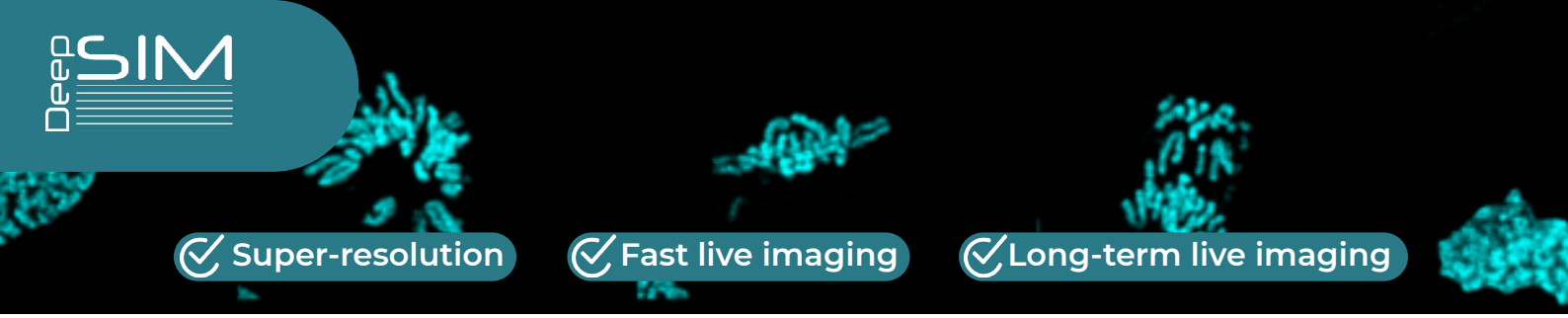

LIVE-CELLS DETAILS AND DYNAMICS WITH STRUCTURED ILLUMINATION MICROSCOPY

One of the biggest challenges for Super-Resolution (SR) techniques is to enhance the subcellular details of living cells while following the dynamics of biological events. The SR commercial solutions applicability is nowadays limited to a tiny fraction of the whole live-cell imaging requests due to their slow acquisition rate, inadequate depth penetration and necessity of special probes.

With the DeepSIM we have succeeded in the major mission to make available a reliable, easy-to-use and affordable solution to extend the current X-light V3 spinning disk imaging capacity to get SR data from living heterogeneous specimens.

In this Application Note, we demonstrate that thanks to a temporal resolution of >10 fps, with the Deep SIM it is possible to follow live-cell dynamics at a cellular and sub-cellular level. In particular, we monitored fast biological events, focusing on lysosomal vesicles movements, as well as on slower and lasting phenomena such as cellular mitotic divisions.

LIVE SUPER-RESOLUTION TRACING OF LYSOSOMAL VESICLES

To demonstrate the DeepSIM optimal performance in following fast biological events, we performed live imaging on HeLa cells permanently expressing GFP-alpha tubulin and stained for lysosomes with **Spirochrome SiR Lysosome probe**.

In **Figure 1** we compared Widefield (WF), Confocal (CF) and SR images demonstrating a significant improvement in terms of resolution over traditional microscopy. Moreover, in **Figure 2**, a parallel comparison between deconvolved spinning disk and SR data allows appreciating how some details of lysosomal vesicles are detectable, sharper and certainly clearer in SR images with respect to deconvolved CF images.

Thanks to the possibility of using **the CrestOptics X-Light V3 spinning disk** together with the **DeepSIM SR module**, it was possible to perform fast live imaging on a large field of view (FOV) (**Figure 3A**), obtaining significant data from a large number of cells, and then focus on a single cell to follow lysosomes dynamics at subcellular level (**Figure 3B**).

The DeepSIM high-speed acquisition modality allows the capture of relevant data at high resolution, minimizing light exposure and the resulting risk of phototoxicity. This functionality, as nicely reported in **Figure 4**, allowed to explore the dynamics of delicate specimens and trace lysosomal vesicles over time. In particular, a continuous 30 seconds DeepSIM SR time-lapse was carried out (Figure 4A), monitoring fast events without

worrying about bleaching. Image quality and lysosomes motility were preserved over time and this made it possible to extract important data from the tracking of lysosomes such as the distance travelled (um) and the speed (um/s) of vesicles (Figure 4B).

Altogether these data demonstrate that the DeepSIM combines high-speed imaging with light efficiency and sensitivity. Fast biological events can be monitored over time at a subcellular level without bleaching problems giving the opportunity to capture relevant data at high resolution also on live specimens.

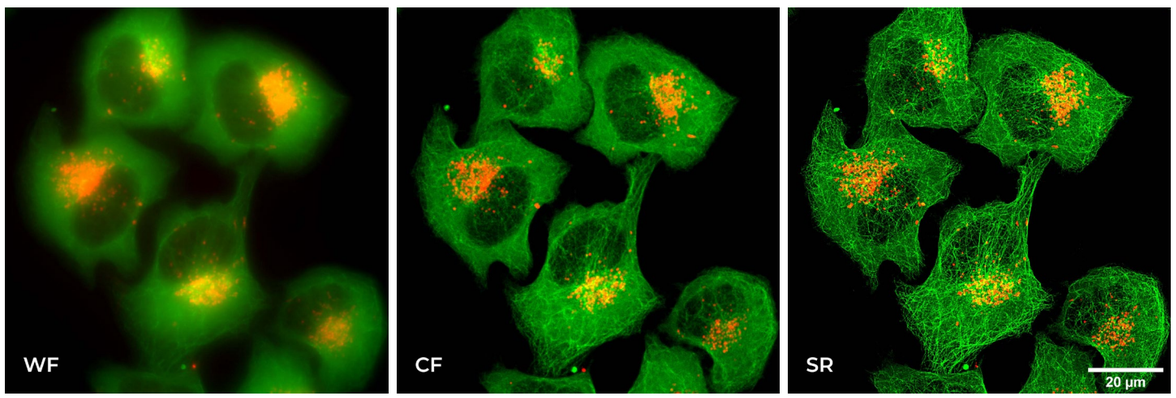

Figure 1: Comparison of WF, CF spinning disk and DeepSIM SR images of HeLa cells expressing GFP-alpha tubulin (green) and stained for lysosomes (red). These images were acquired with **CrestOptics X-Light-V3 CF spinning disk system** coupled with **DeepSIM SR add-on** and equipped with CFI Plan Apo Lambda 60X oil objective.

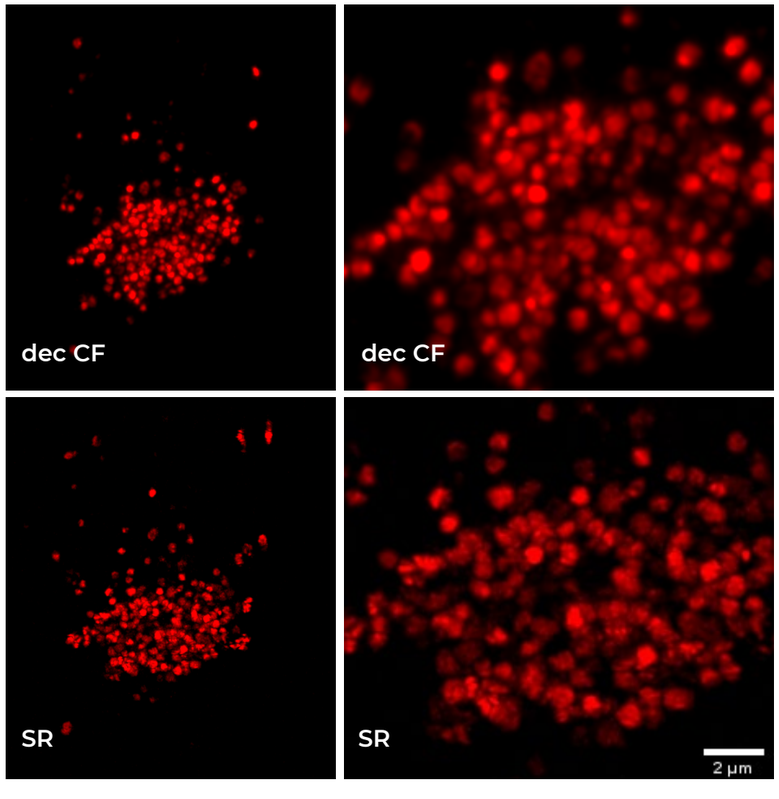

Figure 2: Parallel comparison between deconvolved CF and SR data showing lysosomal vesicles details; deconvolution of CF images was performed by 3D Richardson-Lucy algorithm provided by NIS Elements software. These images were acquired with **CrestOptics X-Light-V3 CF spinning disk system** coupled with **DeepSIM SR add-on** and equipped with CFI Plan Apo Lambda 60X oil objective.

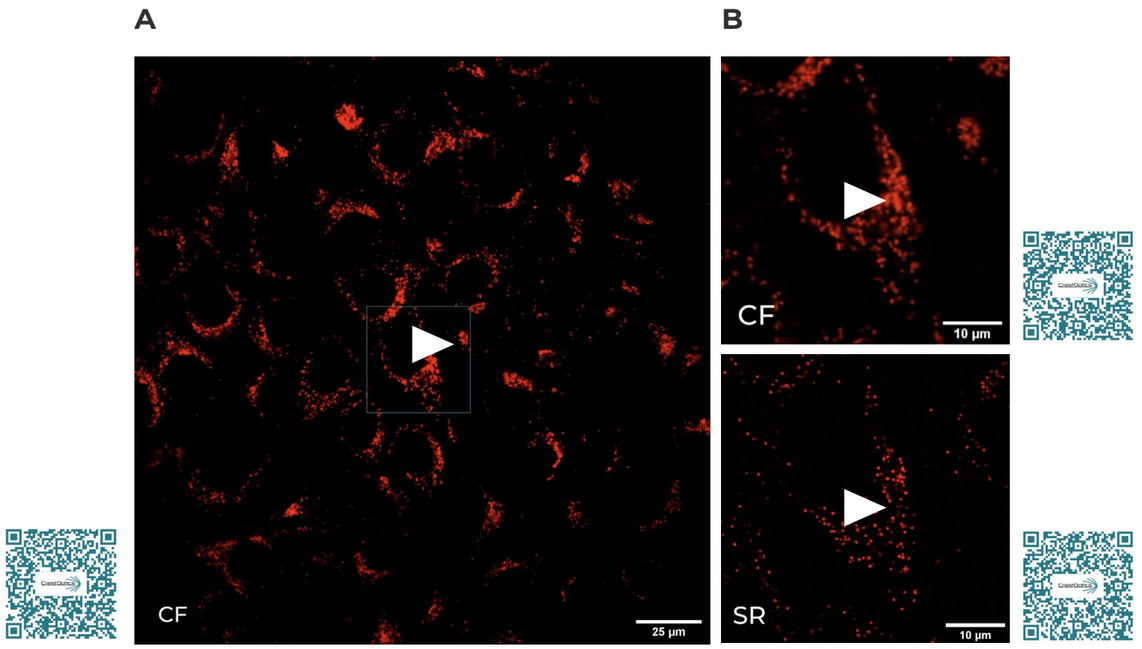

Figure 3: Fast live imaging of HeLa cells stained for lysosomes. (A) CF acquisition on large FOV; (B top) CF acquisition and single-cell focus; (B bottom) SR acquisition and single-cell focus. These images were acquired with **CrestOptics X-Light-V3 CF spinning disk system** coupled with **DeepSIM SR add-on** and equipped with CFI Plan Apo Lambda 60X oil objective.

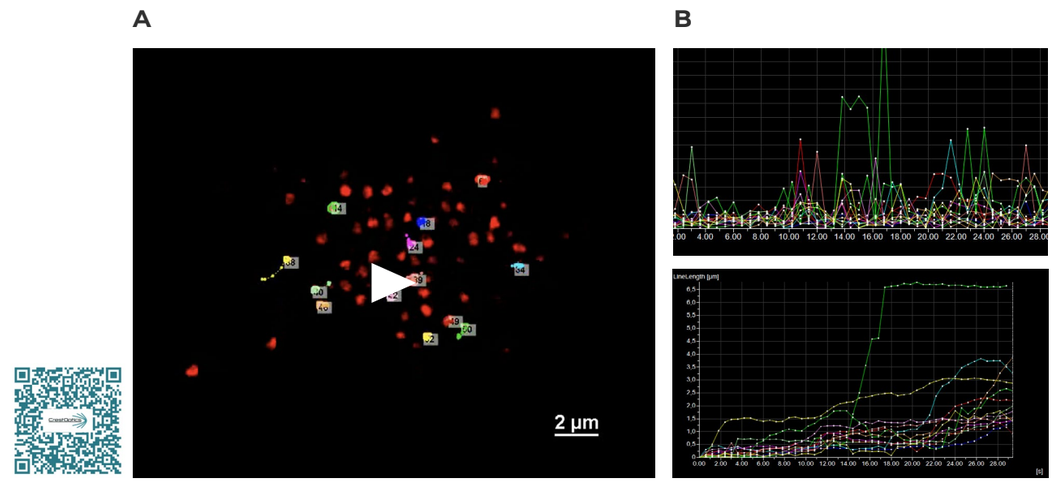

Figure 4: SR real-time lysosomes tracking. (A) Continuous 30 seconds DeepSIM SR time-lapse; (B) distance (um) travelled by lysosomes vesicles and their speed (um/s). These images were acquired with **CrestOptics X-Light-V3 CF spinning disk system** coupled with **DeepSIM SR add-on** and equipped with CFI Plan Apo Lambda 60X oil objective.

Long-term live Super-Resolution imaging of cellular divisions

To demonstrate the DeepSIM optimal performance also in following long-term biological events, we performed live imaging on HeLa cells stained with **Spirochrome SPY 505-DNA** and **SPY 555-Tubulin** probes for DNA and tubulin respectively.

Taking advantage of using the **CrestOptics X-Light V3 spinning disk** together with the **DeepSIM SR module**, we had the chance to perform a CF acquisition to have a general overview of the sample (**Figure 5A**). Once the area of interest was identified, thanks to the fast and easy switch between different imaging modalities, it was possible to spot a dividing cell and follow its entire mitotic process through SR imaging. As a matter of fact, 33 minutes of live SR time-lapse is reported in **Figure 5B** showing different mitotic phases with very crisp details.

One of the main drawbacks of the conventional structured illumination microscopy (SIM) is the photon dosage and its compatibility with delicate biological events; a lasting exposure to light can compromise a biological phenomenon in progress and this can be a problem especially if we consider the multiple images necessary for SIM image reconstruction.

In this regard, to prove DeepSIM's ability not to trade-off sample vitality and physiology, we monitored cells for more than 12 hours and, as reported in **Figure 6**, the health of the cells was not compromised at all, as evidenced by cell divisions and absence of cell death. Notably, image quality is preserved over time, allowing for longer time-lapse experiments without phototoxicity and photobleaching issues.

In conclusion, altogether these data demonstrate that with a temporal resolution greater than 10fps and a low photon burden, the DeepSIM SR module enables the effortless study of live-cell dynamics and provides structured localization imaging using routine sample preparation protocols. Both long-term and fast biological events can be monitored over time without worrying about bleaching, giving the opportunity to trace biological dynamics at the subcellular level and capture meaningful data with SIM also on live samples.

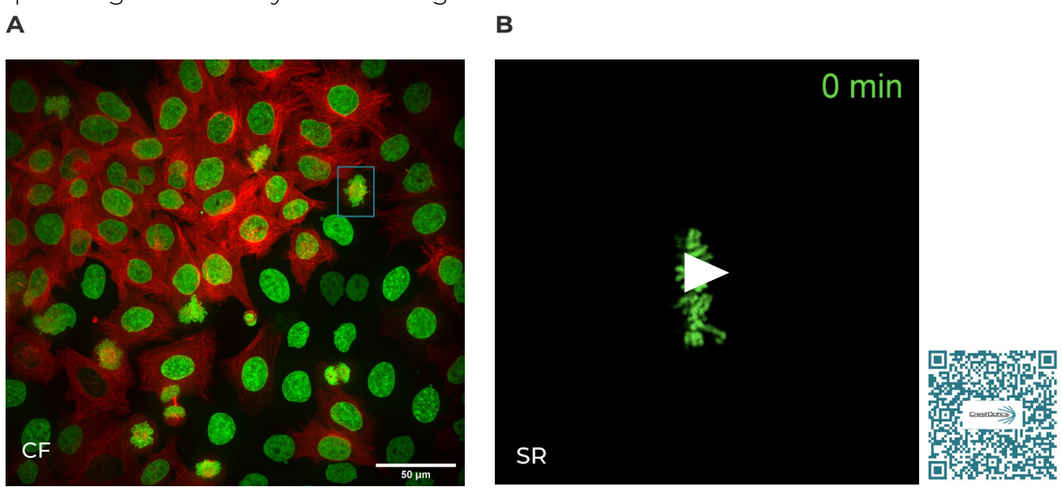

Figure 5: Live imaging on HeLa cells stained with Spirochrome SPY 505-DNA (green) and SPY 555-Tubulin (red) probes. (A) CF acquisition on large FOV; (B) single-cell focus showing 33 minutes of live SR time-lapse. These images were acquired with **CrestOptics X-Light-V3 CF spinning disk system** coupled with **DeepSIM SR add-on** and equipped with CFI Plan Apo Lambda 60X oil objective.

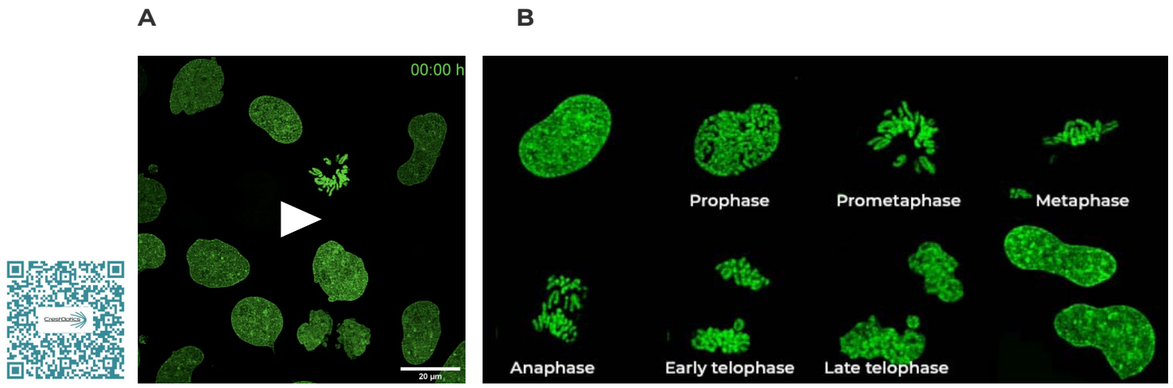

Figure 6: Long-term SR imaging of cellular division. (A) 12 hours time-lapse SR experiment; (B) mitotic phases shown in SR. These images were acquired with **CrestOptics X-Light-V3 CF spinning disk system** coupled with **DeepSIM SR add-on** and equipped with CFI Plan Apo Lambda 60X oil objective.

Microscopy Methods

All the acquisitions of this Application Note were performed through a Nikon Eclipse Ti2 microscope equipped with **CrestOptics X-Light-V3 spinning disk system** coupled **with DeepSIM super-resolution add on**, LDI laser illumination (89 North), Kinetix sCMOS camera with 6.5 um pixels (Photometrics) and CFI Plan Apo Lambda 60X oil objective (NA 1.4, WD 0.13).

Cells staining was performed with Spirochrome (https://spirochrome.com/) fluorescent probes for live bioimaging (Spirochrome). In particular, we labelled cells with SPY 505-DNA, SPY 555-Tubulin and SiR Lysosome probe.

Movies for lysosomes tracking were recorded for 30 seconds with no delay; movies for cellular division imaging were recorded for 33 minutes (3 minutes delay) (Figure 5B) and for 12 hours and 15 minutes (15 minutes delay) (Figure 6A).

HeLa cells shown in this application note were kindly provided by **Dr. Lia Asteriti** and **Dr. Giulia Guarguaglini**, Institute of Molecular Biology and Pathology, **Consiglio Nazionale delle Ricerche** c/o **Sapienza Università di Roma**.