Spectral off-axis phase shifting in transmission optical coherence tomography

Martyna Mazur,* Wojciech Krauze, Arkadiusz Kuś, Aleksandra Piekarska, and Małgorzata Kujawińska

Institute of Micromechanics and Photonics, Faculty of Mechatronics, Warsaw University of Technology,
Sw. A. Boboli 8 Street, 02-525 Warsaw, Poland

Received November 14, 2024; accepted December 30, 2024; published December 31, 2024

Abstract—Transmission optical coherence tomography (tOCT) is gaining interest for imaging biological samples; however, so far, quantitative phase information has not been directly obtained. This study introduces SOPHAST-OCT, an approach for integrated 2D phase recovery in Off-Axis Full-Field Swept-Source tOCT. It uses phase-shifting to leverage OCT's inherent phase changes between recorded interferograms. SOPHAST-OCT demonstrates promising phase agreement with the simulated ground truth phase. This approach represents a first step toward quantitative imaging in tOCT.

Quantitative phase imaging (QPI) plays a crucial role in biomedical research, offering non-destructive and label-free quantitative measurements of biological samples. Standard QPI techniques provide excellent results for *in vitro* investigations, assuming low scattering of the test object [1−2]; however, imaging of thick, multiple-scattering biological specimens, such as organoids, embryos, and microorganisms, remains a major challenge. To address this issue, several research groups are focusing on introducing and developing approaches enabling quantitative measurements in optical coherence tomography (OCT), a widely adopted qualitative and non-invasive imaging technique that has the property of being able to analyze millimeter-thick samples [3−4]. While OCT traditionally operates in reflection mode, there is growing interest in transmission OCT (tOCT), partly due to the high anisotropy parameter g of biological samples [5]. One approach to quantitative phase recovery involves multi-angle tOCT methods [6], where phase information is obtained by identifying the position of a ballistic light peak in the reconstruction. Achieving accurate results in this approach requires a high level of precision in peak localization which can be difficult partly because of weakening of the ballistic signal when analyzing highly scattering samples, such as tissues. Another developed method is angle multiplexing based on the principle of holoscopy [7]; however, this technique does not address the issue of strong scattering. A potentially useful tool for phase retrieval from tOCT data is the phase shifting (PS) technique, widely used in interferometry [8], due to the inherent property of OCT imaging, namely, the naturally introduced phase delays between spectrally distributed sample images for each wavelength. To the best of our knowledge, no PS techniques have been proposed for tOCT, and there are only approaches employed for reflection OCT [9−10], mainly for Spectral Domain OCT. Furthermore, they are not aimed directly at recovering the quantitative information but rather at suppressing Fourier Domain OCT artifacts and achieving full imaging depth.

Here, we introduce a method for retrieving quantitative phase information from Off-Axis Transmission Full-Field Swept-Source OCT (OAT-FF-SS-OCT) data using inhomogeneous generalized phase shifting (IGPS) [11−12], called Spectral Off-axis PHAse Shifting in Transmission OCT (SOPHAST-OCT). This approach does not require any additional measurements or phase-shifting optical components, offering a simple and easy-to-use tool for phase recovery in quantitative tOCT imaging.

The optical system utilized to capture OAT-FF-SS-OCT data is a custom-build transmission OCT system working in an off-axis Mach-Zehnder configuration, the schematic diagram of which is depicted in Fig. 1a. The light from a swept-source laser (BS-840-1-HP, Superlum) with 803-878 nm wavelength range is divided into object and reference beams. The former is traveling through the sample, and as a result, it becomes phase-delayed. The reference beam is tilted at a small angle yielding a low carrier frequency in the resultant interferogram. The interference signals are recorded for N wavenumbers by 2048x2048 full-field CMOS camera (acA2040-180-km, Basler) with 5.5 μm pixel size. The numerical aperture of the imaging optics is 1.3, and the magnification is -71.8.

The OAT-FF-SS-OCT data consists of a series of low-carrier-frequency interferograms (see Fig. 1b) that carries the phase information regarding the investigated sample.

---

[*] E-mail: martyna.mazur.dokt@pw.edu.pl

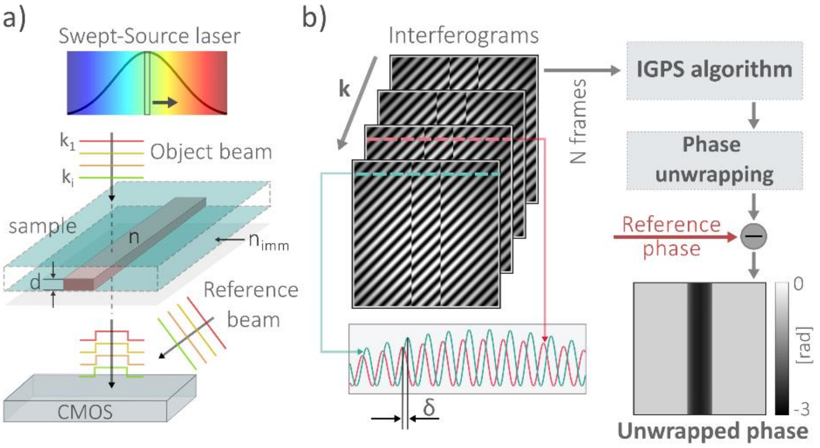

Fig. 1. a) Schematic diagram of data acquisition in Off-Axis Transmission Full-Field Swept-Source OCT system, b) Pipeline of SOPHAST-OCT approach for phase recovery; k – wavenumber, d – thickness of the sample, n, n_{imm} – refractive indices of sample and immersion medium, respectively, δ – phase shift between two interferograms in (x, y) position, reference phase – unwrapped background phase obtained with IGPS.

Assuming a homogenous object with refractive index (RI) n and thickness d, the phase φ can be described by the following equation:

$$\Delta\varphi(x,y,k) = k[n(k) - n_{imm}(k)]d(x,y), \qquad (1)$$

where x, and y represent coordinates of interferograms, k is the wavenumber equal to 2π/wavelength, and n_{imm} describes the RI of the immersion medium.

According to Eq. (1) phase values depend on the wavenumber, which in the case of OAT-FF-SS-OCT data with multiple interferograms acquired for different k gives a naturally introduced phase shift δ between sequential interferograms (see Fig. 1b):

$$\begin{aligned}\delta(x,y,k_i) &= \Delta\varphi(x,y,k_i) - \Delta\varphi(x,y,k_{i-1}) \\ &= [k_i\Delta n(k_i) - k_{i-1}\Delta n(k_{i-1})]d(x,y),\end{aligned} \qquad (2)$$

where $i = 1,\ldots,N$ is the interferogram number, and Δn is the difference between the RI of the sample and an immersion medium.

The resulting phase shifts form the basis for recovering the phase information. However, it should be noted that the determination of a local δ is not a straightforward task, especially when dealing with inhomogeneous specimens with unknown optical properties. Moreover, the tilt angle of the reference beam and optical path difference between interferometer arms affect δ. Therefore, we use the inhomogeneous generalized phase shifting (IGPS) algorithm [11], which does not require specifying the value of δ and instead estimates it for each position (x, y, k) using the least squares method. As depicted in Fig. 1b, N interferograms are processed with IGPS. This approach includes 3 steps: interferogram normalization, estimation of δ, and wrapped phase calculation. This is followed by 2D phase unwrapping [13], the result of which is then corrected by subtracting the reference phase (without a sample in the field of view), also processed by the IGPS algorithm, finally providing an integrated 2D phase of an analyzed sample.

The effectiveness of the SOPHAST-OCT was assessed using OAT-FF-SS-OCT data captured for a 23.5 µm thick microsphere made of poly(methyl methacrylate) (PMMA) (microParticles GmbH) and covered with immersion oil (Zeiss 518F) (Fig. 2a). To recover the 2D phase, 188 low-carrier-frequency interferograms were used (Fig. 2a, b). This number was determined experimentally to obtain the best achievable result. The phase retrieved by SOPHAST-OCT was validated against the ground truth generated by numerical calculation of the 2D integrated phase (Fig. 2b). The ground truth was computed for 839 nm wavelength with the -0.0226 RI contrast (RI$_{839\text{nm}}$ of the PMMA sample and the immersion oil equal to 1.4841 and 1.5067, respectively).

A universal image quality index (Q) [14] was employed to evaluate the SOPHAST-OCT phase quality in two-dimensional space. The obtained Q value is at the level of 0.9, which yields a sufficiently accurate representation of the actual phase of the investigated sample; however, some noise is visible in the phase. The 1D phase analysis was performed by comparing 1D cross-sections marked with blue and red dashed lines in Fig. 2b. The determined 1D profile of the phase follows the shape of the 1D ground truth profile with slight deviations as depicted in Fig. 2b. The mean squared error (MSE) calculated for these 1D cross-sections is approximately 0.02, which, in the context of the entire range of phase values, yields an error of about 0.5%, further confirming the good agreement between SOPHAST-OCT phase and the simulation. However, it

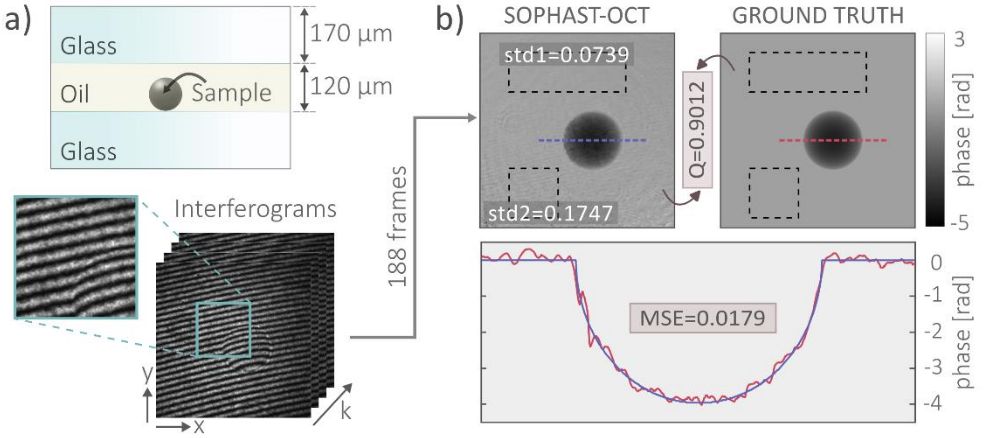

Fig. 2. SOPHAST-OCT performance evaluation: a) from the top: the investigated sample made of poly(methyl methacrylate) microsphere with a size of 23.5 µm and recorded low-carrier-frequency interferograms, b) comparison of the 2D phase obtained by the SOPHAST-OCT with the numerically generated 2D phase (ground truth). k – wavenumber, std - standard deviation, Q – universal image quality index, MSE – mean squared error for 1D cross-sections.

should be noted that despite sufficiently good phase agreement within the PMMA sample, the noise level is relatively high, as indicated by a standard deviation reaching approx. 0.18 rad (see black dashed square with *std2* value in Fig. 2b).

In this paper, we present the SOPHAST-OCT approach for 2D integrated phase recovery from Off-Axis Transmission Full-Field Swept-Source OCT data (OAT-FF-SS-OCT). Our approach exploits the nature of OCT signal acquisition by applying a phase-shifting algorithm – IGPS to OAT-FF-SS-OCT data.

The proposed method's feasibility was evaluated by comparing the determined 2D phase of the PMMA sample with the numerically generated ground truth 2D phase. Results reveal relatively good phase agreement in the 1D and 2D analyses, however with noticeable background noise.

In conclusion, SOPHAST-OCT is the first to our knowledge, off-axis transmission OCT phase recovery algorithm based on phase shifting. This approach is the early stage for the further development of quantitative OCT imaging.

The research was funded by POB Photonics of Warsaw University of Technology within the Excellence Initiative: Research University (IDUB) programme. We would like to thank Rigoberto Juarez-Salazar from *Instituto Politécnico Nacional*, Mexico for sharing the code for inhomogeneous generalized phase shifting (IGPS).

References

[1] Y. Park, C. Depeursinge, G. Popescu, Nature Photon **12**, 578 (2018).
[2] K. Alm, H. Cirenajwis, L. Gisselsson, A.G. Wingren, B. Janicke, A. Mölder, et al., in *Holography, Research and Technologies* (IntechOpen, 2011).
[3] W. Drexler, J. Fujimoto, editors , *Optical Coherence Tomography: Technology and Applications*, 2nd ed. (Springer Cham, 2015).
[4] W. Krauze, P. Ossowski, M. Nowakowski, M. Szkulmowski, M. Kujawińska, in *Quantitative Phase Imaging VII*, **11653** (SPIE, 2021), pp. 19–24.
[5] B.C. Wilson, S.L. Jacques, IEEE J. Quantum Electron. **26**, 2186 (1990).
[6] J. van der Horst, A.K. Trull, J. Kalkman, Optica **7**, 1682 (2020).
[7] P. Hosseini, Y. Sung, Y. Choi, N. Lue, Z. Yaqoob, P. So, Opt. Expr. **23**, 19752 (2015).
[8] P. de Groot, in *Optical Measurement of Surface Topography*, edited by R. Leach (Springer, Berlin, Heidelberg, 2011), pp. 167–186.
[9] M. Wojtkowski, A. Kowalczyk, R. Leitgeb, A.F. Fercher, Opt. Lett. **27**, 1415 (2002).
[10] Q. Zhang, S. Zhong, J. Lin, W. Chen, M. Luo, J. Zhong, Y. Yu, Z. Peng, S. Cheng, Opt. Lasers Engin. **124**, 105794 (2020).
[11] R. Juarez-Salazar, C. Robledo-Sanchez, F. Guerrero-Sanchez, A. Rangel-Huerta, Opt. Express **22**, 4738 (2014).
[12] R. Juarez-Salazar, C. Robledo-Sánchez, C. Meneses-Fabian, F. Guerrero-Sánchez, L. M. Arévalo Aguilar, Opt. Lasers Engin. **51**, 626 (2013).
[13] M.A. Herráez, D.R. Burton, M.J. Lalor, M.A. Gdeisat, Appl. Opt. **41**, 7437 (2002).
[14] Z. Wang, A.C. Bovik, IEEE Signal Proc. Lett. **9**, 81 (2002).