

James B. Pawley

1944 - 2019



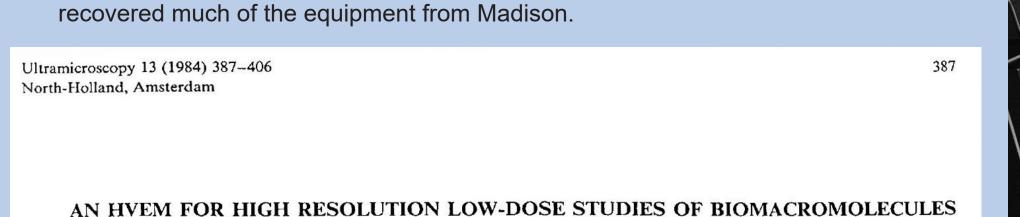
Biography

James Pawley was born January 15th 1944 in Gerrard's Cross, England. He immigrated to Canada with his parents in 1946 first to Cloverdale, BC, and then to Vancouver. In the late 1950's, the family moved to California. From 1962 to 1966 he studied electrical engineering at the Carnegie Institute of Technology in Pittsburgh, and in 1972 he got his Ph.D. in biophysics at the University of California-Berkeley. After a series of postdoctoral positions, in 1978 he took a faculty position in the department of Zoology at the University of Wisconsin-Madison. One of his main responsibilities was to run the three-story million-volt electron microscope, part of a national microscopy facility (an NIH P41 BTTR, started by Hans Ris).

For sixteen years he also directed the "3D Microscopy of Living Cells" course on the University of British Columbia's campus, with a faculty of internationally known scientists and cutting-edge equipment loaned by manufacturers. The "3D Microscopy of Living Cells" motto was "It's not just diffraction; it's not just statistics; It's biology!". The course attracted participants from all over the world. The course provided the foundation for his best-known publication, the Handbook of Biological Confocal Microscopy, now in its third edition and still an essential resource.

He was especially active in the Clean Air Society and the Sunshine Coast Community Solar Association; his letters often appeared in local papers. He was a founder of the Doriston Music Festival. In 2012 he moved to the house he and his wife built in Sechelt., British Columbia.

HVEM As mentioned, Pawley's first responsibility at UW Madison was the AEI EM-7 Mk II HVEM. He made many improvements to the HVEM, e.g. cryo-TEM and low-dose data collection. Along with Gatan, he developed high-resolution cryo-transfer (and an accompanying anticomtaminator) for the HVEM. Although the Madsion HVEM has been scrapped, another AEI EM-7 Mk II is still intact in Albany, NY and Albany group



This paper was the culmination of all of Pawley's HVEM improvements. Each item was first presented as an EMSA submission.

Stevens Arnold

Supply

#C-24-T-15-165-X

Counter Chain

Power Over/Under

Reset Reset

Display Controller

Digital fine control for the objective lens current of the EM-7. (1981

Reference . V BEAM

Medium Focus

Wiper

16 bit Mult DAC

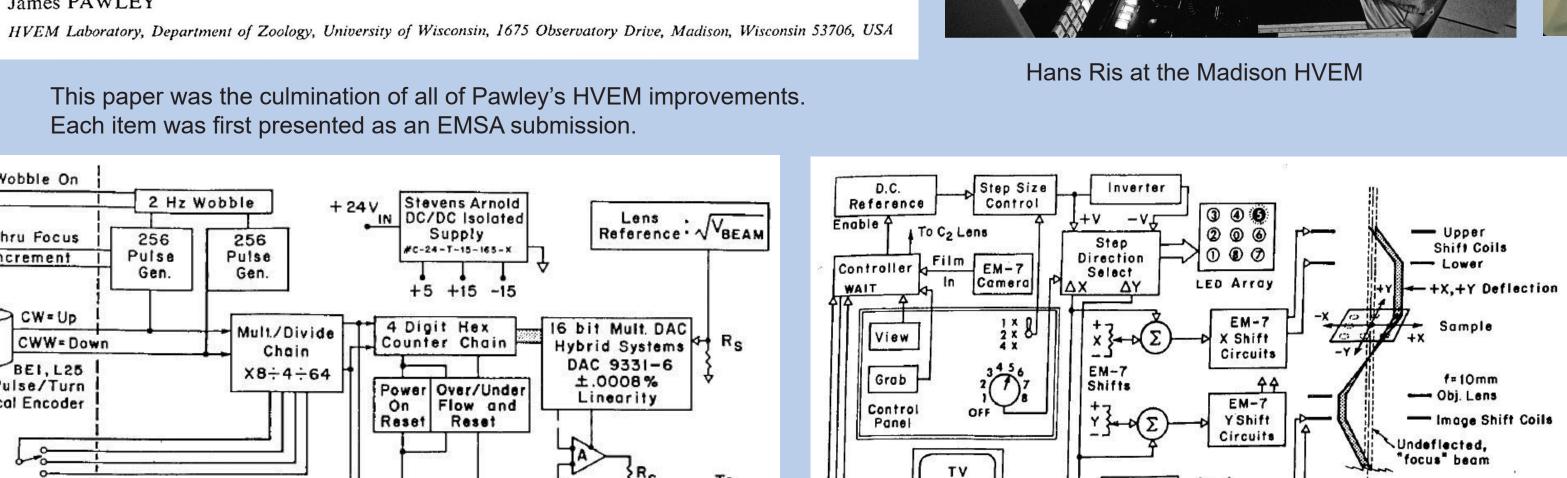
Hybrid Systems

DAC 9331-6

±.0008%

AD517KH

Linearity





- P2 Lens

- P₃ Lens

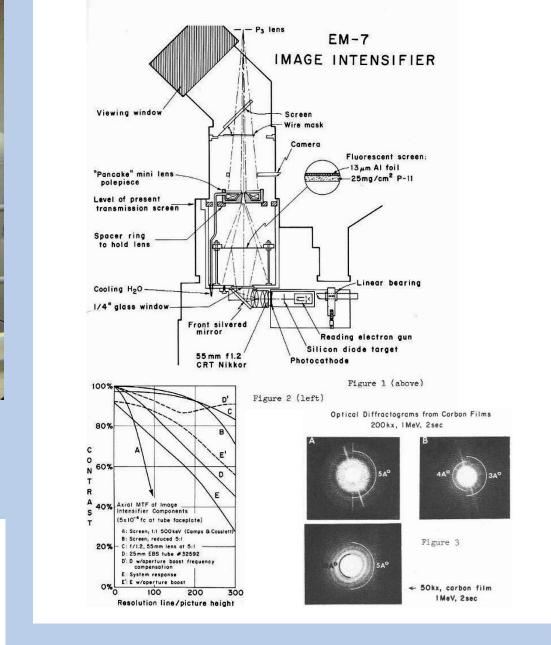
Camera

Transmission Screen





The Albany AEI EM-7 HVEM

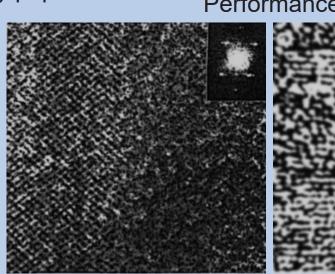


An image intensifier using a mini-lens to optimize its spatial resolution (1979 EMSA submission).

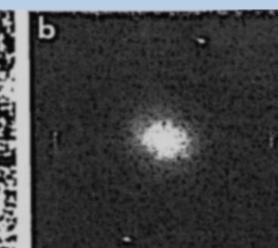
The ACD was only described in the 1984 Ultramicroscopy paper.

EM7 LOWER ANTICONTAMINATOR

Performance of the modified HVEM







resolution studies at 1 MV (1984 EMSA sub-

ultramicroscopy

Au crystal, ~0.2 nm

Gr Carbon

Gr carbon ~0.3 nm

The laser unit (Figure 1A) is the illumination source, and

wavelength affects optical performance, and through the ab-

sorption spectrum of the dye, it determines the amount of flu-

Power output instability is usually noise; its instability is

usually less than 1%, but lasers can become increasingly un-

stable as they age. Because dust, misalignment or mechanical

instability can cause random changes of 10-30%, the effi-

ciency of the optical coupling to the connecting fiber (if

The alignment and reflection characteristics of laser

mirrors can be the source of long-term drift in laser output.

Since the source of the laser light is determined by the laser

mirrors, beam-pointing error/alignment is important. Insta-

bility here will show up as changes in brightness because

changes in the apparent source position will alter the efficien-

cy of the optics coupling the laser light into the single-mode

affects the fraction of the light emitted by the specimen that can be collected. This is also true for light from the laser.

Objective magnification is inversely related to the diame-

with exciting laser light. Underfilling will reduce spatial reso-

lution and the peak intensity. Overfilling will cause some

Cleanliness is important and dirty optics produce much

varies strongly with the coverglass thickness and the refrac-

effectively enlarges the image of objects smaller than the dif-

fraction limit, making them appear dimmer than they should be.

zoom magnification control, determines the size of a pixel at

the specimen. For Nyquist sampling, the pixel should be at

least two times smaller than the smallest features that you ex-

pect to see in your specimen. Assuming a Rayleigh Criterion

resolution of 200 nm, the pixels should be less than 100 nm.

Larger ones produce undersampling, reducing the recorded

The scanning system (Figure 1C), and especially the

Diffraction is the unavoidable limit to optical resolution. It

The numerical aperture of the objective lens (Figure 1B)

optical fiber used in most instruments.

orescence produced.

LV SEM

Electron optical design of a high-resolution low-voltage scanning electron microscope with field emission gun

Department of Radio-Electronics, Peking University, Beijing 100871, People's Republic of China P. S. D. Lin Bellcore, Red Bank, New Jersey 07702-7020

Integrated Microscopy Resource, University of Wisconsin, Madison, Wisconsin 53706

2 Hz Wobble

256

Pulse

Gen.

256

Pulse

Gen.

Amray Inc., Bedford, Massachusetts 01730-1491

EMSA submission).

James PAWLEY

□ Wobble On

Thru Focus

CW=Up

4k Pulse/Turn

Range Switch

HP-5082

Desk Panel

5 Digit Display

Optical Encoder

CWW= Down

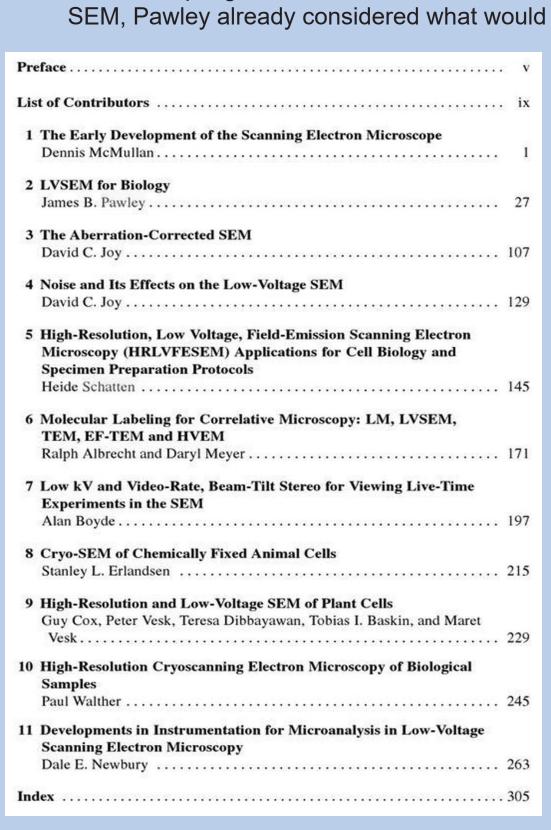
BE1, L25

Increment

(Received 9 March 1993; accepted for publication 18 June 1993)

In the present study, a comprehensive description and a complete process for designing a high-resolution low-voltage scanning electron microscope with a field emission gun have been discussed, including the design of two types of magnetic immersion lenses optimized for low-voltage operation, the evaluation of their resolution, the design of the magnetic lens/ secondary electron collector system, the calculation of three-dimensional trajectories for both secondary electrons and backscattered electrons, and the estimate of their collection efficiency. The computed results and the primary experiments indicate the possibility of achieving nanometer resolution, which basically approaches the electron optical predictable probe size for the low-voltage scanning electron microscope.

Before adopting use of the Hitachi 900S immersion-lens FEG-SEM, Pawley already considered what would be needed.



Heide Schatten • James Pawley **Biological Low-Voltage Scanning Electron Microscopy** 🖄 Springer

As was becoming his custom before moving on to a new area of interest, Pawley edited a book on LVSEM, inviting prominent authors. In 2008, Jim was invited to edit Heide Schatten's book on LVSEM, in which could be imparted what was known about the subject.

Scanning Microscopy Supplement 3, 1989 (Pages 163-178) 0892-953X/89\$3.00+.00 Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA THE CASE FOR LOW VOLTAGE HIGH RESOLUTION SCANNING ELECTRON MICROSCOPY OF BIOLOGICAL SAMPLES James B. Pawley1,* and Stanley L. Erlandsen Integrated Microscopy Resources, University of Wisconsin, ²Department of Cell Biology and Neurosnatomy

- Rotation

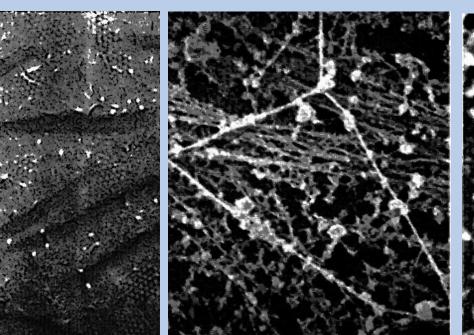
Rotation

Monitor

0000

Quantex DS20

512 x 512 x 8 bit Image Memory



Confocal

Microscopy

Pawley's institution (IMR

with this title in 1989, as a

Press) published the first work

workshop Proceedings at the

1989 EMSA meeting. Inviting

a carefully selected collection

scopy as contributors, in 1990

of the leaders in light micro-

Press) appeared. This was

followed by second (2006)

and third (2008) editions,

(both Springer).

Cytochem. 38(12):1781-1785.

the first edition (Plenum

Light

Jim often worked with former MSA President Stan Elandsen, advocating high-res. LVSEM for biological applications, such as intramembrane particles (I), intermediate filaments (ctr) and nuclear pores (r).

The paper by Joy and Pawley summarized the stateof-the-art. These pictures of the subunits of actin filaments by Ya Chen attracted much interest.

A side-entry cold stage for low-dose high-

High-resolution scanning electron microscopy

David C. Joy 1,2 and James B. Pawley

Received at Editorial Office 11 May 1992

¹ EM Facility, University of Tennessee, Knoxville, TN 37996-0810, USA

² Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

mission).

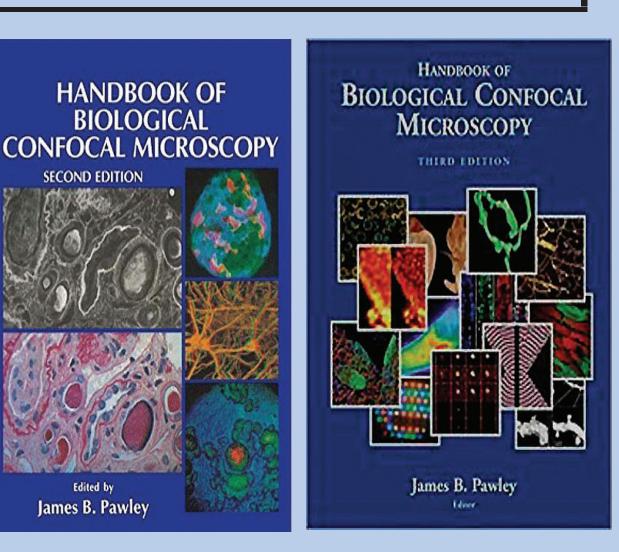
Ultramicroscopy 47 (1992) 80-100

North-Holland

HANDBOOK OF HANDBOOK OF

BIOLOGICAL

SECOND EDITION



CONFOCAL LM

Bialmaging The new BioImaging column of BioTechniques will feature short articles devoted to microscopy and in general, the fluorescence measured is proportional to the laser power level. Although total laser output power is usudigital imaging. The subject matter will address deally regulated, the amount of power in each line of a multitails of the methods used to produce images of cells line laser may not be, and may vary widely with time. The

and tissues at any magnification and resolution, and might include "tricks-of-the-trade", novel methods of specimen preparation, practices of image collection, tips on the digital manipulation and publication of images and historical perspectives.

The 39 Steps: A Cautionary Tale of Quantitative 3-D Fluorescence Microscopy

Jim Pawley University of Wisconsin-Madison,

der/ladic/course/bulletin.html)

884 BioTechniques

Madison, WI, USA We all know that fluorescent micrographs reveal the locater of the objective lens entrance pupil. The objective will tion of the labeled molecules in a tissue, right? Well, maybe not. In fact, all you can be really sure of measuring with most only function properly if the entire entrance pupil is filled laser-scanning confocal microscopes in the fluorescence mode is some feature of the number of photons collected at a particular time. We can hope this is an accurate measure of laser light to strike the metal mounting of the objective and be one or two interesting parameters-the local analyte concenlost, also reducing the intensity of the spot. tration or the local ion concentration. In fact, many factors affect the numerical values actually stored in the computer larger and dimmer spots. Transmission (the fraction of light memory at any given moment. Over the years, students taking incident on the objective that can be focused into a spot on the The 3D Microscopy of Living Cells course held each June at other side of it) varies with wavelength. Beware of using the University of British Columbia, compiled a list of these some relatively new, multicoated optics in IR range. Chroextraneous factors. In the first year, the list grew to 39 entries, matic and spherical aberration both make the spot bigger, and so we borrowed the name of the Alfred Hitchcock film and vary with wavelength. In addition, spherical aberration for our list. Since then, the list has continued to grow! (More information on the course can be found at www.cs.ubc.ca/spitive index of the immersion and embedding media.

Although this article can't fully describe each term, brief and useful explanations are included. The terms appear in bold lettering and many interact with other terms in bold lettering. Note that many of these variables are usually thought of in terms of their effect on spatial resolution. They are listed here because reduced resolution translates into "putting the same number of exciting photons into a larger spot" This lowers the excitation intensity—the number of photons produced by a given molecule—and the fraction of these that are detected. It is often forgotten that normal signal levels in fluorescence confocal microscopy correspond to only 10-20 photons/pixel in the brightest areas. Under such conditions, statistical noise is a more important limitation on spatial resolution than that defined by the Abbé equation (1). A flow diagram of a generic laser scanning confocal microscope with the locations of many of the "39" features mentioned in the text is shown in Figure 1.

The effects of the bleaching rate are proportional to the square of the zoom magnification. As for scan speed, the longer the dwell time on a particular pixel, the more signal will be detected and the less it will be distorted by Poisson Noise (3). At high scan speeds (less than 100 ns/pixel), signals from dyes with fluorescent decay constants that are longer than this dwell time can be reduced. Vol. 28, No. 5 (2000)

brightness of small features (2).

This was Jim's last paper, published posthumously. It brought together the knowledge and techniques covered over the 16 years of his "The 3D Microscopy of Living Cells" course, which was held at the University of British Columbia yearly in June.

Bibliography

BOOKS Pawley, J.B (Ed.) (1989) Handbook of biological confocal microscopy.

HVEM

1989, IMR Press, Madison. 1990 1st edition Plenum Press, NY 2006 2nd edition Springer 2008 3rd edition Springer

Schatten, H., Pawley, J.B. (Eds.) (2008) Biological low-voltage scanning electron microscopy

GW, Claitors, Baton Rouge), pp 304-305.

Biological low-voltage scanning electron microscopy. Springer, NY.

Early TEM

Hayes, T.L., Glaeser, R.M., Koehler, J.K., Pease, R.F.W., McDonald, L.W., Everhart, T.E., Pawley, J.B. (1970) Information content of electron microscope images. J. Ultrastr. Res. 30(1-2): 238-.

Jones, S.J., Boyde, A., Pawley, J.B. (1975) Osteoblasts and collagen orientation. Cell Tiss. Res. 159(1):73-80.

Boyde, A., Pawley, J.B. (1975) Transmission electron-microscopy of ion erosion thinned hard tissues. Calcified Tissue Research 23(Suppl. S) 21:117.

Pawley, J.B.,, Fisher, G.L. (1977) Using simultaneous 3-color x-ray mapping and digital-scan-stop for rapid elemental characterization of

coal combustion by-products. J. Microsc. 110:87. Fisher, G.L, Pawley, J.B., Hayes, T.L., Tyler, W.S. (1978) Multi-element mapping of fly-ash particles in lung cells. Am. Rev. Respiratory Disease 117(4):231-231.

Pawley, J.B. (1979) An image intensifier using a mini-lens to optimize its spatial resolution. 38th Ann. Proc. Electron Microscopy Soc. Am. (Bailey GW, Claitors, Baton Rouge), pp 592-593.

Pawley, J.B. (1980) Recent improvements to the EM-7 at the Madison Biotechnology Resource. 38th Ann. Proc. Electron Microscopy Soc. Am. (Bailey GW ,Claitors. Baton Rouge), pp 8-9.

Ann. Proc. Electron Microscopy Soc. Am. (Bailey GW ,Claitors, Baton Rouge), pp 302-303. Pawley, J.B. (1981) Digital fine control for the objective lens current of the EM-7. 39th Ann. Proc. Electron Microscopy Soc. Am. (Bailey

Pawley, J.B. (1981) Minimal exposure, rotary hollow-one illumination and focus wobble added to the deflection system of an HVEM. 39th

Pawley, J.B. (1981) Thin foil viewing screens and on-line computer as HVEM focusing aids. 39th Ann. Proc. Electron Microscopy Soc. Am. (Bailey GW ,Claitors, Baton Rouge), pp 306-307.

Pawley, J.B. (1982) Can HVEM contribute to high-resolution biological studies? J. Cell Biol. 95:A467-A467.

Pawley, J.B. (1984) An HVEM for high resolution low-dose studies of biomacromolecules. Ultramicroscopy 13(4):387-405. Pawley, J.B. (1984) A side-entry cold stage for low-dose high-resolution studies at 1 MV. 42nd. Ann. Proc. Electron Microscopy Soc. Am

(Bailey GW, San Francisco press), pp 279-279. Pawley, J. B. and Albrecht, R. M. (1988) Structure of the cytoplasmic filament system in freeze-dried whole mounts viewed by HVEM. In:

Advances in Electronics and Electron Physics - Computers, Plenum, New York. LVSEM

Pawley, J.B. (1972) Charging artifacts in the scanning electron microscope. Scanning Electron Microsc. 1(1):153-160.

Pawley, J.B. (1972) Dual needle piezoelectric micromanipulator for scanning electron-microscope. Rev. Sci. Instr. 43(4): 600-602. Pawley, J.B., Hayes, T.L. (1972) Micromanipulator for scanning electron-microscope. J. Ultrastr. Res. 39(1-2):214.

Pawley, J.B., Belton, J.C., McLaughlin, R.F. (1975) Scanning electron micrographs - another look at early emphysema lesions. Chest

Pawley, J.B., Boyde, A. (1975) Robust micromanipulator for scanning electron-microscope. J. Microsc. 103: 265-270.

Pawley, J.B., Norton. J.T. (1978) A chamber attached to SEM for fracturing and coating frozen biological samples. J. Microsc. 11(12):169 Pawley, J.B. (1978) Design and performance of presently available tv-rate stereo SEM systems. Scanning Elect. Microsc. (SEM inc., SFM O'Hare, II 1978):157.

Pawley, J.B., Hook, G., Hayes, T.L., Lai, C. (1980) Direct scanning electron-microscopy of frozen-hydrated yeast. Scanning 3(3):219-226. Pawley, J.B., Hayes, T.L. (1980) Early use of color-modulation SEM. Scanning 3(3):161-164.

Pawley, J.B. (1988) Quantitative compositional mapping. Scanning 10:211.

Hayes, T.L., Pawley, J.B., Fisher, G.L., Goldman, M. (1980) A model for the exposure of individual lung cells to the foreign elements contained in fly ash. Environmental Research 22(2):499-509.

Echlin, P., Hayles, T.L., Pawley, J.B. (1980) Low-temperature SEM and x-ray-microanalysis of developing of lemna-minor. Mikroskopie 36(9-10):296-297.

Lewis, E.R, Pawley, J.B. (1981) Direct SEM study of frozen inner-ear. Scanning 4(3): 1310140.

Pawley, J.B. (1984) Low-voltage scanning electron-microscopy. J. Microsc. 136:45-68.

Pawley, J.B. (1985) Strategy for locating and eliminating sources of mains frequency stray magnetic fields. Scanning 7:43-46. Pawley, J.B., Sepsenol, S., Ris, H. (1986) Four? Dimensional Microscopy of Ascaris Sperm Motility. Ann. NY Acad. Sci. 483:171-180.

Pawley, J.B. (1987) Use of pseudo-stereo techniques to detect magnetic stray field in the SEM. Scanning 9(3):134-136.

HANDBOOK OF

BIOLOGICAL CONFOCAL

MICROSCOPY

Edited by

James B. Pawley

Pawley, J.B. (1988) Beam deflection for stereo imaging and for compensation of misalignment caused by the secondary-electron collector field in the Hitachi S-900. Institute of Physics Conference Series 93:233-234.

Pawley, J.B., Winters, M.P. (1983) Low-voltage scanning electron microscopy. 41st Ann. Proc. EMSA (Bailey, GW, ed.) San Francisco

Pawley, J.B. (1988) Quantitative compositional mapping. Scanning 10(6):211-211.

Pawley, J.B. (1988) The promise of low-voltage SEM. Scanning 10(1):1-1.

Pawley, J.B., Albrecht, R. (1988) Imaging colloidal gold labels in LVSEM. Scanning 10(5):184-189.

Pawley, J. (1988) Quantitative compositional mapping. Scanning 10(6): 211-211.

Scanning Microscopy 2(3):1215-1230. Haggis, G.H., Pawley, J.B. (1988) freeze-fracture of 3T3 cells for high-resolution scanning electron microscopy. J. Microsc. 150(3):211-218. Pawley, J.B., Erlandsen, S.L. (1989) The case for low-voltage high-resolution scanning electron-microscopy of biological samples. Scanning Microscopy (Suppl.3):163-178.

Erlandsen, SL., Bemrick, W.J., Pawley, J.B. (1989) High-resolution electron-microscopic evidence for the filamentous structure of the cyst wall in Giardia-muris and Giardia-duodenalis. J. Parasitology 75(5):787-797.

Erlandsen, S.L., Gould, R.P., Frethem, C., Wells, C.L., Pawley, J.B., Hamilton, D.W. (1989) Membrane fixation for high-resolution lowvoltage SEM - studies on giardia, rat spermatozoa, and mouse macrophages. Scanning 11(4):169-175.

Erlandsen, S.L., Bemrick, W.J., Schupp, D.E., Shields, J.M., Jarroll, E.L., Sauch, I.F., Pawley, J.B. (1990) High-resolution immunogold localization of giardia cyst wall antigens using field-emission SEM with secondary and backscatter electron imaging. J. Histochem. Cytochem. 38(5):625-632.

Pawley, J.B. (1990) Practical aspects of high resolution LVSEM. Scanning 12:247-252. Pawley, J.B., Albrecht, R.M., Simmons, S. R. (1990) Optimizing parameters for correlative immunogold localization by video-enhanced light-microscopy, high-voltage transmission electron-microscopy, and field-emission scanning electron-microscopy. J. Histochem.

Pawley, J.B., Walther, P., Shih, S.-J. (1990) Early results using high-resolution. low-voltage SEM. J. Microsc. 161:327-355.

Erlandsen, S.L., Bemrick, W.J., Schupp. D.E., Shields, J.M., Jaroll, E.L., Sauch, J.F., Pawley, J.B. (1990) High-resolution immunogold localization of Giardia cyst wall antigens using field emission SEM with secondary and backscatter electron imaging. J. Histochem.

Cytochem. 38(5):625-632.

Pawley, J.B. Walther, P., Shih, S.J., Malecki, M. (1991) Early results using high-resolution, low-voltage, low-temperature SEM. J. Microsc. 161:327-335.

Walther, P., Autrata, R., Chen, Y., Pawley, J.B. (1991) Backscattered electron imaging for high-resolution surface-scanning electron micros copy with a new-type YAG detector. Scanning Microscopy 5(2):301-310.

Albrecht, R.M., Simmons, S.R., Pawley, J.B. (1991) Low-voltage, high-resolution SEM of colloidal gold probes. J. Histochem. Cytochem.

Joy, D.C., Pawley, J.N. (1991) High-resolution scanning electron microscopy. Ultramicroscopy 47(1-3) 80-100.

Walther, P., Chen, Y, Pech, L.L., Pawley, J.B. (1992) High-resolution scanning electron microscopy of frozen-hydrated cells. J. Microsc. 168(2):169-180. Pawley, J.B. (1992) LVSEM for High Resolution Topographic and Density Contrast Imaging in: Advances in Electronics and Electron

Physics (ed. Hawkes, P.). Academic Press, New York, 83:203-274. Ximen, J.Y. Lin, P.S.D., Pawley, J.B., Schippert, M. (1993) Electron-optical design of a high-resolution low-voltage scanning electron microscope with field-emission gun. Rev. Sci. Instr. 64(10):2905-2910.

Booy, FP., Pawley, J.B. (1993) Cryo-crinkling: what happens to carbon films on copper grids at low temperature. Ultramicroscopy 48(3):273-280.

sea-urchin embryos. Scanning Microscopy 7(4):1283-1293.

Pawley, J.B. (1996) Symposium on High-Resolution Field-Emission Scanning Electron Microscopy (FESEM) in Biology. Ann. Proc. EMSA Pawley, J.B. (1997) The development of field-emission scanning electron microscopy for imaging biological surfaces. Scanning 19(5):3224-336.

3rd Edition (J.B. Pawley, Ed.) Springer, NY. Pawley, J.B. (2008) LVSEM for Biology. In: Biological Low-Voltage Scanning Electron Microscopy (H. Schatten and J.B. Pawley, Eds.)

Hafner, M., Petzelt, C., Nobiling, R, Pawley, J.B., Kramp. D., Schatten, G. (1988) Wave of free calcium at fertilization in the sea urchin egg visualized with fura2. Cell Motel. Cytoskeleton 9(3):271-277. Pawley, J.B., Hasko, D., and Cleaver, J., 1993, A standard test and calibration specimen for confocal microscopy II. In: Proceedings of the

Pawley, J.B. (2008) Biological low voltage field emission scanning electron microscopy. In: Handbook of Biological Confocal Microscopy

1993 International Conference on Confocal Microscopy and 3-D Image Processing (CJR Sheppard, ed). Sydney, Australia, p. 35. Pawley, J.B., Amos, W.B., Dixon, A., and Brelje, T.C. (1993), Simultaneous, non-interfering, collection of optimal fluorescent and backscattered light signals on the MRC-500/600. Proc. Microsc. Soc. Am. 51:156–157.

Volume Investigation of Biological Specimens (J. Stevens, ed.), Academic Press, New York, pp. 47–94. Pawley, J., Blouke, M., and Janesick, J., 1996, The CCDiode: An optimal detector for laser confocal microscopes. Proc. SPIE 2655:125-

Pawley, J.B. (1994) The sources of noise in three-dimensional microscopical data sets. In: Three Dimensional Confocal Microscopy:

Pawley, J.B., and Centonze, V., 1998, Practical laser-scanning confocal light microscopy: Obtaining optimal performance from your

Hermann, R., Pawley, J.B., Nagatani, T., Muller, M. (1988) Double-axis rotary shadowing for high-resolution scanning electron-microscopy. instrument. In: Cell Biology: A Laboratory Handbook (J.E. Celis, ed.) Academic Press, New York, pp. 149-169. Pawley, J.B. (1999) Three-Dimensional Microscopy: Image Acquisition and Processing III (Proceedings Volume). Proc. Focus on Multidimensional Microscopy, Madison, WI. Pawley, J.B. (2002) Structures Inside Living Cells Limit Optical Sectioning Precision. Microsc. Microanal. 8(S.2):1050CD.

Pawley, J.B., 2002, Limitations on optical sectioning in live-cell confocal microscopy, Scanning, 21:241–246.

Pawley, J.B. (2002) Confocal and two?photon microscopy: Foundations, applications and advances. Microsc. Res. Tech. 59(2). Pawley, J.B. (2002) Limitations on optical sectioning in live?cell confocal microscopy. Scanning 24(5):2421-246.

Pawley, J.B. (2006) When Light Microscope Resolution Is Not Enough: Correlational Light Microscopy and Electron Microscopy. In: Handbook of Biological Confocal Microscopy 2nd Edition (J.B. Pawley, Ed.) Springer, NY.

Pawley, J.B. (2006) Points, pixels, and gray levels: digitizing image data. In: Handbook of Biological Confocal Microscopy 2nd Edition (J.B. Pawley, Ed.) Springer, NY.

Toomre, D., Pawley, J.B. (2006) Disk-scanning confocal microscopy. In: Handbook of Biological Confocal Microscopy 2nd Edition (J.B.

Pawley, Ed.) Springer, NY.

Pawley, J.B. (2006) Fundamental limits in confocal microscopy. In: Handbook of Biological Confocal Microscopy 2nd Edition (J.B. Pawley, **Ed.) Springer, NY.**

Pawley, J.B. (2006) Non-Laser Light Sources for Three-Dimensional Microscopy. In: Handbook of Biological Confocal Microscopy 2nd

Edition (J.B. Pawley, Ed.) Springer, NY.

Pawley, J.B. (2006) Tutorial on practical confocal microscopy and use of the confocal test specimen. In: Handbook of Biological Confocal Microscopy 2nd Edition (J.B. Pawley, Ed.) Springer, NY.

Pawley, J.B. (2008) The intensity spread function (ISF): a new metric of photodetector performance. In: Handbook of Biological Confocal Microscopy 3rd Edition (J.B. Pawley, Ed.) Springer, NY.

Pawley, J.B. (2008) More than you ever really wanted to know about charge-coupled devices Connections. In: Handbook of Biological Confocal Microscopy 3rd Edition (J.B. Pawley, Ed.) Springer, NY.

Pawley, J.B. (2010) The 39 steps: A cautionary tale about "quantitative" 3D fluorescence microscopy, BioTechniques, 28:884.

Multi-Methods

Hayes, T.L., Glaeser, R.M., Koehler, J.K., Pease, R.F.W., McDonald, L.W., Everhart, T.E., Pawley, J.B. (1970) Information content of electron microscope images, J. Ultrastr. Res. 30(1-2):238.

Schatten, G., Pawley, J.B. (1988) Advances in Optical, Confocal, and Electron Microscopic Imaging for Biomedical Researchers. Science 12;239(4841 Pt 2):G164,G48. Albrecht, R. M., Prudent, J, Simmons, S.R., Pawley, J.B., Choate, J.J. (1989) Observations of colloidal gold labeled platelet microtubules high-voltage electron microscopy and low-voltage high-resolution scanning electron microscopy. Scanning Microscopy 3(1):273-278.

Albrecht, R. M., Prudent, J, Simmons, S.R., Pawley, J.B., Choate, J.J. (1989) Observations of colloidal gold labeled platelet microtubules high-voltage electron microscopy and low-voltage high-resolution scanning electron microscopy. Scanning Microscopy 3(1):273-278. Walther, P., Chen, Y., Malecki, M., Zoran, S.L.S., Schatten, G.P., Pawley, J.B. (1993) Scanning electron-microscopy of high-pressure-frozen Simmons, S.R., Pawley, J.B., Albrecht, R.M. (1990) Optimizing parameters for correlative immunogold localization by video-enhanced light microscopy, high-voltage transmission electron microscopy, and field emission scanning electron microscopy. J. Histochem. Cytoichem. 38(12):1781-1785.