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October 3, 1966

Grants and Research Contracts
Code SC
Office of Space Sciences
National Aeronautics and Space Administration
Washington, D.C. 20546

Dear Sir:

This is the tenth quarterly progress report submitted in accordance with the requirements of NASA Contract NASr-169 covering the period January 1, 1966 through June 30, 1966.

Work during the first six months of 1966 on this project has been directed toward the completion of hardware procurement and construction. Design specifications were put into final form for negotiation of a sub-contract for construction of the mechanized microscope to be used as the input for our automatic chromosome analysis system. It was determined that the Perkin-Elmer Corporation was best suited to carry out the construction of the instrument under sub-contract. Accordingly, negotiations were entered into and the sub-contract was developed in final form and signed in March 1966.

During this time period the necessary digital equipment was procured for development of the control logic system to operate the mechanized microscope in our laboratory. In addition, work was completed on the alternative input system for 35 mm. photographs using a mechanical scanning system. The instrument was delivered in the Spring of 1966 and further effort was devoted to its integration into the automatic analysis system.

The precision scanning system to take the data presented by the alternative input systems and subject it to conversion to digital form for computer analysis is now under construction. Detailed system design studies were carried out and necessary plans drawn for the completion of this component. No effort was devoted to the computer programming for chromosome counting and analysis because of our concentration on the completion of the hardware phase of the work.

In order to clarify and summarize the automatic analysis system at its present stage of development, a detailed system description is appended as part of this report.

Respectfully Submitted,

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AUTOMATIC CYTOGENETIC ANALYSIS SYSTEM

I. System Description

The system is composed of a digital computer, an ultra-precision flying spot scanner and a mechanized microscope using either an incoherent or a coherent light source. The functions to be performed are:

1. Detection of mitotic cells
2. Placement of mitotic cells under optical microscope
3. Focussing of microscope
4. Classification of mitotic cells into two categories:
   a. Suitable for chromosome count only
   b. Suitable for chromosome count and karyotype (matching of appropriate parts of chromosomes)
6. Analysis of the cell within the limits set by 4, a and b, and output of results of analysis by means of photograph, printed page or magnetic tape. (The purpose of the magnetic tape is for additional processing by a larger computer or at a later time by the small computer.)

A. Subsystems and their functions

The digital computer is a Model PDP 7, manufactured by the Digital Equipment Corporation in Maynard, Mass. The Precision Flying Spot Scanner is modeled after the Model 31 of the Digital Equipment Corporation. However, it is under manufacture at the University of Pittsburgh using flip chip modules, a Celco deflection system and a Litton Micropix tube. The complex microscope system was designed and developed by the Perkin-Elmer Corporation of Norwalk, Connecticut. Delivery of the instrument is due in November, 1966.

1. Computer: The purpose of the computer is to perform three functions.
   a. Control of the mechanized microscope
   b. Control of the flying spot scanning system
   c. Analysis of the data obtained by the flying spot scanning system.

2. Flying spot scanning system: This subsystem is capable of three operations.
   a. Scan of the microscope slide with a .2 micron spot
   b. Display of a computer generated character or characters on photographic film in a camera located in the data chamber of the microscope.
   c. Scan of photographic film, putting the information into digital form for processing by the computer.

3. Mechanized microscope: This instrument will be discussed in three general areas because of its complexity. The first is that of mechanics, the second, the coherent light optical path, and third, the incoherent light optical path.

The mechanical portion of the microscope consists of a rotating platen, a radial slide and an autofocus assembly. The rotating platen holds six standard 2-inch microscope slides. It can be operated in two modes, a scan mode and a reposition mode. The radial slide is positioned by a step motor which increments the slide assembly approximately 50 microns at the completion of each revolution of the platen. The autofocus assembly consists of two parts. The first is a step motor which increments the focus assembly approximately .5 microns on each increment. The second is a piezo electric crystal which is capable of incrementing the autofocus assembly approximately .1 micron per increment.

The coherent light microscope consists of a helium-neon laser emmiting light at 6,328 angstroms. This coherent light source is focused into a 50 micron spot on the microscope slide. The coherent light passing through this slide is filtered by one high and one low frequency spatial filter. Light output from the spatial filters is then monitored by two photomultiplier tubes. The electrical signal from the photomultiplier tubes is converted into digital form by two 8 bit successive approximation type analog to digital converters. From this data the computer then determines if a mitotic cell is or is not present.

With an incoherent light source, the microscope can be used as a conventional microscope, that is, to view microscope slides in a magnification of 1000X. Like a conventional microscope it can be used to photograph whatever is in the field of view. In addition to the conventional functions in the incoherent light mode, the microscope also acts to demagnify the 25 micron spot size of the flying spot scanning system to a .2 micron spot size which scans the microscope slide. In addition to demagnifying the spot, there is an identical optical path referencing the light output of the cathode ray tube.

The incoherent light microscope also differs from the conventional microscope in that its X,Y and Z coorinates can be automatically controlled, i.e., positioned and monitored by computer. Also, unlike the conventional microscope, this microscope can be automatically focused without human intervention.

The flying spot scanning system in conjunction with the incoherent light microscope also permits us to write pertinent data about the photograph directly on the photograph.

II. Sequence of operation of the system

Step 1. Loading Mode

a. Loading of six standard 2" x 1" microscope slides onto the platen.
b. Calibration of the analog portions of the system.
c. Loading of the control and analysis programs into the digital computer.

Step 2. Scan Mode

During this mode the platen is rotated at approximately 20 rpm by a synchronous motor. With each revolution of the platen the radial arm is incremented by approximately 50 microns. During this period the light output of the spatial filters is monitored by the photomultiplier tube and is used as a data input to the computer. This data is constantly being compared for given criteria. Should the criteria be met, the scan operation is then terminated automatically and a reposition mode is then automatically initiated.

Step 3. Reposition Mode

The reposition mode automatically initiated at the end of the scan mode

proceeds as follows:

a. Upon the detection of a mitotic cell, the absolute angle of revolution of the platen from a 17 bit shaft encoder is transfered into the memory of the computer.
b. The radial stepping motion is inhibited.
c. The synchronous motor drive is disconnected and a step motor drive is connected.

Once the rotating platen has been stopped, the next step is to position the mitoric cell which has just been detected by the coherent light microscope, under the objective lens of the incoherent light microscope. This is accomplished by an incremental step motor driven through two separate gear trains. The gear trains are selected by the computer. The first gear train is a one to one ratio which enables the step motor to make large incremental change until the present output of the shaft encoder agrees to within the four least significant bits of the number stored in the computer from the initial contact. When all bits, except the four least significant bits, compare, the direct drive, i.e., the one to one step ratio, is disengaged and a 200 to one gear ratio is inserted in the mechanical path. The step motor is then incremented until all but the least significant bits compare to the numbers stored from the initial contact. This completes the repositioning mode and places a mitotic cell directly in the center of the field of view of the incoherent light microscope. The instrument then proceeds to the automatic focus step.

Step 3. Automatic focus

As is the case with any conventional optical microscope, the depth of field is directly related to the magnification factor. With a magnification of 1000X and 5000 angstroms light, the depth of field is of approximately .3 microns. This fact makes automatic focus a nontrivial problem which has not definitely been solved as yet, although bread board trials suggest that it is solvable.

Our approach to the problem of automatic focus is as follows: The ultra-precision scanning system is activated. It scans the area of the microscope slide under the objective. The number of density changes weighted by the magnitude of the change for several scan lines is stored in memory along with the corresponding focus position. This process is performed storing only the highest number and its corresponding position for every .5 microns until a distance of ten microns has been traversed. The step motor is then reversed and stepped until the position of the highest number of density changes that occurred during the 10 micron travel has been reached. This completes the autofocus function.

Step 4. Photograph

When an acceptable cell has been detected, the photograph of the cell is taken on 35 mm film. Using the data obtained during the autofocus function we can now make a decision as to the validity of the cell recognition and the probability of its satisfactory analysis. Since this photograph will serve as future reference it must be clearly marked as to origin. This photograph must also contain enough data to permit reexamination of the cell on the microscope by a human observer should it be necessary. The ultraprecision scanner is used in the second mode at this time, that is, it is used to display computer generated characters which are focused onto the data area of the film plane. The photography sequence is as follows:

1. Various mirrors and shutters are positioned that allow a tungston lamp source to illuminate the microscope slide and to provide an optical path to a pre-focused automatic 35 mm camera.

2. Mirrors and shutters are repositioned to provide an optical path between the face of the ultraprecision cathode ray tube and the data area of the film plane.
3. A set of computer-generated characters are then written onto the data area of the photograph.

This completes the photographic phase of the operation.

Step 5. Analysis

The microscope has now in its field of view a mitotic cell. We would now like to analyze this cell for several parameters.

a. Simply count the number of chromosomes present in the cell.
b. Compare each chromosome with its best possible mate, noting any and all deviations from the normal chromosome pattern.

Wide variations of size, shape and density of the chromosomes require each cell to be analyzed within its own frame of reference. The variation due to the biological nature of the system are compounded by overlapping or touching of two or more chromosomes of the cell. Assuming that there is no overlapping of chromosomes within the cell, and no extraneous particles are in the field of view, then counting chromosomes is relatively easy. Pairing the chromosomes or karyotyping, as this process is called, is at least several orders of magnitude more difficult based on the same initial assumptions as the counting problem. Pairing algorithims must be capable of the following:

a. Distinguishing shape, i.e., X or Y configuration.
b. Determing size, i.e., the length of arms.
c. Finding location of constrictions of arms, i.e., primary or secondary.
d. Recording more than one constriction in the same chromosome, i.e., a dicentric chromosome, or one with a chromatid gap.
e. Determing the origin of, and recording, disjointed parts, i.e., fragments.
f. Accepting as normal, crossed arms but not dicentric chromosomes. This is the situation in which arms that are simply overlaying each other by reasons of chance.
g. Recording ring-shaped chromosomes, i.e., that is an X-shaped chromosome with the very ends touching, but one pair of arms.
h. Pairing each chromosome with its best possible mate.
i. Placing paired chromosomes in appropriate groups in accordance with cytogenetic convention.
j. Recording the group in which an abnormal chromosome appears.

The programs to carry out the above ten functions have not as yet been written and may indeed be impossible. Although the problem appears to be extremely difficult, we believe that the system which we are developing, with its microscope, scanner and computer gives us the best possible chance to reach a solution to the problem. This approach allows us to have access to any point in the image plane with a maximum access time of 50 microseconds. This is equivalent to having a random access memory of 1,048,576 words of 8 bits each. This technique, we hope, will allow us to perform a rather complex analysis on a computer with a small magnetic core random access memory.

Another major problem only lightly touched upon is that of unscrambling overlapping of just touching chromosomes. The technique of unscrambling overlapping chromosomes is as follows. The density scale that we are using covers a range of 2.4 optical density units above basic badkground density of the slide. These 2.4 optical density units are divided into 256 linear and equal segments. If two chromosomes are overlapping the net density should be equal to the sum of the two densities of the two chromosomes. Should this be the case a linear gray

scale should prove to be the effective way to handle the problem.

We are confident at this time that although the automatic karyotyping operation is difficult it is possible when each chromosome of the cell is discrete. Therefore, if we were looking at normal and technically ideal chromosome patterns only, we would have a very good probability of making a machine capable of performing this function without human intervention. However, unscrambling crossed or touching but, otherwise normal chromosomes and identifying them by their individual forms may not be possible. It may be necessary for the program to cull out all seemingly atypical forms for human final decision.

This is not a real difficulty since we are not as interested in simply processing the normal cells in any event as we are in finding the abnormal ones containing morphologically distorted or damaged chromosomes. Thus, we are looking for the proverbial needles in the haystack. If our system can find all of them and present them for our examination automatically, and rapidly, we can evaluate their significance from a biologic standpoint much more easily than our present manual methods permit.

III. Auxillary functions of the system

1. The precision flying spot scanning system is capable of converting photographic information into a computer compatible form.
2. The complex microscope, although designed primarily for automatic operation, can be used in both a semi-automatic and a completely manual mode. In the semi-automatic mode, the desired coordinates are simply typed into the computer. The computer will then automatically position the platen and the radial arms to the given coordinates. Focussing can then be carried out in either an automatic or manual mode. A completely manual mode of operation can be carried out by controls in easy reach of the operator.
3. The system has been so designed as to permit complete reviewing of data obtained in the automatic mode. Photographs are to be taken of every cell that is analyzed. In addition, the coordinate location of every cell that is analyzed is to be recorded on a printed page so that the cell may be viewed by a human observer if desired.

4. When the system has been fully assembled and should the chromosome recognition problem be adequately solved, then additional work should be undertaken to improve the ability to classify cells using the coherent light system rather than the incoherent light system. This would require that the spatial filtering system be replaced by a device such as an image disector. This device in conjunction with the computer could then analyze the coherent light patterns for salient features of the mitotic cells. This technique can also be extended to observe other biological specimens. This would give us a powerful research tool in the investigation of other biological and medical problems.

APPENDIX

Computer: PDP-7 Manufactured by the Digital Equipment Corporation

Word length - 18 bits
Memory - 8192 words; cycle time 1.75 μsec.
Addition time - 3.5 μ sec.
Division time - 9.1 μ sec.
Multiplication time - 6.1 μ sec.

Optional Equipment

*1. Expanded memory (4K standard + 4K additional)
**2. Extended arithmetic element
3. Expanded I/o device selector and information distributor
4. Two 8-bit successive approximation type analog to digital converters (See note 1)
Δ5. Two IBM type 727 magnetic tape units
6. Control unit for magnetic tape units (See note 2)

* Purchased from Digital Equipment Corporation
** Gift from Digital Equipment Corporation
Δ Gift from IBM

Note 1: Constructed at University of Pittsburgh using DEC flip-chip modules
Note 2: Constructed at University of Pittsburgh using DEC system modules in addition to some special circuitry

Ultra-Precision Flying Spot Scanning System

I. Operational Features

GEOMETRY
4096 X 4096 computer addressable points
2048 X 2048 unique addressable points
Maximum raster size 8.082 X 8.082 cm.
Spot diameter: minimum - 20 μ (half amplitude) at center of screen, never more than 30 μ (half amplitude) at any point. Spot diameter can be enlarged on command by the computer
Spot intensity: controlled by 3-bit code from the computer
Dynamic correction: high carder 8-bit correction to both focus and position by wired hybrid logic
Automatic grid line generation on command from the computer

II. Sybsystem Description

A. Cathode ray tube - Litton micropix model L-4123 with p-11 phospitor; phosphor moise ± 5%
B. Deflection system - manufactured by Constrantine Engineering Laboratory (Celco) Mahwah, New Jersey
1. Deflection amplifier: Model No. DAPP3N - 2520

a. Sensitivity: 1.0 volts input = 2.0 amps output
b. Linearity: 0.05%

2. Focus amplifier: Model No. I-DA-PP20
   a. Sensitivity: 1.0volts input + 1.0 amps output
   b. Linearity: 0.25%

3. Micro positioners - Model No. -CI628-2

4. Centering coils - Model No. KC 430-S320

5. Focus coil - Model No. -HAF 334-3201560
   a. Static focus coil - R= 63.0 ohms; L= 517 M.H.
   b. Dynamic focus coil - R = 1.21 ohms; L = 253 M.H.

6. Deflection yoke - Model No. HAD 428 - S570

C. Power Supplies

25KVDC anode supply - Walden Electronics Model 545A
2KVDC second grid supply - Keithley Instruments Model 242
200VDC first grid supply - Kepco. Inc. Model SM-325-0.5M
30VDC deflection amp. supply - Kepco, Inc. Model KSM-36-10M
10VDC ladder network reference supply - Walden Electronics Model 543A
-15, + 1-VDC logic power supply - Digital Equipment Model 728
+20, -20VDC operational amp. supply - not selected at this time
+15, -15VDC operational amp. supply - not selected at this time

D. Control logic and interface of analog and digital devices

Digital: Flip-chip modules - Digital Equipment Corporation
Mounting panels - Digital Equipment Corporation
Analog: Operational amplifiers - a. Nexux Model FSK-6
b. Analog Devices - not selected

Special circuits and circuit modifications are designed and built when necessary by the Radiation Physics and Engineering group of the Radiation Medicine Department at Presbyterian-University Hospital.

E. Cabinets: Digital Equipment Corporation - Models CAB-5 and CAB-6

Automatic Microscope

1. Electro-Mechanical

A. Rotating platen drive and encoder
   1. 20 rpm synchronous motor
   2. Step motor - United Shoe Model HDUM-16-172
   3. 10:1 and 200:1 gear ratios between step motor and platen, Computer Selectable
   4. 17-bit absolute angle encoder with Gray Code output Wayne George Model No.RD-17

B. Slide arm assembly drive and encoder
   1. Step-motor - United Shoe Model HDUM-16- 172
   2. Gear ratio between motor and slide is fixed
   3. 12-bit absolute angle encoder with Gray Code output Wayne George Model No. RD-12

C. Focus assembly drive and encoder
   1. Step-motor - United Shoe Model HDUM-16-172
   2. Piezo electric crystal
   3. Gear ratio between motor and focus assembly is fixed
   4. 12-bit strobe type shaft encoder Wayne George Model No. RI-12B27

II. Electro-Optical

A. Light sources
   1. Helium-neon laser - 1MW, 6328A Perkin Elmer Model No. 5200
   2. Cathode ray tube - 5" Litton Model L4123
   3. Tungsten lamp

B. Coherent light path
   1. Helium-neon laser
   2. Magnifying optics - 1000X
   3. Beam splitting mirror
   4. Spatial filters - frequency and band width can be manually changed
   5. Light diffuser
   6. Multiplier photo tube RCA type C70042CPI

C. Incoherent light path for flying spot scanning of the microscope slide
   1. Demagnifying optics 1/375X
   2. Beam splitting
   3. Data channel - stained microscope slide
      Reference channel - clear microscope slide
   4. Light diffuser
   5. Multiplier photo tube RCA type 8575

D. Incoherent light path for photograph of CRT face
   1. Demagnifying optics - 1/3X
   2. Movable front surface mirror - computer operated
   3. Camera shutter - computer operated
   4. Film advance - computer operated

E. Incoherent light path for photograph of microscope field of view
   1. Tungsten lamp source
   2. Magnification optics - 1000X
   3. Movable front surface mirror - computer operated
   4. Camera shutter - computer operated
   5. Film advance - computer operated

F. Incoherent light path for observation station
   1. Tungsten lamp source
   2. Magnification optics - 1000X
   3. Movable front surface mirror - manual or automatically operated

III. Operational Features

A. Platen will accommodate up to six 1" x 2" microscope slides and contains calibration patterns for system testing

B. Speed
   1. Minimum scan time - 25 minutes
   2. Maximum scan time - determined by number of cells to be observed and type of analysis to be performed

C. Data review by human observer
   1. Fully automatic
   2. Automatic except for focus
   3. Manually positioned and focused by electrically operated positioners

D. Data output
   1. Photograph
   2. Complete computer analysis - results given on printed page
   3. Data in reduced form for complete analysis at a later time or by another computer