Upgrade Your Imaging System to NIR

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Dual-color SIM images of liver cells obtained with a pco.edge 26 NIR – courtesy of Dr. Henning Ortkrass, Bielefeld University, Germany

Upgrade Your Imaging System to Include Near-Infrared (NIR)

Over the last decade, the bioimaging field has seen rapid advancements in fluorophores, enabling new imaging modalities in the far-red and infrared spectrum. These advancements allow deeper tissue penetration, gentler imaging with lower energy excitation, and increased channel counts. This article explains how to upgrade your microscope for Near-IR fluorescence imaging, addressing common barriers and simple solutions.

Challenges

Researchers often face several obstacles when incorporating new NIR fluorophores:

1. **Excitation Source:** Your microscope may not have an LED that covers the required longer wavelengths.
2. **Fluorescence Filters:** Existing filter sets might not be suitable for the new fluorophore.
3. **Camera/Detector:** Your current camera or detector may lack sensitivity in the needed range.

Addressing these issues is quite simple and does not necessarily require a major microscope overhaul. This Application Note aims to help users achieve NIR imaging using the microscope they currently own, with a few simple additions.

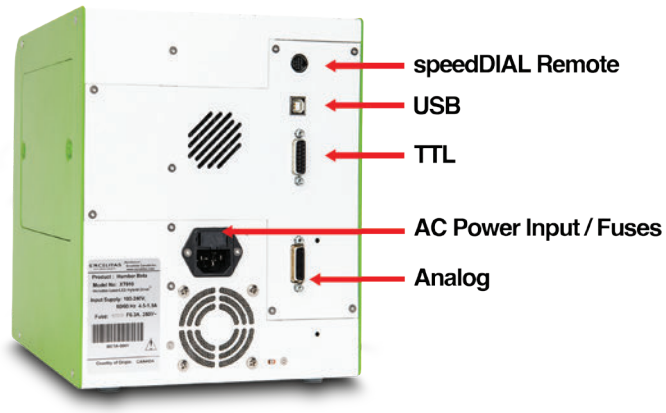
In partnership with

Excitation Source

LED technology has revolutionized Near-IR imaging, offering significant advantages over traditional light sources like Xenon lamps, mercury arc lamps, and lasers. The advancements in LED technology have made it a more efficient, cost-effective, and environmentally friendly option for NIR applications.

The X-Cite NOVEM™ is an excellent example of advanced LED technology for NIR imaging. This nine-channel fluorescence illuminator provides:

- Wavelengths up to 800 nm, enabling NIR imaging capabilities
- Multi-channel functionality, allowing for versatile experimental setups
- Improved control over light output and spectral range
- Liquid light guide delivery of light for seamless integration with any microscope
- Control through 3rd party software or X-Cite GUI
- Ability to trigger all LEDs (simultaneously or individually)



Triggering capabilities

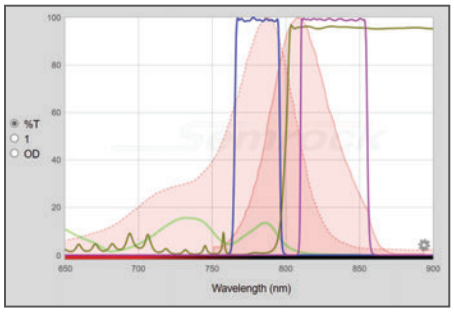
- Individual control for each LED separately
- Trigger multiple LED's simultaneously

NOTE: It is also useful to note that the PCO® cameras are able to trigger X-Cite® light sources without need for additional triggering equipment hardware.

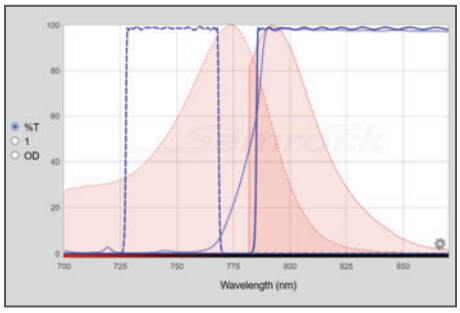
Filter Sets

Optical filters sit at the heart of every fluorescence microscope and NIR fluorescence imaging requires fluorescence filters suitable for these fluorophores. Below are the most popular NIR fluorophores with filter sets used for NIR imaging overlaid with the NOVEM-IR spectrum.

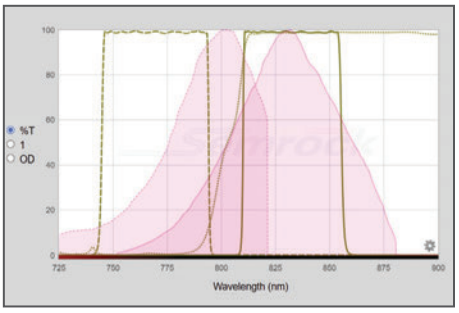
Depending on your microscope and the other fluorophores used in your research, custom filter sets might be needed.



Cy7.5



IR800



ICG

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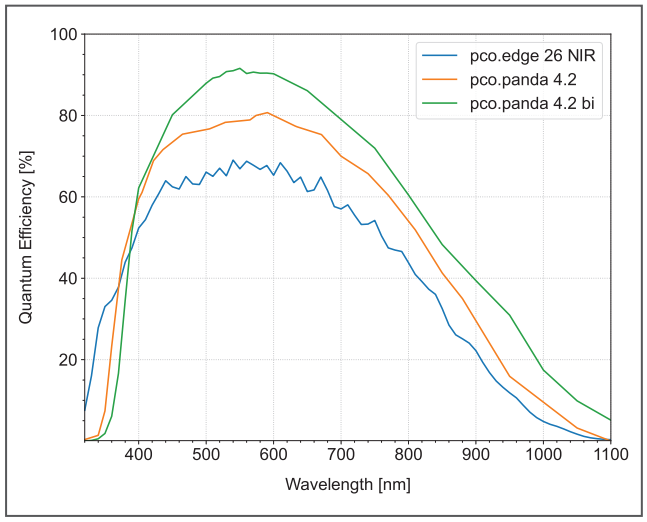
Camera

Choosing the right camera suitable for NIR imaging is crucial and depends on the detector sensitivity. Most silicon-based camera chips are sensitive up to 1100 nm and some can even detect light as high as 1300 nm, but the quantum efficiency at these longer wavelengths is considerably lower than in the visible range.

The pco.panda family of cameras from Excelitas® are compact sCMOS cameras with optimized passive thermal management enabling the high performance of sCMOS image sensors with a small form factor. For example, the pco.panda 4.2 bi offers up to 90% peak quantum efficiency with a spectral range of 370 nm out to 1100 nm, 4.2 Mpixel resultion (2048 x 2048 pixel) at 6.5 µm x 6.5 µm pixel size. These cameras are an ideal and cost-effective choice with optional features like low light or line scanning mode.

Further, our pco.edge cameras feature temperature-stabilized, high-performance sCMOS sensors, delivering ultra-low readout noise, a wide dynamic range, high frame rates, and exceptional sensitivity across a broad wavelength spectrum. This makes them ideal for expanding your imaging capabilities into the NIR regime. A perfect fit in our portfolio is the pco.edge 26 CLHS, equipped with a true charge domain global shutter 26 MP sensor (5120 x 5120 pixels, 2.5 µm x 2.5 µm pixel pitch) for which we also offer a dedicated NIR-enhanced version.

Integration with your system is usually straightforward due to standard coupling options for microscopy as well as a broad coverage of third-party software integrations and our easy to use software development kit offerings.



Quantum Efficiency curves of the pco.panda 4.2 bi, pco.panda 4.2 and pco.edge NIR (right).

Other Considerations

Older microscopes might have hot-mirrors to block NIR light, originally designed to protect samples from excessive heat. If using an older frame, these optics may need to be removed.

Please feel free to contact your account manager to discuss any of these options or to get help with making your microscope NIR friendly.

Conclusion

With the right light source, optical filters, and camera, most fluorescence microscopes can be upgraded for NIR fluorescence imaging. Updating individual components can also benefit visible imaging. The Excelitas and AVR Optics teams are ready to help you navigate these upgrades and develop an effective imaging strategy.

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