Semi-Annual Progress Report

NASA Grant NsG 441-63

Title of Project: Integrated Research and Training in Space-Molecular Biology

Period of Project: April 1, 1966 through September 30, 1966

Institution: The University of Chicago

Principal Investigator: Humberto Fernández-Morán, M.D., Ph.D.
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Our activities during the past six-month period have been carried out according to the program set forth in our renewal proposal for Grant NsG 441-63 (April 1, 1966 to December 31, 1966) and include:

I. Specific Research Program.

A. We have continued a comprehensive research program on electron microscopy with high-field superconducting solenoid lenses. As a result of the first successful experiments we became aware of numerous methodological and conceptual problems. The major difficulties included: (a) incorporation of superconducting solenoid lenses and associated cryogenic components into high performance electron microscope systems (b) precise control and reproducible current settability for focusing superconducting solenoid lenses (c) satisfactory specimen mounting to prevent temperature drift and achieve high degree of stability during irradiation (d) stabilization of lens excitation currents and accelerating voltage, and improved electron source characteristics for low-temperature microscopy (e) reduction of magnetic, electrical and mechanical perturbations under carefully controlled conditions in the requisite cryogenic environment (f) adequate continuous recording of images without breaking high vacuum under cryogenic conditions.

A major development and research program which has been in progress at our laboratory during the past few years has recently enabled us to successfully meet these requirements. As described in our paper entitled "High Resolution Electron Microscopy with Superconducting Lenses at Liquid Helium Temperatures" and published in Proceedings of the National Academy of Sciences, 56,3 (September 1966), p. 801, our research program involves two approaches:

1. Cryo-electron microscope optical bench system using high-field superconducting niobium-zirconium solenoid lenses in liquid helium Dewars, operating at 4 to 32 kG without pole pieces, and with modified objective, and objective-projector pole pieces. An additional current vernier control circuit for the superconducting objective solenoid is used in conjunction with a 25 A regulated power supply to permit adjustable current changes of 10^{-9} for achieving reproducible "superfine" focusing, orders of magnitude better than conventional systems.

   Improved point cathode sources and highly stabilized 50 kV accelerating potential were used, taking special precautions to minimize mechanical, magnetic and electrical field perturbations. Once correct focus of test specimens at electron optical magnifications of 200x-20,000x is attained, the superconducting solenoid system is switched into persistent current mode.

   The high quality images (50-100Å resolution) thus maintained without any external lens current source are of an unprecedented degree of stability, permitting exposures of up to several minutes with low intensity illumination for direct photographic recording on high resolution film. The same area can be continuously recorded at 5-15 minutes intervals over a 10 hour period under carefully controlled conditions without detectable image-changes, demonstrating typical long-term superstability. Combination of this unique stability of superconducting lenses with coherent microbeam illumination appears promising for practical realization of Gabor's wavefront reconstruction microscopy.

2. Special superconducting objective lens in liquid helium cryostat which may be used as an integral part of cryo-electron microscopes or replace the objective lens in modified high resolution commercial electron microscopes. The superconducting objective lens in a special Dewar designed and built to our specifications by Westinghouse Co., comprises a main Niobium-Zirconium coil (27,220 ampere-turns) with vernier coil, superconducting a stigmators, persistent current switches and improved current control devices.

Pointed filaments are made of etched, oriented single-crystal tungsten tips, spot-welded on zone-refined tungsten wire. Because of the high purity and selected crystal orientation these filaments have longer average life and operational stability than standard or sharpened hairpin cathodes. This also reduces specimen damage by energetic ions commonly originating from impurities in normal tungsten sources.

Similar pointed filaments of high purity tantalum, rhenium and niobium have specific properties particularly useful for low-temperature microscopy. The best results were consistently obtained with new types of etched molybdenum or stainless steel caps, the key element of which is a replaceable disc aperture of thin (10-30μ) molybdenum foil with a precisely centered small hole of 0.5mm. The Mb foil is stable under electron bombardment which retards contamination and prolongs operational life of filament tips.

Thin film configurations permit critical centering and height adjustment of filament tips determining adequate bias voltage and emission control. This practical cap design is applicable to both cathode and anode structures, combining optimum micro-geometry and operational conditions for obtaining space-charge-free emission and significant field enhancement.

When used with compensated double condenser systems of high resolution instruments (Siemens Elmiskop I, Hitachi Hu-11) these point sources provide improved microbeam illumination with high specific brightness and high coherence. Enhanced contrast and improved image quality, especially when dealing with biological specimens, is also achieved.

2. With short focal length objective lenses (f=1.8mm) of improved stability (and with circuitry for the Elmiskop developed by H. Armbruster) we confirmed and extended the results of R. Heidenreich by recording phase contrast high resolution images of carbon atom arrays (hexagonal cells of 5 Å) in ultrathin (10-20Å) single-crystal graphite specially prepared by a technique that we described previously. Other pauciatomic structural patterns of organic systems were resolved under similar conditions.

Specimens are mounted on microstages of special design maintained at 4.2°K together with pole pieces of different types including short focal length, single field condenser-objective pole pieces of iron or dysprosium, and trapped-flux Nb_3Sn lenses.

Our cryo-electron microscope is mounted on a 10-ton vibration isolated base and features extensive magnetic shielding, ultrahigh vacuum ion pump system, improved field and T-F emission source, image intensifier and photgraphic recording.

It is primarily designed for high resolution electron microscopy of biological specimens, examined preferably in the frozen hydrated state under ideal low-temperature conditions of minimized specimen contamination, radiation damage and thermal noise.

This work was presented at the Sixth International Congress for Electron Microscopy in Kyoto, Japan September 1966. We learned here that Prof. DuPouy, Laboratoire d'Optique du C.N.R.S. at Toulouse, considered our achievement noteworthy and emphasized the value of superconducting solenoid lenses for electron microscopy because of their unique stability, the unprecedented high fields, and the possibility of now being able to consider other types of magnetic field configuration for obtaining much higher performance in high resolution electron microscopes.

Our work was verified by the Japanese group of Dr. Watanabe and colleagues. In addition, Dr. A. Laberrigue, College de France in Paris, confirmed our work and expressed his view that a new chapter has been opened up in electron optics with super-conducting lenses which should prove to be of particular significance to high voltage electron microscopy.

B. Other instrumentation improvements include:

1. Extending the earlier work of T. Hibi, K. Yada, S. Takahashi, S. Maruse, Y. Sakaki, and our laboratory, improved point cathode sources have been developed and used routinely in high resolution microscopes.

High resolution phase contrast was also achieved by precise alignment of multi-hole or annular condenser apertures and objective phase plates of composite ultrathin single-crystal films (graphite, silver, mica) with adjustable electrical fields, or ferromagnetic thin-film apertures permitting phase shift control

C. Our continued experimentation with preparation techniques included:

1. Systematic use of ultrathin carbon films (10-20Å) prepared by evaporation in ultrahigh vacuum (10^{-8}torr) on special supports. Even thinner lamellae (10Å) of single-crystal graphite and diamond have been used which are extremely smooth and stable under intense irradiation, and can be used for high resolution shadow casting of DNA with carbon. These single-crystal substrates may be ideal atomic monolayer substrates for high resolution electron microscopy.

2. Specimens were mounted without background support on asbestos filaments or related substrates. DNA strands were also mounted on thin single-crystal mica with regular holes (80-100Å ∅) produced by fission tracks.

3. Following earlier work, wet or hydrated biological specimens were examined by using special vacuum-tight microchambers, low intensity microbeam illumination, and cryogenic devices to minimize dessication and radiation damage.

4. Thin frozen sections of native, unfixed tissues can also be examined directly after ultra-thin sectioning (20-100Å) with a diamond knife in microtomes operating in liquid nitrogen or helium cryostats, and transferred without thawing to the low temperature electron microscope stage.

D. We have planned a follow-up of the Preliminary Examination by Electron Microscopy and Electron Diffraction of Materials Collected by the Luster Sounding Rocket Experiment on November 16, 1965.

We are participating in the next Luster flight which is scheduled for launch on October 21, 1966 during the peak of the Orionid's meteor shower. Mr. Lou Sherman, a graduate student in the Biophysics Department, personally carried the specimens to Ames

Research Center and plans to collaborate with us in the investigation.

Three special high vacuum containers and specimen assemblies were designed and constructed to transfer the sampling surfaces. Two units will serve as controls and one will be carried on-flight. The several slides provide different supporting substrates for the specimens to be collected. Some contain several hundred platinum specimen holders coated with different types of thin film substrates, some are of freshly-cleaved single-crystal mica, and some of lucite.

As in the previous experiment, we made systematic efforts to conform to rigorous requirements for contamination control. On the basis of the previous investigation, we plan to carry out by electron microscopy and electron diffraction a comparative analysis of the new specimens, keeping in mind the possible interpretations set forth in our Preliminary Report in the Annual Progress Report for period April 1, 1965 to March 31, 1966.

II. Continued Maintenance of the Special Electron Microscope Laboratories with Clean Room Facilities:

With funds provided by The University of Chicago, NASA Grant NsG 441-63, NIH Grants B-2460, NB-04267, Gm-13243, and AEC Grants AT 30-1-2278 and AT 11-1 1344, a special laboratory facility for high resolution electron microscopy has been completed and put into operation in the Research Institutes. The facility as it now stands is considered by some to be the best electron microscope installation in the world. These laboratories occupy a total of about 4,000 square feet and comprise:

A. 2,500 suare feet of remodeled space in the basement with installation of special floors, wall partitions, ceiling panels, air conditioning of the type used in "clean rooms" for modern electronic industrial facilities. These laboratories are equipped with three electron microscopes with attached electron diffraction units. The facilities include ultra-high vacuum (Varian) evaporation units, four ultra-microtomes, light microscopes, and complete preparation and photographic darkroom facilities. All of the critical equipment has been installed on individual vibration control mountings of special design. Corresponding precautions were taken in the installation of non-magnetic stainless steel ventilation ducts, incandescent lights, and electrical conduit to minimize electrical and magnetic perturbations.

B. Adjoining laboratories of 920 square feet located on the second floor of the Research Institutes have been remodeled:

1. Room 203B (230 sq. ft.)

   This room has been prepared for storage of specimens, equipment, and laboratory apparatus. A Harris Cascade Refrigeration Biological Storage Machine was installed which operates at temperatures as low as -120°C.

2. Room 205 (230 sq. ft.)

   This room has been prepared as a site for superconducting experiments. A Siemens Elmiskop EM-II with accessories was installed. Darkroom equipment has also been installed to expedite development of plates taken during experiments in this room.

3. Room 207 (460 sq. ft.)

   An X-ray diffraction unit with Kratky Camera was installed. Two vacuum puming units were developed and installed which pre-pump photographic plates (at a rate of 912 plates per 2 hours), also with capacity to pre-pump 70mm film and camera. The efficiency of these pumps is such that it reduces working time by several hours. Plates, film, and camera were previously pre-pumped in the microscope itself which involves a much longer time.

C. An additional laboratory (Room P-III) was constructed in the clean room laboratories of the basement to house a Hitachi Perkin-Elmer electron microscope and accessories. This microscope was installed and includes a double condenser lens, electron diffraction chamber, hot and cold stages, and image intensification system.

D. A special highly regulated power supply is located in an air conditioned enclosure on the fifth floor of the Research Institutes. This 50-kilowatt motor generator set, specially designed and manufactured by Westinghouse Company, is equipped with a new solid-state regulator, giving better than 0.1% voltage stability and very low harmonic distortion.

E. A special vibration-free room is now being completed where a new "cryo-electron microscope," adapted from the Hitachi 11-B electron microscope, has been installed. The ten-ton floating foundation and other unique features of this facility should make it possible to expoit unusual stability of superconducting lenses operating in the persistent current mode for long-term exposures: of the order of minutes to hours, instead of the 5 to 15 second exposures presently possible.

III. Training Program

Our laboratory has served as a center of information exchange to other societies and institutions as well as to our own university.

A. The course is Cell Ultrastructure is being offered on Tuesdays and Thursdays, 1-3 PM. The enrollment for this course numbers 30 people and includes students and faculty from the Biophysics, Microbiology, Zoology, Botany, Pathology and Medicine departments of the University of Chicago, in addition to visitors from the Downey Veterans Administration Hospital.

B. Participation in the following sessions and conferences has brought about domestic and international exchange:

1. High Voltage Electron Microscope Workshop at Argonne National Laboratory, June 13 to July 15, 1966

   a. Lecture on Superconducting Lenses - June 14
   b. Chairman, Summary Session - July 14

2. The Neurosciences Research Program at Boulder, Colorado, July 17 to August 12, 1966

   Lecture on Membrane Ultrastructure in Nerve Cells - July 27

3. Electron Microscopy Society of America in San Francisco, August 22 - 25

   a. Served as keynote speaker
   b. Lecture on New Approaches in High Resolution Electron Microscopy

4. Sixth International Congress for Electron Microscopy at Kyoto, Japan, August 28 to September 4

   Lectures presented were as follows:

   a. High Resolution Electron Microscopy of Biological Specimens - Opening Session, August 28
   b. Application of Improved Point Cathode Sources to High Resolution Electron Microscopy - August 29
   c. Low Temperature Electron Microscopy with High Field Superconducting Lenses - September 1

   d. Served as chairman on afternoon session, August 29 on High Resolution (2) and Lens Aberrations

IV. List of Publications for period April 1, 1966 to September 30, 1966 (5 copies of each are included with this report)

1. Microscope, Electron; McGraw-Hill Yearbook Science and Technology - 1966 McGraw-Hill Book Co., Inc.

2. Electron Microscopy with Superconducting Lenses; paper presented at High Voltage Electron Microscope Workshop, Summary Session, Argonne National Laboratory, July 14

3. Proceedings of the Sixth International Congress for Electron Microscopy, Kyoto, Japan - September 1966

   a. High Resolution Electron Microscopy of Biological Specimens - p. 13

   b. Application of Improved Point Cathode Sources to High Resolution Electron Microscopy - p. 27

   c. Low Temperature Electron Microscopy with High Field Superconducting Lenses - p. 147

4. High Resolution Electron Microscopy with Superconducting Lenses at Liquid Helium Temperatures; Proceedings of National Academy of Sciences 56, 3 (September 1966) p. 801

5. An account of our work presented at the Sixth International Congress for Electron Microscopy is given in the article: "Voltage, Lenses Aid Electron Microscopy", published in Chemical and Engineering News, September 26, 1966