Matched-Filter Compressive Imaging using a Deformable Mirror for Label-Free Flow Cytometry

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Abstract: We distinguish geometric cell structures using a deformable mirror for high-throughput, label-free flow cytometry, overcoming approaches that can result in undesirable chemical modifications or low throughput.
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1. Introduction

Conventional flow cytometry uses fluorescent or scattered light to identify different cell populations within a fluid sample [1]. It is an important tool in medicine for identifying various diseases (such as Leukemia) [2] and other biological materials; it is also widely used outside of biological systems for analyzing particulates in fluids (such as fresh water, oil, and natural gas) [3], and controlling precise quantities of solute or fluid in a product [4]. In all applications, speed and throughput are critical for efficient analysis. Traditional cytometry techniques, however, require exogenous fluorescence labeling [5, 6], have camera-limited sampling rates [7], or are costly and complicated to implement [8]. We present a cytometry technique that provides label-free discrimination of multiple particle species based on their scattering properties, capable of operating at high speed.

2. Theoretical Basis

Under spatially coherent illumination, microscopic biological materials (such as single cells) scatter light into different regions of the pupil plane. The scatter pattern in the pupil plane is given by the Fourier transform of the particle's refractive index; as a result, particles with different physical structures (and hence different variations in refractive index) will produce different aberrations in the pupil plane. These variations are unique to the cell geometry, and therefore characteristic of the cell species. By using a new tip/tilt/piston deformable mirror (TTP-DM) to generate tilts in the pupil plane [9], we can filter regions in the pupil plane to high-speed photodetectors in the image plane, thereby recording specific combinations of the spatial frequencies of the cell. This matched filtering forms a compressed image of the cell used for discrimination.

2. Experimental Methods

We use a wide-field microscope configuration modified to include a Boston Micromachines Corp (BMC) Hex-337 deformable mirror (DM) in a pupil-conjugate plane. A Thorlabs 625nm LED is used for trans-illumination, and a 20x 0.46 NA Olympus objective is used to magnify the cells. A SensL high-speed quadrant detector is used to detect the four signals from light redirected into four quadrants in the image plane, and a Thorlabs GiGE Vision camera was placed in a conjugate-image plane to verify the shift of different spatial frequencies. Preliminary testing was done with static beads ranging from 1µm to 20µm in diameter, fixed to a glass slide. Flow is provided by a syringe pump to a lab-build microfluidic flow channel. Schematic of setup is depicted in Fig. 1.

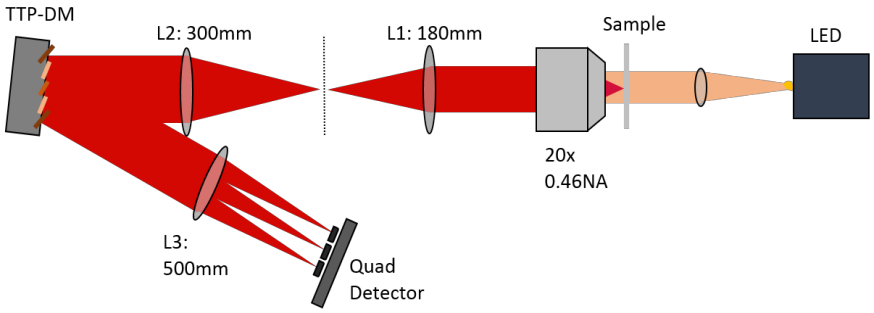

Fig. 1: MFCI flow cytometer system schematic. L1 and L2 chosen to ensure proper matching of 20x objective pupil onto the active area of the TTP-DM

3. Preliminary Data

We demonstrated feasibility of the MFCI flow cytometer by partitioning the TTP-DM into 4 sets of hexagonal rings. Each ring-set is given the same tilt, redirecting light to a different quadrant in the image plane; the resulting camera image was binned into four quadrants to simulate the signals on the SensL detector (Fig. 2).

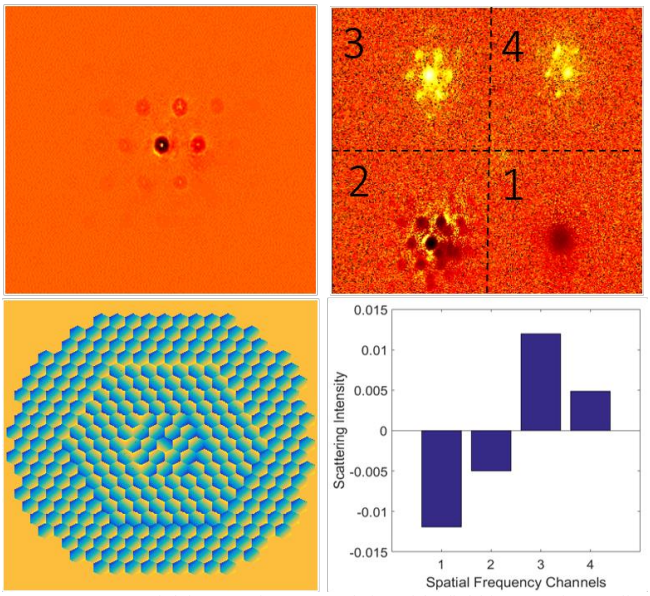

Fig. 2: Separation of single bead into component spatial frequencies. Upper left: Wide-field image of 4μm diameter absorbing bead. Upper right: image of bead after applying the pupil-plane filter; dotted lines indicate different quadrants on the camera. Lower left: shape of TTP-DM used to create filter. Lower right: mean signal in each quadrant of camera image, simulating SensL detector data.

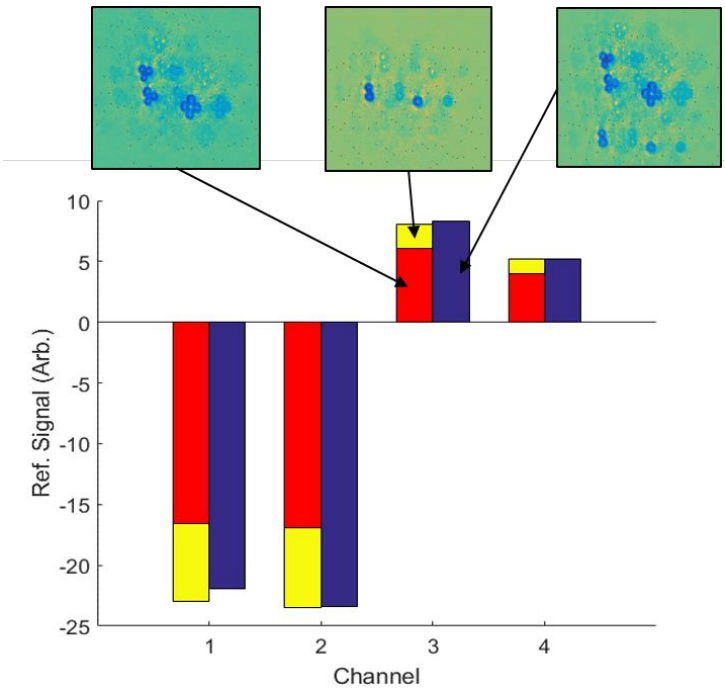

Fig. 3: Linearity of scattering intensity for multiple particle populations. Different arrangements of 4μm beads produce distinct signals in the image plane; red indicates signal from left arrangement, yellow indicates signal from central arrangement, and blue indicates signal from right arrangement. Imaging both the left and center arrangements in one image produces the right arrangement, which the graph demonstrates gives in each channel the sum of the individual arrangements.

We also demonstrated the linearity of mutually incoherent regions (Fig. 3), critical for obtaining extremely high throughput. Since particles from different regions produce intensity patterns that add linearly, we can use a wide field-of-view to detect cells without fear of misidentification due to interference effects. The linearity allows us to formulate the identification process as a linear inversion problem: $N_P(t)=(T_{P\,x\,C})^{-1}S_C$, where N_P is the number of particles of a given population at time t, S_C is the signal in each channel, and $T_{P\,x\,C}$ is the scattering matrix for the specific TTP-DM pattern that connects a particle (or set of particles) to the detector signals.

4. Anticipated Results

We will present high-fidelity identification of particle species of static beads and common bacteria (such as yeast), at linear flow velocities of up to 1m/s. A machine learning algorithm will be used for efficient species identification. We will also demonstrate a machine-learning based optimization process for amplifying the signal of a particular population relative to background cell species, with the goal of creating fixed filters that can be easily fabricated for a single specific application (such as the detection of circulating tumor cells in blood).

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References

[1] Smith et. al., "Development of a Flow Cytometric Method for Quantification of BCL-2 Family Members in Chronic Lymphocytic Leukemia and Correlation with Sensitivity to BCL-2 Family Inhibitors," Cytometry Part B (2016)

[2] Alix-Panabieres, C. and Pantel, K., "Circulating Tumor Cells: Liquid Biopsy of Cancer" Clinical Chemistry **59**(1), 110–118 (2013)

[3] Leme, D.M. et. al. "Genotoxicity assessment of water soluble fractions of biodiesel and its diesel blends using the Salmonella assay and the in vitro MicroFlow_ kit (Litron) assay" Chemosphere **86**, 512-520 (2012)

[4] Mathaes, R. et. al. "Quality Control of Protein Crystal Suspensions Using Microflow Imaging and Flow Cytometry" J. Pharmaceutical Sciences **102**, 3860-3866 (2013)

[5] Wojcik, K. and Dobrucki, J. W. "Interaction of a DNA Intercalator DRAQ5, and a Minor Groove Binder SYTO17, with Chromatin in Live Cells - Influence on Chromatin Organization and Histone—DNA Interactions" Cytometry Part A **73**(A), 555-562 (2008)

[6] Futamura, K. et. al. "Novel Full-Spectral Flow Cytometry with Multiple Spectrally-Adjacent Fluorescent Proteins and Fluorochromes and Visualization of In Vivo Cellular Movement" Cytometry Part A **87**(A), 830-842 (2015)

[7] Blasi, T. et. al. "Label-free cell cycle analysis for high-throughput imaging flow cytometry" Nature Communications (2016)

[8] Lei, C. et. al., "High-throughput label-free image cytometry and image-based classification of live *Euglena gracilis*" Biomedical Optics Express **7**(7), 2703-2708 (2016)

[9] Copland, M. et. al. "GMTIFS: deformable mirror environmental testing for the on-instrument wavefront sensor" Proc. SPIE 9909-990980 (2016)