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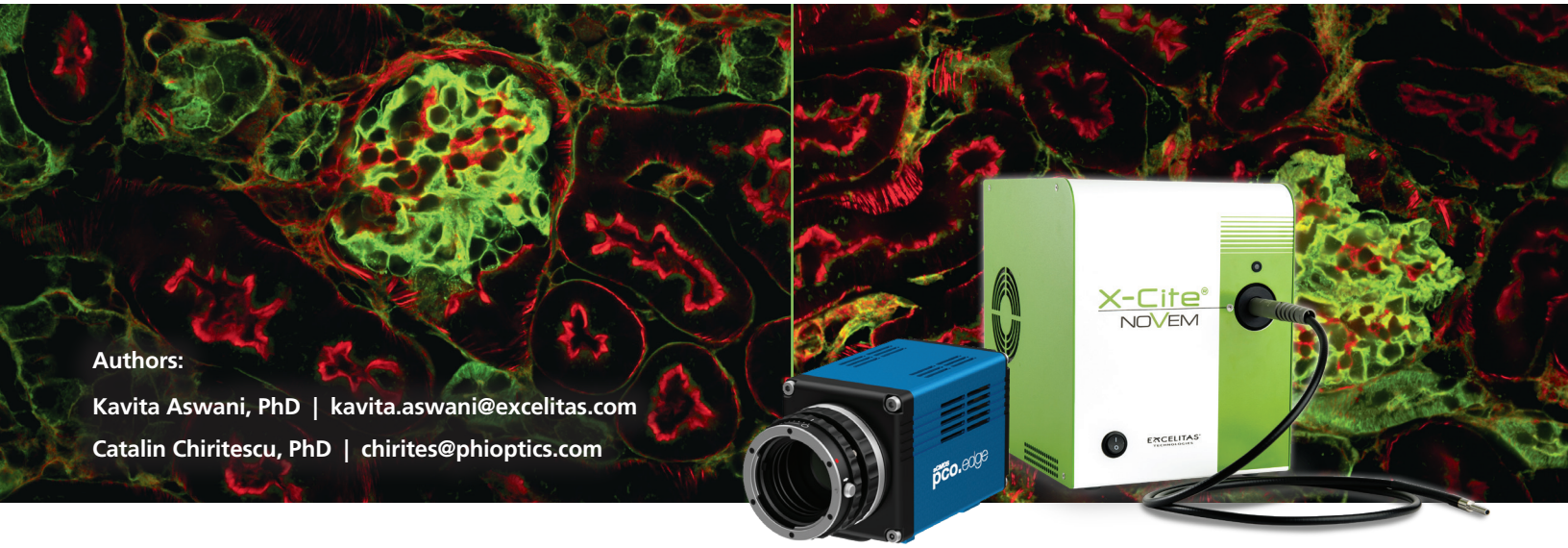
Using the X-Cite NOVEM™ and PCO® sCMOS cameras for quantitative phase imaging with GLIM



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Introduction

Researchers need to image and quantify live, thick samples for long periods of time to observe developmental changes or change in the sample over time. The challenge in imaging thick tissue or embryos is the scattering of light, creating a 'noisy' image. This Application Note illustrates the use of Gradient Light Interference Microscopy (GLIM) to image optically thick specimens such as tissues, embryos, and model multicellular organisms. GLIM yields high contrast images by subduing this light scattering, and also provides optical sectioning for 3D imaging of thick tissues.

What is GLIM?

This Application Note illustrates the use of Gradient Light Interference Microscopy to image optically thick specimens such as tissues, organoids, embryos, and model multicellular organisms. GLIM rejects much of the multiple scattering contributions and yields high contrast of these specimens. Furthermore, the illumination condenser aperture is fully open, which lends GLIM very strong optical sectioning so GLIM can provide 3D tomographic imaging. Phi Optics CellVista-GLIM units are modular and can upgrade any commercial inverted DIC microscopes thus allowing easy overlay and pixel registration with fluorescence channels. GLIM can measure the dry mass, refractive index and morphology over spatial scales from micrometers to millimeters, and temporal scales ranging from milliseconds to weeks. GLIM has applications in areas such as oncology, ART/IVF, crop sciences, developmental biology and in drug discovery/safety.

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Methods

- X-Cite NOVEM light source installed in the transmission light port on a Zeiss Axio Observer Z1 microscope with Phi Optics GLIM module, and a pco.edge 4.2 CLHS
- Near Infrared illumination:
  - Using the X-Cite NOVEM with visible and NIR illumination to image optically thick samples
  - NIR advantage: deeper penetration into the sample ➔ GLIM imaging can provide high SNR (less noise) at longer depths or through highly scattering specimens
- Testing samples:
  - Coronal slices of mouse brain
  - Arabidopsis seedlings fixed and mounted on glass slides with coverslip, PBS buffer with sodium azide, NO CLEARING
- Image acquisition using a pco.edge 4.2 CLHS sCMOS camera (2048 x 2048 full resolution at 6.5 µm pixel pitch) via Phi Optics CellVista Pro - programmed z-stacking with different illuminations
- Two X-Cite NOVEM modes:
  - "White light": 475 nm and 580 nm LEDs at 20% power
  - "NIR": 735 nm and 785 nm LEDs at 20% power
- Z-stack animation using ImageJ/FIJI. GLIM colormaps in radians (equivalent to nanometers optical path length)

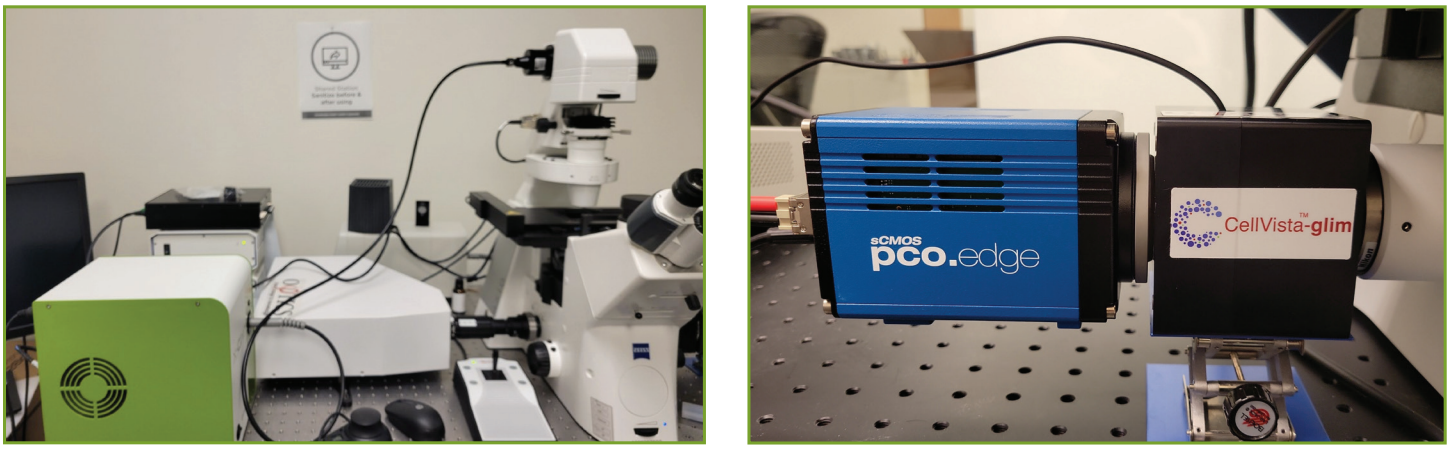

Figure 1 – GLIM setup using the X-Cite NOVEM (left, green box) and PCO camera (right, blue box). The Phi Optics GLIM module is the black box in the right image between the PCO camera and the microscope.

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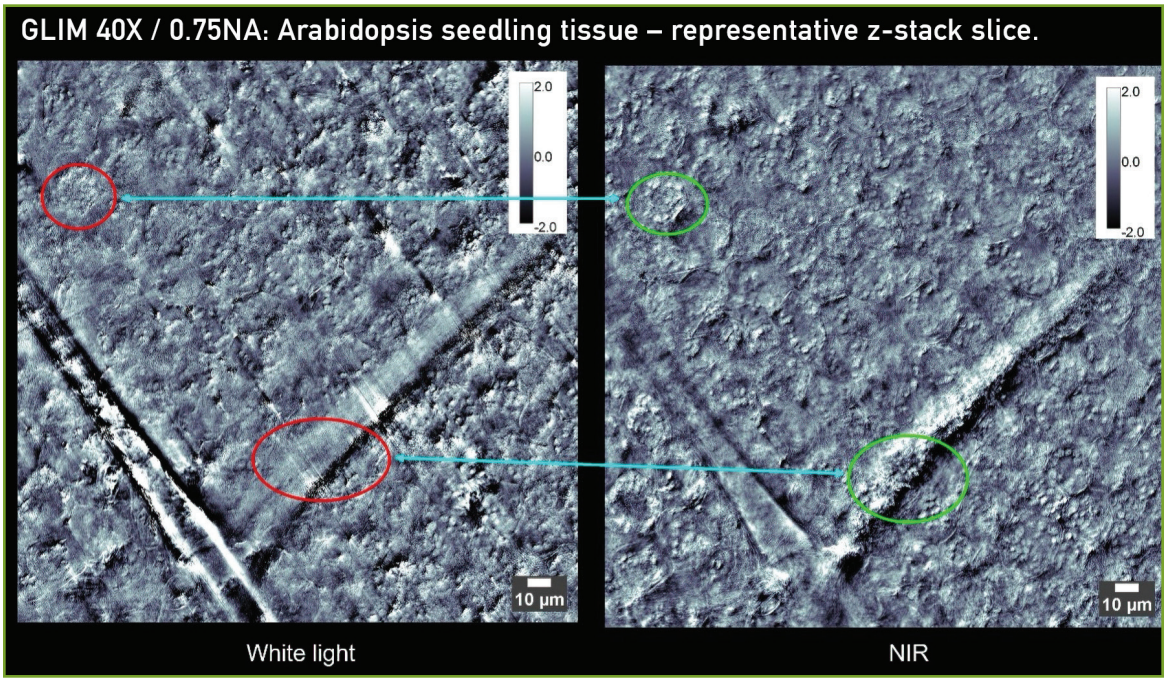
Results

Below images highlight comparison spots in images taken through the more challenging sample (uncleared leaf tissue) with both NIR and white light illumination. The images depict NIR illumination results in better resolving features of the sample.

Detailed movies can be found at this LINK.



Discussion

The Phi Optics GLIM can be used with any inverted DIC microscope, allowing quantitative phase imaging that can also be overlaid with fluorescence images. In this application note, we compare GLIM using visible vs. Near-IR light from the X-Cite NOVEM in transmission imaging.

- GLIM mechanism employs low-coherence illumination with common path interferometry of two imaging beams that although they suffer equal degradation (i.e., the same background noise) due to multiple scattering in the sample, they interfere with high contrast when passing through optically dense specimens hundreds of microns thick. GLIM thus subdues the multiple scattering backgrounds and exhibits strong optical sectioning to suppress the out-of-focus light.

- NIR illumination reduces the noise in GLIM images due to deeper penetration into the sample: the two interfering beams are stronger than when using WL illumination after passing through the sample.

- Sample fixation is not required, **GLIM + X-Cite NOVEM** reveals the internal structure of 3D samples in live samples enabling long term monitoring of live assays.

Cameras with 6.5 µm pixel pitch, such as the pco.edge 4.2 CLHS shown here or the pco.panda 4.2 USB, are very well established for imaging techniques in the life-science disciplines. However, smaller pixel sizes can help to enhance Nyquist sampling whenever low magnifications (e.g. 5-10x) are utilized. Thus, imaging of a whole organoid is possible without the need for stage-rastering. The Excelitas pco.edge 10 bi CLHS (4.6 µm pixel pitch at 4432 x 2368 full resolution) or pco.edge 26 CLHS (2.5 µm pixel pitch at 5120 x 5120 full resolution: available with NIR-enhanced sensor) sCMOS cameras are ideally suited for capturing such scenes in one single field-of-view as both provide small pixels and high-resolution, simultaneously.

Conclusion

Excelitas X-Cite NOVEM illuminator and PCO sCMOS camera combined with Phi Optics GLIM reduces the noise in imaging of 3D specimens (tissues, organoids, embryos, plants and model multicellular organisms) that are thick or scatter a lot, when NIR illumination is used instead of the typical broadband visible (white light) illumination. X-Cite NOVEM still provides the broadband illumination for samples and is an all-in-one solution for researchers who are looking to image 3D specimens live and without invasive preparation.

X-Cite NOVEM features high excitation power for imaging applications across the UV-visible-NIR spectrum (deep sample penetration)

- Phi Optics GLIM provides fast, 3D quantitative and non-invasive imaging of live optically thick specimens (tissues, organoids, embryos, and model multicellular organisms)

- X-Cite NOVEM + GLIM + PCO sCMOS = one-stop solution for quantitative imaging of cleared or uncleared optically thick samples in brightfield AND fluorescence modes

For more information on Excelitas Technologies, visit **www.excelitas.com**

For more information on the Phi Optics GLIM module, visit **https://phioptics.com/**

References

1. Kandel, M.E., Hu, C., Naseri Kouzehgarani, G. et al. Epi-illumination gradient light interference microscopy for imaging opaque structures. Nat Commun 10, 4691 (2019). https://doi.org/10.1038/s41467-019-12634-3

2. Nguyen TH, Kandel ME, Rubessa M, Wheeler MB, Popescu G. Gradient light interference microscopy for 3D imaging of unlabeled specimens. Nat Commun. 2017 Aug 8;8(1):210. doi: 10.1038/s41467-017-00190-7

GLIM is protected by US Patent 10,132,609 owned by the University of Illinois and exclusively licensed to Phi Optics, Inc..

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