Read and Win: Current Protocols Select - Imaging and Microscopy

Microscopy is a visual tool with multiple applications in the field of biology. Designed to serve as a bench-side practical guide for laboratory researchers, this book contains comprehensive methodology covering a wide-range of topics from live cell microscopy and FRET analysis to confocal and super resolution imaging. Supporting protocols are provided to address techniques used in sample and reagent preparation and alternate protocols are discussed to aid wider applicability of the method described. Moreover, an entire section is dedicated towards specifying approaches for digital image analysis to aid image acquisition and processing. Consolidated by experienced scientists and editors, this volume provides an authoritative and detailed compilation of microscopy protocols with stepwise methods and applications that can cater to both, novice and experienced researchers alike.

Win the book!

To have a chance of winning the book read Issue 1, 2016 of Imaging & Microscopy (page 13). As a subscriber you could read the issue already online or order your own copy (as a free trial copy). Take part in our competition and send your answer to contact@imaging-git.com with the subject line Read & Win. All correct answers will be entered in a prize draw and the lucky winner will receive a copy of "Material Characterization".

Closing date: May 24th 2015.

Dr. Watkins is a distinguished professor at the University of Pittsburgh and founder and director of the Center for Biologic Imaging an internationally renowned intellectual nexus for the application of all aspects of microscopic imaging specifically for the study of molecular, cellular and tissue biology. He has published over 550 peer reviewed papers (H-index is 117 lifetime) With 2 million dollars in extramural funding his current research is to develop fast, single molecule imaging tools for use in vitro or in vivo.
**Dr. St. Croix** is an Associate Professor with Appointments in the Departments of Environmental and Occupational Health and Cell Biology at the University of Pittsburgh. Dr. St Croix’s expertise in the application of fluorescence-based probes and state of the art in vivo imaging technologies have led to her appointment as Associate Director of the Center of Biologic Imaging (CBI) at the University of Pittsburgh and invitations to present her work nationally and internationally and to take lead roles in well-respected courses such as Quantitative Fluorescence Microscopy (Mount Desert Island Biology Laboratory).

**Interview with the Editors**

**Is there - in your experience - some things that researchers in microscopy often do incorrectly?**

**Watkins:**

- Probably the most overlooked and common mistake is that users do not set Kohler illumination correctly, hence endless contamination of a beautiful brightfield image with dust and other contaminants in the microscope.
- Images, particularly confocal images are always oversaturated. The user goes for a pretty image in which the colors “pop” but which have close to zero quantitative value.
- Nyquist sampling is ignored or not thought about. This is the sampling frequency to get useful numbers out of samples. It’s about 2.3 times the resolving power of the system and should be included in all sampling calculations. X, Y, Z and T...
- People forget that Z resolution is dependent on the square of NA and so at best half XY so sampling can be half that too.
- When using confocal, people undersample in T and miss so much but massively oversample in XY and Z.
- Images are collected at 8 bit bit depth and then people want to get quantitative data out that actually means something (images should always be collected as 12 bit images).
- The collector lens (between the microscope and camera) is very rarely appropriate for the Magnification and NA being used. Essentially resolution is sacrificed for field of view (things look great down the scope but lousy in the printed image).
- White balancing.. is sooooo easy in bright field but so often ignored.
What is in your opinion the “next big thing” in microscopy?

**Watkins:** If you consider that super-resolution is “done” and whatever you do you cannot beat the basic laws of physics and light, the next big thing(s) are all related to improving the spectral, speed and depth capabilities of microscopy:

- Adaptive optics
- Longer wavelength dyes
- Dyes that are clever
- Detectors that are still faster and have higher QE
- Robotics that faster
- Less moving parts
- Better and continued integration of probes/robotics/external device control into software
- Fast multicolor inexpensive laser launches (much much less expensive than now)

What is your main focus in research, what is your main scientific interest?

**Watkins:**
Developing imaging solutions for problems in biology principally imaging very fast and at depth in living animals. Specifically in Danio

**St. Croix:**
A major focus of my research is the use of advanced optical imaging technologies to dissect molecular signaling pathways controlling vascular function in rodent and zebrafish model systems of disease. An important facet of this work is the development and in vivo application of novel fluorescent molecular reporters to study the biology of reactive oxygen and nitrogen species.

What was the reason to write the book?

**Watkins:**
It was a natural extension of my duties as imaging editor for Current Protocols, I have been accumulating great units for the periodical which all seemed to fit well together as an integrated unit with just chapter headers to tie things together.

What is the target audience for the book?

**Watkins:**
It’s a practicum, the idea is that users can find out how to do things correctly with this book, and not listen to the nearly always erroneous advice of friends
What knowledge is prerequisite for the book?

Watkins/St. Croix:
None. Well being interested in use and application of microscopy would help

What is the structure of the book?

Watkins:

It starts with a series of units describing basic components of the microscope/imaging system and then digs into each imaging technology to give users both an intellectual and practical (with recipes) appreciation of the method.