Supplementary Material

Label-free biochemical quantitative phase imaging with mid-infrared photothermal effect: supplementary material

MIU TAMAMITSU, KEIICHIRO TODA, HIROYUKI SHIMADA, TAKAAKI HONDA, MASAHARU TAKARADA, KOHKI OKABE, YU NAGASHIMA, RYOICHI HORISAKI, AND TAKURO IDEGUCHI

1Department of Physics, The University of Tokyo, Tokyo 113-0033, Japan
2Institute for Photon Science and Technology, The University of Tokyo, Tokyo 113-0033, Japan
3Department of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan
4Department of Neurology, The University of Tokyo, Tokyo 113-0033, Japan
5Graduate School of Information Science and Technology, Osaka University, Osaka 565-0871, Japan
6PRESTO, Japan Science and Technology Agency, Saitama 332-0012, Japan

*Corresponding author: ideguchi@ipst.s.u-tokyo.ac.jp

Published 20 April 2020

This document provides supplementary information to “Label-free biochemical quantitative phase imaging with mid-infrared photothermal effect,” https://doi.org/10.1364/OPTICA.390186. First, we describe the materials used to construct the systems and perform the experiments reported in the main text. Next, we give detailed explanations on the electrical and optical implantations of our MV-QPI method as well as the computational procedures used to retrieve QPIs in DH and ODT. Next, we describe the computational pipeline that we use for retrieving the MV-spectroscopic contrasts in our MV-QPI method. Next, we show the image-processing procedure used for MV-DH method. Finally, we give experimental results that verifies the bond-selective 3D imaging capability of MV-ODT method.

1. Materials and Methods

Light sources
The VIS light source is the second harmonic generation (SHG) of 10 ns, 1 kHz, 1,064 nm pulsed laser beam emitted from the Q-switch laser NL204-1K (Ekspla) produced with the nonlinear crystal LBO-503 (Eksma Optics). The spatial mode of the SHG beam is cleaned by the single-mode optical fiber P3-405B-FC-5 (Thorlabs). The MIR light source is DO418 (Hedgehog, Daylight Solutions) providing access to 1,450 - 1,640 cm⁻¹ region with the pulse duration of ~1 μs.

Measurement of the MIR pulse-energy spectrum
The intensity spectrum of the MIR light source is generally not uniform; hence, the raw photothermal (i.e., MIR ON-OFF) contrast needs to be normalized by the corresponding MIR pulse energy in order to obtain the sample-specific spectroscopic information (see Supplementary Materials for more detail). We use a cadmium-mercury telluride detector PVM-10.6-1x1-T08 (VIGO System) to measure the waveform of the MIR pulse at each wavenumber with an oscilloscope TDS2024C (Tektronix), and use the area of the obtained waveform as a measure of the pulse energy. This measurement is performed independent of the image acquisition.

MV-DH system
The complete description of the optical system is provided in Supplementary Materials. We use the following components to construct our DH microscope: CFC-2X-A (Thorlabs) for collimating the VIS laser beam, IX73 (Olympus) for the microscope housing, LUCPLFLN40X (Olympus) for the objective lens with the NA 0.6, acA2440-75um (Basler) for the image sensor, 66-350 (Edmund Optics) for the diffraction grating with 145 line-pairs (lp)/mm, 36-390 (Edmund Optics) for the pinhole with the diameter of 25 μm, AC508-180-A (Thorlabs) for the two lenses with the focal length of 180 mm used in the 4f system and LA5315 (Thorlabs) for the CaF₂ lens with the focal length of 20 mm used to loosely focus the MIR
beam to the sample plane. The image sensor is operated at 100 Hz with the exposure time of 9 ms. The MIR ON-OFF modulation rate is 50 Hz. The VIS illumination power at the sample plane is ~100 μW which is enough to use the full dynamic range of the image sensor. The MIR pulse energy at the sample plane is ~100 nJ on average but depends on the wavenumber (e.g., ~200 nJ at 1,548 cm⁻¹).

**MV-ODT system**

The complete description of the optical system is provided in Supplementary Materials. We use the following components to construct our ODT microscope: 5007-190 (Beck Optronics Solutions) for the illumination reflective objective lens with the NA 0.65, LCPLFLN100x0.5 (Olympus) for the collection objective lens with the NA 0.85, PS810-A (Thorlabs) for the rotating wedge prism, MirrIR (Kevery Technologies) for the MIR VIS dichroic mirror and acA2440-75um (Basler) for the image sensor. The image sensor is operated at 60 Hz with the exposure time of 1.5 ms. The MIR ON-OFF modulation rate is 30 Hz. The VIS illumination power at the sample plane is ~1 μW which is enough to use the full dynamic range of the image sensor. The MIR pulse energy at the sample plane is ~100 nJ on average but depends on the wavenumber (e.g., ~200 nJ at 1,548 cm⁻¹). The results shown in Fig. 6 of the main text are based on the raw tomographic reconstructions without applying any regularization [1] along the computational ODT reconstruction procedure.

**Materials used to characterize the basic performance of the MV-ODT system**

Series A 1.54000 (Cargille) is used as the liquid oil sample. FT/IR-6800, ATR PRO ONE and PKS-D1F (JASCO) are used to obtain the reference MIR absorption spectrum of the oil.

**Biological samples**

The COS7 cells (Riken) are cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% fetal bovine serum supplemented with penicillin–streptomycin, L-glutamine, sodium pyruvate and nonessential amino acids at 37 °C in 5% CO₂. For the live-cell imaging, the cells are cultured in 35-mm glass-bottomed dishes (AGC Techno Glass) and the medium is replaced by phenol red-free culture medium containing HEPES buffer (2 mL) before imaging. All solutions are from Thermo Fisher Scientific. The HEK293 cells are fixed with 4% paraformaldehyde at room temperature for 5 minutes and immersed in D₂O-based PBS before observation. All the samples are sandwiched between two CaF₂ substrates of 500 μm thickness before observation.

2. **Electrical synchronization of the MV-QPI systems**

The complete description of the electrical synchronization performed in our MV-QPI systems is presented in Fig. S1.

3. **Description of the MV-DH system**

The complete optical implementation of our MV-DH system is presented in Fig. S2. The DH reconstruction procedure is summarized in Fig. S3. First, the raw interferogram [Fig. S3(a)] is Fourier transformed to obtain the spatial-frequency spectrum [Fig. S3(b)]. In this spectrum, the interferometric term and its conjugate are shifted from the center coordinate by the direction and distance defined by the off-axis wavevector of the interferometer. The interferometric term is bandwidth-limited due to the limited NA of the objective lens (0.6 in this case), such that the circular aperture appears in the spectrum as represented by the red circle in Fig. S3(b). This aperture is cropped [Fig. 3(c)] and then inverse Fourier transformed so that the complex field can be recovered. Note that the instrumental background (consisting of the electric-field distributions of the reference and the object waves and the aberration of the interferometer) is independently measured without the sample [Fig. S3(e)] and subtracted from the raw phase reconstruction with the sample [Fig. S3(d)], such that the cell-specific QPI can be finally obtained [Fig. S3(f)].

4. **Description of the MV-ODT system**

The complete optical implementation of our MV-ODT system is presented in Fig. S4. The ODT reconstruction procedure is summarized in Fig. S5. Our ODT reconstruction algorithm is based on the coherent ODT with the Rytov approximation [2]. In this theoretical framework, the 3D scattering potential (which is directly related to the 3D RI distribution) is computationally synthesized based on the multi-angle complex-field measurements. Under the Rytov approximation, the scattered field (i.e., the perturbation term introduced to the plane-wave illumination wavefront by the scattering potential) is estimated by taking the complex logarithm of the phase-unwrapped complex-field measured at the image sensor plane by the principle of, e.g., DH. Then, this scattered field is Fourier transformed to obtain the 2D spatial frequency spectrum as shown in Fig. S5(a). Note that the use of the tilted plane-wave illumination shifts the location of the bandwidth-limited NA of the collection objective lens in the spatial-frequency domain. The Fourier diffraction theorem [3] allows us to map each of the 2D spectra to the 3D frequency space when the illumination is monochromatic. This is because the monochromatic illumination constrains the magnitude of the spatial frequency content carried by the light to be the inverse of its wavelength. Mathematically, this is expressed as the relation: $k_\omega = k_x^2 + k_y^2 + k_z^2 (k_\omega = 2\pi n_m/\lambda)$, where $n_m$ is the RI of the surrounding medium, $\lambda$ the wavelength of the probe light, and $k_x$, $k_y$, and $k_z$ the vector components of the spatial frequency information carried by the light. Essentially, this equation defines a spherical surface in the 3D spatial-frequency space, called the Ewald’s sphere, whose lateral (i.e., $k_x^2 + k_y^2$) extent is limited by the NA of the collection objective lens. Intuitively, the 3D position of the sphere is shifted by the wavevector of the tilted illumination, which can be predicted with the Fourier diffraction theorem. As a result of such mapping of all the 2D spectra, the 3D frequency spectrum of the scattering potential is synthesized as shown in Fig. S5(b). The inverse Fourier transform of this 3D spectrum gives the scattering potential in real space, which can then be converted into the 3D RI distribution as shown in Fig. S5(c).
**Fig. S1.** Complete description of the electrical synchronization of the MV-QPI systems.

**Fig. S2.** Complete description of the MV-DH optical system.

**Fig. S3.** DH reconstruction procedure. (a) Interferogram measured by the image sensor. (b) Spatial-frequency spectrum obtained by Fourier transforming (a). (c) Interferometric term cropped from (b), which is bandwidth-limited due to the limited NA of the objective lens. (d) Raw phase reconstruction with the cell in the FOV obtained by inverse Fourier transforming (c). (e) Raw phase reconstruction without the cell in the FOV, representing the instrumental background consisting of the electric-field distributions of the reference and the object waves and the aberration of the interferometer. (f) Cell-specific phase reconstruction obtained by subtracting (e) from (d).
5. Computational pipeline in MV-QPI

The computational pipeline of MV-QPI is summarized in Fig. S6. For each MIR wavenumber, we acquire several MIR ON and OFF holograms (i.e., interferograms) over certain period of time. These holograms are first averaged in time and then computationally processed, either through DH or ODT reconstruction algorithm, to produce the time-averaged MIR ON and OFF QPI reconstructions (i.e., phase images or RI tomograms, respectively). The MIR OFF reconstruction is subtracted from the MIR ON reconstruction to obtain the time-averaged photothermal QPI, which reflects information about the MIR absorption property of the sample at each wavenumber. Since the MIR pulse energy is generally not uniform throughout the scanned spectral range, its spectrum is independently measured. Then, the raw time-averaged photothermal QPIs are divided in the spectral-element-wise by the MIR pulse-energy spectrum, such that the non-uniform spectral signature of the MIR light source is removed. The resulting spectrally-normalized, time-averaged photothermal QPIs can be considered to reflect the sample-specific MIR absorption information.

6. Image processing procedure in MV-DH

We describe the image processing procedure used in MV-DH, which mainly consists of two steps, i.e., subtraction of the wavenumber-specific photothermal signal and spatial normalization of the photothermal signal.

The raw photothermal contrast obtained by the MV-DH system is contaminated by the MIR photothermal signals originating from the out-of-focus aqueous layers. This effect is visualized in the raw photothermal contrasts of the water-only [Fig. S7(a)] and cell-in-water [Fig. S7(b)] samples. In Fig. S7(a), it is seen that the water
itself shows photothermal contrast reflecting the local fluence of the MIR excitation beam as well as the water's characteristic spectral feature. Therefore, in Fig. S7(b), the raw photothermal contrast of the cell in water is contaminated by the spatial and spectral artifacts due to the water's MIR absorption. To suppress these artifacts, we can, e.g., simply subtract the water-only contrast from the cell-in-water contrast, as shown in Fig. S7(c). Although this procedure does not necessarily provide the true MIR absorption information of the cell, it can remove the background spatial artifact while providing the enhanced spectral contrast. In our case, the spectral signature of the amide II band is more clearly resolved.

The obtained image, however, contains a systematic spatial variation of the photothermal signal due to the MIR optical fluence at the sample plane being non-uniform, which is normalized in the next step. This procedure is based on the assumption that the photothermal signal is linear to the MIR pulse energy; see ref[4] and Fig. 3(a) in the main text to confirm this relation. The MIR optical fluence is estimated by Gaussian-filtering the water's photothermal contrast to remove high-spatial-frequency noise and normalizing it between 0 and 1. The resulting image represents the relative MIR fluence at each spatial point of the FOV. The water-subtracted photothermal contrast of the cell [Fig. S7(c)] is then divided by this relative MIR fluence map to obtain the spatially-normalized contrast, as shown in Fig. S7(d).

Fig. S6. Computational pipeline in MV-QPI.

Fig. S7. Image processing procedure in MV-DH. The photothermal contrast and the MIR spectrum at the spatial location indicated by the arrow are shown for (a) the water-only sample, (b) the cell-in-water sample and (c) subtraction of (a) from (b). (d) Spatially-normalized and water-subtracted photothermal contrast. The image is obtained by dividing (c) by the MIR optical fluence. The MIR fluence is estimated by Gaussian-filtering and normalizing (a) between 0 and 1.

7. Bond-selectivity of MV-ODT
We verify the bond-selective 3D imaging capability of our MV-ODT system using a mixture of porous silica [43-00-503, Sicastar (micromod Partikeltechnologies GmBH)] and poly(methyl methacrylate) (PMMA) [MMA5000 (Phosphorex, Inc)] microbeads immersed in index-matching oil [Series A 1.54000 (Cargille)]. We average 480 MIR ON-OFF measurements to obtain the photothermal tomogram. Figure S8(a) shows the R1 tomogram of the sample at the MIR OFF state with 50 iterations of the non-
positivity constraint [1]. Figure S8(b) shows the photothermal tomogram obtained by irradiating MIR pulses of 2.5 μs at 1,045 cm⁻¹ provided by another QCL [QD9500CM1 (Throlabs)] which is resonant to O-Si-O bond of silica. Indeed, only the porous silica bead shows the decrease in the RI value which is localized in 3D. In Fig. S8(c), we show the differential tomogram between the MIR OFF and OFF states without applying any regularization. The tomogram shows no contrast of either bead, which suggests that the RI change observed in Fig. S8(b) is associated with the MIR irradiation and that the sample’s mechanical drift is sufficiently suppressed such that no relevant noise appears.

Fig. S8. Bond-selectivity of MV-ODT verified with the microbeads mixture sample. (a) RI tomogram of the PMMA and the porous silica beads in the index matching oil (RI = 1.545). (b) Photothermal tomogram where only the silica bead shows the contrast. (c) Difference between two MIR OFF tomograms, where no contrast of either bead is visible.

REFERENCES
1. J. Lim, K. Lee, K. H. Jin, S. Shin, S. Lee, Y. Park, and J. C. Ye, “Comparative study of iterative reconstruction algorithms for missing cone problems in optical diffraction tomography,” Opt. Express 23, 16933-16948 (2015).
2. Y. Sung, W. Choi, C. Fang-Yen, K. Badizadegan, R. R. Dasari, and M. S. Feld, “Optical diffraction tomography for high resolution live cell imaging,” Opt. Express 17, 266-277 (2009).
3. E. Wolf, “Three-dimensional structure determination of semi-transparent objects from holographic data,” Opt. Commun. 1, 153–156 (1969).
4. M. Tamamitsu, K. Toda, R. Horisaki, and T. Ideguchi, “Quantitative phase imaging with molecular vibrational sensitivity,” Opt. Lett. 44, 3729–3732 (2019).