sCMOS Camera Application Note

Scientists like it fast

sCMOS in "light-sheet"-mode enables high-speed imaging

In point of care diagnostics, the so called "Lab-on-a-Chip" devices proved that they have huge advantages compared to normal large scale laboratories. These microsystems are highly miniaturized, complex devices, dealing with only small volumes (a few µl) of reagents and highthroughputs (several ml/s). Consequently, they provide lower turnaround times and costs [1].

In these devices, cells, proteins and small molecules from blood and other body fluids are typically analyzed with the help of fluorescence labeling – a powerful and non-destructive way to track biological molecules. The fluorescent samples are transported in parallel to different analysis regions by pumping them in carrier solutions through microchannels (<1 mm in diameter), enabling the analysis of a large number of cells simultaneously.

Such applications require a camera that is able to detect a weak fluorescent signal in a relatively short time. Some ultra-high-speed cameras achieve several thousand fps utilizing a charge coupled device (CCD) sensor. However, these high-speed sensors have a very low sensitivity for faint light signals and suffer from a low number of pixels (<80,000), which limits resolution.

We demonstrate the capability of the genII scientific complementary metal-oxide-semiconductor (sCMOS) with rolling shutter mode for high-speed fluorescence imaging by tracking small (1 µm diameter) poly-fluorescent microspheres in a laminar flow setup. The camera has very high quantum efficiency, a high resolution of 2048 x 2048 pixels and a high-speed readout mode of 100 Hz. Furthermore, the camera has an additional "light-sheet" readout mode allowing very short exposure times at high frame rates.

Materials and methods

The microfluidic setup was carried out on an inverted microscope (Axiovert 200M, Zeiss) equipped with a sCMOS camera (ORCA-Flash4.0V2, Hamamatsu). Two syringes are connected via tubes (Tygon, Carl Roth) to a ready to buy micro-channel (µ-Slide VI$^{0.1}$, ibidi) in- and outlet. It has dimensions of 1 x 0.1 x 17 mm (width x height, length). For a given fluid height difference Δh the velocity ν of the fluid at a position $\vec{r}$ can be derived by the Stokes-equation

$$\nu(\vec{r}, \Delta h) = \frac{pg\Delta h}{4\eta}(R^2 - \vec{r}^{\,2})$$

here ρ is the fluid density, η the dynamic viscosity, ϑ the gravity constant and $\Re$ the radius of the capillary. The flow can thus be controlled by adjusting the menisci of the two syringes. Polystyrene microspheres having a diameter of 1 µm are used. They have two distinct excitation maxima of 491 and 512 nm, whereas the emission maximum is at 554 nm (Polychromatic (PC) Red Microspheres, Polysciences). Data analysis and tracking is performed by using ImageJ software and TrackMate-algorithm.

Good intensity even at bright backgrounds

Figure 1 (top) shows a bead which is flowing in the micro-channel and its corresponding line graph intensity profile (top). The snapshot was taken using a standard eGFP fluorescent filter set in contrast to Cy3, which is brighter, more photo-stable and introduces less background. The signal to background ratio is still very good (around 2.4). The background signal is given by microspheres flowing in other z-spaces of the microchannel. Note that the signal to noise ratio is around 33333.

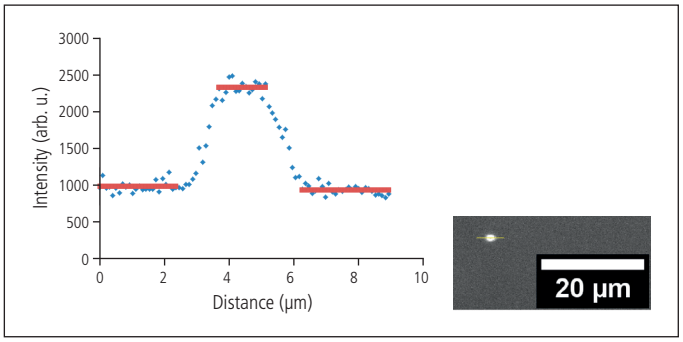

Fig. 1: Line intensity profile of a poly-fluorescent microsphere using a standard eGFP filter set. Even at bright background signals the sample signal is very high yielding a signal-to-background ratio of 2.4.

Spatial distortion of the microsphere

While imaging fast moving samples the recorded object may show a spatial distortion because of two facts: sample velocity and readout time. In general, motion blur occurs if the following equation is not fulfilled.

$$\Delta T \leq \frac{\Delta X \cdot P \cdot 6.5\ \mu m}{M \cdot \nu}$$

In this formula ΔT is the exposure time, ΔX is pixel length of the object, P is the distortion percentage, M is the magnification factor of the lens and ν is the particle's velocity. The factor of 6.5 µm represents the size of a single pixel. In other words, motion blur occurs if the sample moves a significant distance during the exposure time. For example, a 1 µm object flowing at a velocity of 500 µm/s, which is observed under a magnification of 63x, needs to be recorded with exposure times of at least 20 µs to avoid a 10 % motion blur.

However, if the exposure time is already very small there may still be a distortion for large and fast moving samples due to rolling shutter. In comparison to CCD cameras the exposure time for all pixels in the sCMOS image sensor does not start and end concurrently. Moreover, each sensor row is exposed for a certain time and readout separately after a delay of 9.74 µs. The camera has two line readers performing readout from the middle of the image to the top and bottom, respectively.

Consequently, the exposure time has to be smaller than the specimen pixel height multiplied by the readout delay as shown in the following equation:

$$\Delta T < \Delta X \cdot 9.74\ \mu s$$

With reference to the example in question there would be no distortion if the exposure time is smaller than 940 µs.

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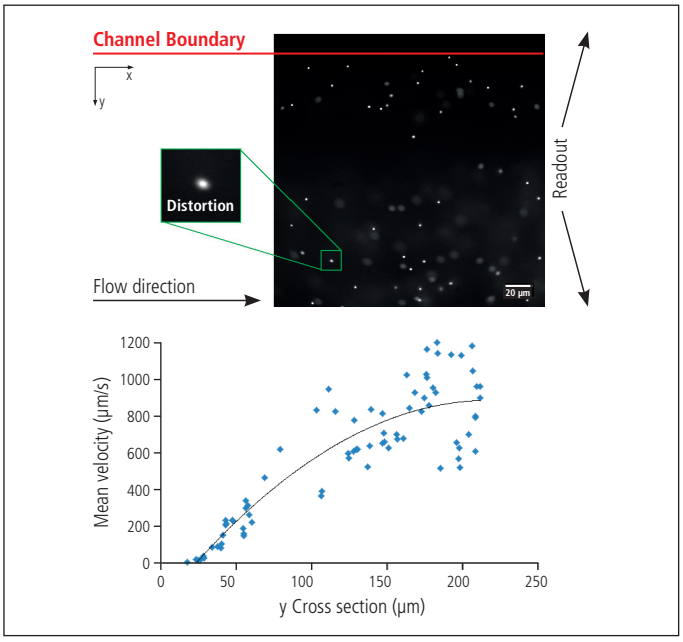

Fig. 2: (top) Snapshot of 1 µm latex beads recorded at full width of view (2048 x 2048 pixels or 211 x 211 µm). (bottom) Average velocity of the particle tracks along the y cross section of the channel.

Particle tracking velocimetry

Microspheres are diluted in water to a final particle density of 0.06 % ($4.5x10^{10}$ particles/ml) and pipetted into the channel. Then, the flow is adjusted by the height difference of the two syringe filling levels and the particles are recorded by the camera under a magnification of 63x.

Figure 2 (top) shows a single snapshot of micro-spheres flowing in the channel at full width of view (211 µm x 211 µm) at an exposure time of 10 ms. The particles are flowing in x-direction. In the bottom of figure 2 the mean velocity of each track versus the channel width is shown. As expected from theory, the velocity increases quadratically with increasing distance from the channel boundary (see fit line). With increasing particle velocity, the distortion becomes larger (maximum: 30 %) as highlighted in the inset. The distortion lowers the tracking quality because the measurement points scatter for larger velocities.

Sub-array readout allows faster image acquisition per second and thus shorter exposure times. In internal mode, the minimum resolution is 2048 x 8 pixels so that only four lines need to be read out by each of the two line scanners resulting in a maximum frame rate of 25,656 fps at exposure times of 38.98 µs.

In addition, setting the camera to "light sheet" mode at the same resolution allows even shorter line exposure times of 9.74 µs at the cost of frame rate (maximum: 12,828 fps) because every line will be readout from top to bottom or inverse.

In figure 3 a time stack of a sub-array video in "light-sheet" mode is shown, where the resolution is lowered to 2048 x 80 pixels (211 µm x 8 µm). This allows high frame rates of 1389 fps at a very low line exposure time of 9.74 µs. The marked bead (purple circle) has a velocity of 1000 µm/s and shows no measureable distortion (<1 %). In theory, distortion larger than 1 % should occur for velocities of 1056 µm/s.

Summary

The presented experiment demonstrates that tracking of small (1 µm) micro-spheres with uniform flow direction inside micro-channels is easily possible up to velocities of 1000 µm/s without any measureable distortion and still maintaining a relatively large field of view. Consequently, the "light-sheet" rolling shutter mode of genII sCMOS enables the imaging of very fast non-uniform fluorescent motions.

This demonstration is also relevant to other research applications demanding high-speed and great sensitivity such as sensing or sorting circulating tumor cells [2].

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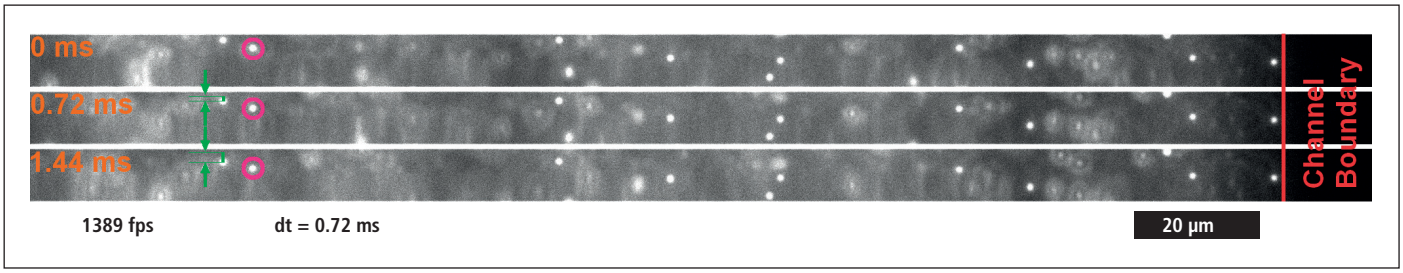

Fig. 3: Time stack of sub-array video using "light-sheet" image acquisition. The exposure time is 9.74 µs and the frame rate is 1389 fps (fps=1/(80 pixels x 9.74 µs)). The purple circle indicates a micro-bead at 1000 µm/s and the green marks shows the distance the bead traveled during image acquisition.

References
[1] W. Westbrook et al.: Lab on a Chip 14.12 1962–1965 (2014)
[2] S. Nagrath et al.: Nature 450.7173: 1235–1239 (2007)

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