



Radiation Dosimetry via Automated Fluorescence Microscopy

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

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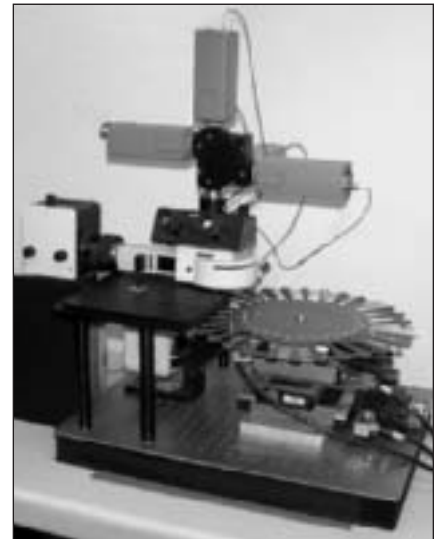
A developmental instrument for assessment of radiation-induced damage in human lymphocytes includes an automated fluorescence microscope equipped with a one or more charge-coupled-device (CCD) video camera(s) and circuitry to digitize the video output. The microscope is also equipped with a three-axis 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-