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Tune in to the Excitation Optimum with White Light Laser

by Rolf Borlinghaus and Scott Young, Leica Microsystems

The Objective

In this section, Leica Microsystems highlights application-oriented stories of value to our readers.

Click on the following link to view a recent CNN report on an innovative green technology in the making:

Farm makes oil from pond scum.

Contents

The Leica TCS SP5 X super continuum confocal provides full freedom and flexibility in excitation AND emission. This allows researchers for the first time to freely choose any excitation line within the continuous range of 470 to 670nm in 1nm increments. The Leica TCS SP5 X adapts precisely to any sample. Any dye can be optimally excited while cross-excitation and sample damage are reduced to a minimum. Up to eight excitation lines can be used simultaneously.

When comparing excitation spectra and available emission lines of conventional lasers, the gap is obvious (see **Figure 1**); most dyes cannot be excited at the position of their maximum cross section. A common dye like Alexa 488, although it is designated as a "488nm dye," has an excitation maximum of 500nm, whereas the absorption at 488nm is only 75%. The white light laser allows users to "steplessly" tune the excitation, no matter what the name of the fluorochrome suggests. So the user may excite the fluorochrome at its maximum cross section; for Alexa 546 this would be 561nm.

Nevertheless, this is not necessarily the best excitation position for Alexa 546. In order to stop excitation light from entering the detector, a certain "security distance"

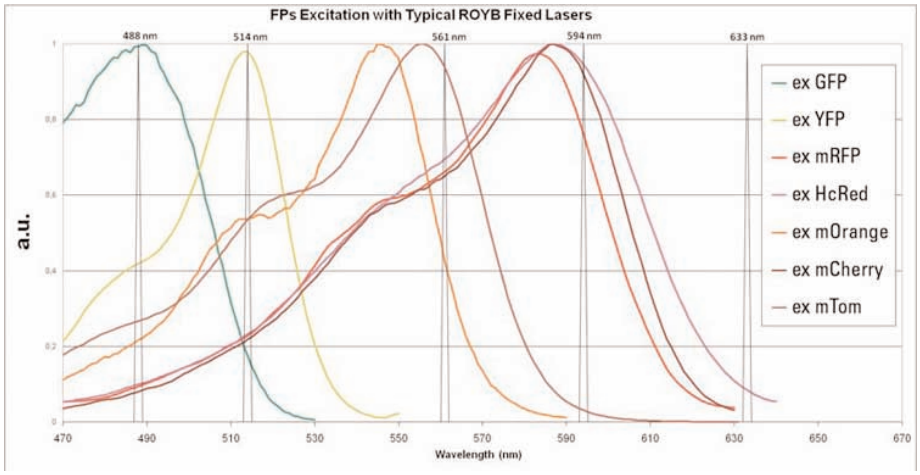

Figure 1: Excitation spectra of various fluorescent proteins and excitation lines of commonly used conventional lasers (ROYB-type). Obviously, most of the shown FPs can be excited optimally with the laser combination indicated.

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White Light Laser

(continued from page 1)

must exist between excitation and the blue edge of the emission band that is collected. If the stokes shift is rather low, then the residual window for emission collection might cut off a significant part of the available photons, which is not desirable. So it sometimes makes sense to excite the dye somewhat off the peak in the blue range (Alexa 488 case) and compensate for the lesser absorption by increasing the laser intensity. Then the user can collect the full emission spectrum.

With the Leica Microsystems tunable laser source, researchers may also find a more efficient excitation for Alexa 546, and get better images at 555nm, for example. (See **Figures 2 and 3**).

The combined operation of tunable excitation and tunable emission can help to find the best setting for excitation and emission. A software tool is available to acquire images at incrementing excitation wavelength (excitation scan) and also automatically adjust the blue cut-off of the emission band; for example, always 10nm off the excitation to stop reflected light from entering the detector.

Furthermore, the environment in which the sample is surrounded will sometimes shift excitation. In some cases, specific dyes have been developed to exploit this phenomenon (pH or Ca^{+2} dyes). Here, with the tunable laser the user can adapt to these alterations and ensure optimal excitation under any circumstances.

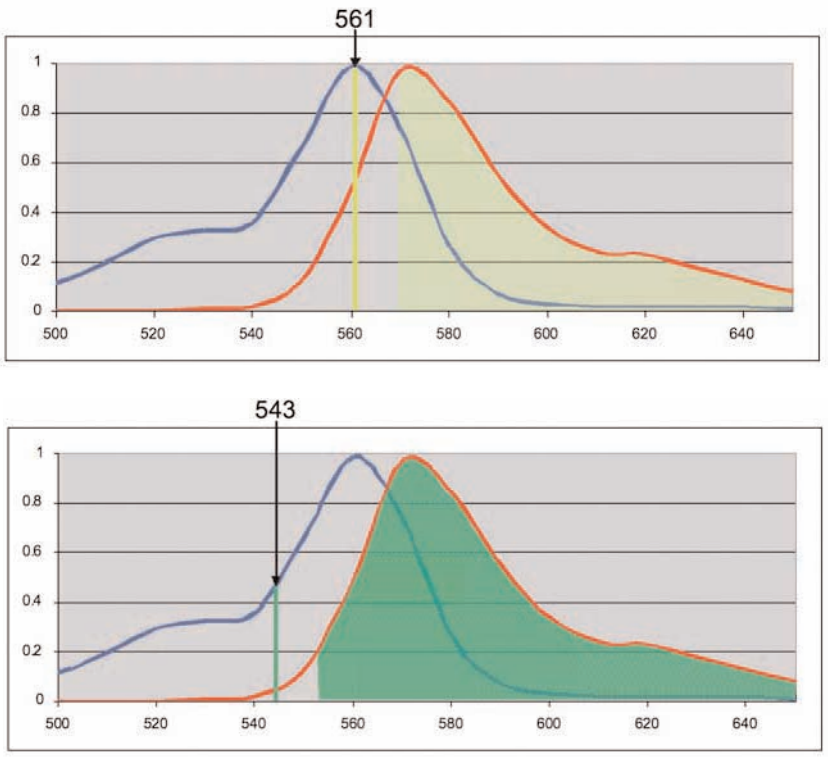


Figure 2: **Top:** excitation at the peak of absorption (here with 561nm from a DPSS laser) is restricting the band for emission collection. Therefore, it might be beneficial to use a line that excites on the blue slope.
Bottom: for Alexa 546, this could be the HeNe 543nm line. Unfortunately, this line is very weak and not efficient for a cross section of only 50%.

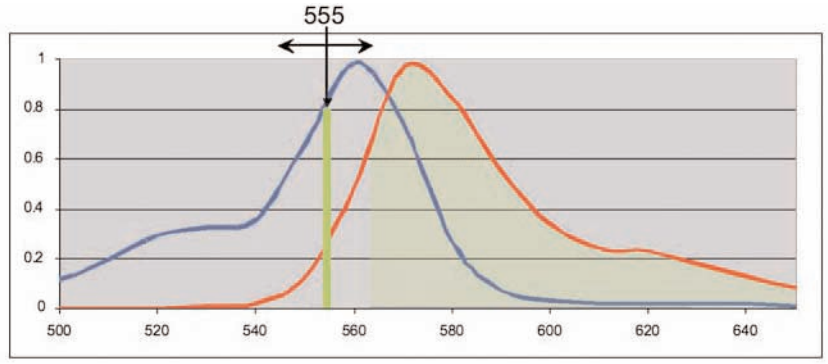


Figure 3: Tunable excitation and ample power density allows optimization of the excitation wavelength and the collection band for emission.

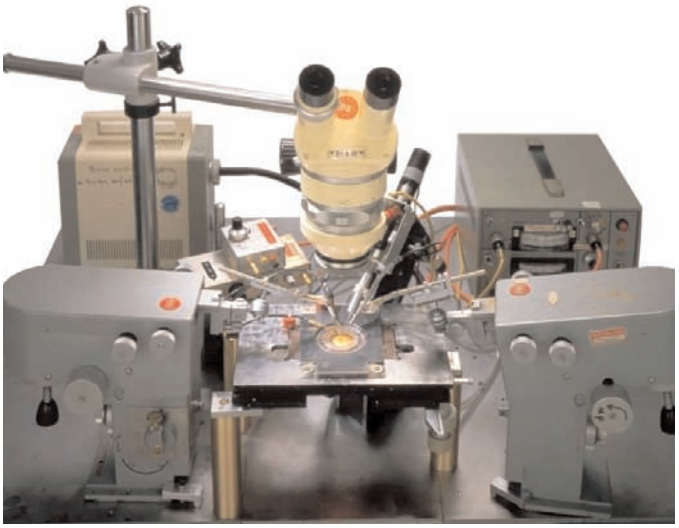
Open Forum

What was the first patch clamp system and who developed it?

*First Patch Clamp, circa 1974**
by Elle Dolgin

In the 1950s, scientists began to suspect that single-ion channels existed, but it took them another quarter century to verify it. In 1974, physicist Erwin Neher and cell physiologist Bert Sakmann at the Max Planck Institute for Biophysical Chemistry in Gottingen, Germany, invented the "patch clamp," the first device to measure the flow of electrical current through single-ion channels, confirming their existence.

In Neher and Sakmann's apparatus, users place a tiny glass pipette filled with salt solution against the plasma membrane of a living cell. A small amount of suction is applied, forming a tight seal between the 0.5µ diameter pipette tip and an ion channel. All ions that pass through the channel then flow into the pipette, and the incredibly small electrical currents – on the scale of a picoampere, or 10^{-2} A, lasting only 10-100 milliseconds – can be recorded. They mounted the device on a 240-Kg tabletop outfitted with antivibration equipment.



Erwin Neher and Bert Sakmann's patch clamp.

"We realized that the main noise source in measuring currents was the leak between the pipette and the membrane," recalls Neher. "The trick was to improve the seal." Neher and Sakmann continued to tinker with the patch clamp, and in 1981, they discovered the "gigaseal," which removed most of the background noise and allowed 10-100X better resolution. In 1991, Neher and Sakmann were awarded the Nobel Prize.

* Courtesy of *The Scientist Magazine*. First published in July 2008.

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Technology Fast Track

Cellvizio® Receives *Wall Street Journal* 2008 Technology Innovation Award

Cellvizio, a new innovation by Mauna Kea Technologies, has been recognized as runner-up in the Medical Device category for breaking ground and demonstrating measurable impact on the medical field. The Cellvizio, a miniature microscope that provides a view of living tissue during endoscopy, potentially eliminates unnecessary biopsies and speeds up gastrointestinal and lung cancer diagnosis.

The Cellvizio product range dedicated to small animal imaging is now distributed by Leica Microsystems under the name Leica FCM1000 with Cellvizio® Technology. The Leica FCM1000 in vivo fluorescence micro-endoscope is used for minimally invasive, longitudinal studies of deep brain and nervous system events with sub-cellular resolution in small animalsSee the *Wall Street Journal* website for more details on all of the winners: **http://online.wsj.com**.

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Your Educational Resource

Christophe Ranger, Scientific Manager/Partner at Explora Nova announces the following **Stereology Course** in collaboration with Vyvyan Howard and Matt Reed the authors of *Unbiased Stereology*, published by Taylor and Francis.

Please find the detailed program here:
http://exploranova.nerim.net. It will be the third edition of this international course. This year all the practice sessions will be done with Leica Microsystems microscopes.

For more information:
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Upcoming Events

Visit Leica Microsystems at the **Society for Neuroscience Annual Meeting**, booth #1903 and booth #2001, November 16-19, 2008.

Visit Leica Microsystems at the **Entomological Society of America Annual Meeting**, booth #400, November 17-19, 2008.

Visit Leica Microsystems at **ASCB**, booth #1300, December 14-16, 2008.

For more events, visit: **http://www.leica-microsystems.us**
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Note: *We are interested in your comments and thoughts about the newsletter. Please feel free to email your comments to:* ***microscience.imaging@leica-microsystems.com***