Optical time-stretch confocal microscopy at 1 μm

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Received May 7, 2012; accepted June 10, 2012;
posted June 18, 2012 (Doc. ID 167968); published August 6, 2012

We demonstrate optical time-stretch confocal microscopy in the 1 μm spectral window for high-speed and high-resolution cellular imaging. In contrast to the prior demonstrations of time-stretch imaging, which all operated in the telecommunication band, the present work extends the utility of this imaging modality to a wavelength regime (~1 μm), which is well known to be the optimal diagnostic window in biophotonics. This imaging technique enables us to image the nasopharyngeal epithelial cells with cellular resolution (<2 μm), at a line scan rate of 10 MHz, and with a field of view as wide as ~0.44 mm × 0.1 mm. We also theoretically and experimentally characterized the system performance. As the low-loss dispersive fibers for the time-stretch process as well as other essential optical components for enhancing the imaging sensitivity are commonly available at 1 μm, time-stretch confocal microscopy in this wavelength range could usher in realizing high-speed cell imaging with an unprecedented throughput.

OCIS codes: 170.3880, 170.7160, 180.1790.

Enabling high-speed and high-throughput optical microscopy is well recognized to be appealing and yet challenging in biomedical diagnostic practice. Higher imaging speed is typically hampered by the technological limitations of the image sensors, namely the CCD and complementary metal-oxide semiconductor (CMOS) [1]. This constraint can clearly be exemplified in that the measurement throughput of the recent advanced imaging flow cytometer (~1000 cells/s), which is equipped with such image sensors, is still far below that which can be achieved in the conventional nonimaging flow cytometers (>100,000 cells/s)—a versatile diagnostic tool routinely used for high-throughput multiparameter analysis in hematology and immunophenotyping [2,3]. In this regard, serial time-encoded amplified microscopy (STEAM) has recently been demonstrated as a new optical imaging modality, without the use of CCDs/CMOSs, which can deliver a record high frame rate (>1 MHz) and sensitivity [1,4]. The essential principle behind STEAM is to map the spatial information of an image into a serial time-domain optical waveform that is stretched in time by group velocity dispersion (GVD), and optically amplified simultaneously. The waveform is then captured with a real-time electronic digitizer for subsequent digital image reconstruction.

Primarily because of the wide availability of the low-loss dispersive fibers—the key element for the time-stretch process—in the telecommunication band (~1550 nm), the prior works on time-stretch imaging were all demonstrated in this wavelength band [1,4–7]. However, its potential use in a more favorable spectral window for biophotonic applications (~1 μm) is yet to be explored so far. In this Letter, we demonstrate, to the best of our knowledge, for the first time *optical time-stretch confocal microscopy* operated in the 1 μm wavelength regime for single-shot high-speed (10 MHz), and high-resolution (<2 μm) bioimaging. Translating the operation wavelength to 1 μm, the present work shows the feasibility of further expanding the time-stretch imaging technology to a wider variety of biophotonic applications. We here show that the time-stretch process can be made possible with sufficiently high GVD (~0.15 ns/nm) by the standard single-mode fiber (SMF) working at ~1 μm—permitting efficient mapping of the information between space and time. Time-stretch imaging at shorter wavelengths can also enjoy the higher diffraction-limited resolution, as in the conventional optical imaging modalities [8].

Detailed descriptions of the time-stretch imaging approach can be referred to elsewhere [1,9]. In brief, the spatial coordinates of the specimen are first encoded in the wavelength spectrum of a broadband pulse with a one-dimensional (1D) (for line scan mode [4,6,7]) or two-dimensional (2D) [1] "spectral shower" created by a spatial disperser, e.g., diffraction grating. This is a process called spectral encoding [10]. The pulse is then time-stretched by GVD so that the image-encoded spectrum is mapped into the serial temporal waveform, which is finally captured by the electronic digitizer. This time-stretch process is also coined as dispersive Fourier transform [11]. In our system (Fig. 1), the broadband source is a supercontinuum (SC) generated from a photonic crystal

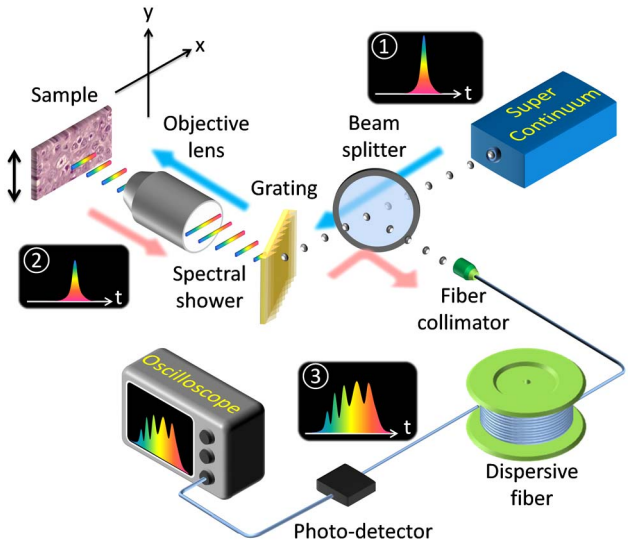

Fig. 1. (Color online) Schematic of the time-stretch confocal microscope. Box 1: Supercontinuum (SC) pulse. Box 2: Spectrally encoded SC pulse. Box 3: Encoded and time-stretched SC pulse.

fiber pumped by a mode-locked laser (center wavelength = 1064 nm, pulse width = 9 ps). The SC pulses are then amplified by an ytterbium-doped fiber amplifier (YDFA), which results in a usable bandwidth of ~60 nm (1060–1120 nm) for time-stretch imaging. The 1D spectral shower is generated by a transmission diffraction grating (1200 lines/mm) and is focused onto the specimen by an objective lens (NA = 0.66). The number of resolvable point is ~400. The backscattered spectrally encoded pulse is then time-stretched using a 5 km SMF (Nufern 1060XP), which has a total GVD as high as ~0.15 ns/nm and a reasonably low loss at 1 μm (<1 dB/km). Finally, the serial data stream is captured by a high-speed photodetector (10 GHz) and a real-time oscilloscope (16 GHz, 80 GS/s). We will show later that the current GVD is sufficient to obtain high-resolution time-stretch images. The 2D images are obtained by line-scanning in the orthogonal direction (y direction). The time-stretch microscope is essentially a confocal imaging system because the aperture of the fiber acts as the pinhole for signal collection. This 1D line scan mode is particularly beneficial for high-speed imaging flow cytometry, in which the unidirectional cell flow automatically collects all the 1D line scans of individual cells without any beam scanning of the spectral shower [6,7].

Because of the two-stage mapping (i.e., space-to-wavelength and then wavelength-to-time), the final image resolution of time-stretch imaging is governed by all the parameters involved in these mappings, rather than solely by the diffraction limit [9]. In general, the resolution in time-stretch imaging can be evaluated by three limiting regimes, each of which is dominated by (i) the spectral resolution of the diffraction grating (spatial-dispersion limited), (ii) the spectral resolution defined by stationary-phase approximation (SPA) in the time-stretch process (SPA-limited), or (iii) the temporal resolution of the digitizer (digitizer-limited) [9]. The spatial resolution of the current time-stretch microscope under these limiting cases is shown in Fig. 2. As expected, the highest resolution, i.e., spatial-dispersion-limited resolution (~1.5 μm), is only achievable for high enough GVD, i.e., $> \sim 0.2$ ns/nm. Having the GVD of 0.15 ns/nm, our current system achieves the resolution of ~1.8 μm (Fig. 2), which approaches to the spatial-dispersion-limited resolution. Because of the low fiber loss (<1 dB/km), we can afford to use a longer fiber to further enhance the GVD, and thus to achieve the spatial-dispersion-limited resolution. More importantly, the intrinsic dispersive loss can be circumvented by optically amplifying the time-stretched signal either using distributed Raman amplification in the same fiber [1,4–6,9,11,12] or the discrete optical amplifiers, which are commonly available in the 1 μm range (e.g., YDFA, and semiconductor optical amplifiers).

The wavelength-to-time mapping was experimentally verified by capturing the spectrally encoded image and the time-stretch image of the smallest feature on a resolution target (group 7 of USAF-1951) (Fig. 3). Both images resolve well the smallest line feature (a linewidth of ~2 μm). By performing 200 line scans with a step size of 0.5 μm (along the y direction), the field of view is as large as ~0.44 mm × 0.1 mm. It should be emphasized

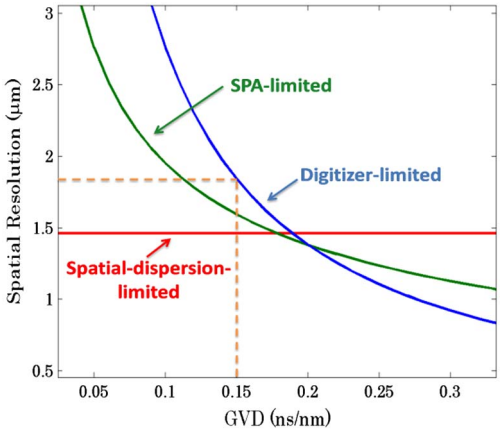

Fig. 2. (Color online) Calculated spatial resolution of the current time-stretch confocal microscope in three limiting cases [9]: spatial-dispersion-limited (red), SPA-limited (green), and digitizer-limited (blue), as a function of GVD. The dashed line (orange) locates the GVD of the fiber used in the system.

that the spectrally encoded image was captured by a spectrometer at a spectral acquisition rate of 5 Hz whereas the time-stretch image, with a comparable image quality, is captured at a dramatically higher spectral acquisition rate (i.e., line scan rate) of 10 MHz, determined by the repetition rate of the SC source—unambiguously demonstrating the strength of time-stretch microscopy. We also note that the single-shot line scan (along the x direction) is obtained only within 9 ns, determined by the GVD of the fiber and the source bandwidth.

Further examining the line scans in both cases [Fig. 3(c)], we can observe the lower amplitude contrast in the time-stretch image than that in the spectrally

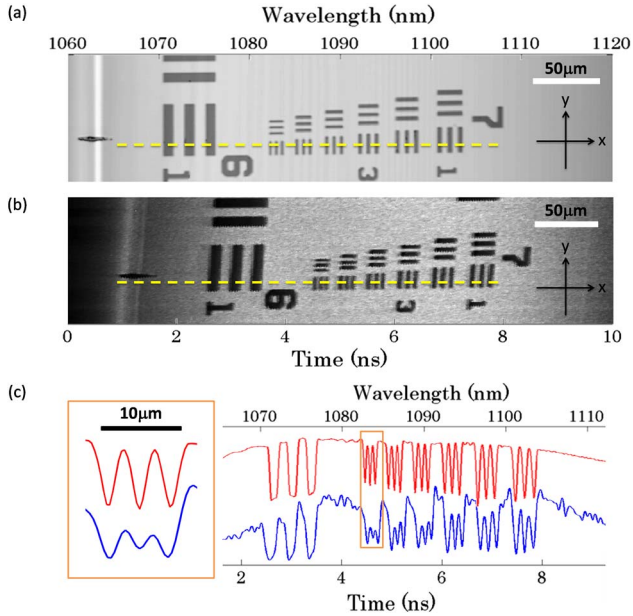

Fig. 3. (Color online) Comparison between (a) spectrally encoded image and (b) time-stretch image of the smallest group (Group 7) of a resolution target (USAF-1951). (c) Spectrally encoded scan (red) and time-stretch scan (blue), as indicated in (a) and (b), respectively. The zoom-in view of the smallest element is shown in the left inset.

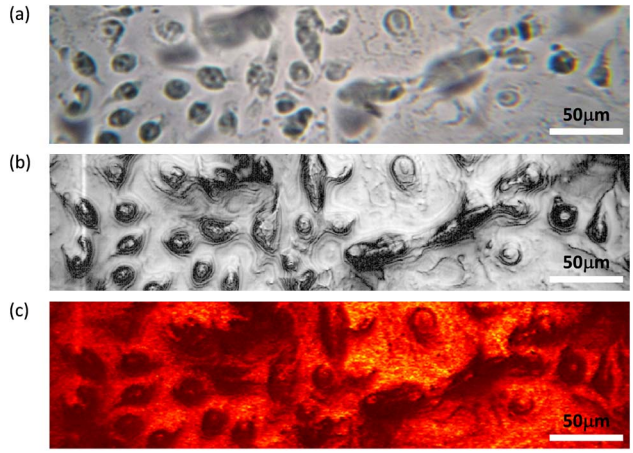

Fig. 4. (Color online) Images of the nasopharyngeal epithelial cells captured by (a) bright-field light microscopy, (b) spectrally encoded imaging, and (c) time-stretch microscopy.

encoded image [see the inset of Fig. 3(c)]. This contrast difference further verifies the theoretical prediction (Fig. 2); i.e., the final spatial resolution in time-stretch imaging is not merely determined by the spectral encoding process, but also the time-stretch processes.

We also demonstrate cellular imaging of the nasopharyngeal epithelial cells by time-stretch confocal microscopy [Fig. 4(c)]. The cell lines were treated with methanol and 6% acetic acid before fixation to increase the image contrast of the nuclei [13]. Figure 4(c) clearly demonstrates the capability of time-stretch confocal microscopy to reveal the cellular structures, such as the nuclei, with an ultrafast single-shot 1D line scan, each of which was captured within 9 ns. Such a high-speed operation is achieved without sacrificing the image quality significantly. This can be evident by comparing the time-stretch image with the spectrally encoded image [Fig. 4(b)] and the bright-field image [Fig. 4(a)] using the standard light microscope (NA = 0.65). This proof-of-principle demonstration validates the feasibility of employing time-stretch microscopy at the 1 μm spectral window for ultrafast bioimaging.

We remark the compatibility of the current time-stretch microscope with the ultrafast flow cell imaging applications in which two key metrics have to be considered: dwell time of the line scan (i.e., shutter speed) and temporal stability of the line scan. The dwell time has to be as short as possible in order to minimize the motion blur. It is particularly relevant to the case of high flow speed (e.g., >m/s in flow cytometers). In our system, the current dwell time is ~25 ps, which is determined by the bandwidth of each spectrally resolvable subpulse (60 nm/400) in a line scan. Given a flow speed of 1 m/s, such an ultrashort dwell time (orders of magnitude shorter than that of the typical CCD/CMOS sensors) corresponds to a flow displacement of ~25 pm, well below the diffraction-limited resolution. It is thus clear that the motion blur in time-stretch microscopy is negligible even at a very high flow speed. It is of great importance to ensure the spectral shower, and hence the SC source to maintain a good temporal stability as the line scan has to be performed continuously at high scan rate (10 MHz in our current system). To this end, we have recently demonstrated a simple triggering scheme to stabilize and enhance the SC pulses [12,14]. This is essential for real-time and high-throughput operation in time-stretch imaging.

To conclude, we have demonstrated for the first time cellular imaging by time-stretch confocal microscopy in the 1 μm wavelength regime. The advantages of operating time-stretch microscopy in this spectral window are threefold: (i) this wavelength range is favorable for a myriad of biophotonic applications, (ii) higher diffraction-limited resolution can be attained compared to the previous demonstrations in the telecommunication band, and (iii) the availability of the off-the-shelf low-loss SMF fibers, the high-power diode lasers, and the optical amplifiers at 1 μm—they are all the necessary elements for achieving high-speed and high-sensitivity time-stretch microscopy, i.e., STEAM. In addition, because of the commonly available near-infrared (NIR) fluorescent probes, operating time-stretching in NIR makes it possible to tap into a myriad of fluorescence imaging modalities. The present work could hold promise for realizing high-throughput cell imaging applications, such as flow cytometry.

The work in this Letter is partially supported by grants from the Research Grants Council of Hong Kong Special Administrative Region, China (HKU 7179/08E, HKU7183/09E, HKU 717510E, and HKU 717911E), and the University Development Fund of HKU. The authors also acknowledge Tony C. K. Chan for his assistance of the cell line preparation.

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