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Structured Illumination Microscopy behind Turbid Media

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1. Abstract

When light from a source travels through a turbid medium, it gets scattered and the image of the source gets scrambled. However, by shaping the incoming light, an optimum wavefront can be constructed which travels through the medium and forms an interferometric focus[1]. It has been shown that the focus can be raster scanned in 3D to obtain image stacks of fluorescent structures behind turbid medium[2]. But, owing to the diffraction limit of the focus, these images are also diffraction limited. Here we demonstrate a way to image fluorescent structures behind turbid medium with resolution beyond diffraction limit. By employing Structured Illumination Microscopy (SIM) [3] we show a resolution enhancement over conventional widefield (WF) microscopy.

2. Introduction

Wavefront Shaping

- Technique to construct a light wavefront that efficiently focuses beind a turbid medium.
- Choosing the right parameters, diffraction limited focus can be obtained **(Fig.1)**
- Due to optical memory effect, turbid focus can be translated in 2D and 3D[2].
- We recently showed that this focus can be transformed into an array of foci of various configurations[4].
- There is an upper bound to the maximum number of foci the turbid focus can be split into.

Structured Illumination Microscopy

- Sinusoidally varying intensity distribution illuminates a fluorescent sample.
- Sample information couples to illlumination structure due to convolution.
- Multiple images at different positions and orientations of illumination are acquired.
- Reconstruction algorithm extracts the high resolution information from these images.
- Lateral resolution upto a factor 2 can be achieved[3].

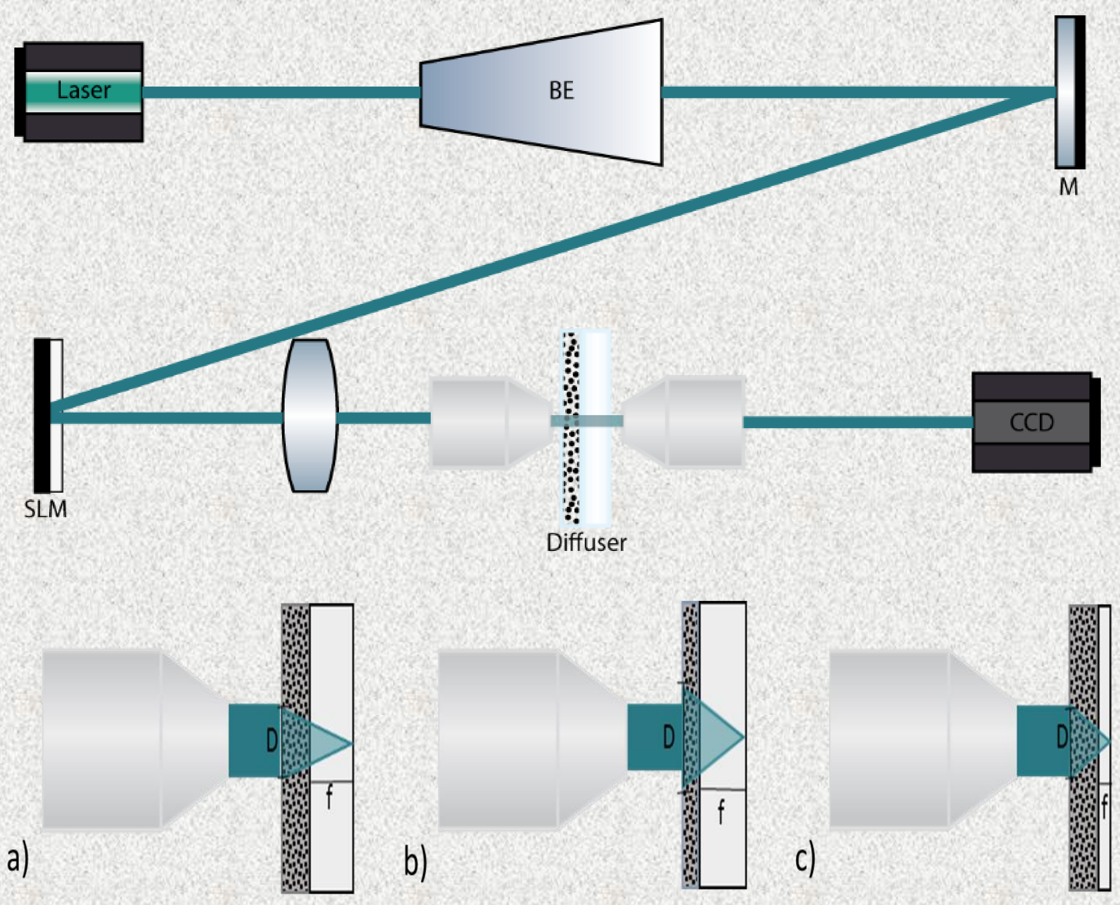

Fig.1:Figure above: shows the experimental setup used to focus light behind turbid medium. Light from laser is expanded by Beam-Expander(BE) unit to fill the entire SLM screen. Modulated light from SLM is imaged onto the scattering surface of the sample. Intensity at a small region on the opposite surface of the sample is monitored using the CCD. An optimisation algorithm determines the optimum wavefront required to generate a diffraction limited focus on the opposite surface of the sample.

Figure below: demonstrates diffraction limit for different "turbid lenses". Numerical Aperture(NA) of a "turbid lens" is approximated to $\approx \frac{D}{2f}$. a) for a ground glass diffuser 'D' before and after the scattering layer is the same. b) For a highly scattering sample such as white paint, 'D' increases with thickness of the paint layer due to diffusion; thus increasing the NA. c) NA can also be increased to some extent by bringing the focal plane closer to the scattering plane.

3. Experimental Procedure

- Input light wavefront is optimised to focus behind a thin diffuser **(Fig.1)**
- Phase mask required to generate a lattice of multiple spots is computed offline.
- Computed phase mask is added to the optimum wavefront on the SLM plane **(Fig.2).** This transforms the focus into multiple foci on the CCD plane.
- By applying a linear gradient on the SLM plane, foci on the CCD plane are translated by precise amounts.
- For each position of multiple foci, an image is acquired.
- Acquired images are processed using SIM Reconstruction Algorithm[5]

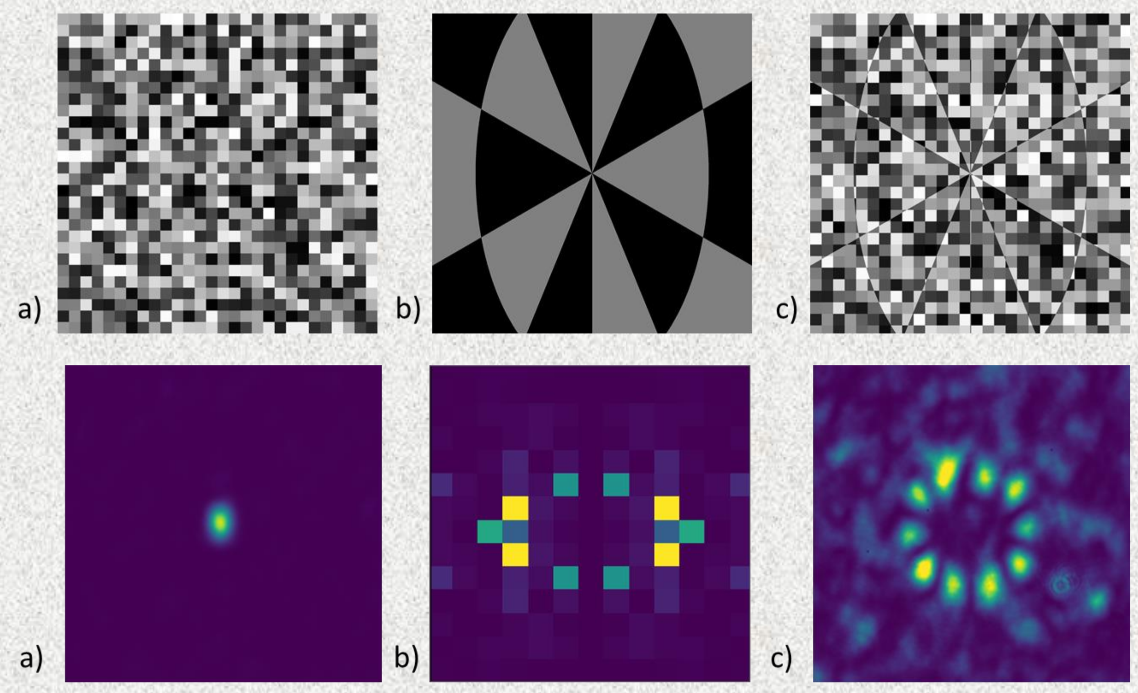

Fig.2: Figure above shows the phase at the SLM plane producing multiple foci. To the optimum wavefront a) leading to a focus behind the turbid medium, when a phase mask such as b) is added, the resulting phase would look like c). Figure below shows the convolution operation on CCD plane.
When the "turbid focus" a) is convolved with a lattice of delta peaks b), it results in the same lattice of multiple foci c).
Also, the bottom subfigures a), b), c) are respectively the Fourier Transforms of the top subfigures a), b), c).

4. SIM Reconstruction

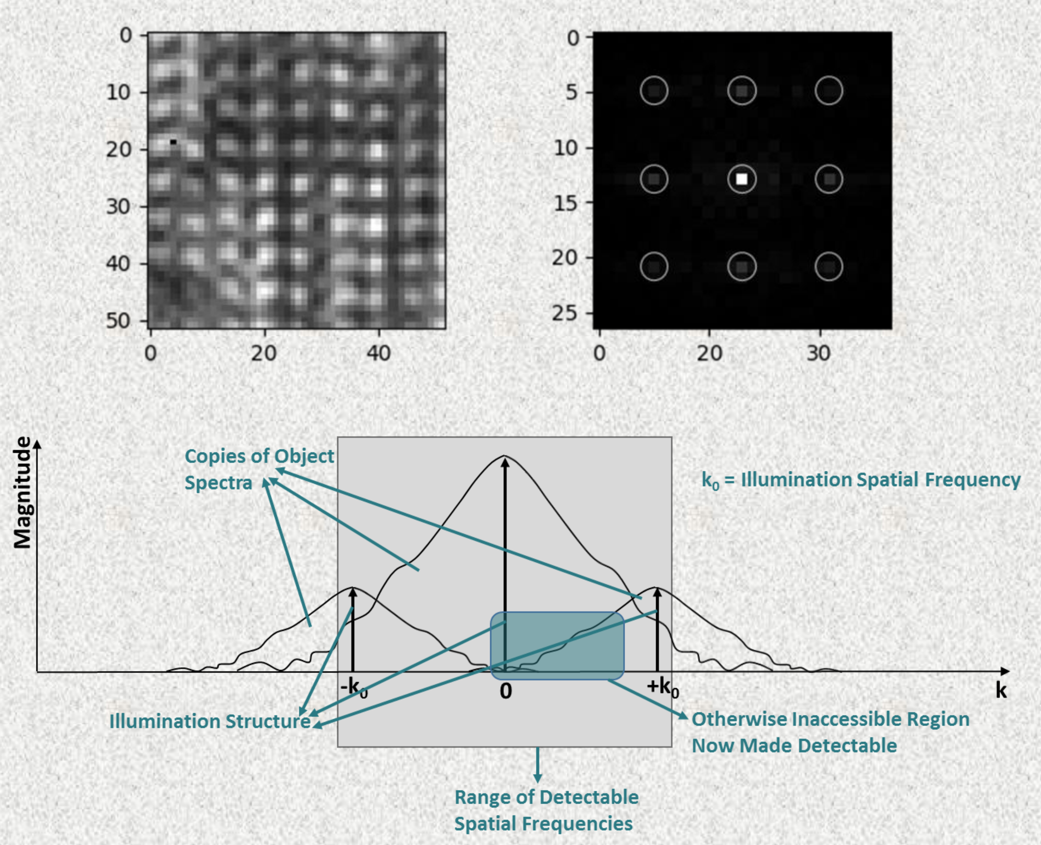

Fig.3: Figure above shows the Structured Illumination behind a diffuser and it's Fourier Transform.
Figure below shows the cartoon of object spectrum due to 1D structured illumination.

- The illumination pattern is shifted to different positions and fluorescence signal at each position is acquired.
- At least nine such acquisitions are required to retrieve the nine components of the object spectrum (marked by gray circles).
- Acquired images are run through a SIM Reconstruction Algorithm to determine the illumination structure *aposteriori*.
- By knowing the k_0 and exact displacements of the illumination pattern, the object components are separated.
- Separated components are further processed and shifted back to origin. This extends the range of detectable spatial frequencies thereby improving the resolution.

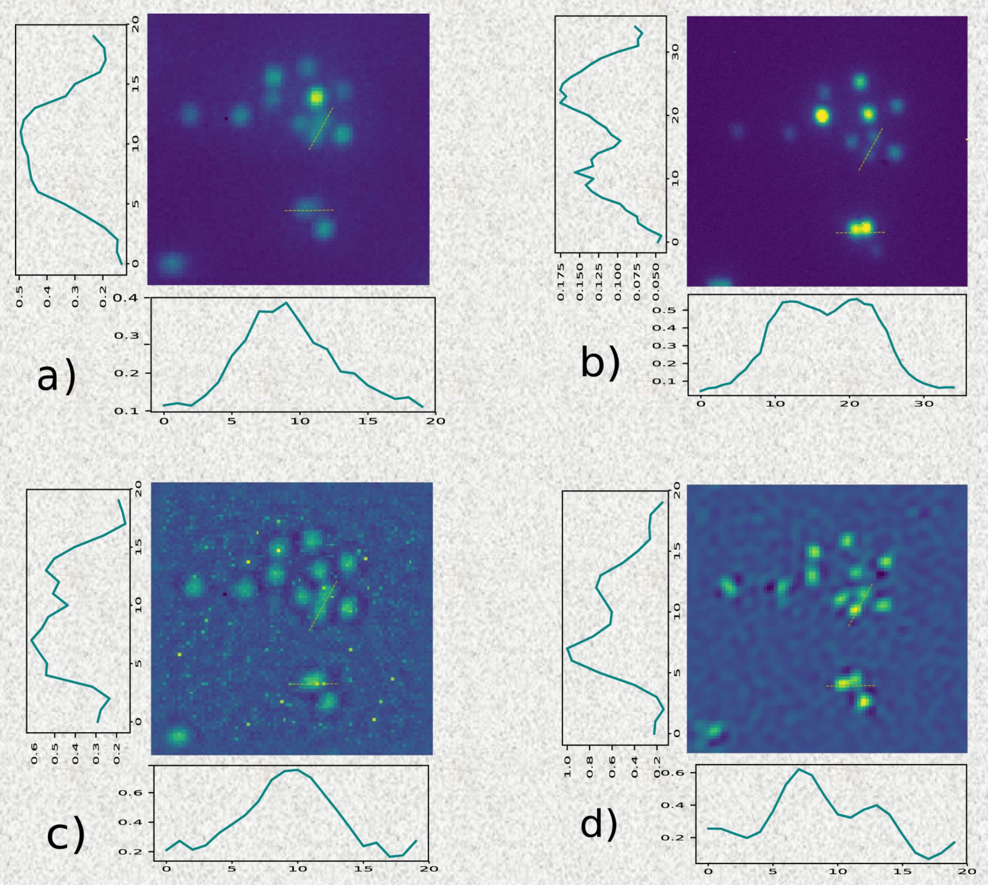

Fig.4: a) Flourescent beads behind the diffuser at the resolution limit of 40X objective. b) same beads seen with 100X objective for reference. c) reconstructed image with just the widefield component. d) SIM reconstructed image with super resolution components. The intensity cross sections along the dashed yellow line are plotted for comparison. Beads that are conventionally un-resolvable (as in a) & c)) are now resolved due to SIM reconstruction (as in d)).

5. Results

- Illumination spatial frequency k_0 is chosen to be at ~48% of the cutoff of the imaging objective (40X, NA=0.7)
- **Fig.4.a)** & **c)** show that the beads are not resolvable with direct image or with widefield reconstruction.
- With a 100X objective (NA=1.32) the same beads are partially resolvable **(Fig.4.b)**
- By acquiring images with Structured Illumination with 40X objective and upon SIM reconstruction, same beads are now resolvable as shown in **Fig.4.d).**
- SIM behind scattering media improves resolution by ~1.6 folds over conventional widefield microscopy.

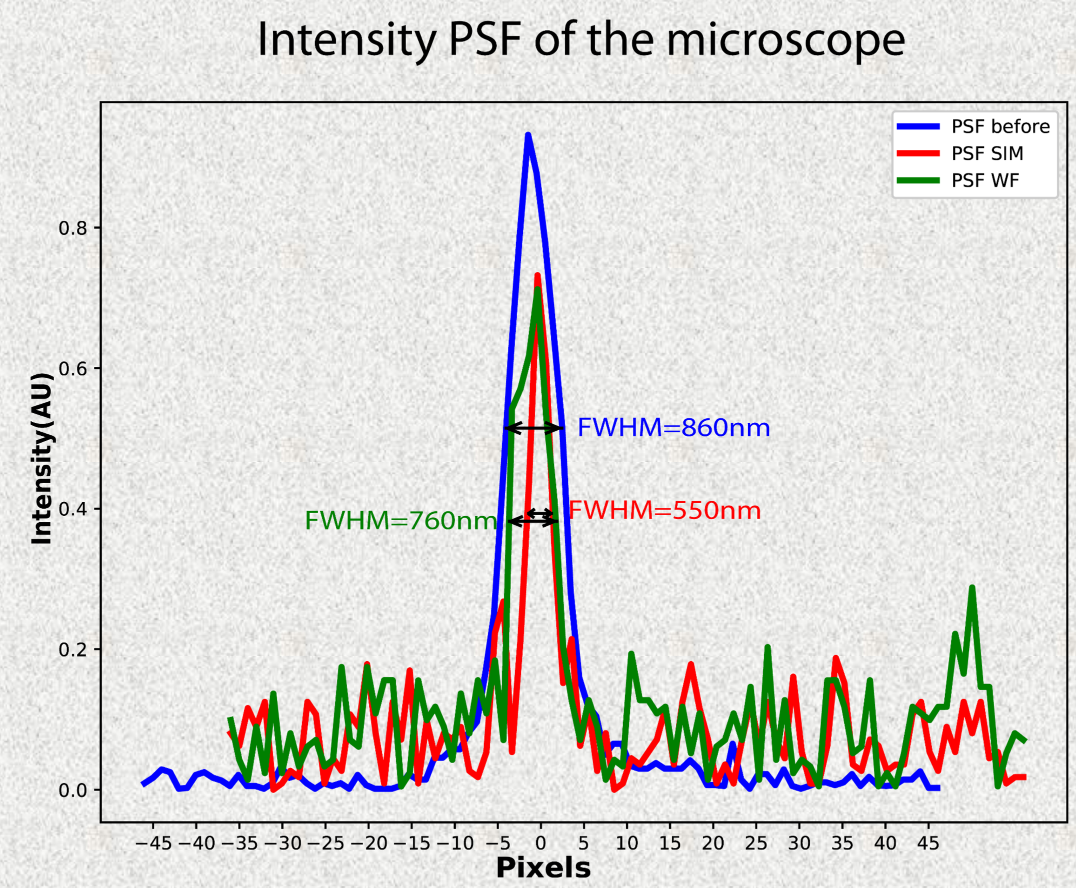

Fig.5: Reduction in width of PSF made possible due to SIM

References

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