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Optical projection tomography via phase retrieval algorithms

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ABSTRACT

We describe a computational method for accurate, quantitative tomographic reconstructions in Optical Projection Tomography, based on phase retrieval algorithms. Our method overcomes limitations imposed by light scattering in opaque tissue samples under the memory effect regime, as well as reduces artifacts due to mechanical movements, misalignments or vibrations. We make use of Gerchberg–Saxton algorithms, calculating first the autocorrelation of the object and then retrieving the associated phase under four numerically simulated measurement conditions. By approaching the task in such a way, we avoid the projection alignment procedure, exploiting the fact that the autocorrelation sinogram is always aligned and centered. We thus propose two new, projection-based, tomographic imaging flowcharts that allow registration-free imaging of opaque biological specimens and unlock three-dimensional tomographic imaging of hidden objects. Two main reconstruction approaches are discussed in the text, focusing on their efficiency in the tomographic retrieval and discussing their applicability under four different numerical experiments.

1. Introduction

Optical biomedical imaging modalities have become essential tools for disease detection and monitoring, occupying an increasing portion of medical imaging needs [1], characterized by the use of non-ionizing radiation, molecular specific contrast agents and minimum or non-invasiveness. Among these methods, *Optical Projection Tomography* (OPT) [2], considered the optical analogue of X-ray *Computed Tomography* (XCT), shares with the latter a broad set of reconstruction approaches, while the measurement scheme might change slightly depending on the experimental architecture. OPT relies on mechanical movement of the specimen rotating it around a fixed axis to acquire the full set of projections, rather than moving the measuring device around the object. However, mechanical translation and rotations are then needed to perform a three-dimensional reconstruction of the sample of interest, thus requiring a correct calibration of the experiment to obtain accurate reconstructions. Such calibration has to be often performed both at hardware and software level and might sometimes limit the acquisition procedure or introduce unwanted artifacts in the reconstructions. Many efforts have been made in finding new algorithmic ways to align, center or reduce misalignment effects at the tomographic level [3–6], but none has the advantage of being fully alignment-free. In this article, we study a more generic approach to confront with misaligned data reconstructions, proposing two algorithmic step-wise protocols that are able to retrieve quantitative reconstructions even in the presence of constant drifts, random vibrations or even overcoming the phase scrambling due to the presence of a scattering layer enclosing the object. The study focuses on two different approaches, both of them relying on the use of phase retrieval algorithms, to generate aligned autocorrelation sinograms that can be inverted in order to obtain tomographic reconstructions. We emphasize the fact that any positional information of the specimen is not important, since the autocorrelation is inherently always centered in its space of definition.

Phase-Retrieved Tomography (PRT) was originally introduced in our previous work [7] to image experimentally the fluorescence distribution of necrotic cells in a human-breast tumor spheroid [8]. In this case the method was compared against classical reconstruction schemes from *Single Plane Illumination Microscopy* (SPIM) [9,10] and OPT, showing promising reconstruction abilities. Here

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we focus on a broader numerical study, better illustrating the potential of phase retrieval techniques applied for tomographic investigations. Alongside PRT, we propose another iterative technique to recover aligned sinograms to which we refer to as *iterative Projection-Retrieved Tomography* (iPRT). The two methods will be tested for the first time in reconstructing corrupted datasets generated by numerical simulations, focusing in particular on their ability to leave the user free of any alignment procedure. The possibility of performing hidden reconstructions will be further considered showing that, under memory effect conditions, blind tomography becomes naturally possible as a result of the incorporation of phase retrieval techniques into classical reconstruction schemes.

2. Material and methods

In this article we generate the datasets numerically, analyzing the possibility offered by the methods under different measurement scenarios in order to have a known reference to compare with. The object under investigation is a previously retrieved reconstruction of the fluorescence distribution in a human-breast tumor spheroid [7], which we will use as a ground truth measurement. This volume has a size of 300 pixels (from now on abbreviated as px) in each spatial dimension, being characterized in the original experiment by a pixel size of 0.8 μm. Starting with this volume, we generate several datasets representing four different experimental measurement conditions. The first dataset is created by rotating the object around an axis parallel to z and positioned at the center of the camera plane, thus acquiring its intensity projections (Fig. 1, row A) as a function of the observation angle. This mathematically corresponds to the Radon transform of the sample volume and, experimentally, to an ideal OPT experiment. The second dataset (row B) is obtained by introducing a random two-dimensional slight vibrations of ±1 px during the rotation, leading to the generation of a fuzzy sinogram. This could represent a situation in which we try to correct the axis of rotation to fit it onto the position of the barycenter of the object, or the case in which the stages slightly vibrate during the rotation. The third dataset (row C) is equal to the above except for the introduction of a constant drift in two dimensions while rotating the object. In fact, in some experimental acquisitions [7], we observed a similar perturbation due to the effect of gravity (sample slowly falling inside the medium) and due to mechanical misalignment of the rotating stage. The fourth, and last, dataset (row D) reproduces a measurement performed when the object is hidden behind a phase scrambling environment [11,12], such as a scattering envelope (e.g. an egg or a cocoon). This dataset is numerically calculated assuming the measurements to be in the memory effect regime [13], which states that a speckle pattern (generated by a single point source) translates in relation to the source movement behind the phase-scrambling layer [14].

All the above described numerical experiments produced different sinograms (Fig. 1, column 2), that can be inverted (with an inverse Radon transformation) to obtain the volume reconstruction in each specific case. Column 3 of Fig. 1 shows, depending on the perturbation introduced in the measurement, how the reconstructed volume differs from the ideal case ultimately producing several kinds of unwanted reconstruction artifacts well known in the literature [4,15]. In the following, we will try to avoid the generation of alignment perturbations, exploiting the mathematical properties of the autocorrelation in combination with the phase-retrieval algorithm.

2.1. Autocorrelation sinogram

From each of the previously obtained sinograms we can calculate the corresponding autocorrelation sinogram A_θ (A-sinogram), which will be used for both the phase-retrieval methods. In this

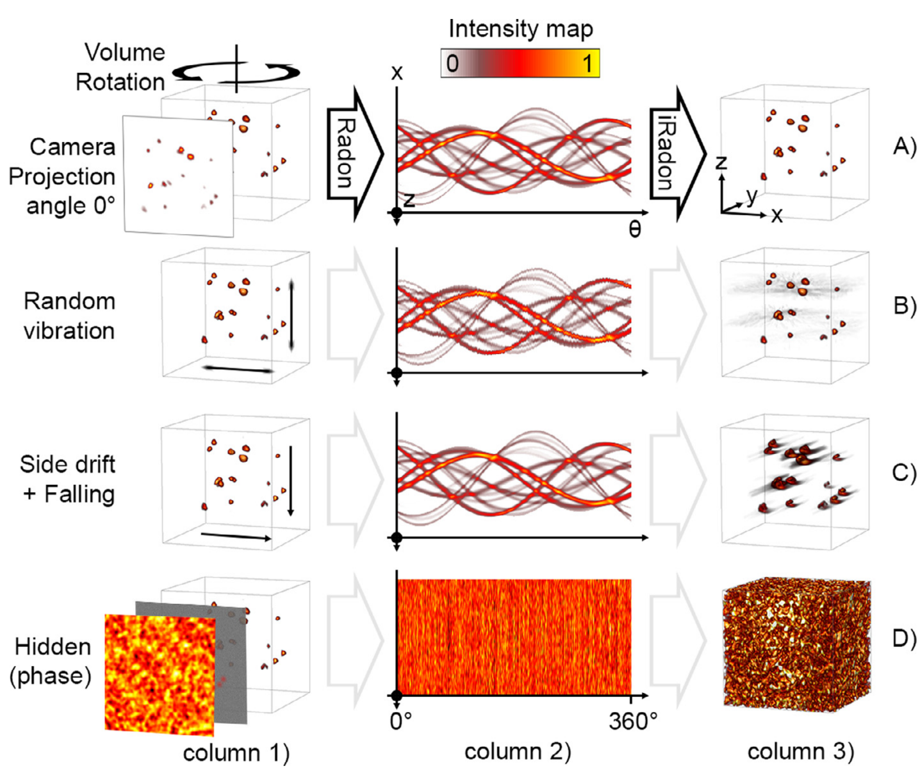

Fig. 1. Four different numerical experiments, showing the resulting sinograms (column 2) of the three different rotations. A) The results of an aligned rotation, leading to optimal reconstructions of the object. B) A one-pixel random vibration was introduced while rotating the object, returning slight artifacts in the reconstruction. C) The measurement was affected by a two-dimensional drift (gravity falling and axis displacement) particularly evident in the corresponding reconstruction. D) Hidden object measurement, impossible to backproject with classical techniques.

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