Methods xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Methods

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

Reconstruction method for extended depth-of-field optical diffraction tomography

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ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form 11 September 2017
Accepted 2 October 2017
Available online xxxx

Keywords:
Optical diffraction tomography
Limited angle tomography
Tunable lens
Tissue analysis
Cell analysis
Extended depth of field

ABSTRACT

In the paper we present a novel method of extended depth-of-field limited-angle optical diffraction tomography, in which the change of a focal plane position is performed with a liquid focus-tunable lens. One sinogram is acquired for each state of a focus-tunable lens. After acquisition process is complete, all sinograms are independently reconstructed and stitched to form the final tomographic reconstruction. The presented solution effectively extends the applicability of the Rytov approximation to relatively thick samples and provides uniform resolution of 3D tomographic reconstructions. The method is also combined with Generalized Total Variation Iterative Constraint algorithm, which minimizes distortion of the results due to the limited angular range of acquired projections. The combined solution is dedicated to investigation of transparent and semi-transparent biological micro-structures, like cells and tissue slices.

1. Introduction

Recent advances in optical diffraction tomography (ODT) have demonstrated its high capability for the study of biological cells and tissues with its unique advantages of label-free and quantitative measurements of opto-geometrical parameters of non-fluorescent micro-samples [1]. Most ODT setups are implemented in digital holographic microscopes (DHM), which differ from classic ones in that they measure both: the phase and amplitude of diffracted fields with numerical defocusing capability [2–4]. Several authors have already implemented tomographic procedures based on digital holography for 3D refractive index (RI) investigations inside cells. The projections of an object are captured based on two main approaches: (1) rotation of a bio-sample [5–8] and (2) scanning of the illumination beam [9–12]. Both solutions have their pros and cons. The first one delivers uniformly distributed data, so tomographic reconstruction is more straightforward and accurate. On the other hand, the experimental difficulties connected with inserting cells into a rotatable capillary, or alternatively rotating a cell by optical tweezers [13] is in many biological applications unacceptable. In the second approach, the data are provided by projections which are captured within a limited angle and therefore it is not possible to recover a part of the 3D Fourier space of an object and the well-known missing cone problem occurs [14]. The illumination scanning method, often called limited-angle optical diffraction tomography (LAODT), results in significant distortion of the reconstructed geometry and 3D RI distribution. This problem has already been addressed e.g. in [15,16] and recently two-stage Generalized Total Variation Iterative Constrain (GTIVIC) algorithm has been proposed [17]. It enables retrieving the accurate 3D external geometry of samples, followed by proper reconstruction of 3D distribution of RI.

In LAODT, a biological micro-structure is placed in a cell culture medium in a stationary Petri dish or a glass plate which is then introduced into the ODT system built in a vertical configuration. This approach has multiple advantages: (a) it simplifies preparation of a sample, since many biological cells are grown in Petri dishes, (b) it minimizes vibrations in the system and (c) increases the measurement speed, since changing a position of laser illumination beam can be carried out much faster than object rotation, thus allowing analysis of dynamic biological phenomena. These experimental advantages have made LAODT popular in numerous biomedical application fields [9,11,12] and in commercial systems [18,19]. However, there are still some important issues to consider with aim to improve the accuracy of quantitative analysis of cells with complex shapes, cell clusters or tissues.

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https://doi.org/10.1016/j.ymeth.2017.10.005
1046-2023/

It has been proven that tomographic reconstruction techniques based on Rytov approximation suffer from the effect of spatially-variant accuracy along optical axis which is associated with an approximated propagation of Rytov fields, which is an inherent part of this technique [20–22]. It is even more observable in LAODT systems, as they utilize high numerical aperture (NA) microscope objectives, for which depth of field (DOF) is extremely shallow [12] and the Rytov approximation, applied in tomographic reconstructions for layers far from the focal plane of a microscope objective, is strongly violated [23]. It should be noted that these errors are present in reconstruction methods that utilize direct inversion algorithm, in which no propagation is directly calculated and instead, filling the spectrum with Rytov fields on Ewald's spheres is carried out, as these two approaches are equivalent, according to [24]. In principle this problem can be solved by proper manipulation of input holographic data and their processing as shown in [21], in which the Extended Depth of Focus Filtered Back-propagation (EDoFFBP) technique has been introduced. In EDoFFBP a complex field from each projection is rigorously propagated to multiple planes that cover the range of the whole sample. In the next step, Rytov fields are calculated from the propagated fields and the final reconstruction is obtained. This method requires capturing of only a single set of projections, which is a significant advantage. However, it involves extensive and time-consuming data-processing and it suffers from variable accessible measurement volume.

The spatially invariant accuracy problem can also be addressed with hardware-based methods, in which a sample stage is scanned in z direction while acquiring projections [25]. Unfortunately, it is difficult to achieve high mechanical stability of such a setup especially if a fast measurement is to be performed. Also, this removes the main advantage of the LAODT, namely stationarity of the investigated biological micro-object, which is usually placed in liquid environment. Therefore, we propose an alternative method of capturing projections for multiple positions of focal planes in the sample by introducing a liquid focus-tunable lens between an imaging microscope objective and a CCD camera. This solution assures stationarity of an object and allows fast, optoelectronic-based selection of focal planes, for which the sequential sets of projections are captured, followed by tomographic reconstruction and stitching of the data volumes. Additionally, to fully address the main downsides in LAODT, that is the so-called missing frequency problem, poor axial resolution and spatially-variant axial accuracy, we present the measurement methodology and full processing chain, which combines the two-stage tomographic reconstruction strategy based on the regularization technique [17] and stitching of the volumes of accurate RI data reconstructed for several focal planes. This general procedure can be easily modified depending on the features of micro-object (its thickness, scattering properties and a character of RI distribution – piecewise constant vs. gradient).

The paper is organized as follows. Section 2 provides the full concept of the proposed method for achieving the spatially invariant axial accuracy. Section 3 describes in detail the measurement system in which we capture the set of projections, while in Section 4 the algorithms that are implemented for data processing and tomographic reconstruction in full volume are presented and explained. Section 5 presents the results of measurements of two types of objects: a certified micro-sphere and two biological micro-structures: a mouse fibroblast cell and a tissue slice. Finally, Section 6 summarizes the findings and draws conclusions.

2. Main concept

In LAODT the projections are captured by illuminating a sample from different directions allowed by the NA of a microscope objective. The basic step in LAODT is acquisition of a single projection. As a result, a hologram with encoded information about integrated complex RI distribution along the illumination direction is recorded by a detector. A microscope objective in the optical setup conjugates a central z-plane (in which z is the optical axis) of the measurement volume with a detector, and thus the part of the integrated complex RI that comes from the surrounding of this z-plane is diffraction-free since it is within the depth-of-field (DOF) of the optical setup. DOF highly depends on the NA of the optical setup, according to the relation:

$$DOF \propto \frac{\lambda n}{\mathrm{NA}^2} \tag{1}$$

where λ – wavelength in vacuum, n – immersion refractive index, NA - numerical aperture.

So, it is clear, that with high NA microscope objectives, utilized in LAODT setups, DOF is extremely small. This is important, because the effect of non-central planes (z-planes that are outside of DOF) on the integrated complex RI is nonlinear. When analyzing the influence of these z-planes, it is necessary to take diffraction into account. What is more, the further the z-plane is from the center of a measurement volume, the more significant the effect of diffraction is.

The two well-known methods that linearize the relationship between RI distribution of z-planes that are outside of the optical system DOF with the recorded projection are 1st order Born and Rytov approximations [26]. It has already been proved, that when biological micro-structures are measured, 1st order Rytov approximation provides superior results. For the sake of simplification, it can be stated, that application of 1st order Rytov approximation creates a synthetic DOF (SDOF) of the optical system, which is significantly higher than the standard DOF. Inside SDOF, diffraction effects are compensated in the reconstruction process. A symbolic relationship between DOF and SDOF is presented in Fig. 1(a). It should be noted, that the value of a SDOF cannot be calculated mathematically, like standard DOF. Instead, it can be estimated for a given class of biological micro-structures based on experiments. If the measurement volume is large, i.e. the thickness of a cell or tissue sample is significant, the SDOF may cover it only partially. As a result, when a tomographic reconstruction is calculated, regions of a sample that are outside of SDOF have lower resolution compared to the parts that are within the SDOF due to violation of the Rytov validity condition. Such anisotropy of resolution deteriorates the measurement conditions and reduces quality of the reconstructed data.

The idea behind focus-tunable tomography is to achieve quasi-uniform resolution within the whole object volume through acquisition of multiple projections for a single illumination direction. Each projection is acquired for a different position of the focal plane in the measurement volume. This way, SDOFs that correspond to different focal planes cover the whole measurement volume (see Fig. 1(b)).

A focus-tunable lens is a liquid-filled membrane, whose optical power is controlled with electric current. Thus, by applying specific values of electric current, different sections of an investigated sample are conjugated to a detector plane. The measurement scenario is presented in Fig. 2. First, the lens is set to conjugate first focal plane (FP1) in the object's space with the matrix detector. Then, object's projections are acquired with the illumination scanning system and a sinogram is created for the given optical power of the lens. Then, the electric current value is modified, and the procedure is repeated for FP2 and FP3. As a result of this stage, multiple sinograms are recorded for different focal planes in the object space.

The number of focal planes within a measurement volume depends on three parameters. The first is thickness of an investigated sample. For thick samples, more focal planes are necessary.

Please cite this article in press as: W. Krauze et al., Methods (2017), https://doi.org/10.1016/j.ymeth.2017.10.005