

## 9.20 Micro-PIV I

*Contributed by:*

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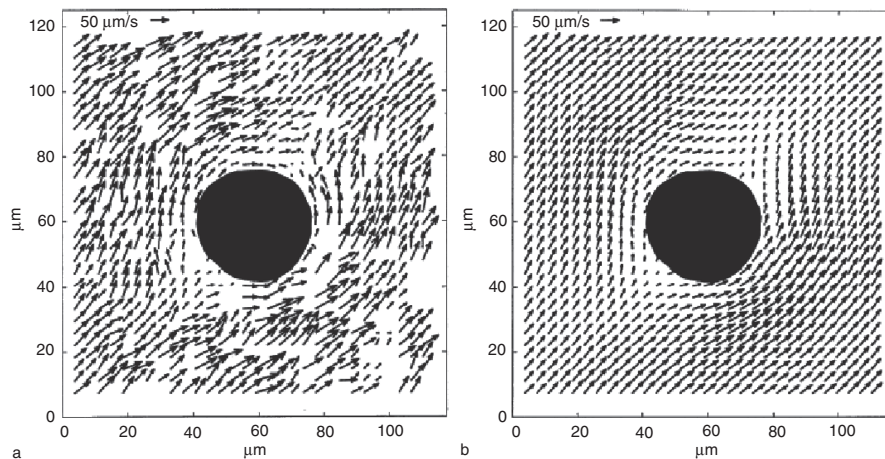
### 9.20.1 Application of PIV to Microscopic Flow

Microfluidics that deals with fluid flow phenomena in microscale is becoming a significant research subject with the development of microfluidic devices or systems [405]. In the fields of chemistry, biochemistry, and biology, various types of microfluidic devices have been designed and demonstrated with the aim of developing the miniature-sized bio/chemical analysis apparatuses that are usually referred to as micro total analysis systems ( $\mu$ TAS) or laboratory-on-a-chip [397, 409]. Downsizing of analytical apparatuses brings us many potential profits, such as reduced amount of sample and reagent, high sensitivity, short analysis time, automation of processes, parallel processing, etc [399, 401, 402]. In order to make effective use of those advantages and work out a hydrodynamically-designed system or geometry, the key issue is better understanding of the fluid flow phenomena taking place in such a microfluidic device.

Over the last several years, microscopic PIV ( $\mu$ PIV) has been studied and developed for use as a diagnostic tool of microscopic flow fields. The idea of that is very simple: an optical microscope is used for imaging of a particle-seeded flow in microscale instead of photographic lenses in macroscale. Nano-sized fluorescent beads are suspended as the tracers within working fluid, and then the distribution patterns of the tracer particles are imaged sequentially with an epifluorescent microscope and a video camera. The captured particle images are analyzed in the same way as standard macroscale PIV.

### 9.20.2 Examples of Micro-PIV

SANTIAGO et al. [410] developed a micro PIV system using an epifluorescent microscope with a high numerical aperture (NA) objective lens and an inten-

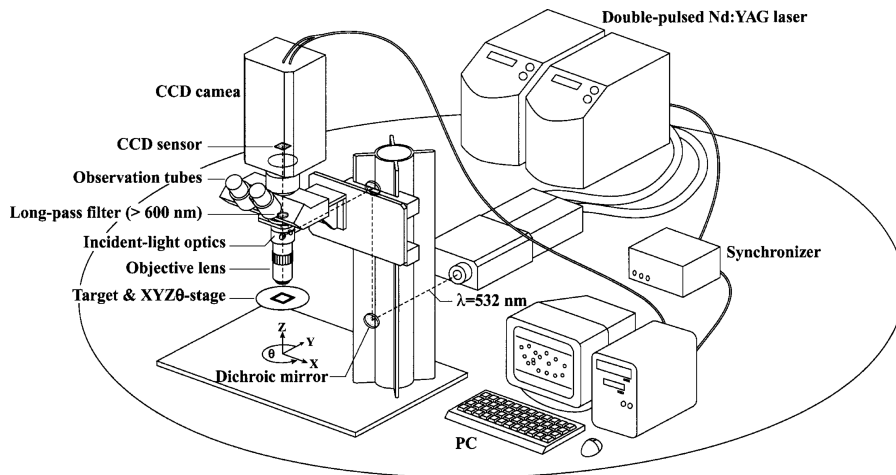


**Fig. 9.93.** Vector fields of a surface-tension driven Hele-shaw flow around a  $30\ \mu\text{m}$  wide obstacle (left: instantaneous, right: time-averaged) [410].

sified CCD camera. The system employed a continuous-illumination mercury lamp and a low frame-rate camera to capture the particle images, so that the time interval between images was relatively large. They successfully measured the low-speed Hele-Shaw flow with a velocity of approximately  $50\ \mu\text{m/s}$  using their micro PIV system (see figure 9.93). The combination of continuous light and CCD video camera is suitable for low-velocity flow measurement, as in the case of electroosmotic flow [411].

MEINHART et al. [406] achieved the frame-straddling micro PIV system using reflected-light microscopy with a double-pulsed Nd:YAG laser by improving the lighting system. Figure 9.94 shows a standard  $\mu\text{PIV}$  system using a reflected-light illumination with a high-power double-pulsed Nd:YAG laser [403]. The dynamic range of PIV can be extended by applying the frame-straddling method, which is often used in conventional PIV. Frame-straddling  $\mu\text{PIV}$  can be also conducted by illuminating the flow field directly from the outside with a pulsed Nd:YAG laser beam rather than using reflected-light illumination.

PTV technique can also be applied to the microscopic particle images [412] instead of PIV algorithm based on the image correlation method. In microscale, tracking algorithm such as PTV makes a significant contribution to investigation of not only fluid flows but also the behavior of solid particles such as cells since PTV enables us to detect the movements of individual particles in time series.



**Fig. 9.94.** Schematic of a micro PIV system. A high-power pulsed Nd: YAG laser is used to illuminate fluorescent tracer particles through an epi-fluorescent microscope [403].

### 9.20.3 Differences from Macroscale PIV

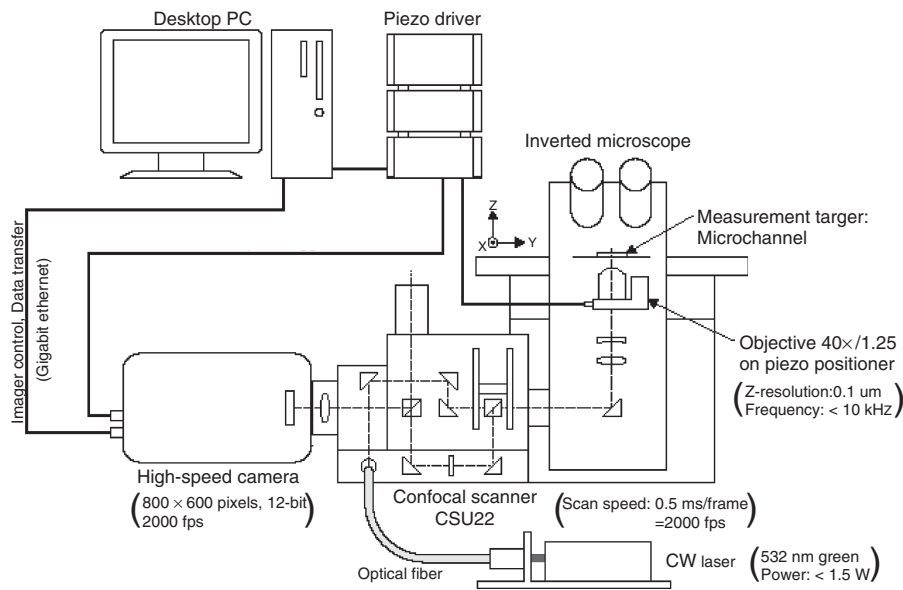
Micro-PIV differs from standard macroscale PIV in a few significant respects. One of the differences is the effect of Brownian motion of tracer particles. In micro-PIV, the effect of Brownian motion is not negligible because the diameter of tracer particles is smaller than  $1\ \mu\text{m}$ . Those sub-micron particles show relatively large random movements due to Brownian motion under a microscope. The Brownian motion of tracer particles significantly affects the basis of velocity estimation in PIV since the PIV method itself is based on the assumption that the tracers follow the fluid motion rigorously. In order to reduce the effect of Brownian motion, the time averaging procedure such as time-averaged correlation method [398, 407] is often conducted. Although time averaging method is useful for reduction of the measurement errors associated with Brownian motion, it is not available for unsteady flow phenomena.

Another major difference lies in the illumination method when taking pictures of tracer particles. In conventional macroscale PIV, the seeding particles in the flow are usually illuminated by a thin planar light sheet so as to visualize the cross-sectional cut plane of the measurement volume. Under a microscope, on the other hand, the sheet lighting proves to be impractical because the flow channel and observation region are certainly smaller than  $1\ \text{mm}$  and it is difficult to produce the light sheet with the thickness of less than  $100\ \mu\text{m}$  and to align the sheet precisely with the focal plane of microscope. For this reason, almost all micro-PIV systems employ the fluorescent microscopy with volume illumination method such as reflected- or

transmitted-light illumination instead of the sheet lighting. Therefore, the depth-of-field (DOF) of the microscope plays a significant role in micro-PIV measurement. MEINHART et al. [408] particularly defined the measurement depth (MD) as the out-of-plane measurement resolution of micro-PIV, which depends on the tracer particle diameter and NA of objective lens. Only the particles within the MD affect the PIV analysis, whereas the out-of-focus particles do not contribute to the evaluation. Consequently, the obtained velocity data is regarded as two-dimensional projected planar velocity field in the focal plane.

#### 9.20.4 Advanced Technique: Confocal Micro-PIV

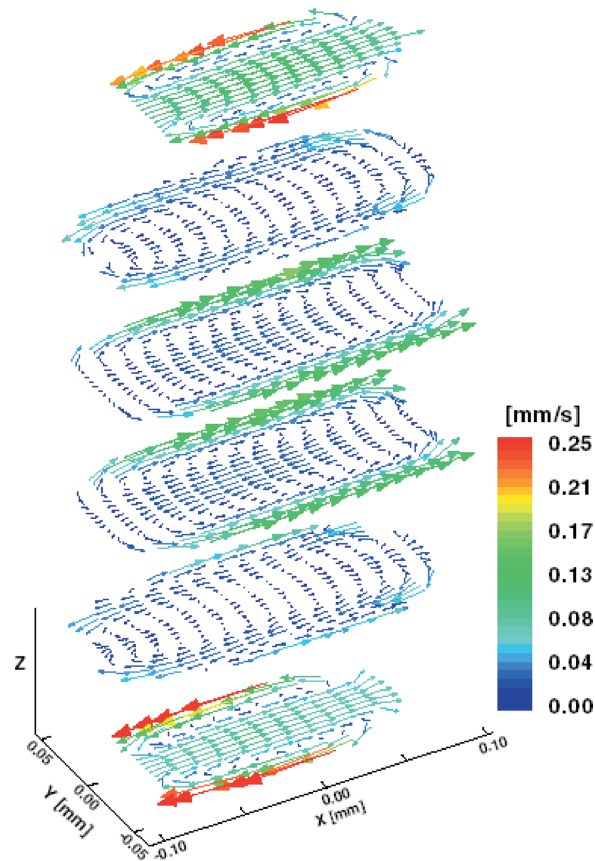
Recently, a new micro-PIV technique has been developed with the aim of resolving the problem associated with the volume illumination and DOF of micro-PIV. It is “confocal micro-PIV”. Confocal microscopy [414] is an advanced technique that offers some advantages over conventional optical microscopy, such as shallow DOF and optical cutoff of out-of-focus light. Application of confocal imaging technique to micro-PIV allows us to slice the seeded fluid volume as if using a thin light sheet in macroscale PIV. Confocal microscopy had scarcely ever been used for micro-PIV till lately because of



**Fig. 9.95.** Schematic diagram of confocal micro-PIV system. The system consists of an inverted-type microscope, a confocal scanner, a high-speed camera, CW laser, and PC. The target device is fixed on the microscope and observed from the bottom side.

its too slow scanning rate. Although PIV needs at least two subsequent exposures of the seeded fluid flow at a short time interval from nanoseconds to milliseconds, conventional confocal microscopy requires time from seconds to minutes to complete the scanning of a whole planar area. But recently, a high-speed confocal scanner has been developed [413], which is capable of scanning a single cross-sectional plane only in 0.5 ms at 2000 frames per second.

Figure 9.95 shows the schematic diagram of confocal micro-PIV system. The measurement target is fixed on the mechanical stage of the inverted-type microscope and the operator observes it from the bottom side. Confocal imaging unit consists of a high-speed confocal scanner, a CW diode laser and a high-speed camera, which is assembled with the side camera port of the inverted-type microscope. The confocal images produced by the confocal scanner are recorded on the high-speed and high-sensitive camera with  $800 \times 600$  pixel, 12-bit monochrome CMOS image sensor. In this case, the frame rate is fixed at 2000 frames per second and the exposure time of each



**Fig. 9.96.** Velocity distribution relative to the moving velocity of a moving droplet.

frame is 0.5 ms. This system enables us to measure cross-sectional velocity distributions of micro flows in the region of  $228 \times 171 \mu\text{m}^2$  with the confocal depth of  $1.8 \mu\text{m}$ . The confocal depth indicates the out-of-plane measurement resolution of the confocal micro-PIV system, which has been measured actually by means of imaging the actual tracer particles at different focus positions. The key advantage of confocal micro-PIV lies in the fact that the light from out-of-focus particles is cut off optically and only the particles in quite shallow depth centering on the focal plane are visualized at high contrast level.

Confocal micro-PIV has been applied to the internal flow of a small droplet that is transported in a square microchannel [404]. Figure 9.96 shows the instantaneous velocity distributions in each cross-section. In order to elucidate the flow phenomenon inside the droplet, the velocities relative to the moving speed of the droplet are estimated and mapped in Fig. 9.96. The axisymmetrical circulation flow is observed in any cross-section, although, its direction differs for the top/bottom wall region and the center of the channel. This result suggests that the fluid inside a closed droplet circulates in the three-dimensions by the drag force on the contact surface with the surrounding channel walls when the droplet passes through a square microchannel.