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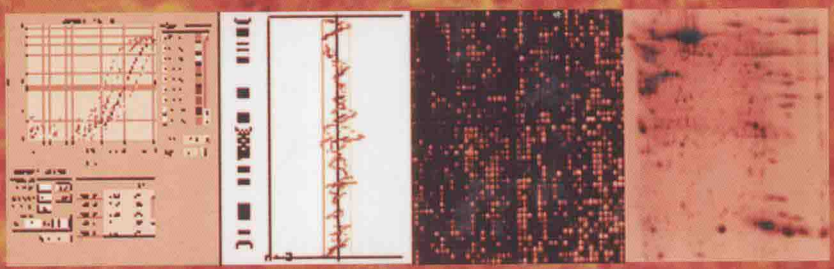
Laser Capture Microdissection

Methods and Protocols

Edited by

Graeme I. Murray

Stephanie Curran



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Graeme I. Murray

and

Stephanie Curran

Department of Pathology,
University of Aberdeen, Aberdeen, UK

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Laser Capture Microdissection

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Preface

Laser microdissection techniques have revolutionized the ability of researchers, generally, and pathologists in particular, to carry out molecular analysis on specific normal and diseased cells and fully utilize the power of current molecular technologies, including polymerase chain reaction (PCR), microarrays, and proteomics. The primary purpose of *Laser Capture Microdissection: Methods and Protocols* is to provide readers with practical advice on how to carry out tissue-based laser microdissection successfully in their own laboratories using the microdissection systems available and how best to apply a wide range of molecular technologies. The individual chapters encompass detailed descriptions of each of the laser-based microdissection systems. Applications of the laser microdissected tissue described in the book include PCR in its many different forms and gene expression analysis involving microarrays and proteomics.

The editors are especially grateful to all the contributing authors for the time and effort they have put into writing their chapters.

The series editor, John Walker, has expertly guided us through the editorial process, while Craig Adams of Humana Press has been very helpful in dealing with all the publication related issues.

We are particularly pleased to acknowledge the excellent secretarial support of Ms. Anne McMillan of the Department of Pathology, University of Aberdeen who helped us deal efficiently with all the correspondence relating to this book. We hope the readers will find this volume valuable.

Graeme I. Murray
Stephanie Curran

Contributors

Jill Bayliss • *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*

Rainer Maria Bohle • *Department of Pathology, Justus-Liebig-University, Giessen, Germany*

Guilherme Brandao • *Division of Pathology, Yale University School of Medicine, New Haven, CT*

Carlos Caldas • *Cancer Genomics Program, Department of Oncology, University of Cambridge, Cambridge, UK*

Grace Callagy • *Cancer Genomics Program, Department of Oncology, University of Cambridge, Cambridge, UK*

Xiao-Ping Chen • *Department of Microbiology and Immunology, Tong-Ji University School of Medicine, Shanghai, China*

Kheng Chew • *Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen, UK*

José Costa • *Department of Pathology, Yale University School of Medicine, New Haven, CT*

Margaret E. Cruickshank • *Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen, UK*

Stephanie Curran • *Department of Pathology, University of Aberdeen, Aberdeen, UK*

Wolfgang Dietmaier • *Institute of Pathology, University of Regensburg, Regensburg, Germany*

Deborah Dillon • *Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*

Colleen M. Feltmate • *Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*

David J. Fink • *Department of Neurology, University of Michigan, Ann Arbor, MI*

Ludger Fink • *Department of Pathology, University of Giessen, Giessen, Germany*

Edward A. Fisher • *Division of Cardiology (Department of Medicine), Department of Cell Biology, NYU School of Medicine, New York, NY*

Jean-François Franetich • *INSERM U-511, Immunobiologie Cellulaire et Moléculaire des Infections Parasitaires, Centre Hospitalier-Universitaire Pitié-Salpêtriere, Université Pierre et Marie Curie, Paris, France*

NADINE T. GAISA • *Institute of Pathology, University of Regensburg, Regensburg, Germany*
LISE METTE GJERDRUM • *Institute of Pathology, Aarhus University Hospital, Aarhus, Denmark*
STEPHEN HAMILTON-DUTOIT • *Institute of Pathology, Aarhus University Hospital, Aarhus, Denmark*
ARNDT HARTMANN • *Institute of Pathology, University of Basel, Basel, Switzerland*
VICTORIA HOLTSCHLAG • *Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO*
LUCY JACKSON • *Cancer Genomics Program, Department of Oncology, University of Cambridge, Cambridge, UK*
LONG JIN • *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*
JUN-YA KAIMORI • *Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, Osaka, Japan*
RUTH KNUECHEL • *Institute of Pathology, University Hospital Aachen, RWTH, Aachen, Germany*
IKUO KOBAYASKI • *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*
HANS KREIPE • *Institute of Pathology, Medizinische Hochschule Hannover, Hannover, Germany*
RENE C. KRIEG • *Institute of Pathology, University Hospital Aachen, RWTH, Aachen, Germany*
LAURA C. LAWRIE • *Department of Pathology, University of Aberdeen, Aberdeen, UK*
ULRICH LEHMANN • *Institute of Pathology, Medizinische Hochschule Hannover, Hannover, Germany*
RICARDO V. LLOYD • *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*
JOAKIM LUNDEBERG • *Department of Biotechnology, KTH, Stockholm, Sweden*
VERONICA I. LUZZI • *Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO*
DANIELE MARRAS • *Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, NY*
MARINA MATA • *Department of Neurology, University of Michigan, Ann Arbor, MI*
DOMINIQUE MAZIER • *INSERM U-511, Immunobiologie Cellulaire et Moléculaire des Infections Parasitaires, Centre Hospitalier-Universitaire Pitié-Salpêtriere, Université Pierre et Marie Curie, Paris, France*

PATRICK MICKE • *Cancer Center Karolinska, Department of Oncology and Pathology, Karolinska Institute, Stockholm, Sweden*
SAMUEL C. MOK • *Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*
GRAEME I. MURRAY • *Department of Pathology, University of Aberdeen, Aberdeen, UK*
BRINA NEGIN • *Department of Pathology, Brigham and Women's Hospital, Boston, MA*
KOUSAKU OKUBO • *Center for Information Biology, National Institute of Genetics, Shizuoka, Japan*
ARNE ÖSTMAN • *Cancer Center Karolinska, Department of Oncology and Pathology, Karolinska Institute, Stockholm, Sweden*
CLOUD P. PAWELETZ • *Department of Anatomy, Physiology, and Genetics, Institute for Molecular Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD*
FREDRIK PONTEN • *Institute for Genetics and Pathology, University of Uppsala, Uppsala, Sweden*
XIANG QIAN • *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*
PATRICK H. ROONEY • *Department of Pathology, University of Aberdeen, Aberdeen, UK*
KATHARINA RUEBEL • *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*
JEAN-PHILIPPE SEMBLAT • *INSERM U-511, Immunobiologie Cellulaire et Moléculaire des Infections Parasitaires, Centre Hospitalier-Universitaire Pitié-Salpêtriere, Université Pierre et Marie Curie, Paris, France*
MAKOTO SHIBUTANI • *Division of Pathology, National Institute of Health Sciences, Tokyo, Japan*
OLIVIER SILVIE • *INSERM U-511, Immunobiologie Cellulaire et Moléculaire des Infections Parasitaires, Centre Hospitalier-Universitaire Pitié-Salpêtriere, Université Pierre et Marie Curie, Paris, France*
ROBERT STOEHR • *Institute of Pathology, University of Regensburg, Regensburg, Germany*
MASARU TAKENAKA • *Graduate School of Life Sciences, Kobe Women's University, Kobe, Japan*
GIOVANNI TALLINI • *Division of Pathology, Yale University School of Medicine, New Haven, CT*
EUGENE TROGAN • *Graduate School of Biological Sciences, Mount Sinai School of Medicine, New York, NY*

CHIKAKO UNEYAMA • *Division of Pathology, National Institute of Health Sciences, Tokyo, Japan*
ANNE VERSMOLD • *Institute of Pathology, Medizinische Hochschule Hannover, Hannover, Germany*
MARK A. WATSON • *Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO*
PETER J. WILD • *Institute of Pathology, University of Regensburg, Regensburg, Germany*
SHUYA ZHANG • *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*
KARL ZHENG • *Columbia University School of Medicine, New York, NY*

Contents

I

Introduction

1

An Introduction to Laser-Based Tissue Microdissection Techniques

Stephanie Curran and Graeme I. Murray

Summary

The development and application of laser-based tissue microdissection techniques has provided a major impetus to the sensitive and specific molecular analysis of solid tissues and tumors. This chapter provides an overview of the different laser-based microdissection systems and an introduction to the principles involved in the function and applications of these individual systems.

Key Words: Laser capture microdissection; laser microbeam microdissection; molecular analysis.

1. Introduction

Tissues, especially diseased tissues, are complex three-dimensional structures composed of heterogeneous mixtures of morphologically and phenotypically distinct cell types. The meaningful molecular analysis of morphologically and/or phenotypically distinct cell types from such tissues requires rapid, efficient, and accurate methods for obtaining specific population of cells.

The molecular investigation of solid tissues, especially tumors, has been revolutionized over the past decade by the development of accurate, rapid, and effective laser-based methods of tissue microdissection *(1,2)*. This has provided an extremely valuable and sophisticated tool to fully utilize the power and sensitivity of modern molecular analytical technologies in the detailed investigation of many different diseases and provided significant new insights into the pathogenesis of these diseases. Many of the investigations using laser microdissected cells have focused on specific types of cancers, where the morphological and phenotypic heterogeneity and complexity of tissues is often the greatest *(1–3)*.

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Individual studies have usually based cell selection on specific morphological criteria of stained histological sections, but phenotypic characteristics as defined by immunohistochemistry of antigen expression *(4)* or genotypic features as demonstrated by *in situ* hybridization have also been used as selection criteria *(5)* and demonstrate the power of laser-based microdissection techniques. One of the major advantages of using laser microdissection methods to obtain specific cells for molecular analysis, especially from the viewpoint of the pathologist, is that the procedure is carried out under direct-light microscopic visualization of the cells. Whereas other technologies used to isolate specific cell populations for molecular analysis—e.g., fluorescent-activated cell sorting or magnetic bead-based cell separation—are indirect techniques with no microscopic visualization of the cells and require the availability of suitable antibodies to aid cell selection. Moreover, the methods used to prepare single cell suspensions (e.g., proteolytic enzyme digestion) that are necessary for antibody-based cell separation techniques from solid tissues may result in alteration or modification of cellular constituents. There is no doubt that the availability of laser-based microdissection technologies has provided a major impetus to molecular pathology research and this technology is now found in many laboratories worldwide (as represented in the diverse geographic locations of contributors to this volume). The wide availablity of this easy-to-use technology has allowed many questions in a range of research disciplines to be answered that previously could not be asked or answered using manual methods of tissue microdissection because of the imprecise nature of manual methods of microdissection or the time required to obtain tissue.

There are two major systems that have been developed for performing laser-assisted tissue microdissection—namely, laser capture microdissection and laser microbeam microdissection. Both types of systems have now been commercially available for several years. An overview of the principles, advantages, and potential disadvantages of each of the systems will be provided in this introductory chapter, detailed descriptions and applications of the individual systems are given in the relevant chapters in this volume.

2. Overview of Laser Microdissection Systems

2.1. Laser Capture Microdissection

The laser capture microdissection system was developed in the mid-1990s at the National Institutes of Health by Emmert-Buck and colleagues *(6,7)*, who recognized the need to develop a microscope-based microdissection system for accurately and efficiently microdissecting cells from histological tissue sections to fully exploit emerging molecular analytical technologies. They developed this system primarily to facilitate the molecular analysis of solid tumors.