We appreciate the response to this publication feature and welcome all contributions. Contributions may be sent to José A. Mascorro, our Technical Editor, at his e-mail address: jmascor@tulane.edu. José may also be reached at the Department of Structural and Cellular Biology, Tulane University Health Sciences Center, 1430 Tulane Ave., New Orleans, LA 70112 and Ph: (504) 584-2747 Fax: (504) 584-1687

On The Limits Of Limits

Tobias I. Baskin
Division of Biological Sciences, University of Missouri
BaskinT@missouri.edu

In these days of the digital universe, discussion of resolution is mostly couched in pixels per inch, but it is useful to revisit the analog universe occasionally. Most of us retain in the attic a memory about the resolution limit of the microscope, which for an optical microscope is in the neighborhood of a quarter micron. But, most of us too have seen light micrographs where objects much smaller than that are shown in all their glory. Has the digital revolution superceded the old analog limits? What is going on?

Those with physics backgrounds may remember a formula for the resolution limit, d, typically given in the form of:

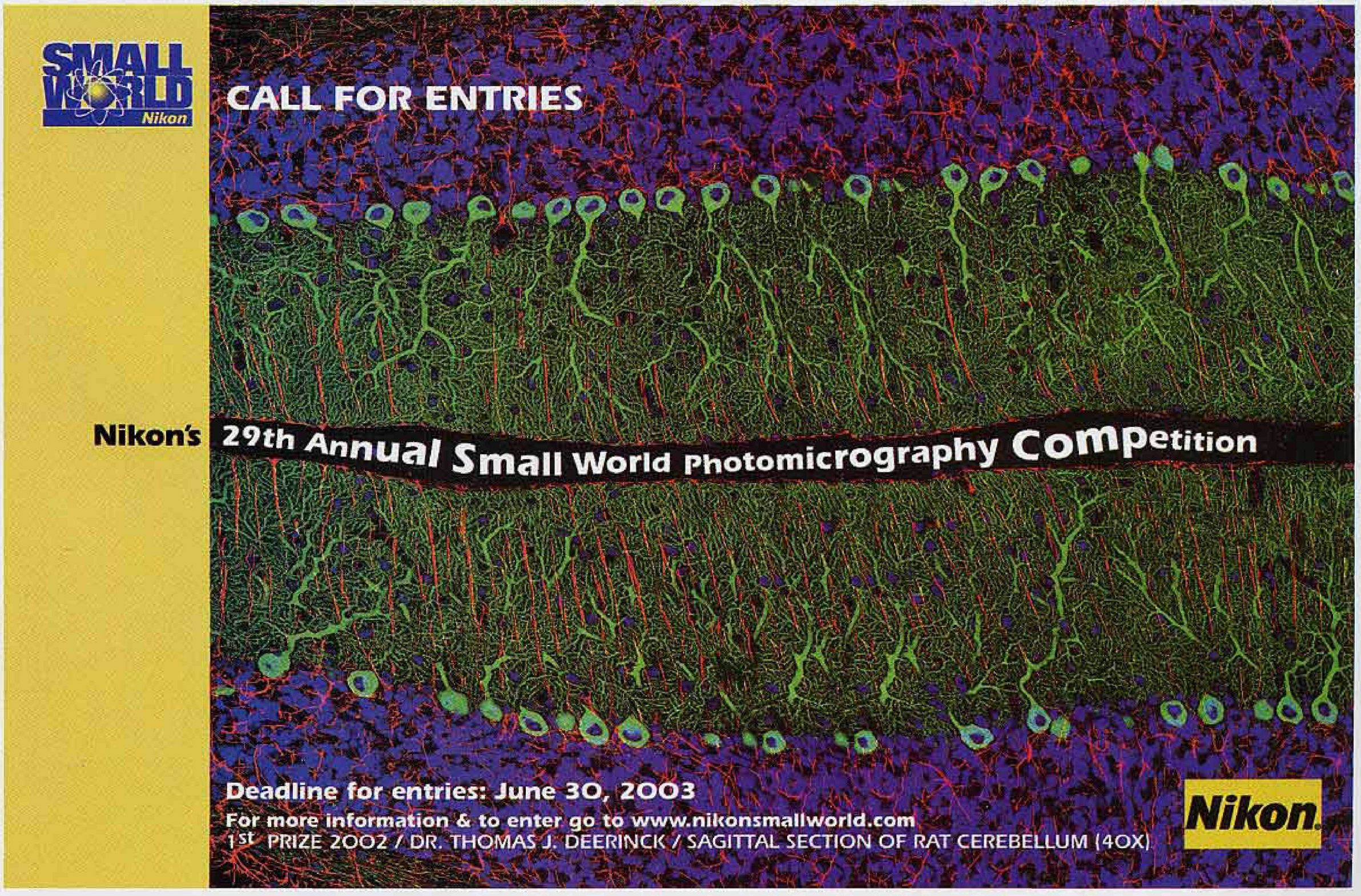
$$d = a\lambda/NA \qquad (1)$$

where NA is the numerical aperture of the lens, λ is the wavelength of light, and *a* is a constant, typically taken around 0.5. For the canonical green light and the highest available NA (1.4), this gives a resolution of limit of 0.2 microns. Physicists will know that equation 1 is an approximation, but the various more exact solutions still produce about the same value. Anyone who has spent time looking down the tubes of a light microscope is certain to have seen things smaller than that, without aid of any digital intermediary. Among many examples, centrioles were drawn by cytologists more than 100 years ago, and I have seen microtubules by eye in dark field. These structures are all smaller than 0.2 micron. How can this be?

The answer comes in understanding what '*d*' in equation 1 really means. The choice of the letter '*d*' puts one in mind of 'diameter' and so it is natural to think that the 0.2 microns calculated equals the diameter of the smallest resolvable object. Natural this may be, but it is entirely wrong. In fact, the term '*d*' stands for 'distance'. The resolution limit formula gives the smallest distance that two objects can be separated and still be seen as two; if they come any closer, they are seen as a single object. This minimum distance arises because images are in essence diffraction patterns, and is given by the greatest overlap of diffraction patterns that allows them to be seen as two separate patterns—rather than one single pattern. That explains why equation 1 is an approximation, because the exact size of the diffraction pattern depends on the geometry of the lens set up and the character of the illumination.

Once the resolution limit is understood to involve spacing between objects, then it comes as no surprise that a single object can be seen even though smaller than the resolution limit. Any object will produce a diffraction pattern and the question is only whether that pattern can be detected. That is an issue of contrast, not of resolution. There is no theoretical limit to the smallness of a pinhole that could be imaged, provided enough light could be driven through it to reach the detector. But interestingly, if one were to image a series of pinholes of decreasing diameter, and measure the size of the pinhole in each image, as soon as the relevant diffraction limit were reached, the measured size would stabilize and would not decrease further, even though size of the actual pinholes continued to decrease. For the best performing optical microscope, as soon as the pinholes dropped below about 0.2 microns, they could still be imaged, but the measured size of the pinhole in the images would stay at 0.2 microns.

Thus, the paradox is resolved: Classical resolution limits are spacings, not sizes.



https://doi.org/10.1017/S1551929500052664 Published online by Cambridge University Press

Comprehensive microscopy information on the web

www.microscopy.info

Product Guide Laboratory Links

Manufacturers Directory

Microscopy News Product Technology

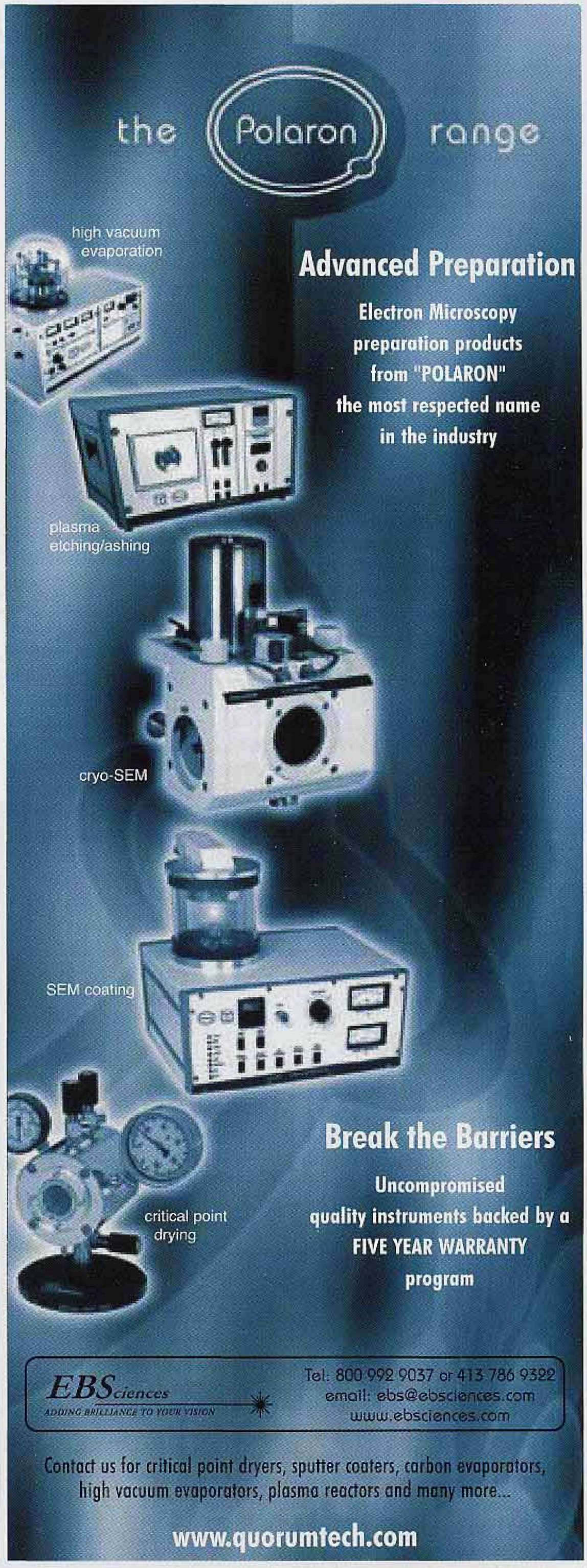
Career Resources

info@microscopy.info
978-456-3100

Electron Microscopist Wanted

LSU Health Sciences Center, Department of Ophthalmology, is seeking an experienced Electron Microscopist to work in a high level academic environment. The candidate will work in an established electron microscopy facility and should have evidence of expertise in all aspects of both scanning and transmission electron microscopy. Previous work in the area of ophthalmology is desired. A bachelor's degree with at least two years of experience is required, or a master's degree and one year of experience. This position will interact with funded faculty and quality of work will be of high priority. LSUHSC is an EEO/AA employer.

Please forward CV and three letters of reference to Wendy Roark, LSU Eye Center, 2020 Gravier St., Suite B, New Orleans, LA 70112.



https://doi.org/10.1017/S1551929500052664 Published online by Cambridge University Press

POSITIONS AVAILABLE FOR EXPERIENCED MICROSCOPY TECHNICIANS

The Analytical Imaging Facility of the Albert Einstein College of Medicine, a comprehensive microscopy core facility offering technologies that include TEM, SEM, electron cryomicroscopy, digital light microscopy, confocal and multiphoton microscopy, has two openings for experienced microscopy technicians.

Electron Microscopy Technician:

Duties will include: sample preparation for biological electron microscopy; operation, maintenance, user training and user assistance of light and electron microscopes; EM photography; computer image analysis and data presentation; solution and reagent preparation; inventory control and ordering of supplies.

Candidates must be well versed in a variety of microscopic methods and image analysis, with at lest two years of experience in electron microscopy. BS degree minimum, MS degree and MSA certification are desirable. Proficiency in biological sample preparation techniques for electron microscopy is essential. The candidate must be able to operate transmission and scanning electron microscopes. Operating experience with digital light microscopes and laser confocal microscopes is desirable. The applicant must have a working ability in traditional photographic techniques, digital image analysis and digital data presentation.

Digital Fluorescence and Confocal Microscopy Technician:

Duties will include: operation, maintenance, user training and user assistance of digital light and laser confocal microscopes; computer image analysis and data presentation; writing and editing user instructions and experimental protocols; inventory control and ordering of supplies.

The successful candidate must be well versed in a variety of microscopic methods and image analysis, with at lest two years of experience in digital fluorescence and confocal microscopy. BS degree minimum, MS degree is desirable. The ability to operate digital light microscopes and laser confocal microscopes is essential. Have working ability in commercially available software for digital image analysis and digital data presentation.

It is essential that applicants for either position have excellent communication skills since user training is a primary responsibility. Finally, the technicians must be able to work well with many people in a multi-user environment. The positions are full time union positions, with a University benefits package. EOE

For consideration send your CV and the names of three references to: damiani@aecom.yu.edu

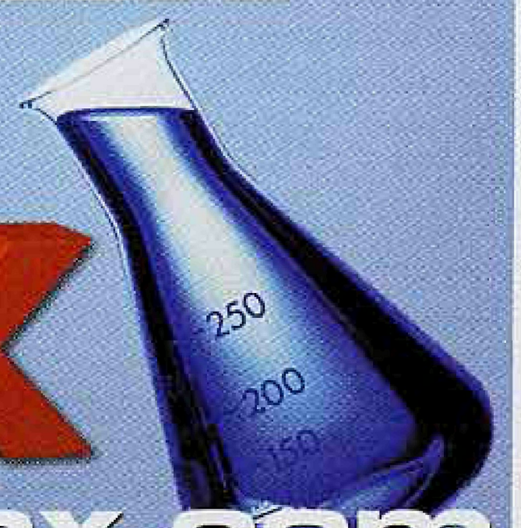
Albert Einstein College of Medicine
Analytical Imaging Facility
1300 Morris Park Avenue
Bronx, New York 10461, USA
http://www.aecom.yu.edu/aif

LabX Online Auctions

- Over 30,000 Registered Users
- Over 200 Microscope Ads Online Daily
- All Major Brands
- Free Equipment Wanted Ads

New - Surplus - Used
Microscopes, Accessories, Objectives

June Special !
Microscope Month
Reduced Ad Rates



labX
www.labx.com

https://doi.org/10.1017/S1551929500052664 Published online by Cambridge University Press