

# New RMS Microscopy Handbook

## Introduction to Immunocytochemistry (2nd Edition)

### Current Techniques and Problems

J.M. Polak & S. Van Noorden, Royal Postgraduate Medical School, London

Describes the most common immunocytochemical methods in detail with explanatory diagrams and photomicrographs. Intended readership includes biomedical undergraduates, postgraduates, researchers and technicians.

Paperback, 72 pages, 8 line illus.; 10 half-tones, 4 tables. ISBN: 0198564155

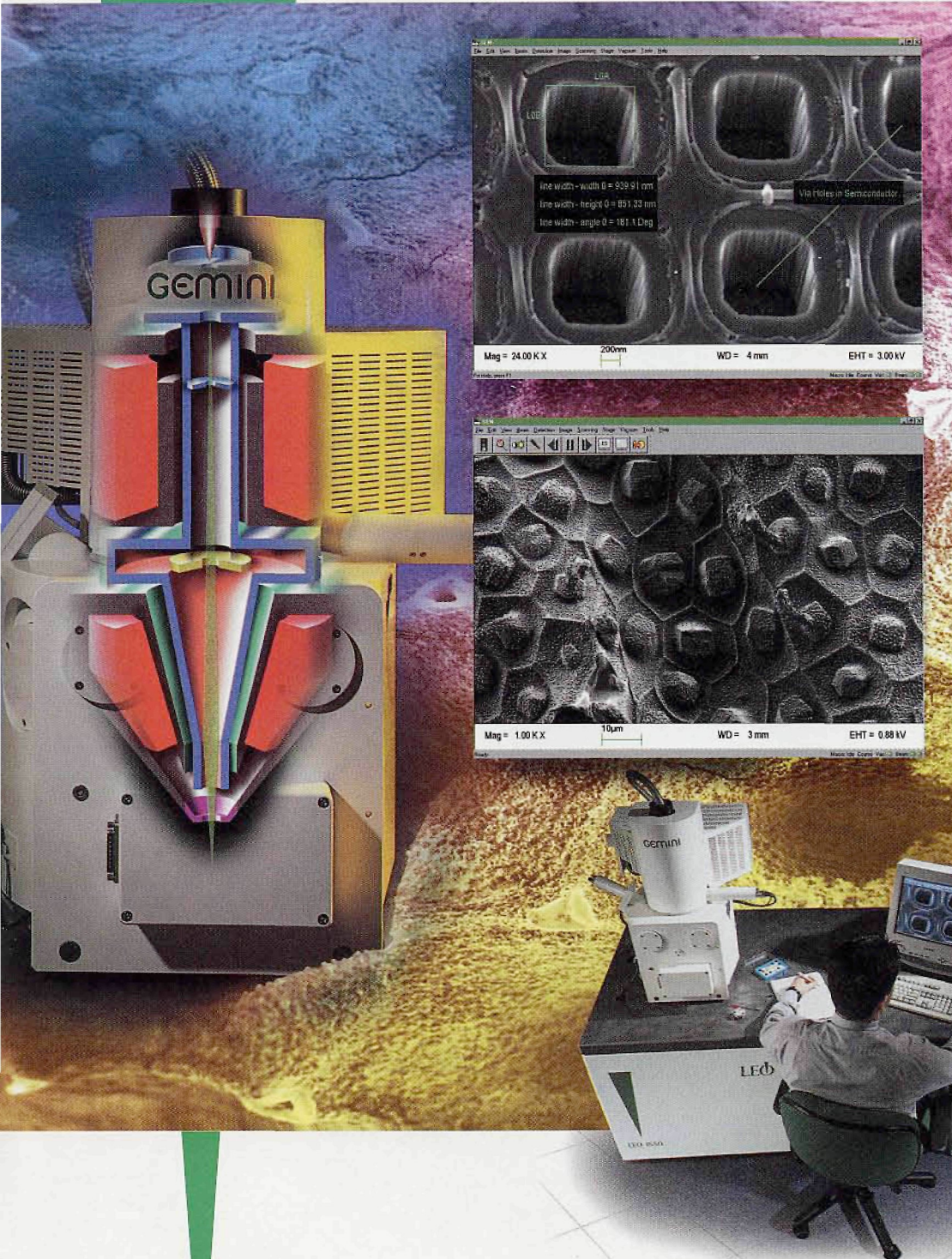
Available from *Microscopy Today* at a price of \$20.00 plus \$5.00 S&H (U.S.)

### Table of Contents

1. **Introduction**  
Definition  
History and development  
References
2. **Production of Antibodies**  
Immunization  
Testing  
Region-specific antibodies  
Monoclonal antibodies  
Characteristics of a "good" antibody  
References
3. **Requirements for Immunocytochemistry**  
Fixation  
    Cross-linking fixatives  
    Precipitant fixatives  
    Combination fixatives  
    Fresh tissue  
    Pre-fixed, non-embedded material  
    Freeze-drying  
    Tissue storage  
    Adherence of sections and cell preparations to slides  
Antigen retrieval in fixed tissues  
    Washing  
    Protease treatment  
    Heat-mediated antigen retrieval  
Visualizing the end-product of reaction  
    Fluorescent labels  
    Enzyme labels  
    Colloidal gold  
    Other labels  
Absence of non-specific staining  
    Causes and prevention of non-specific staining  
    **Color Plates**
4. **Methods**  
General considerations  
    Buffer  
    Antibody diluent  
    Antibody dilution relative to reaction time, temperature and technique  
Methods  
    Nature of antibodies (IgG)  
    Application of antibodies to preparations  
    Direct method  
    Indirect method  
    Three-layer methods  
    Avidin-biotin methods  
References
5. **Specificity Problems and Essential Controls**  
Testing for non-specific binding due to tissue factors  
    Testing for non-specific binding by the primary antibody  
    Testing for non-specific binding of second and third reagents  
    Non-specific or unwanted specific staining due to antibody factors  
    Unwanted specific staining of unknown antigens  
    Non-specific binding of antisera to basic proteins in the tissue  
    Unwanted specific cross-reactivity of anti-immunoglobulin  
    Cross-reactivity of the primary antibody with related antigens  
    Remedies for non-specificity due to tissue factors  
    Blocking binding sites with normal serum  
    Absorption with tissue powder  
    Remedies for non-specificity due to heterogeneity of the antibody  
    Dilution  
    Affinity purification  
    Remedies for non-specificity due to cross-reactivity  
    Controls  
    Negative controls  
    Positive control  
    Experimental controls  
References
6. **Enhancement of Standard Methods**  
Build-up methods  
Intensification of the peroxidase/DAB/H<sub>2</sub>O<sub>2</sub> product  
    Post-reaction intensification  
    Intensification during the peroxidase reaction  
    Tyramine signal amplification(TSA)  
References
7. **Multiple Immunostaining**  
Primary antibodies raised in the same species  
    Separately labelled primary antibodies  
    Unlabelled primary, labelled secondary antibodies  
    Indirect double staining without elution  
Double immunostaining with primary antibodies raised in different species  
    Triple immunostaining  
References
8. **Post-embedding Immunocytochemistry for the Transmission Electron Microscope**  
Principles  
Fixation  
Processing to resin  
Labels  
Sectioning  
Immunolabelling procedure  
Pre-treatment  
Immunolabelling  
Contrasting  
Multiple labelling  
References
9. **In Vitro Methods for Testing Antigen-Antibody Reactions**  
Radioimmunoassay  
Enzyme-linked immunosorbent assay (ELISA)  
Western blotting  
Dot blots  
References
10. **Applications of Immunocytochemistry**  
Histopathological diagnosis  
    Controls  
Research  
Quantification  
    Confocal microscopy  
    Flow cytometry and fluorescence antibody sorting (FACS)  
cell  
    Simpler methods of quantitation  
Non-immunocytochemical uses of labelled probes  
    Receptor localization  
    Lectin histochemistry  
    *In situ* hybridization of nucleic acids  
References
11. **Microscopy**  
References  
**Appendix: Technical Notes**  
Buffers  
    Phosphatase-buffered normal saline (PBS)  
    Tris-buffered normal saline (TBS)  
Antibody diluent and storage of antibodies  
Adherence of preparations to slides  
    Coating slides with poly-L-lysine  
    Coating slides with silane  
Blocking endogenous peroxidase reaction  
    Paraffin sections  
    Milder methods for cryostat sections and whole-cell preparations  
    Blocking endogenous biotin  
Enzyme pre-treatment  
    Trypsin  
    Protease  
    Pepsin  
    Neuraminidase  
Heat-mediated antigen retrieval using a microwave oven  
Enzyme development methods  
    Peroxidase  
    Alkaline phosphatase  
    Glucose oxidase  
    β-D-Galactosidase  
Intensifying the peroxidase/DAB reaction product  
    Following standard development  
    During development  
Immunostaining methods  
    Initial procedure  
    Immunostaining - all preparations  
Immunogold staining with silver enhancement  
    Silver acetate auto-metallography  
Double immunoenzymatic staining  
Post-embedding electron microscopical immunocytochemistry using epoxy resin-embedded tissue and an indirect immunogold method  
Absorption specificity control (liquid phase)  
References

Index

# Seeing is believing The LEO 1500 series is here...



The LEO 1500 series, featuring the unique GEMINI electron optical column and a totally new control system has arrived. With a new generation of LEO's powerful software control running in Windows 95, the LEO 1500 series offers you the following powerful capabilities:

- ▼ Set up different users' access levels, with a log-on procedure using the built-in Administrator facility.
- ▼ Save images to disk or network at pixel resolutions up to 3k x 2k, so that you can even zoom the magnification on stored images.
- ▼ Choose the beam voltage (200V to 30kV) to suit the sample or the analysis, because the resolution is superb at any voltage.
- ▼ Fast and accurate analysis with EDX, EBSP, BSD thanks to the exceptionally stable high probe current.
- ▼ Produce hard copy results in a format that you need using any Windows™ compatible printer - laser prints, colour dye sublimations, photographic etc.

*Best of all, astonishing image quality is at your fingertips. Call your local LEO representative for information or a demonstration and you'll find that seeing really is believing.*

LEO Electron Microscopy  
 UK:  
 Telephone (44) 1223 414166  
 Fax (44) 1223 412776  
 E-mail info@leo-em.co.uk  
 Website www.leo-em.co.uk  
 Germany:  
 Telephone (49) 73 64 94 6137  
 Fax (49) 73 64 94 4851  
 E-mail info@leo.de  
 France:  
 Telephone (33) 1 41 39 92 10  
 Fax (33) 1 41 39 92 29  
 E-mail LEO\_France@compuserve.com  
 USA:  
 Telephone (1) 914 747 7700  
 Fax (1) 914 681 7443  
 E-mail 70142.504@compuserve.com  
 Plus a worldwide network of dealers

Visit us at the MSA Conference - Booth 210 - 215

# LEO

*The power to resolve*

# Twice As Precise

## JSM-5800 Scanning Microscope Features Two Options for Optimum Control.



**JSM-5800**  
Dual  
versatility –  
Scanning  
Microscopy  
via mouse or  
knobset.

- Large Specimen Stage
- High/low vacuum capability
- Super Conical Objective Lens for high resolution

Suitable for a wide range of applications, the JSM-5800 from JEOL represents a new era in scanning microscopy. Now you have the option to choose either mouse or knobset control, while taking advantage of the super conical objective lens designed for the highest resolution (3.5nm) and large sample tilting.

- ▶ Easy-to-use unit has a wide range of built-in automatic functions.
- ▶ Large specimen stage allows room for up to an 8-inch sample.
- ▶ Archiving enables temporary or permanent storage and retrieval in standard TIF format.
- ▶ Five axis stage automation makes the JSM-5800 fast and easy-to-use.

Discover the twice as precise alternative that is as unique as your work itself.

To arrange for a demonstration of the innovative JSM-5800 call JEOL today.



JEOL USA, Inc., 11 Dearborn Road, Peabody, MA 01960 Tel: 508-535-5900 Fax: 508-536-2205 e-mail: eod@jeol.com

Circle Reader Inquiry #4