Cost-effective approaches for high-resolution bioimaging by time-stretched confocal microscopy at 1μm

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ABSTRACT

Optical imaging based on time-stretch process has recently been proven as a powerful tool for delivering ultra-high frame rate (> 1MHz) which is not achievable by the conventional image sensors. Together with the capability of optical image amplification for overcoming the trade-off between detection sensitivity and speed, this new imaging modality is particularly valuable in high-throughput biomedical diagnostic practice, e.g. imaging flow cytometry. The ultra-high frame rate in time-stretch imaging is attained by two key enabling elements: dispersive fiber providing the time-stretch process via group-velocity-dispersion (GVD), and electronic digitizer. It is well-known that many biophotonic applications favor the spectral window of ~1μm. However, reasonably high GVD (> 0.1 ns/nm) in this range can only be achieved by using specialty single-mode fiber (SMF) at 1μm. Moreover, the ultrafast detection has to rely on the state-of-the-art digitizer with significantly wide-bandwidth and high sampling rate (e.g. >10 GHz, >40 GS/s). These stringent requirements imply the prohibitively high-cost of the system and hinder its practical use in biomedical diagnostics. We here demonstrate two cost-effective approaches for realizing time-stretch confocal microscopy at 1μm: (i) using the standard telecommunication SMF (e.g. SMF28) to act as a few-mode fiber (FMF) at 1μm for the time-stretch process, and (ii) implementing the pixel super-resolution (SR) algorithm to restore the high-resolution (HR) image when using a lower-bandwidth digitizer. By using a FMF (with a GVD of ~ 0.15ns/nm) and a modified pixel-SR algorithm, we can achieve time-stretch confocal microscopy at 1μm with cellular resolution (~ 3μm) at a frame rate 1 MHz.

1. INTRODUCTION

In practical biomedical diagnostics, high-speed and high-throughput cellular imaging is recognized to be a crucial yet challenging task. Such predicament stems from the fact that high speed imaging is compromised by the processing speed as well as the detection sensitivity of the conventional image sensors, namely charge-coupled device (CCD) and complementary metal-oxide semiconductors (CMOS) [1]. To this end, serial time-encoded amplified microscopy (STEAM), or generally called optical time-stretch confocal microscopy, has recently been demonstrated as a new imaging modality. STEAM can provide the solution to the aforesaid technological challenge with an ultra-high frame rate (> MHz) (by the use of the optical time-stretch technique) and high sensitivity (by the optical amplification) [1-6]. So far, most of the prior works on the time-stretch imaging operated in the telecommunication band (~1550nm), which is not favorable for many biophotonic applications. In contrast, employing time-stretch imaging in the shorter near infrared (NIR) wavelength regime (~ 1μm), which is the well-known spectral window for biomedical applications, has not been well explored. In this regard, our group, has recently demonstrated this optical time-stretch confocal microscopy at 1μm for cellular imaging [7] – an invaluable tool for high-throughput biomedical applications, such as imaging flow cytometry [1,6].

There are two key enabling components in time-stretch imaging – the low-loss dispersive fiber at 1μm and the high-speed electronic digitizer. For the dispersive fiber, it is used for mapping the spatial information of the optical signal to the time-domain by GVD, named as the time-stretch process. Unlike the telecommunication band (~1550nm) in which a wide variety of low-loss and dispersive fibers are available e.g. SMF28, dispersion compensation fibers (DCFs), achieving high GVD with low loss in the shorter NIR range can only be made possible by the high-cost specialty fibers. [7]. On the other hand, in order to accommodate the ultrafast time-stretch imaging frame rate (at >MHz), it inevitably requires the state-of-the-art electronic digitizer with an ultra-high-bandwidth (>10 GHz) and sampling rate (>40 GS/s). It might hinder the widespread utility of time-stretch imaging in biomedical diagnostics, especially in the resource-limited

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environments. Therefore, it would render time-stretch imaging a more appealing high-throughput diagnostic platform if one could identify the cost-effective alternatives of the specialty 1μm specialty SMFs as well as the high-speed digitizer, without significantly compromising the imaging quality. In this regard, we here propose and demonstrate two practical ways for implementing a more cost-effective optical time-stretch microscopy at 1μm – (i) using standard telecommunication-band SMF to act as the FMF in the 1μm wavelength window for the time-stretch process, and (ii) using a pixel-SR algorithm to restore a HR image from multiple low-resolution (LR) images captured by a digitizer with a lower bandwidth.

2. WORKING PRINCIPLES

2.1. Optical time-stretch confocal microscopy

Optical time-stretch microscopy consists of two main parts. The first part is *spectral-encoding* [8] – the spatial information of the sample is spectrally encoded in the wavelength spectrum. The second part is *optical time-stretch*, also coined as *dispersive Fourier transform* [9] – the spectrally-encoded signal is time-stretched by a dispersive fiber such that the spectral information is mapped into time signal via GVD. After these two processes, the spatial information of the samples is mapped into time domain signal. It thus facilitates ultrafast real-time optical imaging by using a high-speed electronic digitizer, instead of the conventional camera, i.e. CCD or CMOS, and achieves an ultrafast line-scan rate > MHz [1-7,10], which is fundamentally not achievable with the conventional imaging modalities.

2.2. Optical time-stretch with FMFs

The main concern in *optical time-stretch* is to prevent ambiguity in the wavelength-to-time mapping. Therefore, multi-mode fibers should generally be avoided in the time-stretch process because the coexistence of modal dispersion and GVD leads to this ambiguity. FMF, however, has been proven to be effective for low-loss and long-distance data transmission in fiber-optic communication with negligible modal coupling [11,12] – a feature particularly favorable for realizing high-dispersion, and thus high-resolution time-stretch microscopy [13]. Therefore, it is possible that by exciting a specific FMF mode, such as the fundamental mode (LP_{01}) or the higher-order mode (LP_{11}), sufficiently large GVD for time-stretch microscopy with cellular resolution can be attained.

2.3. Pixel super-resolution algorithm

Pixel-SR algorithm becomes crucial when the imaging resolution is limited by the camera or the image sensor [14], i.e. when the camera under-samples the image. Hence, such algorithm is particularly well-suited for optical time-stretch microscopy when a high-bandwidth digitizer is not available. In this case, a low-bandwidth digitizer undersamples the time-stretch image signal, resulting in an LR image. Therefore, applying a pixel-SR algorithm on the LR time-stretch images can reconstruct the HR time-stretch images. Pixel-SR algorithm relies on capturing multiple LR images, each of which differs by a sub-pixel shift. The multiples different LR images can provide different information and hence, make HR image reconstruction possible. After acquiring the under-sampled images, three main digital processing steps are required, namely *registration*, *interpolation* and *restoration* [14]. *Registration* is to map the LR images to a HR pixel grid. *Interpolation* is to re-distribute the pixels in the HR pixel grid. *Restoration* refers to the deconvolution and denoising processes which retrieve the final HR images.

3. EXPERIMENTAL SETUP

The experimental setup of optical time-stretch microscopy is shown in Fig. 1. The supercontinuum (SC) laser source is first dispersed by a grating (1200 lines/mm). This creates a one-dimensional (1D) spectral shower. Then, the spectral shower is focused by the objective lens (NA = 0.66 or 0.4) and is illuminated on the sample. As a result, the spatial information of the sample is spectrally encoded in the SC spectrum. The time-stretch microscope operates in the transmission mode, which is in contrast to our previous report [7]. The spectrally encoded signal is then mapped to the time domain signal by GVD of the dispersive fiber. A photo-detector and a real-time oscilloscope (16 GHz, 80 GS/s) are used for acquiring the time signal. By scanning the sample in the orthogonal direction with respect to the spectral shower, a two-dimensional (2D) image can be acquired [2,4-7]. We emphasize that this 1D line-scan mode can readily be applied in high-speed flow cell imaging (at a MHz line-scan rate), in which the unidirectional cell flow automatically facilitates all the 1D line-scans of individual cells without beam scanning of the spectral shower [4,6,15]. This time-stretch

microscope is essentially a confocal microscope as the core of the SMF acts like a pin-hole for rejecting the out-of-focus light [5].

For implementing practical optical time-stretch confocal microscopy at 1μm, a standard SMF, which is commonly used in the telecommunication band (SMF28 or DCF), are employed as an FMF in 1μm, were directly spliced with a short 1μm SMF for exciting the LP_{01} mode. For exciting the LP_{11} mode, we introduced a small offset < 4 μm (shown on the right inset of Fig. (1)). By using the time-stretch technique, the GVD values of the LP_{01} modes in SMF28 and DCF are ~0.15 ns/nm and ~0.17 ns/nm respectively, while the 1μm specialty SMF has a GVD of ~0.15 ns/nm. On the other hand, for acquiring multiple LR time-stretch images, it is required to translate the sample along the spectral shower direction (i.e. along *x*-axis). In particular, we used 4 to 5 sub-pixel shifted LR images for reconstructing a HR image (details will be explained in the *results* section). The corresponding pixel-shift trajectory (for 5 sub-pixel shifts) is shown on the left inset of Fig. 1.

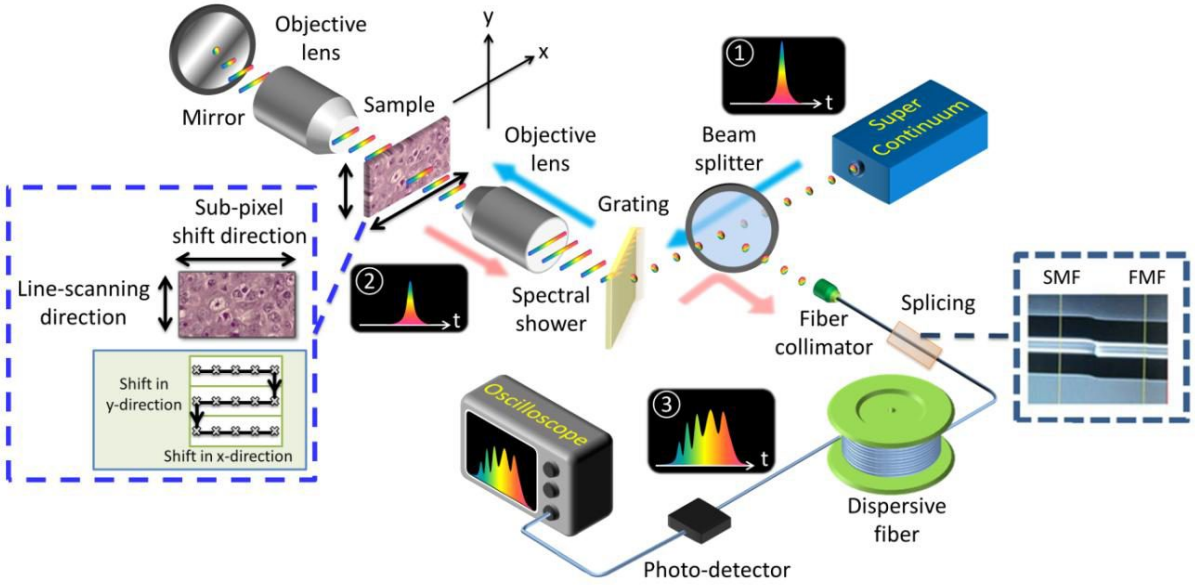

Fig. 1. Schematic of optical time-stretch confocal microscope. Two key parts for implementing cost-effective optical time-stretch confocal microscopy are shown on the left (sub-pixel shift for the pixel-SR algorithm) and right (employing a FMF for the optical time-stretch process. Note that higher-order FMF modes can also be excited, by introducing lateral offset at the fiber fusion facets between the SMF and the FMF).

4. RESULTS

4.1. FMF-based optical time-stretch confocal microscopy

We first performed time-stretch imaging of the smallest features of a resolution target (group 7 of USAF-1951) in order to evaluate the performance of the selected dispersive fibers (SMF28, DCF and 1μm specialty SMF). For comparison, we also captured the spectral encoded image by a conventional spectrometer (Fig. 2(a)). The captured time-stretch images using different fibers, including 1μm specialty SMF, the LP_{01} mode of SMF28, the LP_{01} mode of DCF, and the LP_{11} mode of SMF28 are shown in Fig. 2. These time-stretch confocal images are captured by scanning the sample in *y*-

direction for 200 lines with 0.5μm step size and ultrafast line-scan rate (10MHz). The field-of-view (FOV) is as large as ~0.44 mm × 0.1 mm. The captured time-stretch images based on the LP_{01} modes in the SMF28 and DCF (Figs. 2(c)-(d)) show the similar image quality compared with that using 1μm specialty SMF (Fig. 2(b)) and resolve well the smallest line feature (a line-width of 2μm in Group 7).

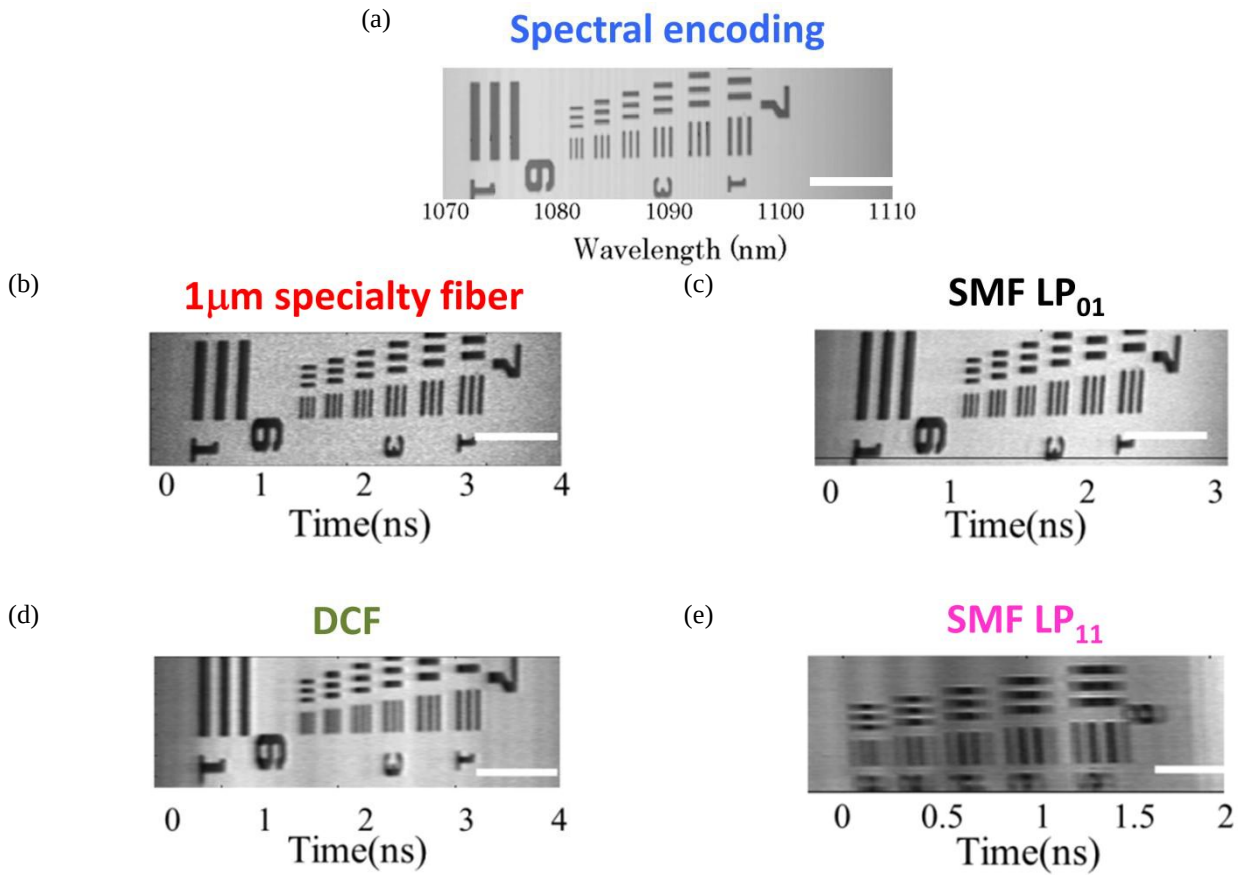

Fig. 2. Comparison of spectral encoded image with time-stretch confocal images based on different FMF modes as well as the 1μm specialty SMF. (a) spectrally encoded image captured by conventional spectrometer. (b)-(e) time-stretch confocal images of USAF-1951 by 1μm specialty SMF (b), LP_{01} of SMF28 (c), LP_{01} of DCF (d) and LP_{11} of SMF28 (e). Note that the scale bars represent 50 μm in (b)-(d), and 100μm in (e).

Time-stretch imaging based on the LP_{01} modes in SMF28 and DCF achieves the resolution of ~2μm, which is primarily digitizer-limited [7,13]. This resolution is high enough for cellular imaging based on time-stretch microscopy (See Fig. 4). The highest resolution, i.e. the spatial-dispersion-limited resolution, can be attained if the dispersion is further enhanced > 180 ps/nm. This can be achieved by using a longer fiber together with the optical amplification to compensate the inherent dispersive loss. Indeed, a GVD as high as > 10ns/nm has been demonstrated in time-stretch spectroscopy [16]. It should be emphasized that all the time-stretch images were captured here at an ultrafast spectral acquisition rate (i.e. line-scan rate) of 10 MHz, determined by the repetition rate of the SC source. This is orders-of-magnitude faster than that achievable in the conventional spectrometers (5 Hz). In addition, each single-shot line scan (along the *x*-direction) of the time-stretch image is obtained only within few ns (i.e. a duty cycle of ~4% given a scan rate of 10MHz), determined by the GVD and the illumination bandwidth. Such duty cycle is already sufficient for achieving high-resolution time-stretch imaging. Interestingly, despite of having the limited dispersion, time-stretch imaging based on the LP_{11} mode in SMF28 (Fig. 2(e)) is also possible with a limited resolving power, i.e. being able to resolve a

minimum line-width of 15μm (Group 6 of the resolution target). The image also shows a ghosting effect. It indicates that the presence of ambiguous wavelength-time mapping during the time-stretch process. We attribute two possible mechanisms resulting in such mapping ambiguity: (1) mode coupling and (2) polarization mode dispersion (PMD) of the degenerate LP_{11} modes due to perturbation along the FMF [17].

The above results thus clearly show the feasibility of selectively exciting and utilizing the LP_{01} mode in the FMF for efficient time-stretch confocal microscopy. Moreover, the fact that the higher-order FMF modes can also be employed for time-stretch imaging offers an additional degree of freedom for realizing time-stretch imaging. Albeit the complication introduced by the modal coupling, we anticipate that careful modal dispersion and GVD engineering of FMFs [17] can open up a wide variety of opportunities for optimizing the time-stretch imaging performance based on either the LP_{01} mode or the higher-order modes in FMFs.

4.2. Optical time-stretch confocal microscopy with pixel-SR algorithm

For evaluating the performance of pixel-SR algorithm with time-stretch imaging, we lowered the bandwidth and the sampling rate of the digitizer down to 8 GHz and 20 GS/s, respectively. This emulates the scenario in which the final image resolution is limited by the digital resolution (imposed by the sampling rate of the digitizer), instead of the optical resolution (i.e. diffraction-limited resolution). We verified it by the theoretically estimated optical resolution and digital resolution of time-stretch microscopy, i.e. ~1.5 μm and ~2.3 μm (with GVD of 0.15 ns/nm), respectively. In this case, the captured image is an LR image, which is under-sampled (Fig. 3(a)). We acquired 4 sub-pixel shifted LR images at 2 MHz line-scan rate (150 line-scans in total). Note that the sub-pixel shift is only performed in the *x*-direction.

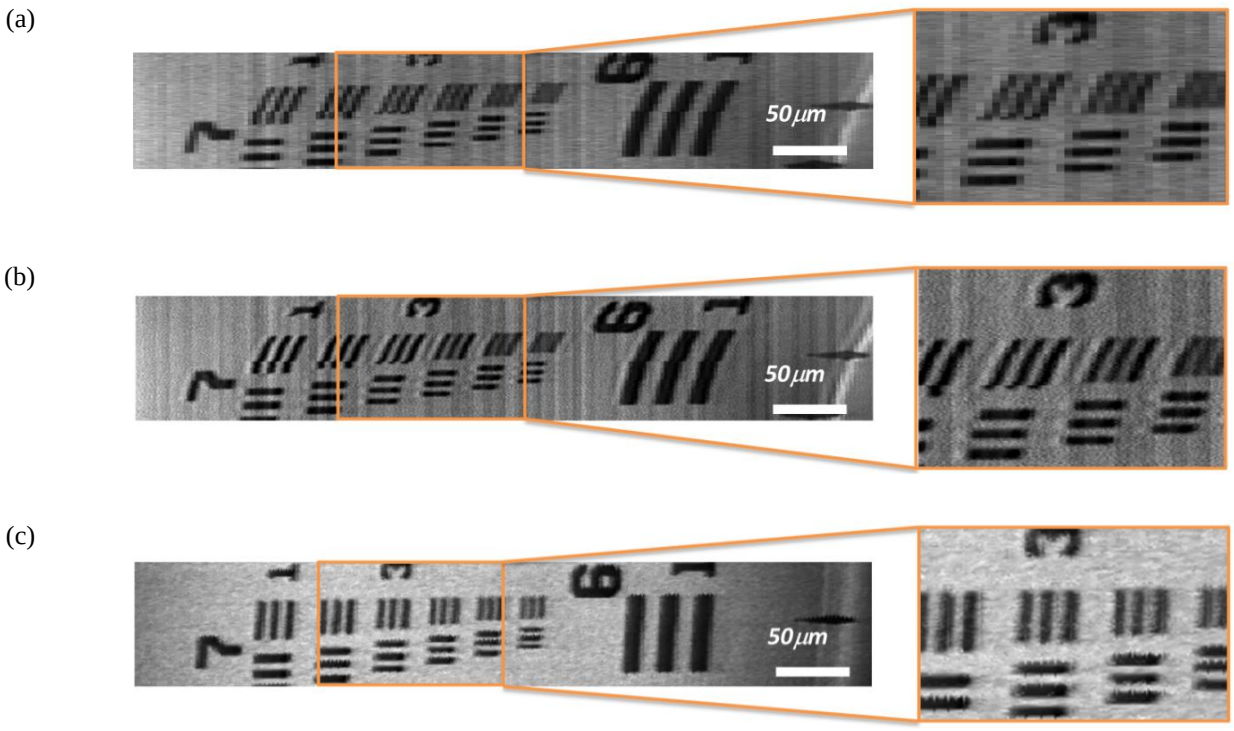

Fig. 3. Time-stretch confocal images (NA = 0.66) of a resolution target (USAF-1951) captured based on different conditions: (a) an LR image captured by a lower-bandwidth digitizer (8 GHz bandwidth and 20 GS/s sampling rate), (b) an HR image reconstructed by 4 sub-pixel shifted LR images, and (c) an time-stretch image captured by a higher-bandwidth digitizer (16 GHz bandwidth and 80 GS/s sampling rate). The insets on the right are the close-up views of the enclosed areas.

The LR multiple images are then undergo *registration*, *interpolation* and *restoration*. In particular, we have adapted *spline* interpolation method and *blind deconvolution* for deconvolution. After these digital image processing steps, a HR time-stretch image is resulted (Fig. 3(b)). For comparison, we have captured a time-stretch image with a higher bandwidth (16 GHz) and higher sampling rate (80 GS/s) at a line-scan rate of 10 MHz (Fig. 3(c)). Comparing the LR image and HR image, we observe that the HR image obviously shows sharping edges and is able to resolve the fine features that are originally not resolvable in the LR image. With a reasonable compromise in speed (a reduced line-scan rate by a factor of 4 because of the 4 sub-pixel shifts), the HR image using a digitizer with 8-GHz bandwidth shows a comparable image quality with the image captured by high-bandwidth digitizer (16 GHz). To apply this algorithm in flow cell imaging applications, we recently proposed a technique based on the use of high-speed acousto-optic device (AOD) to provide ultrafast sub-pixel shift and thus to achieve high-resolution time-stretch microscopy without sacrificing the imaging speed significantly [18].

4.3. FMF-based optical time-stretch confocal microscopy with pixel-SR algorithm

We also demonstrate the feasibility of applying FMF as the dispersive element and pixel-SR algorithm in cellular imaging using time-stretch microscopy at 1μm. Here, we chose the LP_{01} mode of the SMF28 as the FMF mode in the time-stretch process. Using a lower-bandwidth digitizer (8 GHz), we estimated the optical resolution and digital resolution are ~2.5 μm and ~4.2 μm, respectively. Again, the image resolution is digitizer-limited. We acquired 5 sub-pixel shifted LR images at 5MHz line-scan rate (Fig. 4(a)) for HR image reconstruction. Using the same algorithm employed in the previous section, we reconstructed a HR image of the nasopharyngeal epithelial cells (Fig. 4(b)). We also captured a time-stretch image using the digitizer with higher bandwidth (16 GHz) and sampling rate (80 GS/s) and 1μm specialty SMF at the same line-scan rate for comparison (Fig. 4(c)). The image essentially shows a similar image quality with the one using a lower-bandwidth digitizer and a standard telecommunication fiber as FMF. It proves that such cost-effective implementations (i.e. without the needs for high-cost specialty dispersive fiber at 1μm, and the state-of-the-art electronic digitizer) are feasibility to offer reasonable image quality in high-speed time-stretch microscopy.

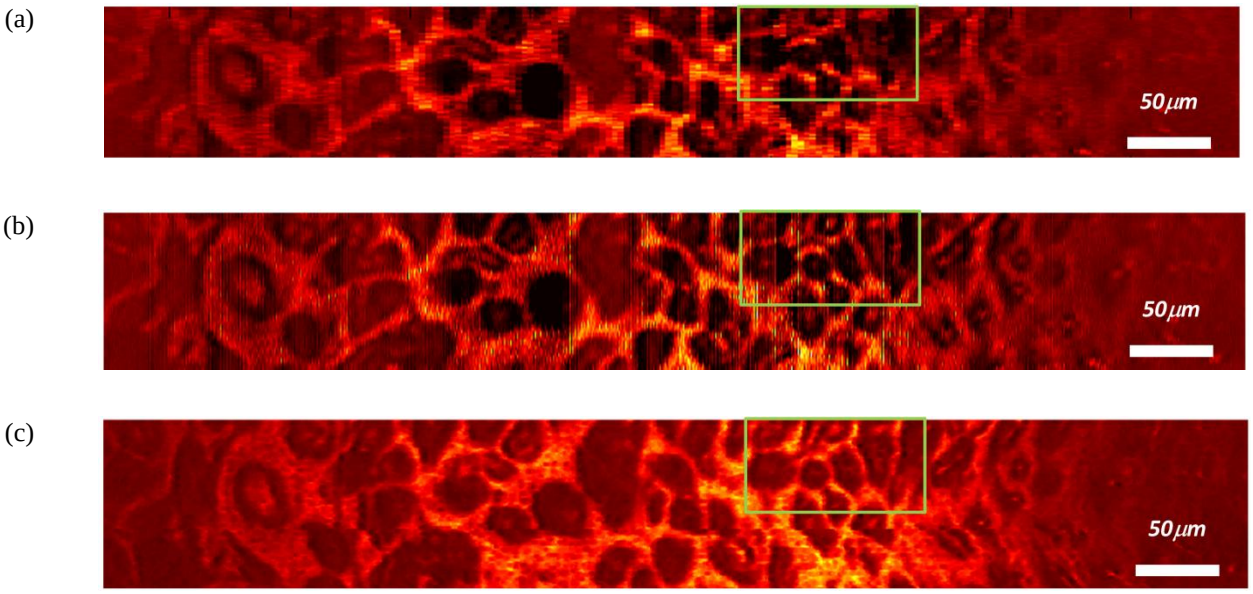

Fig. 4. Time-stretch confocal images (NA = 0.4) of nasopharyngeal epithelial cells captured based on different conditions: (a) LR image captured by a lower-bandwidth digitizer (8 GHz bandwidth and 20 GS/s sampling rate) and LP_{01} mode of the SMF28, (b) HR image reconstructed by 5 sub-pixel shifted LR images, and (c) an image captured by a higher-bandwidth digitizer (16 GHz bandwidth and 80 GS/s sampling rate) and 1μm specialty SMF.

5. CONCLUSIONS

To conclude, we have proposed and experimentally demonstrated two cost-effective approaches for realizing high-speed and high-resolution time-stretch imaging at 1μm. By using the standard telecommunication SMF as FMF, and implementing the pixel-SR algorithm for relaxing the stringent requirement on the considerably high-speed digitizer, we can achieve practical time-stretch confocal microscopy at 1μm with cellular resolution (~3 μm) at a scan rate of ~MHz. Without significantly compromising the imaging quality, the present techniques could facilitate widespread utility of time-stretch imaging in biomedical diagnostics, especially in the resource-limited environments. We expect further improvements in high-speed sub-pixel shift enabled by AOD as well as optimizing the pixel SR-algorithm (especially the restoration steps) would yield enhanced time-stretch image quality. On the other hand, the use of FMF for optical time-stretch also opens up a wide variety of opportunities of further optimizing the time-stretch imaging performance by e.g. modal dispersion engineering as well as the GVD engineering of the FMFs [17].

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