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研究課題名（和文）Full-field holographic optical coherence tomography combined with Raman spectroscopy for in vivo microscopic imaging of biological tissue

研究課題名（英文）Full-field holographic optical coherence tomography combined with Raman spectroscopy for in vivo microscopic imaging of biological tissue

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研究成果の概要（和文）：生物組織の構体と分子を測定できるように新しい非標識顕微鏡を作る。構体計測は光干渉断層像の技術を使い、スキャニングを使わずにフルフィールドを測定する。この顕微鏡は１ミクロメートルの等方的な分解能を果たすために、白いスパーコンティユームで１００ナノメートルの帯域幅を使う。
システムの分光パーツは通常の赤外ラマンのスキャニング技術を使う。この方法は遅い測定しても、ここでは光干渉断層像を使い、関心領域のみ測定すれば、速く試料の分光情報を読み出せる。システムの履行を検証するために、組織ファントムを作り、ゼラチンの中にポリスチレン玉を入れ、顕微鏡の分解能を確かめられ、構体も（肌や筋肉）測定できると確認した。

研究成果の概要（英文）：We developed a system enabling the measurement of both structural and molecular information on tissue purely based on label-free technologies. The structural information is retrieved through optical coherence tomography, developed here in a full-field configuration, which enables parallel detection without requiring scanning. This system employs a wide-band excitation source based on a white-light supercontinuum that provides over 100 nm of bandwidth to reach isotropic micrometer resolution.
The molecular modality is based on a standard point-measurement Raman scanning system with a near-infrared excitation. While this approach typically requires long exposure times, it is here possible to employ the information retrieved from full-field OCT to specifically target regions of interest to rapidly retrieve the molecular information.
The system was characterized with phantoms containing plastic beads and ex vivo tissue.

研究分野：　総合理工

キーワード：　Optical measurement　Raman　Coherent imaging　3D imaging　Label-free　Tissue imaging

１．研究開始当初の背景

(1) There has been in the past decades great development of optical methods that enable the observation of biological specimens *in vivo*. However most of them, such as for instance fluorescence and two-photon microscopy, require extensive sample preparations that are not always compatible with the safety and simplicity required for *in vivo* measurements.

(2) On the other hand, several label-free techniques have been developed for observing tissue samples. The most dominant one is optical coherence tomography (OCT), which makes it possible to retrieve the in-depth information from the signal back-scattered from internal structures in tissue based on the depth discrimination provided by coherence gating. While this approach provides valuable structural information, it lacks molecular specificity to allow the study of specific biological phenomena or the development of functional imaging.

(3) Other methods have shown strong capabilities to extract the molecular information from samples, such as Raman spectroscopy, which can non-invasively measure the vibrational spectrum of biological molecules, giving an insight into the local molecular content of the sample. The measurements with this modality are however inherently slow due to the low Raman cross-section of biomolecules, making it difficult to retrieve molecular images of tissue.

２．研究の目的

(1) The purpose is to develop a multimodal system that can combine structural measurements from OCT and molecular information from Raman spectroscopy. This approach can enable the use of the structural data to specifically target locations of interest with the Raman channel, effectively reducing its drawback of slow acquisition by only measuring the required locations for probing the sample.

(2) The aim is also to develop the OCT system based on a full-field configuration, which does not require spatial scanning as in standard OCT setups where the acquisition is performed through raster scanning in the x-y plane. The approach here is to use a two-dimensional detector to recover the coherent back-scattered signal in parallel from one plane in the sample, based on techniques derived from digital holography that enables one-shot acquisition of the complex field. The three-dimensional acquisition is then performed with a one-dimensional scan in the z direction to acquire the signal from different depths within the sample.

(3) Finally, the project also involves the study of various techniques to reduce the amount of required data in order to form an image. This approach could then enable the measurement of Raman molecular images, based on a significantly reduced amount of data points, making the image formation possible even in the case of the long acquisition times involved in Raman measurements performed in the near-infrared region.

３．研究の方法

(1) The first part of the project involves the design of a wide-band coherent source that can be employed for OCT measurements. Typical sources involve for instance superluminescent diodes, which can provide bandwidths in the order of tens of nanometers. The aim here is to use a larger bandwidth that can provide depth resolution under 10 micrometers. To this end, a white light supercontinuum system is built, that generates coherent light in a very wide range spanning the visible and infrared regions. A suitable band can then be selected through spectral filtering.

(2) In a second stage, this source is injected in a reflection microscope designed to be used for full-field OCT. The large bandwidth employed here for interferometry involves different challenges that are usually not present for standard measurements, such as the very low coherence length which creates vignetting in the field of view. It is also required to accurately compensate the dispersion between the two interferometer arms as well as the dispersion induced by the sample, since the interference from different wavelengths may not additively contribute to the signal without proper compensation.

(3) Based on a working OCT system, the Raman part of the setup can then be added to the microscope by combining the excitation beam of Raman in the object arm of the system. The acquisition can then be independently performed from the OCT channel by employing a switching mirror.

(4) To make the measurements easier to acquire and process, a software interface is also created, in particular to provide fast reconstruction of the hologram stack, which can take several minutes to be reconstructed with standard software. A dedicated code relying on graphics card

acceleration is developed in order to perform close to live reconstruction that enables fast assessment of the sample measurement.

4．研究成果

(1) The white light supercontinuum source has been designed based on a photonic crystal fiber with a zero-dispersion region situated at 780 nm, and pumped with a femto-second laser tuned slightly above the design wavelength of the non-linear fiber. This provides a continuous spectrum of coherent light in the visible and infrared range (typically 400-1200 nm). This large emission makes it possible to then select spectral regions to be employed in the OCT system through the use of bandpass filters. A typical bandwidth of 120 nm was selected, providing around 15-20 mW of optical power injected in the microscope system. Two main ranges were selected; the first one used a central wavelength of 695 nm (red), which provided a convenient visible light for alignment and early assessment. In the second stage of the project, a central wavelength of 875 nm (near-IR) was employed to increase the penetration depth of the measurements.

(2) The full-field OCT system was designed as a Mach-Zehnder interferometer in reflection, which also contains a scanning stage with micrometer precision in the reference arm to enable the retrieval of the back-scattered signal at precise depths for 3D acquisitions. The full-field approach employs an off-axis holographic configuration that enables the recovery of the complex field with one hologram, effectively providing fast acquisition. The vignetting of the field of view induced by the short coherence length of the source is solved by employing a grating to match the propagation planes of both the reference and object arm for direct detection of the full field of view. The dispersion is compensated by inserting a suitable amount of highly-dispersive glass into the reference arm, in order to compensate for the additional components present in the object arm, as well as the sample dispersion.

(3) The system employs a 10x microscope objective, which provides a lateral resolution of 1.5 micron in air. In comparison, the large bandwidth of the source achieves a depth resolution of approximately 2.8 microns in air, effectively reaching close to isotropic resolution in 3D.

(4) As 3D acquisitions imply the reconstruction of a large amount of holograms (typically 100-1000 images per stack), a new reconstruction software was developed to reach close to live reconstructions (approximately 50 holograms per second), even with this large amount of data. Significant speed improvements were achieved by developing a code based on multi-threading and using the heavy parallelization recently provided by graphics cards, as shown in Table 1.

Type	Reconstruction speed [ms]
CPU (1 thread)	136
CPU (4 threads)	85
GPU	19

Table 1: Reconstruction speed of holograms (1024x1024) depending on hardware settings. Tests are averages of 50 reconstruction. CPU: Intel Xeon E31240v3, GPU: Nvidia GeForce GTX960.

(5) The Raman system was developed based on an excitation with a near-infrared diode, and an acquisition relying on a cooled highly sensitive CCD. The system is also employing a 2D scanner that enables the targeting of specific locations within the field of view, and the measurement regions between the OCT and Raman channels were adjusted to be identical. While the x-y position can be selected by properly choosing the voltages of the scanner, the focus in depth can be adjusted with a translation stage placed on the microscope objective. Conversely, the focus in OCT is not strongly affected by the movement of the objective, and can also be adjusted through digital focusing, a capability provided by the acquisition of the complete complex field, so that the focus location can be computed off-line after acquisition.

(6) The performance of the OCT system was first tested with a glass-water interface, providing a refractive index difference comparable to some biological structures, showing a typical sensitivity of 70 dB, and confirming a practical resolution in depth of 3-4 microns. The overall performance was then assessed with phantoms made of gelatin with plastic beads embedded, showing the capability of the system to retrieve the 3D structure of the sample (see Figure 1). Furthermore, the capability of the Raman channel to retrieve molecular information in 3D was confirmed by separating PS and PMMA beads embedded in the gel based on their chemical signature. Finally, the

system was also tested with scattering samples such as onion and *ex vivo* muscle and skin tissue, where typical penetration depths in the order of 1 mm and the ability of retrieving spectral data from biological samples was confirmed.

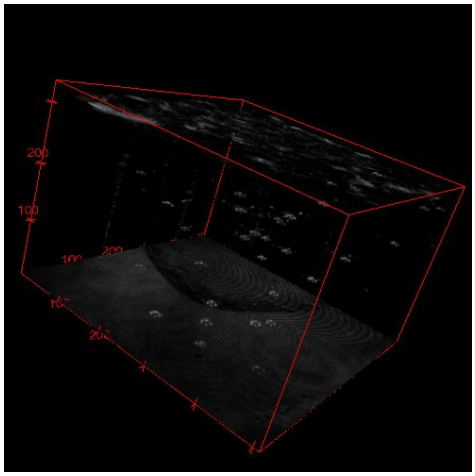

Figure 1: 3D stack of polystyrene beads embedded in gelatin in a 1 mm^3 measurement region.

(7) Finally, an approach to improve the speed of measurements of the Raman channel to enable imaging was developed based on compressed sensing. This approach makes it possible to retrieve an image with much fewer samples than usually required in standard imaging, by relying on specific excitation patterns and a reconstruction algorithm which constrains the continuity of the image. However, this approach is usually not suitable for high local power imaging approaches such as Raman spectroscopy. By adjusting this method to make it usable for standard laser-scanning imaging methods, it was possible to reduce the amount of required samples to acquire an image by a factor 10, making it possible to acquire images within reasonable time frames even in the case of the long acquisition times required for near-infrared excitations. Furthermore, it was shown that this approach, which involves a denoising step based on the assumption of local continuity in the sample, can also improve the signal-to-noise ratio of the retrieved spectra.

５．主な発表論文等

〔雑誌論文〕（計１件）

①Nicolas Pavillon, Nicholas I. Smith, “Compressed sensing laser scanning microscopy,” Optics Express, Vol. 24, No. 26, 2016, pp. 30038-30052. DOI: 10.1364/OE.24.030038

〔学会発表〕（計３件）

① Nicolas Pavillon, Alison J. Hobro, Nicholas I. Smith, “Raman scattering, phase imaging, and nanoparticle-enhanced Raman analysis as methods to probe immune cells,” in Three-Dimensional and Multidimensional Microscopy: Image Acquisition and Processing XXIV, SPIE BiOS, San Francisco CA, USA, 2017 Feb. 2nd, (invited).

② Nicolas Pavillon, Nicholas I. Smith, “Observation of the immune response of cells and tissue through multimodal label-free microscopy,” in Biophotonics and Immune Responses XII, SPIE BiOS, San Francisco CA, USA, 2017 Jan. 31st.

③ Nicolas Pavillon, Nicholas I. Smith, “Full-field optical coherence tomography through digital holography”, in 77th Autumn JSAP conference, Toki Messe, Niigata city, Niigata, Japan, 2016 Sep. 15th.

〔図書〕（計０件）

〔産業財産権〕

○出願状況（計０件）

○取得状況（計０件）

〔その他〕

ホームページ

http://biophotonics.ifrec.osaka-u.ac.jp/

６．研究組織

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None

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None

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None