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Major Goals

The goal of this one-year effort was to develop a new particle velocimetry method, *structured-illumination microscale particle-image velocimetry* (SIμPIV), that exploits structured-illumination microscopy (SIM) approaches originally developed to "optically section" biological samples in fluorescence microscopy, *i.e.*, isolate the signal from the focal plane for a sample illuminated over its entire cross-section. We proposed to evaluate the spatial resolution of SIμPIV by using SIM to image steady laminar Poiseuille flow seeded with neutrally buoyant particles through a microchannel.

The research objectives of this project were to:

- Evaluate and implement recent structured-illumination microscopy (SIM) methods that require only two images
- Use these SIM techniques to visualize fluorescent, neutrally buoyant radius $a < 1$ μm tracer particles convected by steady low-Reynolds number (*i.e.*, laminar) Poiseuille flow through a microchannel
- Determine the characteristics of the particle images (*e.g.* signal-to-noise ratio, particle concentration, time between the images within a "raw" image pair, exposure time) and the characteristics of the structured illumination (*e.g.* spatial frequency, phase) required to obtain optimal results for SIM—specifically, to minimize/eliminate (images of) out-of-focus particles
- Implement SIμPIV: in other words, process a pair of SIM images (where each SIM image is obtained from a pair of "raw" particle images) to obtain velocity profiles in steady laminar Poiseuille flow
- Quantify the spatial resolution along the optical axis of our SIμPIV results based on its known parabolic velocity profile, and compare the spatial and temporal resolutions of SIμPIV with that for "classic" μPIV using correlation averaging (the spatial resolution of μPIV along the optical axis is characterized by the depth of correlation).

Given the limited time for this effort, we decided to only implement one two-image SIM approach, namely double-exposure SIM.[1] In view of the limitations on the particle seeding density that was feasible with double-exposure SIM, the effort focused on particle-tracking velocimetry (PTV), *i.e.*, tracking individual particles, *vs.* cross-correlation based PIV. Finally, the initial studies considered larger $a = 2.4$ μm tracer particles because they scatter more light, and hence produce moer signal.

Double-exposure SIM combines two "raw" images, $I_1(x, y)$ and $I_2(x, y)$, which are both illuminated by light with a sinusoidally varying intensity at a spatial frequency ν with a phase shift (in the illumination) between the images of ϕ_o. Both I_1 and I_2 are a combination of an out-of-focus ("defocused") image $I_D(x, y)$ and an in-focus image $I_F(x, y)$. If I_1 and I_2 are

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[1] X. Zhou, *et al.* (2015) Double-exposure optical sectioning structured illumination microscopy based on Hilbert transform reconstruction. *PLOS One* **10**(3):e0120892

acquired over a short enough interval so I_{D} and I_{F} remain unchanged over this interval, the difference between these two images:

$$\begin{aligned}\Delta I(x,y) &= I_2 - I_1 \\ &= I_{\mathrm{D}}(x,y) + I_{\mathrm{F}}(x,y)\sin(2\pi\nu y + \phi_{\mathrm{o}}) - \left[I_{\mathrm{D}}(x,y) + I_{\mathrm{F}}(x,y)\sin(2\pi\nu y)\right] \\ &= 2\sin\left(\frac{\phi_{\mathrm{o}}}{2}\right)\cos\left(2\pi\nu y + \frac{\phi_{\mathrm{o}}}{2}\right) I_{\mathrm{F}}(x,y)\end{aligned}$$

The in-focus image, or slice, $I_{\mathrm{F}}(x,y)$ is then reconstructed using a Hilbert transform of $\Delta I(x,y)$.

Accomplished under Goals

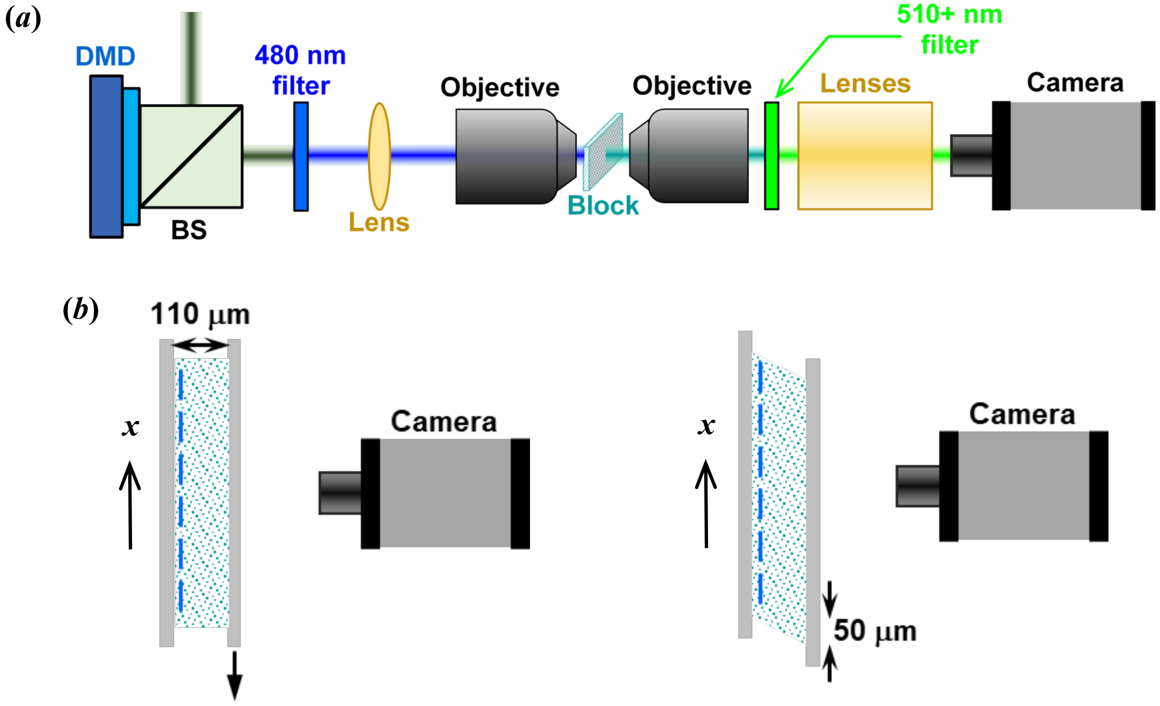

Figure 1 (*a*) The double-exposure SIM setup (BS = beamsplitter cube). (*b*) A top view of the particle-seeded Carbopol block before [*left*] and after [*right*] the block is sheared. The dashed blue line denotes the location where the sinusoidally varying intensity profile is in focus.

The double-exposure SIM system built during this year (Fig. 1*a*) uses the illumination from a 7 W plasma lamp passing through a spike filter (with a transmission band at wavelengths λ = 480±10 nm) and a digital micromirror device (DMD) (Texas Instruments Lightcrafter 6500) to create a sinusoidally varying intensity profile. The spatial structured illumination has a spatial frequency $\nu = 0.0185\ \mu\mathrm{m}^{-1}$ (*cf.* Fig. 2*a*, *b*), and a phase shift $\phi_{\mathrm{o}} = \pi$. In the initial implementation of double-exposure SIM, images were acquired of radius a = 2.4 μm fluorescent polystyrene (PS) particles embedded at a volume fraction of ~0.22% in a block of a transparent gel (Lubrizol Carbopol® 940) sandwiched between glass microscope slides with a spacing (along z) of ~110 μm. To simulate a “flow,” the block was sheared by displacing one of the slides by

~50 μm. The block was illuminated by a sinusoidally varying intensity profile focused 12−13 μm from the stationary slide inside the block (Fig. 1*b*), and images of the embedded particles before and after the block was sheared were recorded by an intensified CCD camera (Princeton Instruments PI-MAX4) (Fig. 2*a*).

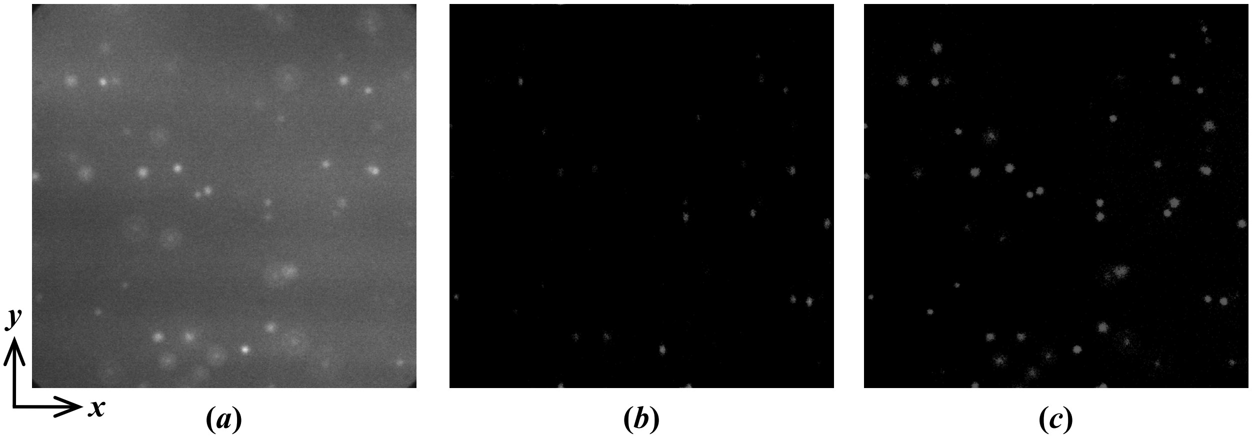

Figure 2 (*a*) A "raw" image of a = 2.4 μm PS particles embedded in the Carbopol block illuminated by a sinusoidally varying intensity profile; (*b*) a reconstructed image of the same particles using double-exposure SIM; and (*c*) a "raw" image of the same region obtained by illuminating the entire block. Images (*b*) and (*c*) were processed so that all pixels with grayscales less than 40% of the maximum grayscale value in the image were considered "background" and reset to a grayscale value of zero (black). All three images have a physical field of view of 245 μm square.

The images were "zero thresholded," where the background of the images, defined to be pixels with grayscales less than 40% of the maximum grayscale in each image, was set to zero (*i.e.*, black). A high boost filter was applied to (a Fourier transform of) the zero thresholded image; particles in these images were then defined to be local grayscale maxima with a negative second derivative (*i.e.*, a concave down peak).

The displacements of each particle in the first ("pre-sheared") image were then determined from the center location of the nearest neighbor in the second ("post-sheared") image along the positive x-direction. In these initial studies, particle displacements were estimated to the nearest pixel (*i.e.*, no attempt was made to determine particle displacments with sub-pixel resolution). The particle displacements obtained from the images reconstructed with double-exposure SIM (Fig. 2*b*) Δx = 5.7±0.89 μm (average ±standard deviation) over 11 particles (with displacements of 4.6–7.2 μm). The displacements extracted from images where the entire block was illuminated (Fig. 2*c*) Δx = 5.5 ± 2.3 μm over 41 particles (with displacements of 0.48–9.4 μm). Assuming that shearing the block leads to particle displacements that vary (nearly) linearly with the distance between the slides from 0 to 50 μm, these initial results indicate that double-exposure SIM can successfully image (*i.e.*, isolate) particles within a thin slice of the block centered at a location about 11% into the 110 μm thick block.

Although the ARO-sponsored portion of this effort ended in early August, we are continuing work on SIM-based flow visualization with support from the American Chemical Society Petroleum Research Fund (ACS PRF). A facility for laminar Poiseuille flow through a 0.5 mm square glass minichannel and driven by a hydrostatically generated pressure gradient was built in August. Double-exposure SIM is currently being implemented in this facility. Figure 3 shows our first images for this flow seeded with a = 2.4 µm fluorescent PS particles. PTV analysis for the SIM images gives an average speed U = 14.2 µm/s with 12% standard deviation based on 7 particles with speeds ranging from 12.3 µm/s to 16.4 µm/s (again, particle displacements were estimated to the nearest pixel). The PTV results for the flow with volume illumination gave U = 12.9 µm/s with 18% standard deviation based on 15 particles, with speeds ranging from 4.1 µm/s to 15.4 µm/s.

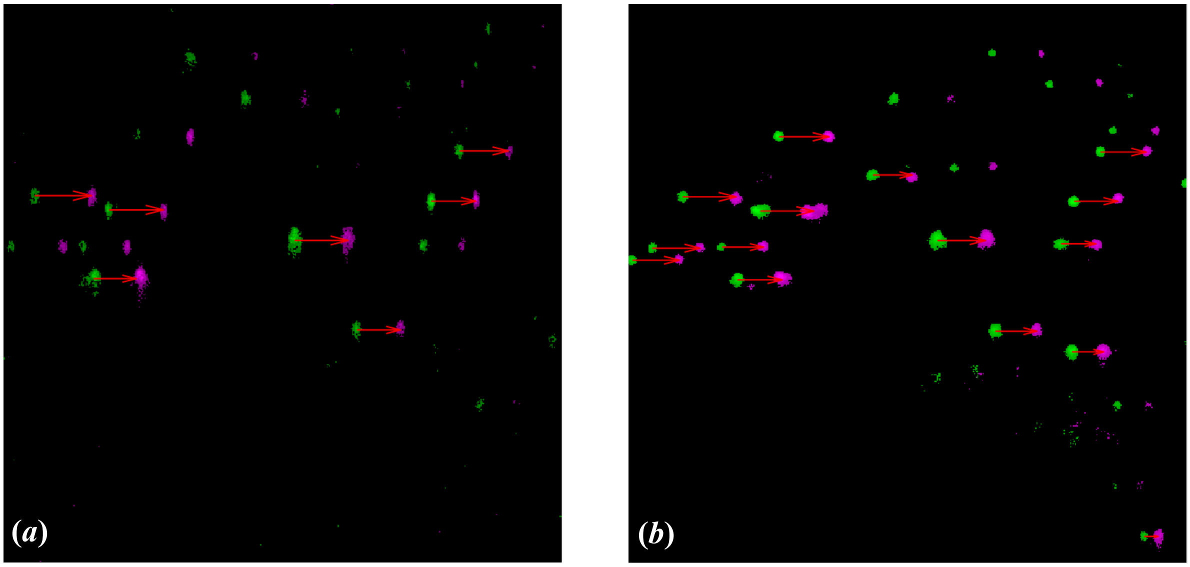

Figure 3 (*a*) Overlay of two reconstructed double-exposure SIM images of a = 2.4 µm PS particles suspended in laminar Poiseuille flow; the particles in the first image are (false) colored green, while those in the second image are colored pink. The arrows denote the particle displacements; the time interval between the images within the pair Δt = 1.52 s. (*b*) Similar to (*a*), but for the same region of the flow visualized instead with volume illumination. Both images have a physical field of view of 245 µm square.

Obviously, the structured illumination needs to be aligned with, and centered within, the illumination volume. Nevertheless, the reduction in the standard deviation of the particle (and flow) speeds suggests that these preliminary SIM results are obtained over a thinner "slice" of the flow. We plan next to acquire more SIM PTV data in this setup, and extend the studies to smaller ($a \approx 1$ µm) tracer particles. Our initial SIM PTV results will be presentedthis November at the 72nd Annual Meeting of the American Physical Society Division of Fluid Dynamics (APS/DFD).

Training Opportunities

A Ph.D. student in Mechanical Engineering (ME), Mr. Michael Spadaro, started this project in September 2017, and was supported for 10 months (July 2018 through March 2019, and July 2019) by this grant. Mr. Spadaro, who passed his doctoral qualifying examinations in November 2018, has taken courses on complex fluids, microfluidics and hydrodynamic stability, as well as optics.

Results Dissemination

An abstract on SIM-based flow visualization has been submitted to the 72nd Annual Meeting of the APS/DFD, which will be held in Seattle, WA on November 23-26, 2019. We plan to submit the initial results on SIM-based PTV, once obtained, for rapid publication as a Letter in *Experiments in Fluids*.