ISIM VERSUS SORA – A DIFFERENT APPROACH TO ANALOGUE-ISM

Since the mid-2010's; the emergence of Image Scanning Microscopy (ISM), for relatively simple and easy to use super-resolution imaging, has increased the catalogue of techniques at the disposal of the research community. The leading ISM technique for live cell imaging is Instant SIM and in particular the VT-iSIM, which allows for super resolution fluorescence imaging to be achieved at high frame rates with reduced photobleaching. Other implementations of this technique, such as the Yokogawa SoRa system, cause significant photo-bleaching and photo-damage to samples when performing live-cell imaging.

In this Technical Note we will detail the fundamental differences between the VT-iSIM and the Yokogawa SoRa systems and how these differences affect the performance of the instruments for live-cell, super-resolution imaging.

As intermated above, the fundamental difference between the SoRa and the iSIM is in the scan architecture. The iSIM utilises a galvanometer scanner to scan a 2-Dimensional (2D) array of points at the sample plane whilst the SoRa utilises a rotating disk.

The 2D-array scan architecture was developed by VisiTech in the early 2000's and became the bases for the analogue based ISM techniques as per York, Shroff, et al (doi:10.1038/nmeth.2687).

For the iSIM, it means that the emission signal is de-scanned by the galvo. Hence, you have a conjugate image plane in an isolated emission path where you can place the re-assignment optic (a µLens array) and then re-scan the emission onto the camera, as per figure 1.

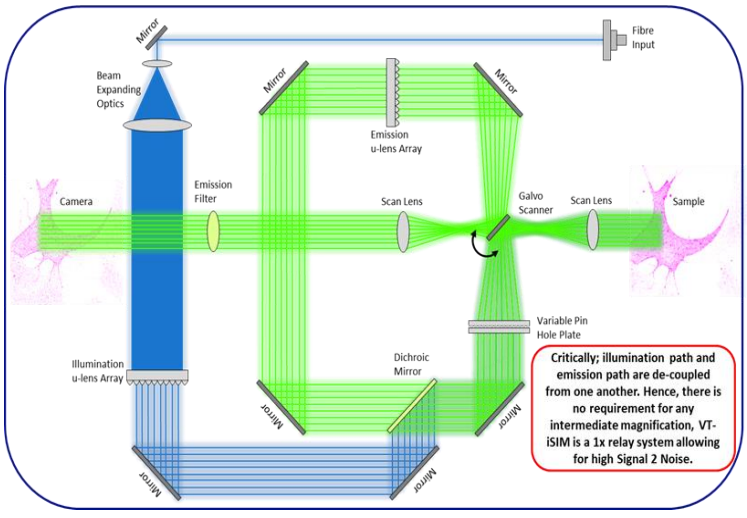

Figure 1: iSIM Scan Architecture

However, the rotating disc architecture in the SoRa leaves no possibility to "*de-scan*" the emission and no conjugate image plane in the emission path. The excitation and emission paths are intrinsically linked to one another, thus the SoRa is forced to place the re-assignment optic between the pin hole array and the sample plane, as shown in fig 3.

This leads to three main issues:

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1. Intermediary Magnification Requirement

The re-assignment optic shrinks each individual emission Point Spread Function (PSF) in the array by 0.5x. This subsequently doubles the Numerical Aperture (NA) of the light in the emission path.

If this optic is also in the excitation path (as per the SoRa), then it will half the NA of the excitation light. This will under-fill the objective back-aperture as well as making the pin-holes appear ~4x smaller at the sample plane. To fill the back aperture of the objective and return the pin-holes to their "*normal*" size, you need to add intermediary magnification.

If you increase the overall magnification, you increase the number of pixels per unit area by the square of the difference in magnification. Given Signal to Noise (S2N) at reasonable exposures is dominated by read noise, it becomes significantly impacted, as shown in figure 2.

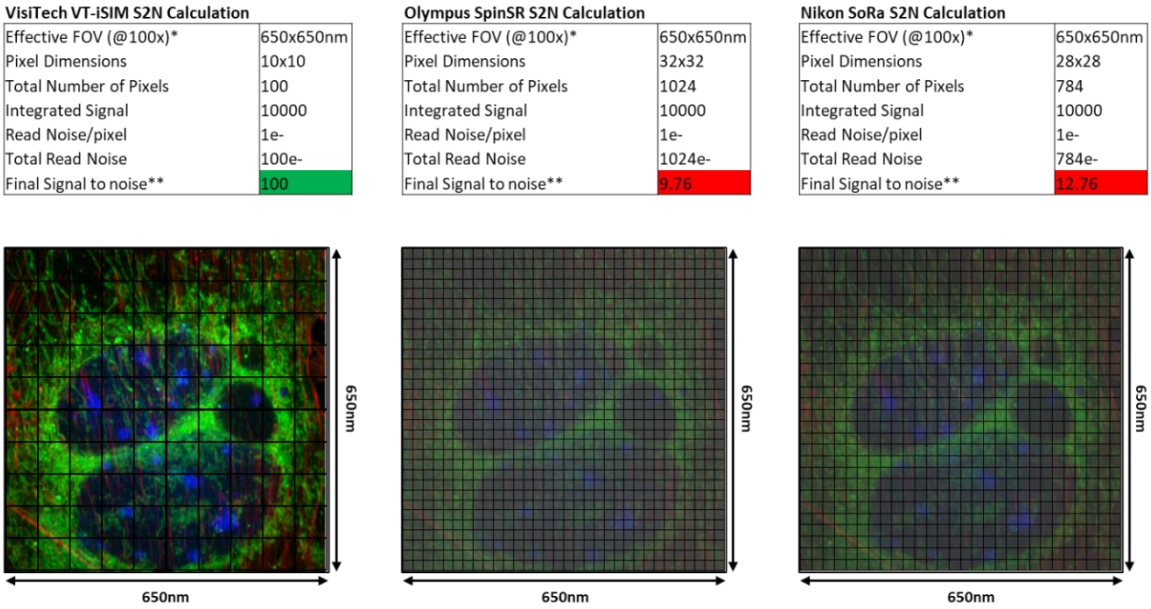

Figure 2: Effect of intermediary mag on Signal to Noise

In the iSIM; as the re-assignment optic is in a conjugate image plane in the emission path (and not in the excitation path), there is no requirement for intermediary magnification and hence much improved S2N compared to the SoRa.

2. Increased Out of Focus Light Collection

As the re-assignment optic in the SoRa has to be placed between the pin hole array and sample plane, the out of focus light is not rejected before conducting the optical re-assignment.

The re-assignment optic is a µLens array. µLens's are designed to collect as much light as possible. If the out of focus light is not removed first, it will be collected by the re-assignment optic and focused through the pin-holes, thus increasing the overall background signal.

Obviously, as the iSIM has the re-assignment optic in a conjugate image plane which is between the pin hole array and the camera, the out of focus light is removed before the re-assignment. This results in much lower levels of background/noise.

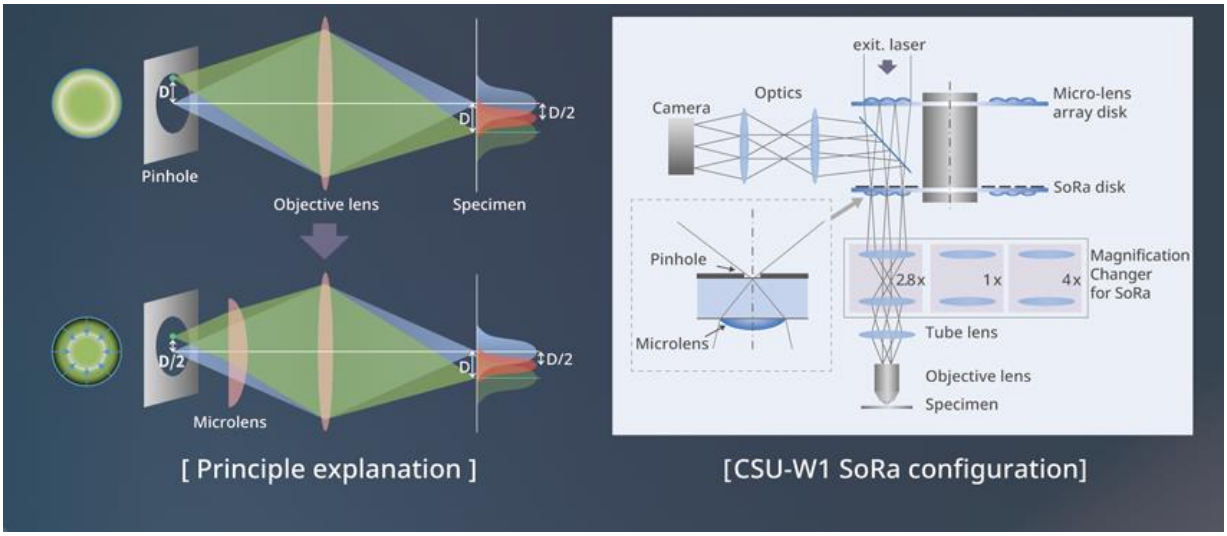

Figure 3: SoRa Scan Architecture

3. Increased Cross-Talk

Where you have thick specimens and/or high refractive index miss-matches, there is increased scatter of emitted photons. If the re-assignment optic is placed between the pin hole array and the sample plane (as in the SoRa), the optic will capture a lot of this scattered light and focus it through neighbouring pin holes. This will produce a hexagon type pattern artefact in the background.

As the iSIM has the re-assignment optic in a conjugate image plane which is between the pin holes and the camera, then this possible artefact is significantly reduced.

In Summary

Isolating the re-assignment optic (emission µLens array) in the emission path only, as in the VT-iSIM, results in lower camera read-noise per unit area, lower background, and less cross-talk. This results in higher signal to noise and lower photo-bleaching/photo-toxicity for your live-cell imaging applications.