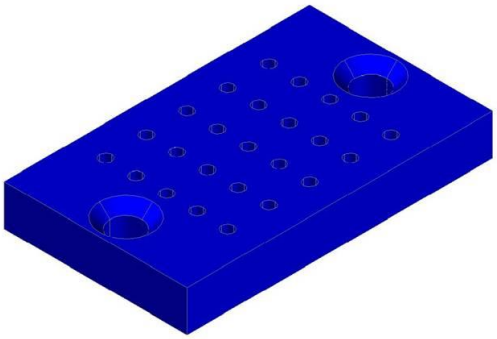
Micro Sample Environments for High Brilliance Small Angle X-ray Scattering

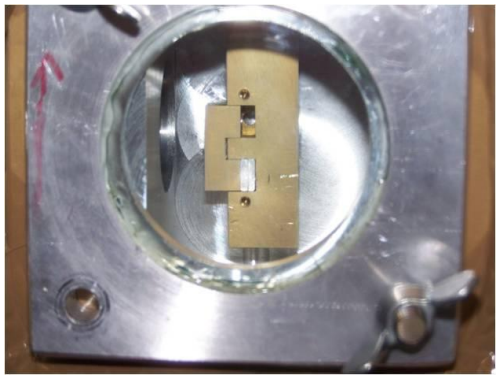
SC 2266

The opjective of this long term project is the development of micro environments for small angle X-ray scattering. This includes cryogenically cooled sample mounts and SAXS measurements in microfluidic devices. This time a optical tweezers experiment is reported and preliminary results presented.

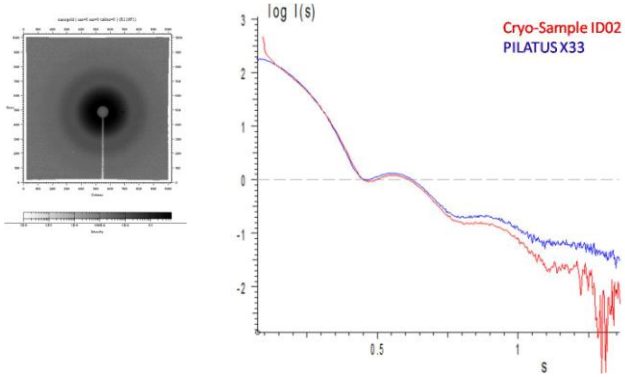
Cryo-SAXS measurements at ID02:

The objective of this beamtime was the further refining of the cryo-SAXS set-up and application for medium focused beamsizes available at ID02. The cryo-flash freezing protocol was slightly changed and samples differently prepared. This time on site prepared high pressure frozen sample were measured as well. For this purpose a high pressure freezing apparatus available at the ESRF was used. A new sample mount was developed and the cold finger cryostat modified and a metal shielding as cryo trap of residual water integrated.





Drawing of the sample mount. In the holes Cu-tubes with high pressure flash frozen samples are mounted. The complete holder is manually transferred to the cold finger (right image) and kept under vacumm. The massive metal around the silver sample holder acts as cryo trap.



A sample preparation protocol involving high-pressure freezing (Leica EMPACT-2) and cryoultramicrotomy (Leica UC-6) has been developed. The resulting samples are frozen aqueous solutions of proteins or other colloids and are 1 mm in length and 0.3 mm in diameter.

Experiments made at ID02, ESRF show that solution scattering under cryoconditions is possible, but it still has considerable challenges. Solid water is fragile and preparing samples which are homogeneous at the length scales probed by SAS has proven to be difficult. Solutions of strongly scattering colloids or concentrated solutions of high molecular mass proteins can be measured with reasonable precision, but the anisotropic background contribution from the aqueous buffer makes measurements of samples with low scattering power hard, although not impossible.

Futher downscaling of the sample volume will be accieved by microdroples and thin solution layers. This experiments are scheduled for beginning of February 2010 as the last experiments in the frame of this LTP.

ESRF microdrop R&D. Work has continued in the reporting period in the context of the PhD work of R. Graceffa (SAXIER). The aim of the project is the development of stroboscopic small- and wide-angle scattering (SAXS/WAXS) for the study of fast mixing reaction reactions of ballistic (several m/s) microdrops. As compared to microfluidic cells, shearing effects, aggregation at cell walls and X-ray background scattering from cell walls can be avoided. A schematic picture of the setup has been included in the last report.

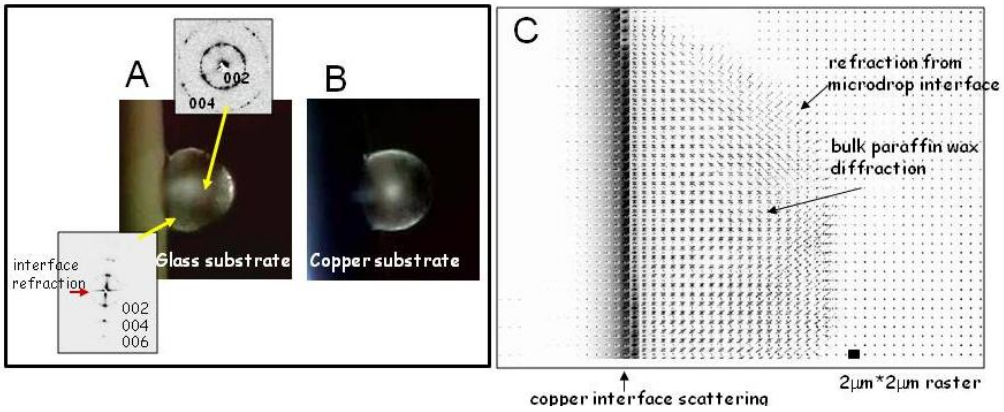


*Figure 1A: Paraffin wax microdrop deposited on glass substrate. Selected diffraction patterns recorded with a 1 μm beam are shown. The 00l peaks suggest a preferential paraffin wax orientation close to the glass substrate. The horizontal streak is due to refraction from the glass interface; B: paraffin wax microdrop on copper substrate. The scale of the microdrops can be derived from Figure 1C; C: Composite diffraction image of paraffin wax drop on copper wire based on a 2*2 μm raster-scan.*

Experiments on paraffin microdrops have been pursued by studying microdrop deposition on glass and copper surfaces. (Figure 1A,B) The deposition temperature of the microdrops was at the paraffin wax melting temperature. WAXS patterns were recorded by 2 □m step-rastering of the solid microdrops using a 1 □m beam at 12.7

keV. (Figure 1C) The patterns show a strong paraffin texture close to the glass interface. For the copper substrate, the texture was observed extending into the bulk. This is tentatively attributed to the factor 400 higher thermal conductivity of copper which is assumed to influence the nucleation step.

Stroboscopic SAXS has been pursued by studying the coalescence of ballistic microdrops. Stable coalescence has been demonstrated for mixing of acid cytochrome C solution microdrops with buffer microdrops. (see Figures below) A composite SAXS image is shown in Figure C. The refraction streak from the microdrop interface allows localizing the microdrops. At 4 ms flight time, the protein has already been distributed nearly homogeneously across the merged microdrop.(Figure below)

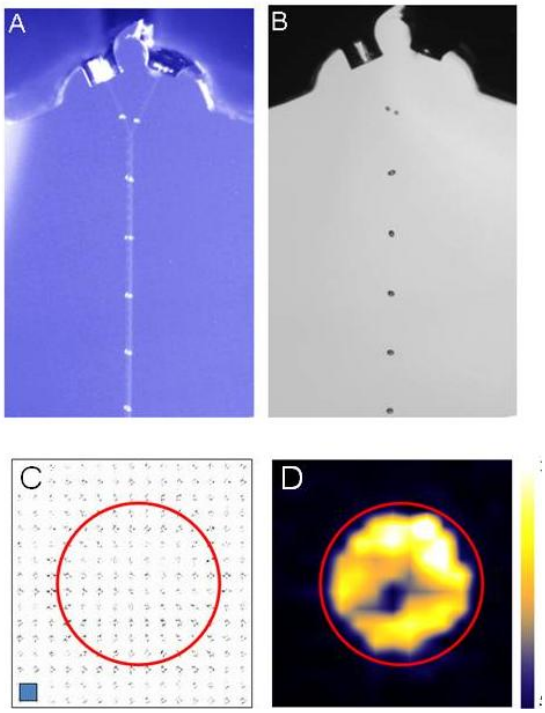
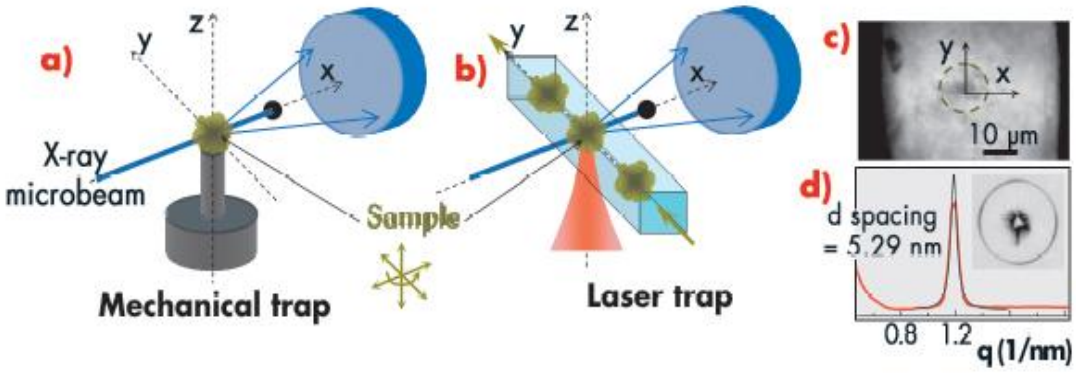


Figure A: Stroboscopic optical images of microdrop coalescence. A: overlap of stroboscopic LED illumination and illumination by continuous light. The sensitivity of the digital camera allows visualizing the drop trajectory. The oscillations are associated with the dissipation of excess kinetic energy in the coalescence. B: stroboscopic LED illumination showing the microdrop shape evolution after coalescence; C: Composite SAXS image obtained from 10μmx10μm raster scan at 4 ms from the coalescence point. The „pixels" correspond to the scattering close to the beamstop. The streaks are due to refraction from the microdrop interface. D: Composite SAXS image; the „pixels" are scaled to the local integrated SAXS intensity. The values were normalized for the beam path length through the microdrop.[3]

Laser Tweezers (LT) and synchrotron nano-beam investigation techniques.

We selected a method to seal SU8 channels by using a UV lamp with shorter wavelength than the ones commonly used for lithography. This allows making the device with two lithographic steps: one conventional lithography to create the channels, one UV lithography with shorter wavelength in order to create the lid. We are still optimizing the process.

In the meantime we have used the alternative circuit based on the square capillary as described in figure 3 at the ID13 beamline at ESRF to trap both starch and liposomes.



(a) Sample manipulation in X-ray microdiffraction experiments: a) Mechanical contact/trap and b) Laser trap without mechanical contact. c) Microscope image of a cluster of liposomes trapped optically (dash line) inside a capillary (the walls of the capillary are seen in black on the lateral sides), scale bar 10 µm. d) Plot of the azimuthally integrated intensity (red) and the Lorentz function (black) fitted to experimental data for the diffraction pattern (inset) at one position of the X-ray beam on an optically trapped POPE cluster.

We also have used the circuit in order to insert lysozyme crystals in the capillary and trap them while still maintaining a buffer flow. In this way, crystals do not stick to the capillary walls and can be manipulated. This experiments were preformed in December 2009 and the data processing is still goning on.