NeuroImage xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Serial optical coherence scanner for large-scale brain imaging at microscopic resolution

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ARTICLE INFO

Article history:
Accepted 23 September 2013
Available online xxxx

Keywords:
Fiber pathways
Nerve fiber orientation
Connectivity
Brain anatomy
Optical coherence tomography
Polarization

ABSTRACT

We describe a serial optical coherence scanner (SOCS) for high resolution imaging of *ex-vivo* brain. SOCS integrates a multi-contrast optical coherence tomography and a vibratome slicer to establish comprehensive brain anatomy and fiber pathways in three-dimensional space. Rat brain images are demonstrated by utilizing intrinsic optical contrasts including back-scattering, birefringence and optic axis orientation, which are simultaneously generated from the same dataset. Volumetric images from serial scans are combined to realize large scale brain maps. Nerve fiber tracts are globally described in 3D by retardance, and delicately delineated by cross-polarization at the resolution of $15 \times 15 \times 5.5\ \mu m^3$. In-plane orientations of the tracts are quantified by optic axis orientation. SOCS offers a new solution for complete reconstructions of macroscopic tissues such as primate and human brains at microscopic resolution. The technique also opens up varieties of opportunities for connectome studies and systematic investigations on neurological diseases and brain disorders.

Introduction

Light microscopy has made tremendous contributions to our knowledge in neuroanatomy including conformation and function of neural cells in the brain over the past century. In recent years, more consensuses arise to realize the role communication pathways play in brain disorders such as autism and schizophrenia. Comprehensive navigation of the white matter routes imposes great challenges to the current imaging technologies. Traditional histology bears serious limitation as single axonal tract can extend up to centimeters long, and the structures of the axonal networks are extremely complicated. Inevitable distortion caused by tissue processing and embedding makes the alignment of sequential microscopy images extremely difficult and laborious (Streicher et al., 2000; Yushkevich et al., 2006). To overcome the problem, plane illumination microscopy (Huisken et al., 2004) and ultramicroscopy in combination with optical clearance technique (Dodt et al., 2007; Jährling et al., 2009) eliminate mechanical sectioning and produce entire brain imaging of young mice with comparable resolution. Nevertheless, better optical clearance has yet to be developed for large scale reconstruction of the axonal networks in adult brain (Chung et al., 2013; Hama et al., 2011). Neural tracers in combination with fluorescent microscopy contribute to sensitive identification of local neural circuits. However, the timeline imposes significant obscure to establish the full connection map in a brain template, considering systematic characterization of thousands of injection sites on multiple samples (Bohland et al., 2009).

Firstly introduced in light microscopy (Odgaard et al., 1990), block-face imaging with electron microscopy (Denk and Horstmann, 2004), confocal microscopy (Sands et al., 2005) and two-photon microscopy (Ragan et al., 2012; Tsai et al., 2003) target large scale synaptic connections or axonal networks in 3D space. The entire tissue block was mounted still and thin slices were removed between consecutive scans, the procedure of which yields automatically aligned image stacks in 3D. However, due to the high density of slicing and the point-scan scheme, complete reconstruction of complicated mammalian brains remains a hardly approachable proposition.

The recent advancements of optical coherence tomography (OCT) may shine a light to deal with the difficulties. OCT is an interferometric technique that uses a broadband light source at near-infrared regime to produce depth resolved images (cross sections) of tissue structures at the micrometer scale resolution (Huang et al., 1991). Fourier domain OCT (Fercher et al., 1995) captures the information at all imaging depths with a single measurement, hence dramatically improving the imaging speed. Since invention, its application in the peripheral nervous system such as retina has been rapidly translated to clinical studies and continuously supported by technical advances (Bernardes and Cunha-Vaz, 2012; Schuman, 2013). In contrast, the applications of OCT in the central nervous system have been sparsely reported until very recently (early review by Arous et al., 2011; Boppart, 2003; Nakaji et al., 2008; Srinivasan et al., 2012; Wang et al., 2011). After demonstrating the feasibility to differentiate the gray matter and white matter in the brain (Bizheva et al., 2004; Jeon et al., 2006), development of OCT probes

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http://dx.doi.org/10.1016/j.neuroimage.2013.09.063

to guide neurosurgical interventions has been presented (Jafri et al., 2005, 2009; Liang et al., 2011). However, systematic studies on large-scale brain structures and white matter organizations have not been reported.

We develop a serial optical coherence scanner (SOCS) to accomplish large-scale volumetric imaging of *ex-vivo* brain and to reconstruct the nerve pathways in 3D. The optical system is a multi-contrast (MC) OCT that uses the polarization-maintaining fiber (PMF) technology and polarization-sensitive measurements to produce multi-parametric images (Wang et al., 2010, 2011). The multiple contrasts provide the anatomical information, the differentiation between the gray matter and the white matter, the fiber architectures, and the quantification of fiber orientations in the brain. With integrated tissue sectioning between serial scans, SOCS reconstructs a rat brain with axial resolution of 5.5 μm and transverse resolution of ~15 μm. The axonal networks are visualized and fiber tracts of various scales are traced.

Materials and methods

Tissue preparation

Three euthanized adult rats were obtained from the tissue sharing program with approval by the Research Animal Resources at the University of Minnesota. Brain was dissected and kept in 10% buffered formalin for 72 h before imaging.

System and data acquisition

Serial optical coherence scanner

SOCS integrates a tissue slicer into a MC-OCT system for reconstructing large-scale biological tissues in 3D. Schematic diagram of SOCS is demonstrated in Fig. 1A. The MC-OCT combines a PMF technique (Al-Qaisi and Akkin, 2008) and the spectral domain measurement to provide morphological and polarization sensitive imaging with high spatial–temporal resolution (Wang, 2010). The light source is a broadband superluminescent diode operating at the center wavelength of 840 nm with a 50 nm bandwidth, yielding an axial resolution (z) of 5.5 μm in tissue (refractive index: ~1.4). Polarized light is directed through PMF coupler into the sample and reference arms. A scan lens in the sample arm ensures consistent imaging quality over a large area. The lateral resolution estimates ~15 μm (xy). Interferometric signals carrying the optical delay gate between reference light and back-scattered light from sample are detected by a customized spectrometer, which consists of a grating to disperse spectral components, a Wollaston prism to separate the PMF channels, and a lens to focus the spectra on a line-scan camera (Basler sprint 140 k). Vertical and horizontal binning was applied on the camera to enhance photon collection. The spectra on the two polarization channels are acquired simultaneously at a rate of 25 kHz. An inverse Fourier transform of the spectral modulations (in k-space) produces a complex depth profile (A-line) for each channel $A_{1,2}(z)\exp\{i\Phi_{1,2}(z)\}$, where A and Φ denote the amplitude and phase, respectively, along the depth z, and 1 and 2 correspond to the cross-coupled and main polarization channels. The imaging contrasts are derived from amplitudes and phases of the depth profiles: reflectivity ($R(z)$), the traditional OCT contrast, is the addition of intensity on the two channels; cross polarization ($C(z)$) only takes the intensity of the cross-coupled channel; retardance ($\delta(z)$) and optic axis orientation ($\theta(z)$) are computed based on the Jones analysis (Götzinger et al., 2005). The equations for the contrasts are extracted as,

$$R(z) \propto A_1(z)^2 + A_2(z)^2 \quad (1)$$

$$C(z) \propto A_1(z)^2 \quad (2)$$

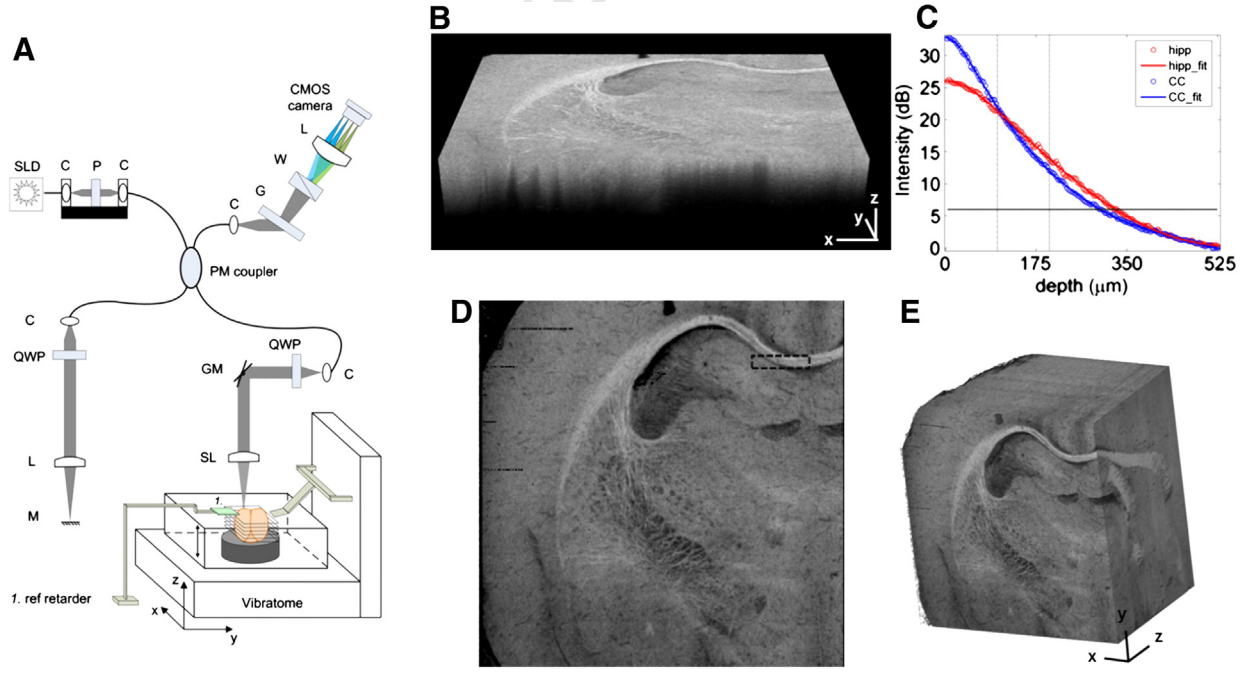

Fig. 1. SOCS and reconstruction of rat brain images. (A) System schematic diagram. SLD, superluminscent diode; P, polarizer; C, collimator; QWP, quarter-wave plate; L, lens; M: mirror; GM, galvo mirror; SL, scan lens; G, grating; W, Wollaston prism; LSC, line scan camera. (B) Volumetric scan of an optical section. *xz*-plane represents the cross section, and *xy*-plane assembles the coronal section. (C) Depth profiles of reflectivity in the hippocampus (red circles) and the corpus callosum (blue circles). The plots present an average of 400 A-lines for each structure within the ROI in (D). Fifth-order polynomial fits were applied for the estimates of light penetration (solid lines). The horizontal line sets the intensity threshold at SNR >6 dB for the estimation. The vertical lines indicate the slice thickness used in the current studies. (D) *En-face* image created from the optical section in (B) resembles the coronal view. The black rectangular box indicates the ROI used for the plots in (C). Scale bar: 500 μm. (E) Large scale brain imaging ($7 \times 7 \times 5.5$ mm^3) is presented by stacking sequential *en-face* images (see also Supplemental Video 1). *xy*: coronal plane; *yz*: sagittal plane; *xz*: axial plane.

Please cite this article as: Wang, H., et al., Serial optical coherence scanner for large-scale brain imaging at microscopic resolution, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.09.063