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Real-time optical imaging and tracking of micron-sized particles

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ABSTRACT

We report real-time imaging and dynamics monitoring of micrometer predefined and random sized particles by time–space-wavelength mapping technology using a single-detector. Experimentally, we demonstrate real-time line imaging of a 5 μm polystyrene microsphere, glass powder particles and patterns such as fingerprints with up to 5 μm resolution at 1 line/50 ns capture rate. By using the same setup, real-time displacement tracking of micrometer-size glass particles with 50 ns temporal resolution and up to 5 μm spatial resolution is achieved. We also show that existing correlation spectroscopy algorithms can be adopted to extract dynamic information in a complex environment.

1. Introduction

Real-time optical imaging and tracking of submicron particles are attractive approaches for in vitro biological sample imaging and capturing transient properties of target objects in electromechanics. Different laser scanning microscopes including fluorescence or scattering based mechanisms have been proposed and illustrated for obtaining high-resolution optical images [1,2]. Unfortunately, temporal resolutions of the above mentioned laser scanning microscopes are between microsecond and second due to mechanical limitation of scanning methods and data acquisition speed of detector arrays [3]. Recently, wavelength-division-multiplexing (WDM) based confocal microscopy has been demonstrated as a useful tool for optical imaging and detection using space-wavelength mapping technique [4–6]. In addition, time-wavelength mapping can provide unique solution for improving temporal resolution to realize real-time optical measurements [7–9] using a single-detector and single-shot measurement. Time domain profile of ultrafast RF (Radio Frequency) signals can be mapped to wavelength domain; thus, the spectral shape can be retrieved directly into time domain by using a real-time oscilloscope after a dispersive time stretching process [10]. Time-wavelength mapping technique also prevails over the slow-speed conventional spectrometers and allows real-time single-shot measurement of dynamic process [11]. This technique has also recently been implemented to detect highly reflective objects with sub-gigahertz resolution [12,13]. In another field of interest, image correlation spectroscopy has proved to be a powerful tool of measuring dynamic processes and providing spatially resolved transient information in biological systems [14,15]. Analysis of temporal and spatial correlation of image series can provide dynamic information such as diffusion coefficients and velocity vectors by using correlation functions.

In this paper, WDM based time–space-wavelength mapping is demonstrated to integrate space-wavelength mapping and time-wavelength mapping configuration into one system to achieve real-time high-resolution optical measurement. Using this technique, we present real-time optical imaging of a 5 μm polystyrene microsphere, which is extensively employed as size-standards for calibration of the system. As a further proof of concept, fingerprint stains are imaged with up to 200 μm/line spatial resolution and real-time, up to 1 line/50 ns, acquisition rate. We also perform single-shot imaging, measurement of consecutive data points captured in a single measurement, of real-time dynamics of micron-size objects and correlated movements by tracking them in a 20 μm imaging range. The image generated by the time–space-wavelength mapping system is compatible with algorithms developed for image correlation microcopy. Here, we illustrate detection and tracking of objects with 50 ns temporal accuracy by applying the algorithms of correlation spectroscopy.

2. Experimental setup

In this system, WDM essentially reduce the two-dimensional scanning to one-dimensional sample scanning while information along the incident beam is provided by wavelength division map-

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ping. As shown in Fig. 1, a supercontinuum source is dispersed by the diffraction optics to produce space-wavelength mapping in one spatial dimension. When the laterally dispersed incoming light encounters with the target object, random amplitude modulation is created on the transmitted signal as in Fig. 1. For two-dimensional micron-sized object optical imaging, sample is scanned only in the dimension normal to the incident beam direction while the lateral information (*x* axis in Fig. 1) is provided by dispersed beam. By comparing the modulated signal with the stored background signal, we can extract the information regarding the position of the object and the density of the scatterers. Imaging and detection of the target object can be realized in real-time by retrieving the single-shot data from the time domain by a detection module of a single-detector and a real-time digital oscilloscope. For particle tracking, dynamic monitoring of micro-particles is achieved by first manipulating the particle moving within 20 μm image frame along the incident beam direction controlled by a piezoelectric stage with the same setup as previously mentioned WDM based system. Transient displacements of the particle induce variation in the temporal waveform due to the amplitude modulation in different wavelengths along the incident beam direction. The trajectory of the particle is recorded and processed using correlation spectroscopy method.

Experimental setup of the real-time high-resolution optical imaging and particle tracking is shown in Fig. 2. A 20 MHz fiber modelocked laser is used to generate a supercontinuum source with 50 nm bandwidth for imaging experiments. The generated light is then chirped by a grating based dispersion compensation module (1300 ps/nm) and hence time-wavelength mapping is produced. The temporally dispersed supercontinuum is subsequently dispersed in space by using a 600 lines/mm diffraction grating. At point "A" in Fig. 2, an elliptical beam where each position along *x* axis is mapped to a different color arriving at different times. Then the beam is focused on the sample at point "B" by using microscope objectives OL1 and OL2 (numerical apertures of 0.65 and 0.85 for desired spatial resolution and the high collection efficiency). Polystyrene microspheres with 5 μm diameter and glass particles are used as samples in this experiment. Samples are embedded in a thin polymer film and attached to a cover glass. Sample imaging is performed by monitoring the intensity of the transmitted light from the sample using an InGaAs detector. The presence of particles on the image plane induces amplitude modulation on different wavelengths, which is captured in time domain by a high-speed real-time oscilloscope with 20 GS/s sampling rate. This setup can also be slightly modified for different-sized object imaging such as fingerprint stain imaging. The objectives lens OL1 and OL2 in Fig. 2 are replaced with spherical lenses (f = 100 mm) for fingerprint object imaging.

3. Experimental results and discussion

Imaging capability of the system is evaluated by scanning different-sized objects in one-dimension. As a proof of concept, we first demonstrate 2D imaging of a 5 μm diameter polystyrene

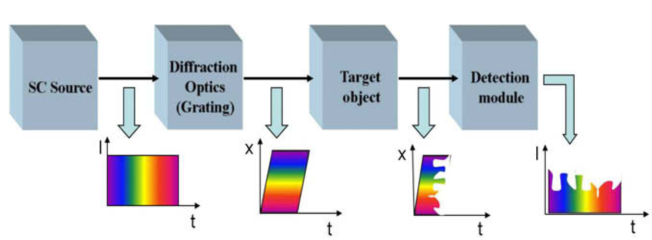

Fig. 1. Conceptual diagram of real-time time–space-wavelength mapping system.

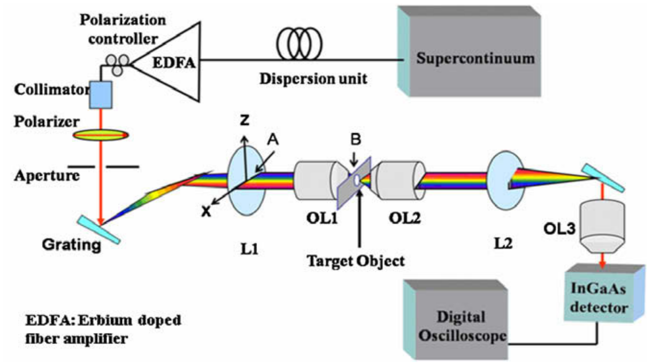

Fig. 2. Experimental setup of real-time high-resolution optical imaging and particle tracking.

microsphere. 2D image of polystyrene microsphere shows that a 5 μm microbead is resolved by the system, Fig. 3a. As a

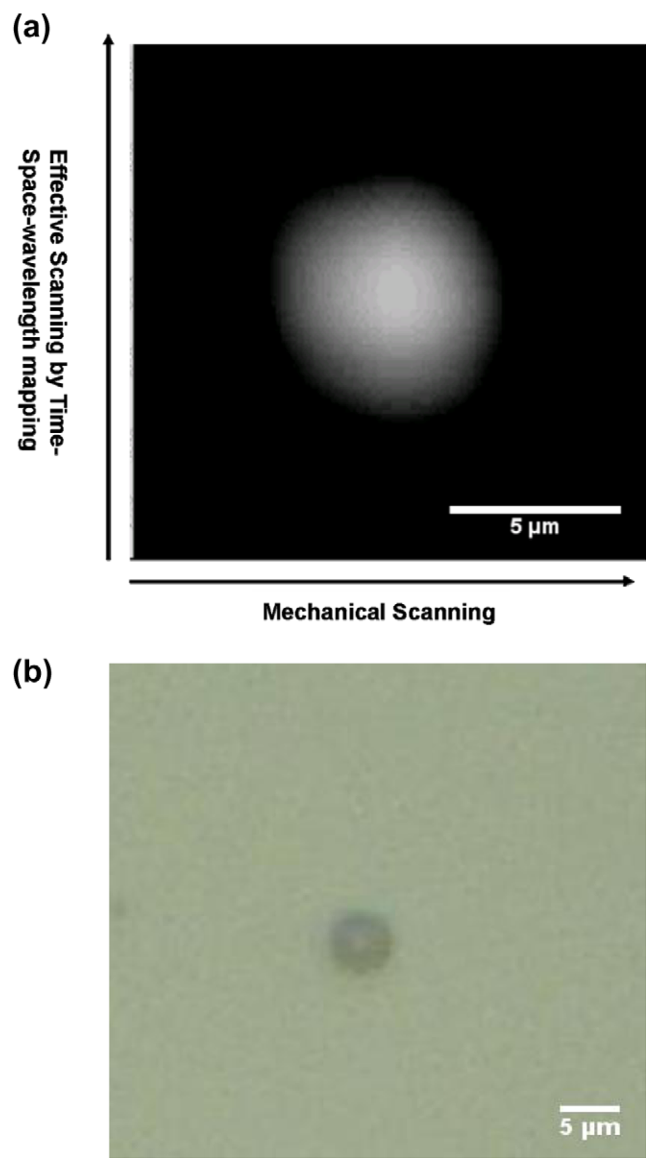

Fig. 3. Imaging of a 5 μm polystyrene microsphere. (a) Image generated by single-shot space–time-wavelength mapping technique, (b) image captured by high-resolution Keyence microscope.

comparison, optical imaging of the microsphere captured by a Keyence digital microscopy is shown in Fig. 3b. In this experiment, microbeads of 5 μm diameter can be detected and imaged with 5 μm resolution. Here, the line capture rate is determined by the laser pulse rate fixed at 1 line/50 ns. Resolution of this system consists of resolution imposed by the diffraction limit and the resolution imposed by the detection scheme in this system. Resolution imposed by the diffraction limit is shown is Eq. (1), NA_{obj} is the numerical aperture of the objectives, and is calculated as 1.5 μm for $\lambda = 1.55$ μm. In addition to diffraction limit, the detection system is limited on the spatial resolution due to RF filtering. The detection resolution of this scanning system shown in Eq. (2) is inversely proportional to the optical bandwidth ($\Delta\lambda$), fiber dispersion (D) and RF bandwidth (BW_{RF}) of the detector and proportional to the beam width at object plane (d).

Resolution of the system is the larger of

$$\text{Diffraction limit} = 0.61\frac{\lambda}{NA_{obj}}[16] \tag{1}$$

and

$$\text{Detection resolution} = \frac{d}{\Delta\lambda \times D \times BW_{RF}} \tag{2}$$

RF filtering can improve the detection resolution to $d/44$ by the optical bandwidth after fiber dispersion (22 ns) and detection bandwidth (2 GHz) used in this system, while beam width at the object plane (d) can be controlled by the numerical aperture of focusing objective lens. Hence, resolution of the system is mainly limited by the diffraction limit as 1.5 μm in this system. By using high numerical aperture lenses such as 80× objective lenses, resolution down to 1 μm is achievable. The imaging system proposed clearly demonstrates that it can resolve sizes of objects with different order of magnitudes. As expected, increasing the scan area is feasible without losing the resolution by using larger bandwidth optical sources. Also, target objects of various profiles can be readily distinguished by this configuration. The application of this proposed scheme may include detection of objects of arbitrary shapes.

To further demonstrate the ability of this image system, a single-shot image of a fingerprint stain of 2 mm × 1 cm area on a glass substrate is also illustrated in Fig. 4 using the same apparatus. Each line ~200 μm can be clearly resolved in the image and one-dimensional information is acquired by mechanical scanning. Random sized glass particles (refractive index = 1.6) are also imaged using the above mentioned imaging system. 2D image of micron-size glass particle is constructed which has a size of approximately 15 um as shown in Fig. 5.

Fig. 6a shows a single-shot image of real-time tracking of a micron-sized glass particle in 20 ms time window with 50 ns temporal resolution and 5 μm spatial resolution. Sample of micro-size particles (refractive index = 1.6) buried in a thin polymer film attached to a cover glass is mounted on a piezoelectric stage that is driven with a 1.5v peak to peak ramp function at 50 Hz. The sample movement is within 20 μm controlled by the stage. The row vectors of the 2D single-shot image correspond to different spatial locations, x, which are generated by a space–time-wavelength mapped single pulse. Column vectors represent evolution of particle at a specific location over time t, where each point is sampled at every T (50 ns), which is the pulse period. The whole image can be captured in a single-shot of N (N as a integer) pulses, which will generate N columns, and M rows, where M is the number of samples per period defined by the sampling rate. The dynamic trace of the sample is clearly captured in the time domain and spatial locations converted from variation in spectral domain. Such data capture rate and tracking can be utilized in applications such as bio photonics and MEMS characterization where detection of correlated events along multiple points is useful.

In order to improve the signal to noise ratio and to extract the dynamic information of the particle, two-dimensional cross-correlation function is utilized in data post-processing. Contour plot of two-dimensional cross-correlation function of the particle movement is shown in Fig. 6b. Cross-correlation function retrieves the prefect line movement of the particle, which suppresses the noise compared to Fig. 6a. We can express each point in single-shot captured image with particle movement as $I_s(x,t)$ while x and t are spatial and time locations and the image has the dimension of $I_s(x_s, t_s)$. Similarly, each point in the single-shot background image without particle occurring can be defined as $I_r(x,t)$ and the reference background image has the dimension of $I_r(x_r, t_r)$. Let $R(x,t)$ in Eq. (1) be the two-dimensional discrete cross-correlation function defined versus respective spatial and time locations x and t.

$$R(\zeta,\tau) = \sum_{x=0}^{x_s-1}\sum_{t=0}^{t_s-1} I_s(x,t) \times I_r^*(x+\zeta, t+\tau) \tag{3}$$

where ξ is the spatial increment and τ is the time increment, and "I^*" means the complex conjugate function. $0 \leqslant \zeta \leqslant x_s + x_r - 1$, $0 \leqslant \eta \leqslant t_s + t_r - 1$.

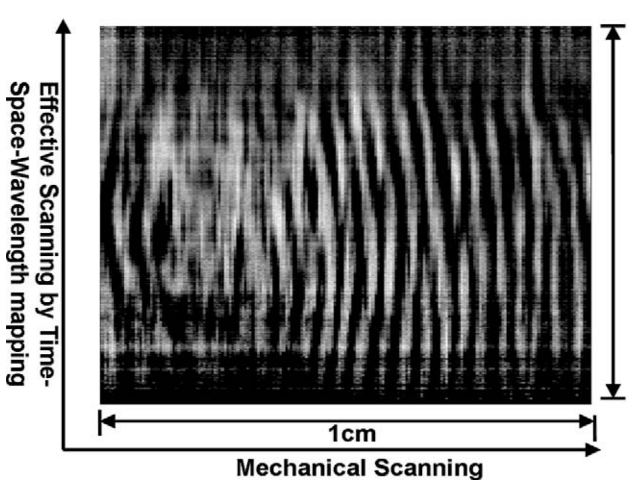

Fig. 4. Single-shot 2D image of a fingerprint stain.

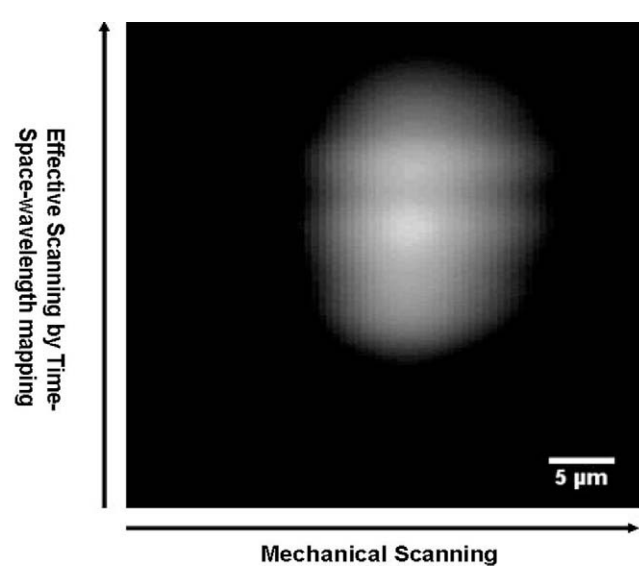

Fig. 5. Image of ~15 μm random glass particles by single-shot space–time-wavelength mapping.

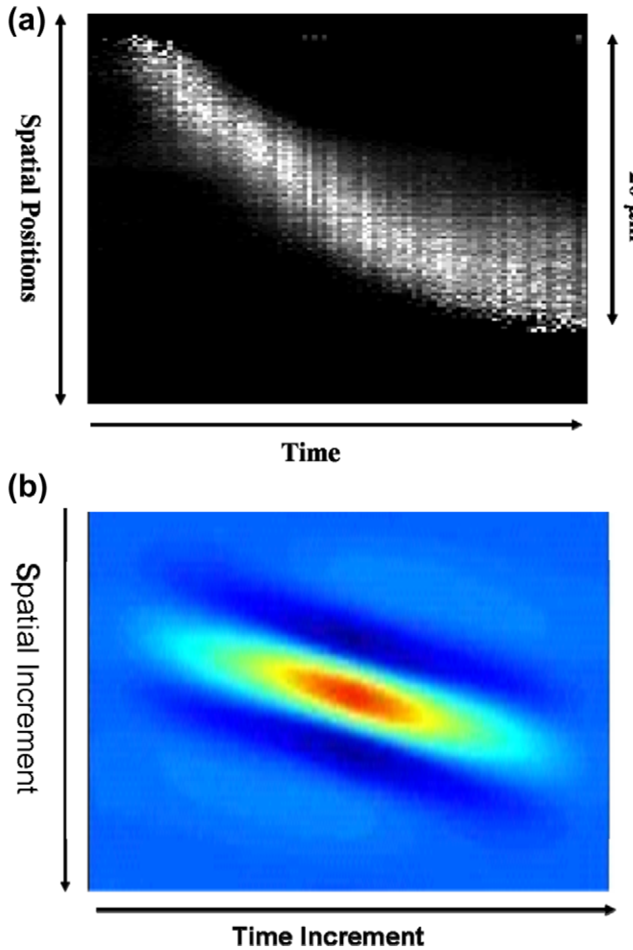

Fig. 6. Image of real-time tracking of micron-sized glass particles. (a) Single-shot retrieved image of real-time tracking of a micron-sized glass particle, (b) contour plot of two-dimensional cross-correlation of the particle movement.

In this system, the particle is moving uniformly; the trace of the shifted correlation peak can clearly represent the position change of the particle ($\xi = v \times \Delta t$, $\tau = \Delta t$). By using correlation algorithms, individual micro-particle is tracked in a single image and its position, trajectory, magnitude of velocity and instantaneous displacement are determined from cross-correlation methods.

These results show that, the demonstrated imaging system is compatible with conventional spectroscopy techniques such as correlation spectroscopy [17,18]. In image correlation spectroscopy, diffusion coefficients as well as spatially resolved dynamic information are provided by correlating between spatial points from a series of images. The space–time correlation function used in correlation spectroscopy can be expressed as in Eq. (2) [19]:

$$r_{ab}(\zeta,\eta,\tau) = \frac{\langle \delta i_a(x,y,t)\delta i_b(x+\zeta,y+\eta,t+\tau)\rangle}{\langle i_a\rangle\langle i_b\rangle_{t+\tau}} \tag{4}$$

where $\delta i_{a(b)}(x,y,t)$ is the intensity fluctuation in channel $a(b)$ at space position (x,y) and time t. The same data can be captured by extracting the intensity variation from the image generated by space–time-wavelength mapping algorithm.

Here, we image dynamic events occurring in a single line over long time duration. Hence, the intensity at spatial location x expressed as $I(t+nT)$ reveals intensity variation at the spatial point with temporal accuracy of T which is the laser repetition rate of 50 ns and n is a integer. Similarly, we can capture information about multiple different spatial locations in a single-shot image. Thus, by using the conventional correlation algorithms such as cross-correlation algorithms, this system can identify correlated events along the image plane and detect instantaneous correlated events with temporal accuracy of 50 ns.

4. Conclusion

We demonstrate the feasibility of single-shot, single-detector high-resolution imaging and tracking by time–space-wavelength mapping technique. We experimentally demonstrate single-shot imaging of 5 μm object at 1 line/50 ns capture rate. Correlation method is implemented to obtain the transient movement of the individual particle. This system can be further optimized for real-time imaging, tracking of multiple micro-particles or arbitrary objects. We also show that the existing spectroscopy algorithms can be utilized to obtain the dynamic flow information of particles. Future work of the proposed work may include single-shot 2D imaging by using combination of multiple gratings and polarization multiplexing.

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