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Pixel super-resolution in serial time-encoded amplified microscopy (STEAM)

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Abstract: We propose pixel super-resolution serial time-encoded amplified microscopy (STEAM) for achieving high speed and high-resolution imaging – relaxing the stringent requirement on the digitizer bandwidth while preserving the ultrahigh frame-rate (>MHz).

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Serial time-encoded amplified microscopy (STEAM) has recently been demonstrated as a fundamentally new optical imaging modality which can deliver ultra-high frame rate (>1MHz) and high sensitivity [1-2]. This is achieved by mapping the spatial information of an image to an optically-amplified serial temporal data stream, which is captured by a high-speed digitizer. Such unique feature makes STEAM as an effective tool particularly for high-throughput imaging flow cytometry. However, capturing high-resolution (diffraction-limited) STEAM images at ultrahigh frame rates is challenging as it strictly requires the state-of-the-art digitizer with high-bandwidth (>10GHz) and high-sampling-rate (>40GS/s) in order to achieve the diffracted-limited STEAM image [1]. In the other words, without the ultrafast digitizer, the spatial resolution of STEAM is mostly limited by the sampling rate of the digitizer. This naturally falls into a common scenario in digital imaging in which pixel super-resolution (SR) imaging technique is required to restore the high-resolution (HR) images [3]. To this end, we here present a technique based on a sub-pixel-shift SR imaging technique and the use of high-speed acousto-optic device (AOD) to restore the HR STEAM images. The pixel SR technique relaxes the stringent requirement on the digitizer’s bandwidth in STEAM, while preserving the diffraction-limited resolution. Our preliminary experiments show that it is possible to recover the HR images by a sub-pixel-shift SR algorithm in space-wavelength imaging – a key intermediate step of transforming the spatial information to the time domain in STEAM.

The basic idea of pixel SR STEAM is to digitally reconstruct a HR image from multiple low-resolution (LR) frames of the same object, each of which with sub-pixel shifts in space. This technique is primarily implemented in the space-wavelength mapping process in STEAM, in which the optical beam of a broadband pulse source is spatially dispersed by the diffraction grating and incident onto the object. Our proposed pixel-SR STEAM configuration (Fig. 1(a)) is designed for microfluidic flow cell imaging, which is widely used in high-throughput screening applications. The sub-pixel shifts in the two orthogonal directions are done with two separate approaches (Fig. 1(b)): (i) the sub-pixel shift in the y-direction ($\Delta d_y$) is accomplished by using an AOD which is placed before the grating. The AOD here produces high-speed and high-precision beam scanning in the y-direction, synchronized with the repetition rate of the source. $\Delta d_y$ is determined by the AOD frequency ($f$) and the repetition rate ($f_{rep}$) of the source. (ii) the sub-pixel shift in the x-direction ($\Delta d_x$) is naturally provided by the unidirectional fluid flow. In this case, $\Delta d_x$ is governed by the fluid flow rate ($v$) and the repetition rate of the source.

The continuous AOD beam scanning in the y-direction together with fluid flow in the x-direction results in a zig-zag sub-pixel shift pattern (Fig. 1(c)). The spatial information of the sub-pixel shifted LR images will then undergo...
the wavelength-to-time mapping (Fig. 1(a)) by amplified dispersive fourier transform (ADFT) process – mapping the spectrally-encoded LR image into a temporal waveform and digitized by the real-time oscilloscope [1,2,6].

While the implementation of the sub-pixel shift in the x-direction is relatively straightforward (only requires proper synchronization between the fluid rate control and the source’s repetition rate), the sub-pixel shift in the y-direction, which involves the AOD, requires careful designs. Based on the specifications of the AOD [4], we theoretically verified that the sub-pixel shift \( \Delta y \) can be precisely controlled by the AOD beam scanning with \( \Delta y \approx 0.1 - 0.4 \) \( \mu m \) in the wavelength range 1000 – 1100 nm when the AOD frequency is varied in the range of 110 – 110.1 MHz. We note that the wavelength-dependent shift has to be digitally registered in the SR reconstruction algorithm, similar to the common SR imaging techniques [6].

The significance of pixel-SR STEAM is to relax the stringent requirement on the digitizer’s bandwidth in STEAM by first capturing the LR images, while preserving the diffraction-limited resolution by then digitally reconstruct the HR images from the multiple LR images (N) (Fig. 2(b)). However, increasing the number of LR images N for obtaining an HR image comes at the expense of the effective frame rate of the HR images (Fig. 2(c)). Nevertheless, we find that this compromise does not severely diminish the effectiveness of STEAM in the high-throughput flow cell imaging applications. Our theoretical model (Fig. 2(b)-(c)) shows that pixel-SR STEAM with an effective HR frame rate of 1MHz can still be realized when N = 10 LR images are captured. The speed is comparable to the recent STEAM demonstrations [1,6] but with the HR imaging capability.

We have also performed a proof-of-concept experiment applying the pixel SR technique in the space-to-wavelength imaging – a key imaging step in STEAM [1]. The resolution of the image here is set to be limited by the pixel-size of the spectrometer, in analogous to the case of pixel-SR STEAM where the resolution is limited by sampling rate of the digitizer. A supercontinuum source (centered at 1064 nm) is first dispersed by a diffraction grating and is then focused on the sample by the objective lens (N.A. = 0.66). Four sub-pixel LR frames (each sub-pixel shift of 0.5 \( \mu m \) in the y-direction (Fig. 3(a)) are captured and digitally processed for reconstructing the single HR frame. The HR frame clearly shows the improved resolution with shaper edges features compared to the LR frame in the y-direction (Fig. 3(a)). The smallest features, which are unresolvable in the LR images, can now be recovered (Fig. 3(b)). Thus, the pixel SR algorithm helps to obtain the HR image close to the diffraction-limited resolution, which is 2 \( \mu m \). We are currently working on further optimizing the reconstruction algorithm which will be compatible to the entire pixel-SR STEAM system – including the ADFT module for wavelength-time mapping and the high-speed AOD for high-speed sub-pixel shift.

![Fig. 2(a) Simulated sub-pixel shift as a function of the frequency of AOD and the bandwidth of the laser. (b) Analysis on the required digitizer sampling rate as a function of number of LR images (N). (c) Trade-off between effective HR frame rate and N.](image1)

![Fig. 3 Comparing the difference between the images and the 1-D cross sections of the LR frame and HR frame. (a) LR image (left) and HR image (right). (b) LR 1-D line cross section (left) and HR 1-D line cross section (right).](image2)