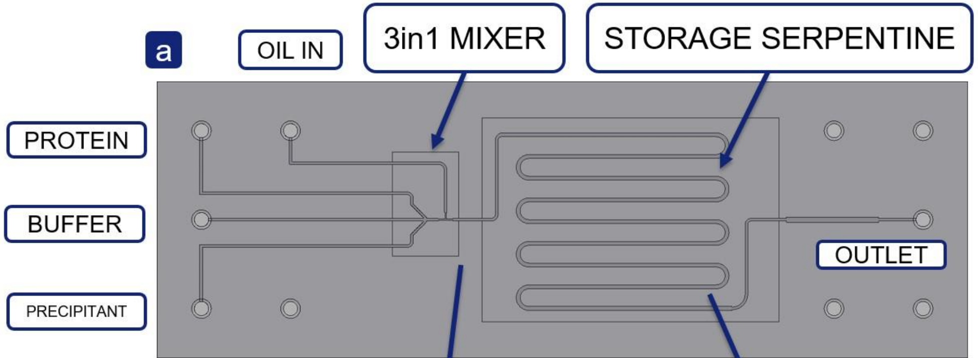
ESRF User Meeting 2022:
SB BAG Meeting

μfluidics for MX/BioSAXS

Anton Popov, 7th Feb 2022

MICROFLUIDIC DEVICE (3D PRINTED) – PART 1

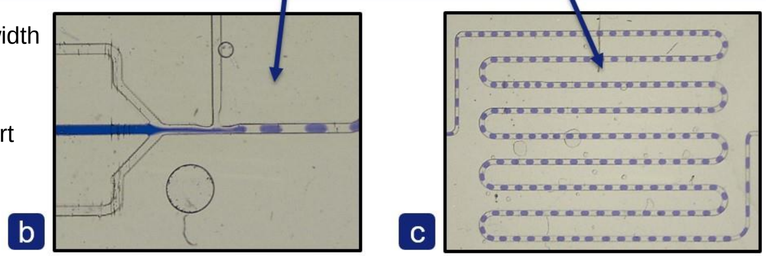


Main channels width and depth

≈ 160 x 150 µm;

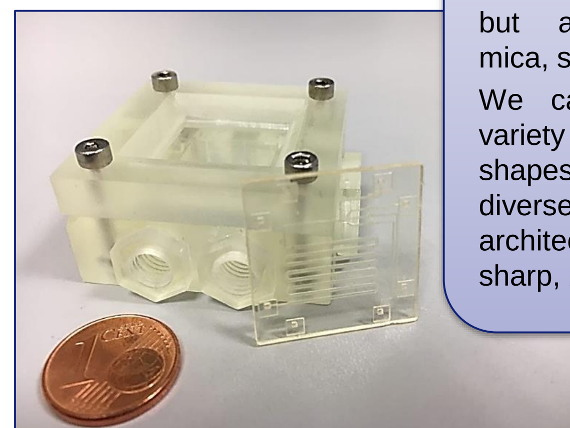
Narrow mixer part

≈ 100 µm;



With a 3D Printer it is possible to build complex systems of micro channels, reservoirs, basins, cascades, sets of inlets/outlets, etc. Using not only different resins, but also glass, mica, silica, etc.

We can print a variety of channel shapes, extremely diverse architecture (deep, sharp, broad, etc.)



In use are both home-made MF equipment (22 x 22 x 1.5 mm) and the Micronit Chip Holder, which determines the size of the chips – 15 x 45 x 1.5 mm.

- Currently, we use two 3D printers: 37 and 27 micron pixel size. The smallest printable structure of a good quality is 2 pixel.
- Smallest practical thickness of a device layer is 25 or 15 microns.
- All chips have high chemical resistance and can be reused.

MICROFLUIDIC DEVICE (3D PRINTED) – PART 2

Through the formation of microbatch droplets, we managed to show the gradient of crystallization parameters inside one chip

(approx. number of droplets inside the chip - 800).

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Test protein – Trypsin from bovine pancreas (T9201).

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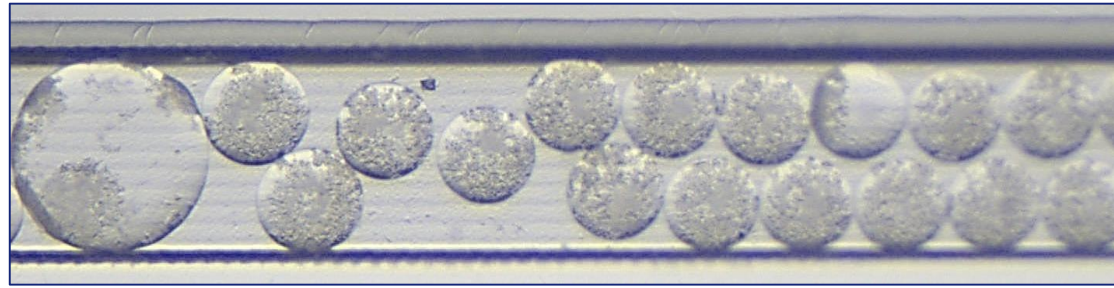
Width ≈ 400 µm

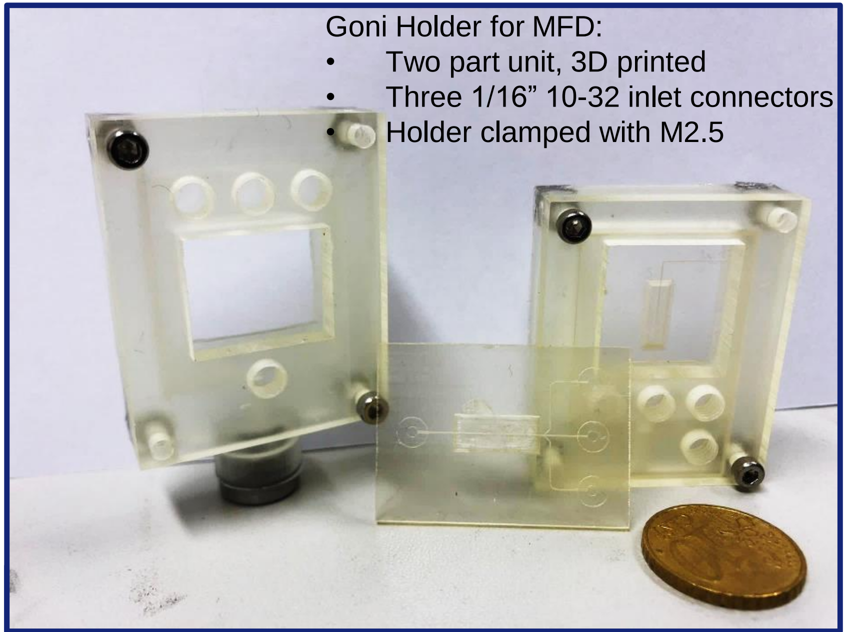
Depth ≈ 150 µm

Estimated crystal size ≈ 70 µm

Estimated droplet volume ≈ 2,5-5 nl

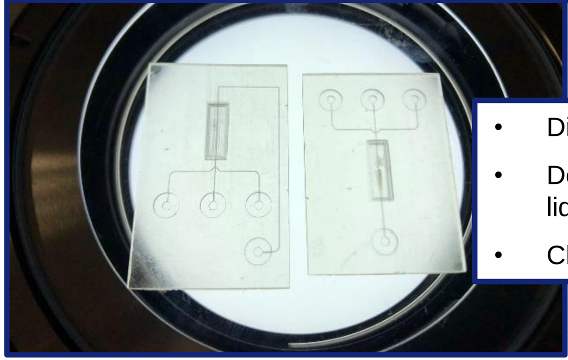


MICROFLUIDIC DEVICE (3D PRINTED) – PART 3

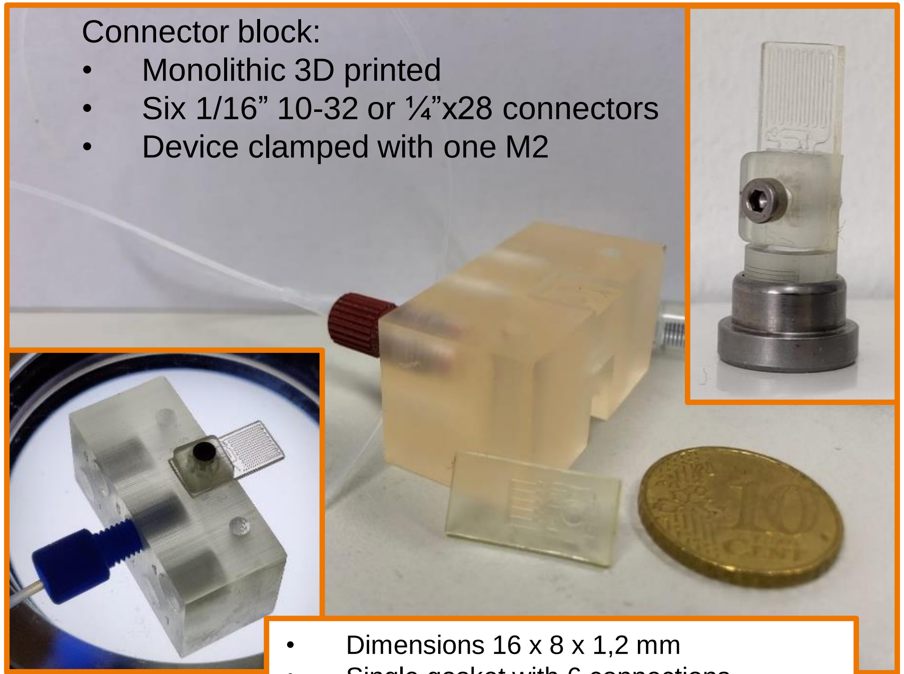


Goni Holder for MFD:

- Two part unit, 3D printed
- Three 1/16" 10-32 inlet connectors
- Holder clamped with M2.5

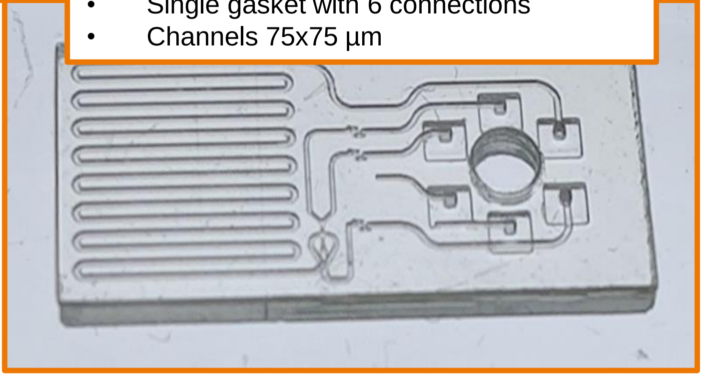


- Dimensions 35 (38) x 25 x 3 mm;
- Devices are sitting very tightly, liquid proof, pressed by fittings;
- Channels 150x150 µm



Connector block:

- Monolithic 3D printed
- Six 1/16" 10-32 or ¼"x28 connectors
- Device clamped with one M2

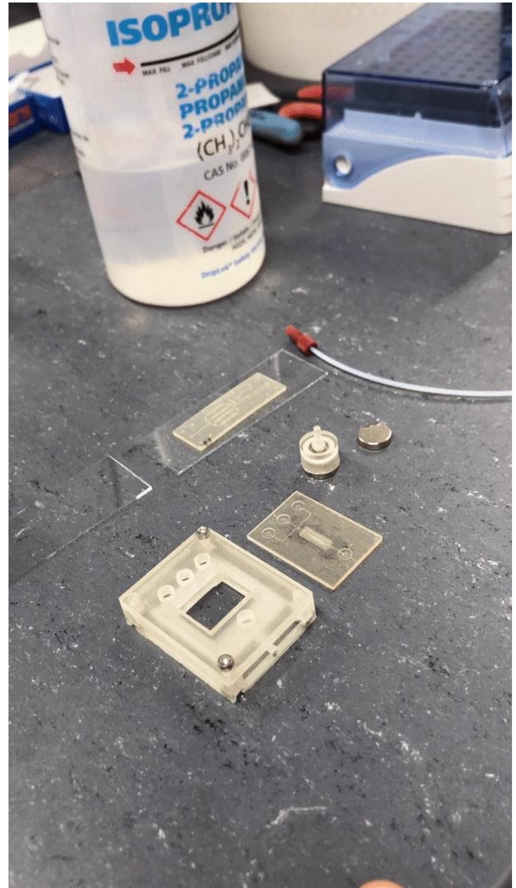


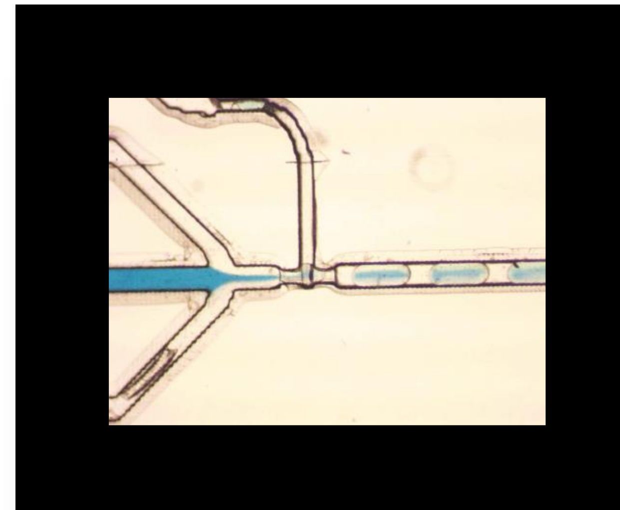
- Dimensions 16 x 8 x 1,2 mm
- Single gasket with 6 connections
- Channels 75x75 µm

MICROFLUIDIC DEVICE (3D PRINTED) – PART 4

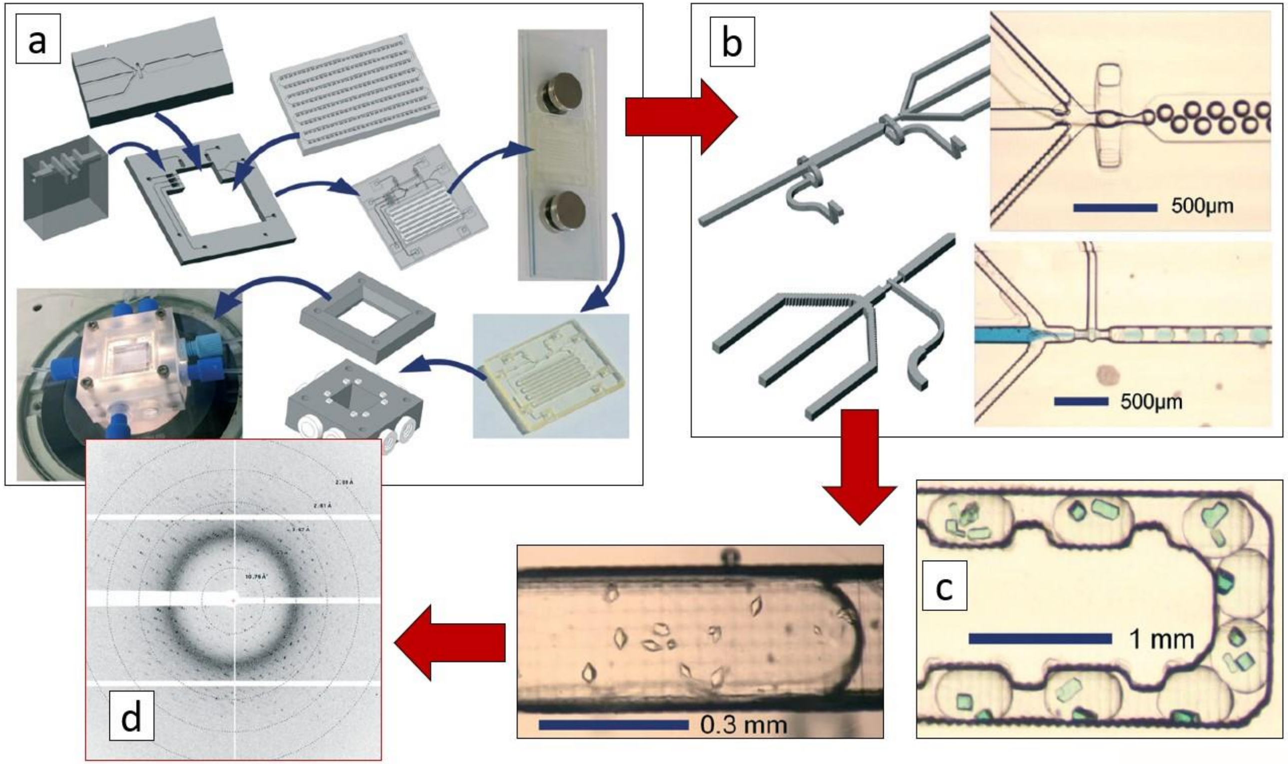
Goniomètre Holder and MFD

Work in progress





ACCURATE AND RAPID 3D PRINTING OF MICROFLUIDIC DEVICES



(a) Different elements such as filters, a droplet generator and droplet traps are digitally assembled into the frame. The completed design is printed, post cured between glass slides, made transparent and clamped in the support for the experiment.

(b) Flow focusing droplet generator design and operation. T-junction droplet generator design and operation.

(c) Channel constrictions with trapped droplets. Lysozyme crystals and thaumatin crystals.

(d) X-ray diffraction of a thaumatin crystal deposited on a resin slab and measured on ESRF beamline ID30-A3.

Lab on a Chip

Peter J.E.M. van der Linden, Anton M. Popov, Diego Pontoni
https://doi.org/10.1039/D0LC00767F

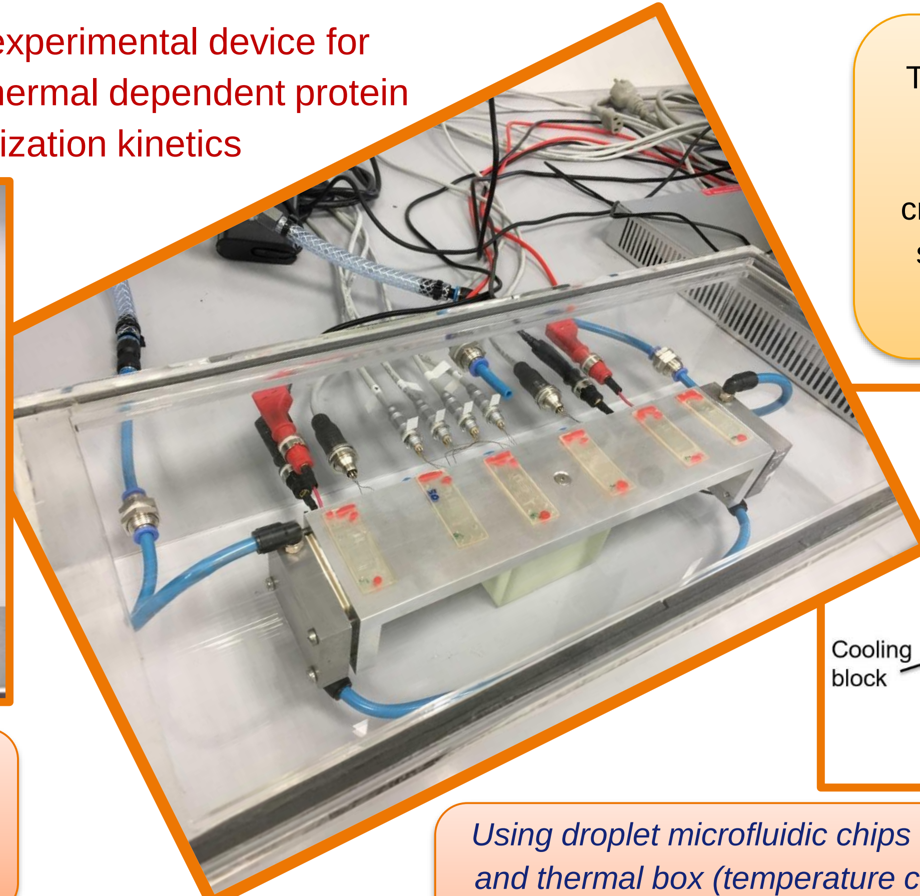
ROYAL SOCIETY OF CHEMISTRY

The European Synchrotron | ESRF

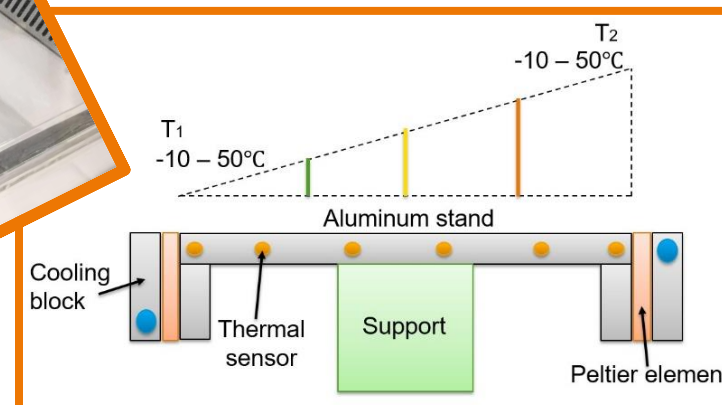
THERMO-BOX PROJECT _ 1

Peltier-based experimental device for investigation of thermal dependent protein crystallization kinetics

MF device
(15 x 45 x 1.5 mm)
in unified goniometer holder

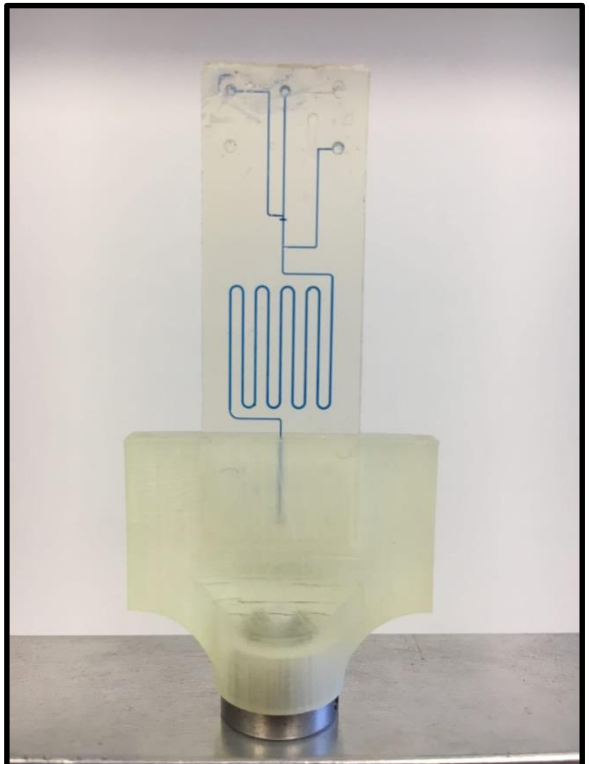


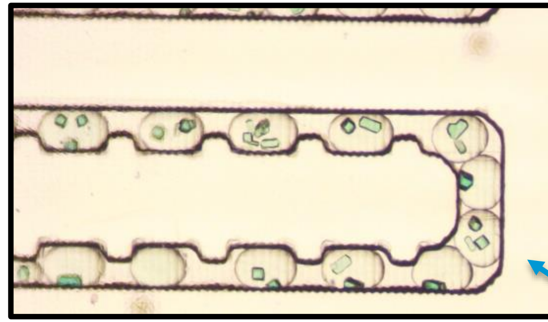
The work platform is a 25 x 6 cm aluminum rectangle that has space for up to 12 MF batch crystallization devices, containing similar or various crystallization conditions.



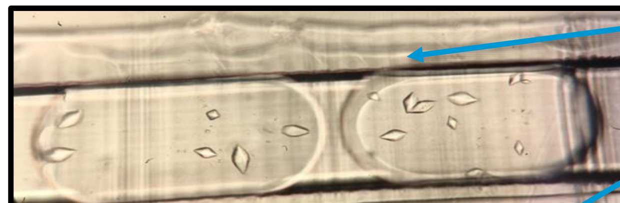
Using droplet microfluidic chips (screening) and thermal box (temperature control), it is possible to determine the optimal crystallization conditions.

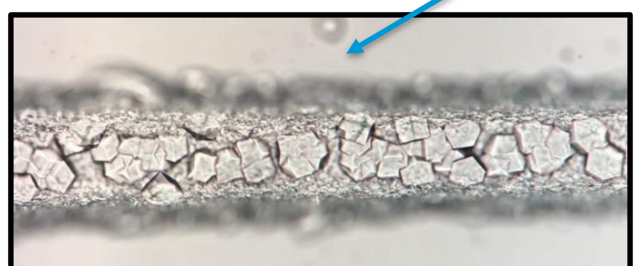
THERMO-BOX PROJECT _ 2





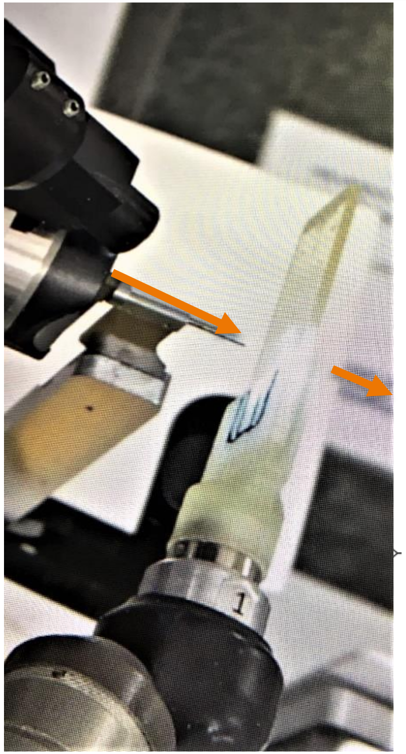
Lysozyme, Trypsin (bovine), Thaumatin, Insulin (bovine), Thermolyzine

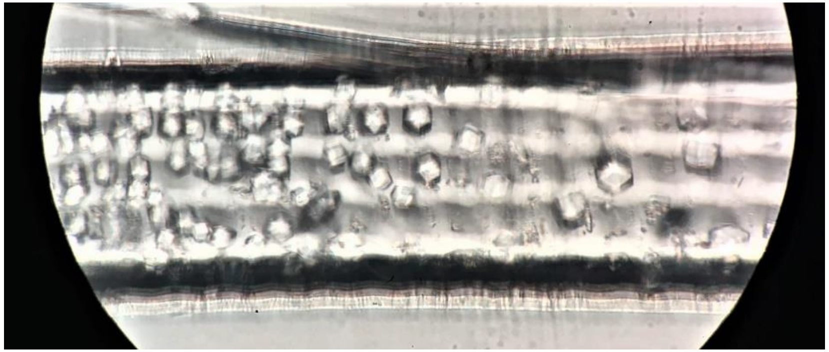


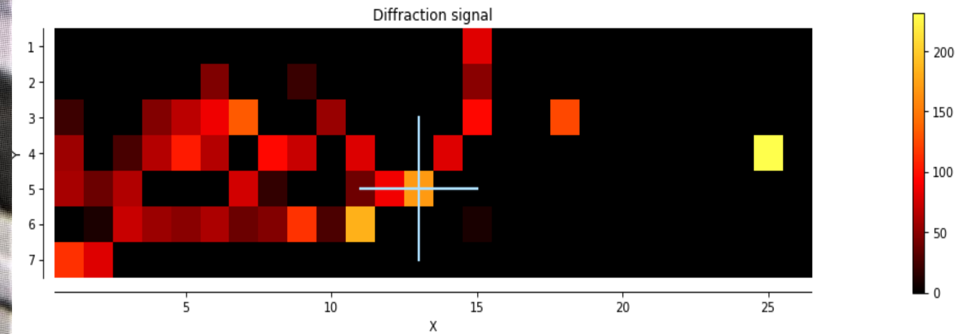


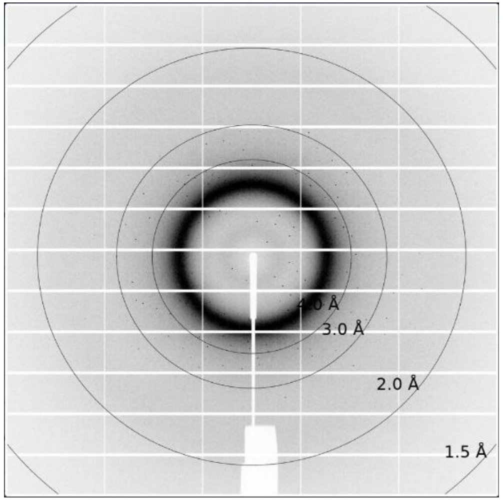
MESH-SCAN ON ID30-B (IN CHIP MEASUREMENTS)

The crystals were inside the microfluidic device. 600 µm of wall thickness at the “front” & 700 µm at “back”.
The background shows a broad scattering ring between 4 and 5 Å, and scattering peaks are visible to a resolution about 1.8-2.5 Å.







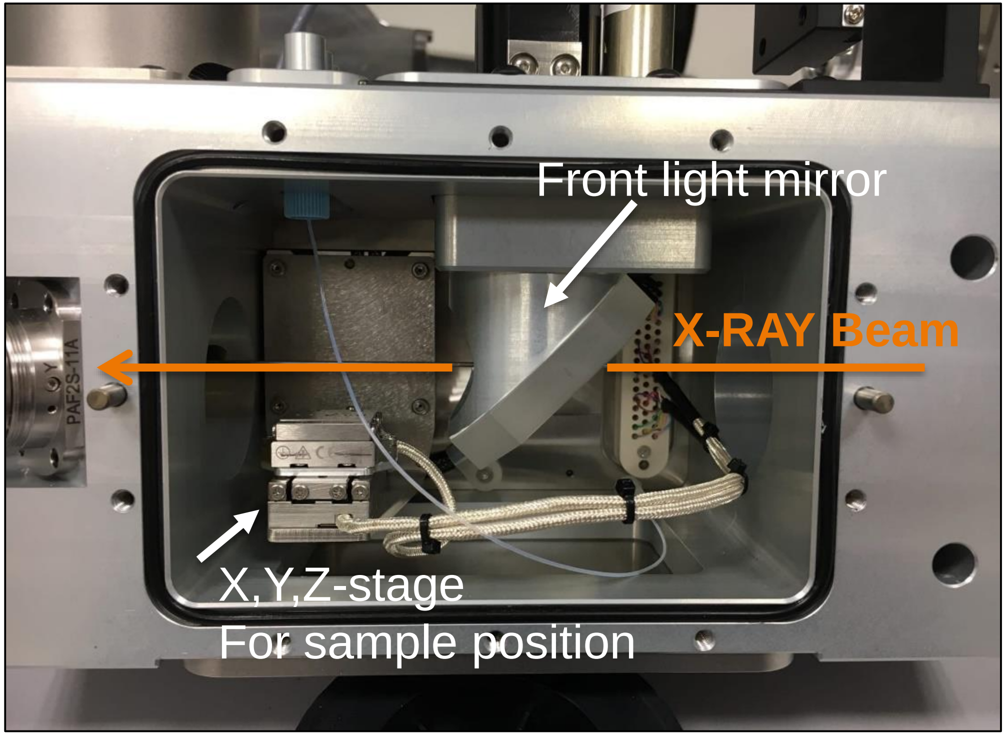


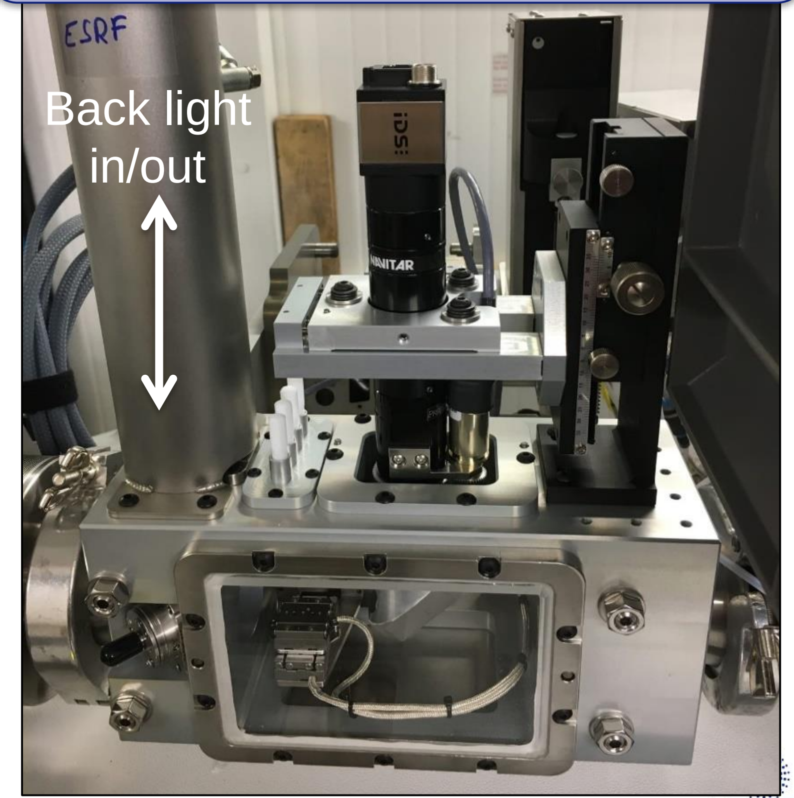
- Photon energy = 12.41 keV
- Wavelength = 0.999 Å
- Focal spot = 10x10 µm
- Diffraction resolution = 2.0 Å
- Diffraction was measured with a Pilatus 6M detector.
- The total resin thickness was 1300 µm

SAMPLE EXPOSURE UNIT @ BM29 _ 1

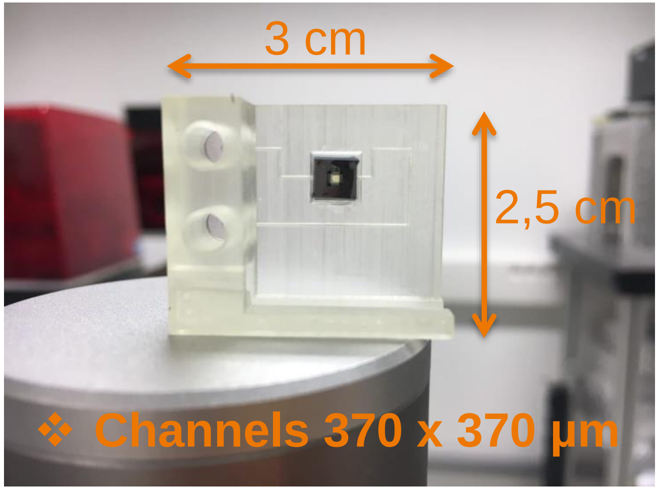
Microfluidic Experimental Chamber will be the third option, besides the BioSAXS Robot and SEC-SAXS (HPLC) to conduct the experiment @ BM29

- Using syringe pumps we have 3 work flow inlets;
- X,Y,Z-stage for precise positioning of the devices;
- Navitar column and IDS camera;
- Upgrade potential.



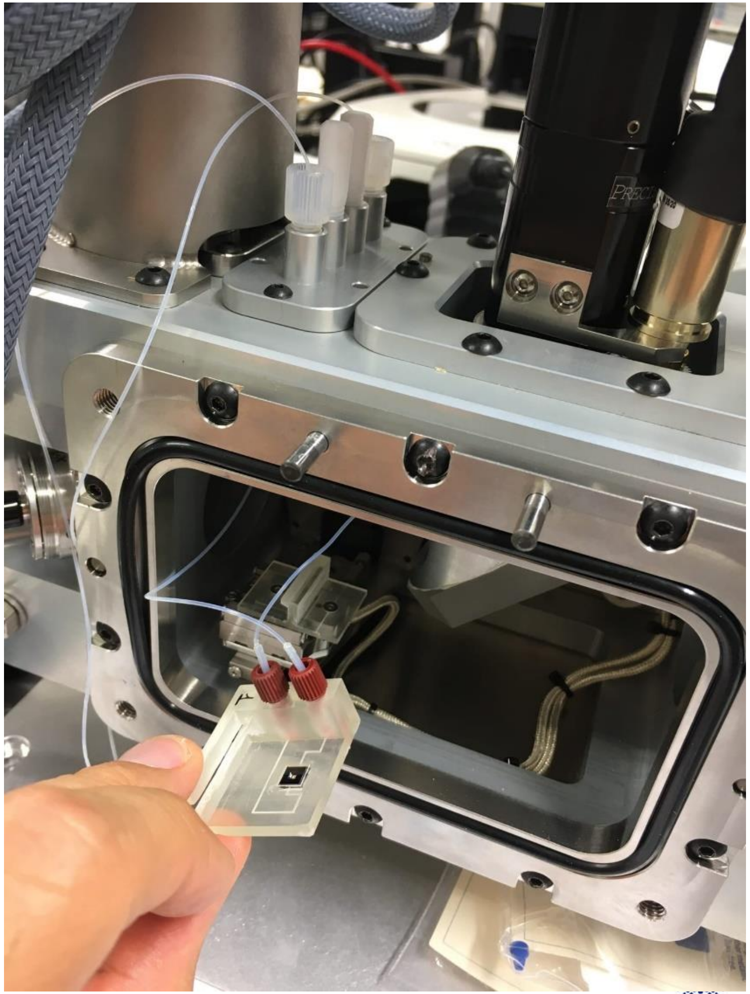


SAMPLE EXPOSURE UNIT @ BM29 _ 2



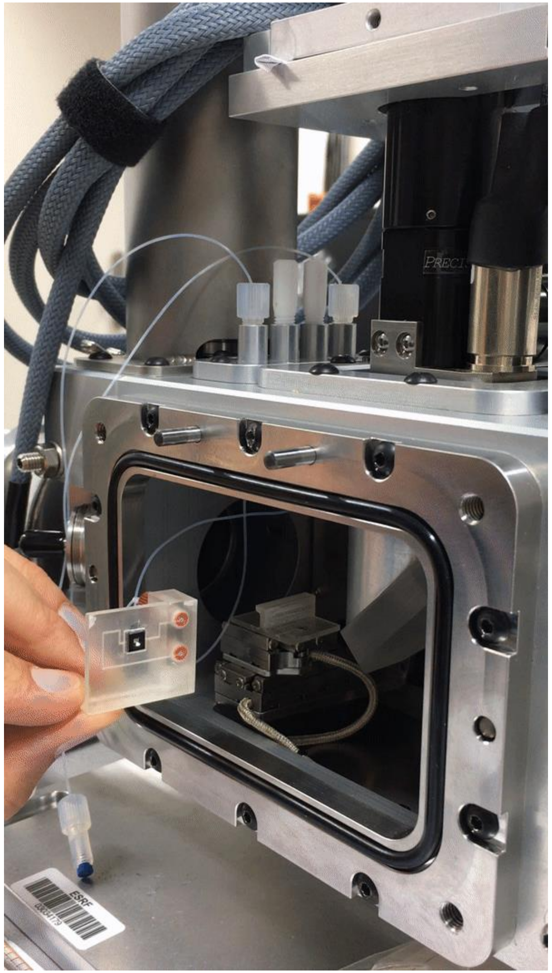
Windows materials – COC film (100 µm) and Silson Silicon nitride (1000 nm)

Permatex Flowable Silicone Glass Sealer - Forms a tough, waterproof, durable, clear seal. Fills surface voids and irregularities.



SAMPLE EXPOSURE UNIT @ BM29 _ 3

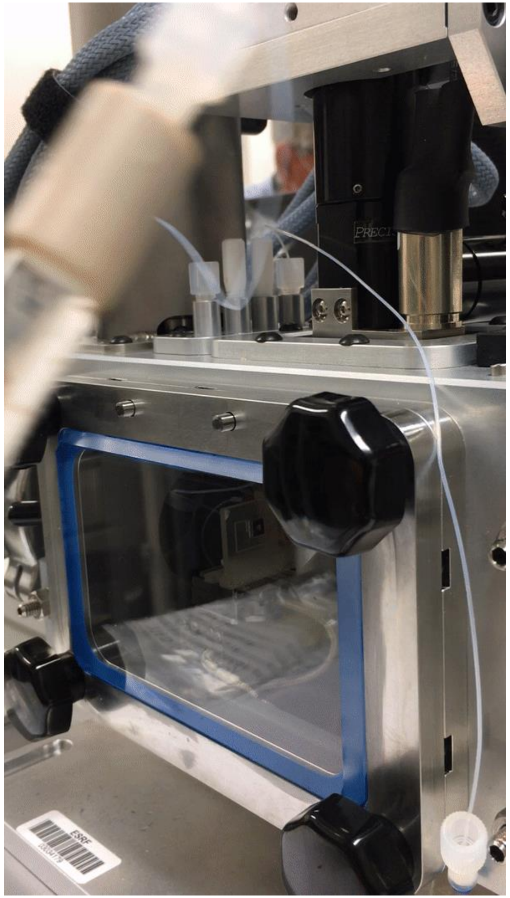
It takes only 40 seconds to change the MFD in the SEU due to fast door and users friendly sample environment.



With non-windows MFD, the Vacutight fittings are holding up to 10-5 mbar.

SAMPLE EXPOSURE UNIT @ BM29 _ 4

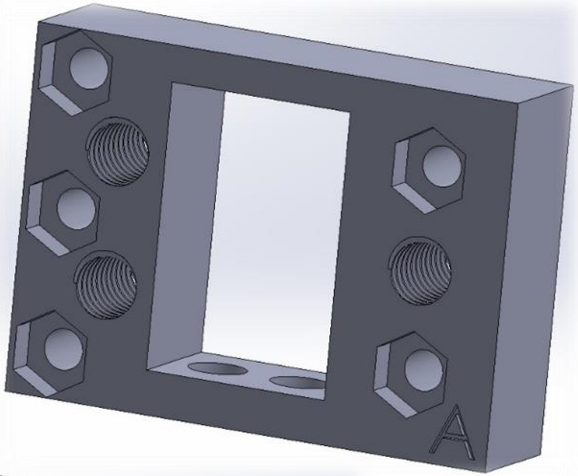
The flow rate (very high!) 25 µl/sec

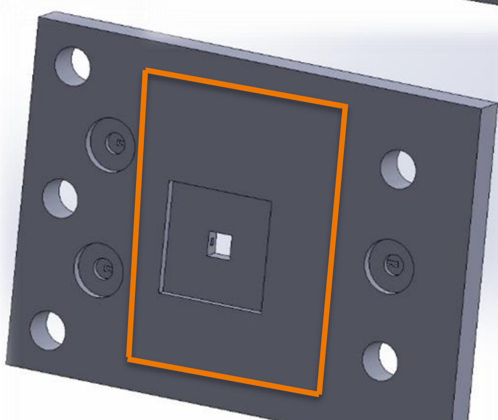


Microfluidic device was in SEU @ 1,4 x 10-3 mbar

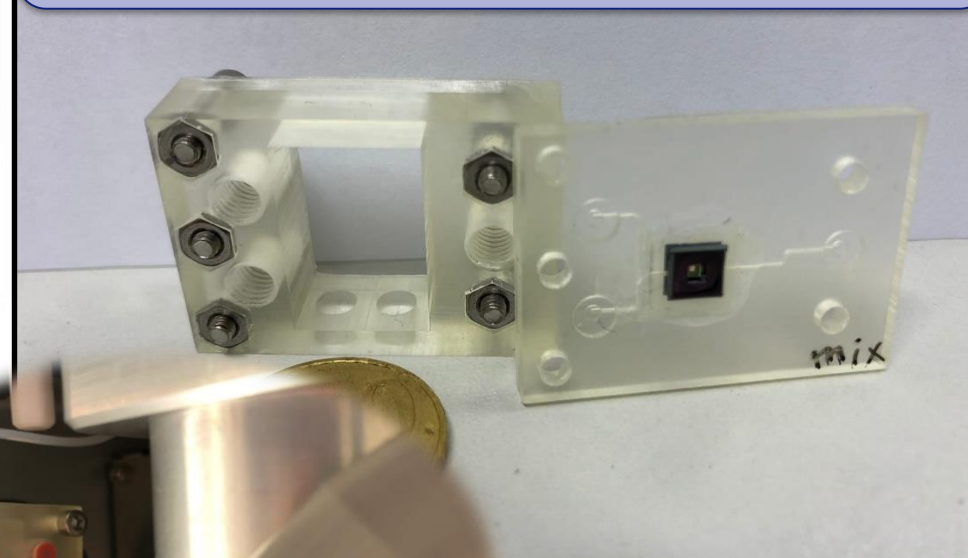
SAMPLE EXPOSURE UNIT @ BM29 _ 5

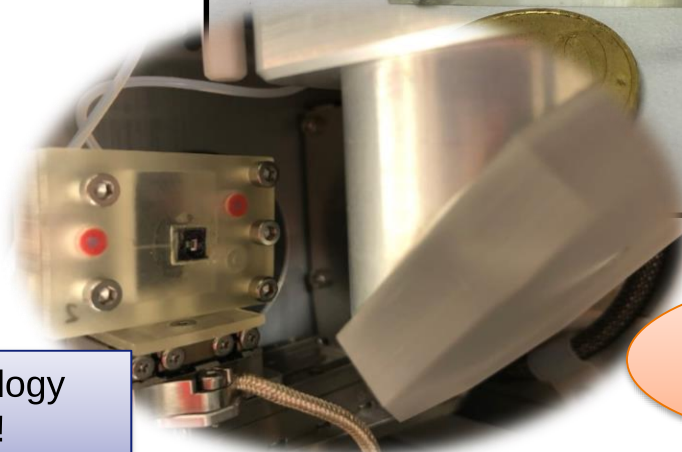
Unified design for all of devices – 25x 35 mm; Placed on a unified holder





Windows glued with Permatex Flowable Silicone Glass Sealer – it forms a tough, waterproof, durable, clear seal; fills surface voids and irregularities.





Create any kind of experimental topology before and after the work window!

Work vacuum:
4,5 * 10^{-3} mbar

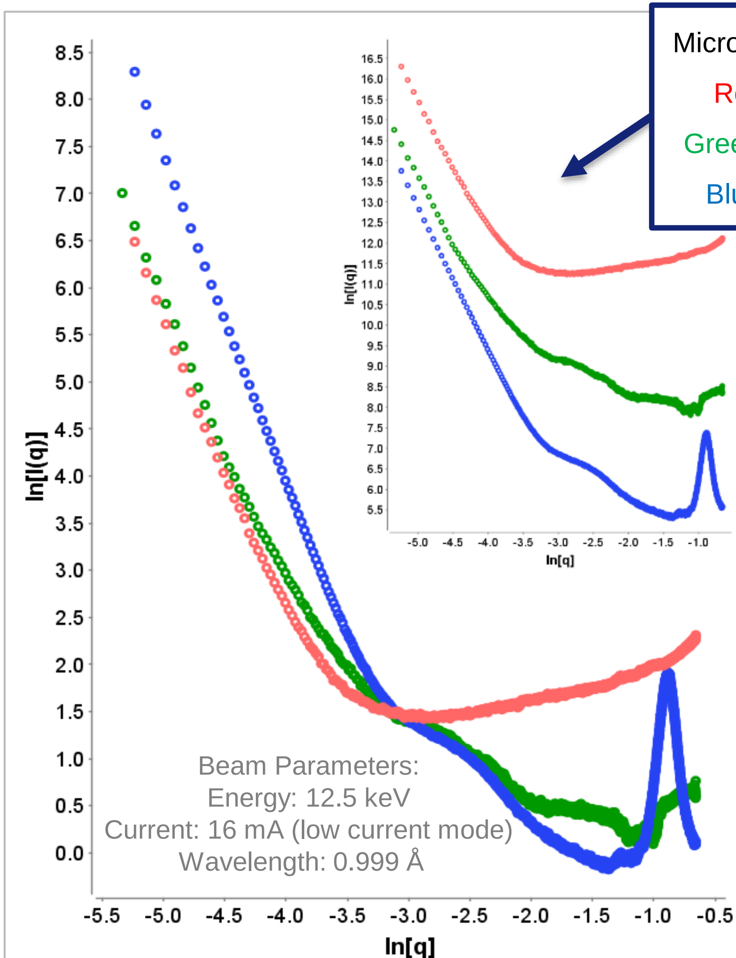
SAMPLE EXPOSURE UNIT @ BM29 _ 6

Microfluidic devices window materials:

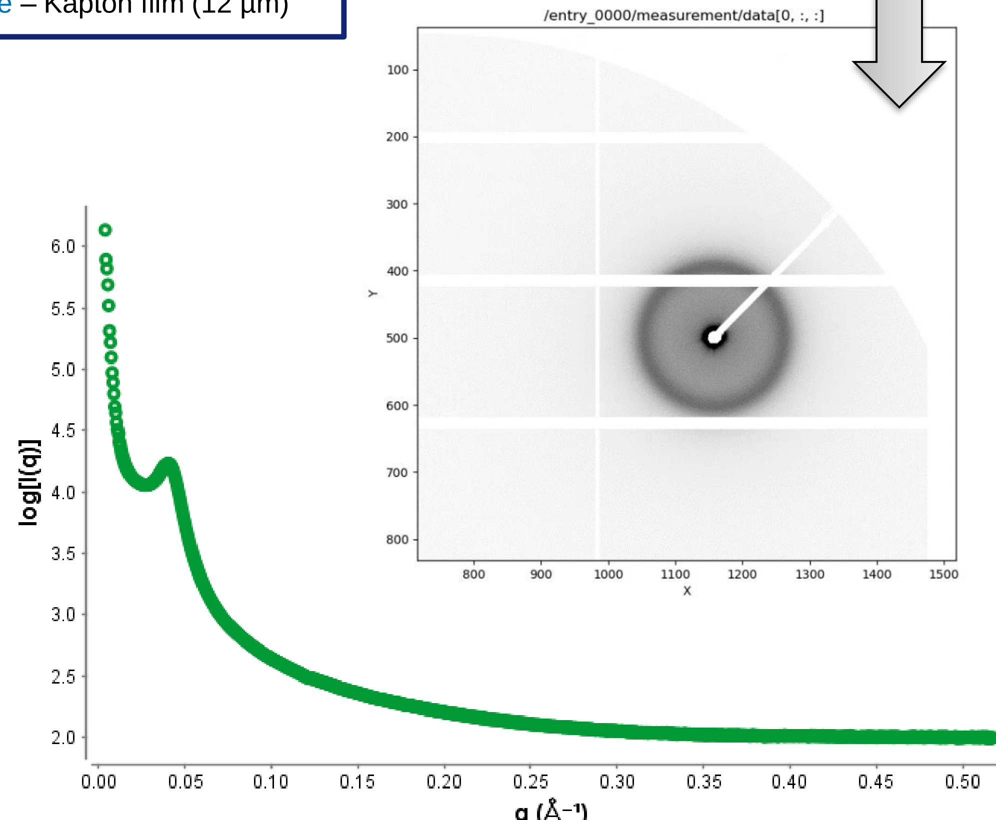
Red curve – COC film (100 µm)

Green curve – Si_3N_4 window (1 µm)

Blue curve – Kapton film (12 µm)

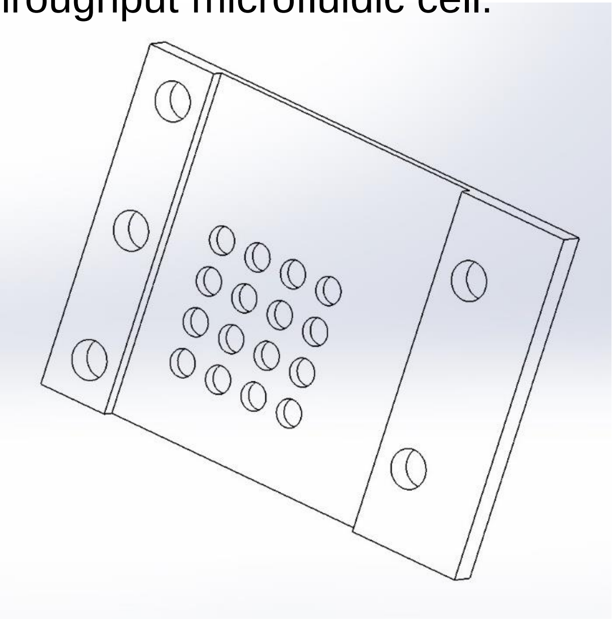


subtracted integration
(of a gel sample in the MFD with Kapton film windows)



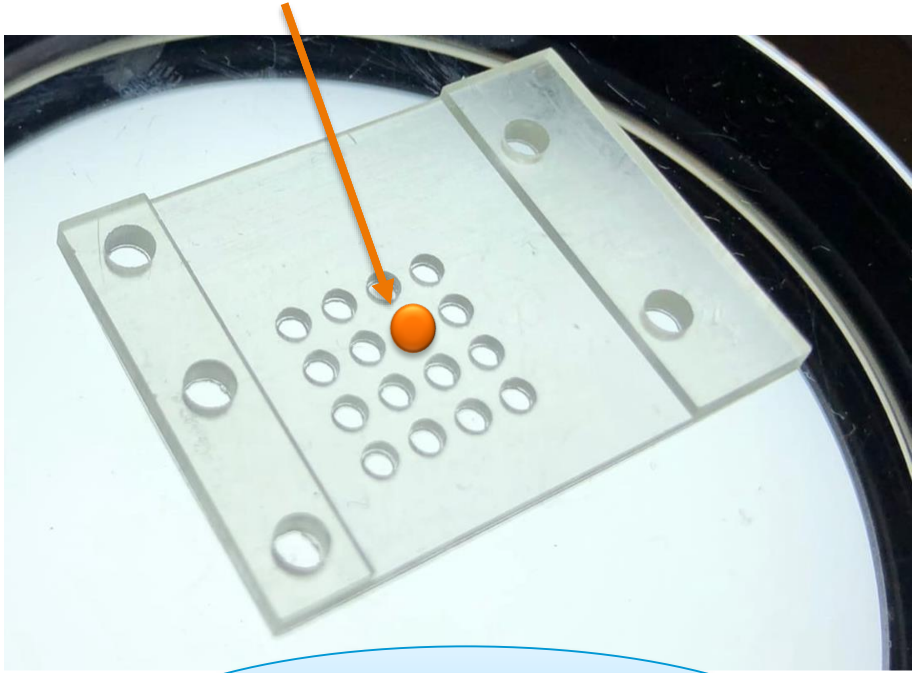
MICROFLUIDIC FOR SAXS – CONCEPTS & REALITY

High throughput microfluidic cell:



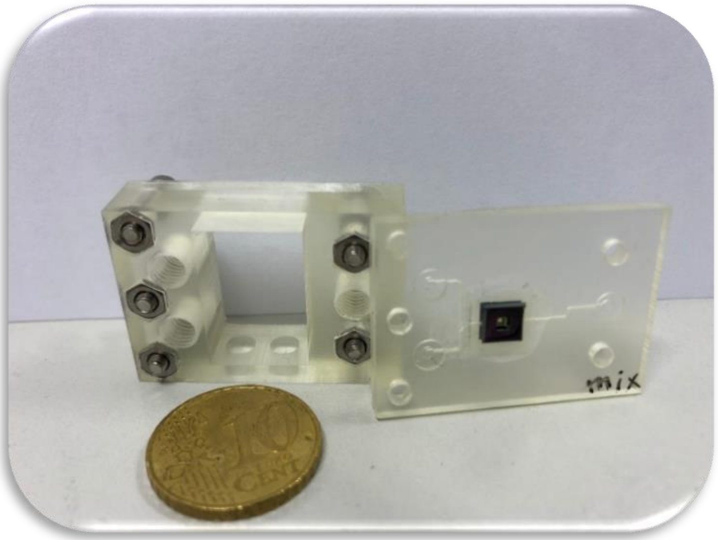
- 3D printed MF Cell (25 x 35 mm) to fit in *x, y, z* stage holder;
- Laminated sandwich, kapton film - 12 µm
- Wells volume are about 1 µl;
- Reusable or disposable.

- X-Ray Zero-position

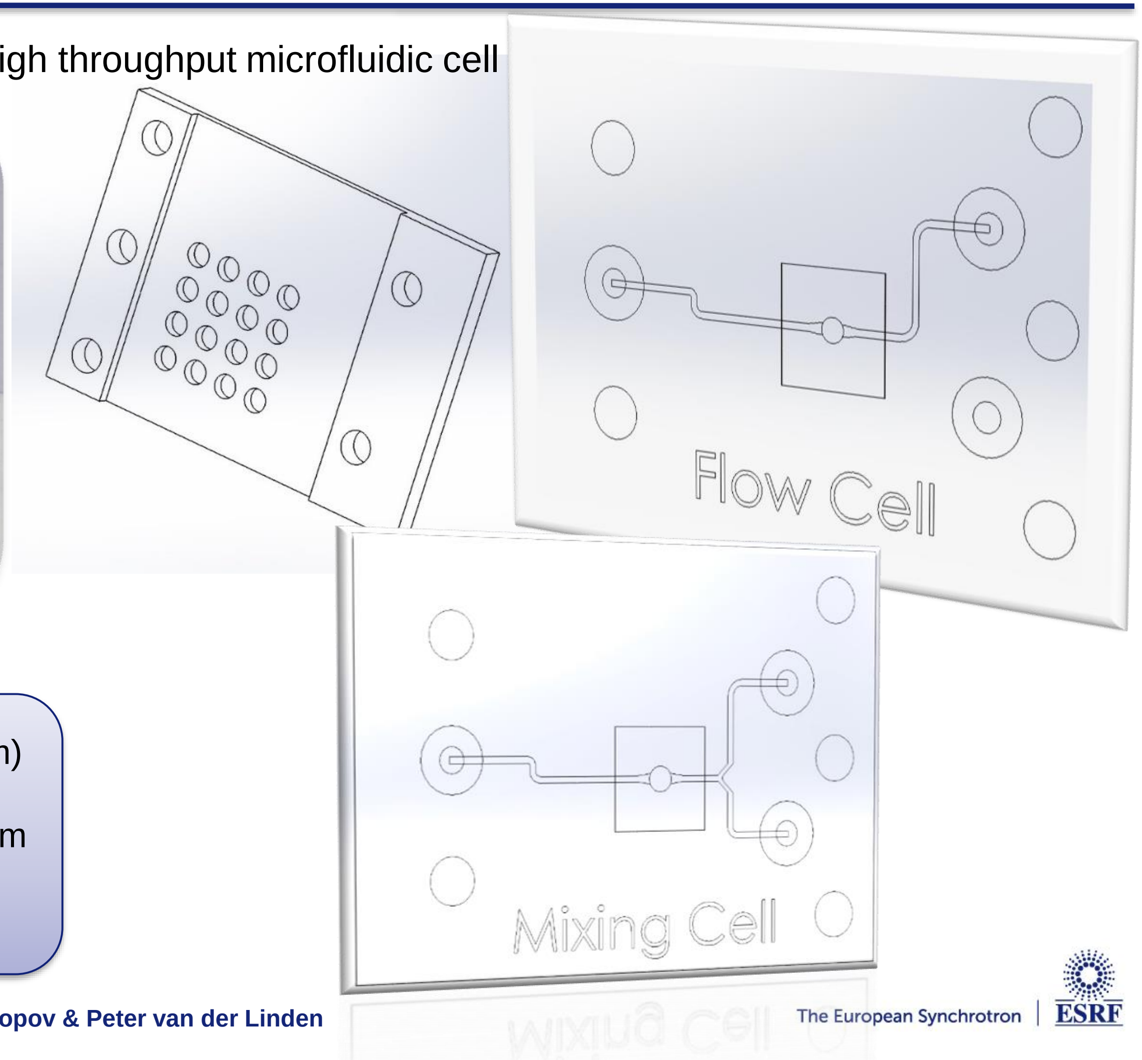


4 x 4 square, you can have 16 different conditions within one experiment

BM29 SEU – SUMMARY

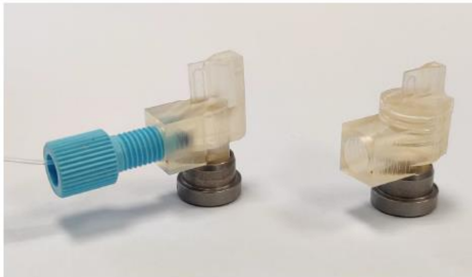


High throughput microfluidic cell

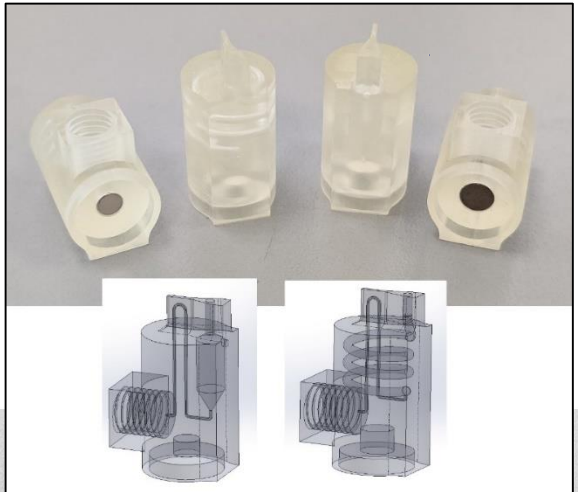


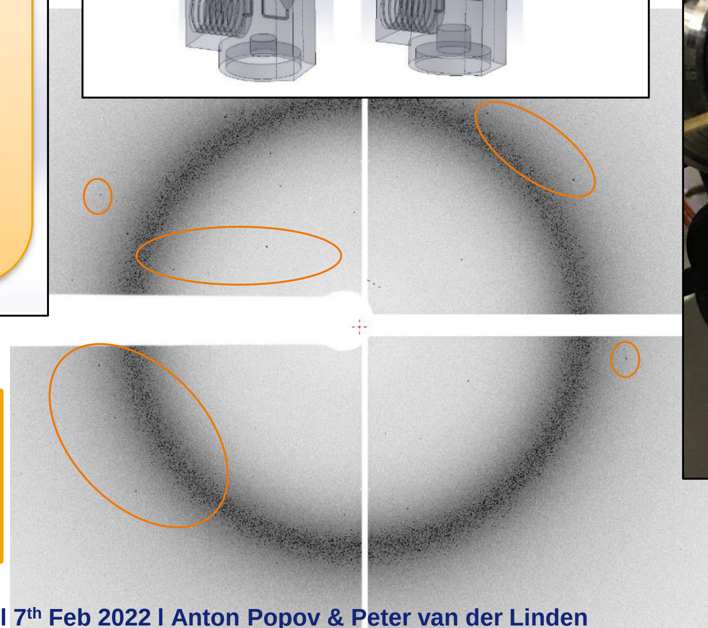
- 3D printed MF Devices (25 x 35 x 2 mm) to fit in *x, y, z* stage holder;
- Laminated sandwich, kapton film - 12 µm
- Wells volume are about 1 µl;
- Reusable or disposable.

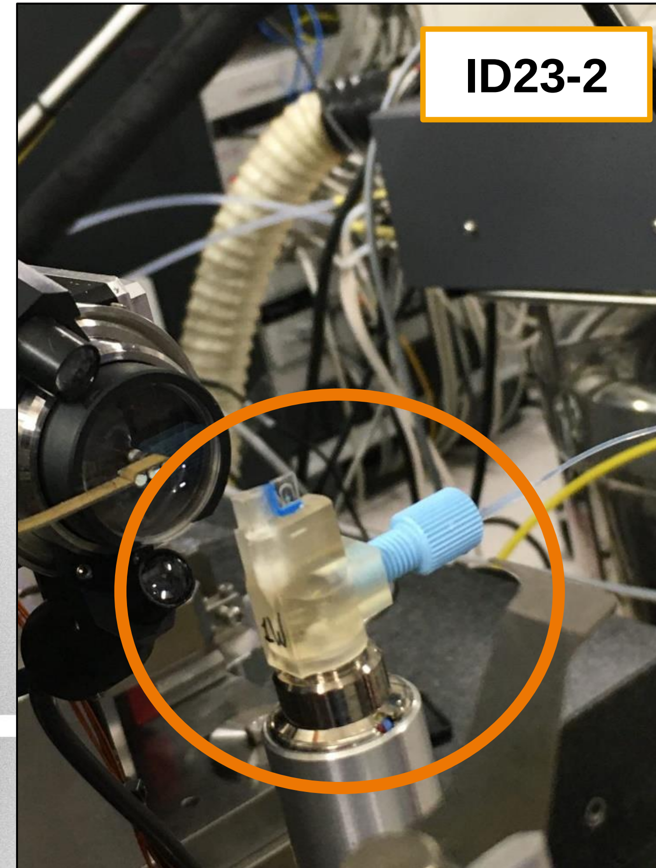
SERIAL CRYSTALLOGRAPHY – TEST



3D printed Injection system for serial crystallography experiments.
Width of the work channel – 150 – 200 µm

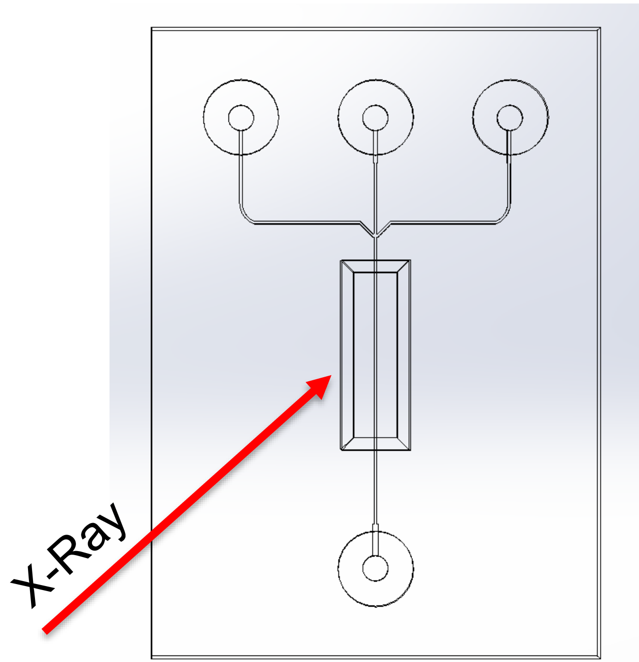


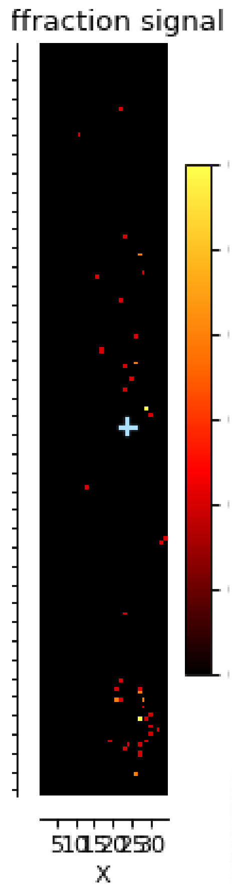




Also used this sample environment @ Massif-3 and ID-30B

MICROFLUIDIC FOR SAXS – CONCEPTS – 2





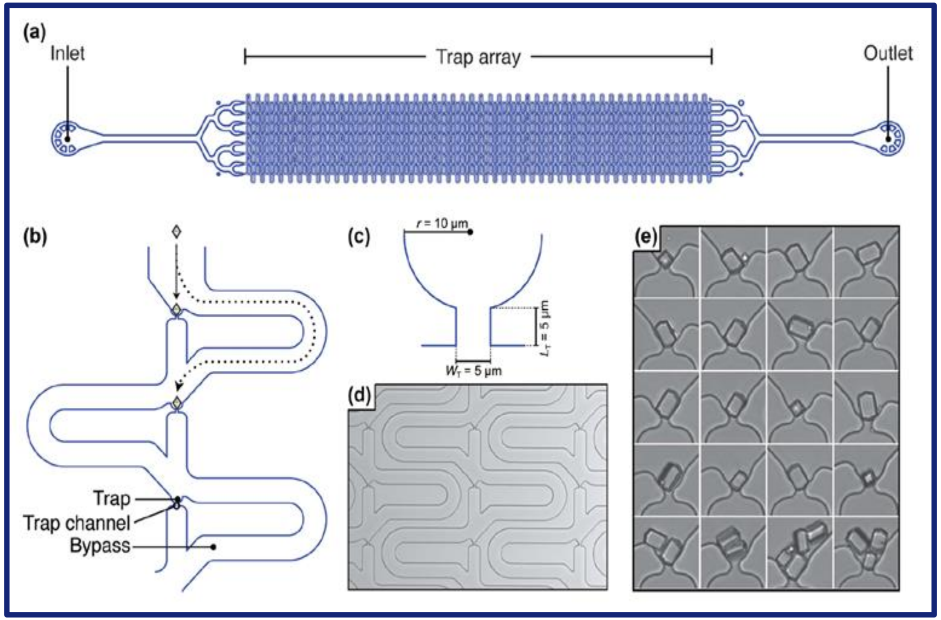
With an exposure time of 30ms, we observed some diffraction images

ID23-2

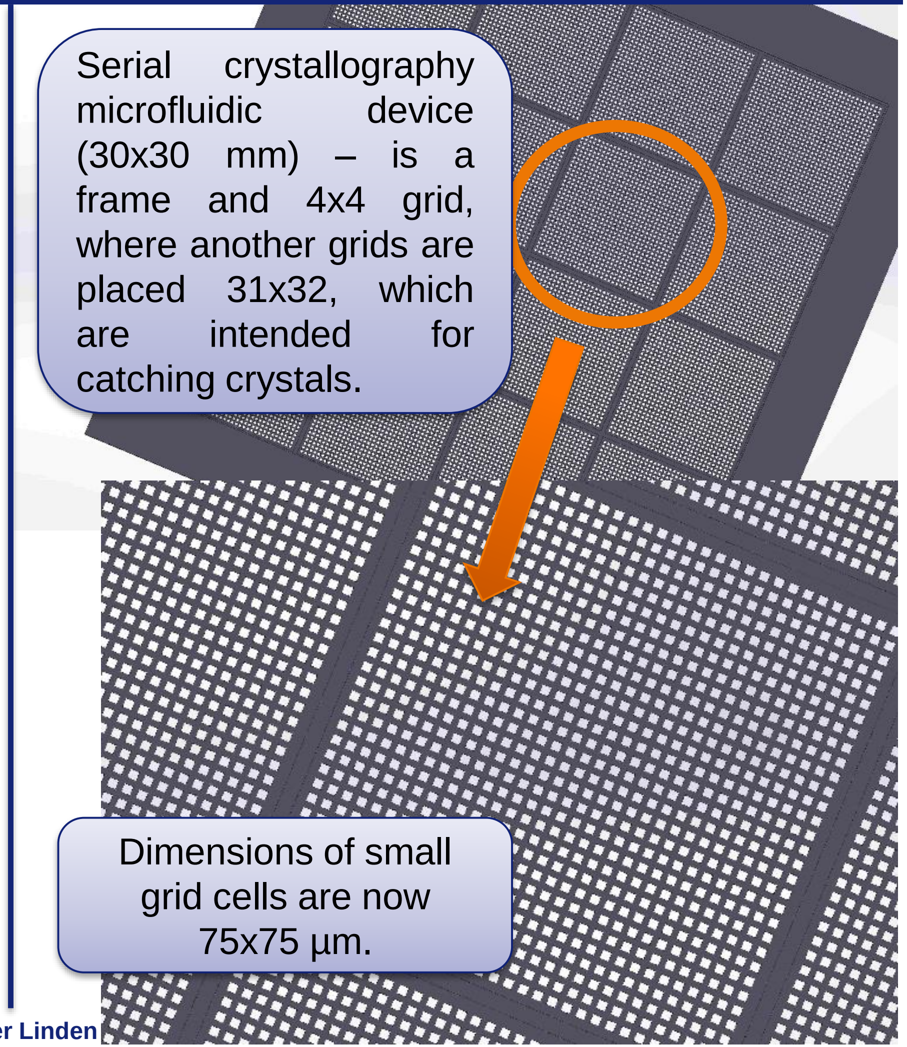
- 3D printed MF Cell (25x35x1.55 mm) to fit in the *unified homemade goni holder* @ ESRF MX Beamlines;
- Channels are 160 x 150 µm;
- Window thickness is 350 µm before and after sample.

SERIAL CRYSTALLOGRAPHY – CONCEPTS – 1

To simplify data analysis it is necessary to select crystals of the same size.



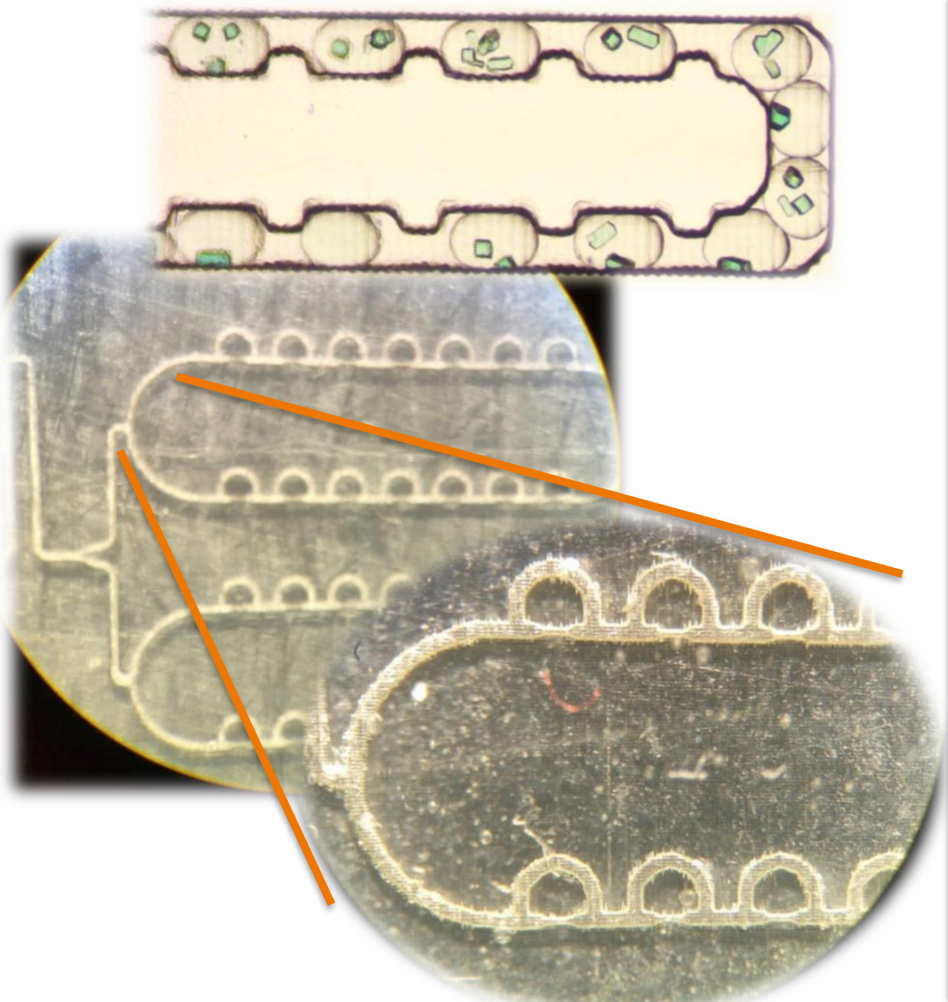
Capture and X-ray diffraction studies of protein microcrystals in a microfluidic trap array
Artem Y. Lyubimov et al.
Acta Crystallogr D Biol Crystallogr. 2015 Apr 1; 71(Pt 4): 928–940.
doi: 10.1107/S1399004715002308

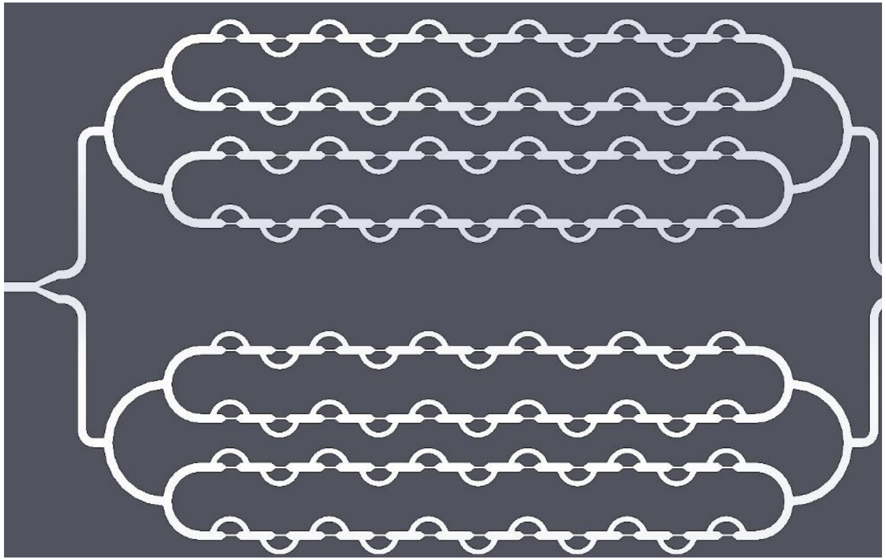


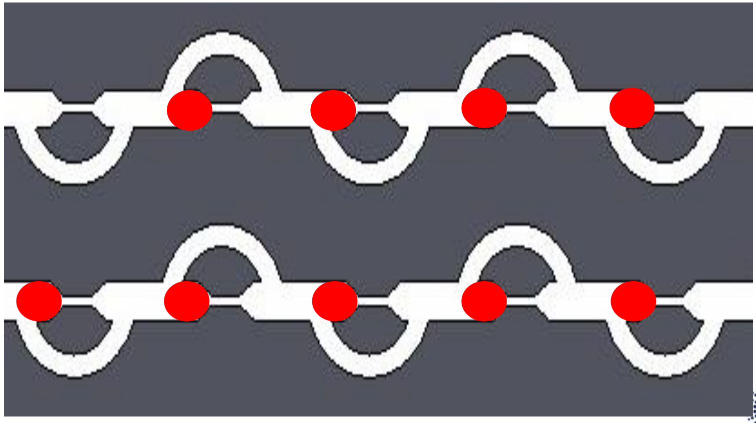
Serial crystallography microfluidic device (30x30 mm) – is a frame and 4x4 grid, where another grids are placed 31x32, which are intended for catching crystals.

Dimensions of small grid cells are now 75x75 µm.

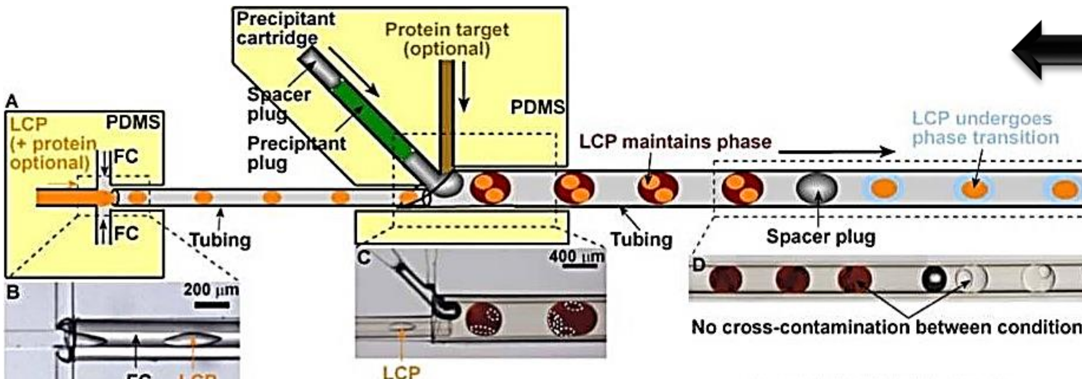
SERIAL CRYSTALLOGRAPHY – CONCEPTS – 2







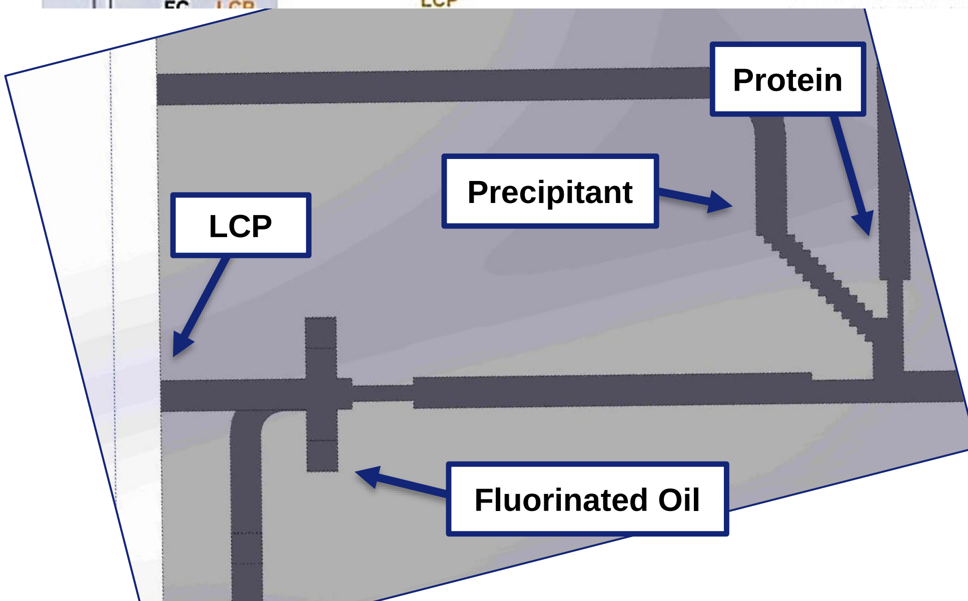
DROPLET-BASED MF FOR MEMBRANE PROTEINS



A Plug-Based Microfluidic System for Dispensing Lipidic Cubic Phase (LCP) Material Validated by Crystallizing Membrane Proteins in Lipidic Mesophases.

Liang Li et al.

Microfluid Nanofluidics. 2010 Jun; 8(6): 789–798.
doi: 10.1007/s10404-009-0512-8



One of the important topics regarding structural biology is membrane proteins. The main difficulty is that all membrane proteins are not soluble in water, for them the native environment is not a solution, but a lipid membrane.

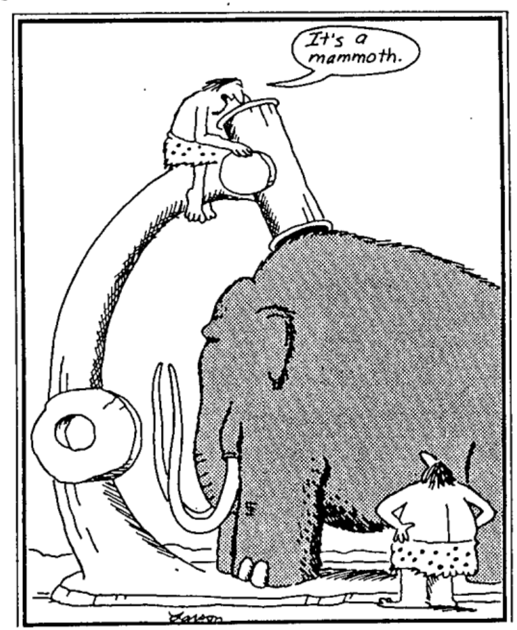
To prevent denaturation and loss of the desired structure, it is necessary to use Lipidic Cubic Phase (a special environment for growth).

LCP forms in area a complex two-dimensional surface along which membrane proteins can reach the growing crystal without leaving the "native" membrane.

CONCLUSIONS AND ACKNOWLEDGEMENTS

Conclusions:

– 3D printing allows to create a device within one day, and if necessary quickly modify it;
– Development of X-ray beamlines sample environment and single microfluidics units with a specific design, could help users to carry out a "bricolage" experiment;
– Use of universal holders for microfluidic chips will allow to make a variety of unique experiments at the beamlines with minor changes in the sample environment.



Early microscope

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diego.pontoni@esrf.fr
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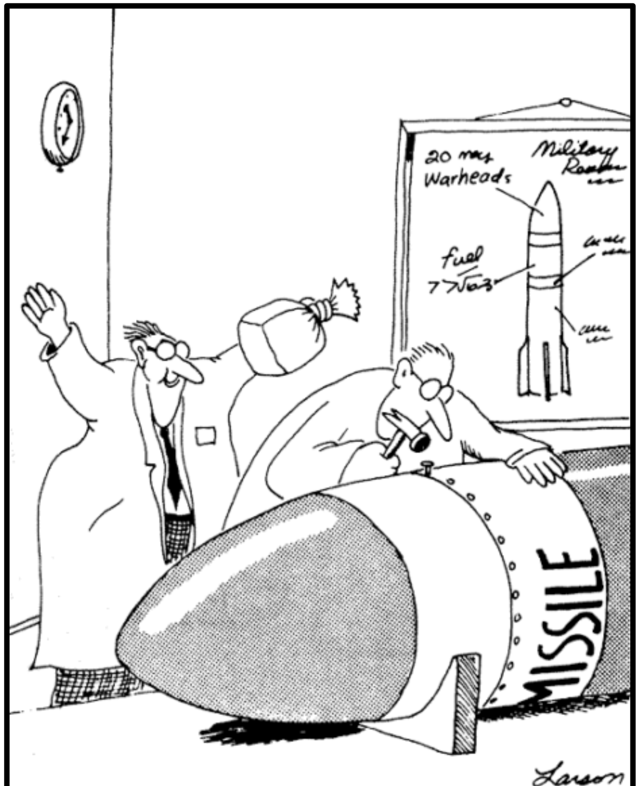
Mark Tully for wise and respectful supervising.
Diego Pontoni and the whole PSCM Group for all possible help and laboratory equipment provided, especially to my closest colleague – Peter van der Linden.
Part of this project received support from Instruct pilot grant, APPID 1557.


Warheads
fuel
MISSILE
Larson

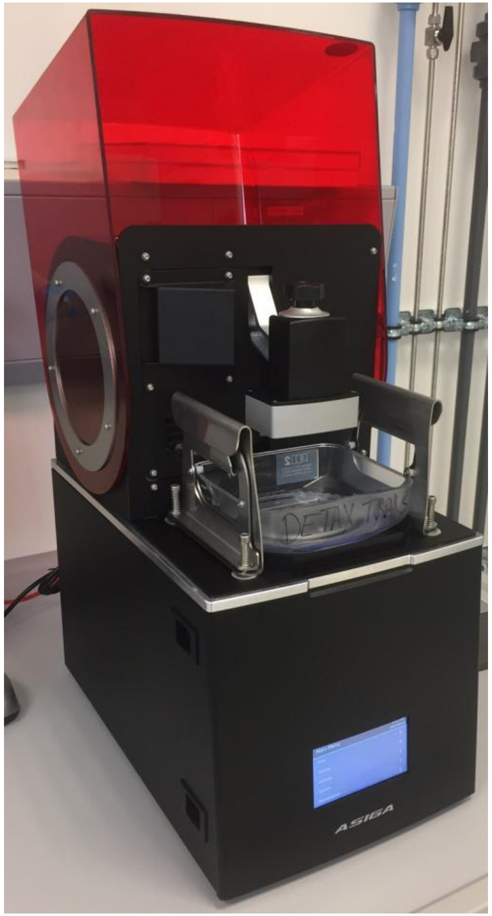

That's all Folks!

THE PSCM DLP PRINTER

Since December 2016:
ASIGA Pico2 HD
DLP = Digital Light Processing

- Pixel size 37 micron
- 1020x1980 pixels
- Build platform 40x70mm
- Min. useful Z step 10 micron
- Build height 70mm
- UV LED 385nm
- Build speed ~6 layers/min →

at 100μm layer thickness 20 mm/hr
at 25μm layer thickness 5 mm/hr



THE SB GROUP DLP PRINTER

Since May 2020:
ASIGA MAX X27
DLP = Digital Light Processing

- Pixel size 27 micron
- Platform size 51.8 × 29.2 mm
- Min. useful Z step 10 micron
- Build height 75 mm
- UV LED 385nm
- Build speed ~12 layers/min → at 25µm layer thickness