Deep-tissue fluroescence imaging with time-reversed light

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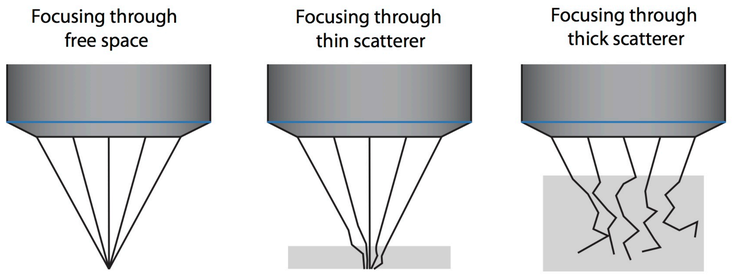
Abstract

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 beyond a depth of several hundred micrometers. Here, we overcome this limitation by using time reversal of ultrasound-encoded light. We 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 a lateral resolution of ~40µm. Finally, we present proof-of-principle results of how this resolution can be further improved to micrometer scale using a new strategy termed variance encoding. Our results pave the way for a range of deep-tissue imaging applications in biomedical research and medical diagnostics.

Motivation

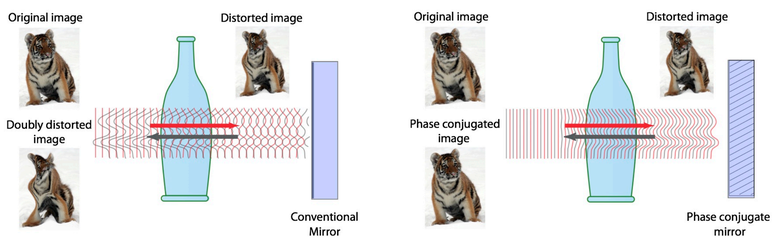
What if all biological tissues were transparent?

The Problem: scattering (limits conventional approaches to superficial layers)



Time-reversal

Conventional focusing methods treat scattered light as noise and rely on unscattered light, which exponentially decreases with depth. However, scattering is a deterministic process and is reversible through optical phase conjugation [1, 2].

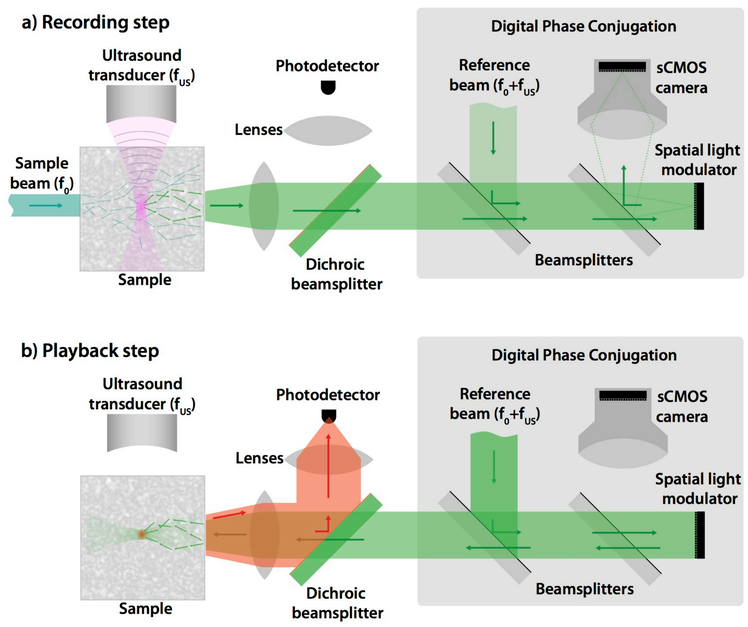


Xu et. al. [3] proposed combining time reversal and ultrasound encoding (short: TRUE) to focus light into turbid samples. To realize high resolution fluorescence imaging, two fundamental challenges still had to be overcome: (a) high gain of the phase conjugate mirror and (b) the undesired background due to incomplete phase conjugation.

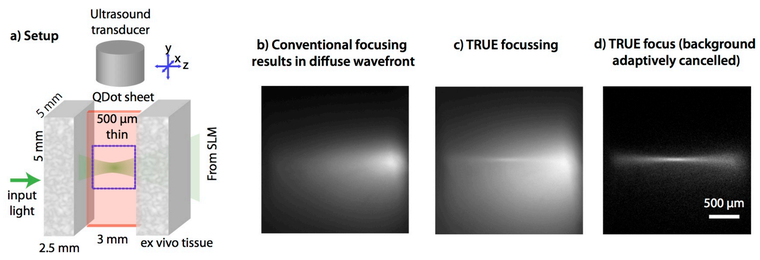
References

(1) Yaqoob Z, Psaltis D, Feld MS & Yang C, Nature Photonics 2, 110–115 (2008)
(2) Vellekoop IM, Lagendijk A & Mosk AP, Nature Photonics 4, 320–322 (2010)
(3) Xu X, Liu H & Wang LV, Nature Photonics 5, 154–157 (2011)
(4) *Wang, YM, *Judkewitz B., DiMarzio CA & Yang C, Nature Communications 3, (2012)
(5) *Judkewitz B, *Wang YM, Horstmeyer R, Mathy A & Yang C, submitted

Schematic of the setup

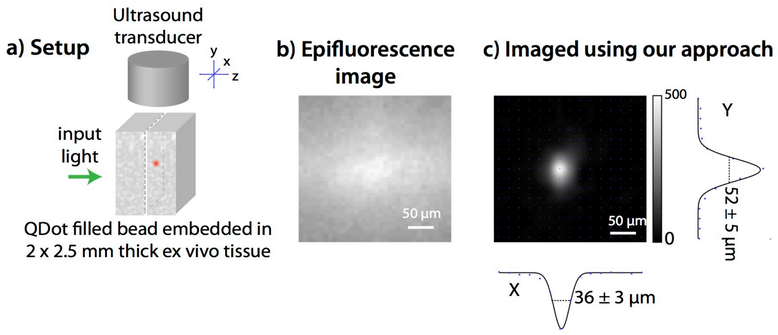


Visualisation of time-reversed focus

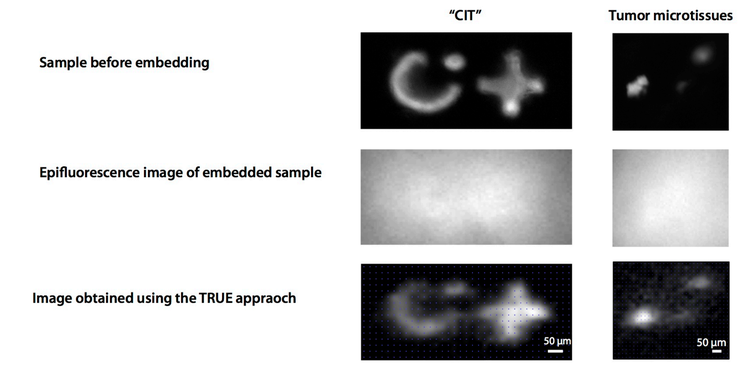


Deep tissue imaging with 'TRUE'

Determining the point-spread function

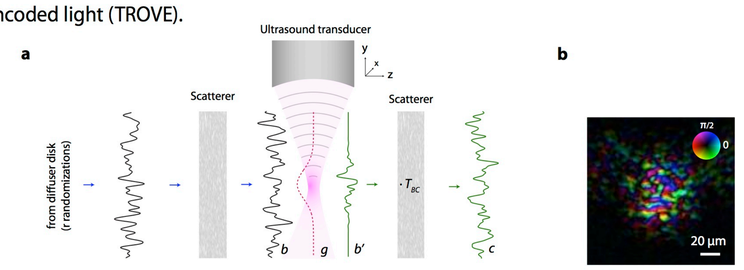


Imaging complex samples



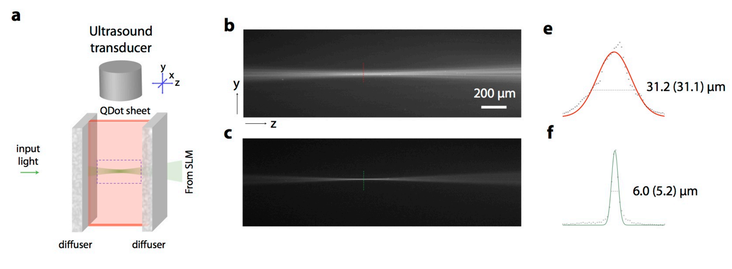
Variance-encoding and 'TROVE'

In TRUE-imaging, the resolution is limited to the size of the ultrasound focus. To break this resolution barrier, we developed a new approach termed time-reversal of variance-encoded light (TROVE).

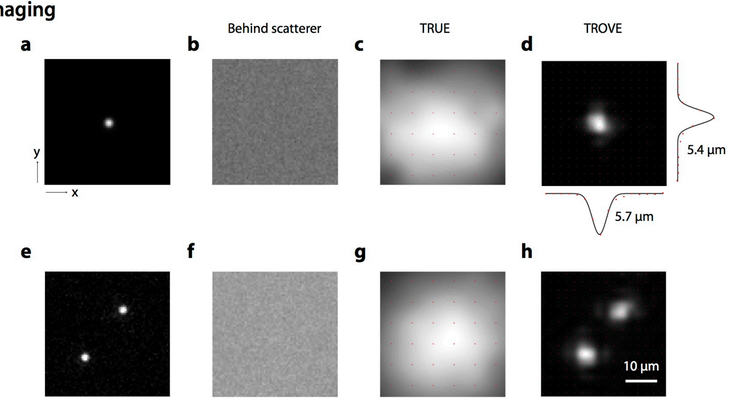


Focusing and imaging with 'TROVE'

Visualization of the focus (comparison between TRUE and TROVE)



Imaging



Conclusions

- By combining ultrasound encoding with digital time-reversal we can focus light at unprecedented depths within scattering samples.
- The theoretical depth limit is the depth llimit of the ultrasound (centimeters). Practically, we are still limited by the sensitivity of currently available detectors.
- The theoretical resolution limit of TRUE imaging is the size of the ultrasound focus (tens of µm). To break this resolution barrier we developed TROVE, a new strategy based on variance encoding that allowed imaging at ~5 µm resolution.
- The theoretical resolution limit of TROVE is sub-µm (diffraction-limited). Resolution depends on illumination geometry.
- Current limitation: acquisition time (minutes to hours) compared to sample stability (sub-second for many biological samples). The advent of high-speed spatial light modulators and more sensitive cameras will enable us to address this challenge in the coming years.
- With these improvements on the horizon, our method paves the way to a range of in vivo applications including deep-tissue imaging, optogenetics and medical diagnosis.

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