Frequency-Time-Division-Multiplexed Single-Pixel Imaging for Biomedical Applications

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Abstract: We demonstrate high-speed single-pixel imaging by integrating frequency-division multiplexing and time-division multiplexing and applying the combined technique, namely frequency-time-division multiplexing (FTDM), to optical imaging. We employ the technique to obtain fluorescence images from biological cells. © 2022 The Author(s)

1. Introduction

Single-pixel imaging [1] is an emerging method for diverse applications where conventional image-sensor–based imaging falls short such as remote sensing, terahertz imaging, X-ray diffraction imaging, and high-speed imaging. This is because a single-pixel photodetector is advantageous over an image sensor in terms of cost, size, simplicity, sensitivity, and imaging speed. The basic principle of conventional single-pixel imaging is to generate an image by illuminating an object with multiple spatially resolved patterns, measuring the correlated intensity with a single-pixel photodetector for each pattern, and patching the measured intensities. The imaging speed of single-pixel imaging can be dramatically improved by employing telecommunication technology. Specifically, frequency-division multiplexing (FDM) is highly effective for high-speed single-pixel imaging in biomedical applications [2, 3]. FDM enables high-speed image acquisition that cannot be achieved by conventional point-scanning imaging methods such as laser-scanning confocal fluorescence microscopy. The high-speed feature is advantageous in cutting-edge biomedical applications such as high-throughput imaging flow cytometry [2] and intelligent image-activated cell sorting [4]. To further enhance the speed, we propose and experimentally demonstrate employing frequency-time-division multiplexing (FTDM) to single-pixel imaging for biomedical applications [5]. As a proof-of-concept demonstration, we use the method to obtain two-color (bright-field and fluorescence) microscopy images of breast cancer cells at a high frame rate of 32,000 fps.

2. Results

The concept of FTDM single-pixel imaging is schematically shown in Fig. 1. FTDM single-pixel imaging is implemented by scanned multiple (n) beam spot arrays, each of which consists of intensity-modulated beam spots having different modulation frequencies. Scattered, reflected, or emitted light from the target field of view (FOV) is collected by a single-pixel photodetector and is converted to an electric signal. The signals from different beam spot arrays are allowed to have spectral overlaps in their intensity modulation frequencies but should be discriminated in the time domain. Therefore, the beam spot arrays are located at different positions such that they scan the target FOV at different timings. Obtained signals are separated into time components in the time domain, each of which is further separated into frequency components in the frequency domain. The inverse Fourier transform of each component becomes a line image of a single beam spot. Then, an array of images at different timings is obtained by a single scan.

Figure 2 shows a schematic of our experimental setup. A continuous-wave laser with 2-W output power at λ = 488 nm (Coherent Genesis CX 488 STM) was employed as the light source. Similar to the previous report of frequency-division-multiplexed single-pixel imaging [2], a pair of beam arrays were generated by interfering beams deflected from two acousto-optic deflectors with 200-MHz bandwidth (AOD, Brimrose TED-300-200-488). The driving signals to the AODs and an acousto-optic frequency shifter (Isomet 1250C) were generated by an arbitrary waveform generator at 1.2 GS/s (Signatec PXDAC4800) and a tone generator (Rohde & Schwarz SMC100A). The modulation bandwidth and frequency spacing of each beam were ~400 MHz and ~4 MHz, respectively. The spatial beam arrays were combined by the polarizing beam splitter with perpendicular polarization states (horizontal and vertical). The combined beam is split l times by l Wollaston prisms (WPs) and l – 1 half-wave plates at an axis angle of 22.5°, generating an array of 2l spatial dual combs. In the following experiments, we used a single WP (Thorlabs WPQ10) and split the combined beam by an angle of 1°, which corresponds to a spacing of 60.5 μm in the sample plane. The scale of the spacing was verified by an image of a test target (Thorlabs R1L3S3P). The beam arrays were scanned by an 8-kHz resonant galvanometric scanner (Cambridge technology CRS8), passed through a slit aperture

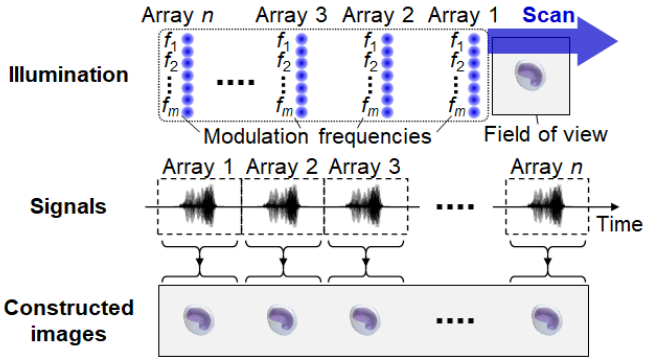

Fig. 1. Concept of FTDM single-pixel imaging.

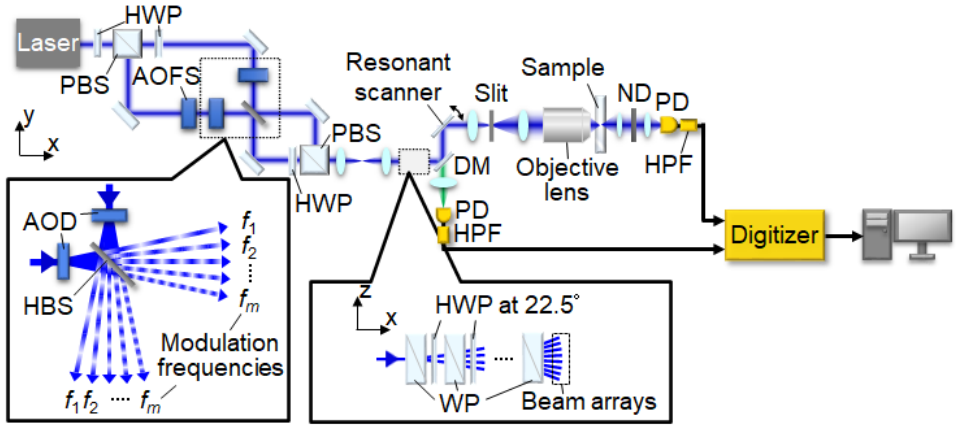

Fig. 2. Schematic of the FTDM single-pixel imaging setup. The left inset shows the details of generating a pair of intensity-modulated beam arrays from acousto-optic deflectors by optical interference. HWP, half-wave plate; PBS, polarizing beam splitter; AOFS, acousto-optic frequency shifter; AOD, acousto-optic deflector; HBS, non-polarizing half beam splitter; WP, Wollaston prism; DM, dichroic mirror; ND, neutral-density filter; PD, photodetector; HPF, high-pass filter.

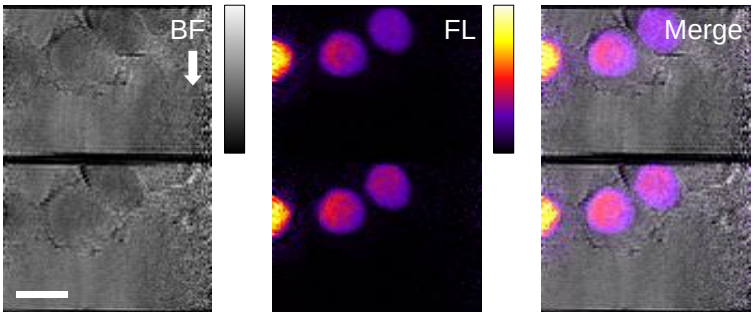

Fig. 3. Images of MCF-7 breast cancer cells stained by SYTO16 obtained by the FTDM single-pixel microscope at a frame rate of 32,000 fps. BF: bright-field images; FL: fluorescence images; Merge: BF + FL. The arrow indicates the scan direction. Scale bars: 20 μm.

placed at an intermediate image plane to guarantee that no light from the outside of the target FOV was collected, and then focused on a target sample through an objective lens (Olympus UPLSAPO20X; NA, 0.75). The transmitted light and backscattered fluorescence light were separately detected by Si avalanche photodetectors (Thorlabs APD430A/M and APD430A2/M). The signals from the photodetectors were converted into digital data by a digitizer with a sampling rate of 1.25 GS/s (Spectrum M4i.2212-x8) and processed by a homemade LabVIEW program. A digital lock-in detection algorithm [2, 3] was employed to construct images.

As a proof-of-concept demonstration, we performed two-line FTDM single-pixel imaging. In this experiment, two beam spot arrays with an identical modulation frequency band were aligned such that they scanned a target FOV at different timings. Then, the FOV was scanned twice by a single scan of the beam scanner, increasing the frame rate by a factor of 2 compared with the case of a single beam spot arrays (namely, frequency-division-multiplexed single-pixel imaging). Fig. 3 shows two-color (bright-field and fluorescence) images of MCF-7 breast cancer cells stained by SYTO16 obtained by the two-line FTDM single-pixel imaging setup. Since a pair of images were obtained at a frame rate of 16,000 fps (a factor of two times the scanning frequency of the galvanometric scanner, as image acquisition occurs in both forward and backward directions), the actual frame rate was doubled to 32,000 fps. The FOV and the number of pixels in each frame were 82 μm × 57 μm and 97 × 110 in the horizontal direction (the alignment direction of each beam spot array) and in the vertical direction (the direction of the galvanometric scanning), respectively. The image distortions caused by the non-uniform sinusoidal scan and variation of the line-by-line intensity level in the frames, which was due to the non-uniform excitation intensity, were numerically corrected. In addition, the slight difference in image intensity between a pair of images due to the difference in the intensity of the beam arrays was also numerically compensated.

3. Conclusions

We proposed and experimentally demonstrated high-speed single-pixel imaging by integrating frequency-division multiplexing and time-division multiplexing for biomedical applications. As a proof-of-concept demonstration, we used the method to show ultrafast two-color (bright-field and fluorescence) single-pixel microscopy of breast cancer cells at an unprecedentedly high frame rate of 32,000 fps.

4. References

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