

# Microfluidic Projection Mask Imaging

## The problem:

There is a need to analyze particles or biological cells at high rates. Flow cytometry (FC) and Imaging Flow Cytometry (IFC) are used to tackle this need. Currently, the capabilities are limited.

Several types of high-throughput instrumentation for analyzing and quantifying different aspects of cell biology are now available. They include plate readers, sequencing platforms, DNA, RNA and protein microarrays, Western blotting, flow cytometers, and so on. However, many platforms allow readouts only on the population level. Technologies that allow readout at the single-cell level include flow cytometry (FC), imaging cytometry, and different automated microscopy setups. These platforms are essential for assaying diversity within cell populations and searching for rare cells with specific features (stem cells, etc.).

Cytometry assays are sensitive, fluorescence-based methods aimed at determining a molecular phenotype of single cells. They can be multiparametric, multiplexed, quantitative, and qualitative. They can also be extracted as a result of kinetic or single end-point measurements. FC allows for the simultaneous quantification of multiple fluorescent emissions and the scattered light of single cells acquired in the laminar flow of cell suspension. FC is a technology that measures the cellular properties (protein expression, siRNA expression, etc.) in a snapshot of the entire population. Imaging cytometry (IC) is represented by two different types of technology: 1) high-throughput microscopy and laser scanning cytometry, which interrogates cells or tissue specimens *in situ* positioned on the microscope slide or in the microplate wells, and 2) imaging flow cytometry (IFC), which interrogates cells and cellular aggregates in the laminar flow.

Some of the applications currently used in a number of fields (e.g., molecular biology, pathology, immunology, synthetic biology, etc.):

- routine cancer screening via the detection and analysis of rare cells (i.e., low rate of occurrence) in peripheral blood, i.e., searching for circulating tumor cells: In cancer screening, the target cells are sloughed into the bloodstream from nascent cancerous tumors.
- hematology
- tumor immunology and chemotherapy
- prenatal diagnosis: In prenatal testing, the target cells are fetal cells that cross the placental barrier into the mother's bloodstream.
- The eukaryotic cell is a highly structured, three-dimensional object containing a wide range of molecular species. The size, shape, and structure of the cell, as well as the abundance, location, and co-location of any of these constituent biomolecules may be of significance in any given clinical situation or research application.
- Cell processing includes the determination of cellular morphology parameters such as overall size, nuclear size, nuclear shape, and optical density, the detection and characterization of numerous fluorescent markers and FISH probes, the quantification of the total amount of DNA in the nucleus, and the detection of other cellular components such as fetal hemoglobin.
- genetics and sperm sorting for sex preselection

- detection of DNA damage, caspase cleavage and apoptosis
- Co-localization of two proteins
- Binding of two cells
- Changes in cell morphological
- Immune synapse formation
- Nuclear translocation
- Cell counting
- Cell sorting
- Detection of biomarkers
- Protein engineering
- battle field monitoring of known airborne toxins
- monitoring of cultured cells to detect the presence of both known and unknown toxin
- non-invasive prenatal genetic testing
- discrimination of cancerous from normal epithelial cells
- the high throughput quantitation of FISH probes in human peripheral blood mononuclear cells.
- characterization of this array of constituent molecules by imaging or flow cytometry provides insight into the physiological function of any particular cell or alternatively, pathological changes that may have occurred or accrued
- cellular evaluation by imaging technologies and flow cytometry provides significant information reflecting the particular cellular phenotype, both normal and pathological.

The current technology used is flow cytometry, which, “flow cytometry is a laser-based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second”

However, improvements are needed. Needed properties:

- high speed measurement;
- the ability to process very large or continuous samples;
- high spectral resolution and bandwidth;
- good spatial resolution;
- high sensitivity;
- low measurement variation

### **Some numbers for the problem**

- The target cells may be present in the blood at concentrations of one to five cells per billion. This concentration yields approximately 20-100 target cells in a typical 20 ml blood sample
- The extreme rarity of the targeted cells demands that any detection and analysis system employed in these applications be capable of processing an enriched sample of approximately 100 million cells within a few hours, corresponding to a minimum throughput of 10,000 cells per second.

- The system must be able to collect cell images with a spatial resolution of approximately 1 micron.
- The system must have high spectral resolution and bandwidth to differentiate four or more fluorescent colors
- Since some probes may label important cellular features with only a few thousand fluorescent molecules, the system must have high sensitivity and good measurement consistency to differentiate very weak signals.

## Plain ol' imaging

Conventional imaging is extremely powerful and well-developed. However, as always, there is a trade-off between acquisition speed, sensitivity, and extracted information. Microscopy provides a wealth of information, but data acquisition rates are slow and analysis is generally subjective. In flow cytometry, data acquisition is rapid and better suited for the evaluation of pathologies present in low frequency, but the data are only intensity-based, thus lacking the morphology that truly lends credence to the analysis

A confocal microscope can produce highly detailed fluorescent cell images, including three dimensional cell representations based on multiple stacked images, but it can take as long as several minutes to produce a high resolution 3D representation of a single cell. Absolute fluorescence sensitivity is also generally lower in confocal microscopy than other techniques because out-of-focus signals are rejected by the confocal optical system and because the image is built up serially from individual measurements at every location across the cell, reducing the amount of time available to collect signal. Sensitivity may be increased in a single image by dwelling over the cell for a longer period of time, but this can cause excessive photobleaching outside of the plane of focus, hindering 3D imaging. The design features associated with confocal microscopy make it well-suited to applications that require the accurate analysis of sub-cellular features in homogeneous samples, but poorly suited to detecting faint fluorescent probes or evaluating statistically significant numbers of cells within heterogeneous samples.

Standard microscopy can image cells in a variety of modes (transmitted light, scattered light, fluorescence, phase contrast, etc.), each of which provides distinct and complementary information about the cell. For a given imaging rate, fluorescence sensitivity is generally better than confocal microscopy and given sufficient time to integrate signal, sensitivity can even exceed flow cytometry. In automated form, standard microscopy can be both quantitative and relatively fast, with a typical throughput of several hundred cells per second. Though the spatial resolution of standard microscopy is not as good as confocal microscopy, it is sufficient to resolve many sub-cellular compartments and structures, making it the standard of practice for clinical cytologic evaluations.

## Flow Cytometer: How it works:

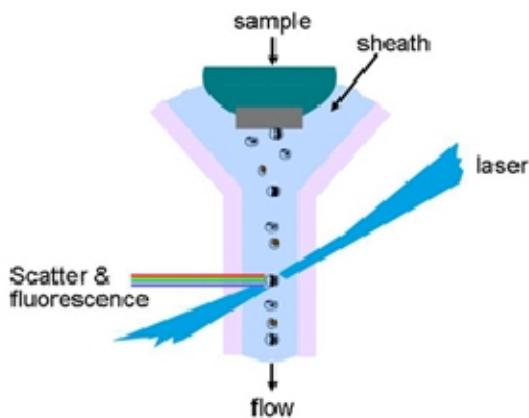
Flow cytometry is the measurement of cells in a flow system, which delivers the cells singly past a point of measurement. Flow cytometry sacrifices imaging entirely in favor of high acquisition rates and fluorescence sensitivity. In flow cytometry, each detection event (cell) is associated with several numerical measurements of fluorescence intensity and the degree of forward and side scatter of laser light. Forward scatter is roughly correlated to the size of the cell and side scatter gives an indication of the cell's granularity, but flow cytometry offers no means of sub-cellular fluorescence localization. The strength of flow cytometry is that it allows the rapid analysis of large populations of cells. Typical analytical throughput is 5,000 cells per second, which means that even rare cell populations (i.e. less than 1 cell in 10,000) can be detected in statistically significant numbers in a reasonable period of time. Fluorescence detection limits are often less than 100 molecules per cell. The value of the technique lies in the ability

to make measurements on large numbers of single cells within a short period of time (tens of seconds to minutes). The heterogeneity of populations can be revealed and different subsets of cells identified and quantified. Selected cell populations can also be physically sorted for further study.

The major disadvantage of flow cytometry is that it requires a suspension of single cells or other particles, with minimum clumps and debris. This means that the tissue architecture and any information about the spatial relationship between different cells are lost when single cells or nuclei are prepared

The first fluorescence-based flow cytometry device (ICP 11) was developed in the year 1968 by Wolfgang Göhde from the University of Münster, Germany (Patent No. DE1815352) and first commercialized in 1968/69 by German developer and manufacturer Partec through Phywe AG in Göttingen. At that time absorption methods were still widely favored by other scientists over fluorescence methods.

The flow chamber lies at the heart of the instrument. It is designed to deliver the cells in single file at the point of measurement. The sample is injected into the center of a stream of liquid (water or buffer), called the sheath fluid. If the flow is unperturbed, the sheath fluid and the sample stream do not mix and the latter is narrowed, typically to a diameter of about  $10\text{ }\mu\text{m}$ , constraining the cells to move through the centre of the chamber. At this point, light is focused



The light beam must be focused onto the sample stream. This can be accomplished by a simple lens giving a beam cross-section of, typically, about  $50\text{ }\mu\text{m}$ . Some instruments use a elliptic lens to produce a  $20 \times 60\text{ }\mu\text{m}$  elliptical beam. An alternative configuration is a crossed cylindrical pair of lenses that can produce an elliptical spot of, typically,  $5 \times 120\text{ }\mu\text{m}$ .

Spherical, or near-spherical, beams are used with stream-in-air systems in which the diameter of the beam has to be less than that of the stream in order to minimize excessive light scatter from the stream-air interface. The higher speed of the cells in this system gives an acceptably fast signal pulse ( $2\text{-}7\text{ }\mu\text{s}$ ) with the wide laser beam. The whole cell is illuminated as it passes through the beam.

The collection lens should have as high a numerical aperture as possible in order to collect as much of the fluorescence as possible. In analysers, with cuvette flow cells, as opposed to cell sorters, it is possible to use a shorter working distance, including immersion objectives, and hence obtain a higher numerical aperture.

## Imaging Flow Cytometer: How it works:

Imaging flow cytometry combines the statistical power and fluorescence sensitivity of standard flow cytometry with the spatial resolution and quantitative morphology of digital microscopy.

A number of techniques have been developed for imaging cells in flow, starting very shortly after the advent of conventional flow cytometry. Approaches have included strobbed illumination techniques, flying spot scanning, mirror tracking, and slit scanning flow cytometry. The challenges associated with imaging cells in flow include achieving sufficient fluorescence sensitivity, producing imagery with high spatial resolution, combining fluorescence imagery with other imaging modes such as brightfield (transmitted light) or darkfield (scattered light), and imaging all of the cells in the flow stream.

The details of the first imaging flow cytometer were published in an article from Leon Wheless' lab at the University of Rochester (Kay et al., (1979) J Histochem Cytochem 27:329). That system was a slit scanner based system with two detectors. The schematic of this system is shown below.

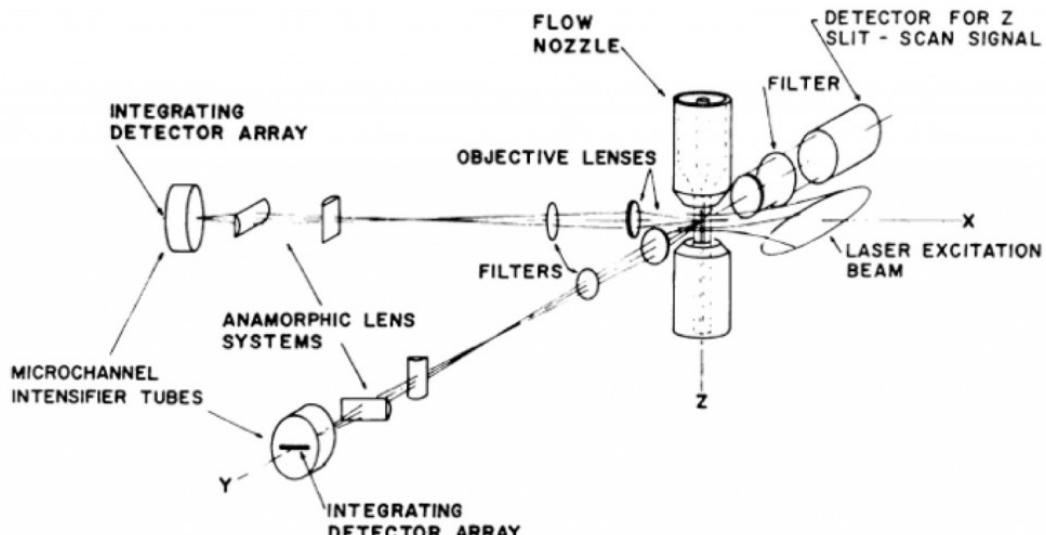
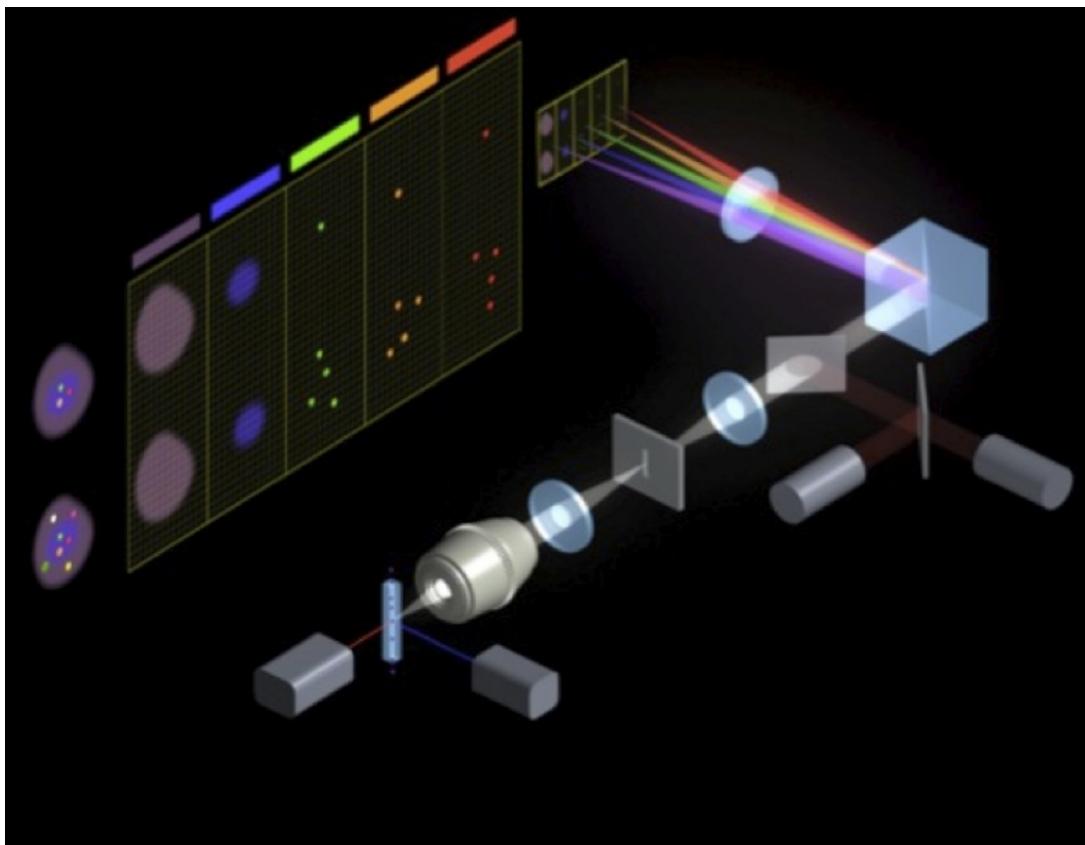


Fig. 5. Multidimensional (X-Y-Z) slit-scan flow optical system.

It took over twenty years before this technology was further developed. The intervening years saw improvements in detection systems, optics, and tools for measuring moving cells allowing the images to remain in focus. With these improvements George et al ((2004) Cytometry A 59:237-245), developed a new instrument, called the ImageStream (marketed by Amnis, now part of EMD Millipore).

The ImageStream system combines a precise method of electronically tracking moving cells with a high resolution multispectral imaging system to acquire multiple images of each cell in different imaging modes. The current commercial embodiment simultaneously acquires six images of each cell, with fluorescence sensitivity comparable to conventional flow cytometry and the image quality of 40X-60X microscopy. The six images of each cell comprise: a side-scatter (darkfield) image, a transmitted light (brightfield) image, and four fluorescence images. The imaging objective has a numeric aperture of 0.75 and image quality is comparable to 40X to 60X microscopy, as judged by eye. The throughput is up to 300 cells per second.

The ImageStream uses a custom CCD camera chip with a fanned filter stack so that specific wavelengths of light are directed to defined areas of the CCD chip. Additionally, to ensure that the cells are in focus, and to get the maximal signal, the ImageStream relies on a technique developed for measuring low light moving objects. This Time Delay and Integration (TDI) relies on a second laser and a co-injected bead. The velocity of the bead is measured to calculate velocity, and is characterized by two PMTs, set slightly ahead and behind the optimal focal plane for the flow cell. By keeping these two signals in balance, the ImageStream keeps the cells in the optical focal plane for the optics. The schematic for the first generation instrument is shown below.



Some of the limitations of the first generation instrument included the fixed lens (40X), capability for only 4 -color fluorescent detection, lack of automation and relatively slow acquisition rate. These were addressed in the second generation ImageStream.

The second generation ImageStream has two cameras, allowing for up to 12 simultaneous images (9 fluorescent). Additionally, it has up to three magnification lenses (20, 40 and 60X), extending the ability to image both larger and smaller cells.

A paper published by Ong et al. [Anal. Quant. Cytol. Histol., 9(5):375-82] describes the use of a time-delay and integration (TDI) detector in an imaging flow cytometer. A TDI detector is any pixilated device in which the signal produced in response to radiation directed at the device can be caused to move in a controlled fashion. Typically, the pixels of a TDI detector are arranged in rows and columns, and the

signal is moved from row to row in synchrony with a moving image projected onto the device, allowing an extended integration time without blurring. The approach disclosed by Ong et al. advanced the art by addressing the need for spatial resolution and high sensitivity for cells in flow. However, this approach does not address the remaining principal characteristics. The authors of this paper cite an operating speed of 10 cells per second and a theoretical speed limitation of 500 cells per second, which is at least an order of magnitude slower than is required for non-invasive fetal testing. In addition, the system has no spectral resolution; laser scatter and fluorescent light are collected by the imaging system indiscriminately.

In more recent developments, U.S. Pat. No. 5,644,388 discloses an alternative approach to an imaging flow cytometer. The patent discloses the use of a frame-based image collection approach in which a video camera views cells in flow, in a freeze frame fashion. This method requires the image collection system to be synchronized with the presence of cells in the imaging area, unlike the case of TDI, wherein the detector readout rate is synchronized with the velocity of the cells. When a cell is imaged with the frame-based method, the integration period must be very short to prevent blurring. A short integration time is achieved either with a strobed light source, or a continuous light source combined with a shuttered detector. In either case, the short integration time reduces the signal-to-noise ratio and the ultimate sensitivity of the approach. Further, frame-based cameras require time to transfer data out of the camera, during which no images are acquired, and cells of interest can escape detection. Finally, like the work of Ong et al., this patent makes no provisions for acquiring data over a large spectral bandwidth and with sufficient spectral resolution to simultaneously resolve numerous and differently colored fluorescent probes and FISH spots.

## Challenges Associated with IFC Studies

Traditional microscopic methods have many advantages compared with IFC; they have better spatial resolution and allow analysis of spatial-temporal organization of the samples, time-lapse experiments with single cells, and so on. However, a limited number of cells are evaluated, and until recent introduction of automated systems, operator bias always had to be considered. IFC has common traits with high-throughput microscopic systems, such as moderate resolution ( $0.5\text{--}1.0\ \mu\text{m}$ ) and the capability of analyzing thousands of cellular events in a reasonable time. On the other hand, high-throughput fluorescent microscopy (HTFM) has advantages of time-lapse analysis of cells and parallel analysis of hundreds of markers, and no perturbation is used for adherent cells.

The acquisition timescale in IFC ranges in minutes, all the way from tenths of a minute to 100 min needed to push through tens of thousands of events from samples with a low cellular concentration. Several issues arise when trying to automatically image multiple live cells with the help of the IS series in IFC, such as the lack of availability of nutrients and oxygen over long time periods, because samples presumably are run in phosphate buffer solution (PBS). Focusing is a large contributor to the length of time required for analysis, because a large percentage of cells will be excluded from analysis due to focusing problems. Moreover, if fluorescence labeling with cell tracking and vital dyes is used, additional experiments are required to exclude phototoxicity as a reason for observed differences between the experimental and control cells.

## State of the art for flow cytometers:

Typical flow cytometers measure a parameter or two, such as the brightness of a fluorescent label applied to a cell.

The performance parameters are typically:

- 25,000-75,000 “events” per second
- Cells flow through the flow chamber one at a time very quickly, about 10,000 cells in 20 seconds or 500 cells per second
- As the cells pass through the laser, the fluorophores attached to the cells absorb light and then emit a specific color of light depending on the type of fluorophores. The fluorophores on the cells are used to label specific things on the cell
- flow velocities: Up to 150  $\mu\text{L}/\text{min}$  (2.5  $\mu\text{L}/\text{sec}$ )
- Quartz cuvette Fused silica with 250  $\mu\text{m}$  square sectioned internal channel

## State of the art for imaging flow cytometers:

The only commercially available imaging flow cytometers are the ImageStream (IS) 100 and X.

- moderate resolution (0.5–1.0  $\mu\text{m}$ )
- Maximal speed (up to 300 events/sec for IS-100 and up to 1000 events/sec for IS-X) can only be achieved with a relatively high sample concentration that is about  $10^7$  cells per milliliter (500,000 cells per 50  $\mu\text{l}$  of volume for IS-100 and 3 $\times$  less for IS-X).

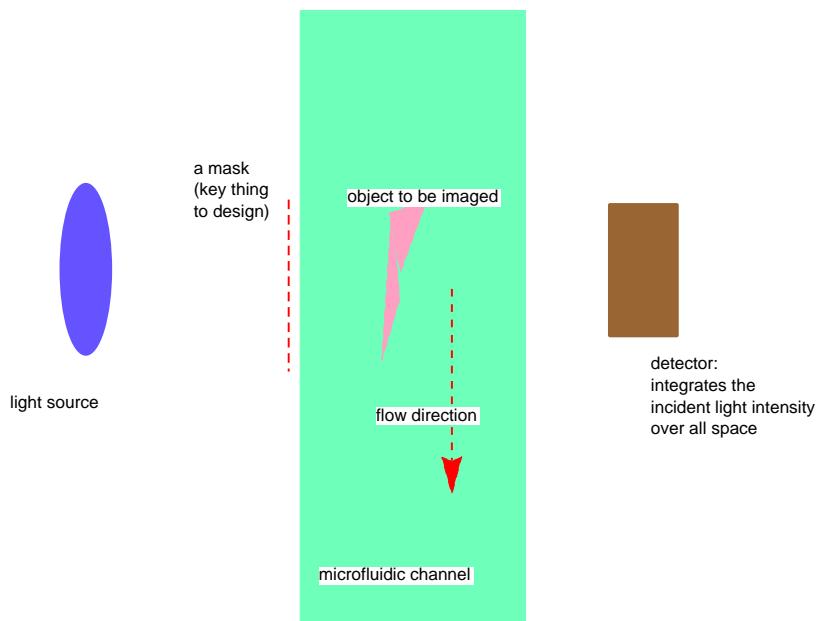
## The need:

There is great value in imaging cells at high speeds: ratio of nucleus to cell size can be an indicator of cancer cells, distributions of protein expression in cells, cell shapes, etc.

We would like to attain full imaging with sub micron spatial resolution at typical FC flow rates.

## The idea:

The basic sketch of the idea that we wish to pursue is shown in the Figure below:



This is a crude sketch, but shows the fundamental idea: objects pass through the microfluidic channel. A spatial mask (a variable intensity transmission mask in 2D) is placed on one side of the microfluidic channel. The mask is illuminated by a light source. Then, a detector collects light intensity (whether transmitted, scattered, or fluorescent light emission from the object). The detector generates a temporal signal,  $s(t)$ , that is used to then reconstruct the object.

We have a number of features:

- The object flows through the channel. The small channel means that the flow is Laminar. This implies that the fluid velocity has a quadratic profile across the channel width. (discussed more below) This parabolic velocity profile is a key factor in our mask design.
- The illuminated mask projects an illumination intensity pattern into the microfluidic channel.
- The detector collects light intensity from the microfluidic channel (could be fluorescent emission, or scattered/transmitted light from the illuminating beam).
- The object flowing through changes the light that is collected by the detector. This time dependent signal will be used to provide an object spatial image.

Why this approach?

- Why not use a camera instead of this proposed approach?
- Suppose we have a fast camera. We are set up with 1500 pixels. The frame rate is 500 Hz (very fast), or a ~2 ms integration time. The resolution of the imaging is set to 1 micron per pixel. If we want the object to translate for less than 1  $\mu\text{m}$  over the integration time, then we have a velocity of 0.05 cm/s. Typical microfluidic systems run at velocities of at least 1 cm/s.
- While cameras are limited to a few hundred Hz in update rates, a photodiode or PMT can have a bandwidth of >1GHz
- High speed cameras cost >\$20k, whereas a photodiode can be a few \$100: total cost will be a fraction of a microfluidic imaging system; the size will be significantly smaller
- The entire system can easily be integrated into a microfluidic chip

- can use LEDs rather than lasers
- MUCH higher imaging speeds will be possible than with current technologies.

The design problem

- Design a spatial mask and an algorithm that can recover 2D spatial images (in the plane of the 2D mask).
- Likely will involve solving for eigenfunctions of the parabolic shift operator.
- Design an optical imaging system to test the design.

## Flow in microfluidic channels:

Under conditions of laminar flow, we may write the velocity profile that is parabolic

$$v(x) = v_{\max} \left[ 1 - \left( \frac{x}{a} \right)^2 \right] \quad (1)$$

Here,  $v$  is a velocity in the  $y$ -direction along the transverse dimension,  $x$ . The total channel width is  $2a$ , so that  $x \leq a$ . We can also define  $\xi \equiv \frac{x}{a} \leq 1$ . We also have the relationship

$$v_{\max} = \frac{\Delta p}{L \mu} \frac{a^2}{2} \quad (2)$$

where  $\Delta p$  is the pressure differential along the channel length,  $L$  and  $\mu$  is the fluid viscosity.

Note that any rigid object at a particular channel position,  $x$ , will have a velocity set by its centroid.

## Axial Position

Here, we assume an incompressible fluid ...

Given the uniform velocity at each  $x$ -position, the position in  $y$  can be written as

$$y(x, t) = y_0 + \int_0^t v(x) dt' \quad (3)$$

This readily gives (assuming  $y_0 = 0$ )

$$y(x, t) = y_0 + v(x) t \rightarrow y = v(x) t \quad (4)$$

Note that the time-window will not be uniform as the transit time along the edges becomes increasingly large. We will assume that the mask has a height (along  $y$ ) of  $h$ , so that we can write a time-window across the modulator as

$$T_w(x) = \frac{h}{v(x)} = \frac{h}{v_{\max} \left[ 1 - \left( \frac{x}{a} \right)^2 \right]} \quad (5)$$

Clearly, we can't image to the edge, and as we will see, we will have an  $x_{\max}$  (with  $x_{\max} < a$ ) that will give us a maximum channel width over which we will observe.

## Microfluidic Mask Imaging Signals

The goal of this imaging system is to design an intensity transmission mask,  $M(x,y)$ , that is used to illuminate an object travelling in a microfluidic channel. The goal is to invert a temporal signal collected

from a photodetector to reconstruct an object image. We will assume that the light illuminating the mask is uniform, and that the light illuminating the object,  $O(x,y;t)$ , is then simply given by the mask, i.e.,  $I_{\text{ill}}(x, y) = M(x, y)$ . Under this assumption, we may write our photodiode signal as

$$s(t) = \int I_{\text{ill}}(x, y) O(x, y; t) dx dy = \int M(x, y) O(x, y; t) dx dy \quad (6)$$

Here, we assume that the object has a spatial pattern that evolves with time. If we consider a set of objects with center locations along the channel cross section coordinate,  $x$ , then we may write the total object as a superposition of rigid objects,  $\circ_j$ , with a center position,  $x_{\text{obj}}$ . This gives

$$O(x, y; t) = \sum_{j=1}^N \circ_j(x - x_{\text{obj}}; y(x_{\text{obj}}, t)) \quad (7)$$

The position in  $y$  is then set by the local center velocity:

$$y(x_{\text{obj}}, t) = y_{\text{obj}} + v(x_{\text{obj}}) t \rightarrow y = v(x_{\text{obj}}) t. \text{ This then yields}$$

$$y(x_{\text{obj}}, t) = y_{\text{obj}} + v_{\text{max}} \left[ 1 - \left( \frac{x_{\text{obj}}}{a} \right)^2 \right] t \quad (8)$$

This results in a signal of the form

$$s(t) = \sum_{j=1}^N \int M(x, y) \circ_j(x - x_{\text{obj}}; y_{\text{obj}} + v(x_{\text{obj}}) t) dx dy \quad (9)$$

In a continuum limit, where we assume that we have a set of very small objects, we can make a transformation:  $O(x, y; t) \rightarrow O(x, y(t))$ , then we simply obtain

$$s(t) = \int M(x, y) O(x, y + u) dx dy \quad (10)$$

where  $u = v(x) t$ . In a continuous limit, we collect these overlap projections for the entire length of the object, so we can take the  $y$  integral bounds to  $\pm\infty$ . The temporal signal will have a finite time provided that we remain away from the no slip (zero velocity) boundary of the channel.

The other version of this to consider is one rigid object, which leads to an integral of the form

$$s(t) = \int M(x, y) O(x, y + v t) dx dy \quad (11)$$

The question remains: can we recover the object information? We need a general 2D image recovery. In the following sections, I present a few intuitively motivated versions that only provide a 1D projection (either along  $x$  or  $y$ ).

Similar discussion from an older document:

Clearly, under laminar flow conditions, a rigid (or nearly so) body will translate at a uniform velocity.

Let's assume this is an evolution that can be described in the following way:

We assume we have narrow stripes along the width of the channel ( $x$ ) with a width given by  $w$ ; this will set the transverse spatial resolution, as the carrier frequency of each mask along  $x$  will map  $y$  information to a unique signal frequency region.

For each channel, we will model the  $y$ -direction imaging in 1D. Let's consider an object in given " $j^{\text{th}}$   $x$  bin" moving with a velocity along the  $y$  direction of  $v_j$ . Then, considering a 2D object,  $g(x,y)$ , it translates as  $g(x,y - v_j t) = g(x,y - y')$ .

The SPIFI signal will be collected by a single-element detector, and a 2D integral must be performed. We will assume uniform illumination of the mask, which has a transverse ( $x$ ) width of  $w$ , and a longitudinal ( $y$ ) length of  $L$ . The signal looks like

$$S(y) = \int_{-\frac{w}{2}}^{\frac{w}{2}} \int_0^L M(x, \psi) g(x, \psi - y) d\psi dx \quad (12)$$

## Chirped mask for imaging $y$ projections of the object that are spatially resolved along the $x$ direction:

The idea here is motivated by our earlier SPIFI imaging: provide a unique modulation frequency at each transverse spatial position along  $x$  so that we can correlate transverse spatial position with a particular modulation frequency.

The mask design motivated by this approach is:

$$M(x, y) = \frac{1}{2} + \frac{1}{2} \cos \left[ 2\pi \frac{\bar{\kappa}}{v_{\max}} \frac{(x + x_c)}{\left[ 1 - \left( \frac{x}{a} \right)^2 \right]} y \right] \quad (13)$$

We choose  $x_c = a$  to make sure that the mask monotonically increases across the channel width (that is we have no redundant modulation frequencies), then

$$M(x, y) = \frac{1}{2} + \frac{1}{2} \cos \left[ 2\pi \frac{a \bar{\kappa}}{v_{\max}} \frac{1}{1 - \frac{x}{a}} y \right] \quad (14)$$

## Chirped mask for imaging $x$ projections of the object that are spatially resolved along the $y$ direction:

This idea is motivated by ultrafast optics techniques. One chirps our the modulation mask along the flow direction.

The mask design motivated by this approach is:

$$M(x, y) = \left( \frac{1}{2} + \frac{1}{2} \cos [\alpha y + \beta y^2 + \phi] \right) \text{rect} \left( \frac{y}{L} \right) \text{rect} \left( \frac{x}{w} \right) \quad (15)$$

See more details in the Matlab simulation files