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”...
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.
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 10⁻¹² A, lasting only 10-100 milliseconds — can be recorded. They mounted the device on a 240-Kg tabletop outfitted with antivibration equipment.

“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.

For You

New Investigator Program
Starting a new lab? Beginning a new research project? If so, you could qualify for thousands of dollars in discounts on Leica Microsystems widefield microscope, stereomicroscope, digital camera, and imaging software products.

Demonstration Microscopes & Accessories Starting at 20% Discount
Some of our best imaging equipment is available at sizeable discounts. All of the demonstration equipment offered at this discount carries Leica Microsystems’ full warranty and is available on a first come, first served basis. Currently available is a completely configured fixed cell, microscope with fluorescence (DM2500 P models) and assorted dissecting microscopes, some with fluorescence (M165 FC, MS5 and MZ9.5 models).

Ultimate Precision Trade-up Offer
Leica Microsystems is pleased to offer a trade-up discount on select EM and LM specimen preparation instruments: the Leica EM UC6 Ultramicrotome; Leica HPM100 and EM PACT High-pressure Freezers; Leica MED020, SD500, and SCD005/SCD050 Sputter-coat Devices; and the Leica CPD030 Critical Point Drying system. This offer is only valid on orders received from August 13, 2008 through December 31, 2008.

Buy 2 Novocastra Abs, Get the Third One FREE!
Purchase 2 Abs at list price (unless by prior agreement) and receive the third one FREE. There is NO limit on repeat orders, but this offer is limited to Novocastra concentrates and RTU primary antibodies. Hurry! This offer is only valid on orders received between November 1, 2008 and February 28, 2009. Please mention promo code ThirdFree when placing your purchase order.

Contact Leica Microsystems today at 800-248-0123 or email microscience.imaging@leica-microsystems.com to find out how these special offers can improve your research and save you money.

Upcoming Events


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

Your Local Team


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

For more events, visit: http://www.leica-microsystems.us (click on Company, then Events)

Editorial Staff

Editor-in-Chief: Molly Baker
Managing Editors: Pam Jandura, Lon Nelson
Graphic Design: M.N. Kennedy
Special Thanks: Rolf Borlinghaus, Scott Young, Elyse Meccio of The Scientist Magazine; Christophe Ranger of Explora Nova; and Mauna Kea Technologies

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