis and go upward after encountering the tube wall. This is a kind of flow control. Siliconed surfaces on plastic tubes would prevent loss of whites on the tube.[479]

The tube is half filled with total sample, blood and diluent. Next the upper half of the tube is carefully filled with diluent only taking care that mixing is at a minimum so that at least the upper 25% of the tube is free of red cells. A long tube is used and is filled so that when in the machine the top of the liquid is near the axis of the centrifuge. This provides a region of low centrifuge action wherein white cells will collect in depth without being packed. After centrifuging the upper 1/4th of the liquid is drawn away for a count.

Will it work?

On BLOOD, July 1952 page 693 reference is made to use of surface active agents to permit complete resuspension of platelets following prolonged centrifugation.[480] Perhaps such agents would help with whites also. They refer to

(To second page.)

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[478] Transcribed from a carbon copy of the handwritten original; WHC Papers.
[479] Here, “reds” refers to erythrocytes and “whites” to leukocytes.
[480] Minor and Burnett, “A method for separation and concentrating platelets.”

use of TRITON WR-1339, state it is non hemolytic and has low toxicity. Other references refer to Tween 80. They are called non-ionic detergents I believe.

I ran across a description of the Boston produced blood cell counter. Made by Jarrel-Ash Company it counts both reds and whites. States that instead of usual ±9% error of microscope count that they have a ±3%. This is the British idea of scanning optically a hemocytometer in two directions using two slits of different widths and subtracting the two to get an answer without edge un-certainties.[481] They *(sic)* answer is difference of 2 large quantities. A 3 to 1 improvements suggests that they may count a net of 5000 about. This would give √(5000/500) statistical improvement about, doing total counts in 2 minutes.[482]

I was slightly in error in my last letter in neglecting the count loss of the 50 micron data when referring to the very close agreement of the 100 micron data to previous data.[483] However except that it did not agree with my first "printed" data fixed to it is very consistent. As you recall I stated that my first "printed" data was partly guesswork. The following day I ran several dilutions carefully and rapidly, using the 100 micron aperture only which agrees very closely with the 100 micron data sent you. The 2 days data give 6 points all of which fall within 1/4% except 2 points which are off 1/2 and 2/3%.

The plot of count loss vs observed count is a straight line thru 20% and 50,000. The 50 micron curve is almost thru 95,000 and 3%. Does this check with your data?

That about covers it.

Yours truly

Wallace H. Coulter

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[481] Wolff, "An apparatus for counting small particles in random distribution," 967; Mattern, Brackett, and Olson noted this in their evaluation report, "The determination of number and size of particles," 57, col. 1, fn 6; Alan Richardson Jones confirmed the origin in, "Automatic instrumentation for hematology," *Annals of Clinical and Laboratory Science* 19 (1989), 77, col. 1.

[482] The numerical expression indicates the square root of 10, or 3.162.

[483] This paragraph does not conform to content of Wallace's February 5 letter (Figure 4.9), so there seems to have been another letter between it and this one.

APPENDIX 14. Robert H. Berg's "authorized reprint," 1958 [484]

According to the citation at the bottom of the cover page (Figure A14.1), this is an "Authorized Reprint from the Copyrighted, Symposium on Particle Size Measurement, *Special Technical Publication 234:* Published by the, American Society for Testing Materials, 1958. However, *Special Technical Publication 234* was not published until August 1959, and this version of Berg's paper differs in a number of respects from the official version (Table 6.2).

In the first text page, the highlighted words in the first column were originally "the Coulter" and "Coulter" has been omitted before "Principle" in the legend for Fig. 1 (Figure A14.2). In Figure A14.3, the highlighted words in the first column were originally "the first embodiment of the Coulter," and "Electric" has replaced the original "Coulter" in both Fig. 2 and its legend. As indicated in the legend for Figure A14.4, no changes or substitutions were noted on the third text page. However, in Berg's Figs. 5 and 7 "Particle" has been substituted for "Coulter" on the fourth page (Figure A14.5). Finally, no changes or substitutions were noted in the fifth page (Figure A14.6), but the Discussion on 256-258 of the published reprint is omitted.

The annotation at the bottom of the fifth page indicates that the exemplar shown here is a later reprinting of the "authorized reprint" done after Berg renamed Particle Data Laboratories, Inc., following his acquisition of Shepard Kinsman's majority interest in late August 1960.

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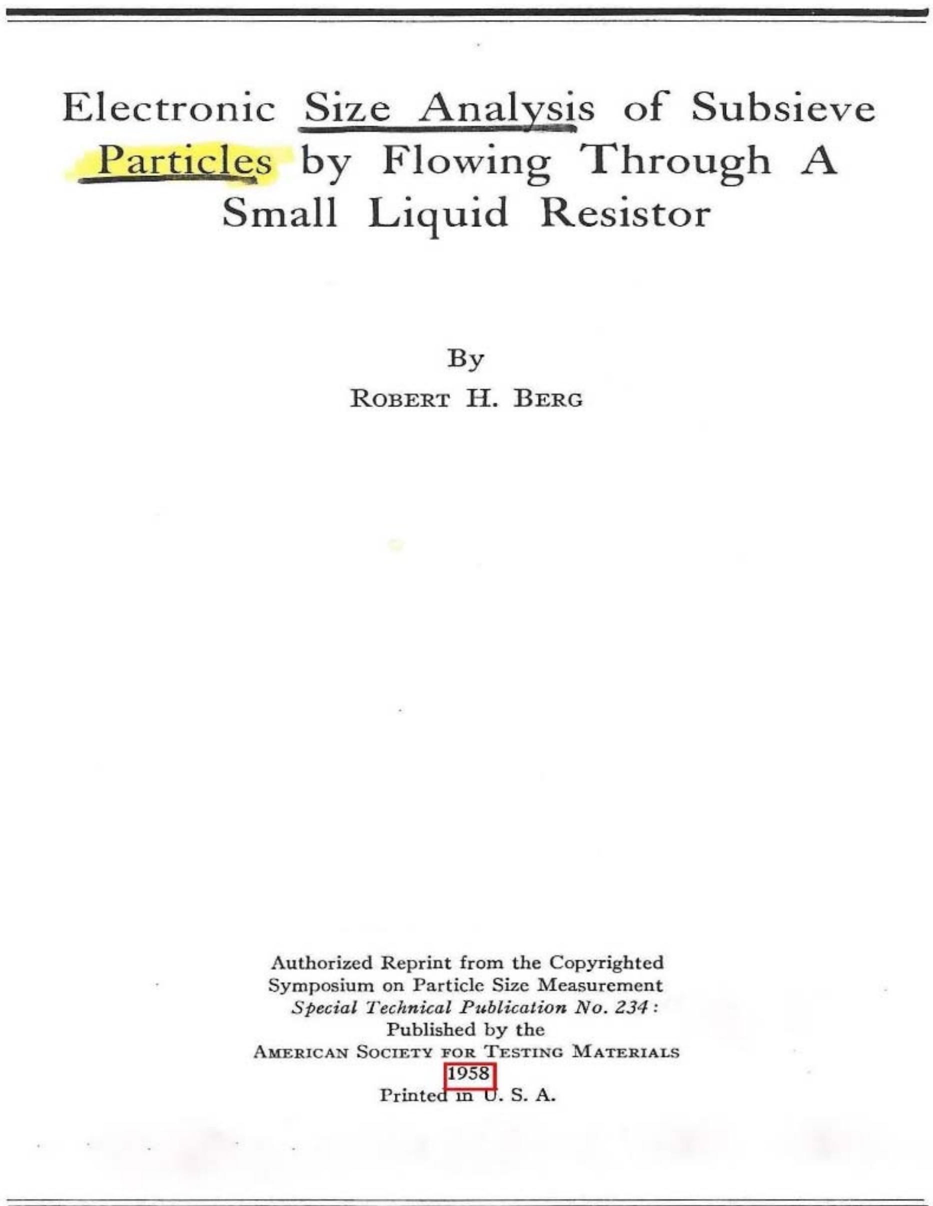
[484] Robert H. Berg, "Electronic size analysis of subsieve particles by flowing through a small liquid resistor" (U.S.A.: Berg, 1958); photocopy, WHC Papers.



Electronic Size Analysis of Subsieve Particles by Flowing Through A Small Liquid Resistor

By

ROBERT H. BERG

Authorized Reprint from the Copyrighted
Symposium on Particle Size Measurement
Special Technical Publication No. 234:
Published by the
AMERICAN SOCIETY FOR TESTING MATERIALS
1958
Printed in U. S. A.

Figure A14.1. Cover page of Berg's "authorized reprint." As indicated by highlighting, this version of Berg's paper differs in a number of respects from the official version (Table 6.2), including omission of the Discussion pages included in the official publication.[485]

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[485] Ibid.

ELECTRONIC SIZE ANALYSIS OF SUBSIEVE PARTICLES BY FLOWING THROUGH A SMALL LIQUID RESISTOR

By Robert H. Berg[1]

Synopsis

This paper describes basically a new principle of particle content and size analysis and reviews the principal theoretical points. The method is applicable to sizes ranging from below 0.6 μ to over 200 μ. A suspension of particles in conductive liquid flows through an aperture with simultaneous flow of electrical current, resulting in a series of electrical pulses, each pulse being proportional in magnitude to the volume of the particle causing it. The pulses are amplified, scaled, and counted to provide direct data for plotting cumulative particle frequency against particle size. Potentials and limitations are discussed, and methods of calibration, sample preparation, and data reduction are emphasized.

Particle-size distribution and concentration are important properties of countless powdered, slurried, or emulsified materials as well as of biological cells, fluid contaminants, and foodstuffs. In processes involving particulate materials, particle size is a critical factor in dynamic process control, in equipment evaluation, in product quality control, and in research and investigation. Present methods of particle-size measurement include microscope counting, sieving, adsorption and permeability, and a number of Stokesian methods. Although most of these methods have been automated to varying degrees in recent years with significant improvements in speed and accuracy, there is still need for instrumentation to reduce frequently inherent tedium, time delay, and error.

During the past ten years a basically new principle has been developed for particle-size analysis. It was first applied to blood cell counting about four years ago (1).[2] Investigations during the past year have shown this new method to be generally applicable to all forms of finely divided material.

Response Principle

The number and size of particles in an electrically conductive liquid are determined by application of a resistance principle. This principle consists of forcing the suspension to flow through a small aperture having an immersed electrode on each side, as shown in Fig. 1. As each particle passes through the aperture, it replaces its own volume of electrolyte within the aperture, momentarily changing the resistance value between the electrodes. This change produces a voltage pulse of short duration having a magnitude proportional to particle volume, and the resultant series of pulses is electronically amplified, scaled, and counted.

[1] Process Control Services Co., Elmhurst, Ill.

[2] The boldface numbers in parentheses refer to the list of references appended to this paper.

Voltage-pulse height is proportional to amplifier gain, aperture current, and resistance change due to particle passage ($\Delta E = G \times I \times \Delta R$). Expressing the particle in electrical effect as a right cylinder, aligned with the aperture axis and shorter than the aperture, it can be

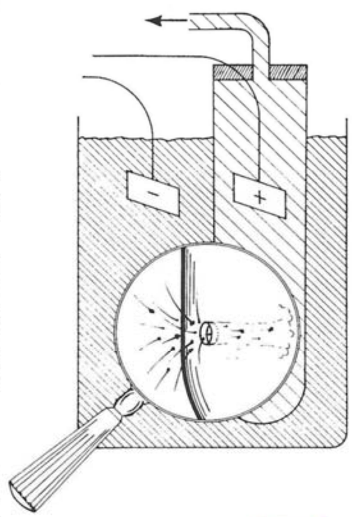


Fig. 1.—Basic Mechanism of Principle.

shown (2,3) that the change in aperture resistance caused by a particle is:

$$\Delta R = \frac{\rho_0 v}{A^2} \times \left(\frac{1}{1 - \rho_0/\rho} - \frac{a}{A}\right)^{-1} \ldots (1)$$

where:

ρ_0 = electrolyte resistivity,
A = aperture area normal to axis,
v = particle volume (see discussion),
ρ = effective particle resistivity, and
a = area normal to aperture axis of equivalent right cylinder for particle as oriented in passage.

Thus, for given electrical conditions and aperture size, response is essentially linear with particle volume, providing high sensitivity of size measurement. (When size is expressed as diameter, error is divided by 3.) Deviations are moderate and usually correctable, and measurement precision within 1 per cent on diameter basis has been commonly experienced.

Changes in electrolyte temperature change electrolyte resistivity by about 1 per cent per deg Fahr for common electrolytes at room temperature; correction is readily made if need be.

Particle resistivity has been found to be effectively many orders of magnitude greater than that of the electrolyte. Metal powders and other apparently good conductors behave like nonconductors. This is hypothesized as being due to oxide surface films and ionic inertia of the Helmholtz electrical double layer and associated solvent molecules at the surfaces of such particles. Electrical charges on the particles have otherwise no apparent effect on response.

Particle density does not affect response but, where gross particle porosity exists (as distinct from sealed internal voids), the pores aligned with the aperture axis may provide a degree of electrical translucency with proportionately lesser pulse height.

As indicated in Eq 1, deviation from linear volumetric response becomes appreciable for nearly spherical particles above 30 per cent of aperture diameter ($a/A \cong 0.09$). This effect is correctable if need be. It is markedly reduced for elongated particles, such as fibers, rods, and flakes, as the prevailing streamline flow in the aperture causes predominant alignment of such particles with the aperture axis.

Thus, it is seen that particle shape and structure have but little effect on response. However, it is recognized that the distortion of the electrical field in the

Figure A14.2. The first page of Berg's "authorized reprint." The highlighted words in the first column were originally "the Coulter" and "Coulter" has been omitted before "Principle" in the legend for Fig. 1. Wallace H. Coulter's NEC paper is cited in the second introductory paragraph, but merely as the first application of the Coulter Principle, not as the introduction of an innovative new instrument by its inventor.

aperture by a particle will conform to the essential surface of the particle rather than follow each crevice and wrinkle, thus sensing the "envelope volume" of the particle much as though it were wrapped in a thin film.

Size Range

Response correction is unnecessary for most particulate systems if the few largest particles do not exceed 40 to 50 per cent of the aperture diameter. This is also a practical maximum to avoid excessive aperture blockage due to coincidence of large particles. The noise limitations of electronic amplification prevent measurement below 1 to 2 per cent of aperture diameter. Thus, a single aperture size provides a diametric measurement range upwards of 20:1, with corresponding volumetric range upwards of 8000:1. This range covers most monocaused particulate systems. Range extension may be obtained by fractionating samples and using two or three aperture sizes.

The maximum size measurable by this method is governed by the ability to keep particles in uniform suspension, using agitation freely (Fig. 2). Aperture sizes now in use range from 10 to 1000 μ. Up to the present, thermal noise generated by electrical heating in the aperture has limited the minimum measurable size to about 0.3 μ. Resolution potential reaches somewhat lower since ionic dimensions are near 0.0002 μ.

Apparatus and Operation

A typical embodiment of the electric principle has been the general purpose laboratory instrument illustrated in Fig. 3, with schematic function as in Fig. 4. When the stopcock is opened, a controlled external vacuum initiates flow from the beaker through the aperture and unbalances the mercury manometer. Closing the stopcock then isolates the system from the external vacuum, and the siphoning action of the rebalancing mercury continues the sample flow.

The advancing mercury column activates the counter by means of fixed start and stop probes, thus providing a constant suspension volume for all counts. The volume between probes may be chosen in a range from 0.02 to 5 ml, and a fresh volume is drawn for each count. A typical count requires from 3 to 30 sec, depending on aperture size and manometer volume requirements.

The voltage pulses are amplified and fed to a threshold circuit having an adjustable screen-out voltage level, and if this level is reached or exceeded by a pulse, the pulse is registered. Thus, each count represents the number of particles larger than the selected threshold-size level in a given volume of suspension By taking a series of counts at various threshold settings, data are directly obtained for plotting cumulative particle frequency or concentration *versus* particle volume (or mass, given constant density).

The threshold level is indicated on an oscilloscope screen by brightening of the pulse segments above the threshold. (The pulse pattern also serves as a monitor for possible instrument malfunction.) The oscilloscope picture, counter cadence, and aperture-observation microscope serve as excellent monitors of aperture blockages which are usually remedied by lowering the beaker and wiping the aperture with a clean finger-tip or rubber-tipped rod.

Agitation of the beaker contents is used freely during measurement to maintain a uniform suspension and to assist thorough dispersion. The momentum of heavy particles may require aperture orientation toward the particle stream. Particles which might accumulate inside the aperture test tube can cause response-signal interference and may be flushed out periodically by means of an auxiliary internal inlet. Otherwise, the suspension

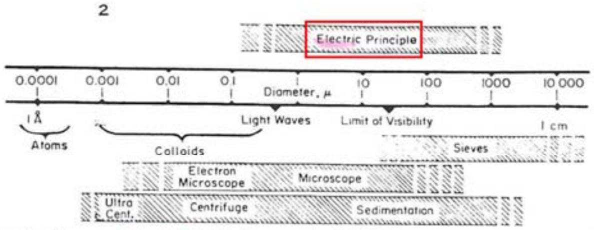


Fig. 2.—Size Range of Electric Method Compared with Coverage of Sieve, Sedimentation, and Microscope Methods, and Overlap of Electron Microscope and Centrifuge Ranges.

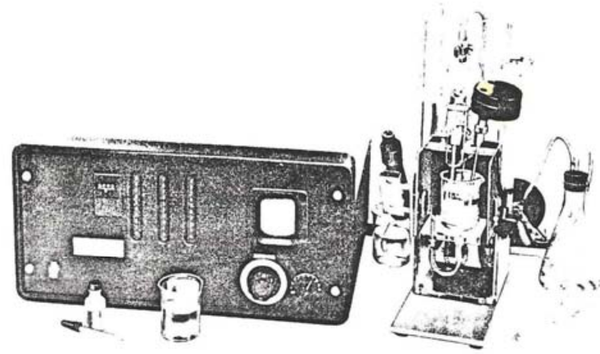


Fig. 3.—Electronic Unit and Sample Stand.

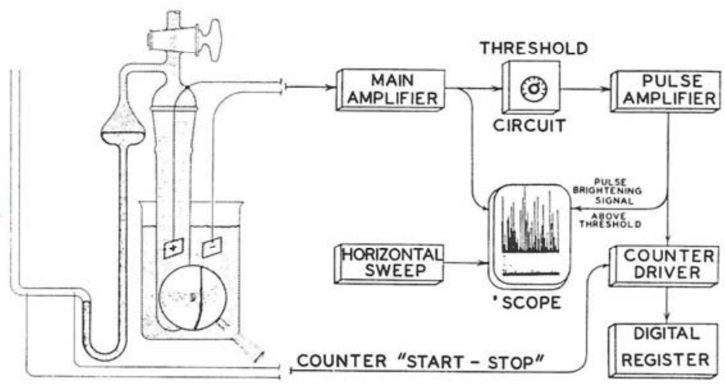


Fig. 4.—Schematic Diagram of Laboratory Model.

Figure A14.3. The second page of Berg's "authorized reprint." The highlighted words in the first column were originally "the first embodiment of the Coulter," and "Electric" has replaced the original "Coulter" in both Fig. 2 and its legend.

downstream of the aperture has no effect on measurement and need only be conductive. Thus, preparation for each successive sample merely involves rinsing the exterior surfaces which are immersed in the sample beaker with a wash bottle of clean electrolyte.

Sample Preparation

As in any analysis measurement, care must be taken that the sample presented to the sensing element is quantitatively representative, and the material from which the test portion is taken must first be thoroughly mixed. Dispersing agents are used as the need arises and do not affect measurement, provided concentrations are reproduced so that electrolyte strength remains constant. A few drops of dispersant solution may be premixed with the sample portion or may be added to the body of the electrolyte in the beaker. Within limits, additives for increasing electrolyte viscosity aid effective suspension of heavier particles. The low particle-concentrations used further aid dispersion and reduce the chances of coalescence or agglomeration.

Particle concentration may range from 1000 ppm to less than 1 ppm by volume and is governed principally by coincidence considerations and the count sizes desired for statistical protection. Variation in repeat counts is a function of counted number, the standard deviation in a number taken from a Poisson distribution being nearly equal to the square root of the number. The amount of repeat data taken may be adjusted accordingly, and sample dilution may be performed during the course of measurement to retain the statistical advantage of larger numbers in the low count-ranges.

For relative size distribution, sample dilution may be done approximately, without weighing, to satisfy coincidence limitations as described. However, where solid content of slurries or data on a significant weight fraction of material beyond the range of measurement are desired, sample weight (or slurry volume) and electrolyte volume used in sample preparation must be recorded.

Electrolyte resistivities generally range from 1 to 1000 ohm-cm, depending on aperture size, and are usually kept as high as noise levels permit to take full advantage of the effect on response as shown in Eq 1. For many purposes a 1 per cent sodium chloride solution in water, having a resistivity of about 55 ohm-cm at room temperature, is quite satisfactory.

Where the particulate material is water soluble or is already suspended in an oil or other such medium, nonaqueous electrolytes may be prepared with polar compounds such as alcohols and ketones, with the ions furnished by materials such as thiocyanates, quaternary ammonium salts, or strong acids. If particles are slowly soluble in a chosen electrolyte, the voltage pulses may be recorded on magnetic tape immediately after adding the sample and then analyzed for height distribution. Solution rates and other time phenomena may be studied in this manner.

Background counts on the blank electrolyte may be taken and correction made if need be. For most purposes, careful filtration and protection from dust contamination will provide negligible background counts. If filtered aqueous saline solutions are to be stored for long, it is well to add 0.1 per cent formaldehyde to prevent the growth of microorganisms.

Coincidence Effects

The primary effect of coincident particle passage is loss in count, and satisfactory numbers of particles may be counted while keeping coincidence corrections below 10 per cent. These corrections are known functions of aperture size and particle concentration and may be checked experimentally by counting a given suspension at successive dilutions. Mattern (4) has shown that this procedure confirms a Poisson distribution of particles within the suspension and that the electrically effective liquid-resistor volume is about three times that of the aperture cylinder.

As a secondary effect, coincident passages involving only particles smaller than threshold size may produce pulses reaching above the threshold level, causing a false increase in count. This effect will thus vary with the size range of the system being analyzed and with the point in the distribution at which measurement is taken. If coincidence-loss corrections are kept low, this secondary effect will usually be negligible, but it may be quite prominent for narrowly distributed systems near the frequency peak. Detection and remedy merely involve sample dilution to reduce coincidence levels.

A further factor in particle concentration involves frequency response and relaxation speeds of electronic components. Six- to seven-thousand random pulses per second may be handled with negligible loss, but when very small pulses are being measured in the presence of very large pulses, the tent-shaped bases of the large pulses may obscure a sizeable fraction of the smaller pulses. Again, the effect depends on the type of distribution and the point at which measurement is being taken. The need for additional sample dilution may be quickly determined by trial and may be indicated by a ragged appearance of the oscilloscope pattern.

Test Procedure

A typical size-analysis on a known material of roughly known size range is begun by placing in a 200-ml beaker about 175 ml of well-filtered electrolyte of suitable properties for the material at hand. The beaker is then placed on the sample stand under agitation, and 10 to 100 ppm by volume (estimated) of particulate material is added (in some cases premixed with a few drops of concentrated dispersant solution on a watch-glass). At times, it may be useful to check the blank electrolyte first for background count.

In the absence of prior experience with similar samples, it is well at first to observe the oscilloscope pattern briefly at various aperture current levels for suitability of choice of aperture size, amplifier gain, and particle concentration. (Any of these may require adjustment which involves a few minutes at most.) At the same time, the aperture-observation microscope is used periodically to check for any occurrence of aperture blockage. If need be, test counts may be taken to obtain indication of the distribution for guidance in taking data and to check for suspension stability (no particle solubility, agglomeration, etc.).

Counts are then taken beginning usually at the largest threshold-setting and progressing downward in successive size settings (single-threshold procedure). Replicate counts are taken as indicated by count size, that is 6 or 8 for counts of 10 or less, 3 or 4 for counts in the low hundreds, and 2 or 3 for counts in the thousands and higher. Satisfactory data result if the size-level settings are chosen such that the points are separated by no more than a factor of 2 to 4 in either particle volume or count. Points may be checked or additional size levels measured at any time, and sample dilution may be made (and noted) in the course of measurement, should coincidence levels so dictate.

The "end" of the particulate system is usually reached when the counts increase by only 10 to 20 per cent or less, each time the particle volume setting is halved. (The smallest particles form only a very small fraction of the total volume or mass of a system.) Thus, a range of 8000:1 in particle volume (20:1 diametrically) is covered by 40 to 50 counts among 12 to 15 size settings, requiring about 15 min. Less time is required for routine tests at a few points on similar

Figure A14.4. The third page of Berg's "authorized reprint." No changes or substitutions were noted.

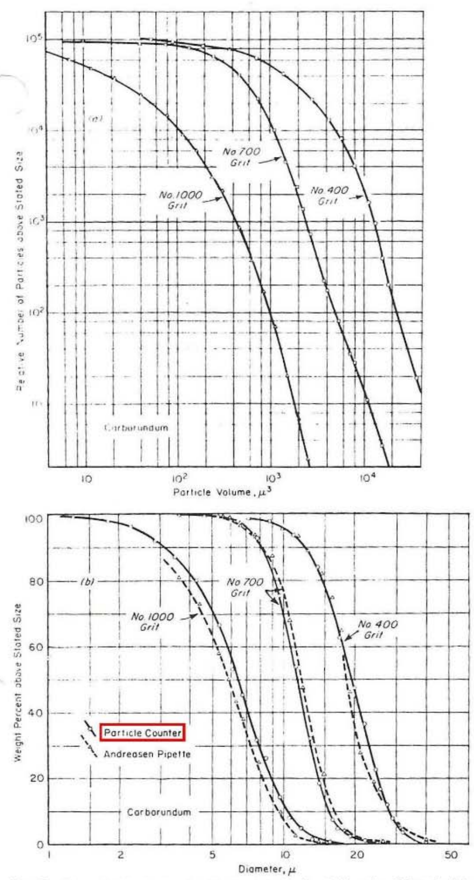


FIG. 5.—Narrowly Sized Abrasive Grades Suitable for Calibration, Plotted with Comparative Sedimentation Data.

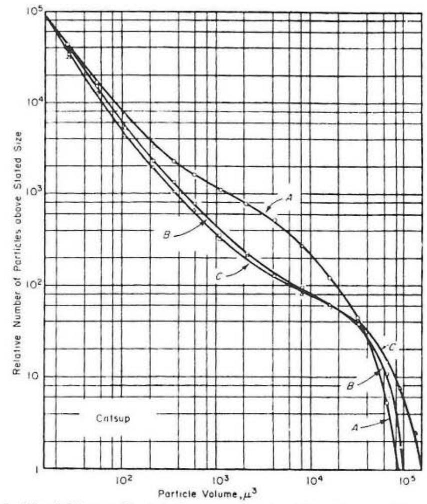


FIG. 6.—Bimodal System (Seeds, Pulp) in Log-Log Cumulative Frequency Plot. Curves B and C represent two totally different sources.

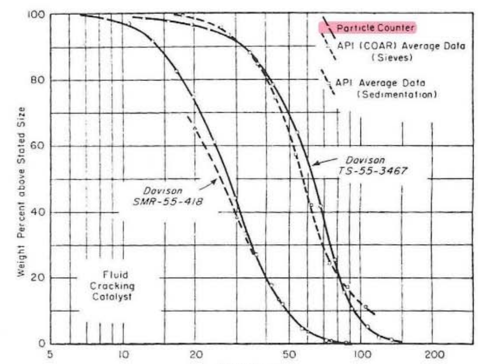
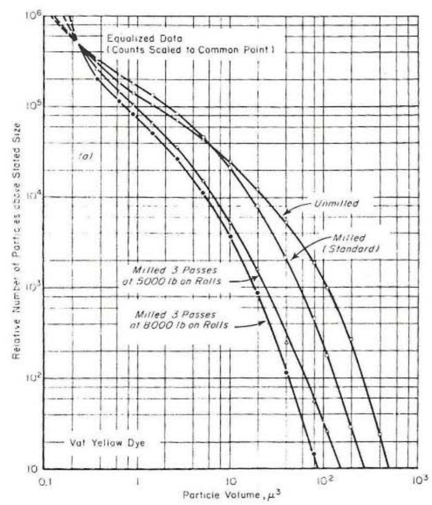


FIG. 7.—Cumulative Weight per Cent Plots of Fresh Catalyst Compared with Average Data of the American Petroleum Institute.



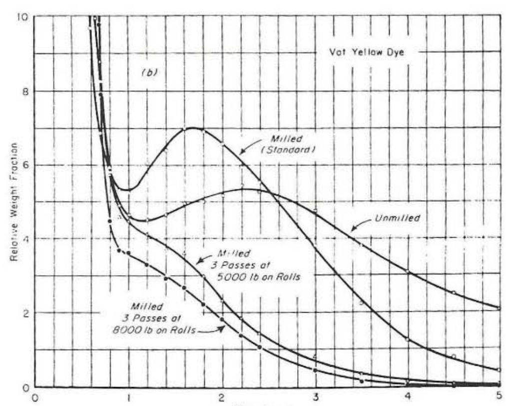


FIG. 8.—Cumulative Frequency (*a*) and Incremental Weight Plots (*b*) Illustrating Effect of Increased Degrees of Milling on Dyestuff.

Figure A14.5. The fourth page of Berg's "authorized reprint." In Figs. 5 and 7 "Particle" has been substituted for "Coulter."

samples, and more time (up to several hours) can be taken on systems requiring multiple aperture-sizes and in exhaustive research investigations.

Data Reduction

The numbers of particles counted range from 1 to 100,000 and more which, with the response range of over 8000:1, makes logarithmic presentation of direct data most effective if not mandatory. Examples are shown in Figs. 5(*a*), 6, and 8(*a*). The upper end of such curves becomes horizontally asymptotic in the region of the finest particles, and the lower end becomes vertically asymptotic in the region of the coarsest particles. With a little experience in interpretation, such curves are often adequate, and further data reduction is not required. This method of presentation is especially sensitive to minor percentages of large particles which is an important consideration in materials such as pigments and abrasives.

As may best suit a given material, data may be converted for plotting particle diameter or volume *versus* frequency, surface area, or weight on either the cumulative or the fractional basis, using linear or semilogarithmic paper. Data may also be presented on probability paper (linear or logarithmic). For the most part, process materials are reported as cumulative or fractional weight (volume) *versus* particle diameter. Examples of these are shown comparatively in Figs. 5(*b*), 7, and 8(*b*).

For various purposes, particularly in application to continuous measurement, data may be reduced automatically by suitable secondary pulse-analysis instrumentation. The more complex digital computers may perform virtually any type of mathematical operation on the pulse signals, but several useful forms of automatic data presentation may be achieved with comparatively simple circuitry. These forms of presentation include the use of multiple threshold-levels, pulse integrators, and rate meters to provide cumulative or incremental frequency rates, frequency ratios, and weight fractions or weight-fraction ratios.

Calibration

Monosized particles are available in a number of diameters and serve as a useful means for calibration. They must first be carefully measured by microscope. Materials used include polystyrene spheres in the range below 2 μ, and blood cells, spores, and pollens in the range from 5 to 80 μ. Rapid calibration may be made by observing the threshold level required to screen out the single-height pulses on the oscilloscope or by observing the threshold level at which half of the total system count is found (such systems being normally distributed). Calibration constants should be established for each combination of electrolyte and aperture size used.

Calibration may also be made by measuring a system of known particle density and narrow distribution. Narrowly classified fractions of glass beads or abrasives similar to those illustrated in Fig. 5 have been used. Since virtually all the particle volume in such systems may be accounted for, integration of the curve of cumulative frequency *versus* relative particle volume provides a relative measure of the total volume of particles in the metered suspension volume used in taking counts. The volume per cent of particles may be determined beforehand by measuring sample weight and electrolyte volume in sample preparation. The factor for converting threshold values to particle volume is thus:

$$F = \frac{(v_s/V_s)(W_p/\rho_p)}{\Sigma(\Delta n)\bar{t}} \quad \ldots\ldots\ldots (2)$$

where:

v_s = metered suspension volume,
V_s = total suspension volume,
W_p = total weight of particles,
ρ_p = particle density,
Δn = integration count increment, and
$\bar{t}$ = arithmetic average threshold value in a given count increment.

Conclusion

Results obtained by this method check closely with those of other methods, as shown in Figs. 5 and 7. There is substantial independence of particle shape, and particle properties other than volume have no influence except for very rare cases of resistivity or porosity. Breadth of measurement range compares favorably with that of any other single method, although the lower size limit does not reach as far as with the use of the centrifuge and the electron microscope. The method has high sensitivity and speed, general applicability, simplicity of sample preparation and data reduction, and low human fatigue element.

References

(1) W. H. Coulter, "High Speed Automatic Blood Cell Counter and Cell Size Analyzer," paper presented at the National Electronics Conference, Chicago, Ill. (1956).

(2) "Theory of the Coulter Counter," Coulter Industrial Sales Co., Elmhurst, Ill. (1957).

(3) H. E. Kubitschek, "Electronic Counting and Sizing of Bacteria," *Nature*, Vol. 182, pp. 234–235, July 26, 1958.

(4) C. F. T. Mattern, F. S. Brackett, and B. J Olson, "The Determination of Number and Size of Particles by Electronic Gating," *Journal of Applied Physiology*, No. 10(1), Jan., 1957.

Compliments Of

Particle Data, Inc.

P. O. BOX 265 ELMHURST, ILLINOIS

Figure A14.6. The fifth page of Berg's "authorized reprint." No changes or substitutions were noted. The annotation at the bottom indicates that this is a later reprinting done after Berg renamed Particle Data Laboratories, Inc., following his acquisition of Shepard Kinsman's majority interest in late August 1960.

APPENDIX 15. The Long Pendency of DuPage County Case 1-61-141

CEI's complaint against Robert H. Berg and his companies in DuPage County Case 1-61-141 proved to be just the first of many convoluted filings while his continued infringing activities prompted a number of additional court cases. Consequent court records indicate that in June 1964 Berg contacted Lars Ljungberg regarding distribution of Celloscope counters and that on June 12 he implemented Ljungberg's invalidation conjecture by originating a court action to have Wallace's U.S. Patent 2,656,508 on the Coulter Principle declared invalid and non-infringed by such activities (Table A15.1, Case 64c1032a), following which Ljungberg attended a trade show at Chicago's Palmer House Hotel where Berg displayed Celloscope counters. Learning of the exhibition, the Coulter brothers on July 1 had CEI file a claim (Table A15.1, Case 64c1148) that Ljungberg and his Celloscope infringed both U.S. Patent 2,656,508 and their U.S. Patent 2,869,078 on the volume-control manometer. They also attempted to have a summons served on Ljungberg at the hotel, but he had fled the premises, whereupon Berg met with him in Stockholm and reached an agreement making PDLI a Celloscope distributor.[486] In response to Berg's Case 64c1032a, CEI filed three counterclaims against PDLI (Table A15.1, Case 64c1032b); the first that Berg's sales of rebuilt second-hand Model A counters infringed three U.S. patents assigned to CEI and the second that those sales without removing CEI's registered trademarks infringed those trademarks.[487] Intended to encourage progress in DuPage County Case 1-61-141, CEI's third counterclaim concerned unfair competition and breach of the CISC franchise agreements by both PDLI and Berg.

[486] Herbert J. Singer and Anthony R. Chiara, *Coulter Electronics Inc. v. A. B. Lars Ljungberg & Co., U.S. Supreme Court Transcript of Record with Supporting Pleadings, October 1967 Session* (LaVergne, TN: MOML Print Editions, 2012); "Sale Agreement (Defendant's Exhibit A)," in Myron C. Cass and I. Irving Silverman, Appendix to Brief for Plaintiff-Appellant, Coulter Electronics, Inc., vs. A. B. Lars Ljungberg & Co., Docket No. 15895, United States Court of Appeals for the Seventh Circuit, 1967, 16-21.

[487] "SN 62,272, Coulter Counter," *Official Gazette* 740 (Mar. 17, 1959): TM 105; "679,591, Coulter Counter," ibid. 743 (Jun. 2, 1959): TM 37.

Table A15.1. Court cases. CEI filed DuPage County Case 1-61-141 January 20, 1961; dismissed May 12, 1970. Berg filed Case 64c1032a to have the U.S. patent on the Coulter Principle declared invalid and non-infringed by PDLI's selling rebuilt Model A and Celloscope counters. CEI filed all other cases because of Berg's infringing activities.

Here, "V and I" indicate the patent on the Coulter Principle was either found to be both valid and infringed or agreed to be so if preceded by "C," which indicates a consent decree; "D" and "Stricken" indicates the case was dismissed without prejudice except for Case 64c1148.

Court Case #	State and Date filed	Coulter Principle [488]	Volume control [489]	Aperture tube [490]	Date of decision
64c1032a	IL 06/12/64	V and I			05/06/70 [491]
64c1148	IL 07/01/64	D; no venue	D; no venue		06/23/66 [492]
64c1032b	IL 11/11/64	V and I	D	D	05/06/70 [493]
65c960	IL 06/11/65	Stricken	Stricken		09/12/68 [494]
65c1150	IL 07/09/65	C; V and I	D	D	05/07/70 [495]
65c272(3)	MO 08/04/65	D	D	D	08/09/66 [496]
66c2256	IL 12/07/66	D	D	D	06/30/67 [497]
66-579	OR 12/13/66 [498]	Shelved	Shelved	Shelved	
46903	CA 04/19/67 [499]	Shelved	Shelved	Shelved	
IH67c298	IN 08/13/67 [500]	Shelved	Shelved	Shelved	
67c1530	IL 09/06/67	C; V and I	D	D	05/12/70 [501]
67c3419	NY 09/06/67	C	D	D	08/31/70 [502]
10-103	ME 07/12/68 [503]	Shelved	Shelved	Shelved	

488 Coulter, U.S. Patent 2,656,508; term expired on Oct. 20, 1970.

489 Coulter and Coulter, U.S. Patent 2,869,078.

490 Coulter, Berg, and Heuschkel, U.S. Patent 2,985,830.

491 "2,656,508," *Official Gazette* 809 (Dec. 22, 1964): 1002 and 876 (Jul. 28, 1970): 813.

492 "2,656,508," *Official Gazette* 809 (Dec. 22, 1964): 1003 and 830 (Sep. 27, 1966): 1330.

493 "2,656,508," *Official Gazette* 814 (May 11, 1965): 370 and 876 (Jul. 28, 1970): 813.

494 "2,656,508," *Official Gazette* 819 (Oct. 26, 1965): 1388 and TM 142, and 859 (Feb. 18, 1969): 1022.

495 "2,656,508," *Official Gazette* 819 (Oct. 26, 1965): 1388 and 876 (Jul. 28, 1970): 813.

496 "2,656,508," *Official Gazette* 819 (Oct. 26, 1965): 1388 and 839 (Jun. 20, 1967): 841.

497 "2,656,508," *Official Gazette* 836 (Mar. 21, 1967): 786 and 843 (Oct. 17, 1967): 783.

498 "2,656,508," *Official Gazette* 836 (Mar. 21, 1967): 786.

499 "2,656,508," *Official Gazette* 840 (Jul. 25, 1967): 1061.

500 "2,656,508," *Official Gazette* 844 (Nov. 14, 1967): 404.

501 "2,656,508," *Official Gazette* 845 (Dec. 5, 1967): 20 and 876 (Jul. 28, 1970): 813.

502 "2,656,508," *Official Gazette* 846 (Jan. 23, 1968): 1033 and 881 (Dec. 15, 1970): 868.

503 "2,656,508," *Official Gazette* 857 (Dec. 17, 1968): 692.

The seven Illinois cases were filed in District Court, Northern District, Chicago, where all but Case 65c960 were decided. Only two of the six cases filed in other states were prosecuted to a decision, the others being shelved against future infringement.

Case 65c960 was against both Lars Ljungberg individually and his company A. B. Lars Ljungberg & Co. regarding Celloscope counters, but neither Ljungberg nor his company had a place of business in the U.S.; on June 23, 1966, Case 64c1148 was dismissed for lack of venue. CEI appealed (Docket No. 15895), but on May 9, 1967, the dismissal was affirmed.[504] CEI then petitioned the U.S. Supreme Court for a review of that decision, but on October 9, 1967, the petition was denied.[505] Then on November 21, 1968, PDLI's argument that CEI was trying to have its third counterclaim in Case 64c1032b adjudged in two courts caused it to be dismissed, with the court finding that CEI's counterclaims should be addressed by resolving the still-pending DuPage County Case 1-61-141. CEI appealed the dismissal, but on December 30, 1969, it would be affirmed.[506] Meanwhile, Berg had continued filing documents in DuPage County Case 1-61-141, and CEI would file ten more infringement lawsuits, two against Berg's distributors [Table A15.1, Cases 65c1150 and 65c272(3)] and the others against purchasers of the counters being distributed. Most made secondary infringement claims regarding the volume-control manometer and the aperture tube with fused watch jewel (U.S. Patent 2,985,830), but if a defendant were willing to stop its infringing activity, infringement of the latter two patents was not actively pursued (Table A15.1).

Case 64c1032a had begun with PDLI's request for a declaratory judgement that Wallace's U.S. Patent 2,656,508 on the Coulter Principle was invalid and non-infringed by Berg's competitive activities. On May 6, 1970, the court found this patent to be valid and infringed and permanently enjoined PDLI from such activities; Cases 65c1150 and

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504 "Coulter Electronics, Inc. v. A. B. Lars Ljungberg & Co.," *Federal Reporter* 376, 2nd series (1967): 743-46, website accessed January 23, 2020, as 376 F.2d 743 (1967).

505 Singer and Chiara, *Coulter Electronics Inc. v. A. B. Lars Ljungberg & Co., U.S. Supreme Court Transcript of Record with Supporting Pleadings, October 1967 Session*; the decision for Case 64c1148 appears at pages 1a-2a, and that for Docket No. 15895 appears at pages 3a-8a. For the October 9, 1967, decision, see "No. 443," *Journal of the Supreme Court of the United States* (1967): 37, website accessed January 23, 2020.

506 "Particle Data Laboratories, Inc., vs. Coulter Electronics, Inc., Docket No. 17442, United States Court of Appeals for the Seventh Circuit, 1969," *Federal Reporter* 420, 2nd series (1969): 1174-79, website accessed January 28, 2020, as 420 F.2d 1174 (1969).

67c1530 were ended by consent decrees to the same effect.[507] Based on this decision, DuPage County Case 1-61-141 was dismissed on May 12, 1970, and that August 31 a consent decree ended Case 67c3419. By then Wallace's U.S. Patent 2,656,508 was nearing its expiry date of October 20, 1970, and there was little practical value in pursuing further court decisions; the four remaining cases were simply shelved (Table A15.1).

After some nine years and seven months, the expensive distraction originating in Berg's breach of CISC franchise agreements meandered to a conclusion. As summarized in Table A15.2, the lengthy pendency of the DuPage County case had enabled him to protect intellectual property closely related to what he had learned during tenure of those franchise agreements. On August 16, 1966, U.S. Patent 3,266,526 on PDLI's "Peri-Lok" aperture tube had issued; the patent allowed Berg to advertise his method as simpler than those of the two CEI patents on which he was named a co-inventor. On August 4, 1970, the Patent Office approved PDLI's "ElectroZone" trademark.[508] Thereafter, Berg filed for five more U.S. patents and to register "Celloscope" as a PDLI trademark. Like his "Peri-Lok" patent, two of the resulting U.S. Patents, 3,502,972 and 3,554,037, were alternatives to two CEI patents suited for industrial processes. When Berg was rebuilding second-hand Model A counters, he became very familiar with their internal glassware; two other U.S. Patents (3,481,202 and 3,523,546) involved modifications of the Coulters' volume-control manometer (U.S. Patent 2,869,078). These two patents and his U.S. Patent 3,345,502 on pulse-analysis apparatus became important once he began modifying Celloscope counters obtained from A. B. Lars Ljungberg & Co. Other U.S. patents and registration of "Elzone," a form of the "ElectroZone" trademark, would result.[509]

With the expiration October 20, 1970, of the term of U.S. 2,656,508, Berg could freely compete with CEI by building or selling implementations of the Coulter Principle that did not infringe CEI's other U.S. patents, and he used this intellectual property to do so.

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[507] "2,656,508," *Official Gazette* 876 (July 28, 1970): 813.

[508] Robert H. Berg, "At Last! economy plus proven performance," *Lab World* 16 (July 1965); 629; "Particle Counter," ibid. 656 (the two CEI patents were 2,985,830 and 3,122,431); "896,151, ElectroZone," *Official Gazette* 877 (Aug. 4, 1970): TM 36.

[509] "SN 166,883, Elzone," *Official Gazette* 1003 (Feb. 3, 1981): TM 25; "1,152,196, Elzone," ibid. 1005 (Apr. 28, 1981): TM 573.

Table A15.2. Berg's accumulation of intellectual property. Applications for protection of the property listed below were made during the pendency of DuPage County Case 1-61-141; the relationship with CEI's existing intellectual property is explained in the footnotes. A number of other applications were made after this case was dismissed May 12, 1970.

Item	Type	Subject	Filed	Issued
ElectroZone	Trademark	Coulter sensing zone.[510]	07/26/61	08/04/70
3,266,526	Patent [511]	Fusing method for aperture disk.	11/26/62	08/16/66
3,345,502	Patent [512]	Pulse analyzing computer.	08/14/64	10/03/67
3,502,972 [513]	Patent [514]	Continuous flow particle analyzer.	03/08/65	03/24/70
Celloscope	Trademark	Ljungberg's particle counter.[515]	04/11/66	03/19/68
3,481,202	Patent [516]	Volume control manometer.	09/27/67	12/02/69
3,554,037	Patent [517]	Continuous flow sampling setup.	05/09/68	01/12/71
3,523,546	Patent [518]	Flushing the control manometer.	07/30/68	08/11/70

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[510] "SN 124,750, ElectroZone," *Official Gazette* 779 (Jun. 19, 1962): TM 121; "896,151, ElectroZone," ibid. 877 (Aug. 4, 1970): TM 36; "ElectroZone – Trademark Details," website accessed December 28, 2019.

[511] Robert H. Berg, "Peripherally locked and sealed orifice disk and method," U.S. Patent 3,266,526, filed Nov. 26, 1962 and issued Aug. 16, 1966. This was an alternative to Coulter, Berg, and Heuschkel, U.S. Patent 2,985,830 and Coulter, Heuschkel, and Berg, U.S. Patent 3,122,431, both of which were assigned to CEI.

[512] Robert H. Berg and Carl Arthur Youngdahl, "Pulse amplifier computer," U.S. Patent 3,345,502, filed Aug. 14, 1964 and issued Oct. 3, 1967. The Coulters attributed this patent on transistorized pulse-analysis circuitry to Berg's awareness of their developing the transistorized Model C counter with its simultaneous multi-bin capability.

[513] Both this patent and 3,523,546 below are related to: Joseph R. Coulter, Jr., "Flow-through sample apparatus for use with electrical particle study device," U.S. Patent 3,340,470, filed Sep. 23, 1964 and issued Sep. 5, 1967 and U.S. Patent 3,340,471, filed Jun. 14, 1962 and issued Sep. 5, 1967.

[514] Robert H. Berg, "Continuous flow particle size analyzer apparatus having suspension level maintaining means, U.S. Patent 3,502,972, filed Mar. 8, 1965 and issued Mar. 24, 1970. This is related to U.S. Patents 3,340,470 and 3,340,471, both assigned to CEI.

[515] "SN 243,164, Celloscope," *Official Gazette* 848 (Mar. 19, 1968): TM 120; "850,186, Celloscope," ibid. 851 (Jun. 4, 1968): TM 44.

[516] Robert H. Berg, "Metering siphon construction," U.S. Patent 3,481,202, filed Sep. 27, 1967 and issued Dec. 2, 1969. This is a modification of the volume-control manometer invented by the Coulter brothers, U.S. Patent 2,869,078, assigned to CEI.

[517] Robert H. Berg, "Continuous flow pipeline sampling orifice arrangement," U.S. Patent 3,554,037, filed May 9, 1968 and issued Jan. 12, 1971. This is also related to U.S. Patents 3,340,470 and 3,340,471, both assigned to CEI.

[518] Robert H. Berg, "Flushing means for fluid metering apparatus and the like," U.S. Patent 3,523,546, filed Jul. 30, 1968 and issued Aug. 11, 1970. This is a modification of the sample-handling glassware used in the Coulter Counter® Model A.

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