Time-Reversal Optical Focusing for Noninvasive Optogenetics

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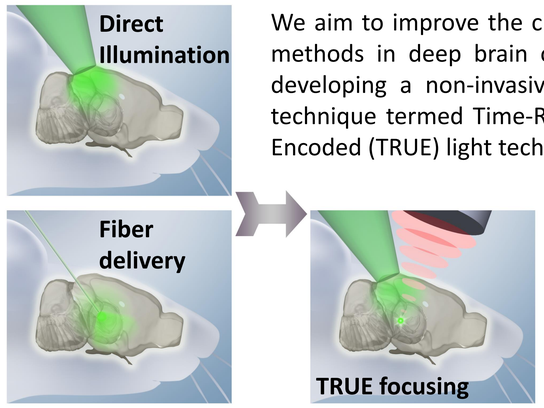
Abstract

Tissue turbidity as a result of light scattering limits the working depth of optogenetics. Thus current optogenetic techniques are limited to either non-specific diffuse optical activation/inhibition, relying solely on genetic techniques for specificity, or invasive light delivery strategies such as implanting optical fibers deep into the brain. The use of Time-Reversal Ultrasound-Encoded (TRUE) light technique would enable the extension of optogenetic techniques to the deep brain for non-invasive, spatially specific, excitation/inhibition.

Aims

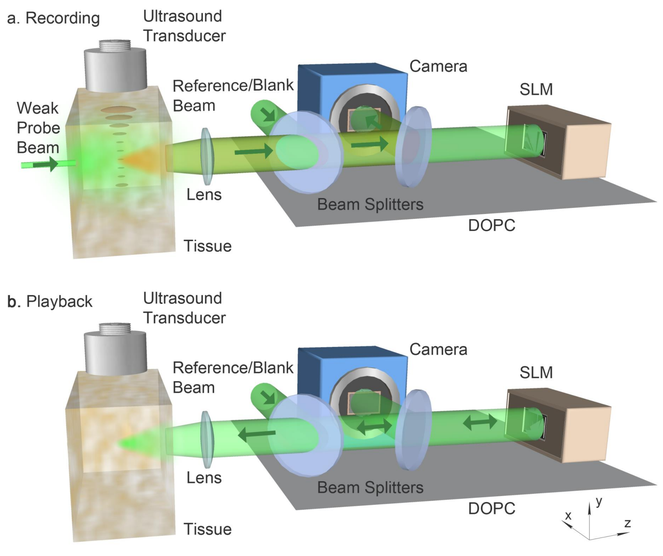


We aim to improve the current light delivery methods in deep brain optogenetics [1] by developing a non-invasive, spatially specific technique termed Time-Reversed Ultrasound Encoded (TRUE) light technique [2, 3].

Time-Reversed Ultrasound-Encoded (TRUE) light

The approach to achieve non-invasive light delivery for optogenetics is based on the TRUE technique [2, 3].

- Through Digital Optical Phase Conjugation (DOPC) technique [4, 5], we are able to measure the optical wavefront and create a phase conjugated copy of light that can travel in the reversed direction.
- If we have an initial point source inside the scattering tissue, its wavefront through the tissue can be detected. Then we are able to couple light to this point source based on DOPC.
- Focused ultrasound is used to create an initial guiding light source inside the tissue. By implementing the recording and playback of the DOPC, light can focus to the focus of the ultrasound.

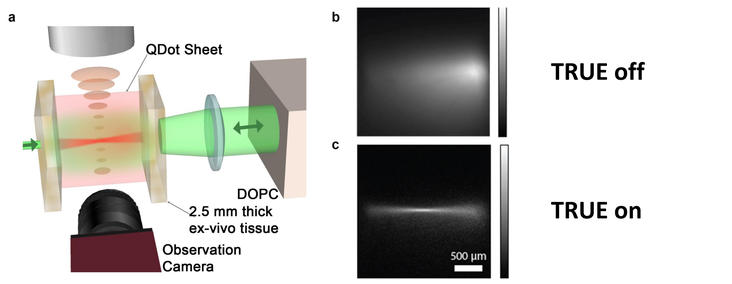


Recording : Ultrasonically-tagged light is detected by the camera of the DOPC system.

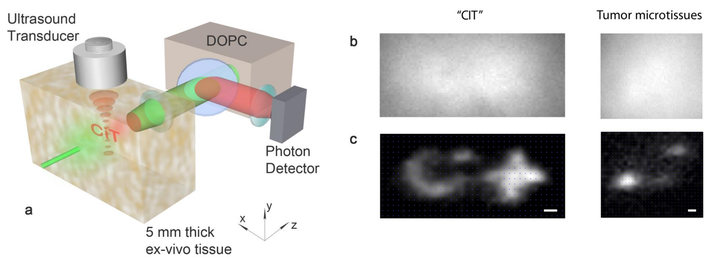
Playback : The SLM inscribes the blank beam with the information measured by the camera during the recording phase. The light will then travel back to the focus of the ultrasound due to time-reversal symmetry.

Light focusing inside biological tissues

We experimentally demonstrated the light focusing through biological tissues [3].



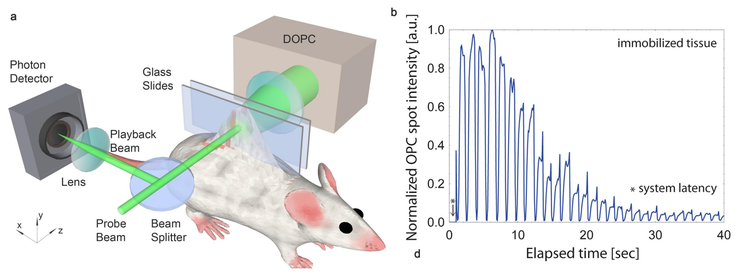
(Top) Direct visualization of the light profile on the quantum dot sheet between two pieces of chicken tissues.



a. **Setup of deep tissue imaging with TRUE. A "cit" fluorescence feature is embedded in a 5 mm thick tissue.**
b. **Epifluorescence image.**
c. **Image with TRUE.**

Tissue decorrelation time measurement

One of the challenges facing the application of TRUE in living animal is the optical decorrelation time due to the motion of tissue scatterers. A DOPC experiment based on a mouse dorsal skin pinch model was studied and shows that, with proper immobilized methods, the DOPC decorrelation time increases to the order of a couple seconds.

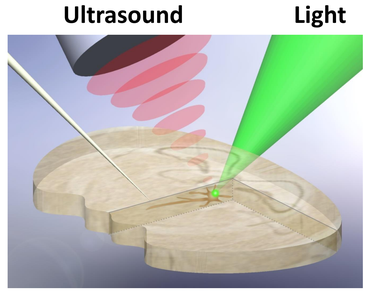


Mouse skin pinch model: DOPC reconstructed light through the mouse skin is detected by a photo detector

Intensity decay of the DOPC reconstructed light through the immobilized mouse skin.

TRUE on brain slices

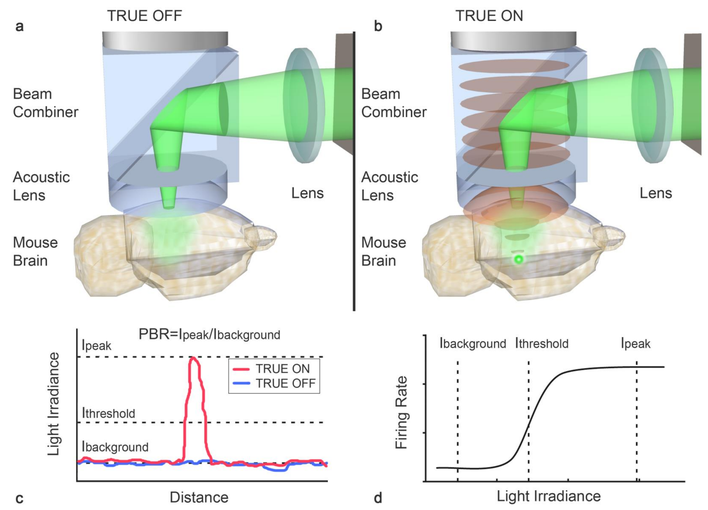
As one of the research aims, we are planning to implement TRUE on acute mouse brain slices.



TRUE creates an optical focus on a acute brain slice. Stimulated signal is detected by electrophysiological recording.

Schematics of focusing light inside rodent brains

A backscattering TRUE scheme is proposed to focus light in a living rodent brain. In this case, a sound-light combiner is used so that both waves incident to the brain via the same cranial window. If successful, this novel approach will enable light focusing up to a depth of 4 mm in a living rodent brain, with a focal minimal width close to the single-cell level (30 µm).



a. Without TRUE, light diffuses inside a mouse brain.
b. With TRUE, a light focus is created at the focus of the ultrasound.
c. A comparison of the light intensity at the ultrasound focus with TRUE on and off as well as the intensity threshold for stimulated neuron firing.
d. A typical opsin neuron activation curve is bipolar with a relatively sharp transition for example [6].

Summary

Research Aim 1: To implement a fast TRUE system with frame rate of 20/sec or higher.

Research Aim 2: Demonstrate the use of fast TRUE in selective excitation of fluorescent targets in fixed thick brain slices and progressing onwards to acute slices in living rodent brains.

Research Aim 3: To study the impact of focus depth and tissue immobilization strategies on digital TRUE performance for living rodent brains.

Research Aim 4: To obtain proof-of-concept for optogenetic modulation *in vivo* using digital TRUE.

References

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[6] Knöpfel T and Boyden ES, *Optogenetics : tools for controlling and monitoring neuronal activity*. Elsevier. Page 224 (2012).

Acknowledgements

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