Instant Volume Microscopy of Organoids with *SOLIS*

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Abstract

Advancements in microscopy techniques have revolutionized our ability to explore the intricacies of biological systems, with engineered human heart tissue (EHT) being a particularly challenging target. In this article, we will have an in-depth look at the award-winning SOLIS technique (scanned oblique light-sheet instant-volume sectioning), a new twist on multifocus fluorescence microscopy. Through its unique capabilities, SOLIS offers a new approach on organoid and tissue imaging, providing unprecedented insights into the cellular architecture of these complex artificial samples. By being able to record optically sectioned volumes during single camera exposures, SOLIS demonstrates remarkable advantages over traditional multifocus microscopy, underscoring its potential to transform our understanding of developmental biology, disease mechanisms, and potential therapeutic interventions.

Organoids – a true challenge for high-resolution fluorescence microscopy

Organoids and artificial tissues have emerged as powerful tools in both research and clinical practice, revolutionizing our understanding and approach to studying human biology and disease. These three-dimensional structures, grown from stem cells or other cell sources, mimic the complexity and functionality of real organs. Their ability to recapitulate the microenvironment and cellular interactions of native tissues makes them invaluable for investigating disease mechanisms, drug discovery, and personalized medicine. Organoids and artificial tissues offer researchers a controllable and reproducible experimental system that bridges the gap between traditional cell culture models and human patients. In clinical practice, these models hold great promise for disease modelling, drug screening, and regenerative medicine, providing a platform to test therapies and study patient-specific responses. With their potential to enhance precision medicine and advance therapeutic strategies, organoids and artificial tissues have become indispensable tools in the quest for improved healthcare outcomes.

One particular area where organoids and artificial tissues have shown exceptional promise is in the study of engineered human heart tissue (EHT). Unlike traditional cell cultures, EHTs are alive and beating, closely resembling the dynamic nature of the human heart. This unique characteristic allows researchers to investigate cardiac physiology and pathology in a controlled laboratory setting, bridging the gap between in vitro models and the complexity of the human cardiovascular system. By utilizing induced pluripotent stem cells and specialized culture techniques, it is possible to cultivate three-dimensional structures that display all the crucial hallmarks of adult cardiac muscle. However, the dense and highly scattering nature of EHTs presents specific challenges in imaging and analysis, making it difficult to capture detailed images without dedicated background rejection techniques.

Imaging living tissue using fluorescence microscopy in general presents challenges, particularly in capturing fast 3D processes on the subcellular level. Existing imaging modalities such as confocal, spinning disk, and light-sheet microscopy rely on sequential recording of points or planes, which can introduce sample movement artifacts and perturbations during focus changes. Additionally, confocal

and spinning disk microscopy techniques pose the risk of photobleaching and light-induced sample damage due to extended illumination light. While computational widefield approaches based on deconvolution and neural networks offer promising 3D capabilities, they also share the same drawbacks of photobleaching and sample damage.

The promises and pitfalls of multifocus microscopy

Multifocus microscopy (MFM) is a technique that aims to capture an entire focal stack in each camera frame, optimizing the use of the illumination volume and maximizing imaging speed. It offers potential benefits for fast-moving 3D samples like EHTs by providing volumetric information and improving the efficiency of data acquisition. MFM encompasses various approaches to capturing an entire focal stack in each camera frame. These include techniques such as beam splitter cascades, multi-plane prisms, and multifocus microscopy based on warped gratings [1-3]. Beam splitter cascades and multi-plane prisms divide the image plane into sub-planes, while multifocus microscopy based on warped gratings splits the image into diffraction orders with order-dependent defocus. Unfortunately, MFM methods are not without drawbacks. These methods suffer from poor axial resolution, making it challenging to accurately capture detailed 3D structures within a sample. Additionally, MFM often lacks proper optical sectioning capabilities, limiting the ability to distinguish and isolate specific features between focal planes. For instance, Figure 1B shows how a cell, which nominally resides in the centre focal plane, blurs substantially into the other focal planes.

One significant limitation of MFM is the degradation of image quality when imaging samples with high scattering properties, such as dense tissues or complex biological structures. The scattering of light within these samples leads to reduced clarity and contrast, affecting the overall resolution and interpretability of the captured images. Moreover, MFM techniques, including those employing gratings, may encounter difficulties in multi-colour imaging applications due to challenges in correcting chromatic aberrations and achieving precise colour fidelity across different focal planes.

These drawbacks of MFM highlight the need for advanced imaging techniques that can overcome these limitations, providing improved axial resolution, enhanced optical sectioning capabilities, better image quality in scattering samples, and efficient multi-colour imaging capabilities. Ideally, an MFM technique is needed that provides clear, detailed imaging, especially of EHTs, without compromising their viability or integrity.

To summarise, conventional multifocus microscopy is severely hampered by strong background haze when imaging EHTs. The tissue's complexity and scattering properties make it challenging to visualize individual structures, such as mitochondria and mitochondria-derived vesicles (MDVs), especially at greater depths within the tissue.

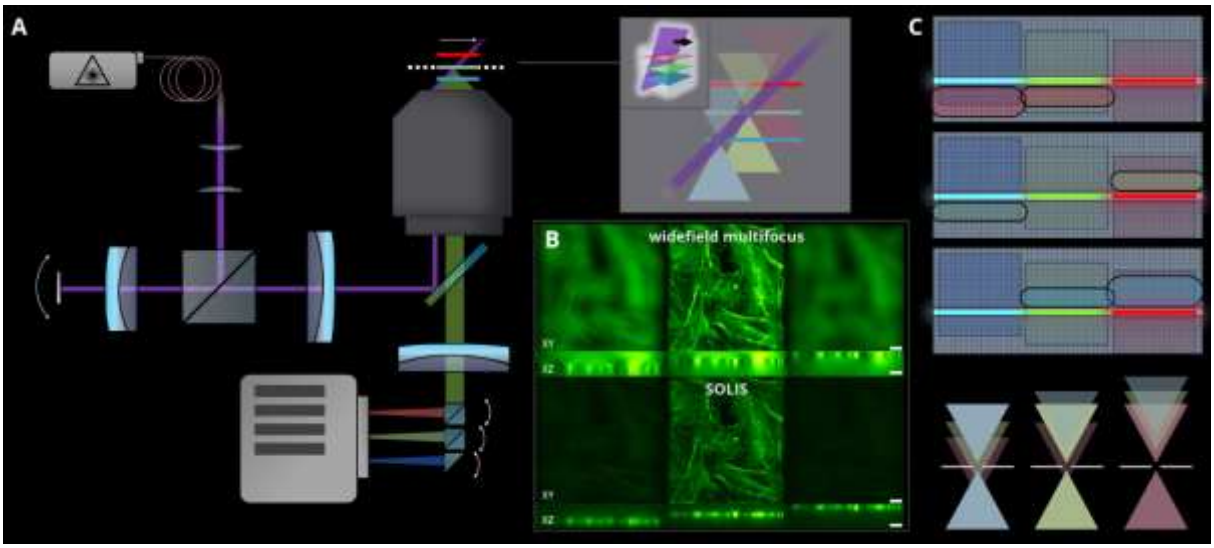

Fig. 1*: (A) A sketch of the optical layout of SOLIS. (B) The actin mesh of bovine pulmonary artery endothelial (BPAE) cells, imaged with a regular multifocus microscope and with SOLIS. (C) Optical sectioning of multiple z planes is achieved by mapping out-of-focus light onto un-exposed parts of the camera at any moment during the scan. Shown are sketches of the light distributions from different planes (colour-coded in red, green, and blue) in XY and XZ views on the camera sensor. Scalebars are 1 µm in B. Images are modified from [4].*

Real volumetric imaging with a tilt

Enter SOLIS (scanned oblique light-sheet instant-volume sectioning [4]), a new volumetric imaging technique and recipient of this year's *Microscopy Today Innovation Award.* SOLIS demonstrates superior performance compared to conventional multifocus microscopy, especially in challenging situations like imaging EHTs. SOLIS allows for single-camera exposure recording of the 3D distributions of cellular organelles like mitochondria and enables the attribution of individual mitochondria to various depth positions within the tissue with great precision. Furthermore, SOLIS achieves higher resolution and even enables visualization of sub-diffraction structures, such as mitochondria-derived vesicles, at significant depths within the tissue, while conventional imaging techniques struggle to provide clear and detailed images. This is how it works.

The key principle of SOLIS involves a light-sheet sweeping through the sample volume, mapping each illuminated oblique plane during its sweep onto separate single rows of a camera sensor with multifocus optics. Unlike traditional widefield imaging with a global shutter camera, SOLIS synchronizes a rolling-shutter camera's read-out with the light-sheet sweep, enabling individual and sequential detection of each sensor line. This synchronized operation creates a pinhole-like effect within the camera, resulting in an effective optical transfer function (OTF) governed by a convolution between the illumination and detection OTFs. In a sense, SOLIS is "plane-scan confocal microscope".

The light distribution in SOLIS is illustrated in a comprehensive animation of Figure 1C, available on YouTube [5]. Light originating from the red, green, and blue "instantaneous intersection lines" is captured by the camera's read-out line only when it is in focus. Conversely, if the light comes from a location outside the nominal focus, it appears blurred in both X and Y directions on the camera. It is important to distinguish between these two aspects: the portion of light that blurs within the rolling shutter line cannot be entirely eliminated, resulting in a minor background haze reminiscent of line-scan confocal microscopy. However, the majority of light, which would accumulate along the rolling

shutter direction, is effectively avoided during the recording process. This arrangement enables SOLIS to efficiently remove haze from the other two image planes in the case of the thin cell depicted in Figure 1B, resulting in an optically sectioned image as expected.

In practice, a SOLIS setup looks like this: a collimated laser beam is shaped into a light-sheet using a cylindrical lens. The light-sheet is then directed through scan lenses and a steerable galvanometric mirror to the sample plane. SOLIS is a single-objective technique, so fluorescence is collected by the same objective that is used for illumination. The emitted light is then split using a beam splitter cascade and a prism and relayed onto the camera, where the synchronization between the light-sheet scan and the camera's rolling shutter enables optimized sectioning capabilities (see Figure 1A).

As the light-sheet is swept at an oblique angle, SOLIS achieves increased axial resolution akin to confocal microscopy. By adjusting the alignment of the multifocus imager under oblique illumination, such as tilting a multiplane grating or by adapting the constituting parts of a beam splitter cascade, SOLIS ensures proper mapping of the light-sheet onto the camera's rolling shutter.

SOLIS represents a significant advancement in microscopy as it combines the advantages of light-sheet illumination, optical sectioning and increased axial resolution, with the speed of multifocus microscopy. This technique offers researchers the ability to capture single-shot, three-dimensional images of samples with enhanced clarity and detail. In addition to preserving the full numerical aperture, a further intriguing aspect is that SOLIS can achieve optimal Nyquist sampling in all dimensions. This sets it apart from other single-objective light-sheet methods, that always need to oversample in at least one direction due to a tilt in the objective’s effective image plane.

By overcoming the limitations of traditional imaging methods, SOLIS holds great potential for applications requiring volumetric imaging, such as studying the complex structures and dynamics within EHTs. SOLIS enables researchers to obtain comprehensive insights into the internal organization and dynamics of such samples, contributing to a deeper understanding of their functional properties and potential clinical applications.

Unveiling the Intracellular Landscape of Artificial Hearts

In a pioneering study, my group from the Arctic University of Norway used SOLIS to explore its imaging capabilities and compare them with conventional multifocus microscopy. The findings demonstrated the remarkable performance and advantages of SOLIS in imaging various biological samples.

Firstly, we employed SOLIS to image sub-diffraction-sized fluorescent beads, a standard sample to assess resolution. Correcting for bead size, we found an impressive lateral resolution reaching beyond the 200 nm mark, which is consistent with simulations that suggested a superior lateral resolution achievable with SOLIS compared to widefield microscopy. To further evaluate SOLIS, we imaged actin-stained bovine pulmonary artery endothelial (BPAE) cells. Using phase decorrelation analysis, they confirmed a resolution down to 240 nm without any post-processing. Additionally, SOLIS demonstrated efficient rejection of out-of-focus light on biological samples, generating clean optical sections of the imaged BPAE cells (see Figure 1B). A volumetric frame rate of 35 Hz allowed for motion artifact-free imaging, enabling comprehensive optical sectioning within the sample.

With these encouraging results in mind, we teamed up with scientists from the cardiovascular research group from the university hospital of Northern Norway and launched a proof-of-concept study in a much more challenging sample: engineered human heart tissue. To our delight, SOLIS

provided exceptionally crisp single-camera exposure recordings of the 3D distributions of mitochondria in cardiomyocytes within the EHTs (Figure 2). Compared to conventional multifocus microscopy, SOLIS outperformed by resolving individual mitochondria with higher resolution (0.3 µm to 0.4 µm versus 0.5 µm to 0.6 µm) and showcased the ability to visualize mitochondria-derived vesicles (MDVs) tens of micrometres deep within the tissue, enabling their characterization with measured sizes of approximately 250 nm lateral and 500 nm axial.

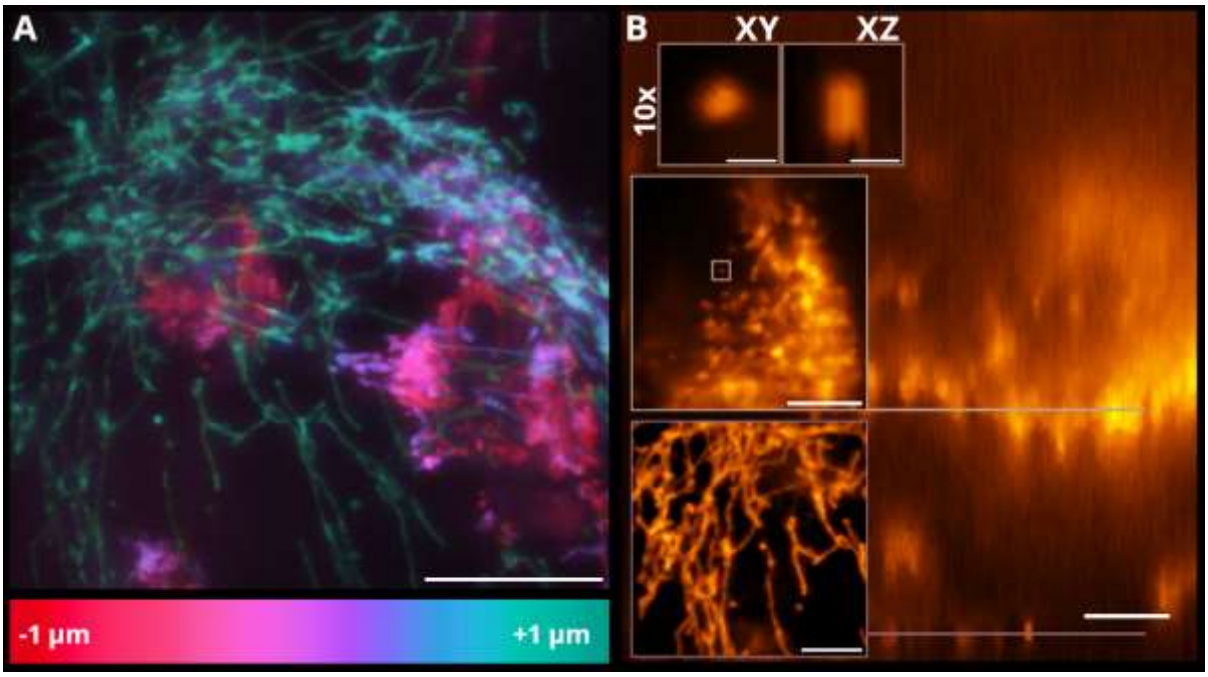

Fig. 1: *Optically sectioned multifocus microscopy with SOLIS of mitochondria in uncleared engineered human heart tissue. (A) Mitochondrial network in a 2 µm volume, displayed via color-coded depth. (B) Optical sectioning with the new method works deep into heavily scattering tissue and allows for instance the recording of sub-diffraction sized mitochondria-derived vesicles more than 20 µm into the tissue. Scalebars are 10 µm in A and B, 5 µm in the inlays of B, and 500 nm in the zoomed views. Images are modified from [4].*

Summary

The results obtained using SOLIS highlight its distinct advantages over conventional confocal, widefield or multifocus microscopy. SOLIS provides enhanced resolution, efficient rejection of background haze, and improved visualization of subcellular structures in complex samples like EHTs at video-rate. By utilizing a light-sheet sweeping technique, SOLIS achieves optical sectioning at or even beyond the widefield resolution limit and increased axial resolution akin to confocal microscopy.

While SOLIS has demonstrated remarkable performance, it is important to acknowledge its limitations and room for development. For instance, as the imaging depth increases beyond 50 µm, the resolution gradually decreases. Additionally, the number of simultaneous planes that SOLIS can capture is currently limited. Nevertheless, by utilizing aberration-corrected multifocus gratings and cameras with multi-line rolling shutters, SOLIS could be scaled up to 25 planes at Nyquist sampled inter-plane distances. It is worth noting that addressing chromatic aberrations would be necessary when imaging spectrally broad fluorophores in such a configuration.

With the increase in the number of planes, there are trade-offs to consider though. Firstly, as light from the sample is evenly distributed among all planes, the achievable SNR per plane decreases. Furthermore, SOLIS' lateral field of view would shrink since space on the camera is repurposed for axially displaced planes. Already now though, it is possible to fit all z-planes of an entire cell – imaged at the diffraction limit – onto a camera chip and, with ever-growing camera chip sizes, this limitation in application imaging is only temporarily limited. Exciting improvements in multifocus elements might offer another way forward by boosting the light efficiency in multifocus microscopy through novel optical elements that are tailored to SOLIS specifically. There is much to look forward to!

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Florian Ströhl is an expert in advanced microscopy at the Arctic University of Norway (UiT) in Tromsø. His research is centred around application-oriented method development of microscopy techniques for the life sciences. Dr Ströhl obtained his PhD in biotechnology on structured illumination microscopy from the University of Cambridge, where he successively took the role of Head of Imaging at the Dementia Research Institute. Since 2019 he is a project and team leader at the Arctic University of Norway in Tromsø, where his work focuses on the development of advanced imaging methods like label-free superresolution microscopy and next-generation 3D live-cell imaging.