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Fluorescent wavefront shaping using incoherent iterative phase conjugation

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Abstract: We extend iterative phase conjugation algorithms, previously derived for coherent illumination. We show they can be used to focus on incoherent fluorescent sources, and the incoherent emission largely expands penetration depth and convergence speed.

1. Extended summary

One of the core challenges when performing linear fluorescence microscopy inside tissue is the fact that biological tissue is highly scattering at visible wavelengths.

A promising approach for overcoming the multiple scattering challenge is wavefront shaping correction: if one reshapes the incoming (or outgoing) coherent wavefront, such that its aberration is conjugate to the aberration that will happen inside the tissue, then after propagation the wavefront will focus into a sharp spot inside the tissue. Our interest in this work is wavefront shaping for linear, single-photon fluorescence feedback.

The practical application of wavefront shaping is hindered by the difficulty of finding the wavefront correction to apply. This wavefront correction varies between different tissue layers, and even between different positions inside the same tissue sample. In the absence of a single guiding star, finding a wavefront shaping correction typically involves optimization strategies relying on a variety of feedback mechanisms.

This optimization is tractable when the wavefront correction can be described by a small number of parameters (e.g., using Zernike polynomials). However, to focus inside thick highly-scattering media, it is desired to use all the degrees of freedom of a modern spatial light modulator (SLM), often in the megapixel range. This is posing non-trivial optimization challenges, limiting any real-time applicability.

For fully coherent imaging systems, an alternative class of techniques for estimating the wavefront correction is *iterative phase conjugation* [3, 4]. These techniques use the observation that a wavefront shaping correction focusing on a single point inside tissue is an *eigenvector* of the transmission matrix of the scattering sample. They then find these eigenvectors using an optical implementation of the power method, which iterates between sending in a wavefront, measuring the scattered wavefront, and using the measurement as the successive input. Often this procedure converges after a very small number of iterations, leading to an order-of-magnitude acquisition speedup compared to standard optimization approaches.

An important assumption underlying the coherent iterative phase conjugation scheme is that light scatters only once. This greatly limits its applicability to thin or sparse volumes. Our goal in this work is to develop an iterative phase conjugation approach that is applicable to linear (single-photon) fluorescent imaging. As the emitted light does not excite the tissue or the particles again, by working with fluorescent sources we can greatly relax the single scattering assumption, making our approach applicable to much thicker volumes, in particular tissue.

The primary technical challenge in this setting is that any uncorrected incident wavefront (such as the wavefronts used during the power method) will excite more than one fluorescing point inside the tissue sample, and the excited points will emit light that sums *incoherently*.

Consequently, we cannot model the relation between input excitation and output fluorescent emission using a linear transmission operator, as fully-coherent iterative phase conjugation techniques do. To overcome this challenge, in [1] we analyze the incoherent case, and report two findings: First, we show that the same power method procedure as in the fully-coherent case can be used to recover the correction pattern also in the incoherent case. Second, we show that, whereas for the fully-coherent case the power method converges at an exponential rate, for the incoherent case it converges at a *doubly-exponential* rate.

In [1] we build a lab prototype and demonstrate that our algorithm can focus on fluorescent beads attached at the back of a few tissue samples. Usually this algorithm converges within $2-6$ power iterations. Each iteration is realized by capturing 5 images of the scene, which are used for phase estimation. Thus overall our technique achieves wavefront correction after capturing as few as $10-30$ images, compared to thousands of images captured by existing optimization-based wavefront shaping strategies for fluorescent imaging [2].

Results. Fig. 1 illustrates the convergence of our algorithm, on one test sample with fluorescent beads attached at the back of a chicken breast slice of thickness $300\mu m$. The imaging setup consists of an excitation laser and an

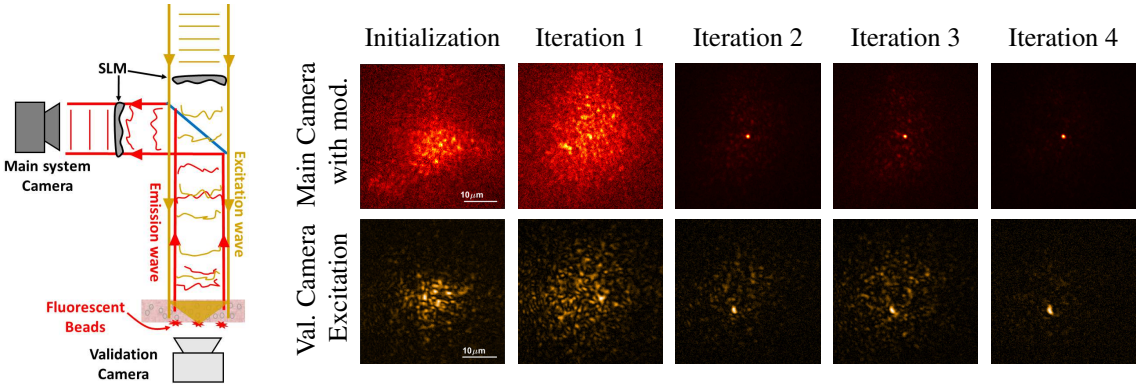

Fig. 1: Demonstrating power iteration convergence. We include a view from the main camera seeing the excitation via the front of the tissue, followed by SLM modulation correction. To better appreciate the focusing we have also used a validation camera behind the tissue that can observe the illumination profile at the plane of the fluorescent beads. This camera does not provide any feedback to the algorithm. In the first iteration we see a speckle image, but as power iterations proceed the illumination wavefront converges and excites a single point. When the same modulation pattern is placed at the imaging arm, imaging aberrations are corrected and one can see a sharp image of the excited bead, despite the fact that the excitation light scatters through the tissue on its way to the camera.

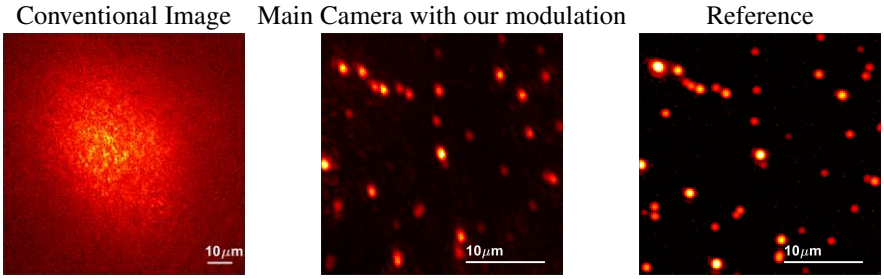

Fig. 2: Using the recovered modulation to image a local region of fluorescent beads. We correct the imaging arm, while using a random illumination to excite multiple beads.

imaging camera, both located at the same side of the tissue sample, in a non-invasive geometry. Both excitation and emission waves can be modulated by a SLM. The algorithm starts with a wide speckle image and uses it to update modulation elements in both arms. Within a very small number of iterations, the aberrated speckle pattern is corrected, and our camera can see a sharp spot. For validation, we also placed a second camera behind the tissue. This camera can observe the fluorescent beads directly and monitor convergence. It is only used for validation and does not provide any feedback to the algorithm. The second row of Fig. 1 shows a view from this camera, demonstrating that while in the beginning the excitation wave is aberrated, within a few iterations we manage to modulate it such that after propagation inside the tissue it focuses into a single spot.

In Fig. 2 we use the recovered modulation to image a region of fluorescent beads rather than a single spot. For that we use the modulation to only correct the imaging arm, while the illumination is random and excites a wide region of beads. Due to memory effect correlations, the modulation recovered at one bead can explain a local neighborhood of fluorescent sources.

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