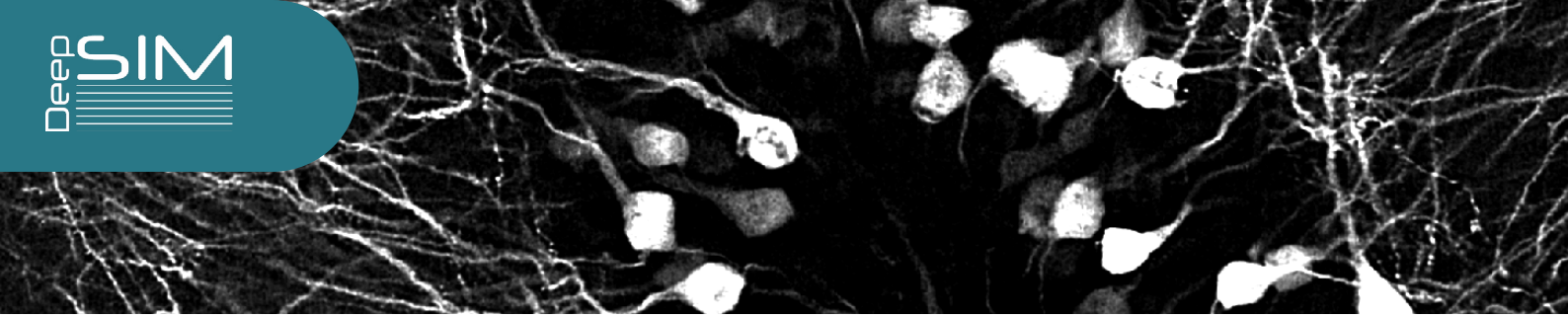


STRUCTURED ILLUMINATION MICROSCOPY WITH LOW MAGNIFICATION OBJECTIVES

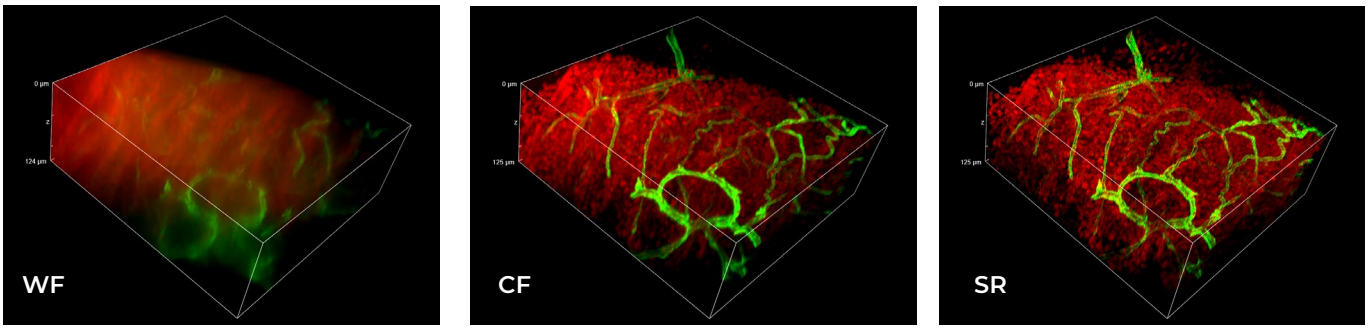
At CrestOptics we are working to make Super-Resolution (SR) accessible to all researchers and to advance their scientific potential and breakthroughs to the next level.
Our newest product, the DeepSIM, is based on a multi-spot structured illumination system and it is able to study cellular structures up to an XY resolution of ~100nm without the need to apply any particular sample preparation protocols.
Structured illumination microscopy, as all SR techniques, is generally associated with the use of high magnification lenses such as 100X and 60X; however, we designed the DeepSIM to work with 40X and 20X as well. **In this Application Note, we demonstrate that two-fold enhanced spatial resolution can also be obtained with a low magnification objective as 20X, making our SR module a reliable, simple to use and affordable solution to study sub-cellular details and expand the range of applications, including complex three-dimensional (3D) models such as tissues, spheroids and organoids.**

Super-Resolution imaging of cleared samples at low magnification

To demonstrate the DeepSIM optimal performance also with low magnification objectives, we compared Widefield (WF), Confocal (CF), and SR images in complex and thick cleared biological samples using a CFI Plan Apo Lambda 20X air objective (NA 0.75, WD 1).
In Figure 1, different visualizations of a cleared mouse intestine section (0.55 mm thickness) with blood vessels in green and nuclei in red are shown. In particular, thanks to the fast switch between three microscopy methods, we report a global comparison of WF, CF spinning disk and SR acquisitions of a volume of 125 um (Figure 1A) and a 3D movie of the DeepSIM SR acquisition showing fine details of intestinal villi (Figure 1B).
In Figure 2, a cleared mouse brain section (0.55 mm thickness) with GFP-expressing neurons is shown. Notably, we acquired a 150 um Z stack of an intricate neuronal network shown as maximum intensity projection (MIP) (Figure 2A) and 3D volume view (Figure 2B).
As nicely appreciated from these images, switching from WF, CF and SR modalities allows reaching an increasing resolution gain that enhances the fine details of the neuronal network even at high thickness.
Moreover, we decide to compare the 20X SR acquisition with a 60X CF acquisition and, as demonstrated in figure 2C and 2D, the acquisitions with the DeepSIM not only allow to have an evident resolution improvement compared to the 20X CF acquisitions, but it enables to reach an image quality

comparable to those obtained with the CF spinning disk equipped with a CFI Plan Apo Lambda 60X oil objective (NA 1.4, WD 0.13). In addition to a noticeable enhancement in neuronal details quality with respect to a 20X CF (Figure 2D), the use of the DeepSIM with 20X objective allows going even deeper into the sample than what is permitted by the maximum working distance of a common 60X oil objective (130 um) (Figure 2C).

A



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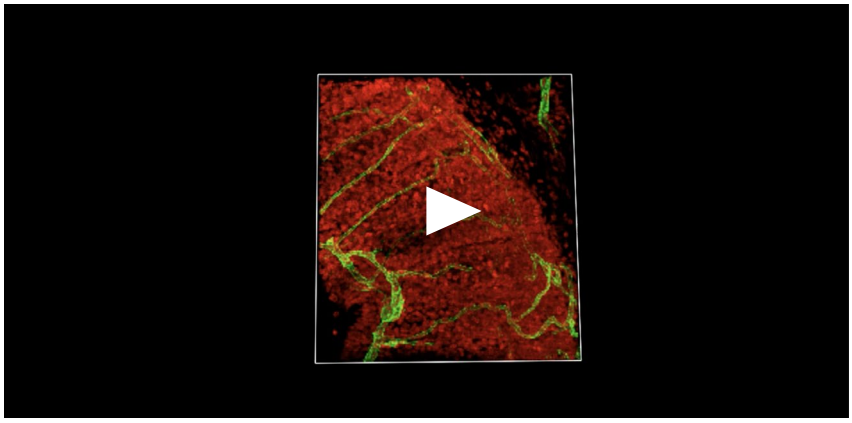
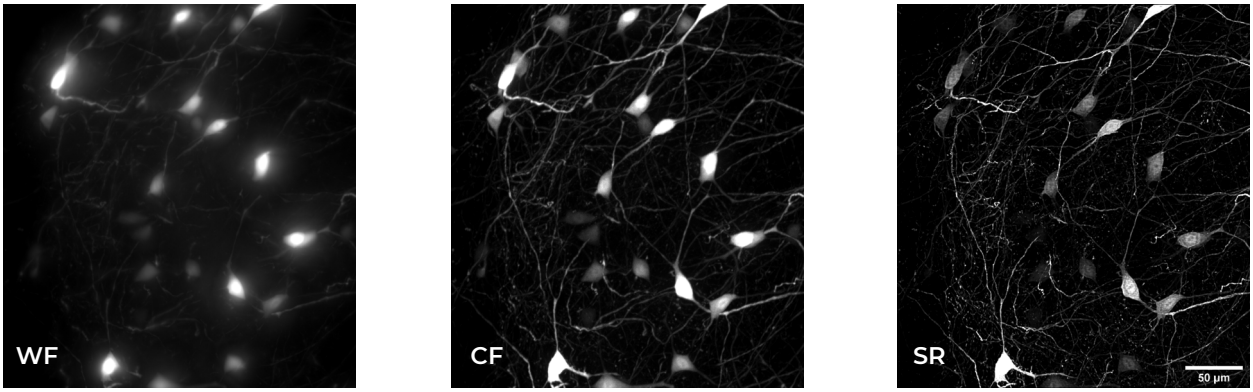


Figure 1: Cleared mouse intestine section showing blood vessels (green) and nuclei (red). **(A)** Comparison of WF, CF spinning disk and DeepSIM SR 3D volume views. **(B)** 3D movie of DeepSIM SR acquisition, 125 um thickness. These images were acquired with CrestOptics X-Light-V3 CF spinning disk system coupled with DeepSIM SR add-on and equipped with CFI Plan Apo Lambda 20X air objective.

A



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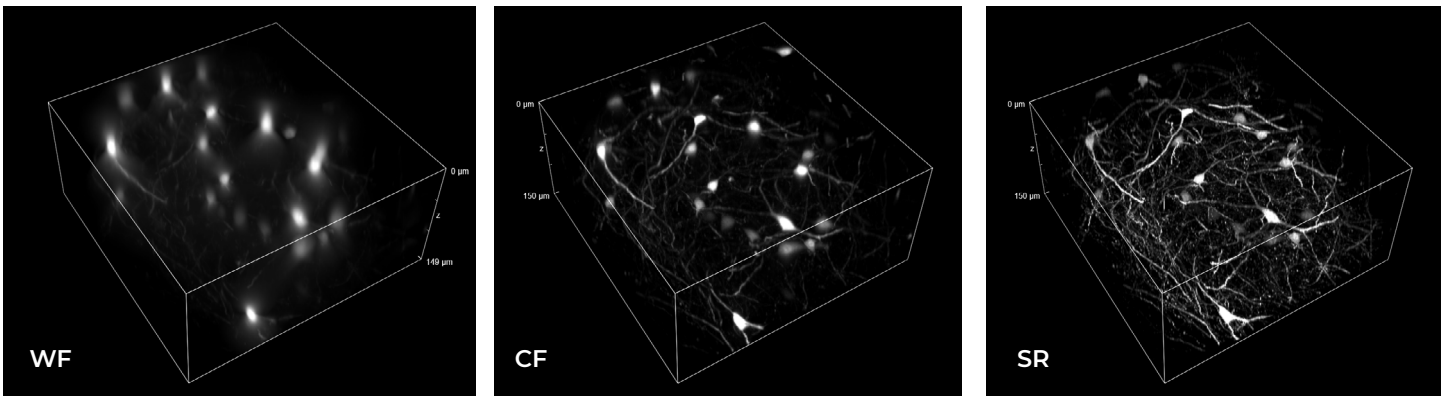

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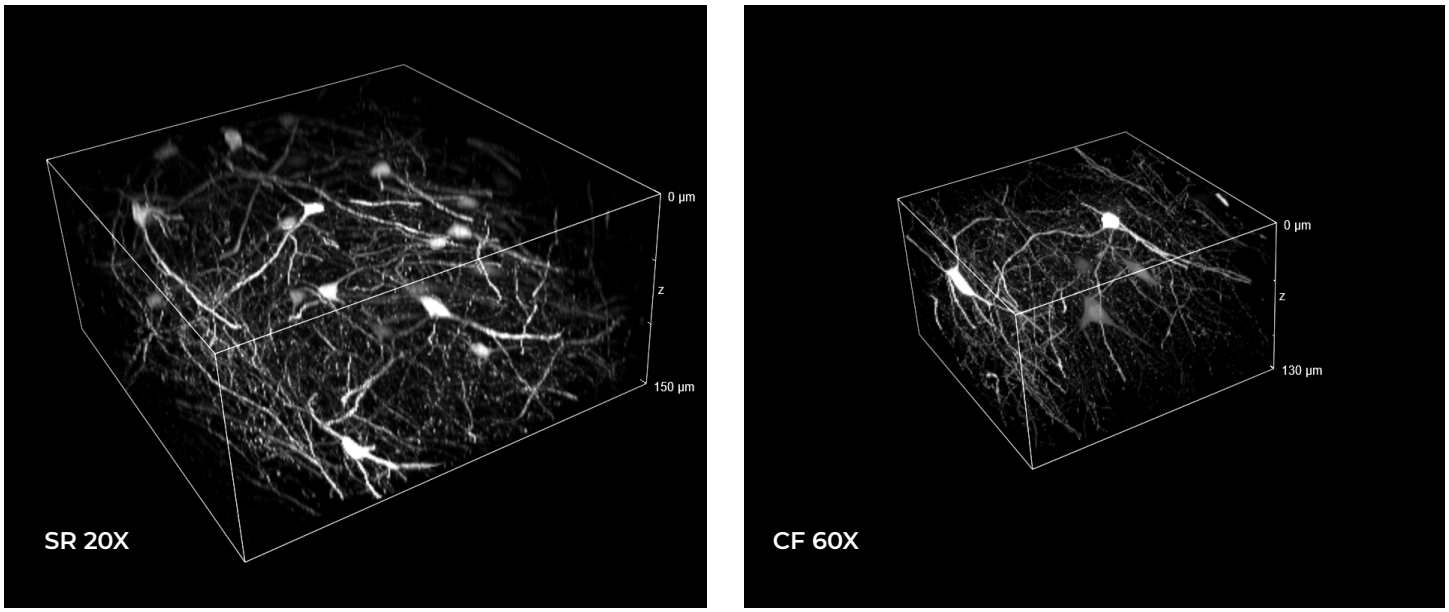

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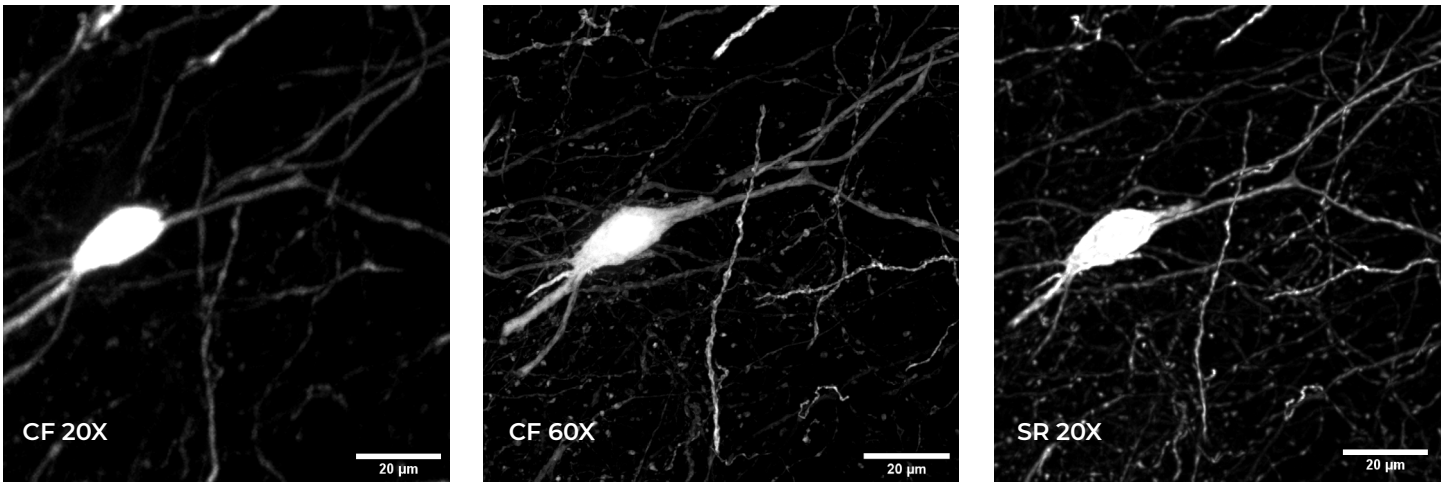


Figure 2: Cleared mouse brain section with GFP-expressing neurons. **(A)** Comparison of WF, CF spinning disk and DeepSIM SR acquisition acquired with 20X air objective and shown as maximum intensity projection from Z stack; **(B)** 3D volume views acquired with 20X air objective, 150 um thickness. **(C)** Comparison between 3D volume views acquired with DeepSIM SR module equipped with 20X air objective (left) and CF spinning disk equipped with 60X oil objective (right). **(D)** Focus on neuronal details and comparison between three different acquisition modalities: CF spinning disk equipped with 20X air objective (left), CF spinning disk equipped with 60X oil objective (middle) and DeepSIM SR module with 20X air objective (right).

Super-Resolution imaging of conventionally prepared samples at low magnification

Clearing reagents can make biological tissues transparent reducing light scattering and enhancing the visualization depth of very thick specimens in depth. However, the DeepSIM can also offer SR also inside routinely prepared samples, where the different refractive index of the main biological components causes a greater scatter of light when it travels through the tissue.
Therefore, we tested some conventionally prepared samples, such as brain organoids and mouse brain sections, making acquisitions that are usually done with a confocal microscope.

Human whole-brain organoids are able to recapitulate the very first neurodevelopmental events in vitro, both in terms of cellular interactions and tissue architecture, mimicking the real 3D organization of the developing human brain. **In Figure 3**, a human brain organoid section (50 um thickness) showing CTIP2-positive deep layer cortical neurons (green) and pan-neuronal MAP2 marker (red) is represented. In particular, we focused on a cortical plate performing a comparison between WF, CF spinning disk and DeepSIM SR images acquired with 20X air objective and shown as MIP from Z-stack (15 um). Again, switching between the three acquisition modalities, we obtained a notable increase in resolution and image quality with the DeepSIM, enhancing fine cellular structures of cortical deep-layer neurons that, differentiating towards the periphery, shape the typical laminar cortical structure.

In Figure 4, we report images of another conventionally prepared sample: a hippocampal coronal slice from Thy1-GFP mouse brain (50um thickness) and, analyzing the acquisitions made with 20X air objective (Figure 4A), the resolution gain obtained with the DeepSIM is evident, also in this very scattering sample. Furthermore, focusing on neuronal details inside the intricate organization of the hippocampal neuronal network, we compared the 20X SR acquisition with a 60X CF image. As nicely reported in figure 4B, the Deep SIM equipped with the 20x air lens allows reaching an image quality comparable to those obtained with the CF spinning disk equipped with the 60X oil objective.

In conclusion, all these data taken together demonstrate the capability of the DeepSIM to bring out fine details in extra-large objects using routine preparation protocols. **Affordable easy-to-use low magnification dry objectives can also be used to acquire both conventional and not-conventional specimens, doubling the objective performance and obtaining an image quality very similar to that reached with immersion lenses.**
The use of the DeepSIM with low magnification objectives allows benefiting all the advantages of an air lens, like the absence of immersion media or a greater working distance, without compromising the quality of the image. **This type of configuration greatly increases the plasticity of the system and gives our customers more flexibility in applications, applying the DeepSIM as a powerful platform for SR imaging and laying the foundations for the use of SR in high-content screening.**

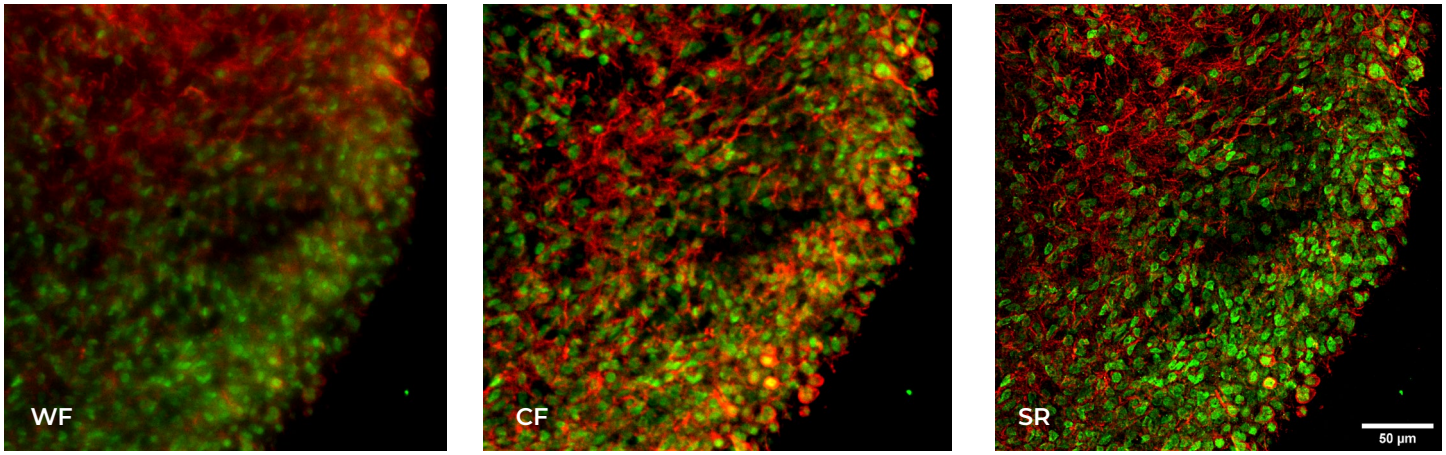
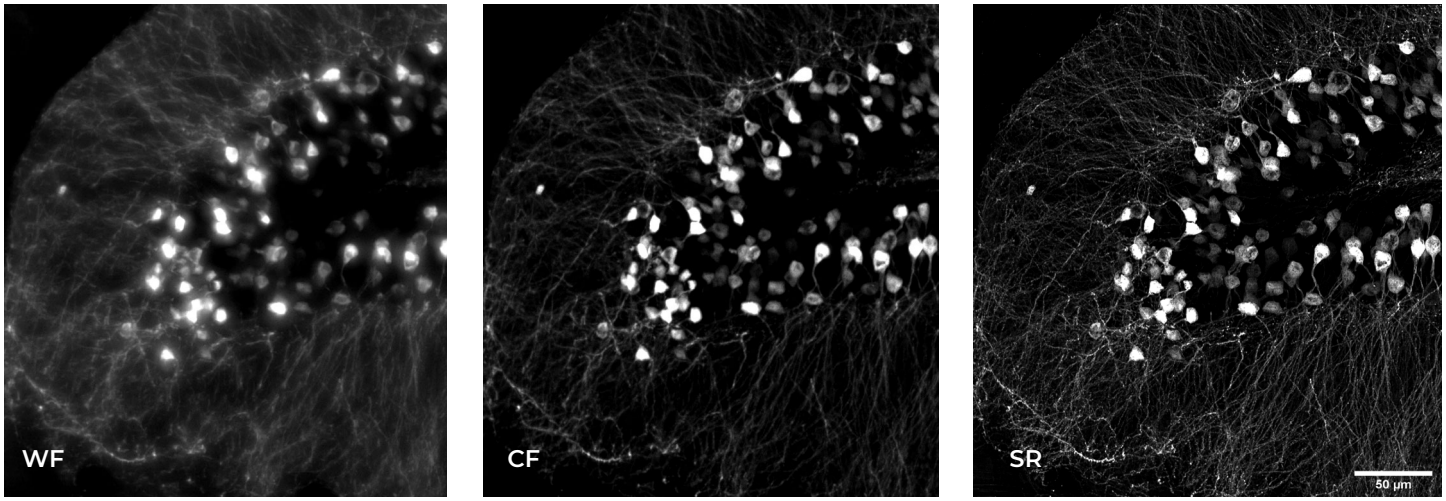


Figure 3: Human brain organoids showing CTIP2-positive deep layer cortical neurons (green) and pan-neuronal MAP2 marker (red). Comparison of WF, CF spinning disk and DeepSIM SR images acquired with 20X air objective and shown as maximum intensity projection from Z stack.

A



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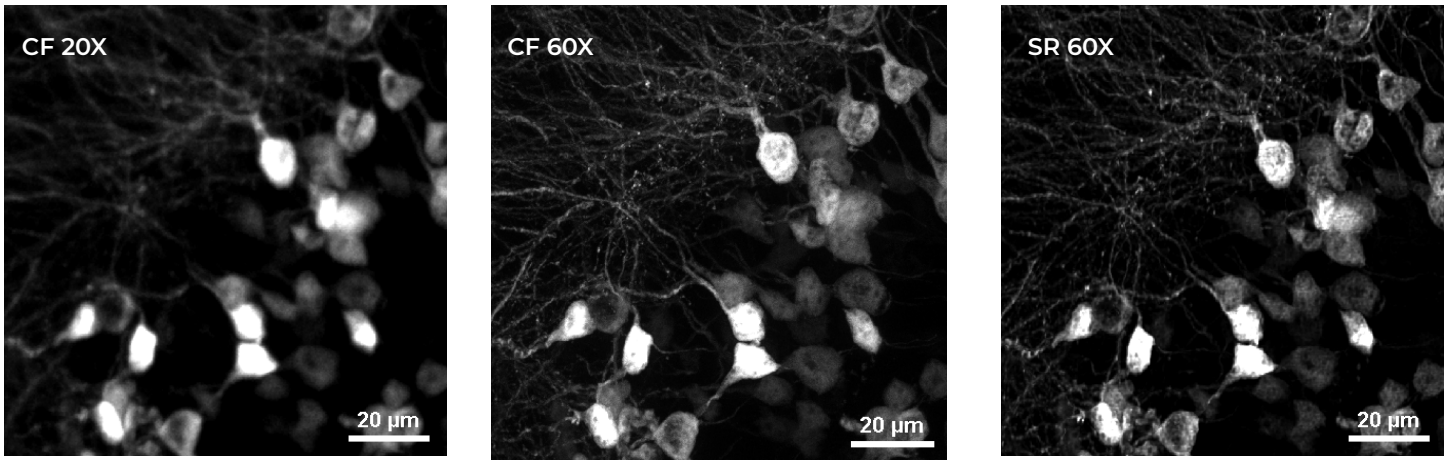

Figure 4: Hippocampal coronal slice from Thy1-GFP mouse brain. (A) Comparison of WF, CF spinning disk and DeepSIM SR images acquired with 20X air objective and shown as maximum intensity projection from Z stack. (B) Focus on neuronal details and comparison between three different acquisition modalities: CF spinning disk equipped with 20X air objective (left), CF spinning disk equipped with 60X oil objective (middle) and DeepSIM SR module with 20X air objective (right).

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) and Prime BSI Scientific CMOS (sCMOS) camera with 6.5 um pixels (Photometrics). We used a CFI Plan Apo Lambda 20X air objective (NA 0.75, WD 1) and a CFI Plan Apo Lambda 60X oil objective (NA 1.4, WD 0.13).

Human cerebral organoids shown in Figure 3 and mouse brain sections represented in Figure 4 were kindly provided by **Prof. Silvia Di Angelantonio**, **Dr Maria Rosito**, **Dr Federica Cordella** and **Dr Caterina Sanchini**, Center for Life Nano- & Neuro-Science (**CLN2S@Sapienza Università di Roma – Istituto Italiano di Tecnologia**).