Fungal Biology

Tanya E. S. Dahms
Kirk J. Czymmek *Editors*

Advanced Microscopy in Mycology

Springer

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Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become the subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet, detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with the living and non-living is essential to underpin effective and innovative technological developments. This Series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, microscopy, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems, and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this Series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students. This volume series tracks the continuous developments in fungal biology with the publication of each volume.

More information about this series at http://www.springer.com/series/11224

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Preface

Fungi have been under a microscope almost since its inception (Zacharias and Hans Jansen 1595), with Hooke (1665) first describing and illustrating *Phragmidium mucronatum* (parasitic rose rust) and the saprophytic *Mucor*, and Malpighi (1675, 1679) documenting a variety of fungi. Every subsequent development in molecular tagging and microscopic instrumentation has impacted mycology. Since 2008, the Nobel Prize in Chemistry has been awarded twice to researchers who have developed advanced tools for microscopic imaging. The most recent was awarded last year for developing super-resolution fluorescence microscopy, which collectively pushed microscopic resolution beyond the diffraction limit, the holy grail of optical microscopy since first defined by Ernst Abbé in 1873. The second involved the discovery and development of the green fluorescent protein (GFP) which has produced a spectral rainbow of fluorescent proteins for tagging and tracking molecules in living cells. This development, revolutionized biological microscopy, and inspired the discovery of mEos fluorescent proteins which have enhanced certain types of super-resolution microscopy. Such advances now allow us to image cells at resolutions that are an order of magnitude better than diffraction limited optical approaches.

This volume is a compilation of the principles underscoring various advanced microscopy methods and how they have been, or have the potential to be, applied to mycology. Chapter 1 begins with Drs. Rosa Mouriño Perez (Centro de Investigación Cientifica y de Educación Superior de Ensenada) and Robby Roberson (Arizona State University) offering a comprehensive overview of the confocal principle, confocal laser scanning microscopy and its application to fungal biology. In some ways, this chapter is a cornerstone of the volume, as so many of the newer sophisticated fluorescence-based methods depend on this technology. Drs. Norio Takeshita from Karlsruhe Institute of Technology and Oier Etxebeste at the University of Basque Country teamed up in Chapter 2 to introduce us to fluorescence-based techniques that have been used to study fungi, including bimolecular fluorescence complementation (BiFC), the so-called four-letter F-words (i.e. FRET, FRAP, FLIM), and novel dyes (mEos) that have been developed for super-resolution methods. This leads us directly to Chapter 3 in which Drs. James Dodgson, Rafael Carazo Salas, Anatole Chessel from the University of Cambridge and Dr. Susan Cox at King's College London describe the various types of recently developed super resolution

microscopy techniques, the subject of this year's Nobel Prize, and how they have been used to uncover minute details within fungi both spatially and temporally. In Chapter 4, Dr. Annette Naumann from the Julius-Kühn Institute describes how Fourier transform infrared (FTIR) microscopy is uniquely poised to image and determine the chemical make-up of fungi alone, and in the context of a common substrate, wood. Chapter 5 turns to Drs. Zhiting Liang, Yong Guan, Shan Chen and Yangchao Tian at the National Synchrotron Radiation Laboratory in the University of Science and Technology in China who describe how full-field hard X-ray tomography has been applied for the first time to reconstruct, at the nanoscale, the three-dimensional (3D) structure of yeast, along with the future potential of this method. Chapter 6 by Dr. Yajing Shen from the City University of Hong Kong outlines a completely novel and clever method for the *in situ* characterization of yeast at the nano scale, using environmental scanning electron microscopy (ESEM) and focused ion beam milling (FIB). Some of the methods from Chapter 6 could also find application to atomic force microscopy (AFM), the subject of Chapter 7 by Drs. Cécile Formosa and Etienne Dague from the Centre National de la Recherche Scientifique. In this chapter, Formosa and Dague tell us how AFM can be used to image and quantify the biophysical properties of live yeast and fungi. Finally in Chapter 8, coeditors Dr. Tanya Dahms from the University of Regina and Dr. Kirk Czymmek Carl Zeiss Microscopy, Inc. describe advances in biosensors, 3D imaging, correlative microscopy, and other recent advances in microscopy methods as applied to fungi.

While most of the methods described in this volume have, at least in principle, mycological applications, there are so many open questions that could be answered using the advanced microscopy described herein. Even for microscopes that are commercialized (confocal, FTIR, AFM), they often remain most effective in the hands of microscopy experts, while other more specialized methods are usually only found in a handful of labs (super resolution and ESEM-FIB) or require a trip to a synchrotron source (X-ray tomography). That being said, those researchers who operate or create specialized instrumentation in their lab are always keen to collaborate (instruments seeking applications) and so I would encourage you to make friends with one or more microscopy specialist. Just like correlative microscopy, the fruit of collaborative research is more like the fruit tree rather than simply a collection of fruit.

In Gratitude

KJC expresses his thanks to Dr. Terry Hammil who let me into his electron microscopy course as an undergraduate and triggered my lifelong passion for microscopy and fungi. Also to Drs. Karen Klomparens, Dr. Stanley Flegler and Dr. Joanne Whallon for the opportunity to learn every advanced microscope and technique I could get my hands on. A special thanks also goes to Drs. Rick Howard and Tim Bourett. Numerous great conversations, numerous experiments, tons of fun over

the years, always with a critical eye and searching for the best representation of cell morphology and reality. A special thanks goes to Drs. Seogchan Kang and Dr. Jeff Caplan, for their long-term and ongoing fruitful collaborations. Finally, my gratitude to Drs. Hye-Seon Kim and Dr. Carissa Young who both spent days optimizing and imaging until the result was as good as it could get.

TESD is grateful to Arthur G. Szabo who taught me all I know about lasers and spectroscopy and Linda Johnston who first introduced me to AFM. Dr. Susan Kaminskyj is the person who guided me into mycological microscopy, having met as postdoctoral fellows at Purdue University. Our collaboration, beginning over sips of scotch, has spanned 15 years and has been a constant source of inspiration—this volume would not exist without her! I thank Drs. Vijai Gupta and Maria Tuhoy for the invitation to create this volume as part of the Springer series in mycology, along other collaborative reviews. Their confidence in me and Vijai's constant encouragement has been invaluable for bringing this volume to fruition. I thank Eric Stannard, Springer editor, for his valuable advice, encouragement and flexibility throughout this project and Gina Kahn, assistant editor, who has kept this project on track, offered her copyediting expertise and who wove the chapters together into this volume. Thanks to the University of Regina (UofR) faculty, students and staff, who put up with me during this process, and the UofR for providing the freedom to pursue such a worthy project of this magnitude. TESD's introductory and advanced microscopy undergraduate and graduate students have been a constant source of inspiration—they are my greatest teachers. Finally, I acknowledge that this volume would not have been possible without the support of my partner in life, Douglas Casimel.

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