A Clear New Vision in Microscopy

A collaborative team overcomes the long-standing challenge of blurred imaging, illuminating once hidden details within the depths of thick samples.

ANYONE WHO HAS USED A CAMERA understands the familiar frustration of losing focus on an object and its image becoming blurred. This seemingly simple issue poses a major hurdle in microscopy, particularly when imaging thick samples such as brain slices, where blurred out-of-focus backgrounds obscure in-focus areas.

Complicating things further, "brain slices are notoriously autofluorescent," noted Alec De Grand, a product manager at Evident Scientific. "You can see what one cell is doing right next to another cell in an XY plane, but you can't always understand what's going on in the Z plane. It's difficult to have a spatial understanding of how the object you're studying interacts with everything around it."

De Grand oversees the development of advanced microscopes, including the VS200 slide scanner. As a versatile widefield imaging system that automatically scans histology and cytology slides, the VS200 has helped researchers focusing on neuroscience, cancer research, and drug discovery to capture high resolution images of whole slides.

To tackle the age-old issue of imaging thick samples in widefield microscopy, De Grand and his team pursued solutions to effectively eliminate out-of-focus blurs. Through optical engineering, they developed an innovative optical sectioning device, named the Speckle Illumination Acquisition (SILA) module, that integrates with the VS200 slide scanner, enabling clear and efficient imaging of thick samples.

Two images, one vision

The concept behind SILA is rooted in HiLo microscopy, introduced in 2008 by Jerome Mertz, a bioengineer at Boston University. The name HiLo comes from combining "high" and "low" spatial frequency. Within an image, every detail, from coarse to fine, holds a distinct spatial frequency, which represents the density of patterns within the given area. The HiLo technique cleverly captures the entire spatial frequency spectrum by acquiring two distinct images using different illumination methods.

"First, it takes a normal picture on a standard wide field," explained De Grand. This uniformly illuminated image contains both in-focus and out-of-focus content. The blurred out-of-focus content primarily consists of low spatial frequency elements. The HiLo microscope incorporates a specialized filter to eliminate these elements, retaining only the high spatial frequency components of the in-focus content.

"Then, it takes a second picture, but it puts a pattern on the image," said De Grand. The pattern, produced by laser light, typically comprises small, scattered speckles. These speckles exhibit distinct intensity patterns on in-focus and out-of-focus regions, offering insight into the level of focus across the sample's different areas. By analyzing the speckle pattern and its variations, researchers can employ computational algorithms to extract the lower spatial frequency components of the in-focus content from specific planes deep within a thick sample.

The fusion of both images leads to a clear, in-focus image that contains the full spatial frequency range. "Rather than looking through a haze of autofluorescence from above and below the object, you can have a clear signal of exactly what you're looking at," said De Grand.

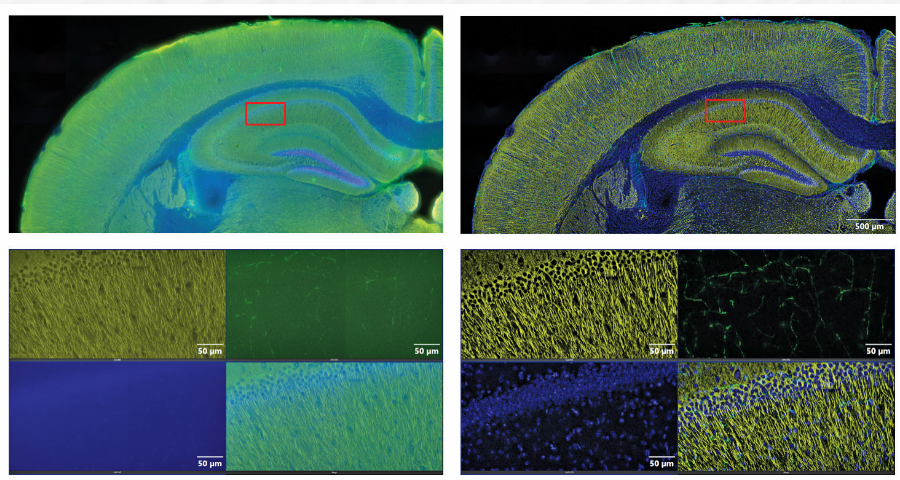
A collaborative journey

In 2017, Bliq Photonics, a microscope manufacturer, first integrated HiLo microscopy into commercial microscopes. This inspired the Evident Scientific team to explore the potential of incorporating the technology into their existing microscopy platforms. To achieve this, they established a collaborative partnership with Bliq Photonics and brought in experts from both companies to join the development project.

Developing a new imaging device required meticulous software and hardware refinement and teamwork across various groups. "We first specified what we wanted in the new software and communicated these requirements to the Evident Scientific engineering team. They then translated our request to Evident Scientific software developers who wrote the code and tested the software to make sure it aligned with our requirements and functioned properly." said Wei Juan Wong, a product manager for the VS200 at Evident Scientific.

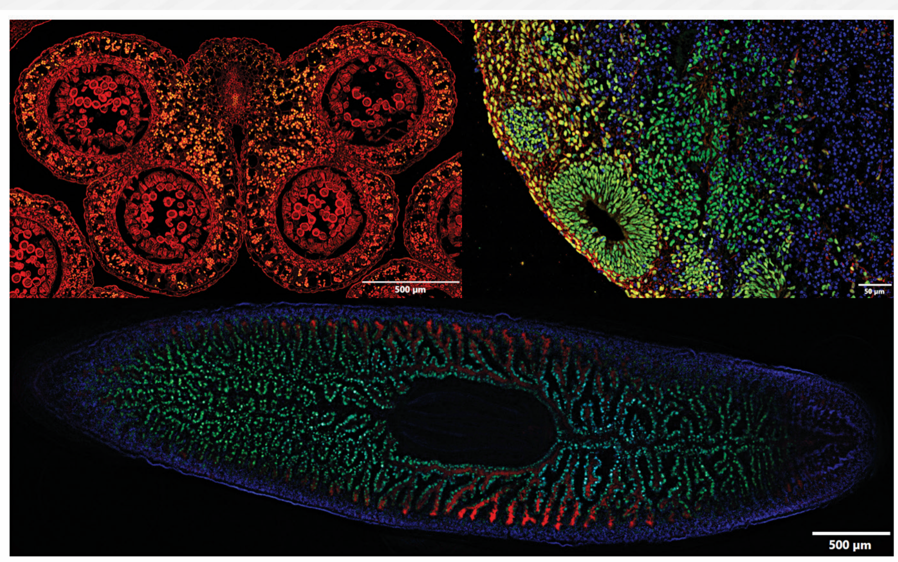
Similar to many product development journeys, this interdisciplinary team faced the challenge of juggling a variety of needs. Despite many new features to consider, it was important to stay focused on the main goal of crafting a compact module that could be conveniently installed into the VS200 system. "We needed to balance what everyone wanted versus what would work best for researchers in different parts of the world," said De Grand.

After three years of iterative development and close discussion with experts at Bliq Photonics, Evident Scientific launched the SILA optical sectioning device in April 2023. "Back then, we didn't know what the final image would look like until we got really nice images. Everything turned out really well," Wong shared.



CREDIT: EVIDENT SCIENTIFIC

A side-by-side display of a 200-micron-thick mouse brain section reveals enhanced clarity and sharpness through SILA imaging (right) compared to regular widefield imaging (left). The close-up views show individual cellular structures, where staining highlights nuclei in blue, glia in green, and neurons in yellow.



CREDIT: EVIDENT SCIENTIFIC

SILA-integrated microscopy allowed the Evident Scientific team to capture detailed images of diverse biological structures, such as a lily flower (upper left), an organoid (upper right), and a whole planarian flatworm (bottom).

Bridging the gap

When considering rejecting out-of-focus light and acquiring high resolution images, confocal microscopy comes to mind. However, researchers often can only access these expensive microscopes by visiting a shared core facility, while navigating a complex and time-consuming imaging procedure. "With a confocal, you have to scan one slide, take it off, put another slide, and do it again," commented De Grand.

The integration of the SILA module with the VS200 slide scanner can manage up to 210 slides, combining quality with efficiency. "You can get confocal-esque images without using a pinhole or specialized detectors, but in a slide scanner," said De Grand. "You just need to set up a single program and load all the slides. It automates the whole process. Researchers can scan massive amounts of datasets and gain insights from them rather than a small subsection because of time constraints."

The software guides users to perform optical sectioning, enabling them to focus on a specific plane within a three-dimensional sample while excluding the contribution from other out-of-focus planes. This eliminates the need to cut micron-thin tissue slices to achieve visual clarity. Using the SILA-integrated widefield microscopy, the Evident Scientific team has captured high-contrast, blur-free images of 200-micron-thick brain sections. These images reveal sharp individual structures of neurons and glial cells, beyond the limit of a regular widefield microscope.

"It doesn't just apply to brain samples. Any thick sample can be used for this technique," Wong emphasized. "For samples that are 30 microns and above, you can see a significant improvement when you go from a regular widefield microscope to SILA. Researchers can easily image up to 500 micron-thick sections."

De Grand and his team shared the SILA images with their customers and colleagues and received positive feedback. The team is now excited about the benefits this breakthrough can bring to the wider research community. "We did a pretty good job for this initial offering," said De Grand, "As microscopy constantly evolves, we plan to expand new features with SILA technology as well. Those will be coming."