High-Speed Confocal Microscopy

Microscope System

Confocal microscopy is a well-developed technique that is widely used in biomedical research because of its advantages over conventional widefield microscopy, which is used in conventional micro-PIV systems. High-speed spinning disk confocal microscopy was first used to obtain microscale flow field measurements in 2004 and was able to show an increase in resolution and accuracy by reducing the signal-to-noise ratio for PIV measurements. This first so-called confocal laser scanning microscopy (CLSM) micro-PIV system was able to achieve high spatial accuracy, but was limited to a low temporal resolution of up to 120 frames per second, which limits the system's usefulness for microfluidic velocity measurements. Since then, advances in camera and spinning disk technology lead to the development of confocal systems capable of up to 1,000 frames per second. This type of set up is now fast enough to visualize dynamic systems with the increased resolution and 3D capabilities of traditional confocal microscopy.

A method for recording high-speed, high-sensitivity, and high-resolution images of fluid flowing through microchannels is critical to enable control and design of novel microfluidic devices. Additionally, if multiphase systems, such as solid/liquid and gas/liquid systems, can be studied, this could provide essential information to assist in the design of microfluidic processes involving liquid-particulate interactions, such as microreactions, particulate sampling, and blood flow. We have developed a high-speed confocal microscopy system with both a high-speed CMOS camera for particle tracking studies and a slower but more sensitive CCD camera for low light biological studies.

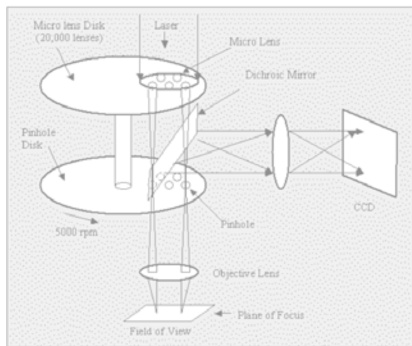

Figure 1. Confocal spinning disk diagram

3D Microfluidic Velocity Measurements

This study demonstrates the use of a high-speed confocal imaging system to generate 3D velocity profiles of fluid flowing in trapezoidal microchannels at speeds of 1000 frames per second, which is five times faster than any results previously reported in literature.

The microchannel has dimensions of 52.9 micron across the top of the channel, 47.0 microns in depth, and 23.8 micron across the bottom. To track fluid motion, distilled water was seeded with 1 micron Fluosphere® tracer particles at 0.07% by volume. A Harvard Apparatus syringe pump was used to deliver 5 microliters per hour of the fluid to the channel. The top and bottom of the channel were found by scanning through the z direction of the channel, and image slices were captured every 4 microns at a rate of 1000 fps.

Experimental particle tracking results were compared to CFD simulations, as shown below.

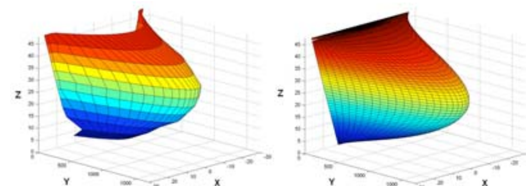

Figure 2. Experimental vs. simulation results

DNA as a Model Polymer

Now we have progressed to work that uses DNA as a not only a velocity tracer, but also a stress tracer in interesting non-Newtonian flows. Important flow phenomena in polymer solutions such as vortex formation, flow instabilities and stress relaxation are still not well understood. Using DNA as a model polymer to observe individual molecular behavior will offer groundbreaking insight into the cause of such interesting polymer flow behaviors.

DNA is ideal for studying polymer flow behavior because of its well-defined structures, which can be synthesized to match specific polymers. DNA is also relatively easy to label for direct observation of individual molecules and gives insight to the behavior of individual polymer molecules. Although DNA can be stained, it still takes a very sensitive optical system, such as our high-speed confocal microscope, to see individual molecules because of their relatively small size and low signal. Few

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labs have the capability to visualize the dynamic behavior of individual DNA molecules.

Large DNA molecules, such as Lambda DNA and Calf Thymus DNA, are stained and used as tracers in DNA solutions. By staining only a small fraction of DNA molecules in a 1% wt. solution of DNA in water, we can effectively watch the individual molecular behavior of the DNA solution.

Non-Newtonian viscoelastic solutions are made by adding 1% wt. unstained salmon DNA to water and adding very small amounts of stained tracer DNA. When these solutions flow through microchannels, they have a low Reynolds number but a high Weissenberg number, which produces some interesting flow patterns. To view the DNA in these flow systems, the high-speed confocal microscope is used with the high sensitivity CCD camera.

Interesting vortex patterns and flow instabilities were observed in the non-Newtonian DNA solutions that were not seen in Newtonian Solutions under identical flow conditions. DNA molecules were observed in the secondary flow region where the flow is slower but were not visible in the main flow region where the flow was much faster. The molecular orientation of individual DNA molecules can be seen during the different flow conditions. The results are qualitative so far but show we have developed a good method for viewing individual molecular behavior in polymer systems. We plan to extend this work to obtain more quantitative results and directly observe molecular behavior, such as chain disentanglement, shear banding, and wall slip.

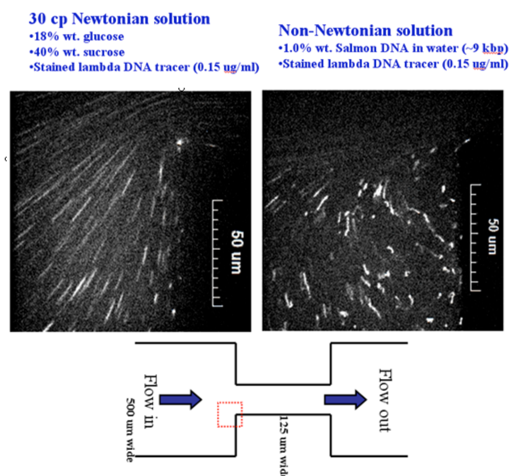

Figure 3. Newtonian vs non-Newtonian flow

Membrane Sandwich Electroporation

The high-speed confocal microscope can also be used to visualize the performance of various biomedical devices. One example is the Membrane Sandwich Electroporation device. We developed a microfluidic platform to interface the cell patch device with our high-speed spin disk confocal microscope. This chip will allow for the evaluation of static and dynamic interactions of cells during membrane-based electroporation. The platform allows users to mount membranes and apply suction or vacuum and carry out electroporation events all under the observation of a high-speed spin disk confocal microscope.

From the images below, we can see the cells that exhibit fluorescence received the plasmids, and we can investigate each cell's interaction with the membrane to see if there are any differences to account for cells that exhibit higher fluorescence or no fluorescence.

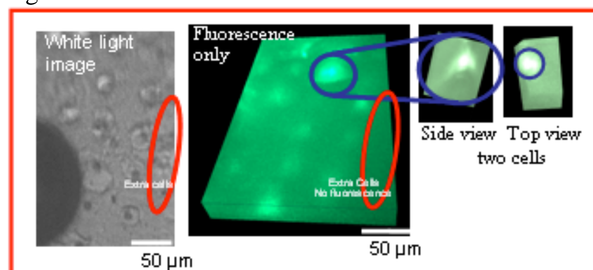

Figure 4. Individual cell analysis of the 3D confocal images

Publications:

1. "Gene Transfection of Mammalian Cells Using Membrane Sandwich Electroporation", Zhengzheng Fei, Wang, S., Zie, Y, Henslee, B.E., Koh, C.G., Lee, L.J., *Anal. Chem*. **2007**, *9*, pp 5719-5722.

2. "Bubbles in Nanofluids", Liang-Shih Fan, Orin Hemminger, Zhao Yu, and Fei Wang *Ind. Eng. Chem. Res.*, **2007**, *46*, (12), pp 4341–4346.

3. "Microfluidic velocity measurements using three-dimensional confocal micro particles tracking velocimetry (CM-PTV)", Hemminger, Orin; Yu, Zhao; Zhang, Chunhe; Lee, L. James; Fan, L-S; International Fluidization conference 12 Proceedings, May **2007**.

4. "Experiment and Lattice Boltzmann Simulation of Two-Phase Gas-Liquid Flows in Microchannels, Fan, Liang-Shih"; Yu, Zhao; Hemminger, Orin, *Chem. Eng. Sci.*, **2007**, *62*, (24), pp 7172-7183.

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