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On-chip multiple particle velocity and size measurement using single-shot two-wavelength differential image analysis

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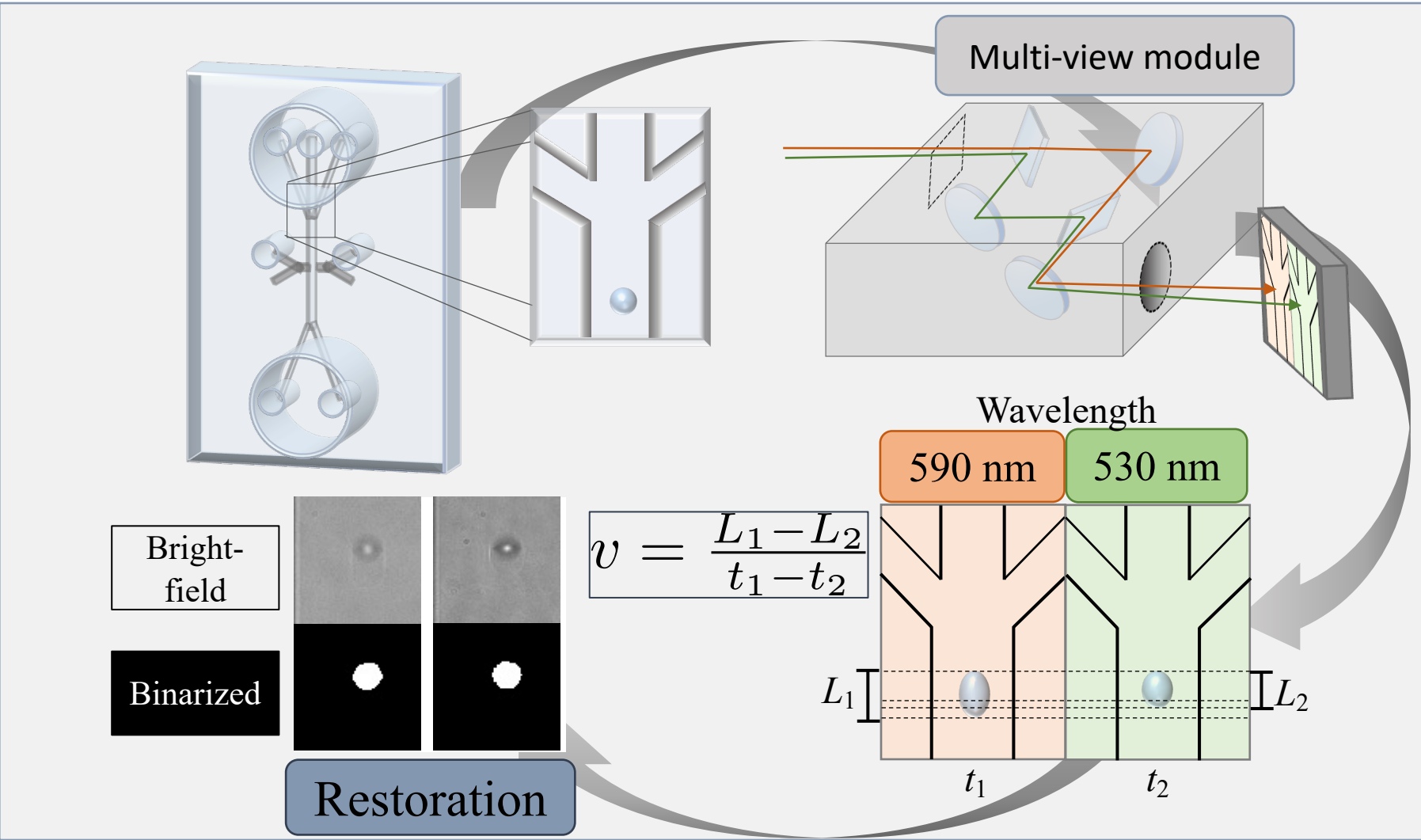
Graphical Abstract

Title: On-chip multiple particle velocity and size measurement using single-shot two-wavelength differential image analysis



Abstract

Precise and quick measurement of samples' flow velocities is essential for cell sorting timing control and reconstruction of acquired image-analyzed data. We have developed a simple technique for single-shot measurement of flow velocities of particles simultaneously in a microfluidic pathway. Microparticles were injected through an imaging flow cytometer and two wavelengths of light with different irradiation times were irradiated to the particles simultaneously. The mixture of two wavelengths transmitted lights was divided into two wavelengths, and the images of the same microparticles for each wavelength were acquired in a single capture. The speed was calculated from the difference in the particles' elongation in an acquired image which arose when applying two wavelengths of light with different irradiation times. The distribution of polystyrene beads' velocity was parabolic and highest at the center of the flow channel, consistent with the expected velocity distribution of the laminar flow. Exploiting the calculated velocity, we restored the accurate shapes and cross-sectional areas of particles in the images, demonstrating the capability of this simple method for improvement of imaging flow cytometry and cell sorter for diagnostic screening of circulating tumor cells.

Keywords: Imaging flow cytometer; precise velocity measurement; particle shape reconstruction; multi-view imaging

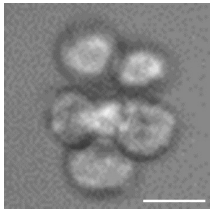
Introduction

- CTC forming clusters have been observed inside bloodstream.

  Please see previously published paper, Odaka *et al.*,Micromachines, 2019, for more detail

- CTC clusters are 23～50 times more likely to cause metastasis

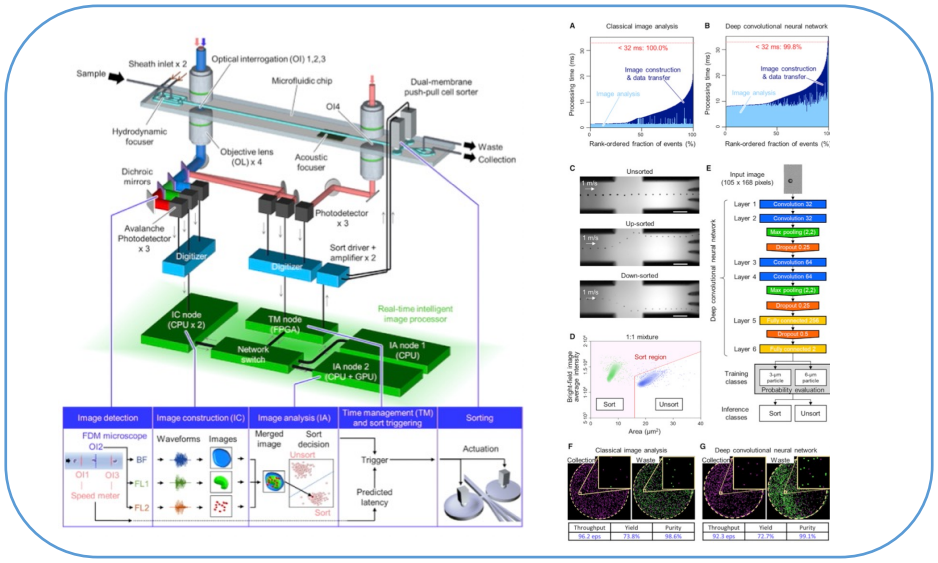
  e.g., A.Fabisiewicz, Medical Oncology, 2016



CTC cluster. Bar, 10 μm

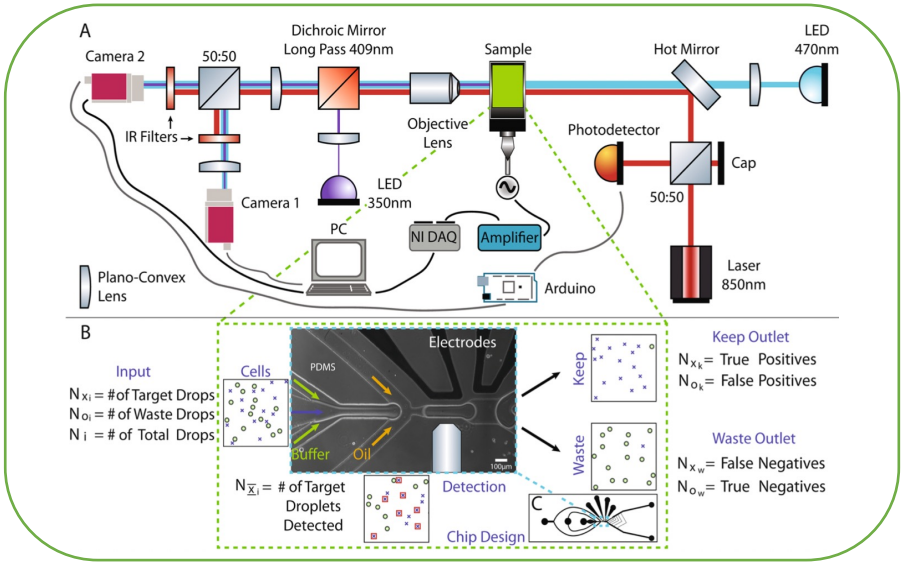
Advancement in image recognition and analysis technologies in the field of imaging cell sorting,

Machine learning based sorting



e.g., N. Nitta, et al., Cell., 2018

Droplet microfluidics



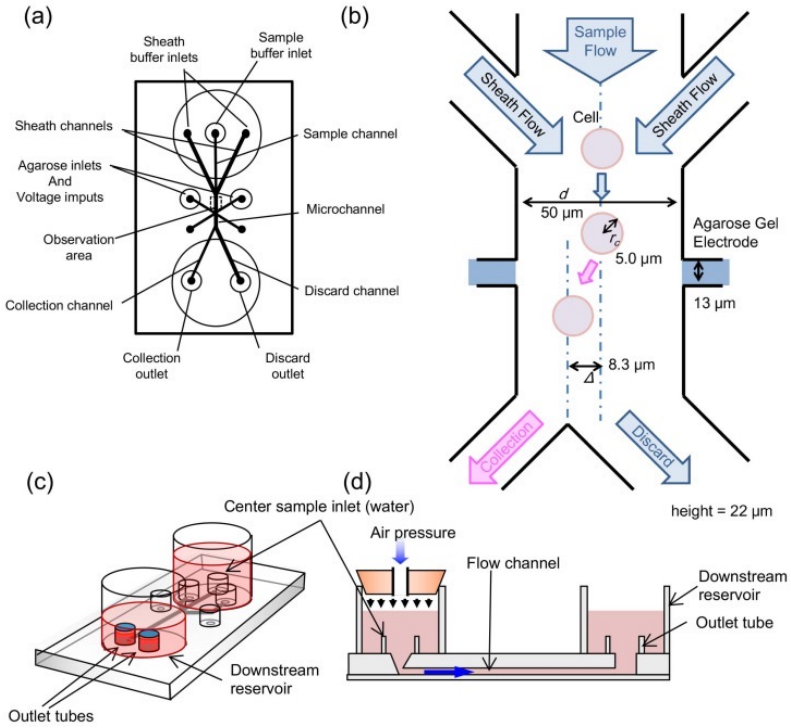
e.g., M. Sesan, et al.,Sci. Rep., 2020

Difficulty overcoming the precise measurement of microparticle flow speed for correct target collection

Introduction

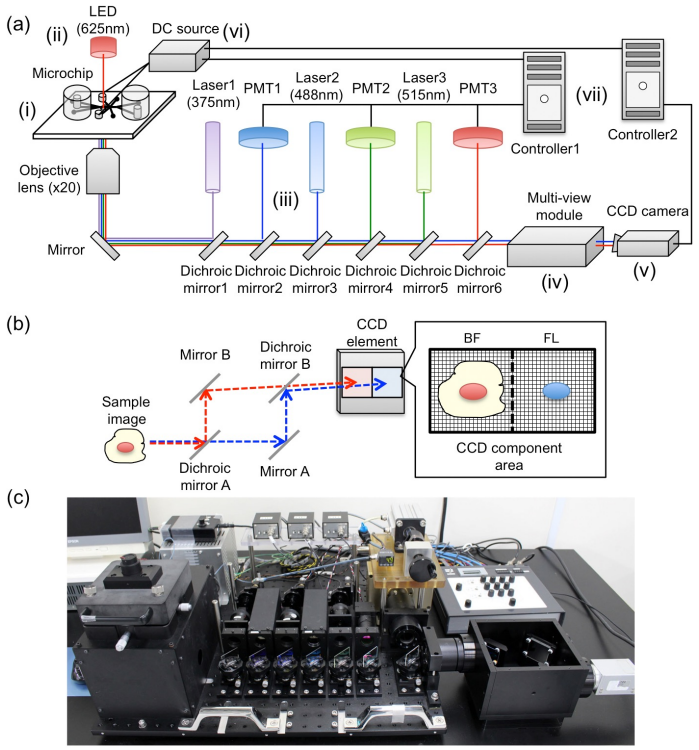
Development of a universal detection method of CTC clusters

Chip design



cf. M., Odaka, et al. Micromachines, 2019

System design



cf. H., Kim, et al. PLOS, 2014

Image recognition＋Applied voltage
Sorting by shape (area)

Fig. 1. Schematic of imaging cell sorter system.

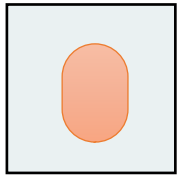
Objective

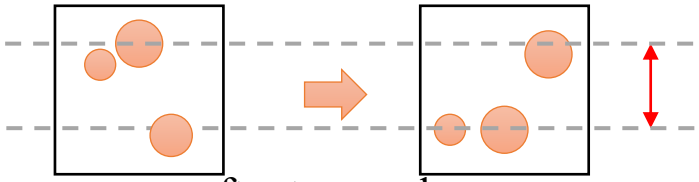
Development of a universal detection method of CTC clusters

Task

- Movement of particles while the shutter is open
- shape is not consistent → not the accurate size measurement

Causes elongation





Difficulty identifying the particles

Solution

- Development of a velocity measurement technology requiring only single-capture of the object.
- Estimation of the elongation and restoration via image processing

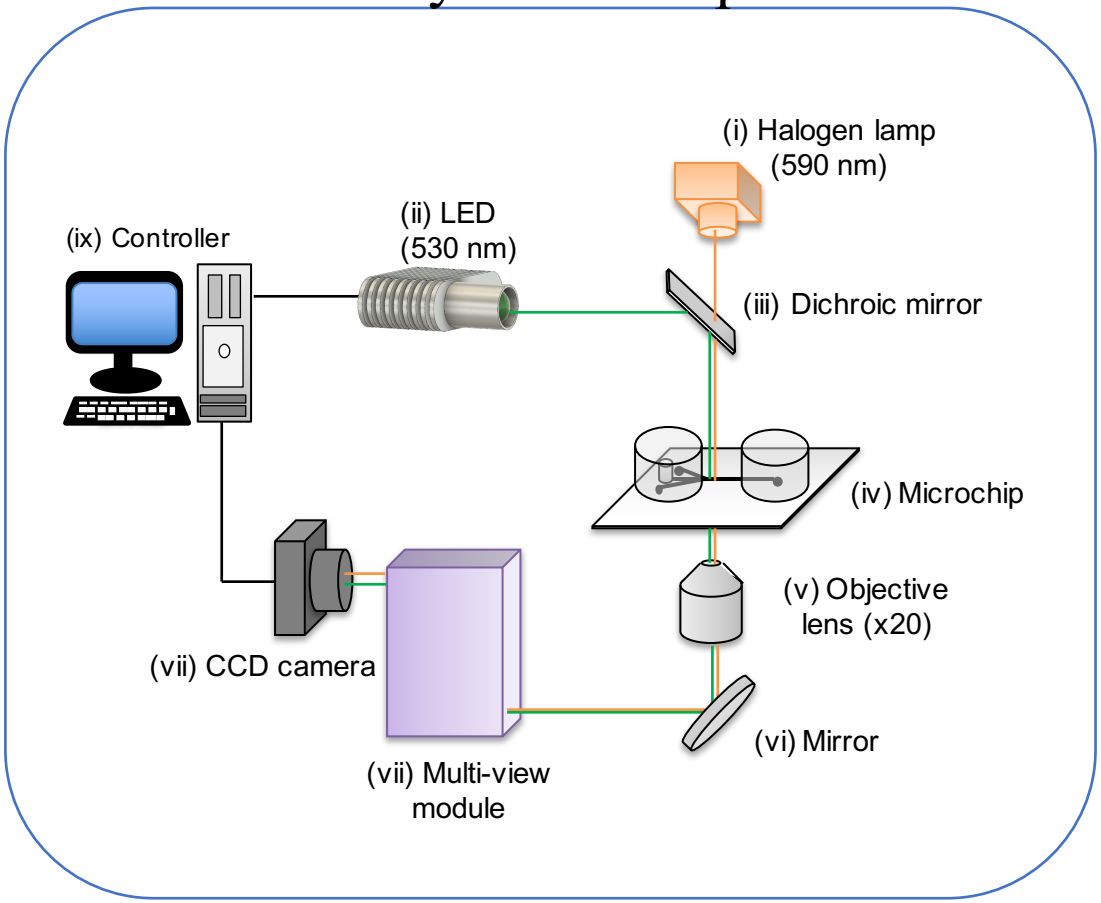
Essential to know the flow velocity

Results

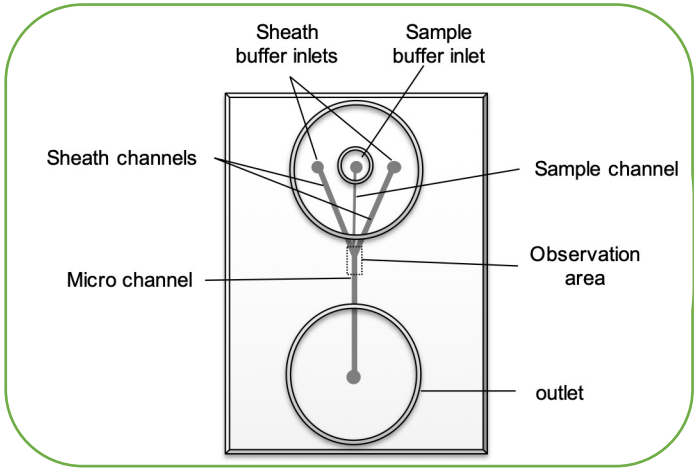
Image acquisition flow cytometer with simultaneous two-wavelength differential imaging

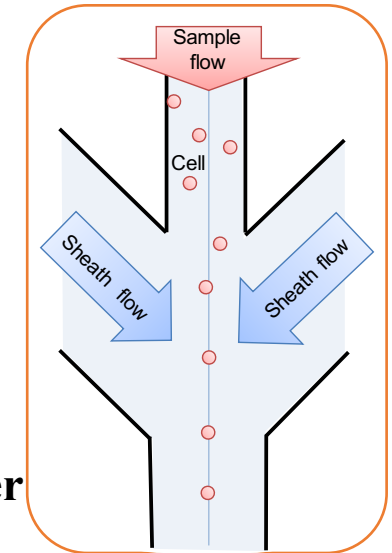
Proof of concept

System set up



Microchip design





Sheath flow to focus the dispersed particles

Fig. 2. Schematic of imaging acquisition flow cytometer

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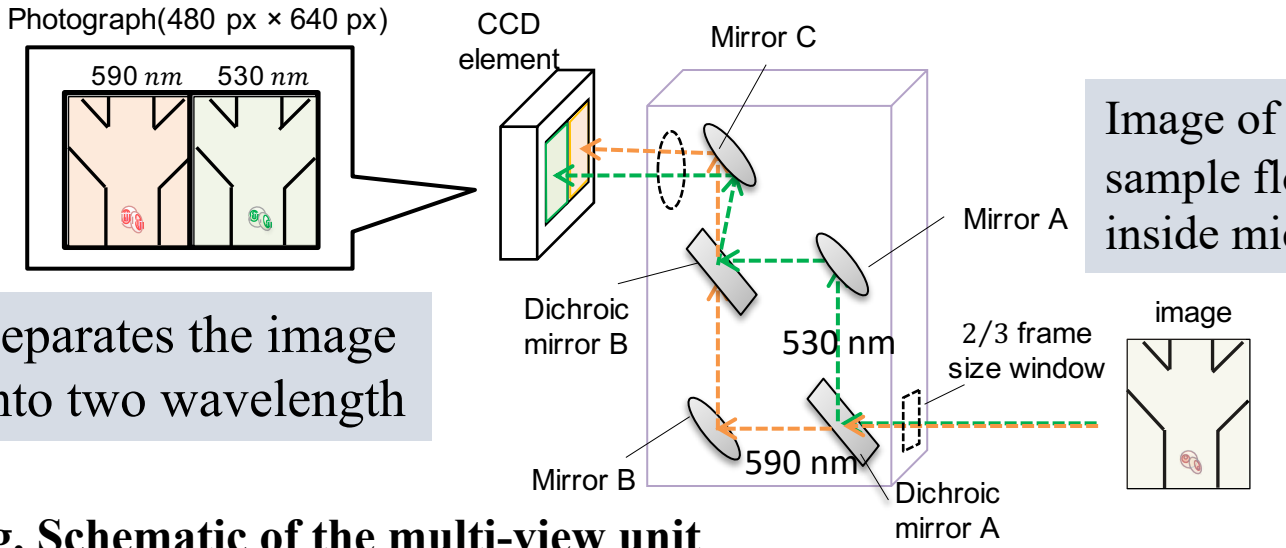
Principle

Multi-view unit

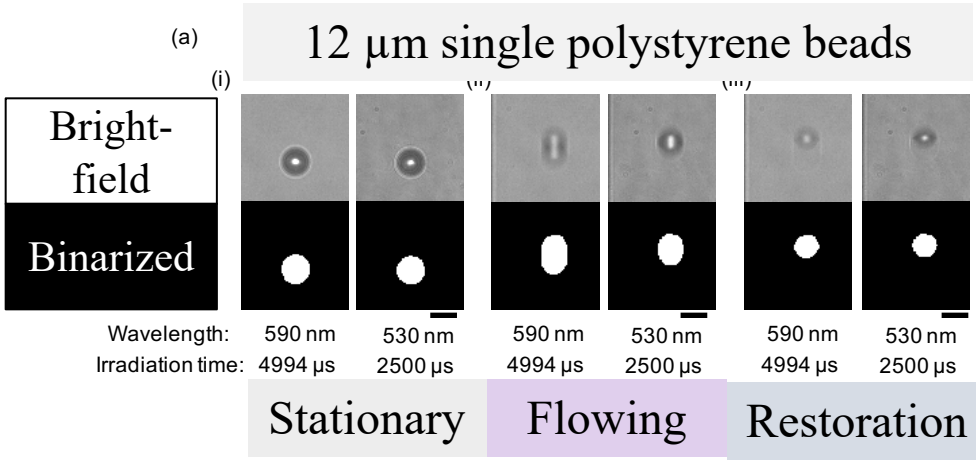


Separates the image into two wavelength

Image of a sample flowing inside microchip

Fig. Schematic of the multi-view unit

Results



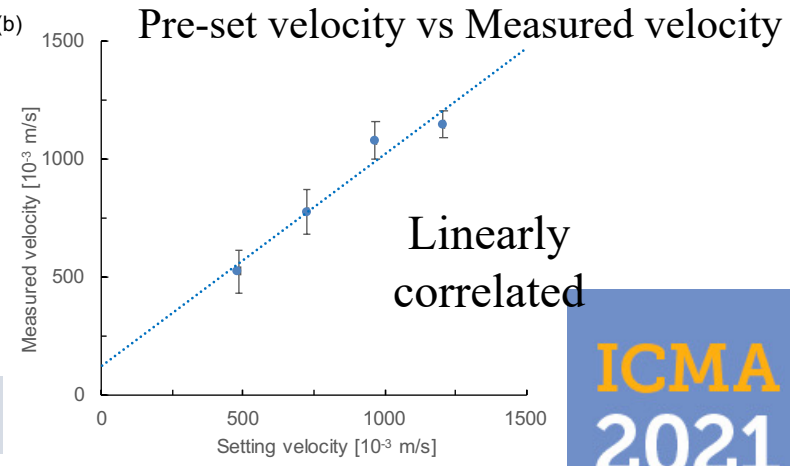


Fig. 3. Original and binarized images of polystyrene beads

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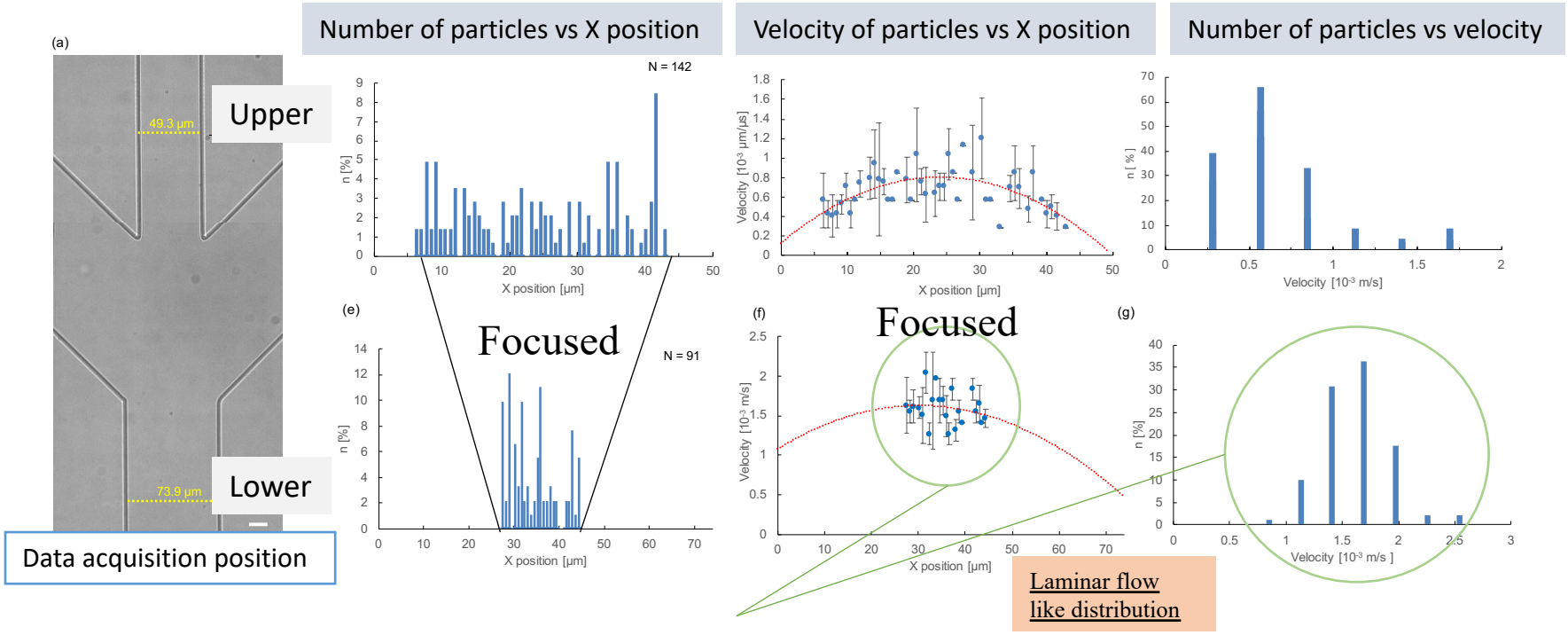
Results

Flow velocity distribution in microfluidic pathway



Even after the hydrodynamic focusing, the variation in flow velocity distribution remained present.

Fig. 4. Flow velocity distribution of particles for upper and lower stream

- Accurate flow velocity measurement is a prerequisite for determining the precise cell sorting timing to shift a target at the sorting point.

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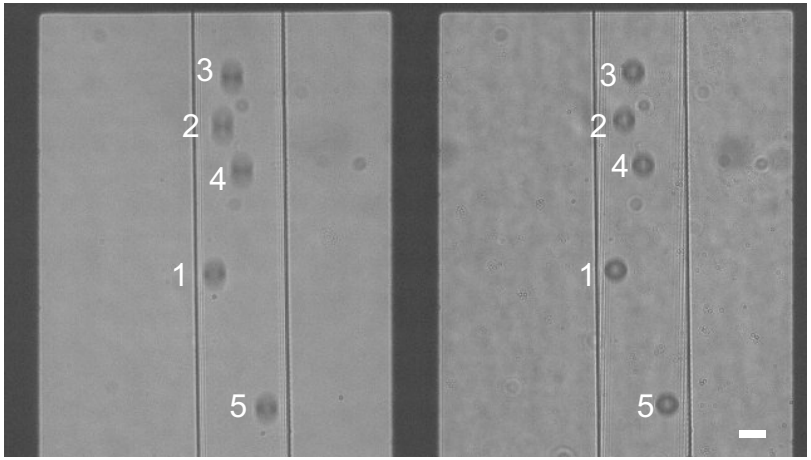
Results

Simultaneous flow velocity measurement of multiple particles

(a) Five particles flowing simultaneously

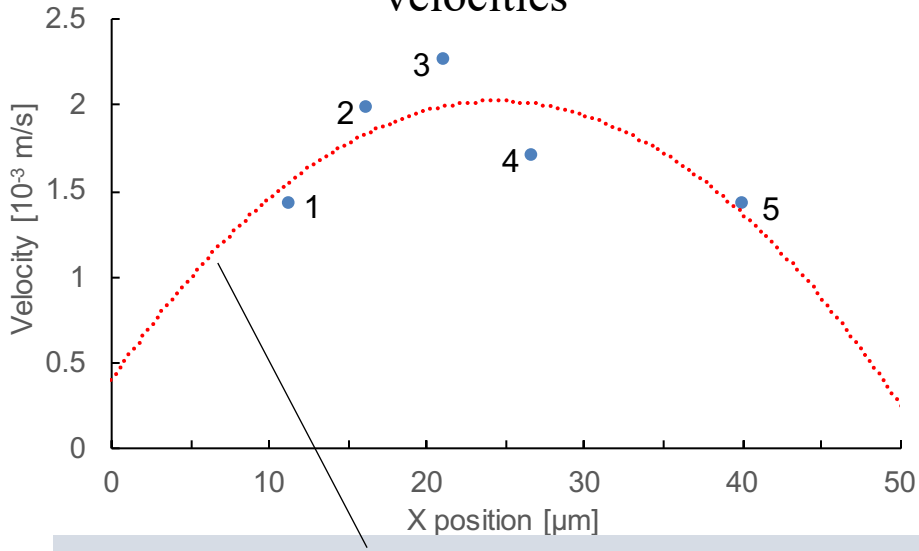


Bar, 15 μm

Wavelength : 590 nm 530 nm

Irradiation time : 4994 μs 2500 μs

(b) Measurements of the five observed particle velocities



Quadratic approximation of laminar flow

Fig. 5。 5. ow velocity measurement of multiple particles and its X positions

- Two-wavelength images of five particles were obtained
- The elongation was different depending on the flow velocities of each particles.
- The velocity was dependent on the location of microchannel.

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Results

Conventional restoration method versus the proposed method

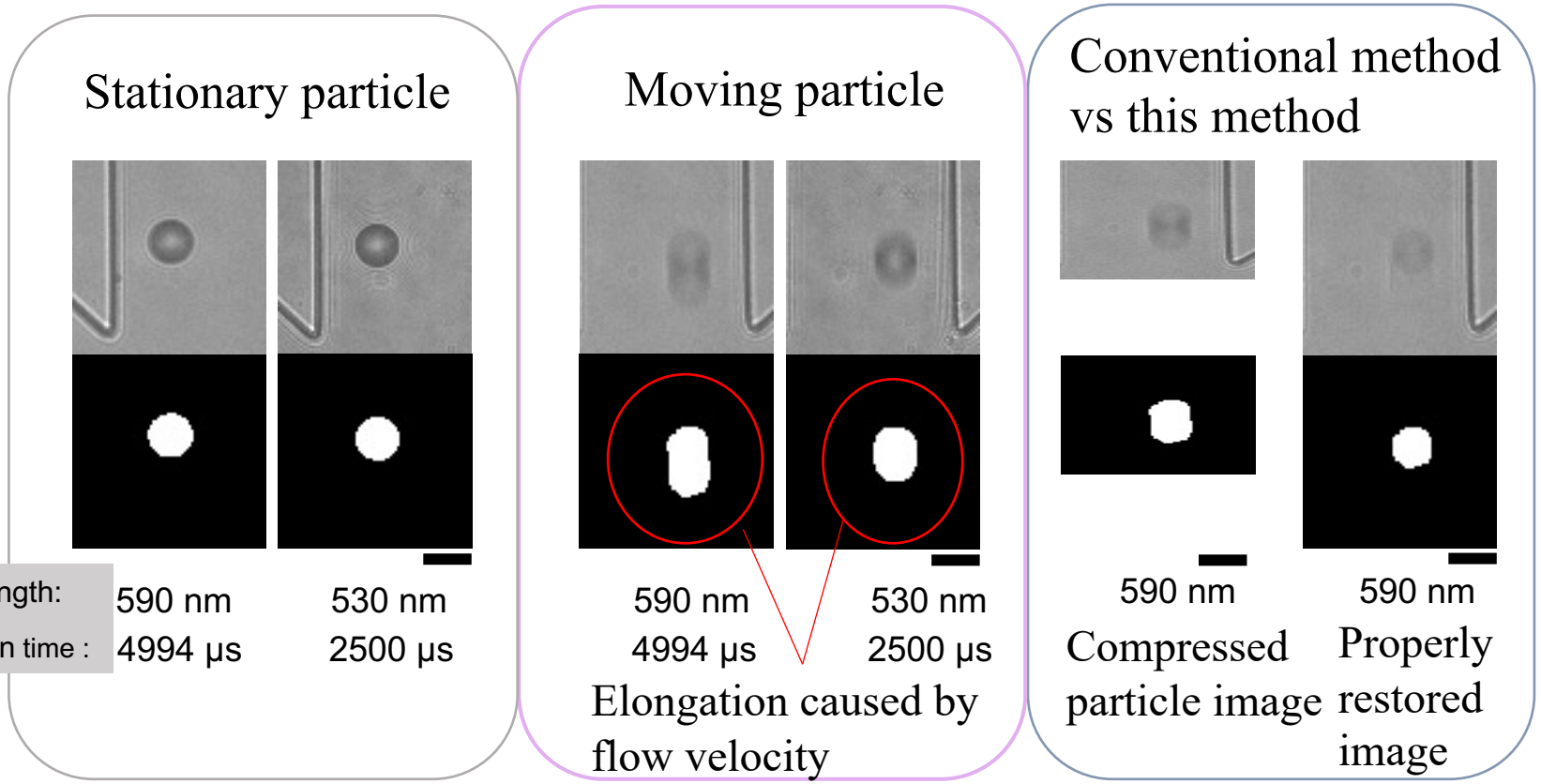


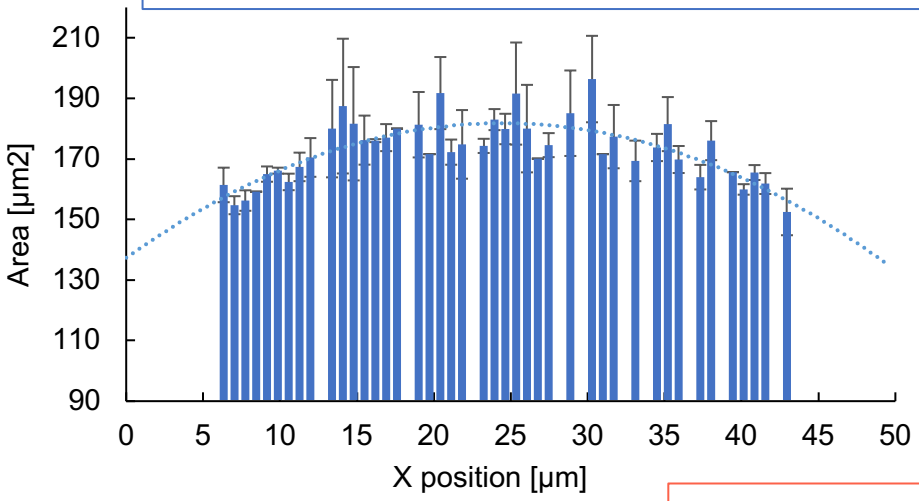
Fig. 6. Shape comparison between conventional and our restoration method

- Conventional method compresses the particle to restore the image
- The proposed method lifts the elongated particle up according to the calculated velocity

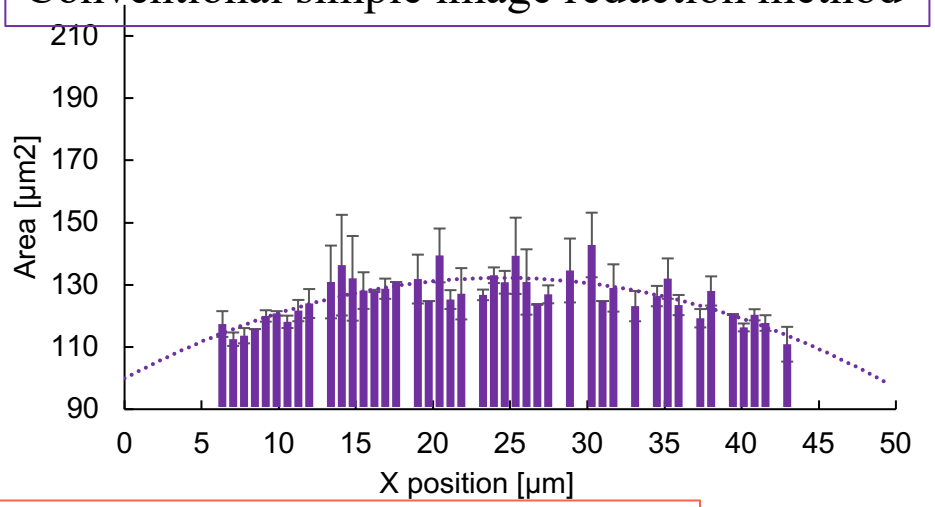
Results

Distribution of the averaged area of the particles at different X positions

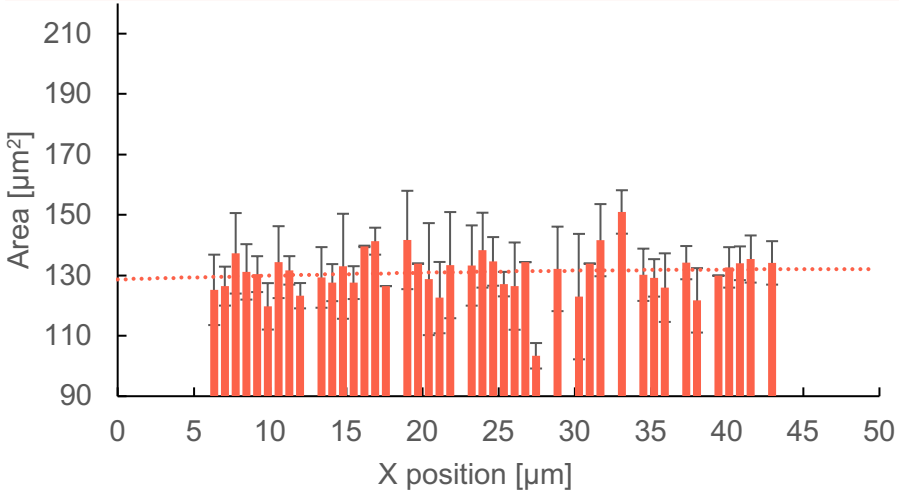
Without using any restoration method



Conventional simple image reduction method



Area of the restored particles using the method



- The size of the polystyrene beads is more consistent at various X positions of the microchannel

Fig. 7. Graph of area of the particles at X positions

Discussion

Limitation of the method for imaging flow cytometry measurement

- Resolution and preciseness of the measurement is reliant on the hardware capability
- The difference in irradiation times of the two wavelengths acquisition must be greater than the curve (left graph) for elongation to appear
- If multiple particles appears within 80x80 pixel image, accurate particle recognition cannot be performed
  - ➢ must adjust the density and the velocity of the flowing particles (less than the linear plot)

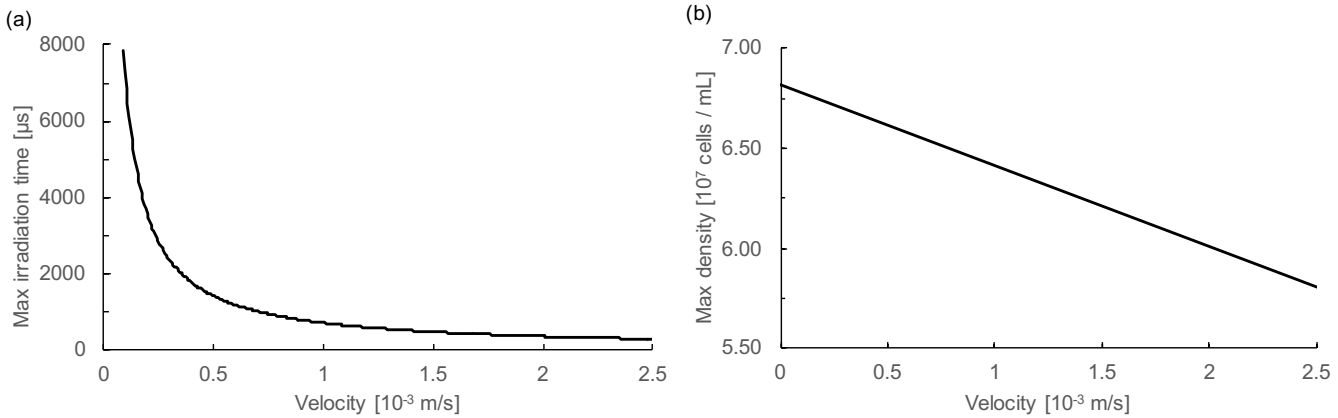


Fig. 8. Relation between velocity and max irradiation time (left) and max density (right)

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Discussion

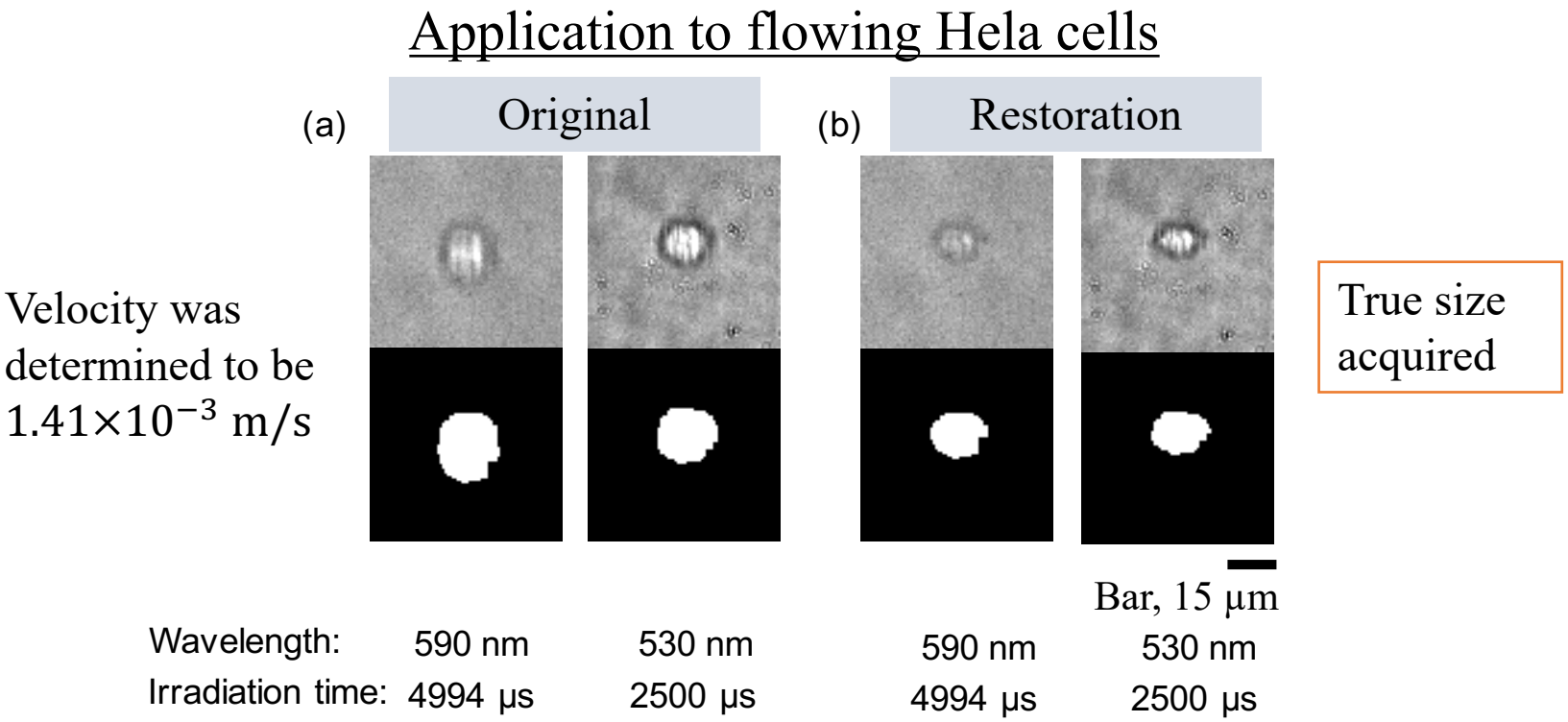

Fig. 9. Images of the flowing Hela cell before and after restoration

- The area of the captured cell image after binarization is 293.5 μm^2
- The area size of the Hela cell is 170.6 μm^2 after restoration.

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Conclusions

1) Developed a system for measurement of flow velocities of each samples and reconstruction of size information from single image acquisition

2) Contribute to precise target recognition and target collection timing for diagnostic screening, such as circulating tumor.

In detail, please visit our publication of this issue:

Sawa, S.; Sentoku, M.; Yasuda, K. On-Chip Multiple Particle Velocity and Size Measurement Using Single-Shot Two-Wavelength Differential Image Analysis. *Micromachines* **2020**, *11*, 1011.

Acknowledgments

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