Structured Illumination Microscopy behind Turbid Media

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Abstract

In turbid media, light gets multiply scattered to an extent that all the information of its propagation is scrambled over a characteristic distance called the transport mean free path. Controlling light propagation through such media is therefore challenging. By using a feedback signal, the input wavefront of light can be shaped such that light gets focused through or even inside a scattering medium[1]. Here we show that such an interferometric focus can be then transformed into an array of multiple focal spots with a desired configuration. Since the position of the focal spots can be precisely controlled, they could be used to illuminate and image the fluorescent structures hidden behind or inside multiply scattering media.

Introduction

When the light traversing through a multiply scattering medium is modulated, it focuses to a point. This focus can be translated in the focal plane by translating the incoming light owing to Memory Effect[2]. Having such a good control over the transmitted light calls for microscopy applications. We explore particularly Structured illumination microscopy[3](SIM) to get high resolution images behind turbid medium.

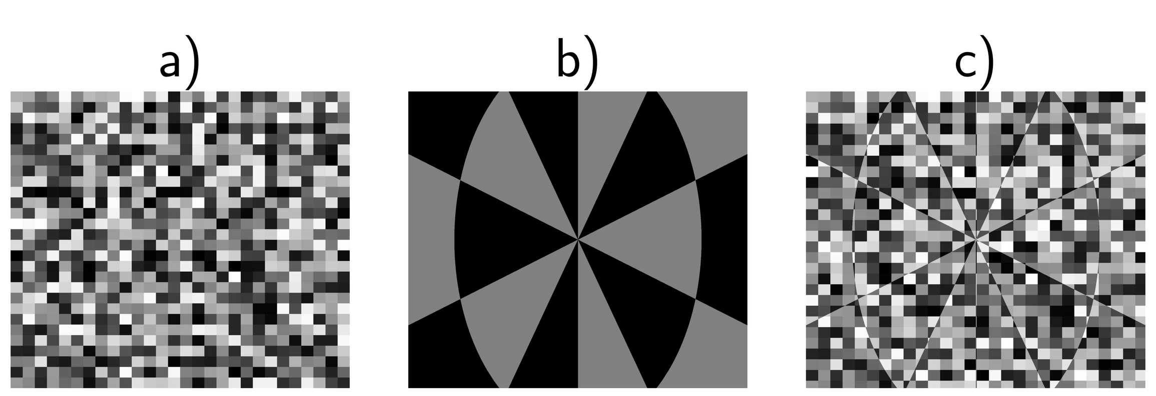


Figure 1: To the optimum wavefront a) leading to a focus behind the turbid medium, when a phase mask such as b) is added, the resulting phase would look like c)

Experiment

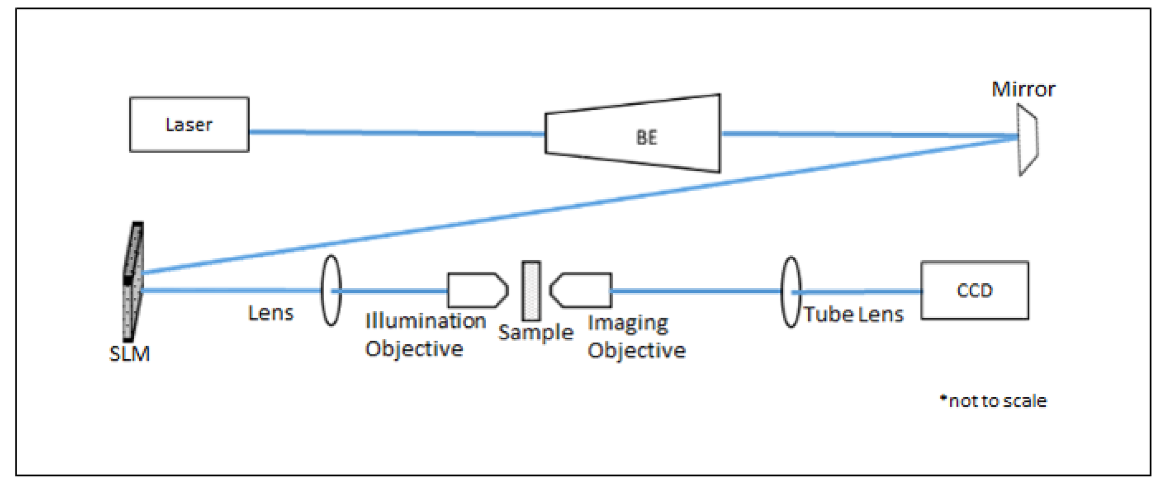


Figure 2: Experimental Setup

The experimental procedure is as follows:

- Feedback based algorithms modulate the phase of input wavefront using SLMs such that transmitted light focuses interferometrically behind the turbid medium.
- Phase masks generated offline are then added to the modulated wavefront to transform the focus into multiple spots as desired.
- In the focal plane images of the fluorescent structures at various positions of illumination pattern are captured and processed.

Theoretical background

After focusing, the combination of SLM and the scattering medium acts as a lens. To this combination resulting in a lens, by adding the phase pattern of a grating such as Fig.3, we bring the far field of the grating onto the focal plane, resulting in multiple spots. This can be understood using the Convolution Theorem, as follows:

if $\mathcal{F}\{\mathcal{E}_f(y,z)\} = E(k_y,k_z)$ and $\mathcal{F}\{f(y,z)\} = F(k_y,k_z)$, then

$$\begin{aligned}\mathcal{F}\{\mathcal{E}_f * f\} &= \mathcal{F}\{\iint_{-\infty}^{+\infty} \mathcal{E}_f(y,z)\, f(Y-y, Z-z)\, dy\, dz\} \\ &= \mathcal{F}\{\mathcal{E}_f(y,z)\}\ \mathcal{F}\{f(y,z)\} \\ &= E(k_y,k_z)\ F(k_y,k_z)\end{aligned}$$

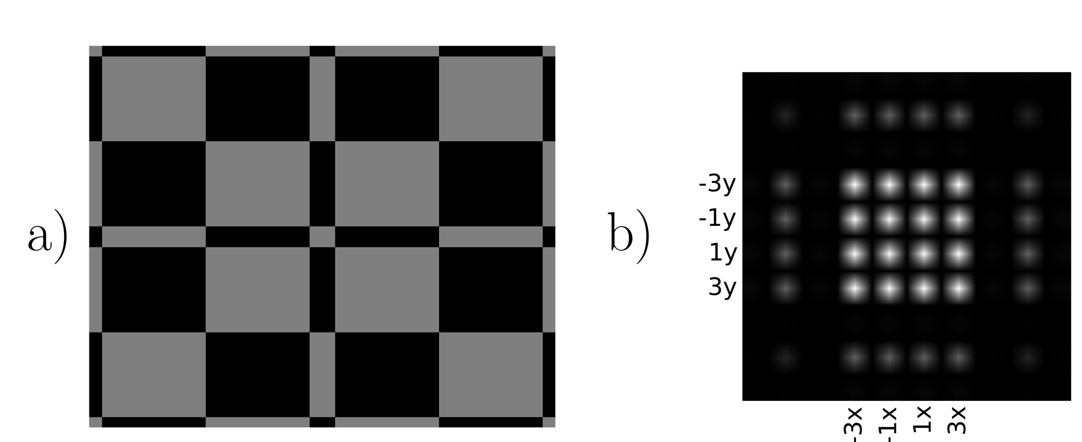
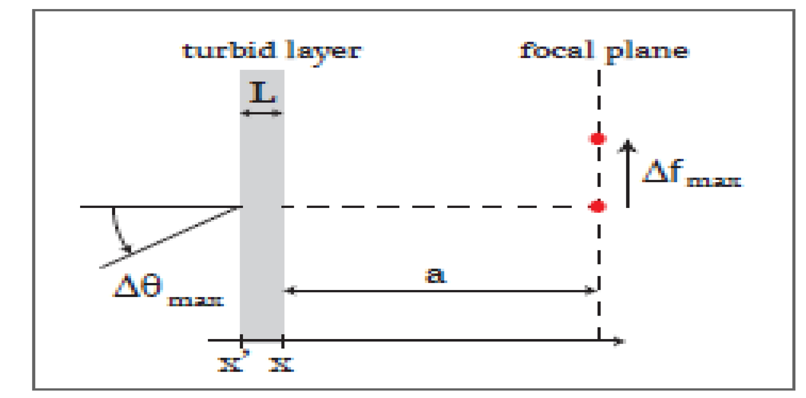


Figure 3: a) Phase mask loaded onto the SLM b) 2D-FFT of a)



Memory effect range: $\Delta\theta = \dfrac{\lambda}{2\pi L}$

where λ is the wavelength of light used and L is the thickness of scattering medium.

Results

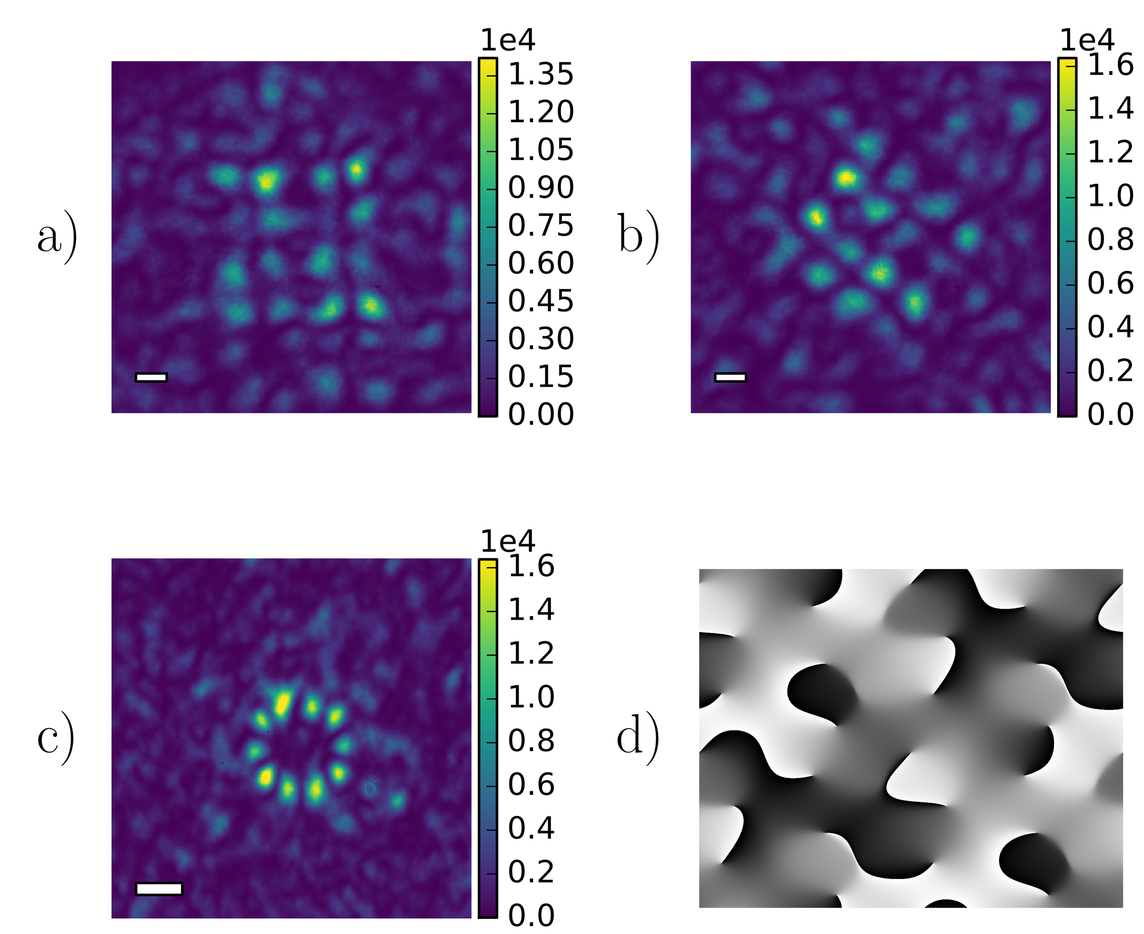


Figure 4: a) Multiple foci on CCD b) Pattern in a) rotated, c) Pattern with a circular symmetry, d) Phase mask generated using iterative methods Scalebar corresponds to 5μm.

Current Objectives

Precise control over transmitted light through turbid medium calls for imaging applications such as SIM. Using SIM high resolution information that is usually blocked by the imaging objective OTF can be reconstructed, leading to a resolution enhancement of a factor 2. A sinusoidally varying illumination such as

$$I(r) = I_0[1 + cos(k_0.r + \phi)]$$

produces a Fourier transform which has 3 components as shown in Fig.5

$$I(k) = \frac{I_0}{2}[\delta(k) + \delta(k+k_0)\exp(i\phi) + \delta(k-k_0)\exp(-i\phi))]$$

By obtaining images at different spatial phase of the illumination pattern, object information corresponding to these 3 components can be extracted.
This method has also been successively extended to imaging 3D samples.

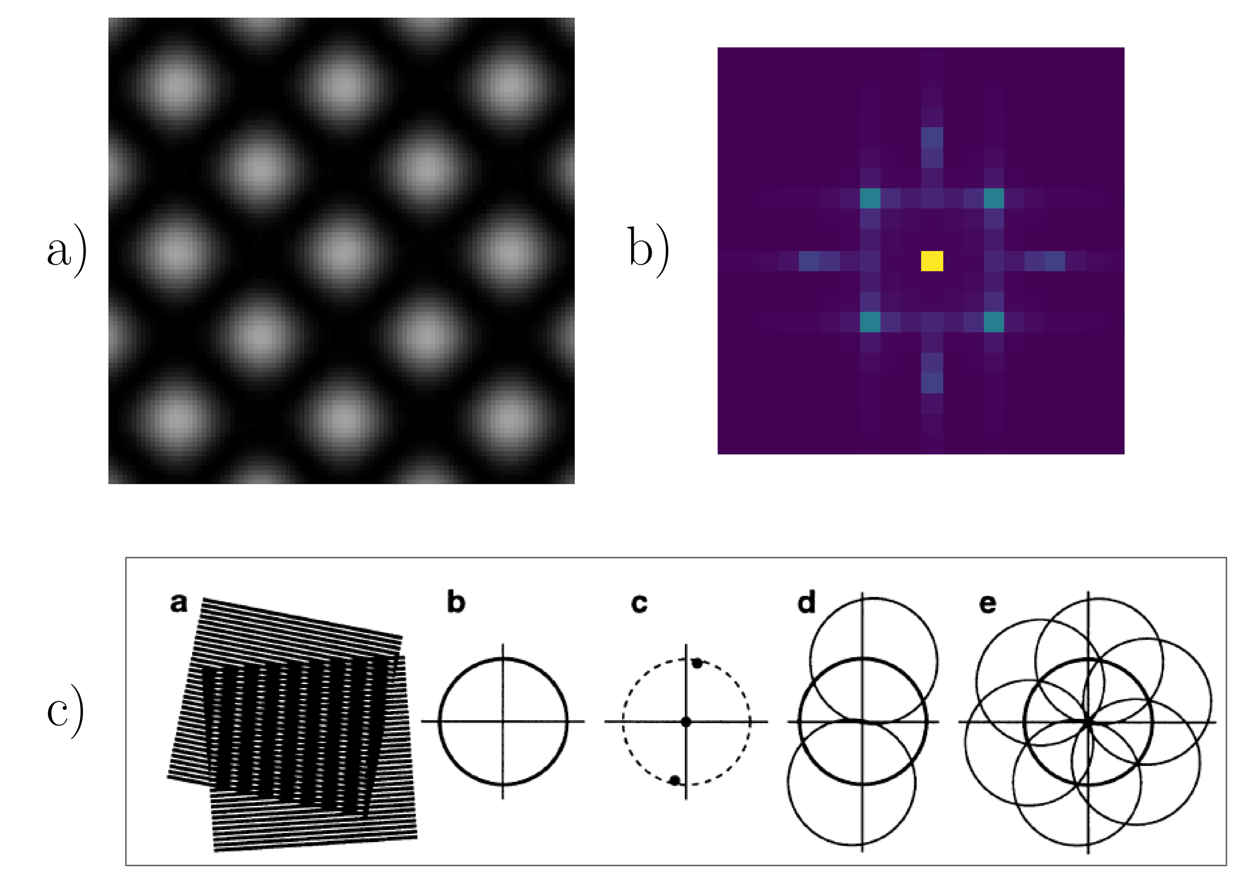


Figure 5: a) Simulated 2D-structured illumination grid, b) Frequency content of a) , c) SIM resolution enhancement

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