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Recent advances in optical diagnostics for the investigation of microfluidic flows

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In this paper we present fundamentals and applications of optical micro-scale flow diagnostics. Starting with classical flow visualization techniques, quantitative optical methods will be discussed. These are molecular tagging velocimetry (MTV) and micro particle image velocimetry (μ PIV) for the determination of velocity vector fields and laser-induced fluorescence (LIF) for the determination of scalar fields like temperature or PH-value. Examples for MTV will be presented using caged fluorophores and photobleaching. The principle of micro-scale particle image velocimetry (μ PIV) will be explained and extensions of the method are presented as well as applications in various fields of microfluidics. The following μ PIV methods will be covered:

- Stereoscopic μ PIV for the determination of all three components of the velocity vectors in a measurement plane uses a perspective distortion of the same observation area from two different observation directions. After a calibration of the two-viewing directions, the out-of-plane component of the velocity vector can be reconstructed from the perspective distortion. We will present a self-calibration procedure on the particle images in a closed microchannel.
- 3D-micro-scale particle tracking velocimetry (3D- μ PTV) using a custom-build four-camera microscope for the measurement of three-dimensional flow in a volume; similar to the stereo- μ PIV principle a volume is observed from 4 different directions and the particle distribution is reconstructed in 3D with the calibration procedure mentioned above. The particle displacements are then determined in 3D with a tracking algorithm.
- Holographic μ PIV for the measurement of three-dimensional flow in a volume uses the phase information of the light for the determination of the particle distribution in 3D. Light refracted on the particles in the flow serves as object beam that interferes with unrefracted light passing through the same measurement volume as the reference beam. Object beam and reference beam form a hologram, which is evaluated digitally to obtain the 3D particle distribution. The particle displacements are then determined with a 3D-correlation-based tracking algorithm.
- Total internal reflection fluorescence microscopy in combination with particle image velocity (TIRFM-PIV) for the observation of near-wall flow in a region of several 100 nm from the surface. Using TIRF-illumination one creates an evanescent wave that penetrates several 100 nm into the liquid volume. The depth of the evanescent wave can be controlled by the TIRF-angle allowing measurements in different distances from the surface.
- Single-pixel particle image velocimetry obtains high spatial resolution velocity data by reducing the size of the interrogation region to an area of one by one pixel². Using ensemble-averaging over a large number of recordings the displacement of tracers passing by the observation area is evaluated. Applications of μ PIV are shown on
 - the flow over endothelium cells in a microchannel, where from measurements in several planes over a cells the topography of the cell layer as well as the shear stress distribution is derived and related to the gene-expression of the cells.
 - in-vivo μ PIV in the beating heart of a chick embryo, in the vitelline vessel network and on cilia in the mouse trachea; the μ PIV technique has been developed for in vivo measurements. Bio-inert, long-circulating tracer particles are added to the organism to visualize the flow by a micro-needle injection mechanism. A fast recording procedure for pulsating flow has been developed that allows a large number of measurements in a short instant of time of the changing biological sample
 - the characterization of the internal circulation in droplets in a pressure-driven channel flow by means of refractive index matching. Trains of water droplets are transported in a continuous oil phase. The size of the droplets is slightly larger than the channel diameter. The water droplets do not completely wet the square channel, which creates a bypass flow in the channel corners. The bypass flow drives a complex circulation pattern in the water droplets as well as in the oil compartments in-between the droplets. These patterns enhance mixing. Using refractive index matching it is possible to simultaneously investigate the flow patterns on both sides close to the liquid-liquid interface.

- flow measurements in an operating fuel cell. We will show first velocity measurements in an operating Direct Methanol Fuel Cell (DMFC). The one channel fuel cell has an optical access for flow measurements. The velocity fluctuations due to the creation of two-phase flow as result of the reaction on the membrane are determined. These fluctuations are expected to be one reason for unstable power output of fuel cells.

Measurements of the temperature distribution by means of two-dye ratiometric laser-induced fluorescence (LIF) will be introduced and the application will be shown for the characterization of the PCR reaction chamber on a BioMEMS chip.

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