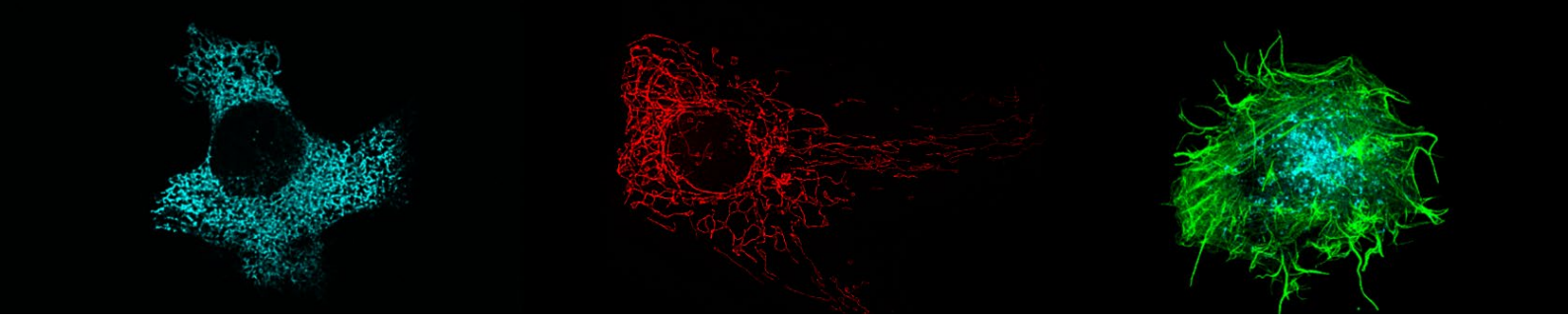


BREAKING THE DIFFRACTION LIMIT: DICOVERING CELLULAR ORGANELLES WITH STRUCTURED ILLUMINATION MICROSCOPY

An organelle is a **subcellular structure** that contributes to a variety of cellular functions through its molecular composition and environmental interactions.
Standard fluorescence microscopy techniques were traditionally used for organelle studies and focused on identifying the unique characteristics of individual compartments. However, the finer structures of organelles, as well as many key sub-cellular details, are smaller than 200 nm and therefore cannot be sufficiently characterized by diffraction-limited conventional microscopy.

Advances in imaging technologies have enabled simultaneous studies of multiple organelles' structure and dynamics, as well as their movements, interaction, and reshaping.
In this context, the development of **super-resolution (SR) microscopy techniques** represents a key driver to exceeding the diffraction limit of light and enables the investigation of cellular structures and organelles at the nanometer scale.

Through the use of a multi-spot structured illumination system, DeepSIM provides reliable, easy-to-use, and affordable solutions to study sub-cellular structures with an XY resolution of 100 nm without requiring any special sample preparation protocol. In this Application Note, we demonstrate that with the **DeepSIM** it is possible to follow live organelles dynamics at a cellular and sub-cellular level. In particular, we focused on different intracellular structures ranging from **endosomes, actin, mitochondria, tubulin, endoplasmic reticulum (ER)**, and **lysosomes** discovering subcellular details under the diffraction limit of light.

SR live imaging follows cytoskeletal and endosomal dynamics

Endosomes are subcellular organelles where internalized cargoes are organized and sorted toward different trafficking pathways; their content recycling is orchestrated by multimolecular complexes promoting new actin filaments polymerization.
The study of **actin dynamics** in the regulation of endosomal recycling, and the interaction between these two cellular components, can undergo considerable improvement thanks to SR techniques that allow studying the intracellular structures at the nanometer scale.
To demonstrate the DeepSIM optimal performance in following these fast biological events, we performed **live imaging of cells permanently expressing cytoskeletal (actin) and endosomal markers.**
In **Figure 1** we compared Widefield (WF), Confocal (CF) and SR images demonstrating a significant resolution increment between the three imaging modalities.

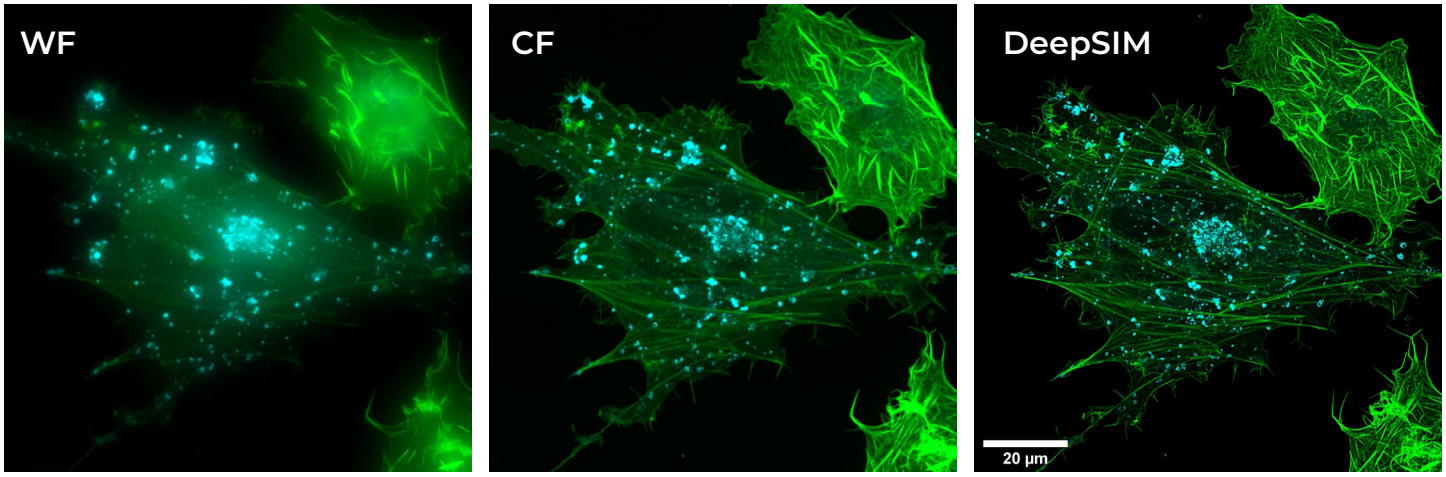


Figure 1: Comparison of WF, CF spinning disk and DeepSIM SR images of cells permanently expressing actin (green) and endosomal (cyan) markers. These images were acquired with **CrestOptics X-Light V3** CF spinning disk system coupled with **DeepSIM X-Light** SR system and equipped with CFI SR Plan Apo IR 60X water immersion objective.

Moreover, thanks to the DeepSIM high-speed acquisition modality, we captured relevant data in a continuous 30-second SR time-lapse. As represented in Figure 2, we were able to monitor fast events in SR without worrying about bleaching and demonstrating how image quality and biological dynamics were preserved over time.

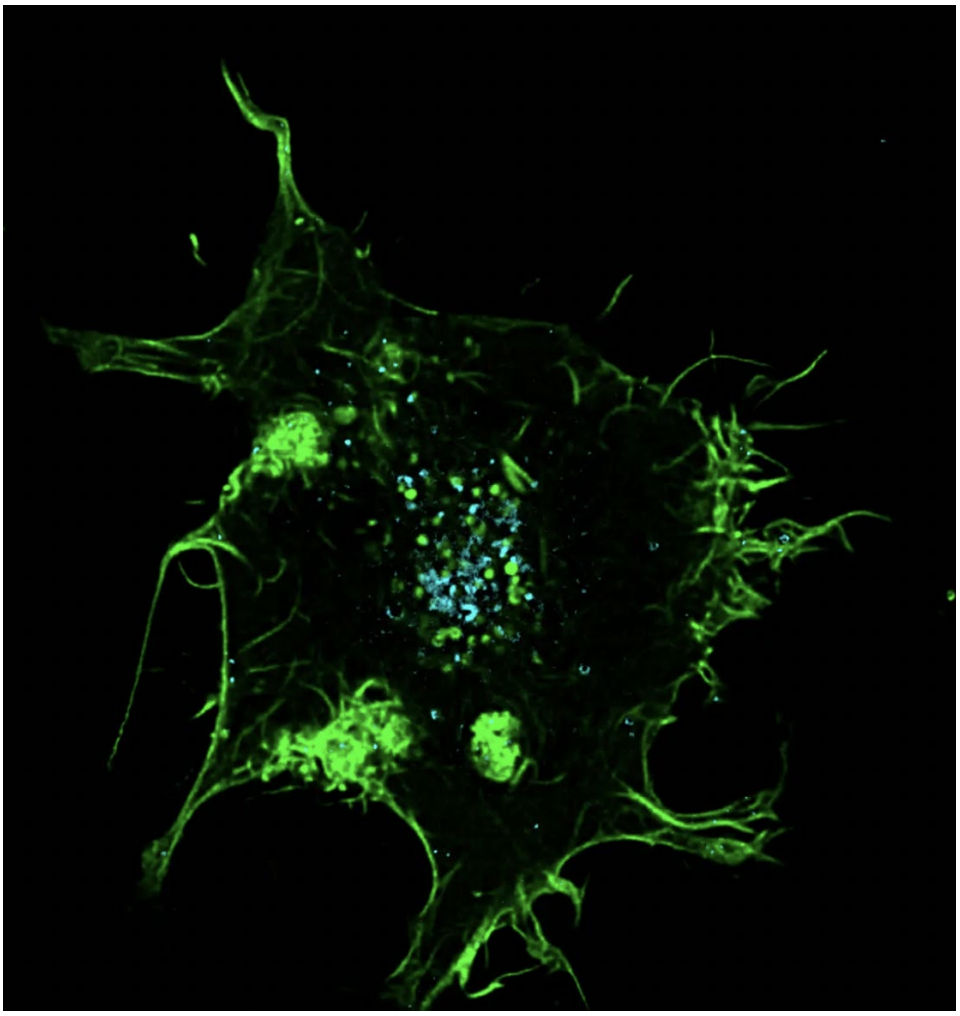


Figure 2: Movie showing 30 seconds fast live imaging of cells stably expressing actin (green) and endosomal (cyan) markers. This time-lapse was acquired with a **DeepSIM X-Light** SR system equipped with CFI SR Plan Apo IR 60X water immersion objective.

SR live imaging reveals mitochondrial meshwork details.

The mitochondria are the cellular powerhouses of most eukaryotes. They are composed of an intricated network of tubules that are tightly integrated with other cellular compartments.
The size of most mitochondrial structures is just at the resolution limit of optical microscopy making their analysis always challenging using diffraction-limited optical microscopes. In this context, **SR microscopy provides additional insight into mitochondrial morphology, distribution, and inter-mitochondrial contacts.**
As evidence of that, in **Figure 3**, we compared WF, CF, and SR images demonstrating a significant resolution increment between the three imaging modalities, especially if we focus on an intricately interconnected network of tubules (Figure 3B).

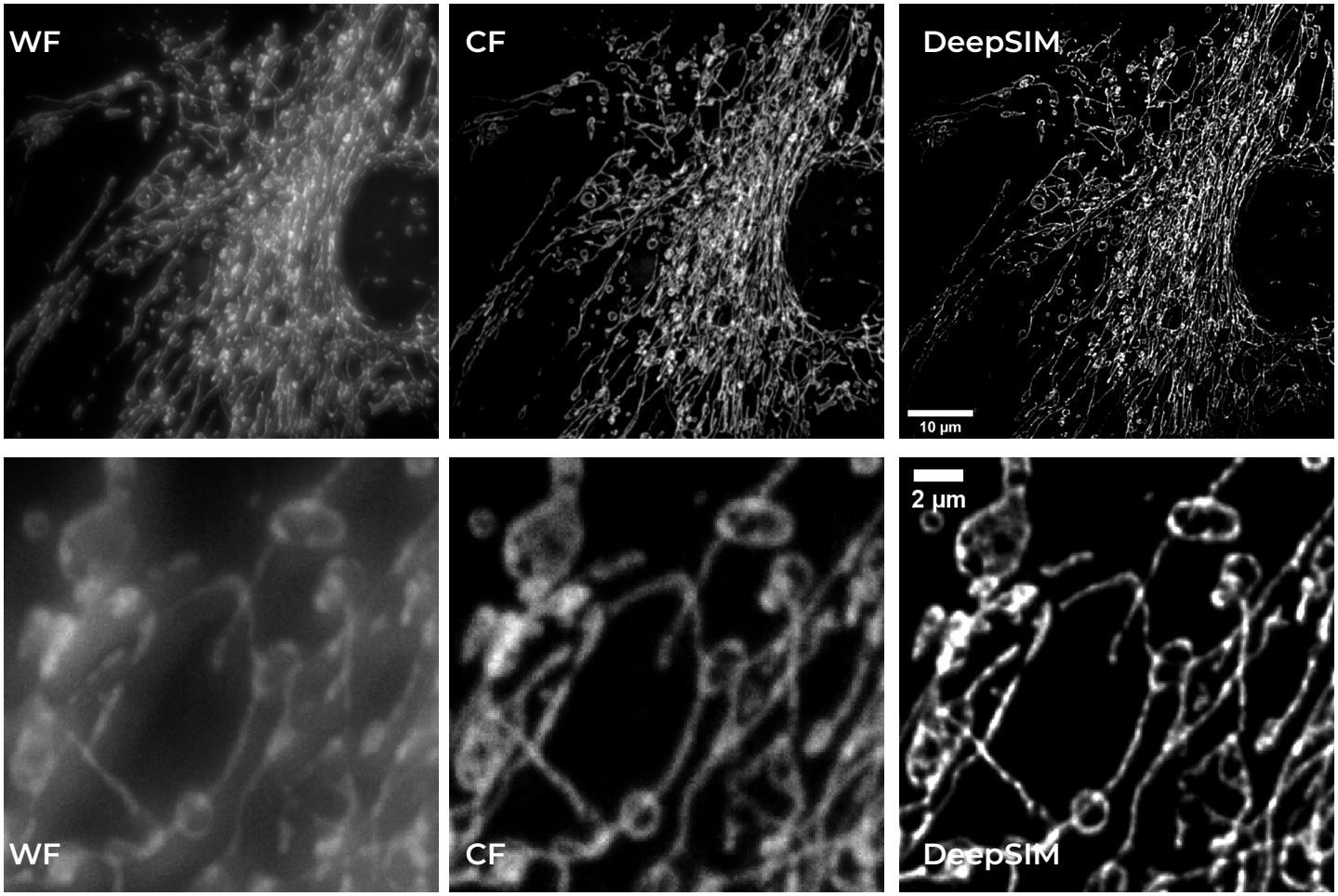


Figure 3: Comparison between WF, CF spinning disk, and DeepSIM SR images of cells permanently expressing a mitochondrial marker. The entire network of a cell (A) and the zoom of mitochondrial details (B) are shown. These images were acquired with CrestOptics X-Light V3 CF spinning disk system coupled with DeepSIM X-Light SR system and equipped with a CFI Plan Apo Lambda 100X oil immersion objective.

By removing out-of-focus and scattered light, CF imaging can provide excellent quality images respect to WF modality; however, to observe biological details below the refractive index, a super-resolution technique is required. The improvement in image quality provided by DeepSIM was compared to the one achieved when applying deconvolution to CF images, as it has been shown to improve mitochondrial structure detection (**Figure 4**).
Although deconvolution on CF acquisition increases image contrast, the improvement in resolution is limited compared to that achievable using SIM (Figure 4A). As demonstrated by the analysis of the intensity

profile (Figure 4B), subtle inter-mitochondrial contacts (160 nm) are only effectively distinguishable through SR microscopy (green line profile), which allows viewing of mitochondrial meshwork structural details.

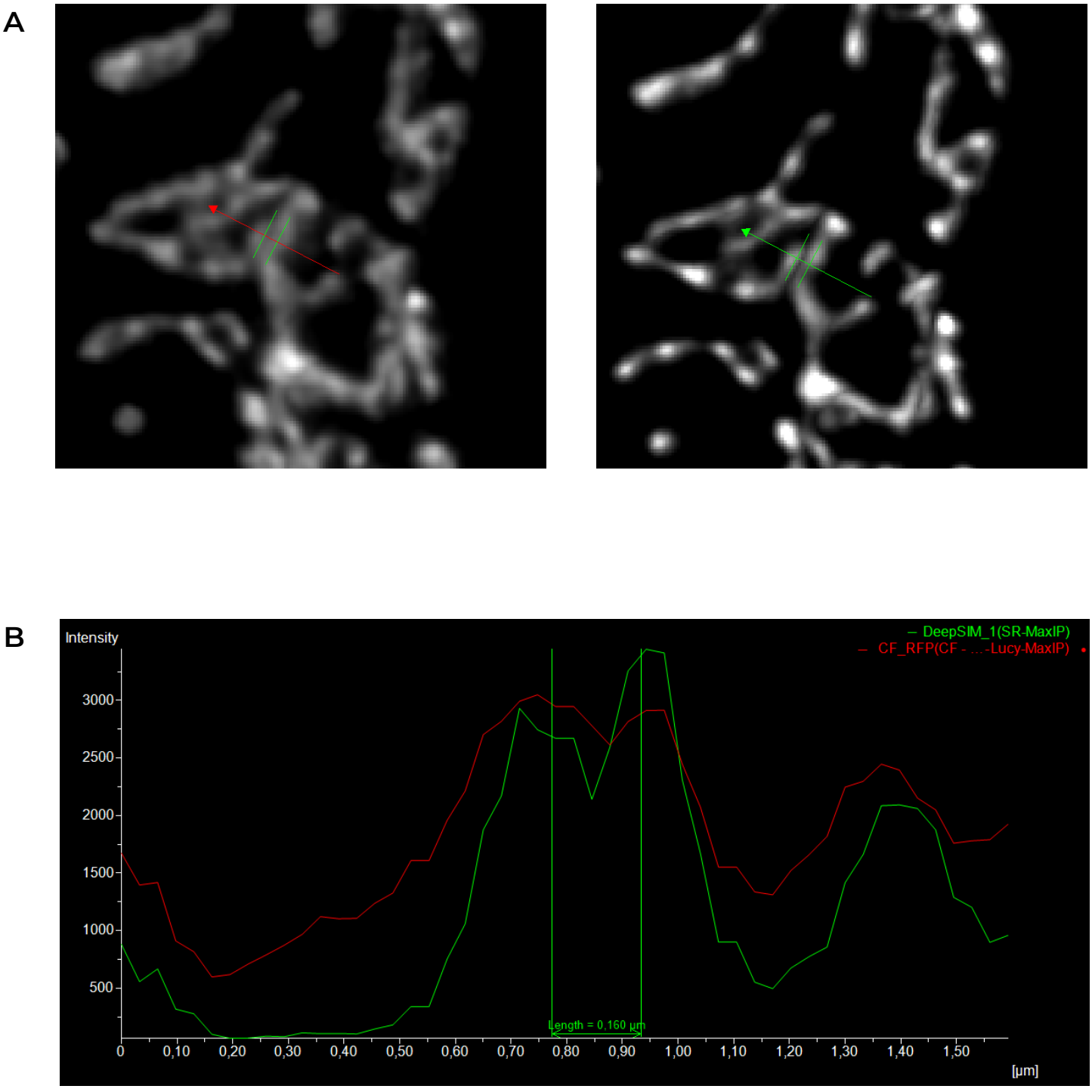
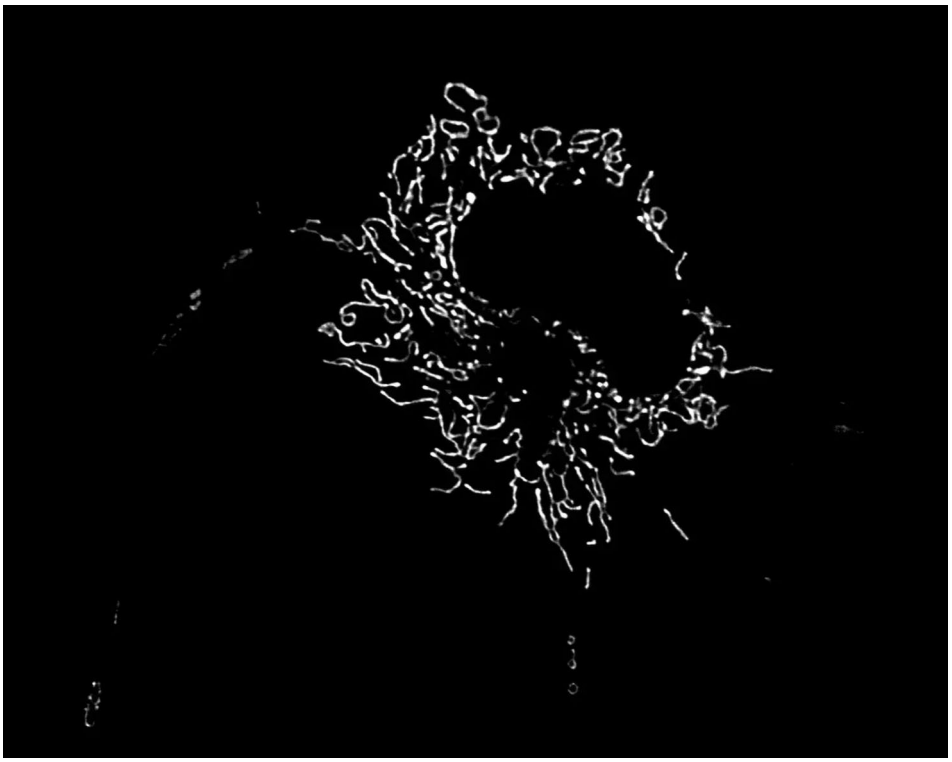


Figure 4: Parallel comparison between deconvolved CF and SR data showing mitochondrial structures. The inter-mitochondrial contacts (A) and the related intensity profiles (B) are shown (the red line and the green line represent the intensity profiles of the deconvolved CF and DeepSIM respectively). Deconvolution of CF images was performed by 3D Richardson-Lucy algorithm provided by NIS Elements software. These images were acquired with **CrestOptics X-Light V3 CF** spinning disk system coupled with **DeepSIM X-Light** SR system and equipped with a CFI Plan Apo Lambda 100X oil immersion objective.

Mitochondria are highly dynamic organelles undergoing fission and fusion cycles in order to maintain their shape, distribution, and size. After demonstrating the need for a gain in terms of resolution for the study of inter- and intra-mitochondrial structures, we leveraged the DeepSIM high-speed acquisition modality for the study of these mitochondrial dynamics.

A



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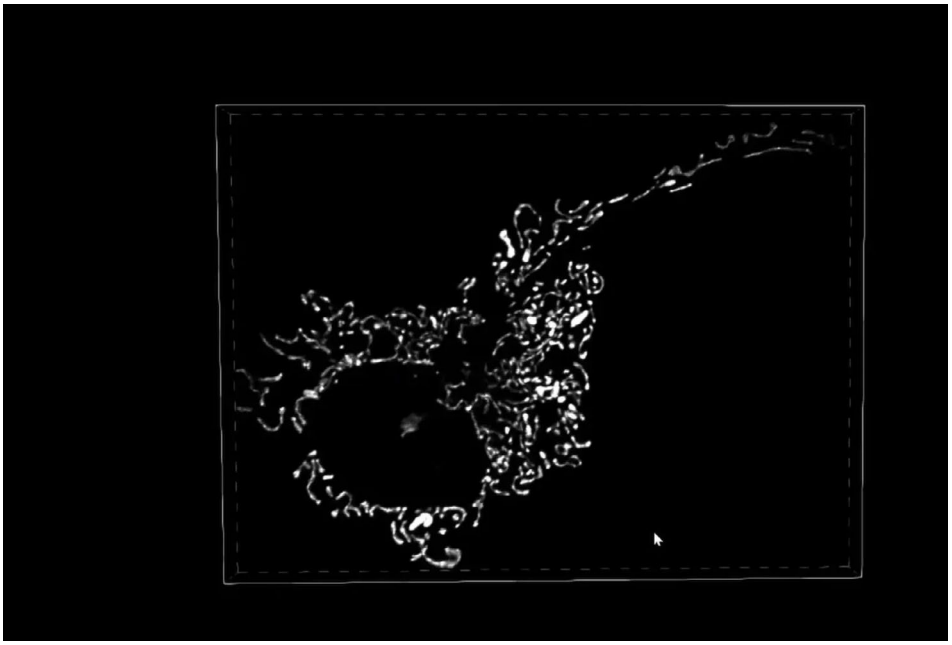

Figure 5: Parallel comparison between deconvolved CF and SR data showing mitochondrial structures. The inter-mitochondrial contacts (A) and the related intensity profiles (B) are shown (the red line and the green line represent the intensity profiles of the deconvolved CF and DeepSIM respectively). Deconvolution of CF images was performed by 3D Richardson-Lucy algorithm provided by NIS Elements software. These images were acquired with **CrestOptics X-Light V3** CF spinning disk system coupled with **DeepSIM X-Light** SR system and equipped with a CFI Plan Apo Lambda 100X oil immersion objective.

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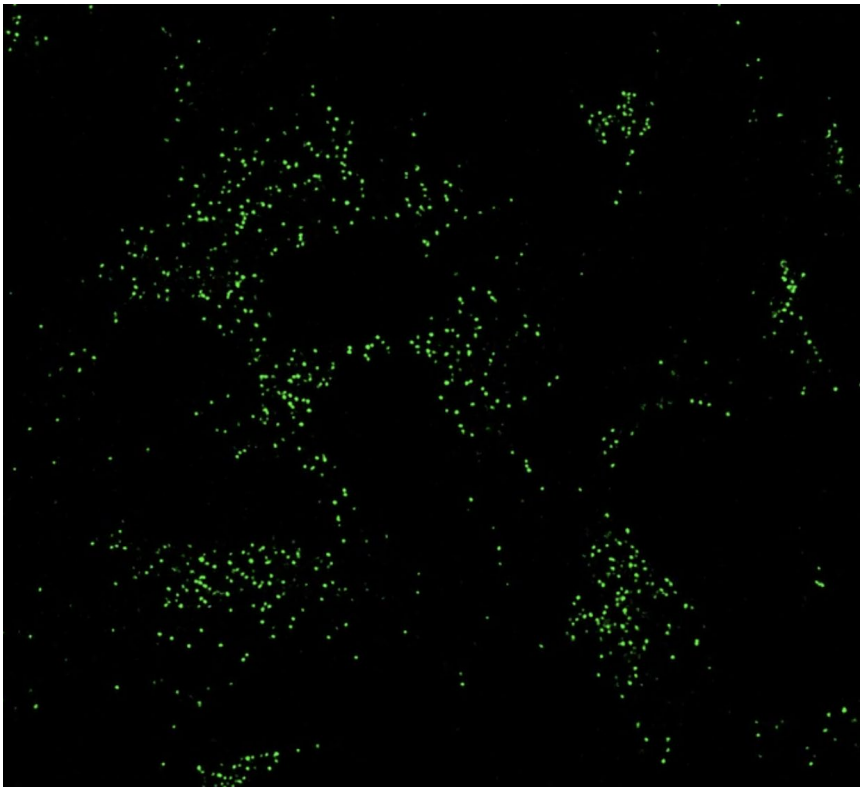

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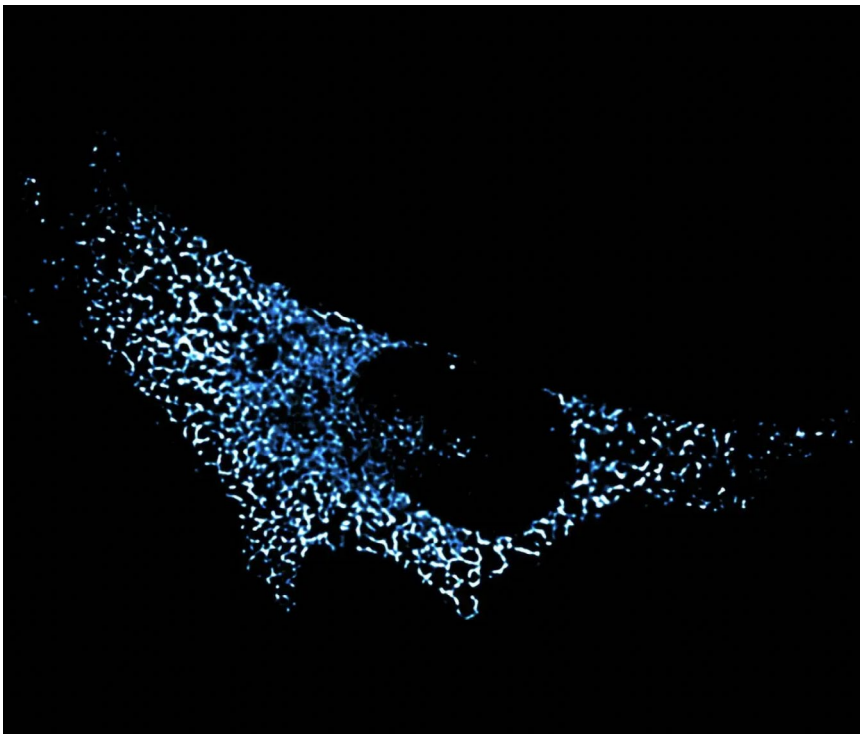

Figure 6: Fast live imaging of cells stained for lysosomes (A) and ER (B). These time-lapses were acquired with DeepSIM X-Light SR system equipped with CFI SR Plan Apo IR 60X water immersion objective.

Thanks to its temporal resolution of 12 fps, the DeepSIM allows for the capture of meaningful data at high resolution while minimizing light exposure and photo-toxicity risk. Delicate and live specimens can be explored in real-time monitoring cellular and subcellular changes. This functionality, as nicely reported in Figure 6, allowed exploring lysosomal vesicles (Figure 6A) and ER movements (Figure 6B) demonstrating how fast biological events can be monitored over time with an impressive resolutio.

Conclusions

Altogether these data demonstrate that DeepSIM combines SR with high-speed imaging, light efficiency, and sensitivity. Imaging membranes in live cells with SR techniques reveals structural dynamics which represent the crucial point for understanding cellular functions. However, many of these intracellular movements occur very rapidly and it is not easy to follow such dynamic events, especially if we consider that SR techniques require the acquisition of a set of raw images necessary for the creation of one super-resolved image. Therefore, it is very important to have an instrument capable of acquiring with a high frame rate, without damaging the sample or blocking the ongoing biological event.
In this context, because of its imaging speed and resolution power, the DeepSIM enables the effortless study of live-cell dynamics giving the opportunity to trace fast biological events at the subcellular level.

Acknowledgments:

The data shown in figures 1, 2, 4, and 5 were acquired during the Imaging Bootcamp 2023 held at Hokkaido University. Thanks to Dr. Fujioka (Hokkaido University) for the invitation and **Yasutomo Kubota** (Givetechs) for installation support.
Beautiful dynamic lysosomes shown in figure 6A have stained with a Spirochrome SiR Lysosome probe; thanks to **Spirochrome** for this excellent fluorescent probe.