Application Note

Large FOV Optical Sectioning & Super-Resolution SIM enabled by the pco.edge 10 bi camera

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Structured Illumination Microscopy (SIM), as a super-resolution widefield imaging technique, benefits from the sCMOS image sensor of the pco.edge 10 bi CLHS camera with a size of 4416 x 2368 pixels, allowing for a super-resolved field of view (FOV) of up to 200 µm x 365 µm — depending on the microscope configuration.

In Super-Resolution Structured Illumination Microscopy (SR-SIM), a sample is illuminated by an interference pattern with a periodicity close to the diffraction limit. The sample is illuminated by rotating and shifting the pattern and, typically, at least 9 images (3 angles with 3 phase steps for each angle) are required in order to computationally reconstruct an image at maximum with a factor of 2 improved spatial resolution. During the reconstruction, the number of pixels in the final image are also doubled for each dimension, resulting in images of up to 8832 x 4736 pixels if the entire sensor of the pco.edge 10 bi CLHS is used for imaging. The high dynamic range and fullwell capacity of the camera in combination with its excellent sensitivity allows for imaging of challenging biological samples with a high staining contrast.

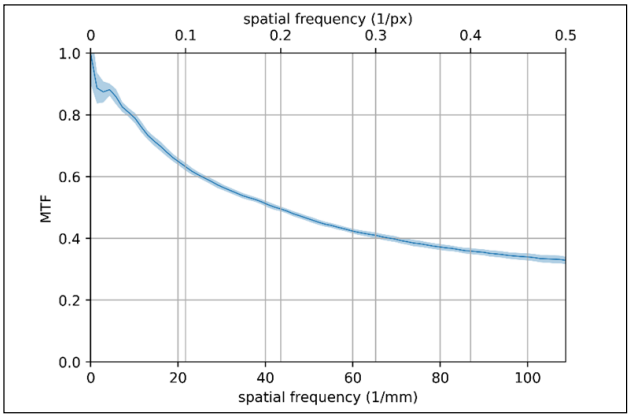

Figure 1: Modulation transfer function (MTF) of the camera sensor. Compared to other cameras, the sensor of the pco.edge 10 bi CLHS still shows a high signal (0.33) at the sampling limit (109 1/mm).

Given the maximum raw image frame rate of 122 fps when reading the full sensor, eight large field-of-view super-resolved SIM images can be acquired per second, which enables researchers to temporally resolve dynamic changes in the sample. The pixel size of 4.6 µm allows for the use of high NA (Numerical Aperture) oil immersion objective lenses without the need for additional image magnification to obtain sufficiently over-sampled images. By using a 1.4x additional magnification, the camera can be used with both 40x and 60x objective lenses, while still sampling the images below the Rayleigh or Nyquist limit in both configurations.

This flexibility makes the pco.edge 10 bi CLHS very well suited for optical sectioning (OS-SIM), 2D-, TIRF-(Total Internal Reflection Fluorescence), or 3D-SIM with a 60x objective lens with a high NA or a 40x lens with a large FOV. The image sensor features a deep trench isolation architecture and additional microlenses on each pixel, and it exhibits a very low pixel crosstalk resulting in a high modulation transfer function (see Figure 1) at the sampling limit (0.33). This provides a high spatial contrast even when sampling close to the Nyquist limit.

Dr. Henning Ortkrass and Prof. Thomas Huser from University of Bielefeld, Germany, applied the camera to 2D-SIM and OS-SIM to immuno-stained samples containing multiple different fluorophores.

Super-Resolution SIM with a large FOV significantly reduces the time that is required to obtain statistically significant information about the sample. Rather than having to acquire approximately 30 separate SIM images from different sample locations, the same area can be imaged within a single SIM image that exploits the full image sensor of the pco.edge 10 bi CLHS. Similarly, if a living sample is imaged dynamically with such a large field of view, statistically relevant information from several cells can be acquired simultaneously, further reducing the amount of time that it takes to e.g. follow the dynamics of mitochondria, vesicle movement or transfer of materials from outside a cell into a cell.

As example, Figure 2 shows a 3-color SR-SIM image of specifically labeled pre- and postsynaptic proteins as well as the cellular plasma membrane in a thin slice of mouse brain tissue, while Figure 3 shows the fine optical sectioning of a liver spheroid slice, both of which nicely illustrate the excellent performance of the pco.edge 10 bi CLHS.

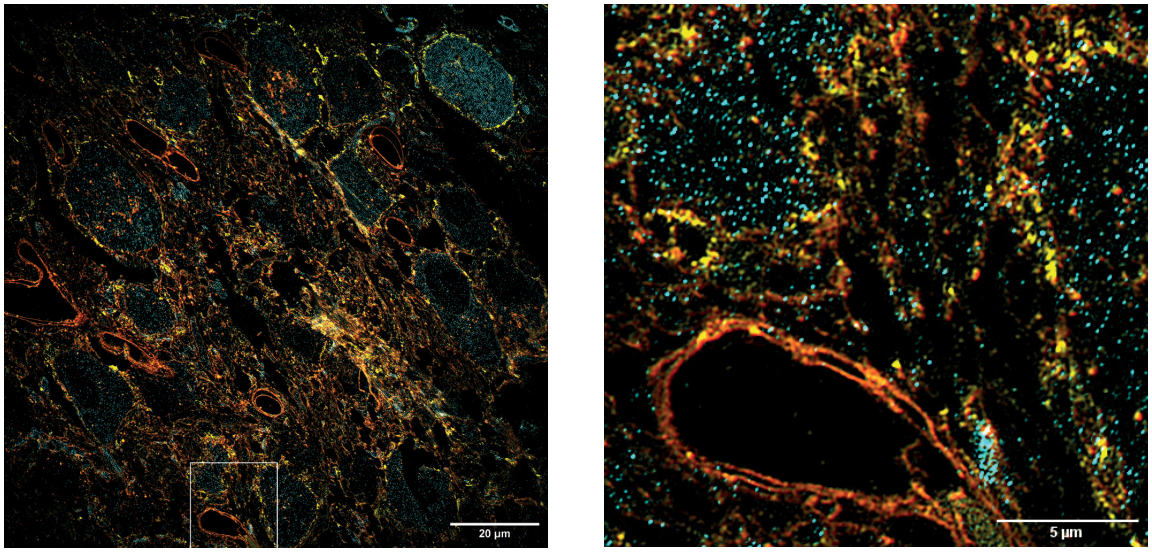

Figure 2: Multicolor 2D-SIM with a field of view of 120 x 120 µm² of a mouse brain slice. The tissue slice is stained against post- and presynaptic proteins as well as the plasma membrane. The spatial resolution is approximately 130 nm.

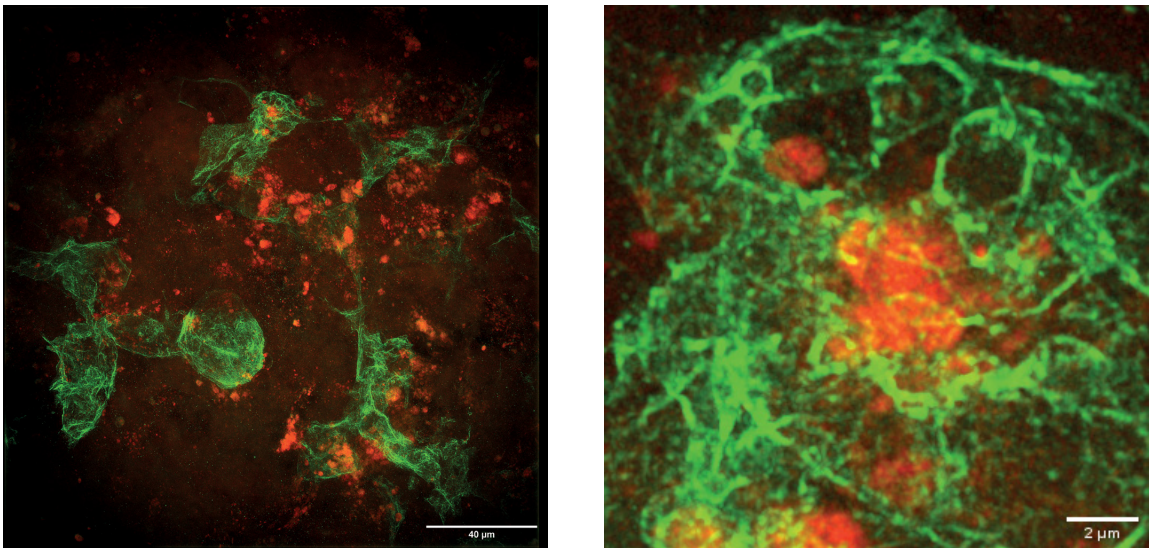

Figure 3: Multicolor optical sectioning SIM with a FOV of 200 µm x 200 µm and a lateral resolution of approx. 160 nm. The image shows a liver spheroid, with a membrane staining of liver sinusoidal endothelial cells (green) and hepatocytes (red). The image is a maximum intensity projection of a 10 µm thick slice, imaged at 50 z-planes.

For a broader applicability, Dr. Henning Ortkrass and Prof. Thomas Huser have integrated the pco.edge 10 bi CLHS into their self-developed tabletop microscope system ***ProSIM***, which is capable of a large variety of different modalities:

- 2D-SIM
- 3D-SIM
- TIRF-SIM
- Multi-Angle TIRF-SIM
- OS-SIM
- Laser Scanning Microscopy
- Brightfield, Widefield, TIRF, Ring-TIRF
- Fluorescence Anisotropy Microscopy

The ***ProSIM*** microscope system has been demonstrated in action at the Trends in Microscopy Conference held in March 2025 in Albgut-Münsingen, Germany (see Figure 4). During each of the workshops, various combinations of imaging modalities were shown in live experiments, and the resulting data were discussed in detail. At the end of the conference, all results and image data were made publicly accessible.

Figure 4: Dr. Ortkrass demonstrates the operational modalities of the ProSIM microscope, based on a pco.edge 10 bi CLHS, to attendees of his workshop at Trends in Microscopy Conference 2025. The instrument was developed by him and the group of Prof. Huser at the University of Bielefeld, Germany.

Acknowledgements

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