Tricks and Treats - Continued from page 18

the finest object detail with no gain of image quality in other respects.

If the intensity of the image is to be varied, *without* change of numerical aperture or spectral distribution of the illumination and without undesirable change of condenser position, the only correct method to accomplish this is by use of neutral density filters. Even the method of varying intensity with a rheostat (varying voltage) is not entirely correct because by such procedure not only the intensity, but also the spectral composition of the light is changed. This is not objectionable in black and white photography, but must be careful avoided when color film is used. Only very small deviations from the correct aperture of the iris diaphram are permissible, for instance, to adjust the illumination to a value indicated by a photoelectric exposure meter, to correspond to an available shutter speed. Let us assume that at the "OOMPH" position the exposure meter reading is 64, and experience has shown that for an exposure time of 1/25 second, a deflection to 75 is required. The slight increase in diameter of the substage iris diaphram necessary to obtain this intensity value is permissible.

The only factor which influences, not only resolving power, but also the entire optical character of the image, is the ratio of the aperture of the objective to that of the illumination.

To summarize the practical aspects of the "OOMPH" position of the aperture iris diaphram; optimum image quality prevails when the field of view is illuminated by light of uniform intensity; when the illuminated area is not larger than the field of view; and when the numerical aperture of the condenser comes closest to matching the numerical aperture of the objective considering the inherent contrast of the specimen.

The microscopist who guides himself by these suggestions will consistently produce photomicrographs which are a real "treat" to everybody who knows how to evaluate them. ■

The *Why* and *What* of Flow Cytometry

The following is the introduction in the Royal Microscopical Society Handbook No. 29 "Flow Cytometry," which is available from Microscophy Today ($27.00 plus S&H).

Why Flow Cytomertry?

In the last 10 years, the applications of flow cytometry have spread through all branches of biological sciences. Most research institutes now have several machines and even quite small research groups expect to have access to an instrument. People have used flow cytometers to measure the properties of, and to sort, mammalian and plant cells, yeast and bacteria and isolated nuclei, chromosomes and mitochondria. The biggest growth has been in the clinical field. Flow cytometers are now being used for routine measurements in immunology, haematology and, to a lesser extend, pathology departments.

In cells, a variety of properties may be measured, for example the DNA content of a nucleus, the expression of a surface antigen, the activity of an intracellular enzyme or the pH. Several properties might be measured simultaneously. Indeed, the scope of the technique is only limited by the fluorescent dyes available and the investigator's imagination - the latter probably being the most important.

What is Flow Cytometry?

As its name implies, flow cytometry is the measurement of cells in a flow system which has been designed to deliver particles in single file past a point of measurement. Although, in theory, many types of measurement could be made, in practice the term is applied to instruments which focus light on to cells and record their fluorescence and the light scattered by them. Electronic cell volume and absorbed light additionally may be measured.

The power of flow cytometry lies in the ability to measure several parameters on tens of thousands of individual cells within a few minutes. The method can therefore be used to define and to enumerate accurately sub-populations. Once identified, such sub-populations can be sorted physically for further study.

Typically, five parameters might be measured on 20,000 cells. Using blue light for excitation, one might record green, orange and red fluorescence and blue light scattered in a forward direction and at right angles to the laser beam. The large amount of data generated cannot be processed adequately without a powerful, well-programmed computer. The computer is not an optional extra but an essential part of the instrument.

The major disadvantage of flow cytometry is that a preparation of single particles (cells, nuclei, chromosomes) is required. Of necessity, tissue architecture is destroyed, so that spatial information about the relationships of cells to each other is lost. Also, no information is acquired about the distribution of entities within a cell and little, if any, information about a cell's shape. Flow cytometry can be contrasted to conventional light microscopy. The eye makes qualitative estimates of large numbers of parameters on a few cells, recording detail within each cell; the cytometer quantifies an average parameter for each cell but measures thousands of cells.

A comparison may also be made with a biochemical measurement in which an average value of, for example, an enzyme activity, is made for all the cells in a sample. Flow cytometry makes a measurement on each cell individually so that, if a small sub-set of cells has a high value, this feature will be recorded. Such a sub-set would not be detected biochemically.

Several recent books have given overviews of flow cytometry (Bauer *et al.*, 1993; Givan, 1992; Melamed *et al.*, 1990; Shapiro, 1988; Watson, 1991) and others have given detailed descriptions of many of the methods in common use (Darzynkiewicz and Crissman, 1990; Macey, 1994; Ormerod, 1994; Radbruch, 1992; Robinson, 1993). ■

For a complete price list of available Royal Microscopical Society Microscopy Handbooks, contact Microscopy Today.

& MICROSCOPY & MICROANALYSIS

KANSAS CITY, AUG. 13–17, 1995

Including the World's Largest Commercial Exhibition of Related Equipment, Supplies, Software and Services

MICROSCOPY SOCIETY OF AMERICA
53RD ANNUAL MEETING

THE HISTOCHEMICAL SOCIETY
46TH ANNUAL MEETING

SYMPOSIA AND CONTRIBUTED SESSIONS

▼*Instrumentation & Methodologies*

- 3-D Imaging & 3-D Reconstruction
- Advanced Computing in Microscopy
- Advances in Light Microscopy
- Auger Electron Spectroscopy
- Biomaterials
- Confocal Microscopy
- Convergent Beam Diffraction
- Correlative Microscopy (Tech Forum)
- Developments in Computer Controls
- Direct Digital Imaging & Microscope Automation
- Energy Loss & Filtering Spectroscopy
- Field Emission
- Image Formation & Recording
- Image Processing, Analysis, & Modeling
- Microbeam Mass Spectroscopy
- Near Field Microscopy
- Optical & Electron Holography
- Optical Microspectroscopies
- Scanning Probe Microscopy & Analysis
- SEM & TEM/STEM
- Surface Analysis Methods
- High-Res Intermediate- & High-Voltage EM in Biology & Materials Science
- Tomography
- X-ray EDS & XRF/XRD Techniques

SHORT COURSES

- Immunocytochemistry & Protein Labeling: Ag-Enhanced Small Au Particles
- Approaches for *in situ* PCR & *in situ* Hybridization in Research & Diagnosis
- Cryotechniques for TEM, & Low-Temperature SEM
- Electron Diffraction in the TEM
- Introduction to Digital Imaging

▼*Biological Sciences*

- Applied Biomedical Microscopies
- Cryotechniques
- Cytochemistry/Histochemistry
- EM Tomography in Biology
- Extracellular Matrix/Cell-ECM Interactions
- Functional Imaging of the Nervous System
- Functional Organization of the Cell Nucleus
- Histochemistry: Nucleic Acid Detection
- Immunology & Immunocytochemistry
- *In Situ* Hybridization
- Macromolecular Microscopy
- Membranes, Microdomains & Transport
- Microbiology
- MRI Microscopy
- Organelles, Chromosomes & Nuclei
- Pathology
- Phytomorphology & Phytobiology
- Reproduction & Developmental Biology
- Scanned Probe Microscopy

IN ADDITION

- Over 130 Commercial Exhibits
- Over 600 Scientific Papers
- Over 50 Scientific Sessions
- Computer Software Exchange
- 8th Annual Golf Tournament
- Postage Stamp Exhibit
- 3rd Annual 5k Run/Walk
- Best Poster & Photomicrograph Awards
- Educational Outreach Program
- Public Education Panel
- Histochemical Society Lillie Awards
- MSA Presidential Student Awards
- EM Facilities Management (Tech Forum)

▼*Physical Sciences*

- Amorphous/Disordered Materials
- Analytical Microscopy Advances: Ceramics
- Approaches to Catalyst Characterization
- Coatings
- Electronic Materials
- Geology/Mineralogy
- High Spatial Resolution Microanalysis & Microscopy
- *In Situ* Microscopy in Materials Research
- Magnetic Materials
- Microscopic Characterization of Materials
- Nano-Phase & Nano-Composite Materials
- Oxidation & Corrosion
- Petrology
- Phase Transformations
- Polymers, Metals & Alloys, Ceramics
- Quantitative Electron Diffraction
- Radiation-Sensitive Materials
- Small Particles/Clusters
- Surfaces & Interfaces
- Superconductors
- Tribology

TUTORIALS

- Exploring Local Properties of Biological Surfaces with Atomic Force Microscopy
- Histochemical Methods for Localizing & Characterizing Glycoconjugates
- Energy Filtering in Biological EM
- Navigating the Internet
- Digital Imaging
- Electron Backscattered Diffraction: An Emerging Probe for Materials Characterization
- Atom Probe Analysis of the Solid–Liquid Interface
- Image Analysis Fundamentals

FOR MORE INFORMATION

Meeting Office ▼ P.O. Box EM ▼ Woods Hole, MA 02543
Toll-Free: 800-538-3672 ▼ Fax: 508-548-9053 ▼ E-mail: lmaser@mbl.edu