3D quantitative phase imaging of neural networks using WDT

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ABSTRACT

White-light diffraction tomography (WDT) is a recently developed 3D imaging technique based on a quantitative phase imaging system called spatial light interference microscopy (SLIM). The technique has achieved a sub-micron resolution in all three directions with high sensitivity granted by the low-coherence of a white-light source. Demonstrations of the technique on single cell imaging have been presented previously; however, imaging on any larger sample, including a cluster of cells, has not been demonstrated using the technique.

Neurons in an animal body form a highly complex and spatially organized 3D structure, which can be characterized by neuronal networks or circuits. Currently, the most common method of studying the 3D structure of neuron networks is by using a confocal fluorescence microscope, which requires fluorescence tagging with either transient membrane dyes or after fixation of the cells. Therefore, studies on neurons are often limited to samples that are chemically treated and/or dead.

WDT presents a solution for imaging live neuron networks with a high spatial and temporal resolution, because it is a 3D imaging method that is label-free and non-invasive. Using this method, a mouse or rat hippocampal neuron culture and a mouse dorsal root ganglion (DRG) neuron culture have been imaged in order to see the extension of processes between the cells in 3D. Furthermore, the tomogram is compared with a confocal fluorescence image in order to investigate the 3D structure at synapses.

Keywords: WDT, SLIM, Tomography, Quantitative phase imaging, Microscopy, Interference, Phase shifting, Neurons, Label-free

INTRODUCTION

Many of traditional investigations on neurons are based on imaging methods, such as phase contrast of fluorescence microscopy, which are 2D plane measurements [1-10]. However, in reality, neurons from a 3D network run the entire living organism. Therefore, it is very important to study neurons using 3D imaging modalities. With the advances in biochemical markers and laser technology, fluorescence confocal microscopy has become the main methods for studying cells or a system of cells, such as embryonic body, in 3D [11, 12]. However, fluorescence confocal microscopy requires fluorescence markers that ultimately modify the live samples. Furthermore, the high-power laser illumination used in the imaging system causes photo-toxicity and affects live cells.

White-light diffraction tomography (WDT) is a recently developed tomographic imaging technique based on quantitative phase imaging (QPI) [13]. This technique is based on a commercial phase contrast microscope upgraded with a QPI module called spatial light interference microscopy (SLIM), therefore, uses low-irradiance white-light illumination and also the environmental control to keep the specimen unaffected by the external stress [14]. Based on a QPI method, this technique provides a 3D map of the refractive index distribution of the sample, which relates to the 3D distribution of the non-aqueous content within the cell. The sub-micron resolution of this technique is suitable for single cell imaging and the sub-cellular structures can be resolved in all three dimensions.

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WHITE-LIGHT DIFFRACTION TOMOGRAPHY (WDT)

Unlabeled biological cells are mostly transparent under visible light because the absorption is low and the scattering is weak [15]. This allow the cells to be assumed as phase objects. Therefore, the structural information in the sample is encoded in the phase of the light that scatters through the sample. Zernike's phase contrast (PC) microscopy utilizes this encoded information to increase the contrast [16]. PC measures the interference between the phase-shifted reference field, $U_i(\mathbf{r},\omega) = A(\omega)e^{i\beta(\omega)z}$, and the scattered field. The scattered field can be calculated as

$$U_s(\mathbf{k}_\perp, z;\omega) = -\frac{\beta_0^2(\omega)A(\omega)e^{iqz}}{2q}\chi(\mathbf{k}_\perp, q-\beta(\omega)), \tag{1}$$

where $\beta = \bar{n}\beta_0 = \bar{n}\omega/c$ with $\bar{n} = \langle n(\mathbf{r})\rangle_{\mathbf{r}}$ being the spatially averaged refractive index, $q = \sqrt{\beta^2 - k_\perp^2}$, and χ the scattering potential of the object [13, 17, 18]. By assuming zero-delay and proper change of variables, the cross-correlation is expressed as a spatial convolution between a characteristic function, Σ, or the coherent transfer function (CTF), of the imaging system and the scattering potential,

$$\begin{aligned}\Gamma_{12}(\mathbf{k}_\perp, z;0) &= \frac{1}{8\bar{n}^2}\int_0^\infty \frac{\left(Q^2+k_\perp^2\right)^2}{Q^3} S\left(-\frac{Q^2+k_\perp^2}{2Q}\right)\chi(\mathbf{k}_\perp, Q)e^{izQ}dQ \\ &= \frac{1}{8\bar{n}^2} FT_Q^{-1}\left[\frac{\left(Q^2+k_\perp^2\right)^2}{Q^3} S\left(-\frac{Q^2+k_\perp^2}{2Q}\right)\right] \circledv_z \chi(\mathbf{k}_\perp, -z) \\ &= \Sigma(\mathbf{k}_\perp, -z) \circledv_z \chi(\mathbf{k}_\perp, -z)\end{aligned} \tag{2}$$

In Eq. (2), $Q = q - \beta = \sqrt{\beta^2 - k_\perp^2} - \beta$, S is the power spectrum of the incident field and $\circledv_z$ represents a convolution in z and multiplication in $\mathbf{k}_\perp$. Now, one can computationally determine the CTF of an optical system using its specifications, and reconstruct the 3D object by deconvolving the measurement with the DTF. This method, called white-light diffraction tomography (WDT), combines a quantitative phase imaging technique with a sparsity based deconvolution technique [14, 19].

For our optical imaging system, the characteristic function, Σ, is calculated based on the specifications of the objective lens (63x/1.4NA Oil immersion). Figure 1 shows the point spread function (PSF) derived from the Fourier transform of Σ. The result is compared with the measured PSF of the system and showed a good agreement.

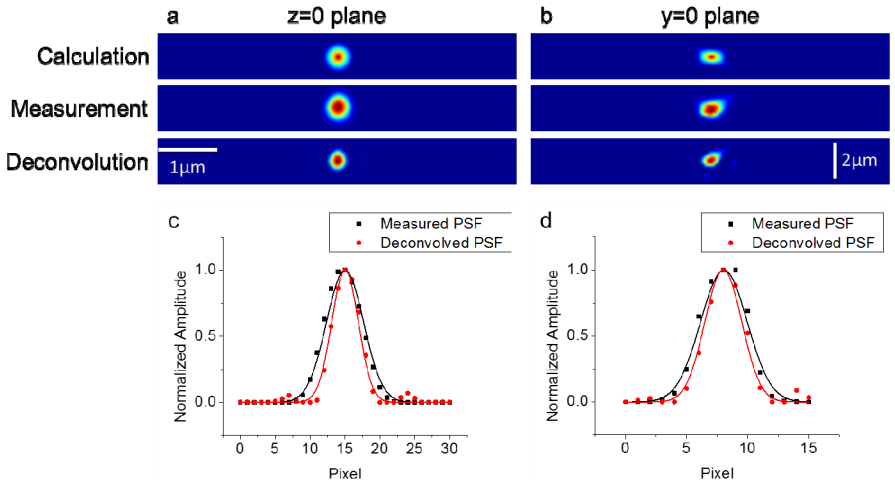

Figure 1. Resolution analysis of WDT. (a, b) Transverse and longitudinal cross-sections of the PSF generated from the theory (top), from the measurement of a polystyrene bead smaller than the diffraction spot (middle) and the result of deconvolving the measured PSF with the calculated PSF (bottom). (c, d) Profile along the center of measured and deconvolved PSFs. (Figure adapted from [13])

The resolution of WDT is determined based the resolution of SLIM. In the transverse dimension (z=0 plane), the width of the measured PSF is 398 nm and the width of the deconvolved PSF is 285 nm. In the longitudinal dimension (y=0 plane), the width of the measured PSF is 1218 nm and the width of deconvolved PSF is 967 nm. Therefore, the resolution is increased by nearly 1.3x in both transverse and longitudinal directions. Furthermore, the calculated PSF itself shows smaller widths in both directions, 350 nm and 890 nm, compared to the measurement.

WDT OF NEURONS

As demonstrated in previous publications, a neuronal network exhibits very complex emergent behaviors [1]. However, quantitative phase imaging, a tool to quantify and analyze these networks and their activities, have only given a two-dimensional data for the cells cultured in a dish. Here, we apply WDT to rat neuron cultures, one with dorsal root ganglion (DRG) neurons and the other with hippocampal neurons.

Figure 2 shows a DRG neuron culture imaged with WDT. Figures 2a and 2b show two different depth position in the sample, as indicated in Figs. 2c and 2d. Conversely, Figures 2c and 2d show two different sections indicated in Figs. 2a and 2b. The process extending diagonally in Figs. 2a and 2b is shown to be extending in the depth dimension as indicated in Fig. 2c. Moreover, Fig. 2d shows that the two process crossing each other in Figs. 2a and 2b are located in different depth position from each other.

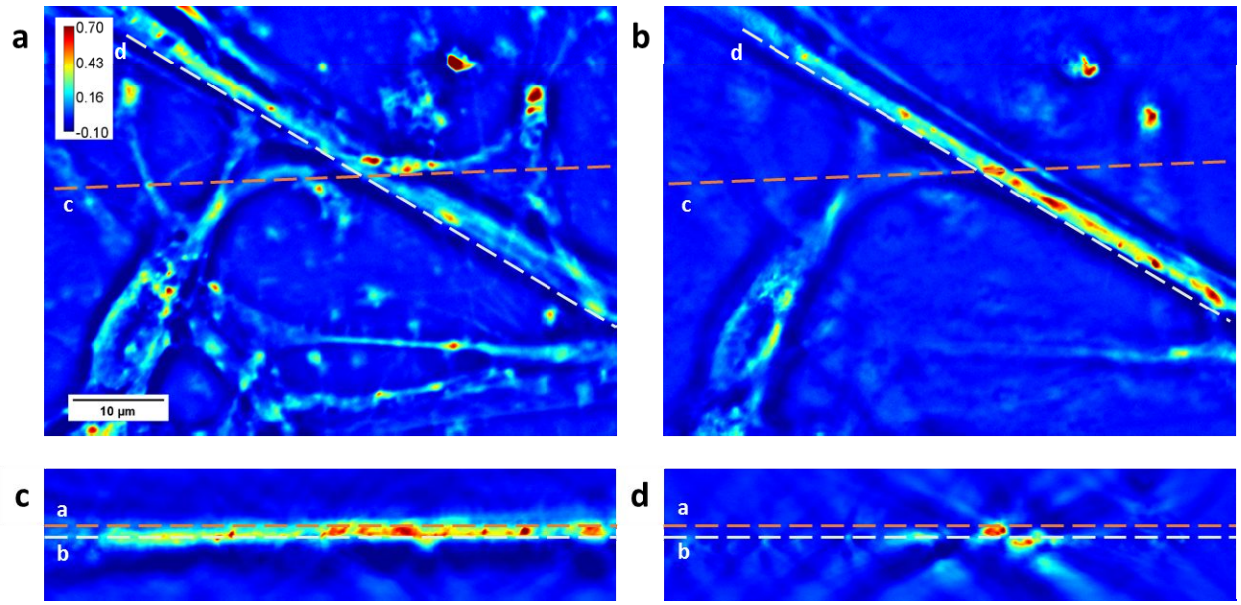

Figure 2. WDT of a DRG neuron culture shown at (a) a z-position near the petri dish surface (white dashed line in 2c and 2d), (b) a z-position further from the bottom of the sample (orange dashed line in 2c and 2d). Vertical slices at each dashed line in 2a and 2b are shown in (c) corresponding to the white dashed line and (d) corresponding to the orange dashed line in 2a and 2b. Color bar is in radians.

Furthermore, in order to see more miniscule structures, a rat hippocampal neuron culture has been imaged using WDT. The culture was four days old at the time of imaging and was kept at 37˚C, 5% CO2. Figure 3 shows the result of imaging this sample. Figure 3a and 3b shows, again, two depth positions of the sample, one near the glass bottom dish and the other well above the dish. Figure 3c is a slice of the stack taken along the dashed line in Fig. 3a. This figure shows clearly the membrane of the soma and also the nucleolus. Figures 3d and 3e show two different perspectives of a 3D rendering of the WDT data. As demonstrated here, WDT is capable of imaging neurons at both single cell level and a network level by showing neurons at different depth planes.

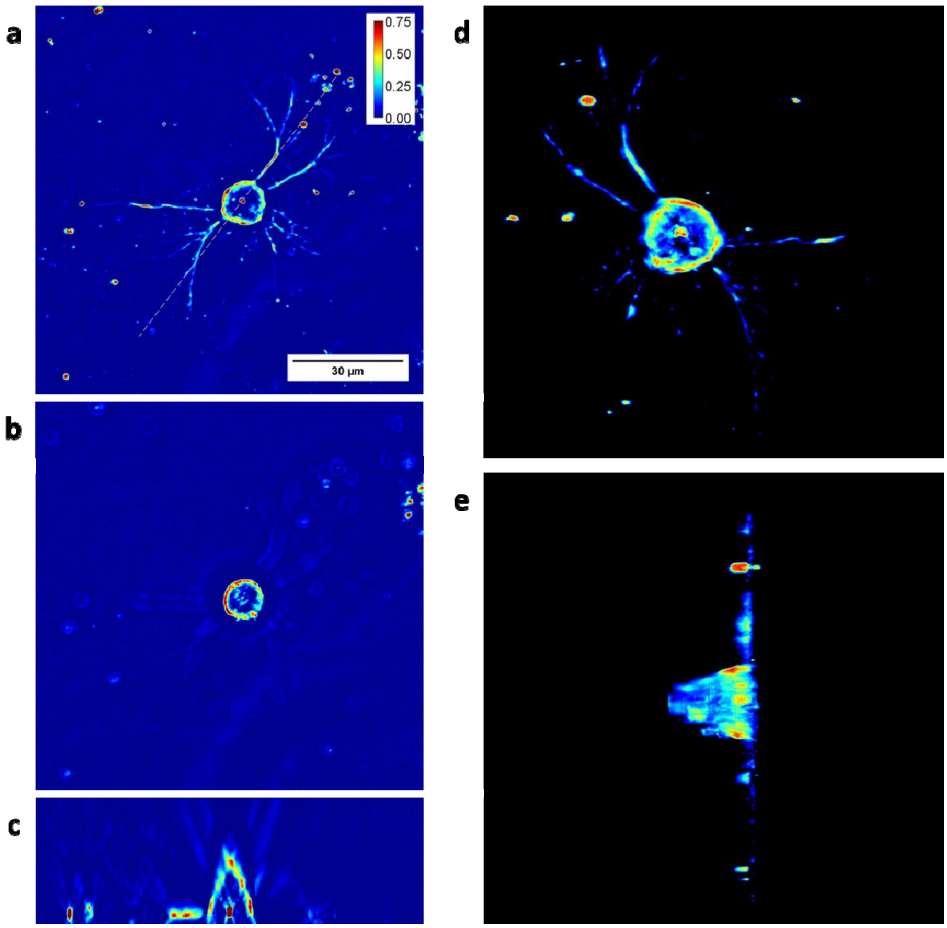

Figure 3. WDT of a rat hippocampal neuron. (a) An x-y plane immediately above the glass bottom dish. (b) An x-y plane well above the dish. (c) An x-z view taken along the dashed line in (a). (d, e) two perspectives of a 3D rendering of the WDT data. Color bar is in radians.

SUMMARY

In summary, we have presented 3D images of rat neuron cultures, by showing single cells and the network of neuronal processes. By using White-light diffraction tomography, which combines spatial light interference microscopy with an inverse scattering solution, the neurons were kept alive during the imaging process in the incubation system installed on the commercial microscope. Therefore, this method provides structural information of a live cell with minimal disturbance applied on them. Furthermore, because the sample is kept alive, this method is expected to provide a 3D dynamics of a neuronal network. Through optimization in speed and resolution, this technique can reveal new and unique insights into the dynamics of the formation of neuronal circuits.

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