Deep-tissue focal fluorescence imaging with digitally time-reversed ultrasound-encoded light

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Abstract

Fluorescence imaging is one of the most widely used research tools in biomedical sciences. However, scattering of light by thick tissues severely limits our ability to study intact specimens at high resolution. Using digital time reversal of ultrasound-encoded light and adaptive background cancellation, we directly demonstrate focusing and fluorescence imaging deep inside biological tissues. We further illustrate the potential of our method for fluorescence bioimaging in the diffusive regime by imaging complex fluorescent objects and tumor microtissues 2.5 mm deep in biological tissues, at an anisotropic lateral resolution of 36 μm by 52 μm. Our results set the stage for a range of deep tissue imaging applications in biomedical research and medical diagnostics.

Motivation

- Focal fluorescence imaging beyond one transport mean free path has not been achieved so far.
- Conventional focusing methods treat scattered light as noise and select for ballistic light, which exponentially decreases with depth.
- However, scattering is a deterministic process and is reversible through optical phase conjugation (OPC) [1, 2].

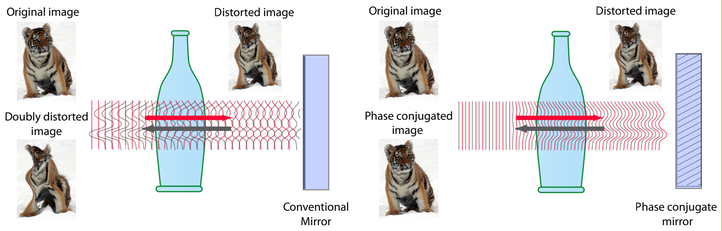

Figure 1 | Conventional mirror versus phase conjugate mirror.

- Xu et. al. proposed combining OPC and ultrasound encoding to focus light into turbid samples at sub-millimeter resolution with improved absorption contrast [3].
- To realize high resolution fluorescence imaging, two fundamental challenges must be overcome: (a) the low gain of the conventional phase conjugate mirror and (b) the undesired background due to incomplete phase conjugation.

Schematic of setup

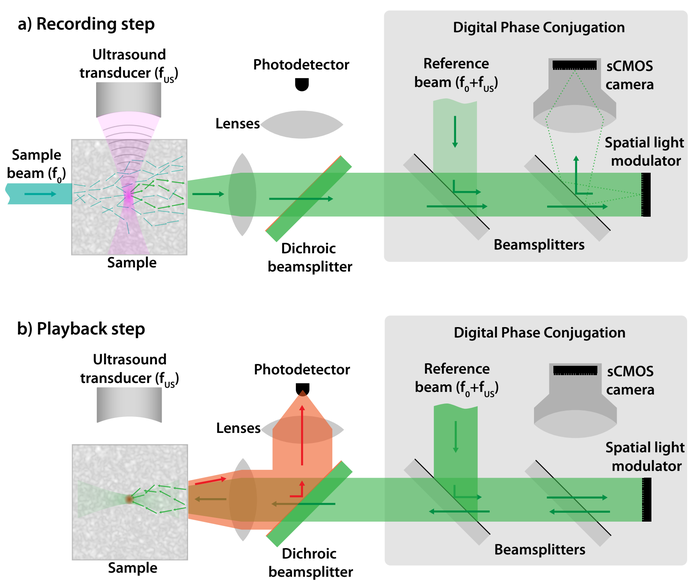

Figure 2 | Light originating from the optical focus is selectively phase conjugated by the digital optical phase conjugation mirror (DOPC) [4]. The new digital OPC scheme allows for simple, repeatable alignment.

Principles: Resolution

- Ultrasound (US) focus dimensions dictate the resolution of the optical focus.
- US and laser are pulsed to confine the resolution along axis of US propagation.
- Calculated US focal width: 34 μm.
- Calculated resolution along the axis of US propagation: 54 μm.

Principles: Gain

- In photorefractive crystal based phase conjugate mirrors (PCMs), the power in the OPC beam (P_{OPC}) is proportionate to the power in the sample beam (P_S). The gain, $G = P_{OPC}/P_S$
- Since the fraction of modulated light is small (~10^{-4} in our setup), $G \gg 1$ is required to excite detectable fluorescence.
- In digital OPC, P_{OPC} is not dependent on P_S. Thus, digital OPC is capable of theoretically unlimited gain (~ 5 x 10^5 used in our experiments).

Principles: Background

- Complete time reversal requires full control over phase, amplitude and polarization of the entire scattered field – which is fundamentally unfeasible.
- Practically, phase conjugation is always accompanied by a background.

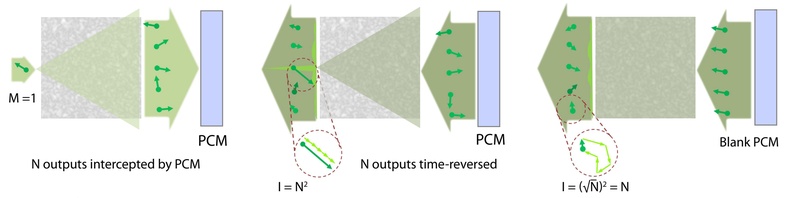

Figure 3 | The peak focus intensity to average background intensity ratio (PBR) is determined by the number of optical modes intercepted by the PCM (N) and the number of modes in the ultrasound focus (M): PBR ~ N/M. [5]

Visualising the time-reversed focus

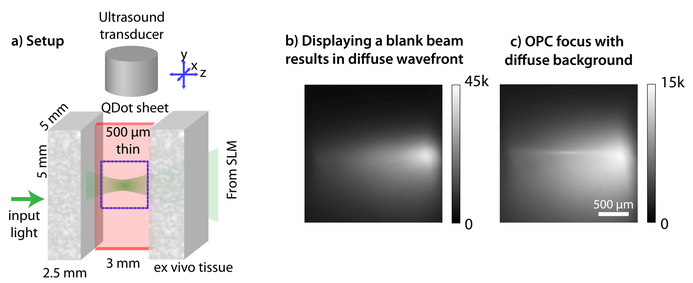

Figure 4 | a) Setup. b) A blank beam fail to form a focus. c) Displaying a phase conjugate map on the spatial light modulator results in a focus at the ultrasound location, albeit on a significant diffuse background.

Adaptive background cancellation

The OPC background excites fluorescence outside focus can drown out desired focal fluorescence signal measured by single pixel PMT. We show that this OPC background can be dynamically reproduced and cancelled.

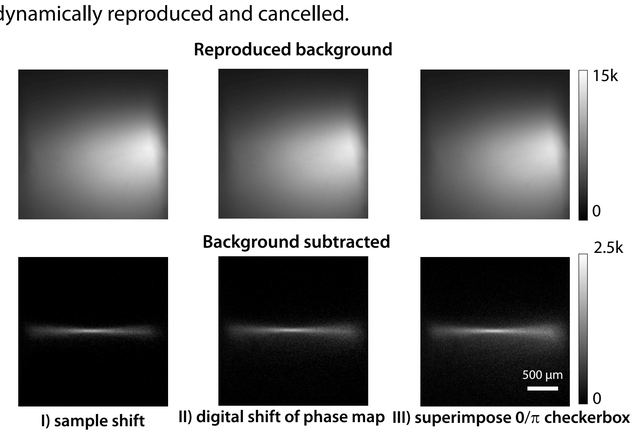

Figure 5 | Background subtraction yields a high contrast OPC focus. These adaptive background cancellation methods effectively uncouple the focal fluorescence signal from that excited by the background. Method III is used in all subsequent experiments.

Determination of point-spread-function

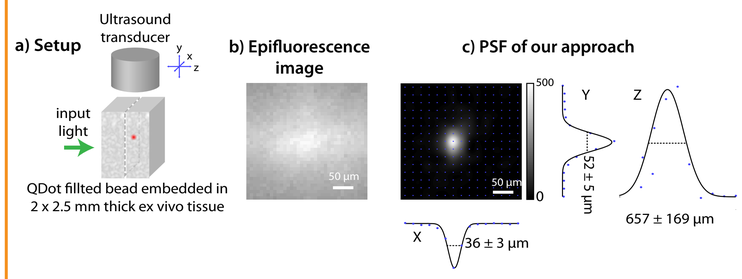

Figure 6 | **(a)** Schematic of the sample. **(b)** Epifluorescence image of embedded bead (QDot 655 nm). **c)** Fluorescence image obtained by scanning the position of the US transducer in X and Y, detecting the fluorescence excited by time-reversed light and using adaptive background cancellation.

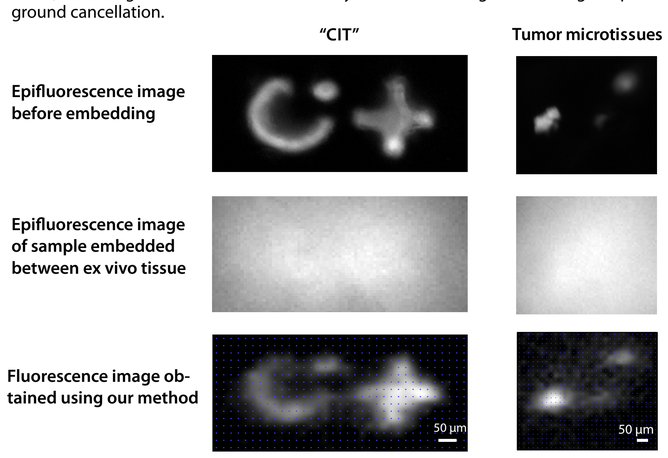

Figure 7 | Images of quantum dot (QDot 705 nm) "CIT" feature and DY-521XL NHS-ester stained HEPG2 cancer microtissues embedded between two pieces of ex vivo tissue.

Conclusions

- We demonstrated high resolution focal fluorescence imaging using time-reversed ultrasound-encoded light.
- Combining a digital background cancellation procedure with the high phase conjugate gain and resolution of our technique, we presented focused fluorescence imaging at an unprecedented depth of 2.5 mm inside biological tissue.
- With the development of faster, larger format spatial light modulators and faster, higher dynamic range cameras, our results set the stage for a range of deep tissue imaging applications in biomedical research and medical diagnostics.

Other potential applications

- Molecular imaging (e.g. for early cancer diagnosis).
- Photodynamic therapy.
- Targeted excitation of optogenetic tools in deep tissues.
- Incisionless optical scalpel.

References

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Acknowledgements

Ying Min Wang is an Agency for Science, Technology and Research (Singapore) National Science Scholar. Benjamin Judkewitz is a Sir Henry Wellcome Postdoctoral Fellow. This work was supported by NIH 1DP2OD007307-01 & R21 EB012255, DARPA W31P4Q-11-1-0008.