Optical sectioning with doubled resolution by means of Structured illumination Scanning Microscopy (SISM)

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1. Theory:

We present a technique capable of surpassing the diffraction resolution limit whilst providing optical sectioning to the host microscope. The system can be considered a **hybrid** between conventional **structured illumination** and **scanning** microscopes. The sample is illuminated with an extended illumination pattern but the SISM image is obtained after a scanning process.:

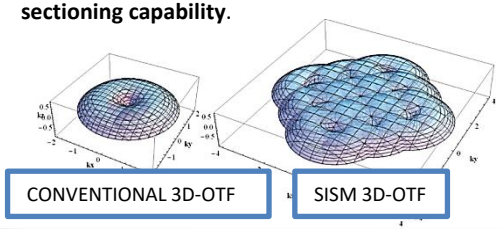
- **Extended illumination** + **Scanning**

$$I_{3D}(\mathbf{r}) = \left[O(\mathbf{r})\, S(\mathbf{r})\right] \otimes_3 h(\mathbf{r}) \qquad I_{\mathrm{SEISM}}(\mathbf{r}_s) = O(\mathbf{r}_s) \otimes \left[S(\mathbf{r}_s) h(\mathbf{r}_s)\right].$$

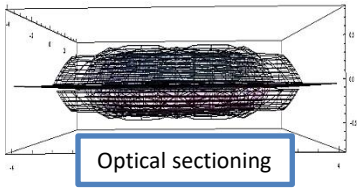
$$\tilde{I}_{3D}(\mathbf{k}) = \left[\tilde{O}(\mathbf{k}) \otimes_3 \tilde{S}(\mathbf{k})\right] H(\mathbf{k}) \qquad \tilde{I}_{\mathrm{SEISM}}(\mathbf{k}_s) = \tilde{O}(\mathbf{k}_s) \left[\tilde{S}(\mathbf{k}_s) \otimes H(\mathbf{k}_s)\right],$$

Concretely, the patterned illumination is created by the interference of **4 plane waves**.

After the scanning, the microscope has a **synthetic optical transfer function** (OTF) **that doubles the bandwith** of the conventional OTF. Furthermore, the missing cone is filled providing **optical sectioning capability**.



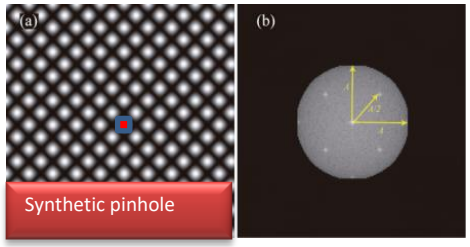
CONVENTIONAL 3D-OTF | SISM 3D-OTF



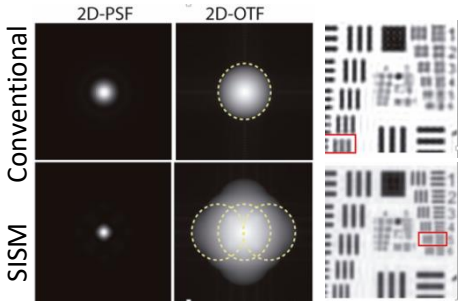
Optical sectioning

2. Simulated results:

Simulated image of a plane fluorescent object in SISM before the scanning process and its corresponding Fourier transform:



Simulated 2D-PSFs, OTFs, and images for a conventional system and a SISM after the scanning process.



3. Experimental results:

Schematic setup:

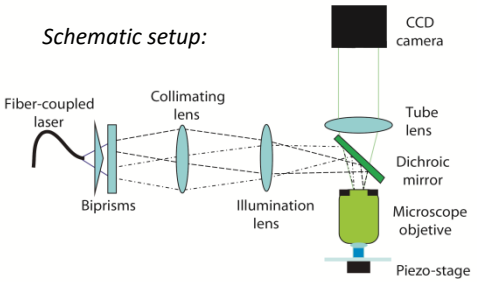
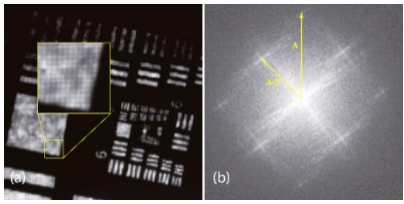
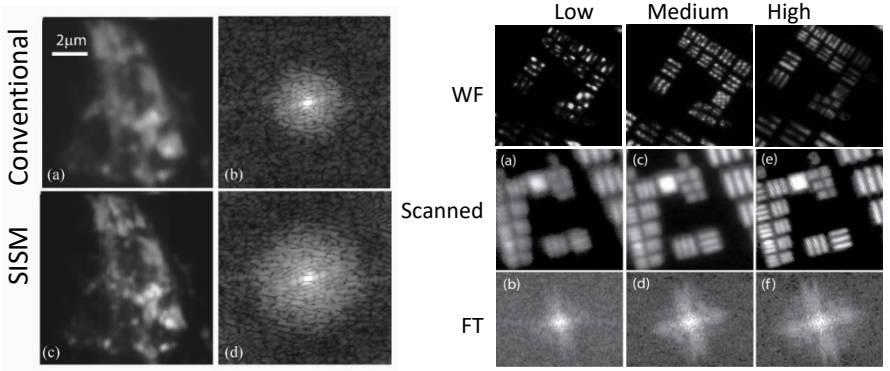
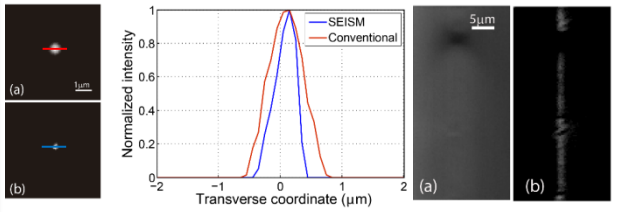


Image of a fluorescent USAF test in SISM before the scanning process and its corresponding Fourier transform:



As expected, the scanned SISM image lateral resolution depends on the spatial frequency of the projected pattern. The enhancement and shape of the synthetic OTF become apparent in the Fourier domain:





Sample:Fluorescent bead (r≈100nm) *Sample: Thin fluorescent layer*

LOW photobleaching due to the extended illumination onto the sample.
Direct recomposition of the orders ⇨ NO reconstruction algorithm required
Simultaneously provides Optical Sectioning and transverse superresolution.