DeepSIM

Visualization of the binding pattern of monoclonal antibodies against *Klebsiella pneumoniae*

Fondazione Toscana Life Sciences (TLS) is a no-profit organization based in Siena (Italy) which supports research activities in the Life Sciences, from basic research to industrial application. Among their projects, the **Monoclonal Antibody Discovery (MAD) Lab** at TLS is focused on identification and production of **monoclonal antibodies (mAbs)** as novel therapeutics against bacteria and viruses. mAbs have various advantages when compared to standard medications: for instance, their specificity, a limited risk of resistance development, and ability to work synergistically with antibiotics. In addition, mAbs help **the identification of vaccine targets and accelerate vaccine development, thus representing excellent tools to fight infectious diseases.**

A relevant aspect of the mAbs research field is the validation of the binding efficiency of the antibody to the bacterial surface. Here we show data of a **488-fluorophore-conjugated mAb isolated at TLS binding to Klebsiella pneumoniae expressing** mCherry. Acquisitions were obtained with **CrestOptics X-Light V3 confocal spinning disk system coupled** with **the DeepSIM Super-Resolution add on**, Celesta laser source (Lumencor) and sCMOS Prime BSI Camera (Photometrics, 6.5 µm pixel size). We used 100x oil objective (Nikon, 1.45 NA) and performed Z-stack acquisitions along the bacterial volume (0.1 µm Z step size).

In **Figure 1** we show the maximum intensity projection (MIP) of a Z stack of bacterial cells acquired with CrestOptics X-Light V3 confocal spinning disk. To be more accurate in the identification of the **mAb binding pattern** on the bacterial surface, we analysed the sample with the **DeepSIM Super-Resolution add on of X-Light V3 spinning disk** and we visualize bacteria as a 3D volume. **The DeepSIM super resolution module is easily integrated into existing microscope systems to provide structured localization imaging of complex biological specimens using routine sample preparation protocols.** In **Figure 2** we show a comparison between the confocal spinning disk and the super-resolution acquisition by displaying 3D volume views and highlighting cellular details appreciable only in the super-resolved image, especially regarding the mAb staining (green).

To demonstrate how **CrestOptics DeepSIM** confers significant gains over traditional microscopy, **we report a global comparison of different microscopy methods ranging from widefield, to confocal spinning disk to super-resolution acquisitions (Figures 3-4).** Of note, the monoclonal antibody 3D organization, the alternation of aggregates and even small, detailed protrusions are detectable and certainly clearer in the super-resolved image compared to widefield,

original and deconvolved spinning disk data. Overall, the screening of the binding quality and efficiency between antibodies and bacteria is an excellent biological example suited for spinning disk microscopy. After selecting the promising antibodies, the possibility to **easily switch to super-resolution microscopy inside the same set-up configuration** helps researchers to deepen the data and make further biological considerations. **CrestOptics X-Light V3 spinning disk configuration with the DeepSIM module is the perfect solution for every scientific need that requires resolution enhancement at specific steps of the experimental protocol, without the need to move to a different microscope.**

Figure 1: MIP image of bacterial cells acquired with a **CrestOptics X-Light V3 spinning disk.** 488-fluorophore-conjugated mAb is shown in green and cytoplasmic mCherry in red.

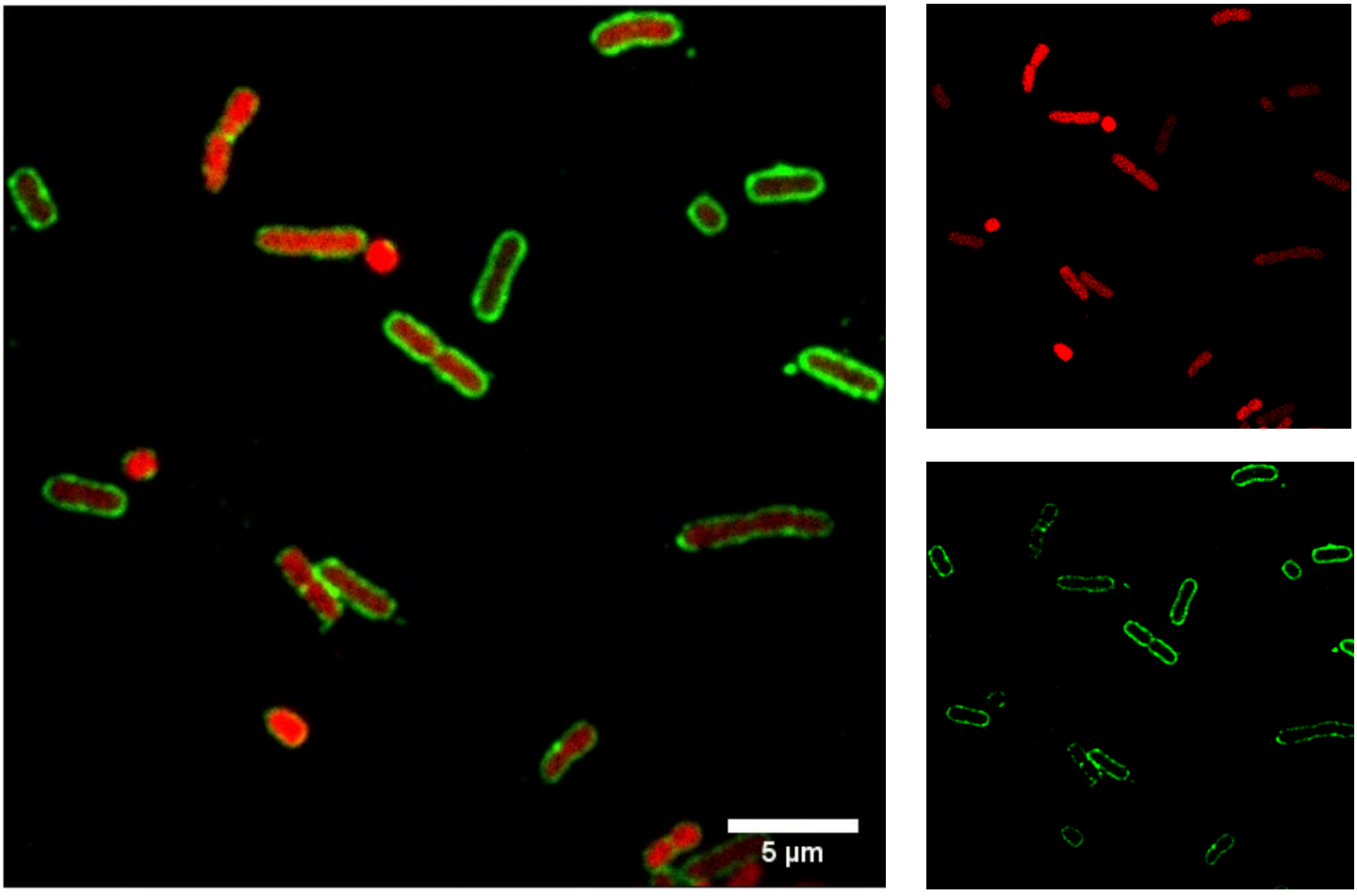


Figure 2: 3D volume views of a region of interest showing bacterial cells acquired with a **CrestOptics X-Light V3 spinning disk (SD) and DeepSIM Super-Resolution (SR) system.** 488-fluorophore-conjugated mAb is shown in green and cytoplasmic mCherry in red. Arrows indicate examples of structural details appreciable only in SR image. SR images were processed starting from 65 raw images obtained with a multi-spot structured illumination by a modified version of the joint Richardson-Lucy (jRL) algorithm.

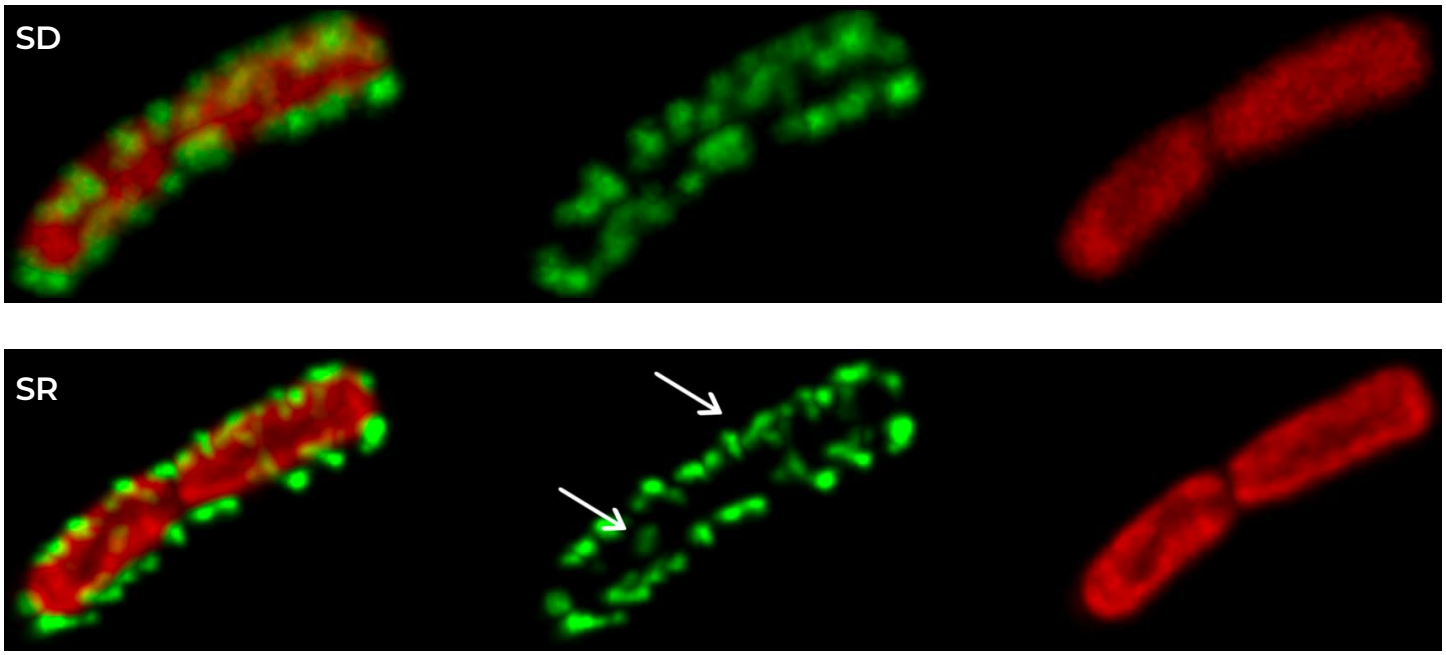


Figure 3: A single Z plan showing mAb staining (green) acquired in different modalities (widefield, WF; spinning disk, SD; deconvolved SD by 3D Richardson-Lucy algorithm (20 ite) provided by NIS Elements software; DeepSIM Super-Resolution, SR) with a **CrestOptics X-Light V3 system**. Arrows indicate a structural detail appreciable only by increasing image resolution.

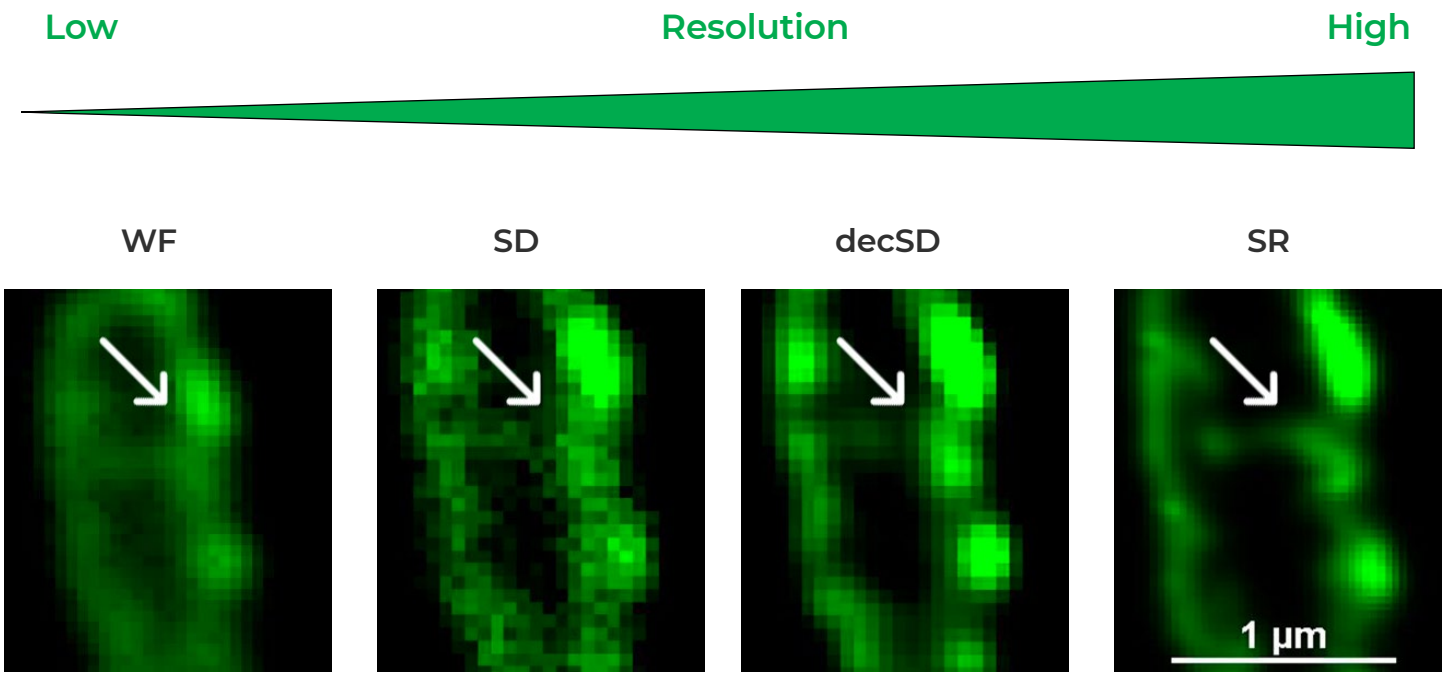
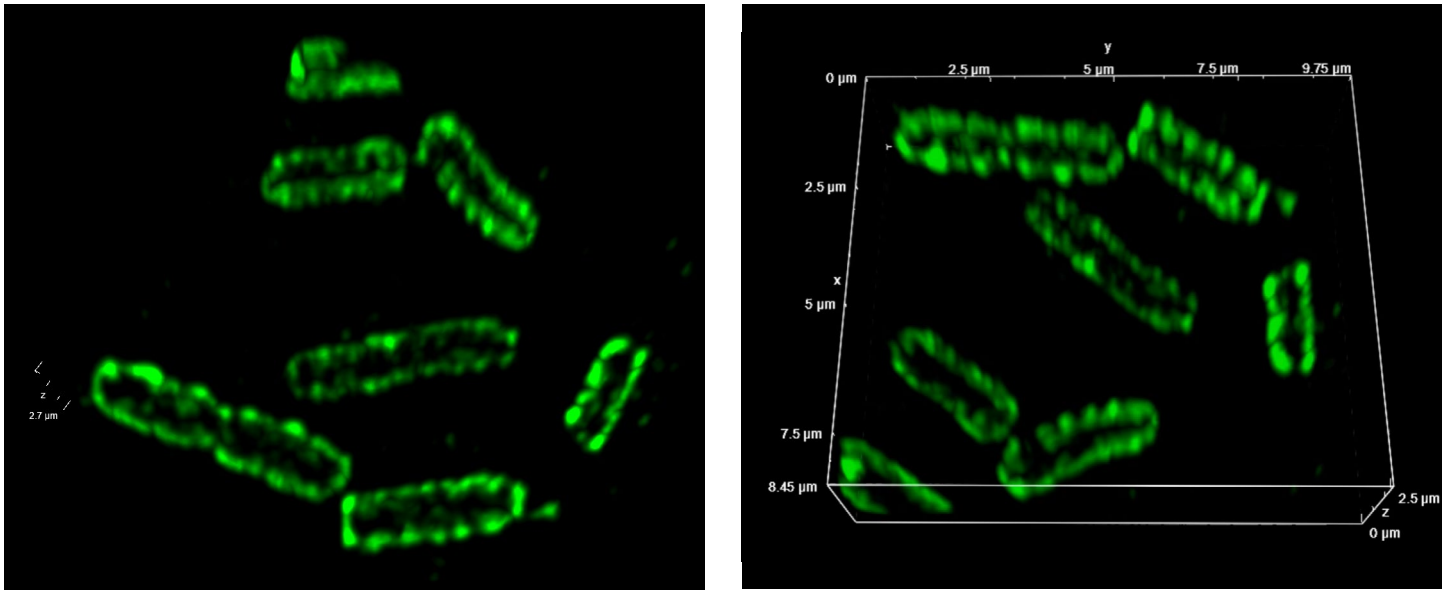


Figure 4: 3D volume view and movie of DeepSIM Super-Resolution (SR) image.



The application note has been prepared in collaboration with **Dr. Emanuele Roscioli**, **Dr. Anna Kabanova** and **Dr. Claudia Sala**. **Fondazione Toscana Life Sciences, Siena (Italy).**

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