

METHODS IN MOLECULAR BIOLOGY

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Histochemistry of Single Molecules

Methods and Protocols

Edited by

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Cover Illustration: Double staining of claudin-5 (red fluorescence) and beta-actin (green) in the glomerulus of mouse kidney in a formalin-fixed paraffin-embedded tissue. Beta-actin and claudin-5 colocalize along the capillary walls showing yellow to orange color, which might correspond to pedicels of podocytes. Beta-actin is also demonstrated in the mesangial cells. Nuclei were stained with the DNA-specific dye DAPI (blue fluorescence). (Courtesy of Shuji Yamashita and Osamu Katsumata.)

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Preface

The story of histochemistry goes a long way back and, in the last 150 years, has evolved in parallel with the operating instruments, from bright field microscopy to super-resolution microscopy. In this evolution resides the uniqueness of this science. Compared to the biochemical and molecular methods, the distinctive feature of histochemical techniques is the ability to localize chemical species in the very place (in a tissue, or a cell or an organelle) where they exist, are produced, or operate *in vivo*; this makes histochemistry an irreplaceable tool in basic and applied biomedical research.

Actually, over the last fifteen years, about 400,000 “histochemical” articles have been published in qualified international journals (according to the *Scopus* database). This demonstrates the impact of histochemistry on a wide variety of research subjects (from cell and tissue biology to anatomy and pathology, from zoology to botany, from ecology to nanotechnology), where histochemistry is essentially used for localizing (and often quantifying) *in situ* single molecules or molecular complexes to relate structural organization and function.

This book aims at providing an (certainly nonexhaustive) overview of histochemical techniques, through a series of lab-tested protocols for the detection of specific molecules or metabolic processes, both at light and electron microscopy. More in detail, the book is divided into six parts covering a variety of chemical targets.

The first part is on *vital histochemistry*, including overviews on single-cell histochemistry and autofluorescence. In these chapters, the detection of enzymatic activities is shown through the protocols for detection of peptidases, and a series of enzyme-histochemical methods are described for investigating functional histology in different invertebrate taxa.

Lectin histochemistry is represented by an overview followed by the use of lectins to detect glycosylation-specific cell types, cancer cells, or apoptotic cells.

Histochemistry of proteins used to be the hardest part of the histochemical course for a student in the 1970s, with a huge number of reactions for different chemical groups; nowadays antibodies have the stage, and in the third part, they are used to detect proteins marking neuronal differentiation, the myogenic progenitors, or autophagy. An essential prerequisite for all these techniques is antigen preservation and detection, which is often made problematic by sample fixation and embedding; a chapter is therefore devoted to the most suitable protocols for retrieving antigens in formalin-fixed paraffin-embedded specimens and frozen sections.

The fourth part on *lipid histochemistry* offers basics in fixation and tissue processing, staining for myelin, lipids in yeast, and single lipid droplets.

Nuclear histochemistry, after an overview of DNA fluorochromes, contains protocols for staining and labeling DNA and RNA at electron microscopy as well as for single- and double-strand breaks detection.

The last part is on *plant histochemistry* and deals with nuclear proteins, plant secretory structures, and acetogenins.

From this short list of contents, covering molecules, tissues, and species very far from each other, a simple conclusion may be drawn: histochemistry can be, and actually is, applied in *all* these cases and with successful results. With their specificity and resolution, histochemical and cytochemical methods are more than alive today and can effectively help scientists in very different research fields to elucidate biological issues through a unique approach to molecular biology in situ.

Pavia, Italy

*Carlo Pellicciari
Marco Biggiogera*

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