

## ABSTRACT :

Flow cytometry has become a critical tool for quantitative cellular analysis over the past 40 years. Current systems detect scatter and fluorescence signal by illuminating a cell with laser spot significantly larger than the cell itself. In this mode, the laser spot is so-called “Top-Hat” shape for uniform illumination which brings a wider tolerance by reducing the spatial resolution. On the other hand, Gaussian laser beam has intrinsically higher spatial resolution and peak intensity. The motivation for this work was to provide a new method for analyzing every cell features by using a smaller laser spot than the cells being analyzed.

The use of the flying-spot concept is well known in areas such as SEM, CRT, confocal microscopy and the optical disk. But in order to apply this concept to cytometry, the most advanced devices are mandatory : 405nm laser, acoustic optical (AO) device & driver, planar-type flow chamber design, custom objective lens, high speed detectors and a huge data acquisition system. Based upon cell size and optical tolerance, 2μm FWHM laser spot, 0.1~1.0m/s flow velocity, 100k~1,000kHz beam deflection were targeted as the proof-of-concept. Further, using 405 nm as the shortest visible wavelength has the advantage of reduced spot size and deflection RF power. In addition 405nm excitation can provide a broad wavelength range for fluorescence detection. We present a system using wavelength stabilized 405nm laser with a plane wave front & good Gaussian profile. Good beam deflection is the key for micro imaging applications combined with TeO<sub>2</sub> crystal for acoustic optical high-speed beam deflection. Finite optics were applied to minimize wave front distortion and satisfy fill conditions. The AO deflection frequency is 250MHz ±50MHz . Combined with an NA=0.16 objective lens, we have confirmed 40μm linear beam deflection scanning at 1MHz. We have also evaluated 2.1μmFWHM laser spot size on focused plane. Because of the planar flow chamber, the use of the small flying spot allows for the simultaneous detection of multiple cells on the focused plane. To achieve this concept, a unique flow chamber with hydrodynamic focused plane was developed. The prototype shows in-focus (hydrodynamic) flow at 0.1 ~ 1.0 m/s mean velocity using a flying spot velocity of up to 40m/s. In order to detect sufficient spatial resolution, we have developed 70MHz bandwidth detector modules. Si PIN diodes were used for 405nm signal detection and μPMTs were applied for high speed fluorescence detection. A new data acquisition system requiring 16bit/160MHz/4CH A/D converter was developed to capture high speed data. Using this data system, it is possible to record raw data on 1.5 TB solid state memory with 10.24Gbps. We have developed a prototype data analysis software allowing initial analysis with more extensive system under development. We have used beads and stained cell mounted on slides and flow chamber for live cell analysis is under evaluation for system performance and biological application.

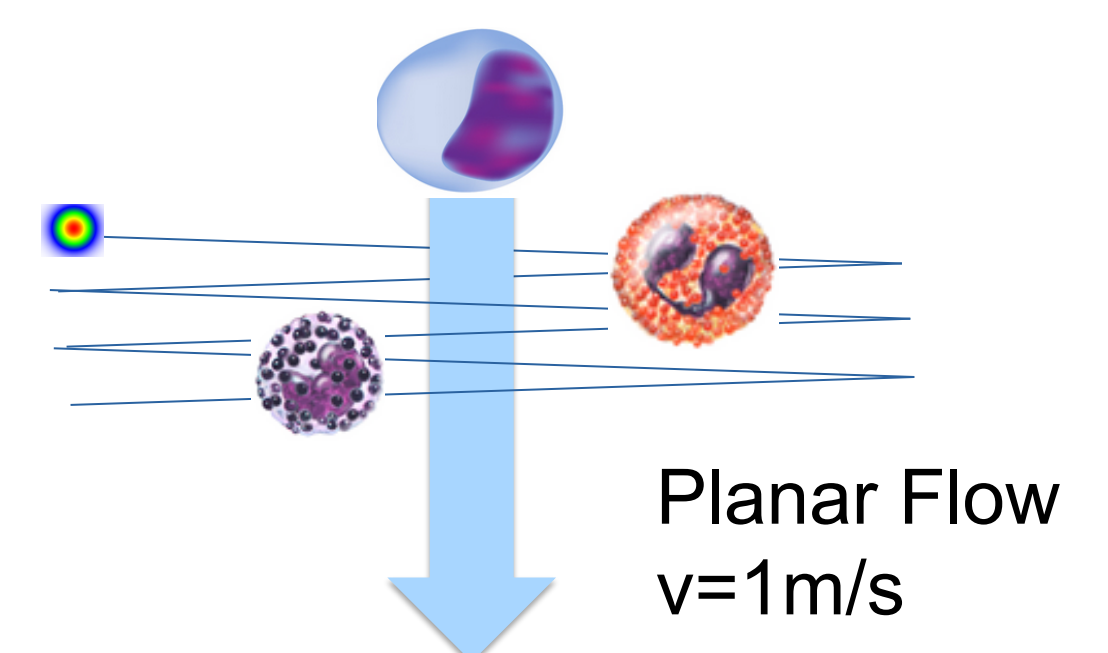
The first proof of Micro Imaging Cytometry is now demonstrated. Future development include migration of laser spatial resolution to 0.25 microns, extending into the UV region and development of unique analysis methods. Transforming from a larger to a smaller spot size than the cell may open new frontiers of cellular analysis for smaller particle detection, polarization analysis, photon fluorescence, time sequential analysis and 3D cell imaging.

## Motivation & Objectives

1. Observe individual cell with accurate dimension
2. Detect smaller structure and particles
3. Analyze Fluorescence photon
4. Integrate Flow and Image cytometry
5. Provide new method for cellular analysis
6. Ultimately, 3D cell scanner

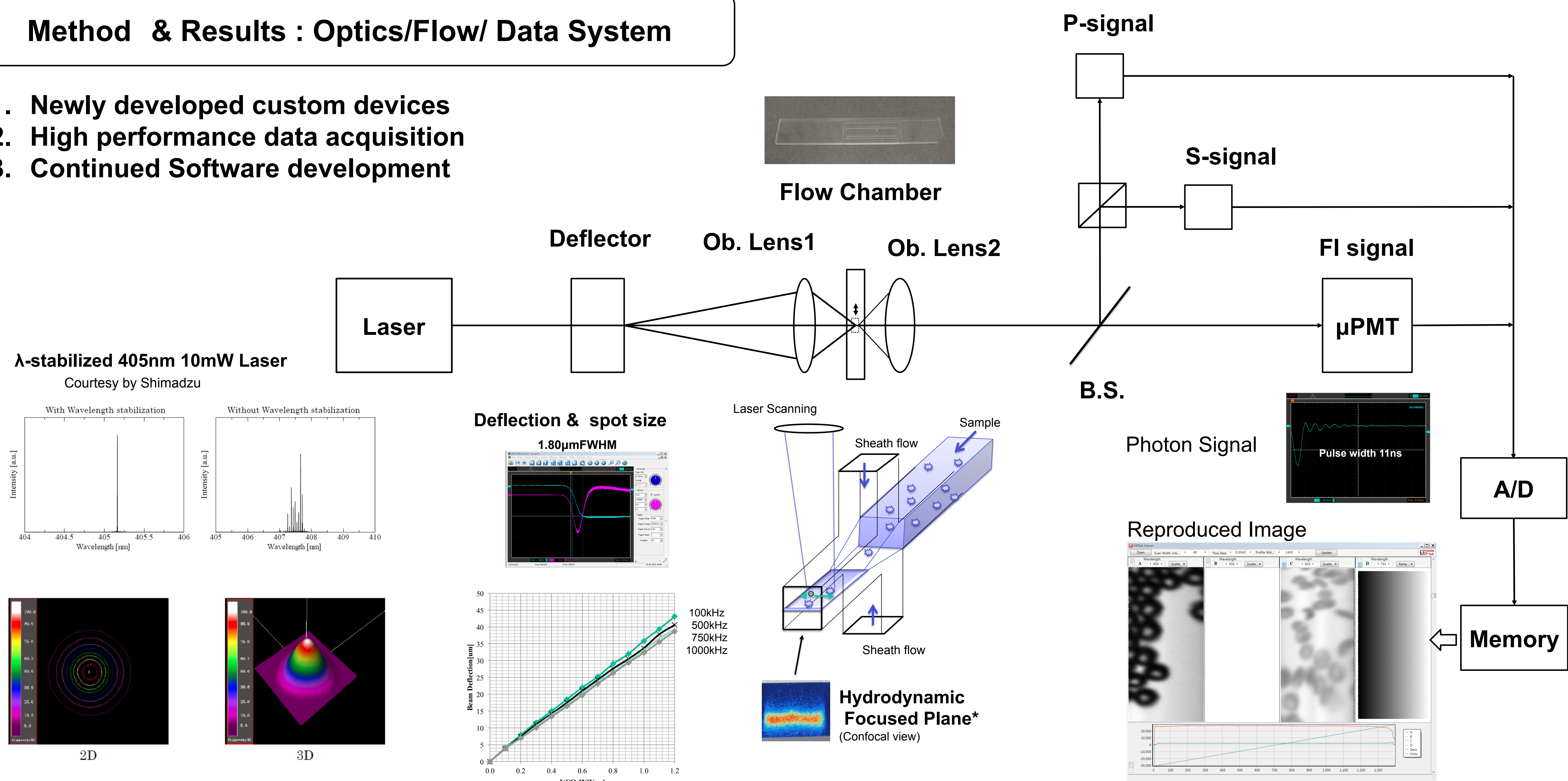
**Key Concept: Flying laser spot smaller than cell**

- |                           |                        |
|---------------------------|------------------------|
| 1. Smaller Spot than cell | < 2 $\mu\text{m}$ FWHM |
| 2. High speed deflection  | 0.1~1 MHz              |
| 3. Deflection Range       | 0 ~ 50 $\mu\text{m}$   |
| 4. Planar Flow Velocity   | 0.1 ~1m/s              |
| 5. High speed detection   | 70MHz BW               |
| 6. Data Acquisition       | 16bit / 160MHz         |



## Method & Results : Optics/Flow/ Data System

1. Newly developed custom devices
2. High performance data acquisition
3. Continued Software development



## Discussion : time domain & analysis

1. Potential capability of spatial resolution  $\sim 0.25\mu\text{m}$  FWHM
2. Parameter trade-off and harmonization for application
3. Contribution for unmet need in cellular analysis
4. Compatibility to conventional flow cytometry
5. Influence to cell analyzed – damage, etc.
6. Fluorescence detection – spatially integrated to localized
7. New function for cellular analysis

## Conclusion

**Micro Imaging Cytometry is developed by smaller spot concept.\*  
Transforming basic parameter for flow cytometry may open new  
frontiers in cellular and intercellular analysis.**

## Acknowledgement

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\* Patents Pending

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