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Experiment Report

Experiment title: In-situ observation of capturing target cells at the inner surface of porous media
Experiment number: LS 2354
Beamline: ID19
Local contact: Dr. Alexander Rack
Number of shifts: 9
Names and affiliations of applicants: Dr. Christoph Blankenburg Jenny Hon and Christopher Helbig (University of Applied Sciences, Darmstadt), André Liebscher, Katharina Losch and Jan Niedermaeyer (Fraunhofer Institute of Industrial Mathematics, Kaiserslautern)
Date of experiment: from October, 29th to November, 1st, 2014

The separation of specific cells out of a suspension of a cell mixture is an important and central task in the treatment of various diseases. Such separation is usually done by centrifugation or fluorescence activated cell sorting (FACS). Chromatographic filtering could be, on the one hand, more conservative than centrifugation and, on the other hand, much faster than FACS. This would be, for instance, an original and efficient solution to separate cancerous and healthy blood cells, i.e. large blood amounts of patients suffering from leukemia must be draw, cleaned using filtering techniques and re-injected. In the chromatographic technique the cell suspension is flown through a porous medium whose inner surface is for instance activated by a cell binder molecule monolayer upon an organosilanized surface. The aim of such a surface coating is to permanently or temporarily bind the specific cells (e.g. cancerous cells in the case of leukemia) at the surface and, as a consequence, to reduce their mobility compared to that of of healthy ones, see Fig. 1. Aside from the coating of the inner surface, the chromatographic process depends on the geometry of the pore space that influences the dynamics of the liquid-solid flow of the suspension and, thus, the deposition rate of cells at the inner surface. In experiments performed at ID19 we investigated partially open foams with respect to their suitability for chromatographic filtering.

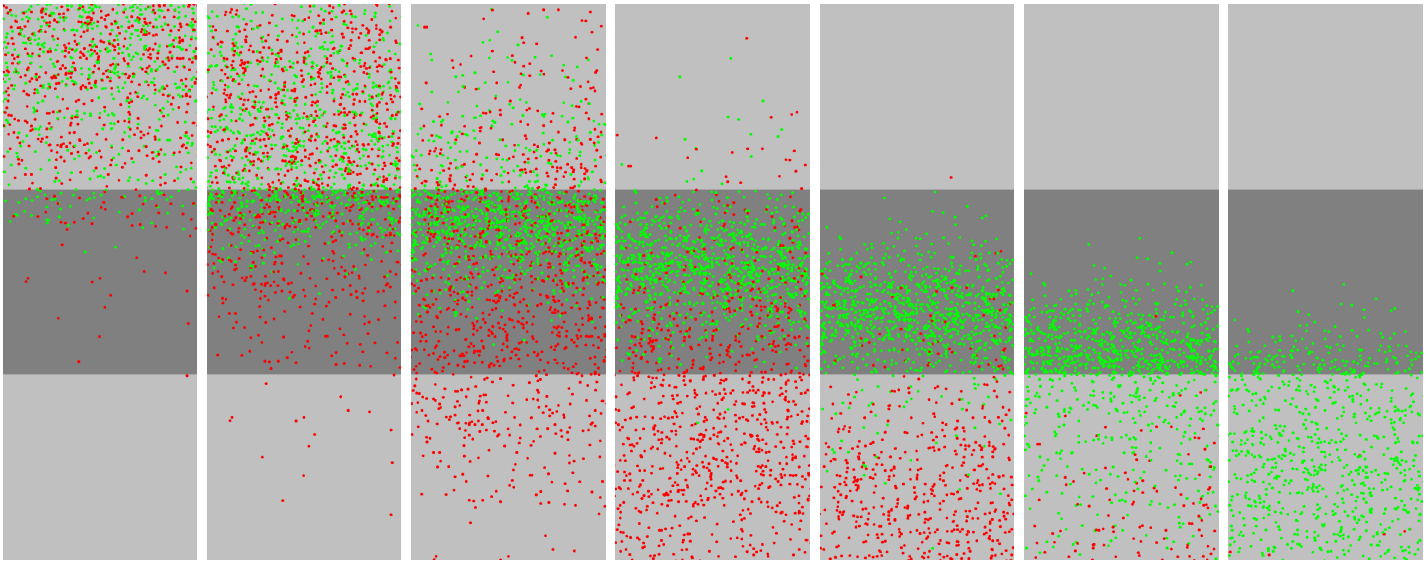

Figure 1: Scheme of chromatographic cell filtering: The image sequence is showing a simulated flow (from top to bottom) of a suspension consisting of two populations of cells (one of them marked red and the other one marked green). The green population is interacting with the inner surface of the filter (dark gray).

In-situ time-resolved µCT has been proven as a powerful technique for observing moving particles in a liquid-solid flow of a suspension through porous media, see Fig. 2 for a sketch of the experimental setup and Fig. 2 for a sequence subimages with particles slowly moving through a partially open foam. As far as we know, the scan rate combined with high spatial resolution and sensitivity for 4D µCT reached at the beam line ID19 of the ESRF is currently one of the best. Using this technique it is possible, for example, to track the motion of particles with diameters larger than 20 µm and a speed less than about $50\,\mu\mathrm{m\,s^{-1}}$. Future developments in this field will lead to much higher scan rates allowing also for tracking of faster particles. Clearly, simulation of liquid-solid flow is a well established supplementary method of investigating chromatographic filtering, where the flow models can include e.g. the formulation of blood rheology depending on the local concentration of the cells as well as its change by selective deposition, which can notably be of interest in the leukemia context. Nonetheless, experiments with real suspensions pumped through porous media and direct observation of particle motion and deposition will also be in future indispensable methods to get deeper insight into filtration processes.

Generally, the estimation of the torsion of particle paths from 4D µCT images has been proven to be a hard problem of image analysis. It needs a careful design of the experimental setup, a choice of estimation methods depending on the specific kind of data sampling, as well as a forecast of the estimation errors to be expected. The core of the estimation method presented in [2] is a new discretization scheme of the differential geometric formulas. This discretization is directly induced by observing moving particles by 4D µCT, where the distances between particle positions (i.e. the step width) in two subsequent 3D images are large compared to the accuracy of measuring these positions which mainly depends on lateral resolution. Furthermore, the true curvature and torsion themselves have a considerably impact on the estimation errors. Simulation studies are helpful to get an overview of the very complex interdependencies.

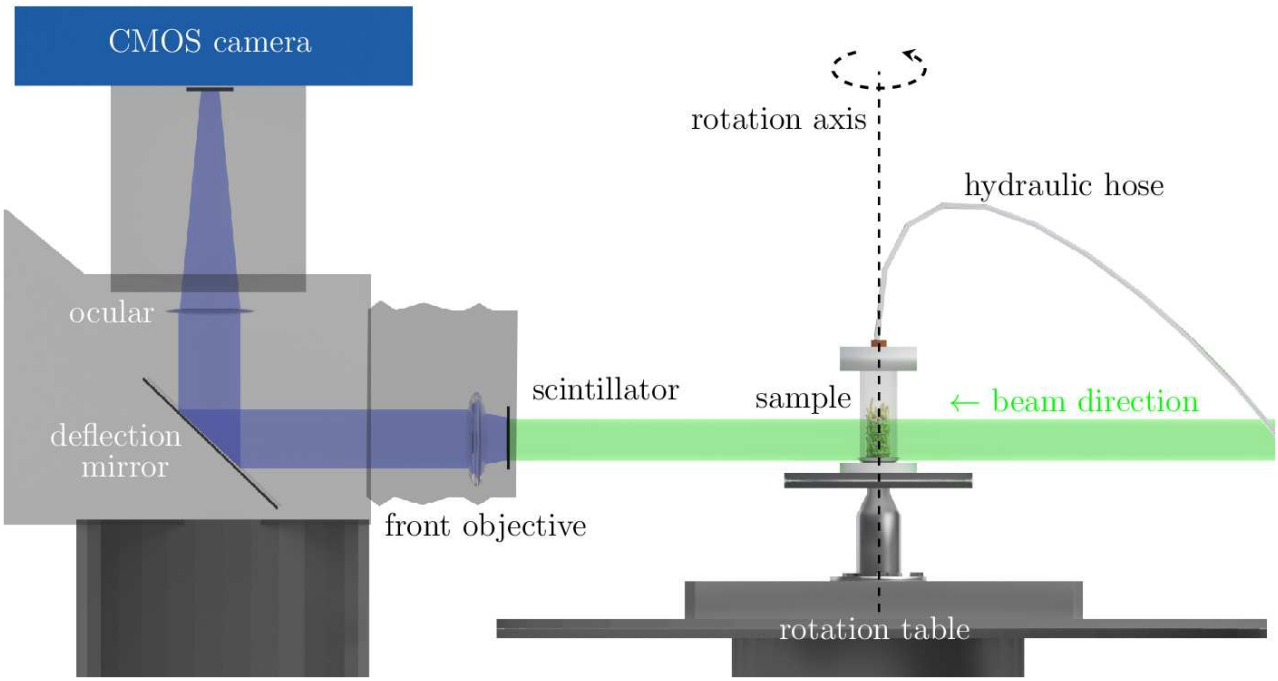

Figure 2: Sketch of the setup for time-resolved µCT with the interior setup of the image acquisition device. The distance between the sample and X-ray source was about 150 m.

The empirical mean curvature was $0.16\,\mu\mathrm{m^{-1}}$ for the slower particles (mean speed of $16.7\,\mu\mathrm{m\,s^{-1}}$) and $0.013\,\mu\mathrm{m^{-1}}$ for the faster ones (mean speed of at least $1.9\,\mathrm{mm\,s^{-1}}$). Hence, the magnitude $\|F\| = m\kappa\|\dot{f}\|$ of the centripetal force F that makes a fast particle of mass m follow a curved path f is much higher than that for slower ones. Nonetheless, the moderate increase of the the curvature (and torsion) for decreasing speed is of high importance for the microcentrifugation effect. It partially compensates the effect of slowing down the centripetal force such that significant microcentrifugation probably appears as long as the particle is not deposited at the inner surface.

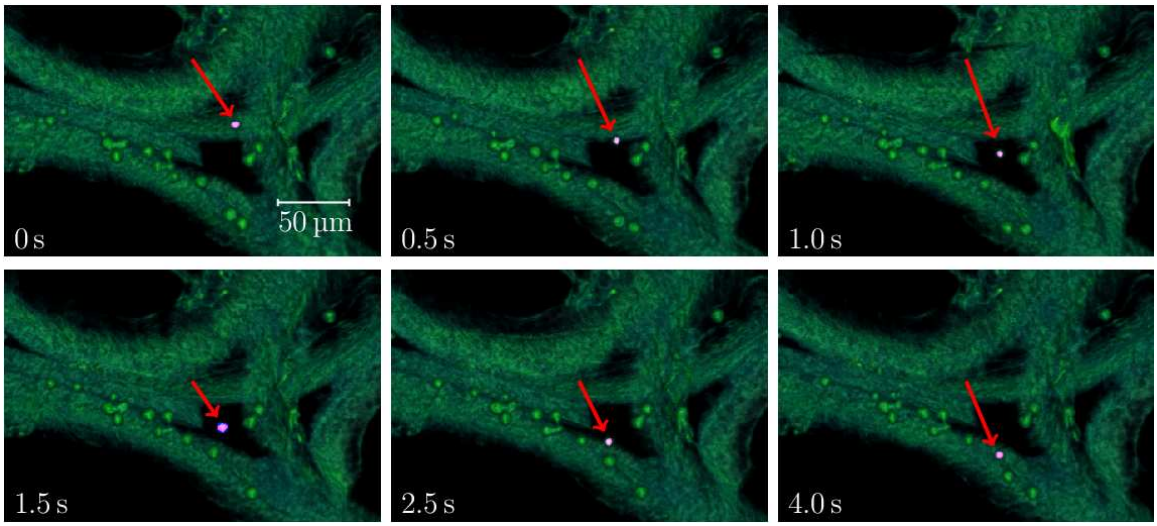

Figure 3: A sequence of small subimages with slowly moving particle marked with a red arrow, volume rendering. The marked particle is slowed down with increasing time. The pixel size of the 3D data set is 1.1 µm.

The foam structures investigated in these experiments are completely isotropic. More precisely, the distribution of the foam is invariant with respect to spatial rotations and, thus, it is also free of orientation. However, orientation can be detected e.g. for fiber fleeces produced by a melt-blown process with a rotating lattice of fiber spinnerets. This is an important aspect for development of porous media applied in cell chromatography, since one can considerably increase the microcentrifugation effect when inducing orientation of the pore space by varying the production process.

The centripetal acceleration caused by the specimen rotation during image acquisition can have a huge effect on curvature and torsion estimation. In our experimental setup, the specimen's radius and the angular speed were $r = 4\,\mathrm{mm}$ resp. $\omega = 2\pi\,\mathrm{s}^{-1}$, which yields a magnitude of the centripetal acceleration of at most $158\,\mathrm{mm\,s}^{-2}$. On the other hand, the magnitude of the centripetal acceleration of a particle moving along the path f is equal to $m\|\ddot{f}\|$. For the slow particles, the mean of this magnitude was about $200\,\mu\mathrm{m\,s}^{-2}$ only. The impact on torsion estimation is not clear, but we can argue as follows: In the case of a significant influence of sample rotation on the torsion of particle paths, the torsion distribution would be shifted (to left or right, depending on the orientation of the specimen rotation). The observed torsion distribution, however, is symmetric (and in particular, the estimated mean torsion is close to 0). Nevertheless, in future experiments with higher temporal resolution the effect of the specimen rotation on the observations should be taken into consideration.

The characterization 3-dimensional images of partially open foams as well as fiber fleeces with respect to their suitability for chromatographic filtering is published in [1]. Results of the analysis of the image sequences obtained by the time-resolved µCT are presented in [2]. The investigations made at ID19 are stimulating the development of new chromatographic filters e.g. at Merck KGaA [3].

[1] C. Blankenburg, C. Daul, J. Ohser (2016) Estimating torsion of digital curves using 3D image analysis. *Image Anal. Stereol.* **35**, 81–91.

[2] C. Blankenburg, A. Rack, C. Daul, J. Ohser (2017) Torsion estimates of particle paths through porous media observed by in-situ time-resolved microtomography. *J. Microsc.*, **266**, 141–152.

[3] www.esrf.eu/home/Industry/industry-news/content-news/esrf-news-list/exploring-chromatographic-filtering-or-how-to-separate-healthy-from-cancerous-cells.html.