Microscopy TODAY

Coming Events

2009

AVS 56th Int. Symposium & Exhibition
November. 8–13, 2009, San Jose, CA
www2.avs.org/symposium

2009 MRS Fall Meeting
November 30–December 4, Boston, MA
www.mrs.org

American Society of Cell Biology
December 5–9, 2009, San Diego, CA
www.ascb.org

2010

Multiphoton Microscopy at SPIE Photonics West
January 23-28, 2010, San Francisco, CA
www.spie.org

PITTCON 2010
February 28–March 5, 2010, Orlando, FL
www.pittcon.org

American Chemical Society
March 21-25, 2010, San Francisco, CA
www.acs.org

Focus on Microscopy 2010
March 28-31, 2010, Shanghai, China
www.focusonmicroscopy.org

SPIE Scanning Microscopy 2010
May 17-19, 2010, Monterey, CA
www.spie.org

Electron Backscatter Diffraction Topical Conference
May 24-26, 2010, Madison, WI
www.microbeamanalysis.org/ebsd-2010

Microscience 2010
June 29-July 1, 2010, London, UK
www.rms.org.uk

Microscopy & Microanalysis 2010
August 1–5, 2010, Portland, OR
www.microscopy.org

2011

Microscopy & Microanalysis 2011
August 7–11, 2011, Nashville, TN

2012

Microscopy & Microanalysis 2012
July 29–August 2, Phoenix, AZ

2013

Microscopy & Microanalysis 2013
August 4-8, Indianapolis, IN

Please check the "Calendar of Meetings and Courses in the MSA journal *Microscopy and Microanalysis* for more details and a much longer list of meetings and courses.

Carmichael's Concise Review

Let the Light Shine Through!

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Fluorescent proteins have been used to great benefit in studies of cell biology. One of the limiting factors in their utility has been that the wavelengths (λ) emitted by most fluorescent proteins are in the visible spectrum and cannot penetrate far through tissues because of absorbance by hemoglobin, water, and lipids, as well as light-scattering. Longer λ can penetrate tissues better, but up until now, proteins that have excitation and emission spectra of longer λ have not been available. Recently, Xiaokun Shu, Antoine Royant, Michael Lin, Todd Aguilera, Varda Lev-Ram, Paul Steinbach, and Roger Tsien [1,2] have engineered a new fluorescent protein that can be excited at 684 nm and has an emission maximum of 708 nm. For reference, visible red has a λ of about 650 nm, thus longer λ that extend into the infrared are generally not visible to the eye but can be visualized with appropriate detectors.

Shu *et al.* thought phytochromes (pigments in plants that detect light, also found in some bacteria) derived from bacteria were promising because they incorporate biliverdin IXα (BV), a bile pigment involved in heme catabolism and commonly found in mammals. The most promising was a phytochrome from *Deinococcus radiodurans*. Interestingly, this bacterium is listed in the *Guinness Book of World Records* as "the world's toughest bacterium." They re-engineered this phytochrome through four generations of "evolution" to maximize desirable characteristics (such as brightness of emission signal) and minimize undesirable features (such as rapid photobleaching). The fourth-generation Infrared Fluorescent Protein was dubbed IFP1.4. It is about four times brighter than the first generation dye and it is stable over a wide *p*H range.

IFP1.4 could be expressed in cultured cells, and exogenously added BV further increased infrared fluorescence of transfected cells. This demonstrated that BV can permeate membranes. As a demonstration that fusing IFP1.4 with other proteins can be functional, IFP1.4 was fused to a protein that is known to become active and binds to membranes after stimulation with growth factors. Unstimulated cultured cells expressing IFP1.4 fused with this protein showed a signal diffusely distributed in the cytosol. Within 10 minutes after stimulation with insulin, this signal was located in the plasma membrane, demonstrating that IFP1.4 can highlight the trafficking of fusion proteins.

Also, Shu *et al.* used routine recombinant technology to combine a fluorescent dye they developed to an adenovirus that specifically infects the liver in mice. Weak infrared fluorescence of the liver was detected five days after this engineered virus was injected intravenously, and the whole liver was easily detected after BV was injected into the mouse. Using software to manipulate the images, the three-dimensional distribution of fluorescence could be seen in the liver. As a control, BV did not generate infrared fluorescence in mice infected with a virus not combined with IFP1.4 and uninfected mice.

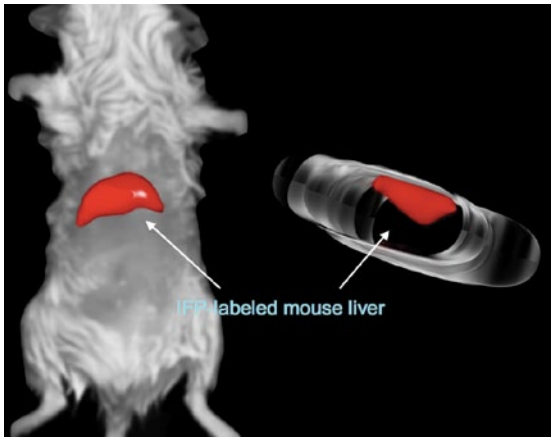
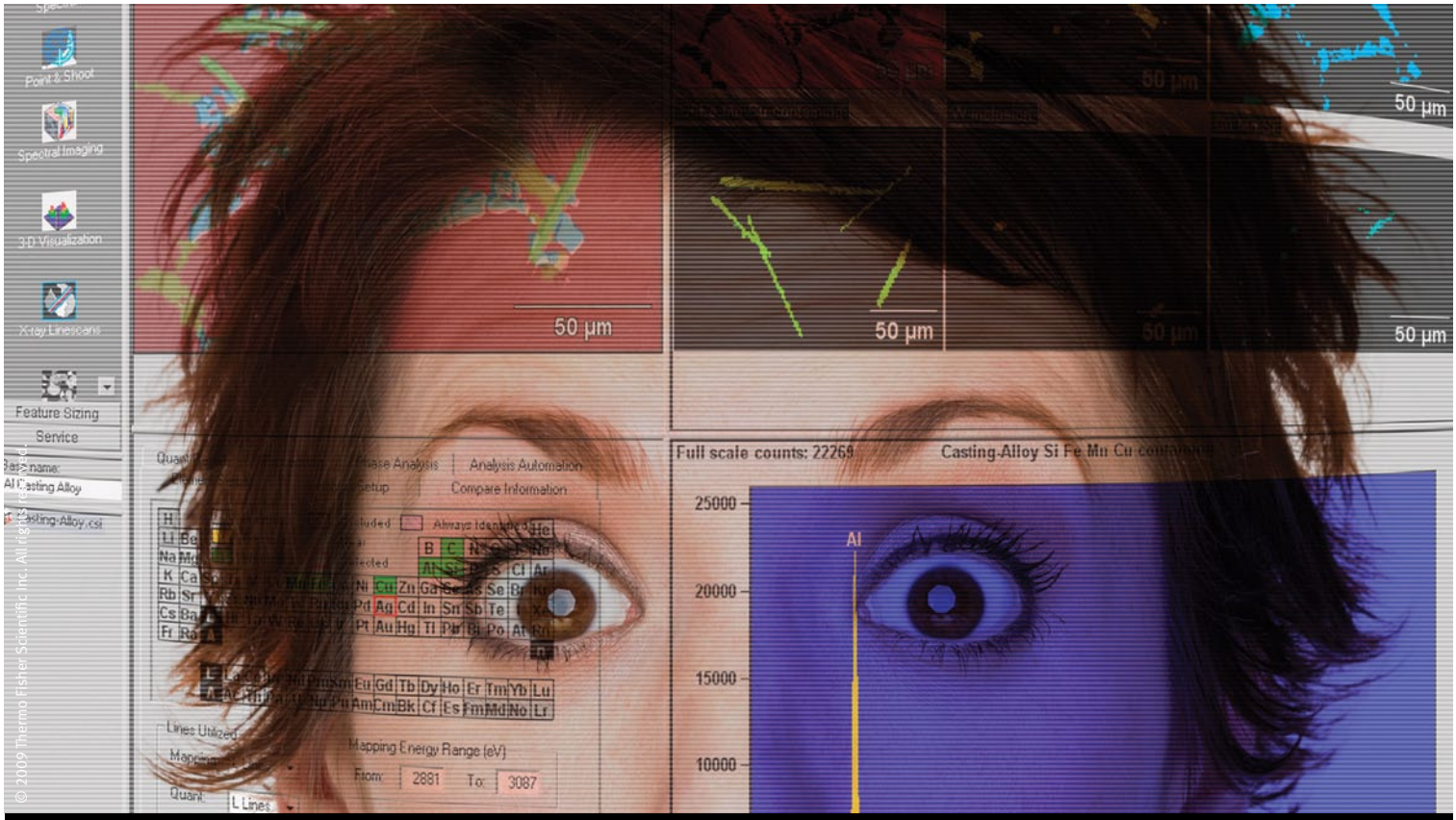


Figure 1: Intact mouse liver infected with an IFP1.4-fused virsus.

doi: 10.1017/S1551929509990976



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In additional studies, livers from mice infected with the IFP1.4-fused virus were processed for histology. The infrared fluorescence remained detectable and in fact was brighter than other fluorescent proteins such as green fluorescent protein. IFP1.4 and related dyes can be imaged over spatial scales extending from subcellular resolution up to strongly pigmented organs within intact animals (see Figure 1). No other class of fluorescent proteins can do this. Even for microscopic imaging where other fluorescent proteins are highly effective, IFPs present some advantages such as reducing cellular autofluorescence. The λ, however, is particularly well-suited to optical tomographic reconstruction, even in intact animals. Also, for several reasons BV is particularly advantageous as a cofactor.

Shu *et al.* concluded by pointing out that more than 1,500 bacteriophytochrome-like materials are already available in accessible databases. There is great potential for novel fluorescent proteins that can increase the useful armamentarium of cell biologists. MT

References

[1] X Shu *et al.*, *Science* 324 (2009) 804-807.

[2] The author gratefully acknowledges Drs. Xiaokun Shu and Roger Tsien for reviewing this manuscript. The author also congratulates Dr. Tsien on winning the 2008 Nobel Prize for Chemistry.