

METHODS IN MOLECULAR BIOLOGY

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Plant Cell Morphogenesis

Methods and Protocols

Second Edition

Edited by

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Cover Illustration Caption: Growing epidermal cells color-coded according to several growth parameters (from Chapter 18)

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Preface

Five years after the publication of the first edition of this volume, the focus of post-genomic land plant biology is definitely leaving the confines of a handful of major models, such as *Arabidopsis thaliana* or *Physcomitrella patens*, thus opening new spaces for defining and solving major questions of basic plant biology. Collecting wisdom and skills accumulated mostly from work on the founding molecular biology models can undoubtedly guide applications in other species, including crop plants. As editors of this volume, we strive to reflect this development in order to inspire future research in cellular aspects of land plant morphogenesis.

Studying the dynamics of plant shapes, starting from the cellular level and advancing via tissues to organs and onward to the whole plant, is a truly fascinating perspective that we share with the nineteenth- and twentieth-century founders of our field. Here in Prague, we acknowledge continuous inspiration by Jan Evangelista Purkyně and, in our field especially, his disciple Julius Sachs, who started his career in the German-speaking part of the Charles University in Prague and became the father of modern plant physiology (including pioneering studies of the processes of plant morphogenesis). We have worked on this volume in a building constructed in 1898 for the German Plant Physiology department, directed in those years by Professor Hans Molisch, author of *Mikrochemie der Pflanzen* (published after his move to Vienna in 1909). Several of the Czech contributors to this volume consider themselves “academic grandchildren” of Professor Bohumil Němec, one of the fathers of experimental plant cell biology. When Němec discovered the decisive role of starch statoliths in root columella for root gravitropism (1900), he immediately understood that, to function in graviperception, columellar cells need to be not only internally dynamically polarized but also connected in a communicative (i.e., signaling) network with other root cells. This indicated an intricate internal cellular structure and intercellular communication, beyond the imagination of scientists of those times. Němec taught us, via his students (our teachers) and his impressive published volumes on plant biology, to understand tissues and cells as products, not mere constituents or “bricks,” of a living plant body as a whole.

As in the first edition of this book, the first eight chapters of this volume (Chapters 1–8) focus on the visualization of plant cell structures, since seeing the objects of interest is an obvious prerequisite of understanding the processes that brought them into being. Chapters 1 and 2 present a contemporary take on light microscopy, the classical approach that was instrumental in establishing the plant cell biology field. These chapters are directly linked to a classic plant histochemistry methods book published by Bohumil Němec—*Botanical microtechnique* (“Botanická mikrotechnika” in Czech, Prague 1962).

While light microscopy remains a central visualization method in plant cell biology, electron microscopy provides exciting insights into cellular ultrastructure. Chapters 3 and 4 describe a collection of useful electron microscopy techniques, including immunogold localization procedures. Chapters 5 and 6 then present techniques for in situ qualitative analysis of plant cell wall composition.

Modern cell biology is dominated by digital data. This is reflected by the recurrent inclusion of digital image analysis protocols in several of the following chapters. Chapters 7 and 8 provide generally applicable techniques for quantitative image analysis, as well as for the only seemingly mundane task of presenting image data *lege artis*.

The second part (Chapters 9–13) is devoted to qualitative and quantitative detection of the organization and dynamics of individual intracellular structures responsible for the generation of cell shape, in particular the two cytoskeletal systems, as well as endomembranes, whose structure and behavior can be studied using in vivo fluorescent markers.

The third section (Chapters 14–19) is devoted to exciting new possibilities of manipulating intracellular structures by means of optical tweezers, and probing their mechanical features by Cellular Force Microscopy. It also covers detailed monitoring and quantifying structural dynamics of meristems and developing organs on the cellular level.

The choice of the experimental model is, as a rule, tightly linked with the choice of questions that can be studied. It is hard to find a field where this would be more obvious than the study of cell morphogenesis. In the final six chapters (Chapters 20–25), we present specific techniques for studying model cell types such as filaments of the moss *Physcomitrella patens*, *Arabidopsis* root hairs, pavement cells, developing xylem vessels, pollen tubes, or plant cell lines.

We decided to leave out several topics addressed in the first edition of this book. Several previously included methods received excellent coverage in recent volumes of the *Methods in Molecular Biology* series. This is, in particular, the case for additional electron microscopy techniques, such as cryofixation with freeze substitution and subsequent immunodetection [1], electron microscopy tomography and 3D reconstruction [2, 3], or analyses of nuclear morphology [4]. Omission of other topics, such as automated microscopy application in forward genetics screens, use of laser microdissection to study gene expression, microfluidics applications, general techniques for transient gene expression in plant systems, or heterologous expression in yeast, was in effort to keep the present volume focused and its size manageable.

We approached our task in editing this collection of protocols with the hope that this volume may become a source of inspiration for further research into the morphogenesis of plant cells, tissues, and organs. We are especially grateful to many colleagues—the best experts in their fields from all over the world—who accepted our invitation and contributed chapters to this volume, for making it more likely that our hope may be fulfilled.

Prague, Czech Republic

*Fatima Cvrčková
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Contents

<i>Preface</i>	<i>v</i>
<i>Contributors</i>	<i>ix</i>
1 Essential Methods of Plant Sample Preparation for Light Microscopy	1
<i>Aleš Soukup and Edita Tylová</i>	
2 Selected Simple Methods of Plant Cell Wall Histochemistry and Staining for Light Microscopy	27
<i>Aleš Soukup</i>	
3 Chemical Fixation, Immunofluorescence, and Immunogold Labeling of Electron Microscopical Sections	43
<i>Ilse Foissner and Margit Hoefftberger</i>	
4 Essential Methods of Plant Sample Preparation for High-Resolution Scanning Electron Microscopy at Room Temperature	63
<i>Jana Nebesářová</i>	
5 Fluorescence Lifetime Imaging of Plant Cell Walls	77
<i>Christine Terryn and Gabriel Paës</i>	
6 Raman Spectroscopy in Nonwoody Plants	83
<i>Dorota Borowska-Wykręt and Mateusz Dulski</i>	
7 Image Analysis: Basic Procedures for Description of Plant Structures	109
<i>Jana Albrechtová, Zuzana Kubínová, Aleš Soukup, and Jiří Janáček</i>	
8 From Data to Illustrations: Common (Free) Tools for Proper Image Data Handling and Processing	121
<i>Fatima Cvrčková</i>	
9 Visualizing and Quantifying In Vivo Cortical Cytoskeleton Structure and Dynamics	135
<i>Amparo Rosero, Denisa Oulehlová, Viktor Žárský, and Fatima Cvrčková</i>	
10 Quantitative and Comparative Analysis of Global Patterns of (Microtubule) Cytoskeleton Organization with CytoskeletonAnalyzer2D	151
<i>Birgit Möller, Luise Zergiebel, and Katharina Bürstenbinder</i>	
11 Using FM Dyes to Study Endomembranes and Their Dynamics in Plants and Cell Suspensions	173
<i>Adriana Jelínková, Kateřina Malínská, and Jan Petrášek</i>	
12 Transient Gene Expression as a Tool to Monitor and Manipulate the Levels of Acidic Phospholipids in Plant Cells	189
<i>Lise C. Noack, Přemysl Pejchar, Juraj Sekereš, Yvon Jaillais, and Martin Potocký</i>	
13 The Photoconvertible Fluorescent Protein Dendra2 Tag as a Tool to Investigate Intracellular Protein Dynamics	201
<i>Alexandra Lešková, Zuzana Kusá, Mária Labajová, Miroslav Krausko, and Ján Jásik</i>	

14	Cellular Force Microscopy to Measure Mechanical Forces in Plant Cells	215
	<i>Mateusz Majda, Aleksandra Sapala, Anne-Lise Routier-Kierzkowska, and Richard S. Smith</i>	
15	Optical Trapping in Plant Cells	231
	<i>Tijs Ketelaar, Norbert de Ruijter, and Stefan Niehren</i>	
16	Sequential Replicas: Method for In Vivo Imaging of Plant Organ Surfaces that Undergo Deformation	239
	<i>Dorota Kwiatkowska, Sandra Natonik-Białoń, and Agata Burian</i>	
17	Time-Lapse Imaging of Developing Shoot Meristems Using A Confocal Laser Scanning Microscope	257
	<i>Olivier Hamant, Pradeep Das, and Agata Burian</i>	
18	Quantifying Plant Growth and Cell Proliferation with MorphoGraphX.	269
	<i>Soeren Strauss, Aleksandra Sapala, Daniel Kierzkowski, and Richard S. Smith</i>	
19	Kinematic Characterization of Root Growth by Means of Stripflow	291
	<i>Tobias I. Baskin and Ellen Zelinsky</i>	
20	Automated Image Acquisition and Morphological Analysis of Cell Growth Mutants in <i>Physcomitrella patens</i>	307
	<i>Giulia Galotto, Jeffrey P. Bibeau, and Luis Vidali</i>	
21	Live Cell Imaging of Arabidopsis Root Hairs	323
	<i>Tijs Ketelaar</i>	
22	Morphological Analysis of Leaf Epidermis Pavement Cells with PaCeQuant	329
	<i>Birgit Möller, Yvonne Poeschl, Sandra Klemm, and Katharina Bürstenbinder</i>	
23	Imaging of Developing Metaxylem Vessel Elements in Cultured Hypocotyls	351
	<i>Takema Sasaki and Yoshihisa Oda</i>	
24	Antisense Oligodeoxynucleotide-Mediated Gene Knockdown in Pollen Tubes	359
	<i>Martin Potocký, Radek Bezvoda, and Přemysl Pejchar</i>	
25	Plant Cell Lines in Cell Morphogenesis Research: From Phenotyping to -Omics	367
	<i>Petr Klíma, Vojtěch Čermák, Miroslav Srba, Karel Müller, Jan Petrášek, Josef Šonka, Lukáš Fischer, and Zdeněk Opatrný</i>	
	<i>Index</i>	377

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