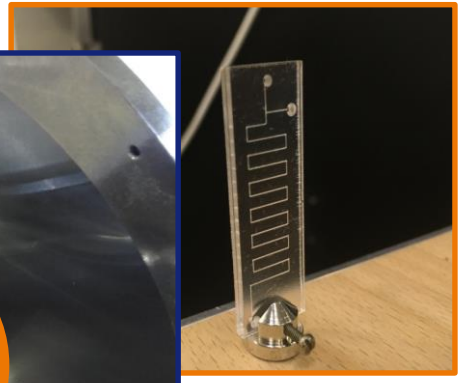
ESRF Users' Meeting 2021: MX BAG Meeting

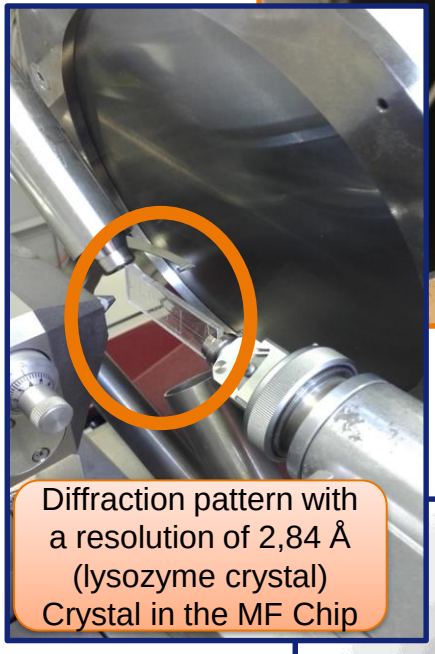
The European Synchrotron

Microfluidics for MX/BioSAXS and other recent ideas

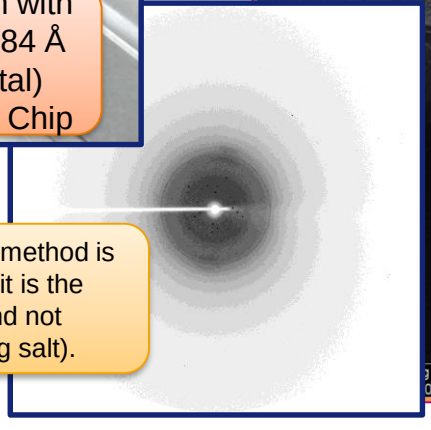
Anton Popov, 8th Feb 2021

MAIN GOAL – “ONE-DAY MICROFLUIDICS”





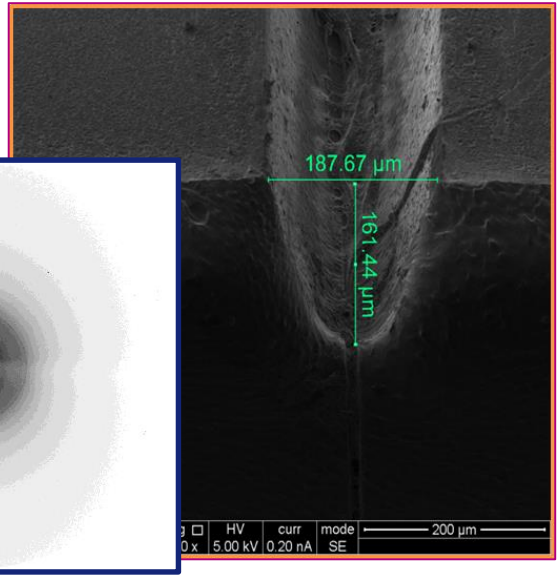
Diffraction pattern with a resolution of 2,84 Å (lysozyme crystal) Crystal in the MF Chip



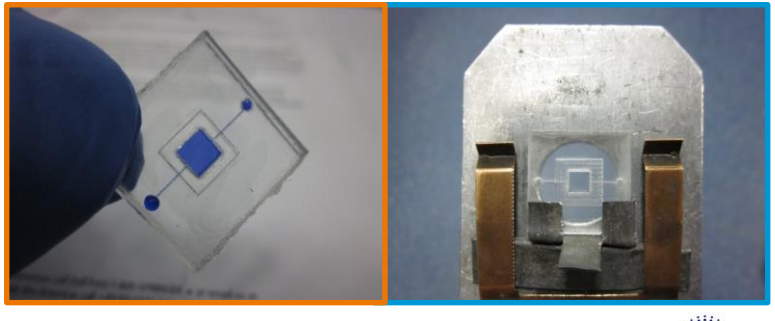
The main task of this method is to understand that it is the protein crystal and not something else (eg salt).

«Microfluidic devices for studying proteins structure and crystallization processes at a synchrotron radiation source»

Main Goal #1: From idea to realization is just 1 step.
Main Goal #2: Flexible channel shapes.
Main Goal #3: Easy bonding & easy unsoldering.
Main Goal #4: Multilayer systems.
Main Goal #5: Metal deposition.
Main Goal #6: External and internal surface treatment.

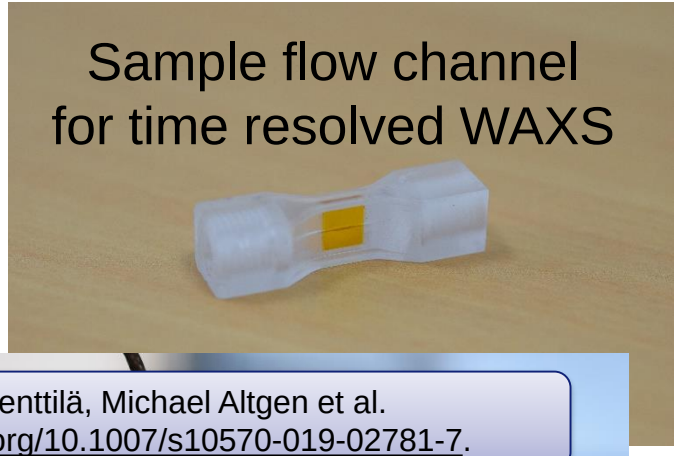


It is proposed to use the SAXS method to determine the oligomers formation of protein subunits in the early phases of crystallization, such oligomers are the building blocks of a crystal, by combining such oligomers the whole crystal is created.



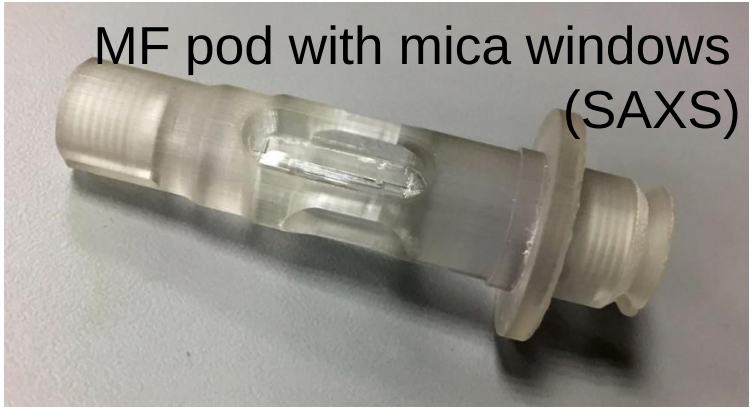
SOME 3D PRINTED DEVICES

Different 3D printed microfluidics were used at ESRF Beamlines:
- ID30A-3 (MASSIF3);
- BM29 BioSAXS;
- ID09 - White Beam Station - Time-resolved Beamline;
- BM02 - D2AM a French CRG Beamline for in situ material characterization.

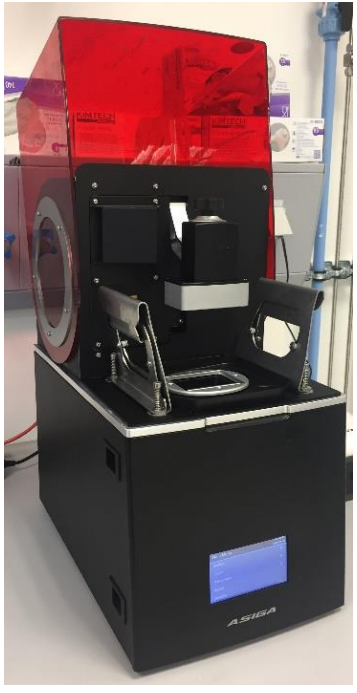


Diana C.F. Monteiro, Martin Trebbin et al. https://doi.org/10.1107/S2052252519016865.

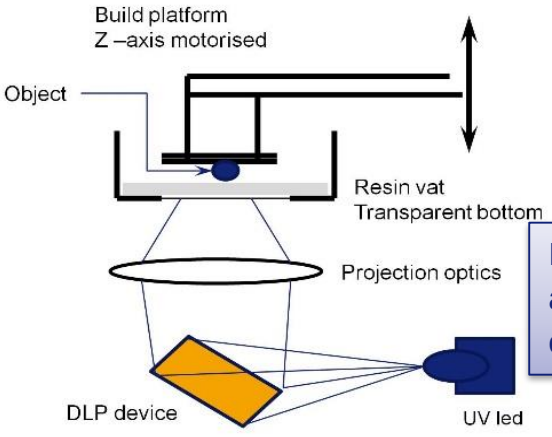
Paavo A. Penttilä, Michael Altgen et al. https://doi.org/10.1007/s10570-019-02781-7.



MICROFLUIDIC CHIP (3D PRINTED) – PART 1

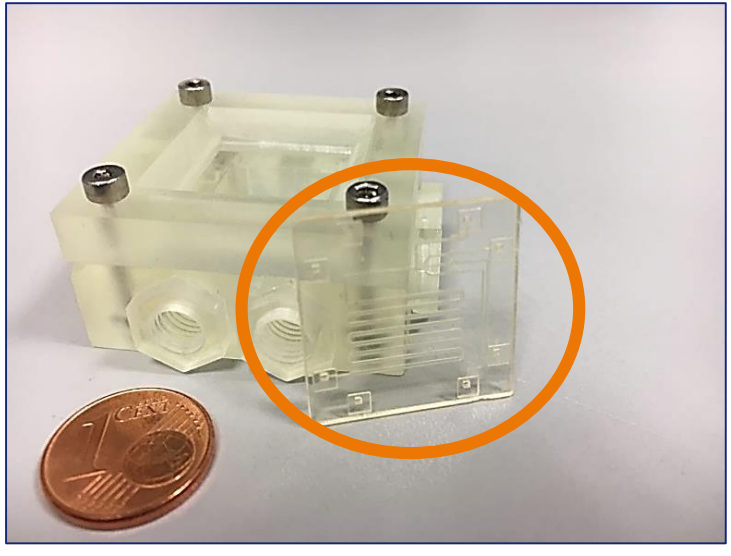


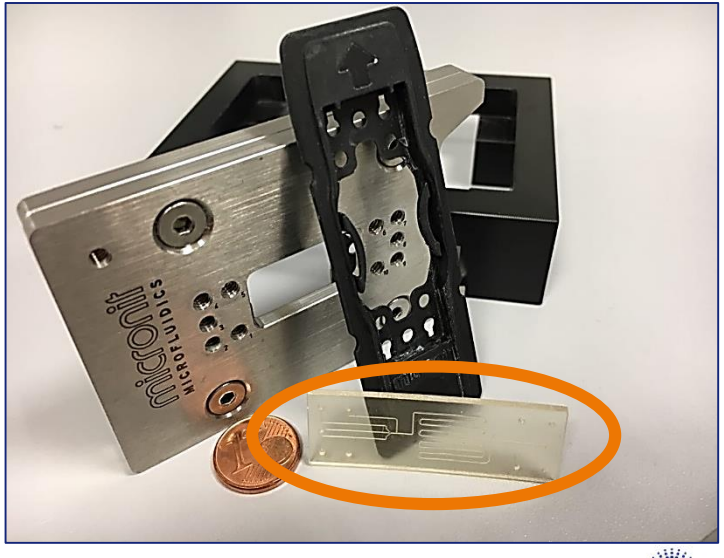
Digital Light Projection (DLP) 3D Printing



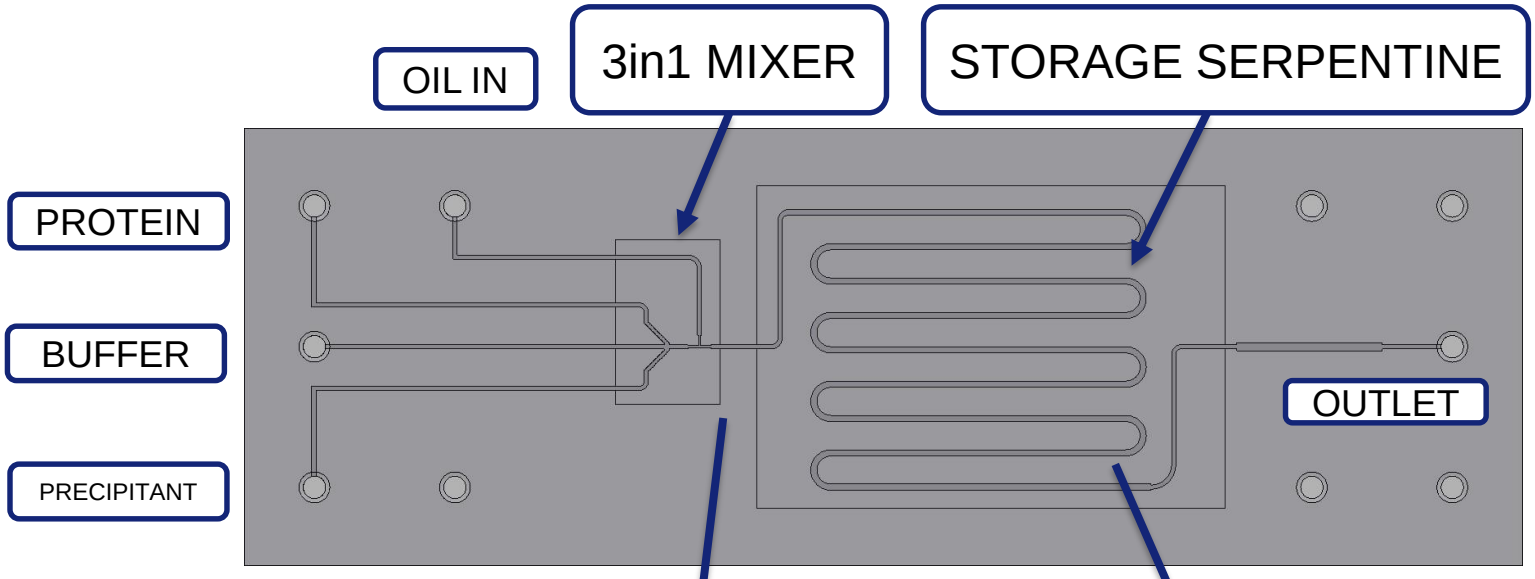
- Currently, the minimum possible printed width is two pixels – 75 microns.
- Smallest practical thickness of a microfluidic structure is 75 microns.
- All chips have high chemical resistance and can be reused.

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.



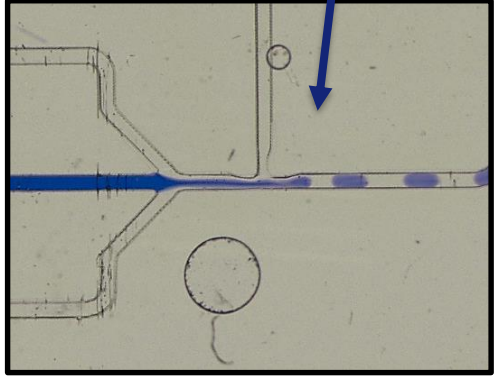


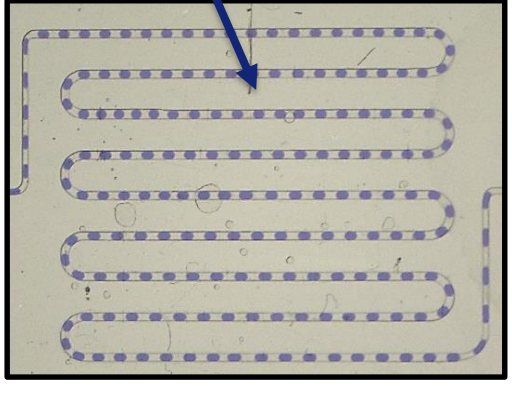
MICROFLUIDIC CHIP (3D PRINTED) – PART 2



Main channels width and depth
≈ 160 x 150 µm;

Narrow mixer part
≈ 100 µm;

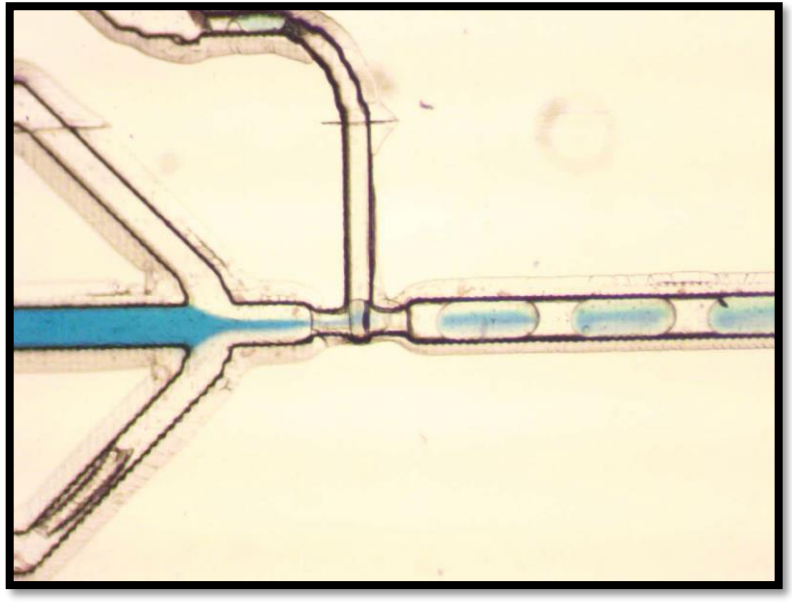


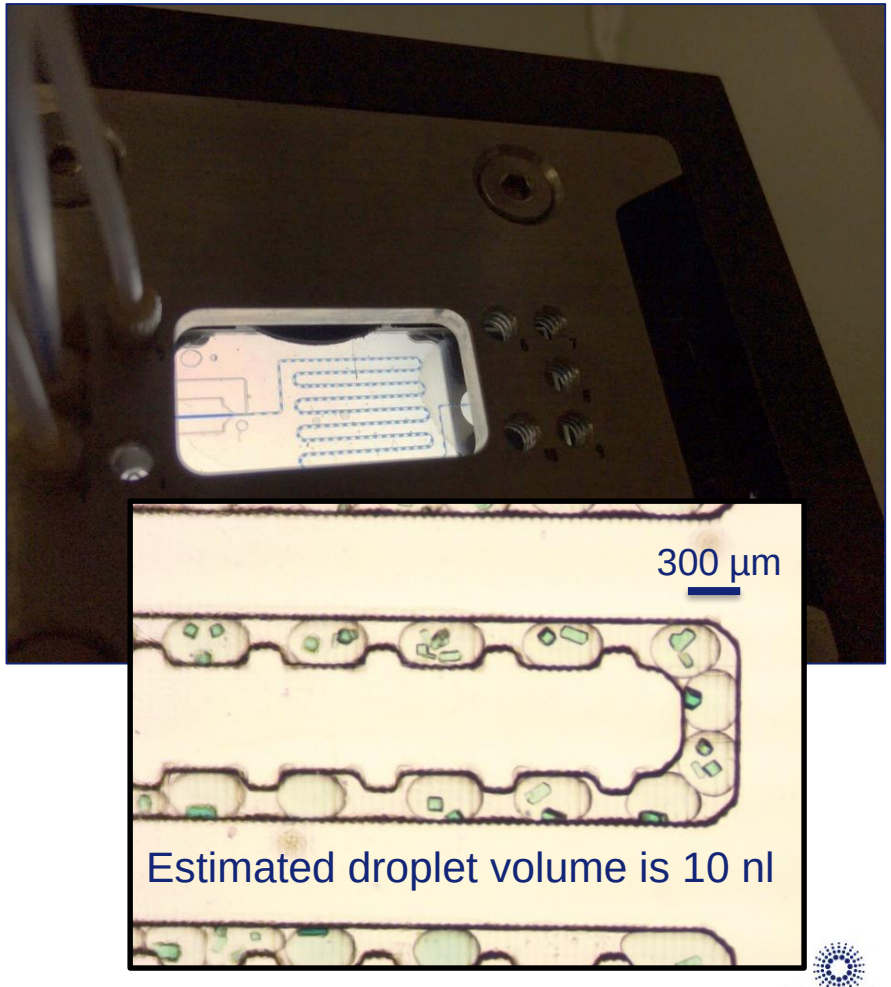


With a 3D Printer it is possible to build complex systems of micro channels, reservoirs, basins, cascades, sets of inlets/outlets, etc. Using not only different resins, but also glass, mica, silica, etc.

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

MICROFLUIDIC CHIP (3D PRINTED) – PART 3





Creating a system of droplets (microreactors) with different volumes and wide range of protein-precipitant concentrations within one chip.

It is possible to make a preliminary rating of the quality of the obtained crystals directly from the chip, followed by extraction of these crystals and full data X-ray diffraction analysis.

MICROFLUIDIC CHIP (3D PRINTED) – PART 4

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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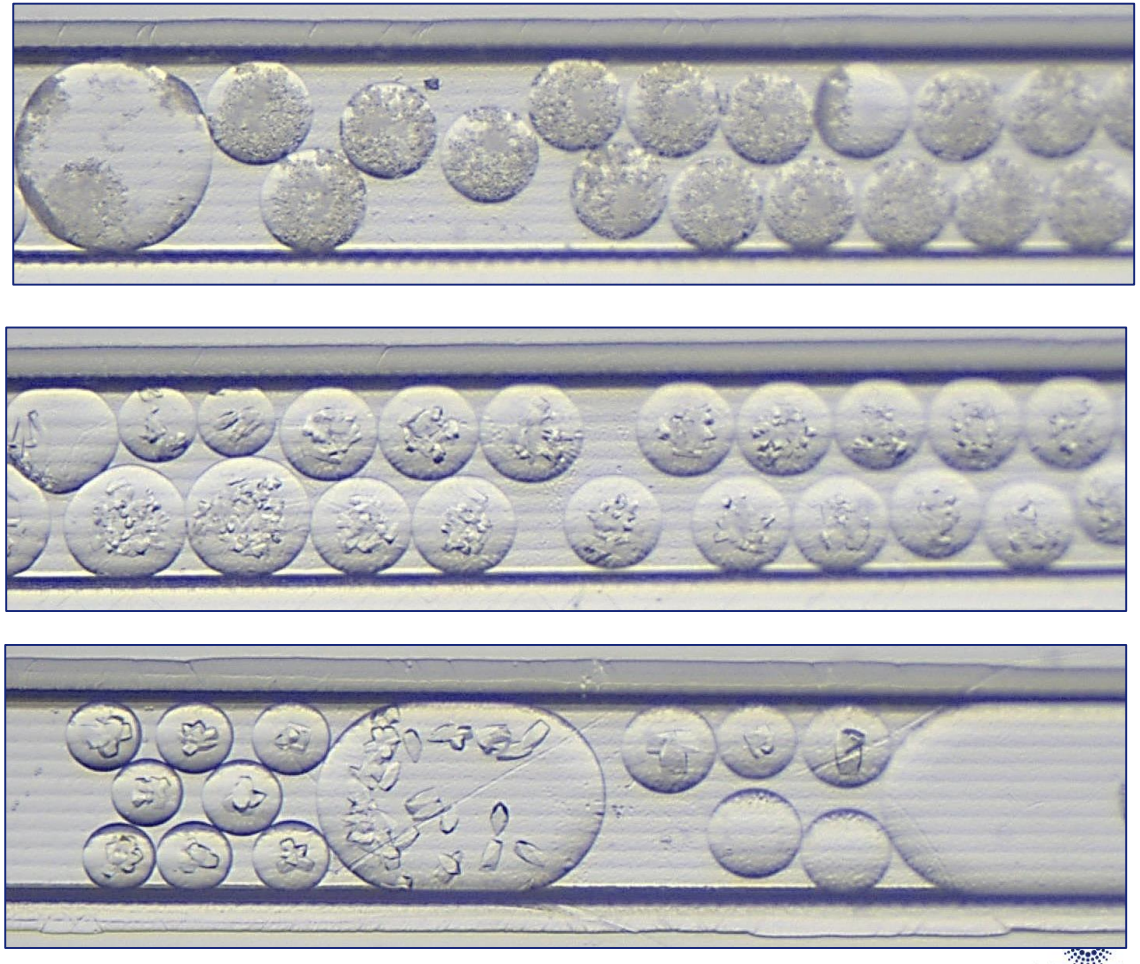
Width = 400 µm

Depth = 150 µm

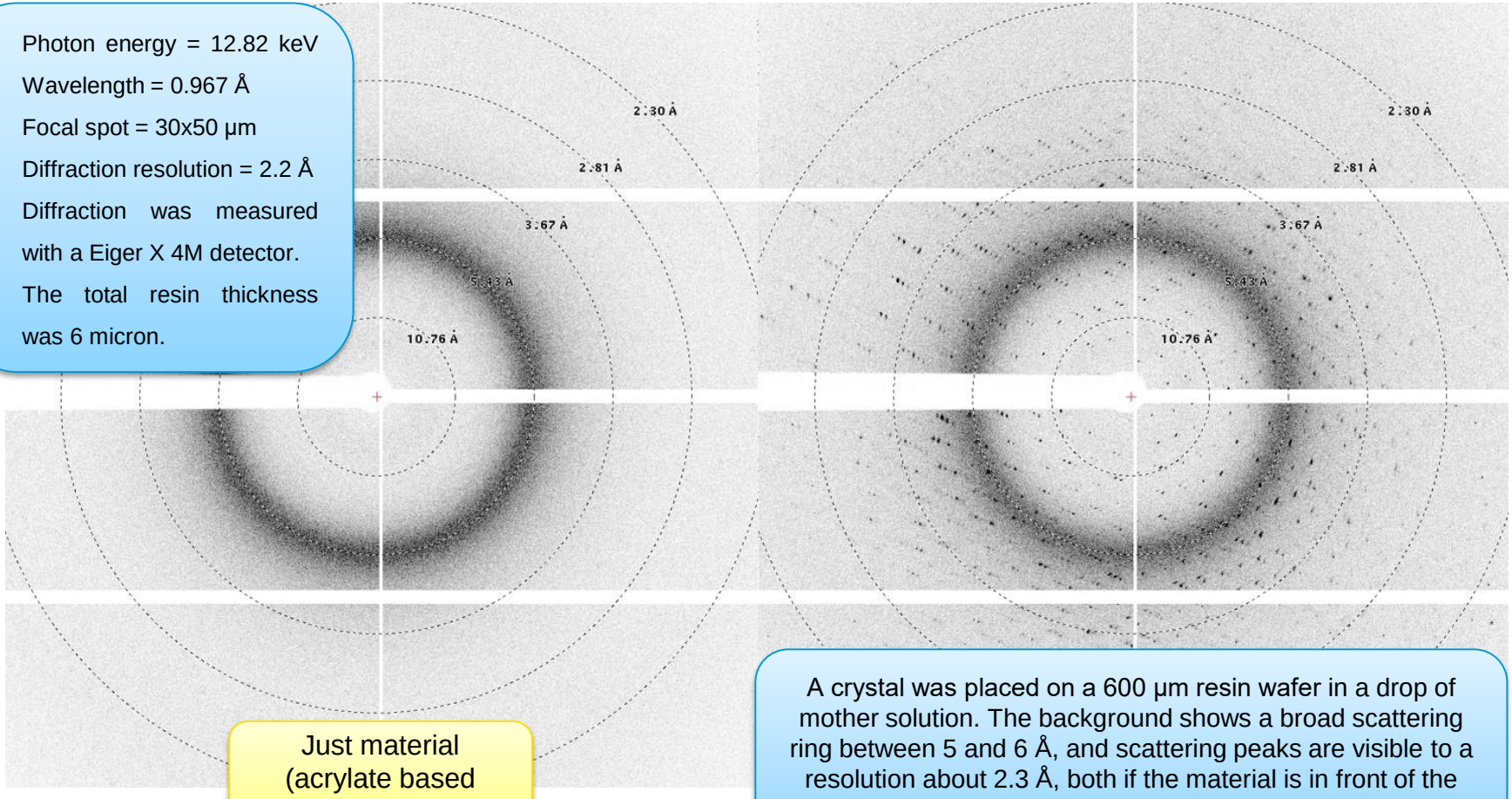
Estimated crystal size = 70 µm

Estimated droplet volume = 2,5-5 nl



TEST EXPERIMENTS ON MASSIF-3

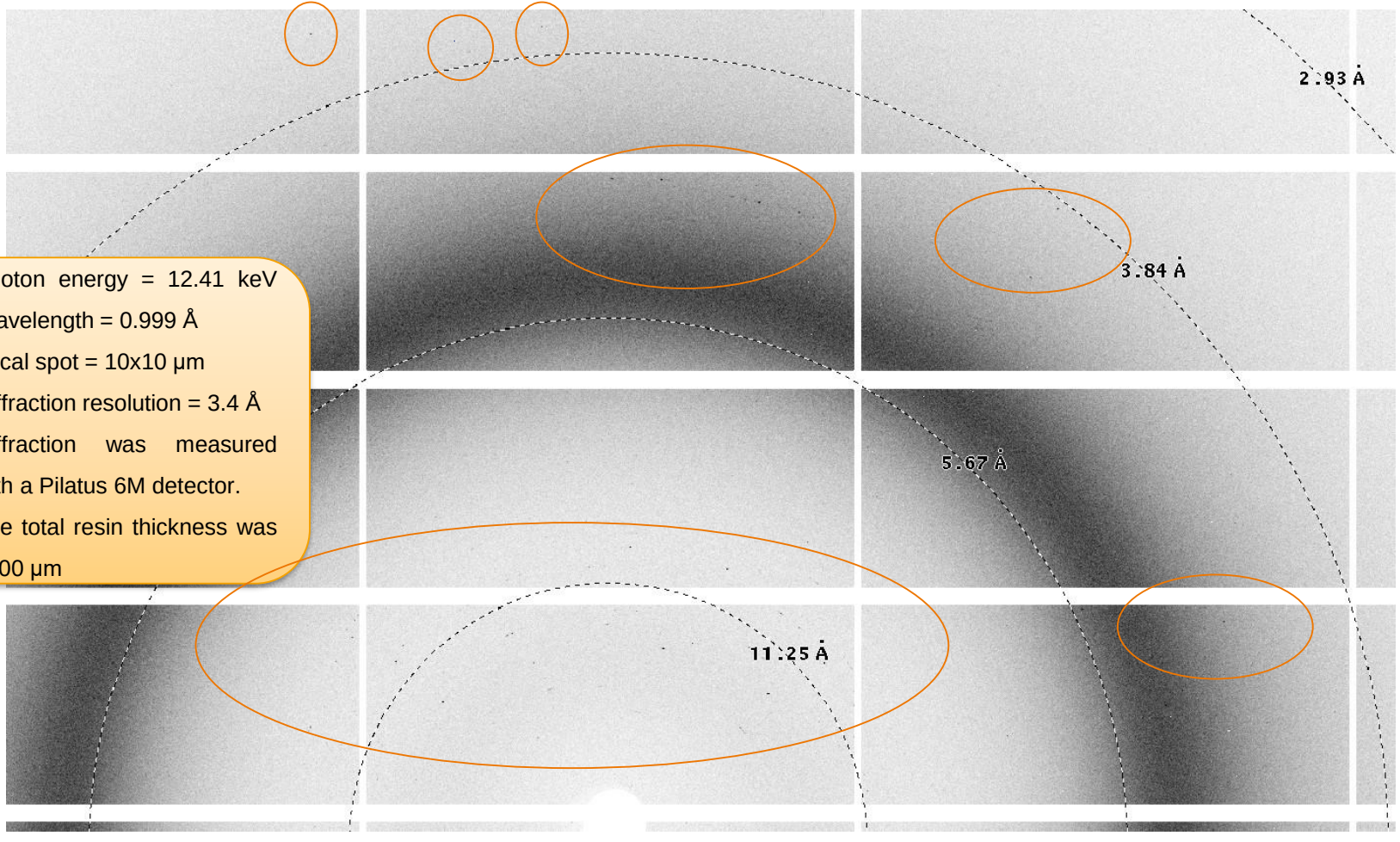
- Photon energy = 12.82 keV
  Wavelength = 0.967 Å
- Focal spot = 30x50 µm
- Diffraction resolution = 2.2 Å
- Diffraction was measured with a Eiger X 4M detector.
- The total resin thickness was 6 micron.



Just material (acrylate based resin)

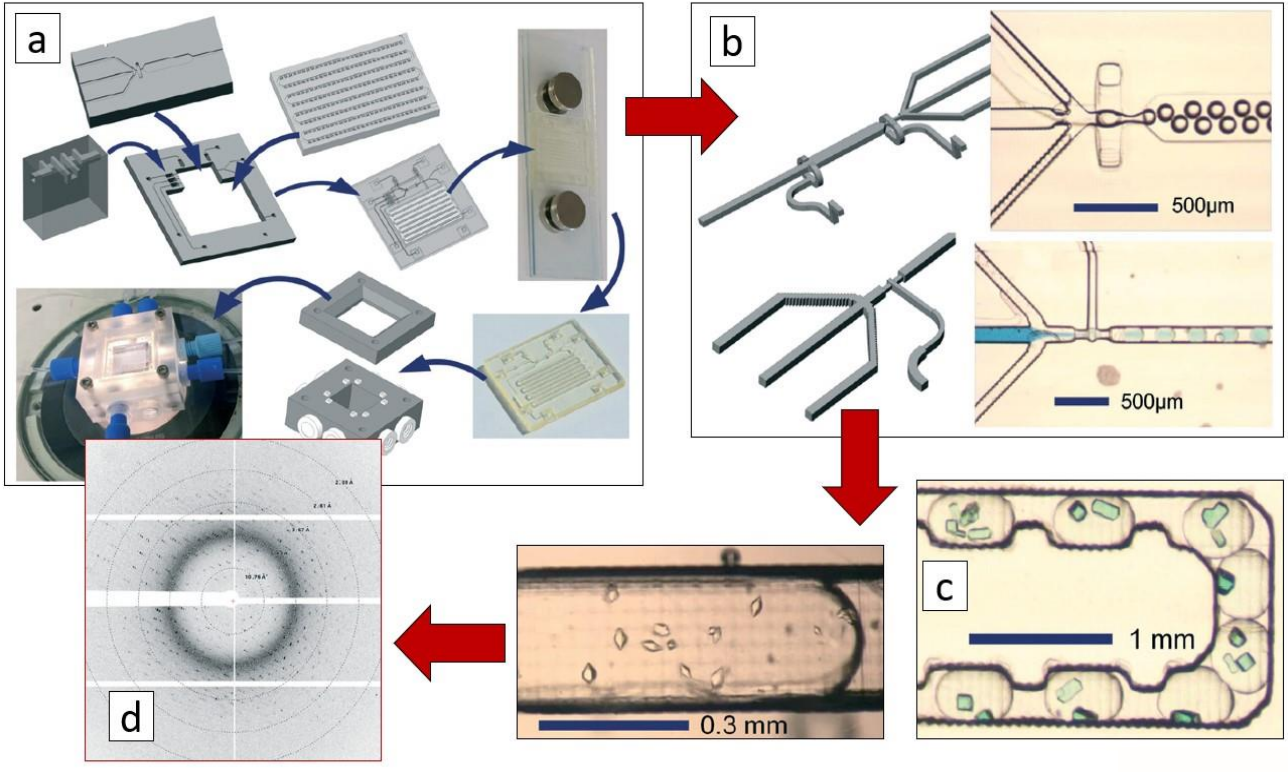
A crystal was placed on a 600 µm resin wafer in a drop of mother solution. The background shows a broad scattering ring between 5 and 6 Å, and scattering peaks are visible to a resolution about 2.3 Å, both if the material is in front of the crystal, and if behind.

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



- Photon energy = 12.41 keV Wavelength = 0.999 Å
- Focal spot = 10x10 µm
- Diffraction resolution = 3.4 Å
- Diffraction was measured with a Pilatus 6M detector.
- The total resin thickness was 1300 µm

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

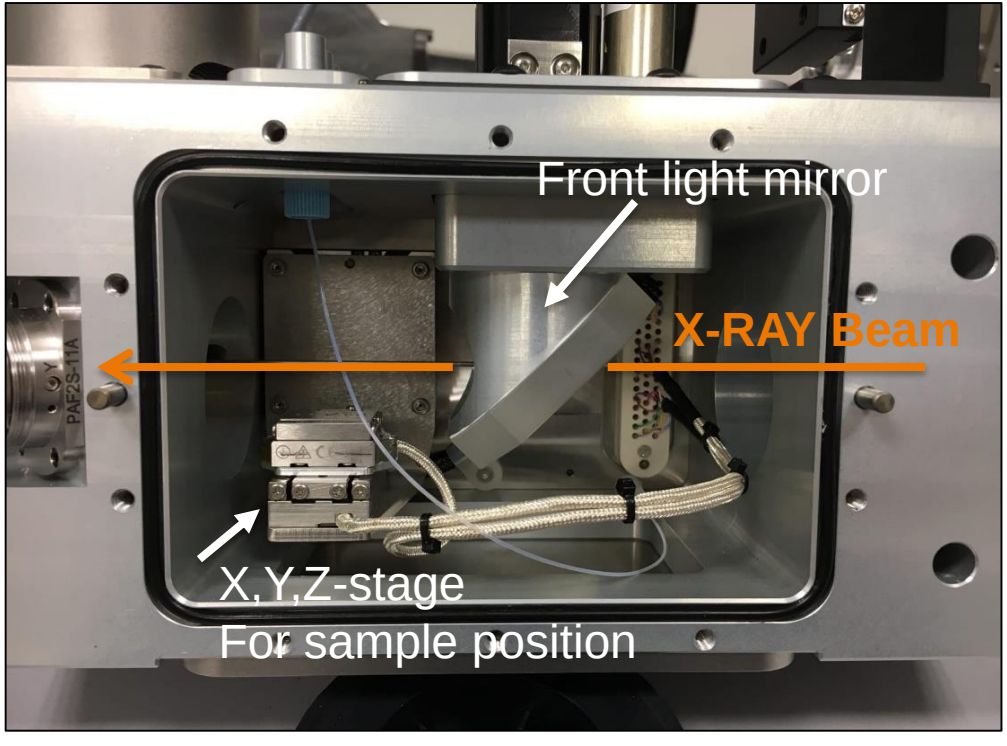
Peter J.E.M. van der Linden, Anton M. Popov, Diego Pontoni
https://doi.org/10.1039/D0LC00767F

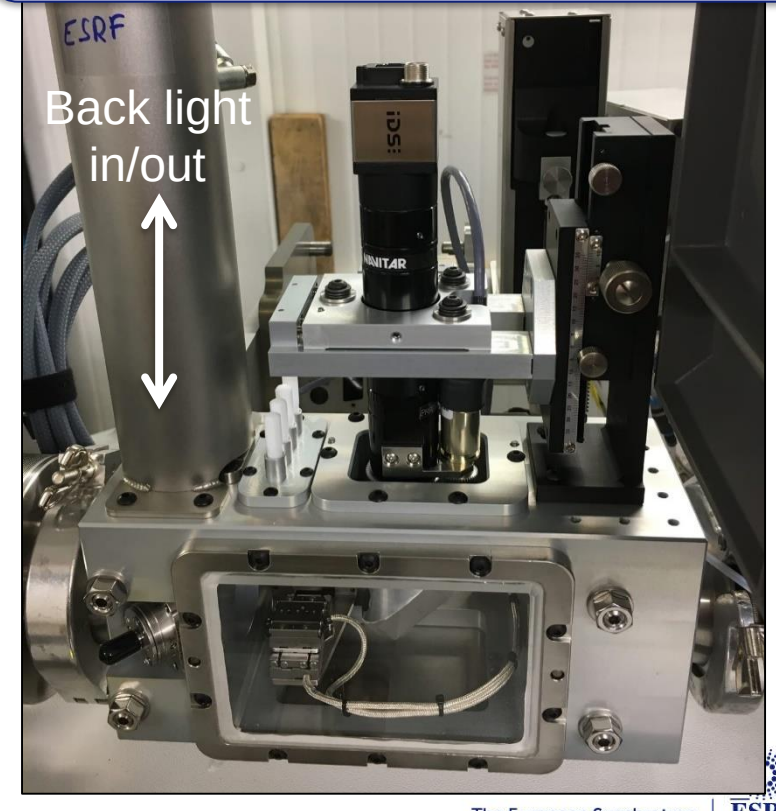
ROYAL SOCIETY OF CHEMISTRY

BM29 – MICROFLUIDIC SAXS CHAMBER

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

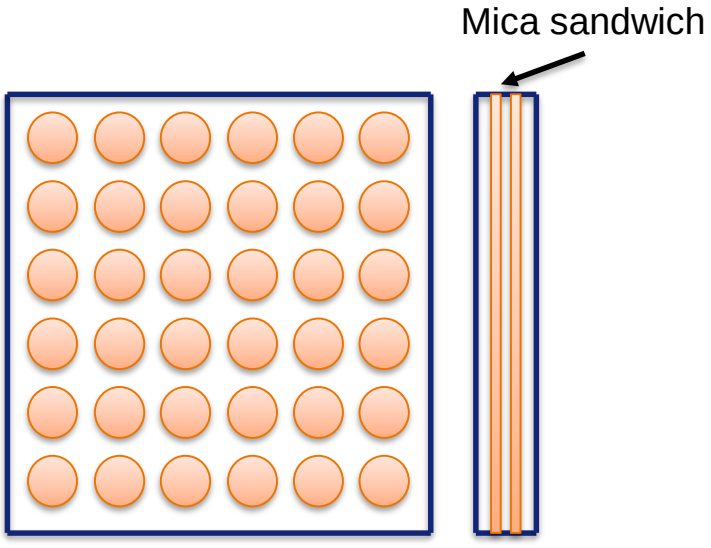
- Using syringe pumps we have 3 work flow inlets;
- X,Y,Z-stage for precise positioning of the devices;
- Navitar column (Pylon Viewer software);
- Upgrade potential.





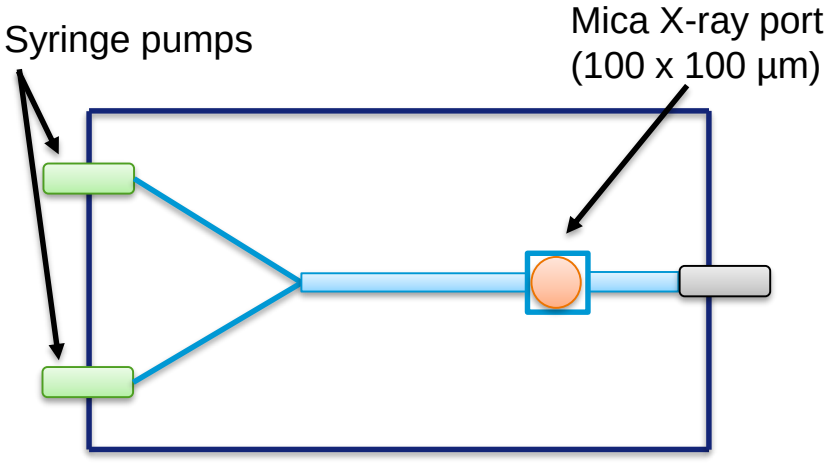
MICROFLUIDIC FOR SAXS – CONCEPTS

❖ High throughput microfluidic cell



- 3D printed MF Cell (45 x 45 mm) to fit onto *x, y, z* stage
- Laminated sandwich, mica thick. 10 – 50 µm
- Wells volume are about 10 µl
- Disposable

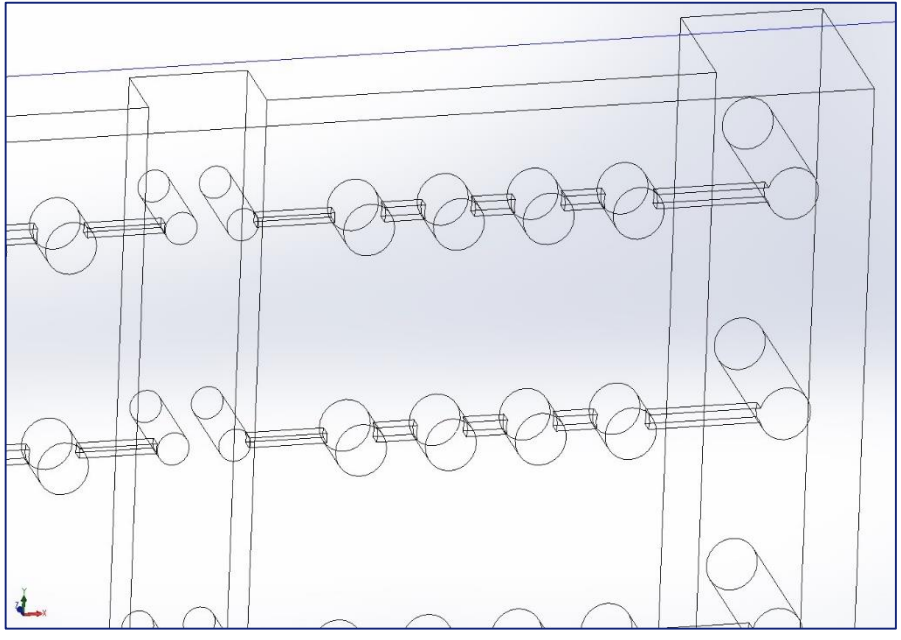
❖ Ligand cell

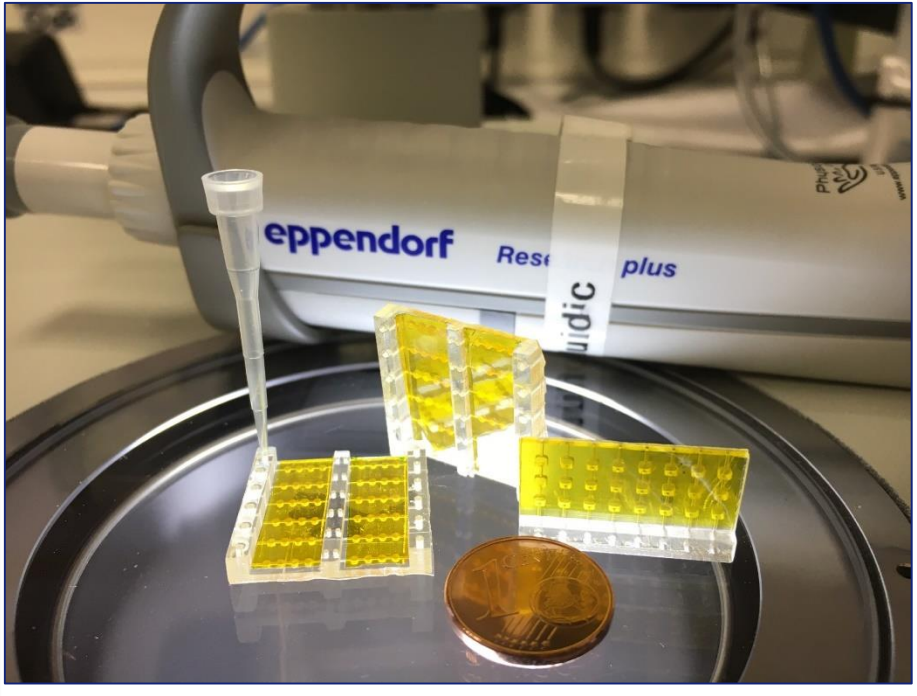


- 3D printed MF Cell (25 x 45 mm) to fit onto *x, y, z* stage
- Mica window, thick. 10 – 50 µm
- Flow cell for protein investigation, then addition of ligand, monitoring changes
- Disposable

MICROFLUIDIC FOR SAXS – CONCEPTS – 2

❖ High throughput microfluidic cell



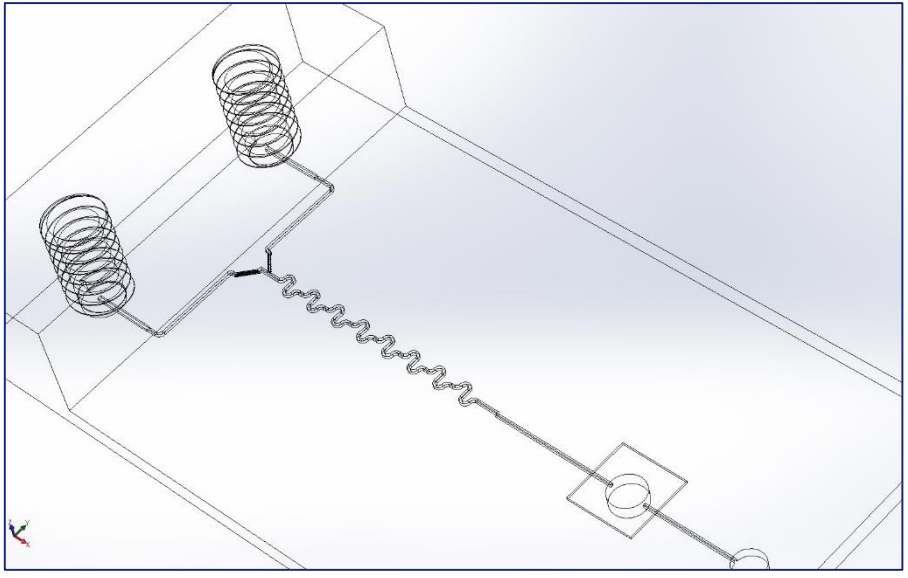


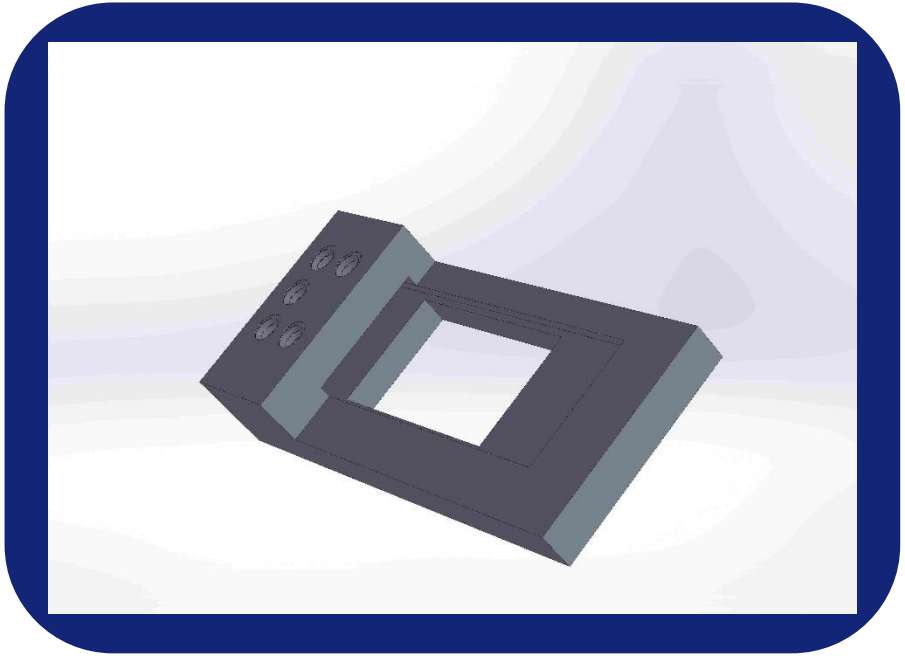
- 3D printed MF Cell (20 x 20 mm) to fit onto *x, y, z* stage @ BM29

- Eppendorf friendly (10 µl nozzle)
- Volume of a well = 500 nl
- Kapton film (50 - 100 µm)
- Disposable

MICROFLUIDIC FOR SAXS – CONCEPTS – 3

- High throughput microfluidic cell



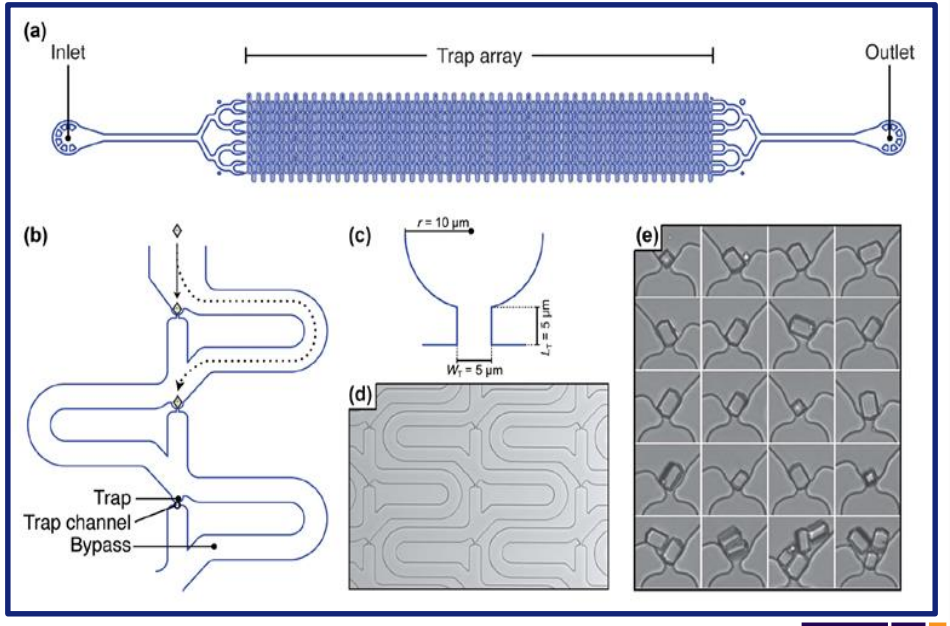


- 3D printed MF Cell (25 x 45 mm) to fit onto *x, y, z* stage @ BM29
- Small ferrules in use
- Volume of work vessel = 2,3 µl
- Kapton film (25 - 100 µm)
- Disposable

This is a developed concept of a chip holder for a vacuum chamber, which will be one of the options for sample examination on BM29.
Possible to use microfluidic devices of diverse architecture.

SERIAL CRYSTALLOGRAPHY – CONCEPTS – 1

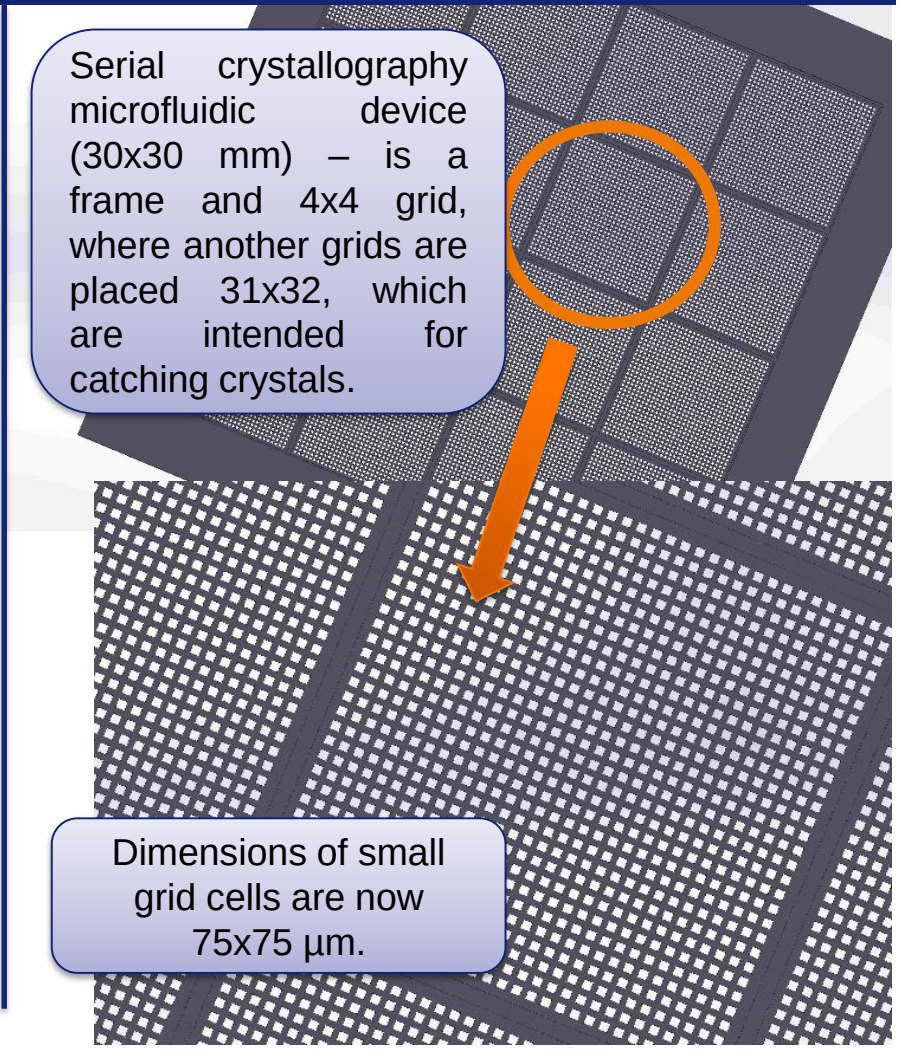
To simplify data analysis it is necessary to select crystals of the same size.



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

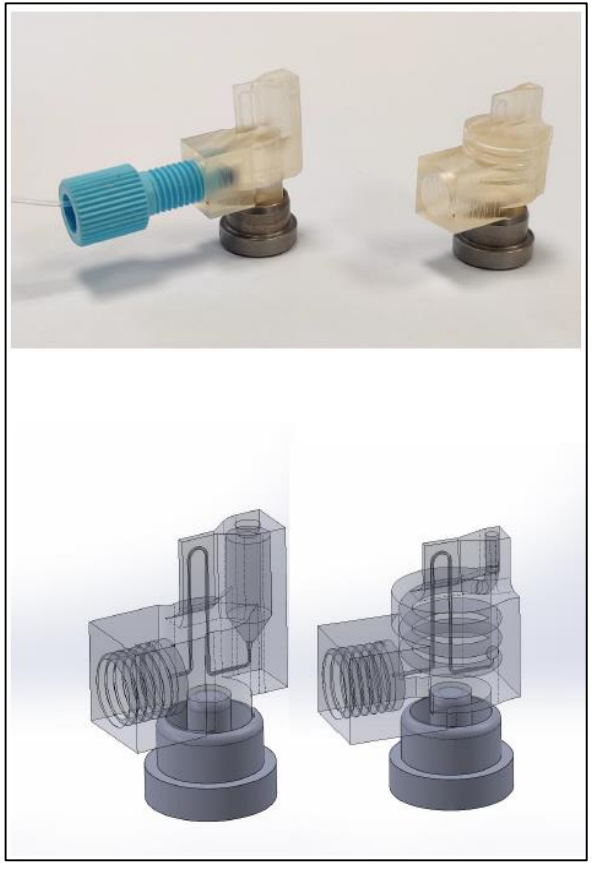
European XFEL

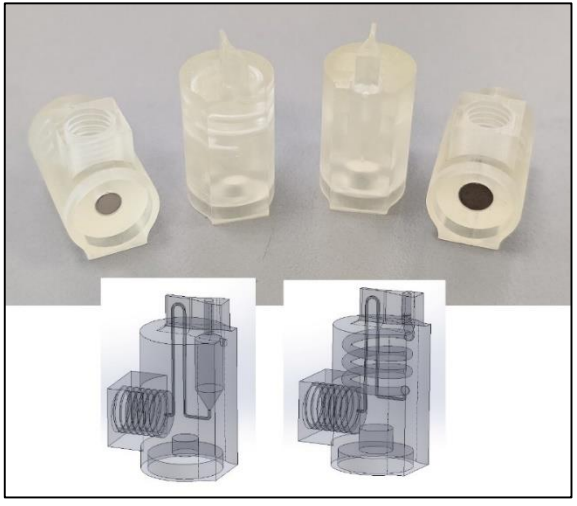
Serial crystallography microfluidic device (30x30 mm) – is a frame and 4x4 grid, where another grids are placed 31x32, which are intended for catching crystals.



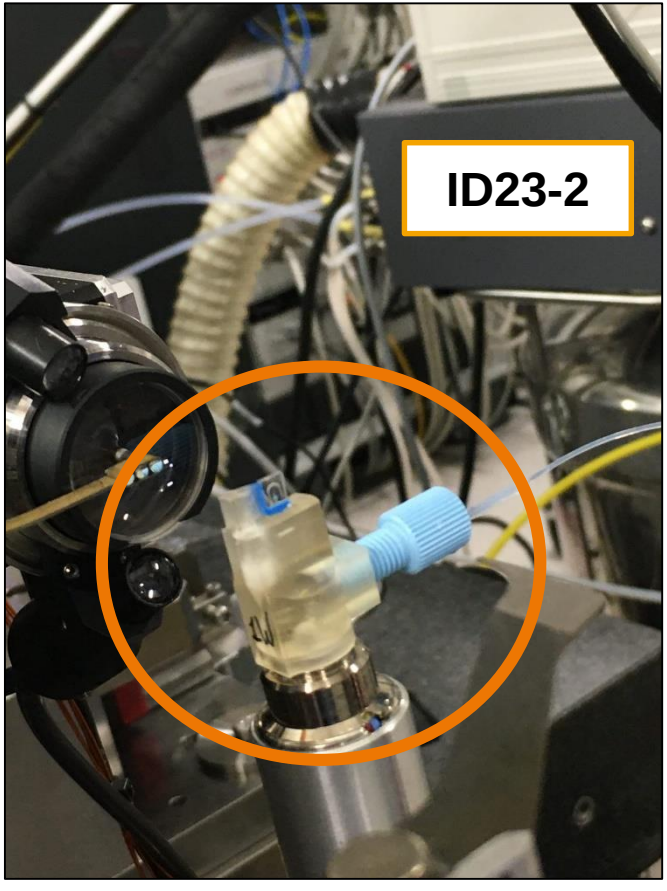
Dimensions of small grid cells are now 75x75 µm.

SERIAL CRYSTALLOGRAPHY – CONCEPTS – 2



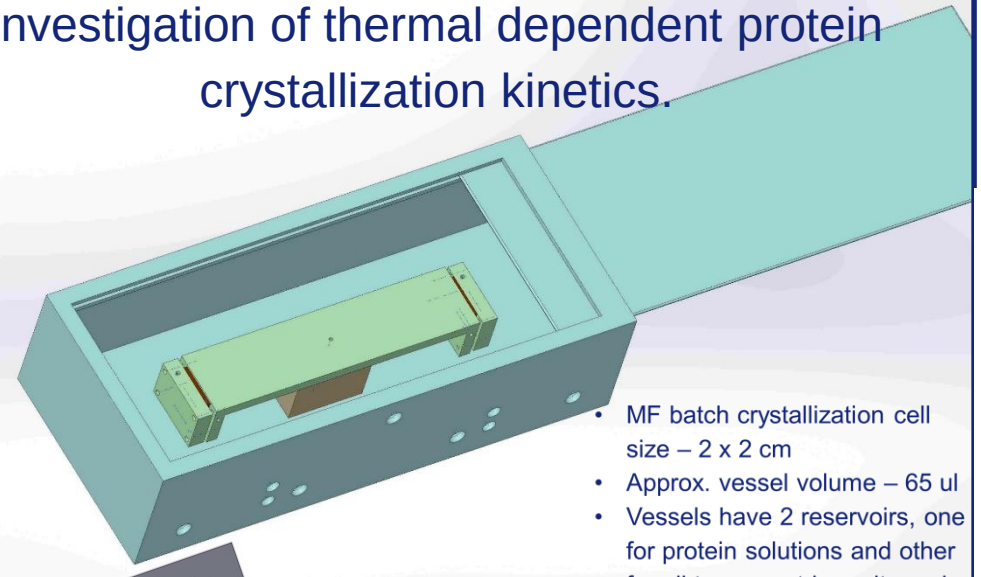


3D printed Injection system for serial crystallography experiments.
Width of the work channel – 150 – 200 µm

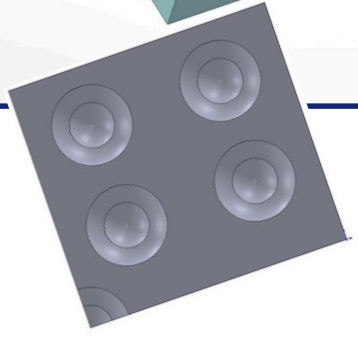


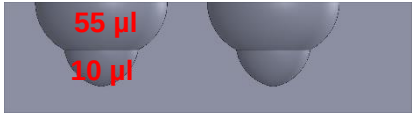
TEMPERATURE DEPENDENT CRYSTALLIZATION – 1

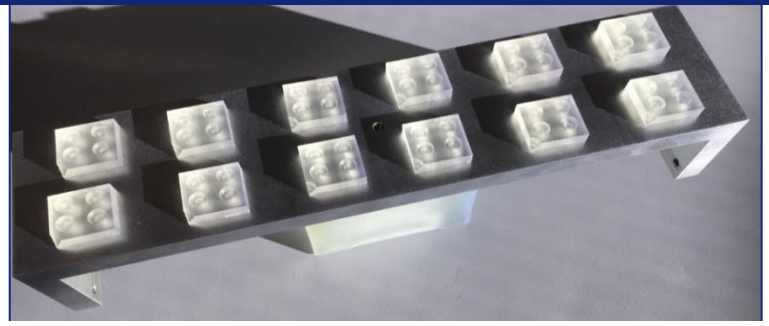
Peltier-based experimental device for investigation of thermal dependent protein crystallization kinetics.

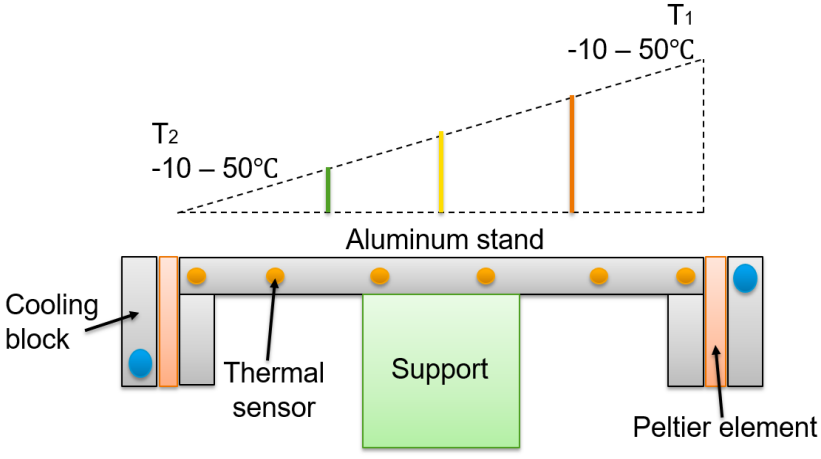


- MF batch crystallization cell size – 2 x 2 cm
- Approx. vessel volume – 65 ul
- Vessels have 2 reservoirs, one for protein solutions and other for oil to prevent impurity and evaporation of the sample.





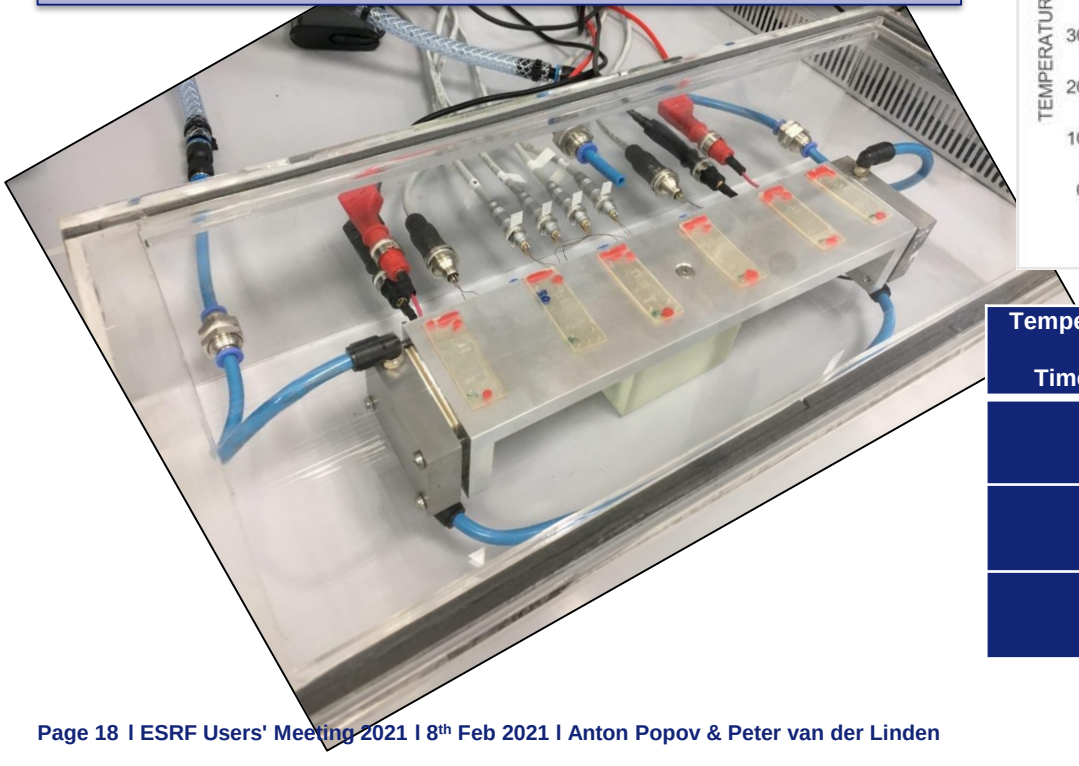


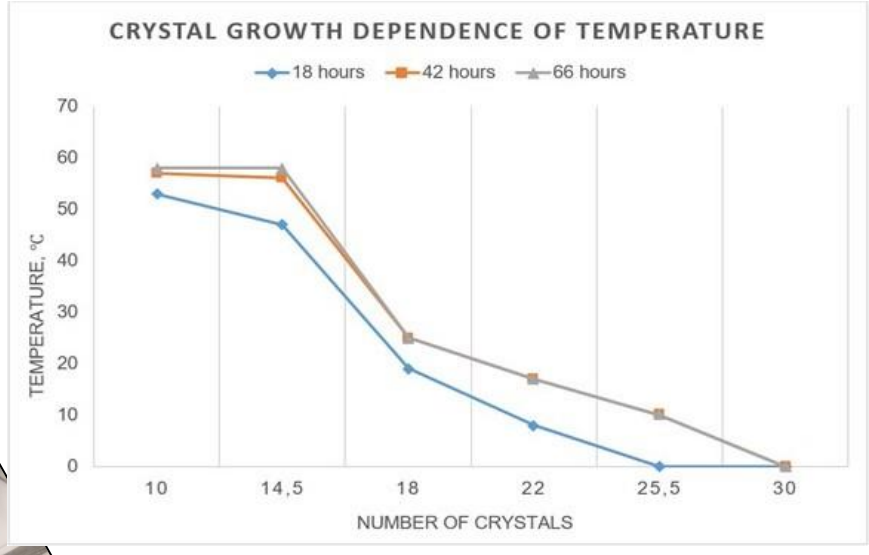


The work platform is 25 x 6 cm aluminum rectangle and has space for up to 12 MF batch crystallization cells, containing 4 similar or various crystallization conditions each.

TEMPERATURE DEPENDENT CRYSTALLIZATION – 2

Peltier-based “Thermo-box” was tested with the Lysozyme from chicken egg white (SA №62971). Six microfluidic chips were filled with microdroplets of same solution and then stored in the box at different temperatures. The crystal growth depended strongly on the storage temperature; over time, the number of crystals grew.



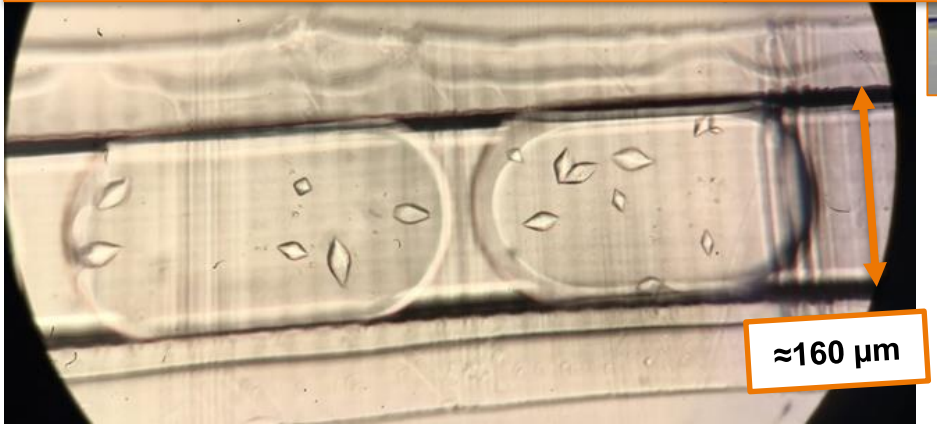


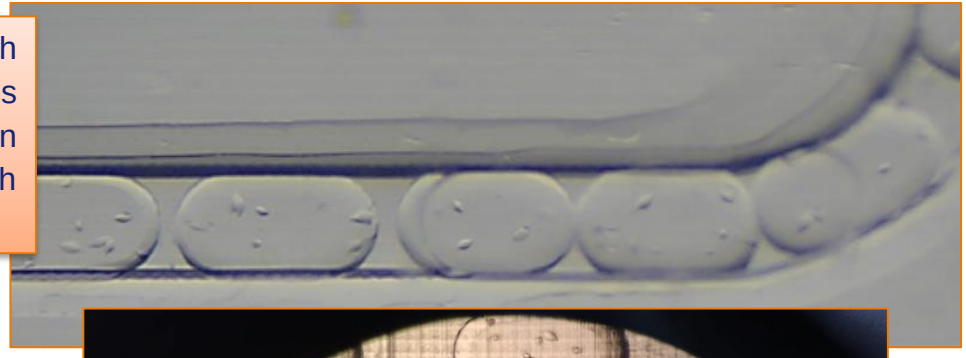
Temperature, °C Time, hours	10	14.5	18	22	25.5	30
18	**53***	47	19	8	0	0
42	**57**	56	25	17	10	0
66	**58**	58	25	17	10	0

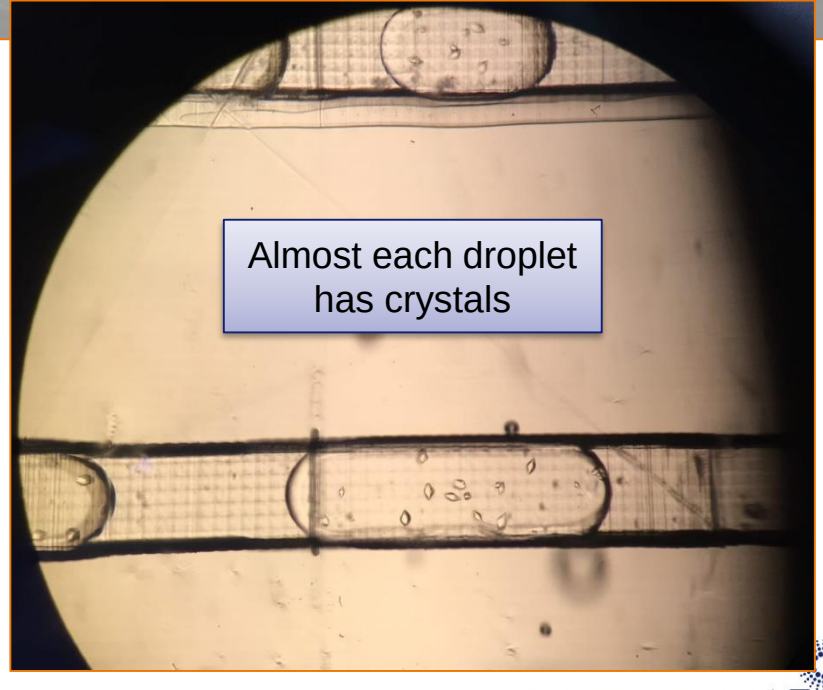
*** Number of crystals inside the chip**

TEMPERATURE DEPENDENT CRYSTALLIZATION – 3

The success of first experiment was consolidated through experiment on Thaumatin (SA №7638). Six microfluidic chips were filled with microdroplets of different solution and then stored in the box at 5°C in 96 hours. The crystal growth depended strongly on protein/precipitant concentration.

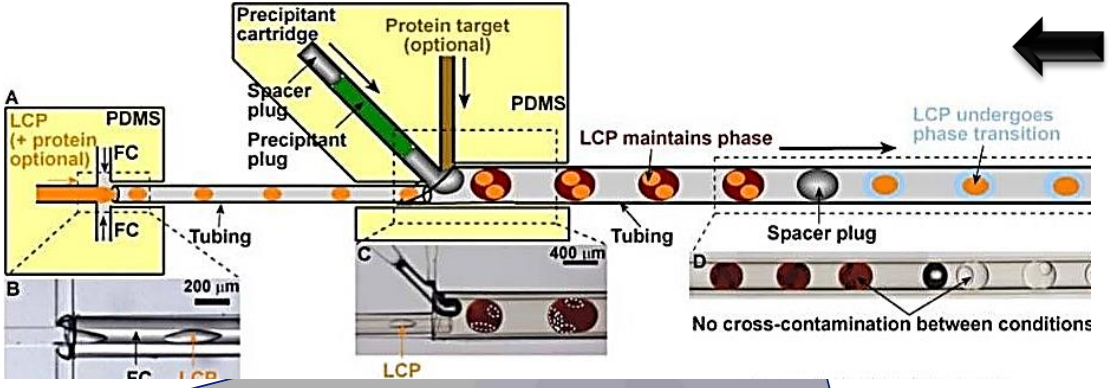




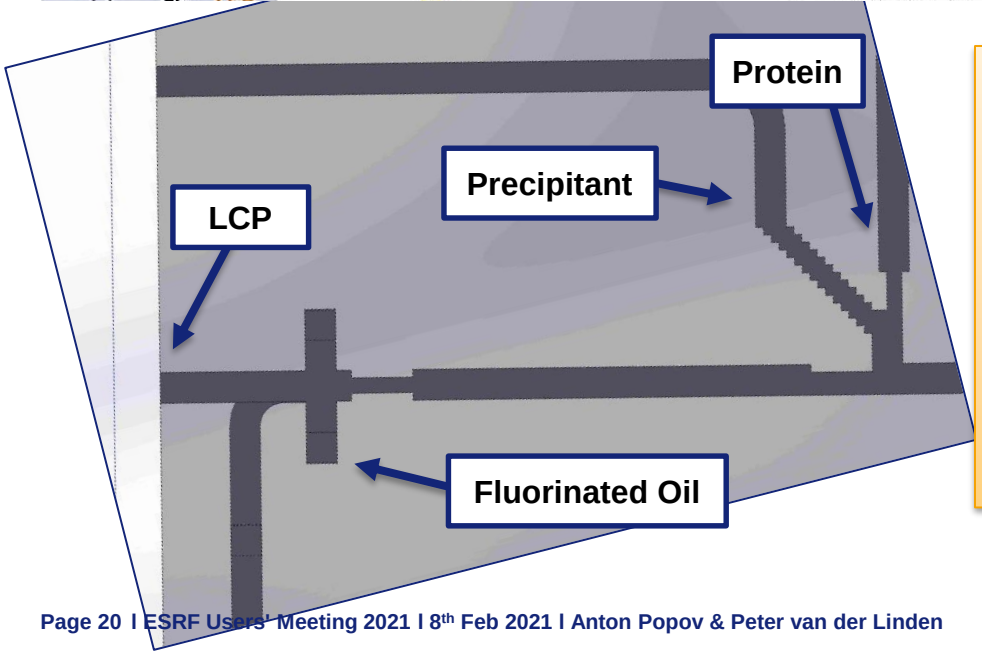


Using droplet microfluidic chips (screening) and thermal box (temperature control), it is possible to determine the optimal crystallization conditions.

DROPLET-BASED MF FOR MEMBRANE PROTEINS



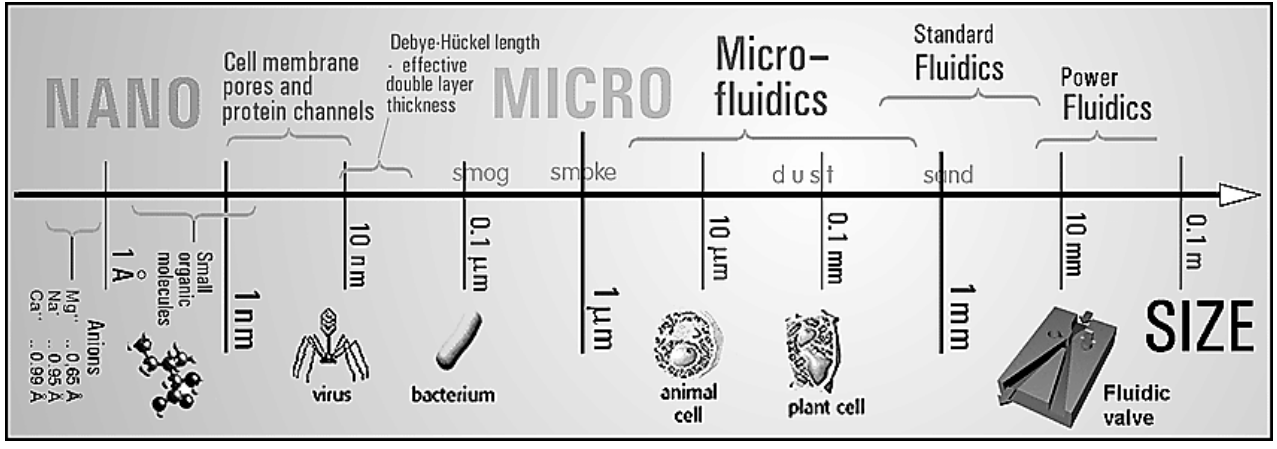
A Plug-Based Microfluidic System for Dispensing Lipidic Cubic Phase (LCP) Material Validated by Crystallizing Membrane Proteins in Lipidic Mesophases.
Liang Li et al.
Microfluid Nanofluidics. 2010 Jun; 8(6): 789–798.
doi: 10.1007/s10404-009-0512-8

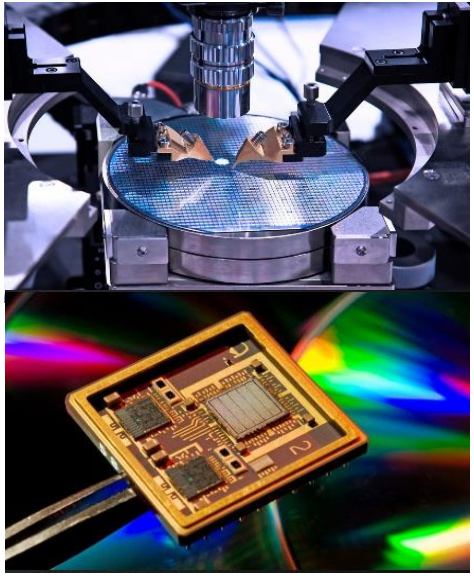


One of the important topics regarding structural biology is membrane proteins. The main difficulty is that all membrane proteins are not soluble in water, for them the native environment is not a solution, but a lipid membrane.
To prevent denaturation and loss of the desired structure, it is necessary 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.

MICROFLUIDIC STANDARDS

From microelectronics some technologies were transferred to microfluidics. Now both are evolving to nano scale.





One of the most important tasks is unification – the definition of x and y, standardization of dimensions, positioning of input / output holes, sample environment, etc.

Common sizes: credit card – 86 x 54 mm, microscope slides – 76 x 26 mm, this sizes were common to scientific society.

Nowadays, more standard sizes are considered devices with sides multiples of 15 mm (15x15, 15x30, 15x45, 30x30 etc.).

"Design Guideline for Microfluidic Device and Component Interfaces" by Henne van Heeren (et al.) was supported by the Microfluidic Consortium (UK, France and the Netherlands), the MFManufacturing project and many more.

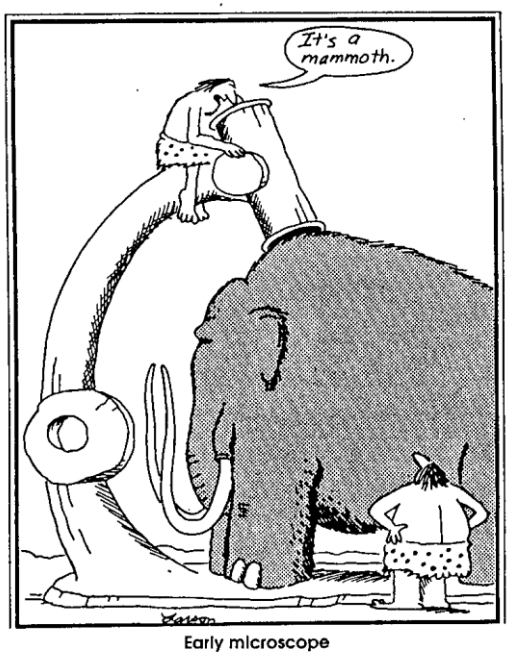
CONCLUSIONS AND ACKNOWLEDGEMENTS

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

Authors to whom correspondence should be addressed:
anton.popov@esrf.fr
vanderlinden@esrf.fr
diego.pontoni@esrf.fr
leonard@esrf.fr

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Structural biology group science meeting