



Radiation Dosimetry via Automated Fluorescence Microscopy

With further development, this instrument could enable biodosimetry on a large scale.

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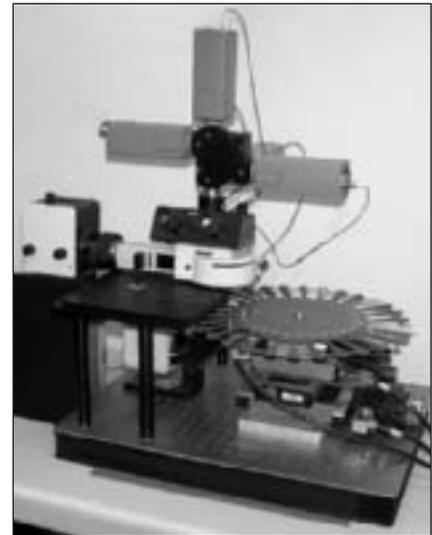
A developmental instrument for assessment of radiation-induced damage in human lymphocytes includes an automated fluorescence microscope equipped with a one or more charge-coupled-device (CCD) video camera(s) and circuitry to digitize the video output. The microscope is also equipped with a three-axis translation stage that includes a rotation stage, and a rotary tray that holds as many as thirty specimen slides. The figure depicts one version of the instrument. Once the slides have been prepared and loaded into the tray, the instrument can operate unattended. A computer controls the operation of the stage, tray, and microscope, and processes the digital fluorescence-image data to recognize and count chromosomes that have been broken, presumably by radiation.

The design and method of operation of the instrument exploit fluorescence *in situ* hybridization (FISH) of metaphase chromosome spreads, which is a technique that has been found to be valuable for monitoring the radiation dose to circulating lymphocytes. In the specific FISH protocol used to prepare specimens for this instrument, metaphase lymphocyte cultures are chosen for high mitotic index and highly condensed chromosomes, then several of the largest chromosomes are labeled with three of four differently colored whole-chromosome-staining dyes. The

three dyes, which are used both individually and in various combinations, are fluorescein isothiocyanate (FITC), Texas Red (or equivalent), and Cy5 (or equivalent); The fourth dye — 4',6-diamidino-2-phenylindole (DAPI) — is used as a counterstain.

Under control by the computer, the microscope is automatically focused on the cells and each slide is scanned while the computer analyzes the DAPI-fluorescence images to find the metaphases. Each metaphase field is recentered in the field of view and refocused. Then a four-color image (more precisely, a set of images of the same view in the fluorescent colors of the four dyes) is acquired. By use of pattern-recognition software developed specifically for this instrument, the images in the various colors are processed to recognize the metaphases and count the chromosome fragments of each color within the metaphases. The intermediate results are then further processed to estimate the proportion of cells that have suffered genetic damage.

The prototype instrument scans at an average areal rate of 4.7 mm²/h in unattended operation, finding about 14 metaphases per hour. The false-alarm rate is typically less than 3 percent, and the metaphase-miss rate has been estimated to be less than 5 percent. The counts of chromosomes and fragments thereof are 50 to 70 percent accurate.



The Original Version of the Automated Fluorescence Microscope included four CCD cameras and dichroic beam splitters for acquiring images in four fluorescence wavelength bands. The instrument was then modified to incorporate a single, more sensitive video camera and a filter wheel for taking a sequential exposures of each microscope field in the fluorescence wavelength bands of interest.

This work was done by Kenneth R. Castleman and Mark Schultze of Perceptive Scientific Instruments, Inc., for Johnson Space Center. For further information, contact the Johnson Technology Transfer Office at (281) 483-3809. MSC-23072

Multistage Magnetic Separator of Cells and Proteins

Purifications and separations can be carried to higher degrees than were previously possible.

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The multistage electromagnetic separator for purifying cells and magnetic particles (MAGSEP) is a laboratory apparatus for separating and/or purifying particles (especially biological cells) on the basis of their magnetic susceptibility and magnetophoretic mobility. Whereas a typical prior apparatus based on similar principles offers

only a single stage of separation, the MAGSEP, as its full name indicates, offers multiple stages of separation; this makes it possible to refine a sample population of particles to a higher level of purity or to categorize multiple portions of the sample on the basis of magnetic susceptibility and/or magnetophoretic mobility.

The MAGSEP includes a processing unit and an electronic unit coupled to a personal computer. The processing unit includes upper and lower plates, a plate-rotation system, an electromagnet, an electromagnet-translation system, and a capture-magnet assembly. The plates are bolted together through a roller bearing that allows the plates to rotate with re-

spect to each other. An interface between the plates acts as a seal for separating fluids. A lower cuvette can be aligned with as many as 15 upper cuvette stations for fraction collection during processing.

A two-phase stepping motor drives the rotation system, causing the upper plate to rotate for the collection of each fraction of the sample material. The electromagnet generates a magnetic field across the lower cuvette, while the translation system translates the electromagnet upward along the lower cuvette. The current supplied to the electromagnet, and thus the magnetic flux density at the pole face of the electromagnet, can be set at a programmed value between 0 and 1,400 gauss (0.14 T). The rate of translation

can be programmed between 5 and 2,000 $\mu\text{m/s}$ so as to align all sample particles in the same position in the cuvette.

The capture magnet can be a permanent magnet. It is mounted on an arm connected to a stepping motor. The stepping motor rotates the arm to position the capture magnet above the upper cuvette into which a fraction of the sample is collected.

The electronic unit includes a power switch, power-supply circuitry that accepts 110-Vac input power, an RS-232 interface, and status lights. The personal computer runs the MAGSEP software and controls the operation of the MAGSEP through the RS-232 interface. The status of the power, the translating electromagnet, the capture magnet, and the rotation of the

upper plate are indicated in a graphical user interface on the computer screen.

This work was done by Ken Barton, Mark Ainsworth, Bruce Daily, Scott Dunn, Bill Metz, John Vellinger, Brock Taylor, and Bruce Meador of Space Hardware Optimization Technology, Inc., for Johnson Space Center.

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:

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Refer to MSC-23124, volume and number of this NASA Tech Briefs issue, and the page number.

Elastic-Tether Suits for Artificial Gravity and Exercise

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Body suits harnessed to systems of elastic tethers have been proposed as means of approximating the effects of normal Earth gravitation on crewmembers of spacecraft in flight to help preserve the crewmembers' physical fitness. The suits could also be used on Earth to increase effective gravitational loads for purposes of athletic training. The suit according to the proposal would include numerous small tether-attachment fixtures distributed over its outer surface so as to distribute the artificial gravitational force as nearly evenly as possible over the wearer's body. Elastic tethers would be con-

nected between these fixtures and a single attachment fixture on a main elastic tether that would be anchored to a fixture on or under a floor. This fixture might include multiple pulleys to make the effective length of the main tether great enough that normal motions of the wearer cause no more than acceptably small variations in the total artificial gravitational force. Among the problems in designing the suit would be equalizing the load in the shoulder area and keeping tethers out of the way below the knees to prevent tripping. The solution would likely include running tethers through rings on

the sides. Body suits with a weight or water ballast system are also proposed for very slight spinning space-station scenarios, in which cases the proposed body suits will easily be able to provide the equivalency of a 1-G or even greater load.

This work was done by Paul Torrance of Johnson Space Center, Paul Biesinger of Science Applications International Corp., and Daniel D. Rybicki of Lockheed Martin Corp. For further information, contact the Johnson Technology Transfer Office at (281) 483-3809.

MSC-23145

Multichannel Brain-Signal-Amplifying and Digitizing System

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An apparatus has been developed for use in acquiring multichannel electroencephalographic (EEG) data from a human subject. EEG apparatuses with many channels in use heretofore have been too heavy and bulky to be worn, and have been limited in dynamic range to no more than 18 bits. The present apparatus is small and light enough to be

worn by the subject. It is capable of amplifying EEG signals and digitizing them to 22 bits in as many as 150 channels. The apparatus is controlled by software and is plugged into the USB port of a personal computer. This apparatus makes it possible, for the first time, to obtain high-resolution functional EEG images of a thinking brain in a real-life,

ambulatory setting outside a research laboratory or hospital.

This work was done by Alan Gevins of SAM Technology, Inc., for Johnson Space Center. For further information, contact the Johnson Technology Transfer Office at (281) 483-3809.

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