Automated Micro-PIV measurement in Lab-on-a-Chip systems

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Abstract

Flow rate and wall shear stress are important parameters for perfused cell culture systems and should be monitored. An easy and non-invasive method is the particle image velocimetry (PIV). In this work PIV was used to characterize a cell culture system with included peristaltic pump. The time-dependent flow profile was measured on several points of the chip for different pumping speeds to figure out which forces are applied to dissolved and adherent cells. The results can be used to improve the developed pump in respect to its layout, the excitation and the position within the chip.

1 Introduction

Cell culture systems and lab-on-a-chip systems are used for in vitro drug screening or as bioreactor in medical synthesis. With perfused 3D systems it is possible to achieve much better oxygen and media supply than in static cell culture [1] allowing higher cell densities and the cultivation of small micro-organoids [2]. Those living systems on a chip may improve the significance of drug testing in comparison to animal testing. However some cells are damaged if they are affected by a high shear stress, making a continuously monitoring of the flow necessary. Hereby presented is a method for automatic and non-invasive measurement of the flow rates within small micro fluidic devices. This method is applied to a cell culture system with an included micro pump.

2 Methods

2.1 Cell culture system

Within the last years a micro fluidic platform including a peristaltic micro pump as well as several valves, manifolds and micro channels has been developed [3]. The flow cell is produced with a casting process using polydimethylsiloxane (PDMS). We used *Sylgard 184* from *DowCorning*, mixed ten units of monomer with one unit curing agent, steered them and degassed the silicone with a desiccator. Afterwards the flow cell was casted from a lithographically produced negative master (channel height 100 µm) by injecting the PDMS in a casting station and baking the chip at 90 °C for 90 minutes. Thereafter the micro fluidic system is put out of the casting station and plasma-bonded to a glass slide which allows easy access by optical microscopy. The biochip is now fixated on an electrically heated support. Both the pneumatically actuated peristaltic pump and the temperature control are performed by a devised control device. Figure 1 shows a cross-sectional view of the developed biochip.

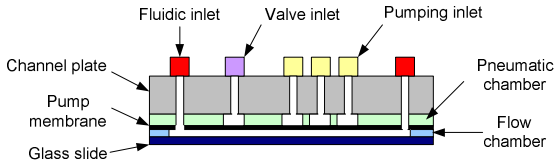


Figure 1 Cross-sectional view of the multi-organ-chip (MOC)

2.1.1 Peristaltic pump

A peristaltic pump is a displacement pump with three pump chambers in a row, whereby the first and the last chambers act as valves [4]. The volume of each chamber can be increased or decreased by applying vacuum or pressure to the pneumatic chamber on top of the separating PDMS membrane [5]. The membrane has a thickness of 500 µm and can be operated with pressures ranging from 900 mbar to 2000 mbar. Figure 2 shows the pumping cycle consisting of 6 steps which is used for the experiments.

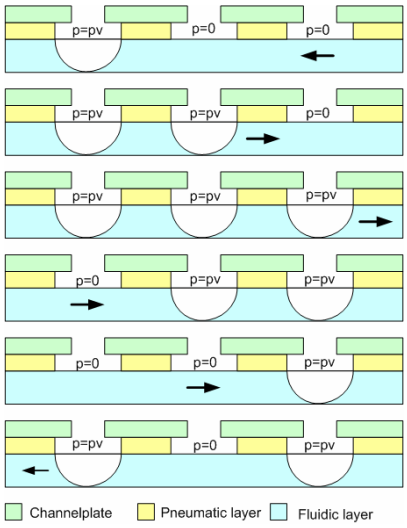


Figure 2 principle of the peristaltic pump

The pumping cycle can be easily changed by modifying the firmware of the controller. It is obvious that the flow in the micro channel depends on the following parameters:

- Applied pumping pressure (mbar)
- Applied filling vacuum (mbar)
- Pumping speed (Hz)
- Position at the biochip

Furthermore the peak velocity depends on the membrane speed when pressure or vacuum is applied. Therefore throttles are mounted on each pneumatic output of the control device to reduce the driving force from controller to micro pump. A mass airflow sensor (*Honeywell AWM 5000 series*) is used to measure the adjusted value.

2.2 Micro-PIV system

PIV is a non-invasive method to measure the flow by simply tracking solved particles, e.g. latex beads in a fluid [6]. A high speed camera takes a series of pictures of the channel. Afterwards two images are compared with a correlation algorithm to calculate the movement distance of the particles between both frames.

We developed a new µPIV system consisting of a *Zeiss Axiovert 40C* inverting microscope coupled to a Gigabit-Ethernet camera (*UI-5240CP-M-GL*). The velocity tracers are detected via phase-contrast imaging using the standard halogen lamp of the microscope as continuous light source. Finally the correlation is done by the open-source software *URAPIV* [7].

Due to the pumping principle the resulting fluid flow is very pulsatile with particle speeds ranging from 0 mm/s to 200 mm/s. At highest velocity a particle moves approximately 600 µm if the movement is captured with a frame rate of 300 fps. Most PIV algorithms cannot analyse those big differences. In order to detect the peak velocity one can either:

- Increase the frame rate (camera settings)
- Choose a lower magnification to inspect a larger region which induces bigger tracking particles
- Analyse a greater interrogation window for correlation algorithm.

The frame rate can be easily increased by reducing the picture size and using binning. The experiments were done with a magnification of 2,5x and polystyrene beads with a size of 15 µm (*Invitrogen*) as tracking particles. Their density (1.05 g/cm³) matches that of water reducing sedimentation processes. To detect the highest velocities the z-focus was set to the middle of the fluidic channel (50 µm above glass slide). Only stripes with the width of the fluidic channel (500 µm) were observed achieving frame rates up to 800 fps. Image 3 shows a frame shot at 312 fps with a picture size of 1248x140.

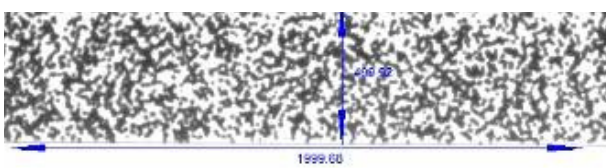


Image 3 Microscopic image of micro channel

After correlation the processed image pair includes the vector field of particle movement. Different resolutions, frame rates and interrogation areas have been tested. Lower resolutions are leading to higher frame rates but also to smaller interrogation areas. Best results are achieved when stripes with a size of 1248x140 pixels are detected with a frame rate of 312 fps and an interrogation window of 128x128 pixels with 50 % overlap and a grid size of 40 to 10 pixels (x- and y-direction).

The calculated vector field is then saved as text file for further processing. Preliminary tests show that the PIV system is able to detect peak velocities up to 25 mm/s. Therefore throttling should be used for the peristaltic pump to reconstruct the flow profile for a whole pump cycle.

2.3 Data analysis

For cell culture systems the wall shear stress and the maximum velocity are relevant parameters. The examined fluidic channel has a width of 500 µm (y-direction) and a depth of 100 µm (z-direction). If we consider a laminar flow in the micro fluidic system then a parabolic flow profile can be detected in z-direction and a more flat profile shows in y-direction. Adherent cells are only cultivated at the top and at the bottom of the channels that is why it is sufficient to calculate the shear stress only at these walls. Following formula can be used to calculate this value (viscosity µ, channel height h):

$$\tau = \frac{-4\mu \cdot \mathbf{v}_{max}}{\mathbf{h}}$$

Laminar flows have their velocity maximum (vmax) at the centre of the micro channel. The flow is considered as laminar because the Reynolds number in micro channels is below the critical value (2320) even at a velocity of 200 mm/s. This has been proven with a Laser Doppler Profile Sensor (LDV-PS) measurement done at TU Dresden [8].

A series of 2,500 image pairs have been captured and analysed. Afterwards the produced vector files have been read by a developed program calculating the averaged maximum velocity and comparing it to the frame number.

3 Results

The evolution of the averaged maximum velocity is measured and calculated at three points of the fluidic system (1, 2, 3) shown in figure 4.

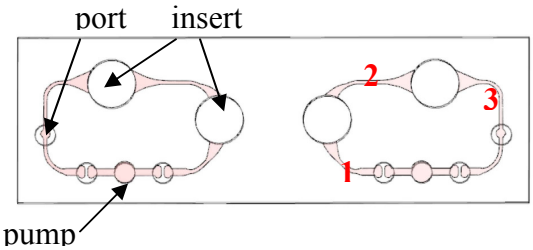


Figure 4 Micro fluidic with three measure points

Due to the pumping principle a periodic velocity-time dependency should be expected. Therefore the pumping period is calculated and several succeeding cycles are averaged to reduce artefacts. Furthermore the dependency of the flow profile for different pumping speeds is analysed at the channel position between both inserts.

3.1 Flow profile at different points on the biochip

There is a pressure drop throughout the whole biochip caused by the compressible PDMS flow cell and the wall shear stress which leads to a velocity reduction depending on the distance to the pump. Due to the fact that the micro fluidic system is totally closed the mean velocity should be the same everywhere on the chip but the profile near the pump has to be much more pulsatile with higher peak velocities and smaller pumping pulse durations. Depending on the current valve state the flow is positive or negative. Figure 5 shows the averaged maximum velocity for the three points on the MOC chip.

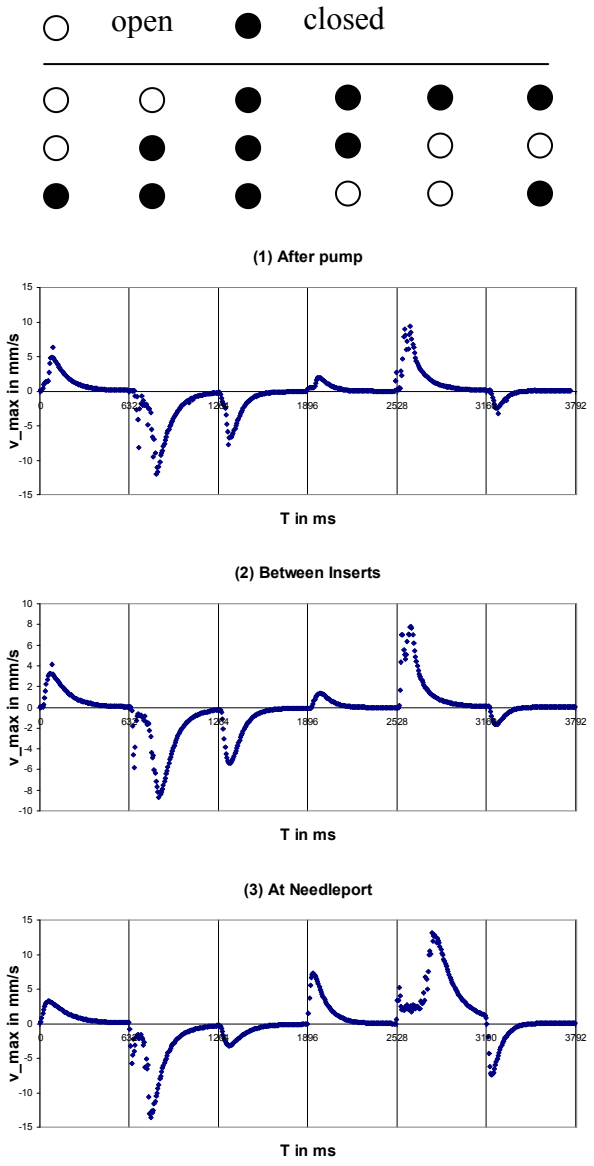


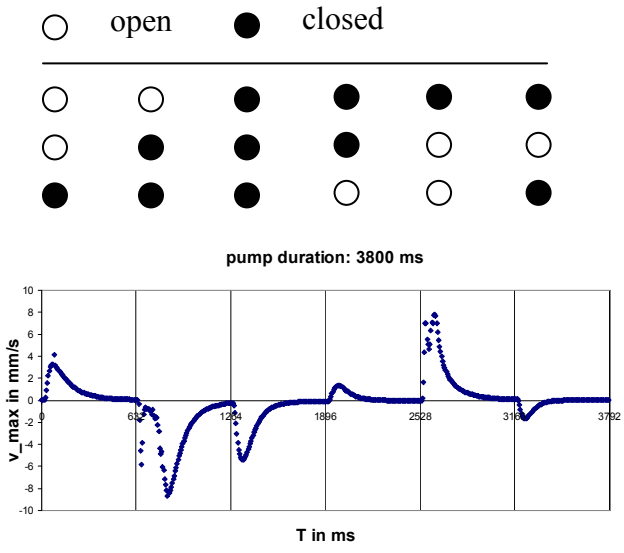
Figure 5 Flow profile for three different points

The measurement was done at 313 fps with cycle duration of 3.8 s (whole pump cycle). Therefore one pump phase is about 630 ms. As predicted, the damping effect of the PDMS walls leads to a smoother flow profile between the inserts. Furthermore the graph shows the limit of the actual measurement system. Especially when the pump chamber in the middle of the peristaltic pump is actuated or filled the particles are affected by a very high acceleration resulting in a random distribution of the velocity vectors. Besides the measurement shows that the developed pump does not work correctly. Normally only during the first and the last pump cycle a back-flow should occur, but also at the filling phase the fluid is pushed into the opposite direction which is caused by slightly open valves. This additional back-flow dramatically reduces the performance of the pump. The highest wall shear stress occurs near the pump and has a value of 8 dyn/cm² (h = 100µm, µ = 1 mPa*s, vmax = 20 mm/s).

3.1 Flow profile for different pumping speeds

As shown in figure 6 the mean velocity of the particles increases with the pumping speed but only to a certain point. This effect is in accordance to the results presented by *Wunderlich* [9]. The flexible PDMS channel walls act as damping elements, comparable to capacitors in electronic circuits, leading to a much smoother flow profile for higher pumping speeds.

The last graph also shows that there is a maximum working speed for the peristaltic pump. This is caused by the throttling of the controller outputs. If the output is throttled it takes some milliseconds until there is pressure or vacuum applied to the membrane of the pump. If this delay is higher than the cycle duration then the valves are not completely closed or fully opened resulting in a reduced velocity.



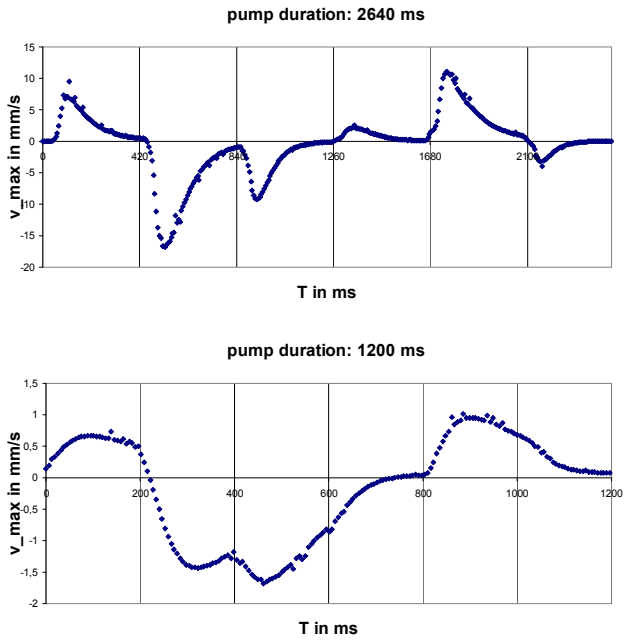


Figure 6 Flow profile for three different pump speeds

4 Conclusion

The presented µPIV measurement is suitable to detect the flow of fluids within small cell culture systems and micro channels. The flow profile of the developed peristaltic micro pump can be analysed on several points of the multi-organ-chip. To detect particle velocities higher than 25 mm/s a camera with a much higher frame rate, e.g. with a CMOS instead of a CCD chip should preferably be used. The measurement of the flow profile near the pump points out that the valves are not completely closed if pressure is applied to the membrane. In further steps their layout will be improved to reduce the resulting back flow. Furthermore the damping effect of PDMS can be used to produce a more steady flow by actuating the pump with higher pumping speeds and including reservoirs before and behind the peristaltic pump. At the moment the PIV measurement is done by taking a short video of the flow, extracting the frames and afterwards analysing them with *URAPIV* software. In the future all steps should be included in one package by using the open source algorithms of the camera and *URAPIV*. The goal is to develop a software solution which automatically detects several measurement points on the chip and then performs a PIV analysis for several seconds, saving the data in a log file. This allows online monitoring of the MOC chips, so that leaks, air bubbles or membrane damages can easily be detected.

Acknowledgements
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