IN SITU DIFFRACTION EXPERIMENTS AT THE NAGOYA UNIVERSITY BL2S1 BEAMLINE

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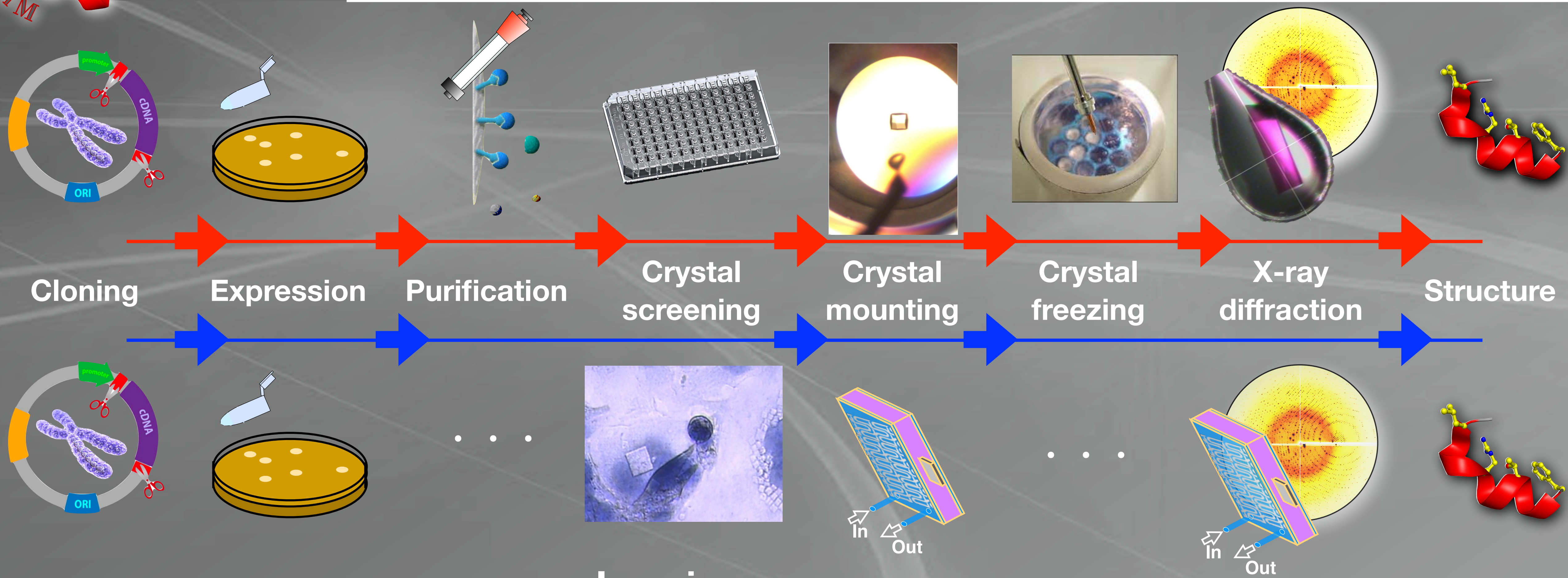
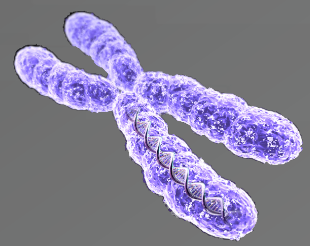
Nagoya University, Japan

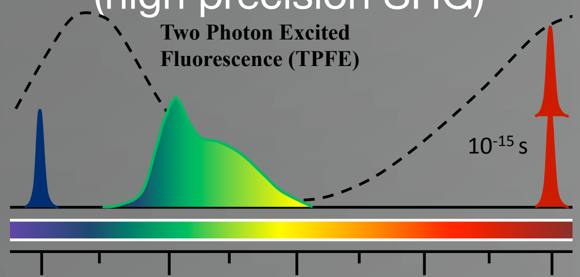
Motivations: easier sample handling



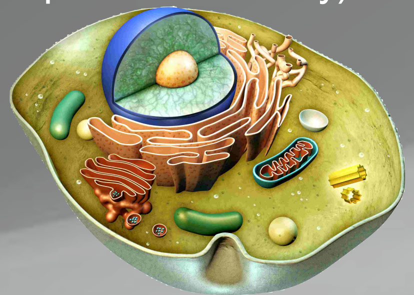
Cloning
(*iv*MX plasmid library)



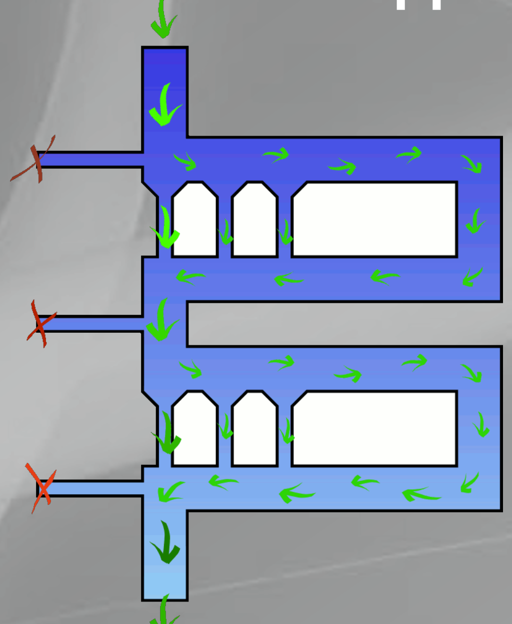
Imaging
(high precision SHG)



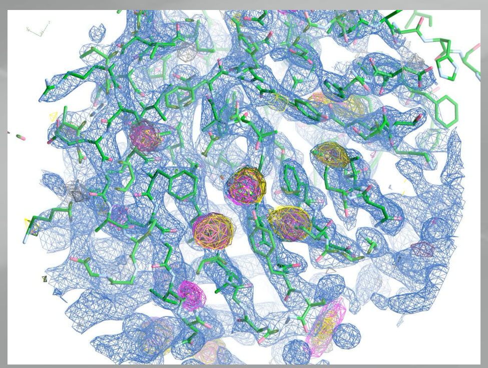
Cell biology
(expression facility)



Serial trapping



(Structure solution)
MX

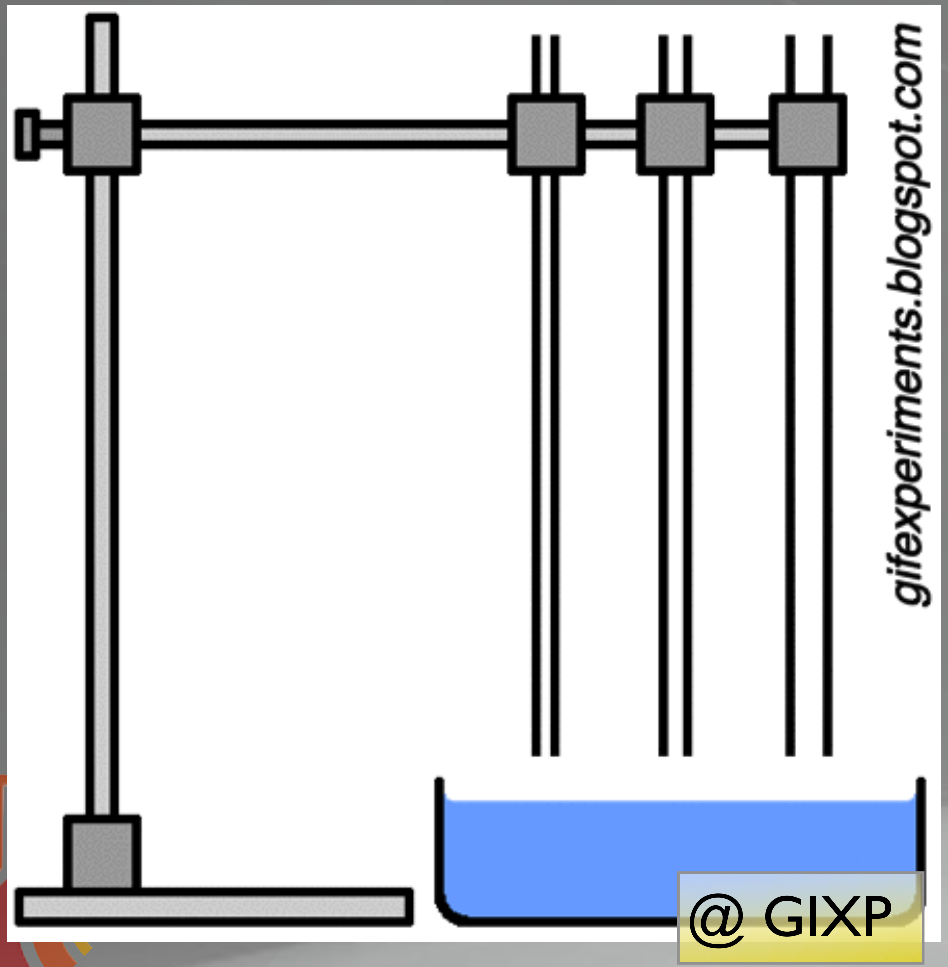


Why microfluidics?

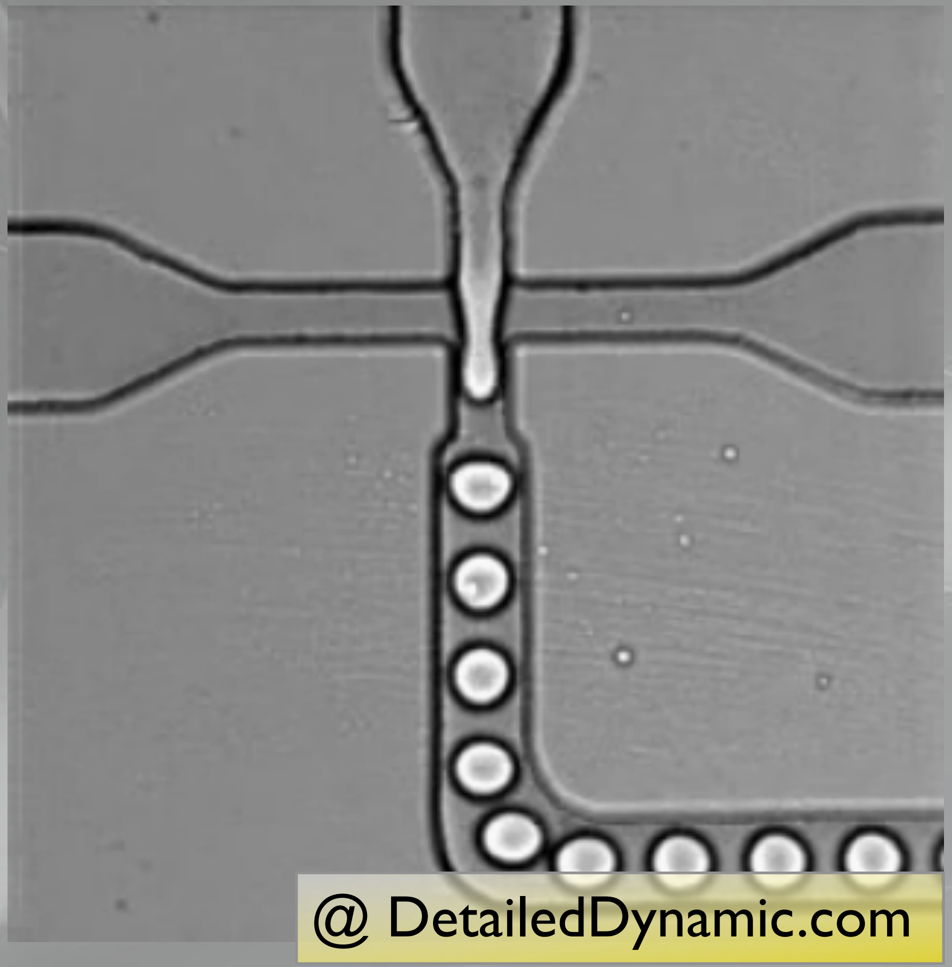
Microfluidics = handling of small-scale fluids (typically sub-millimeter)

There are two distinct processes practically applied:

passive fluid control
uses capillary forces



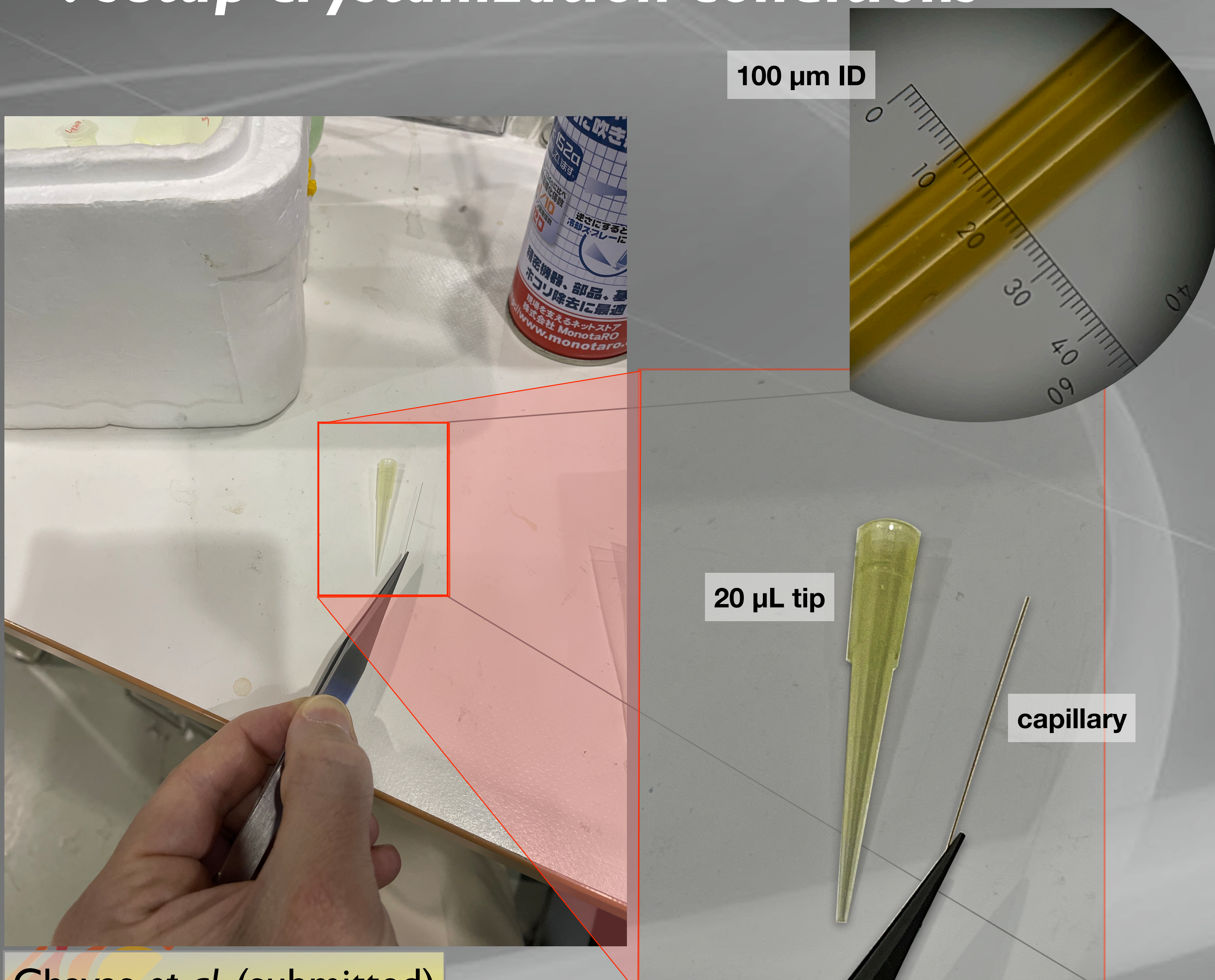
active fluid control
uses active components such as pumps

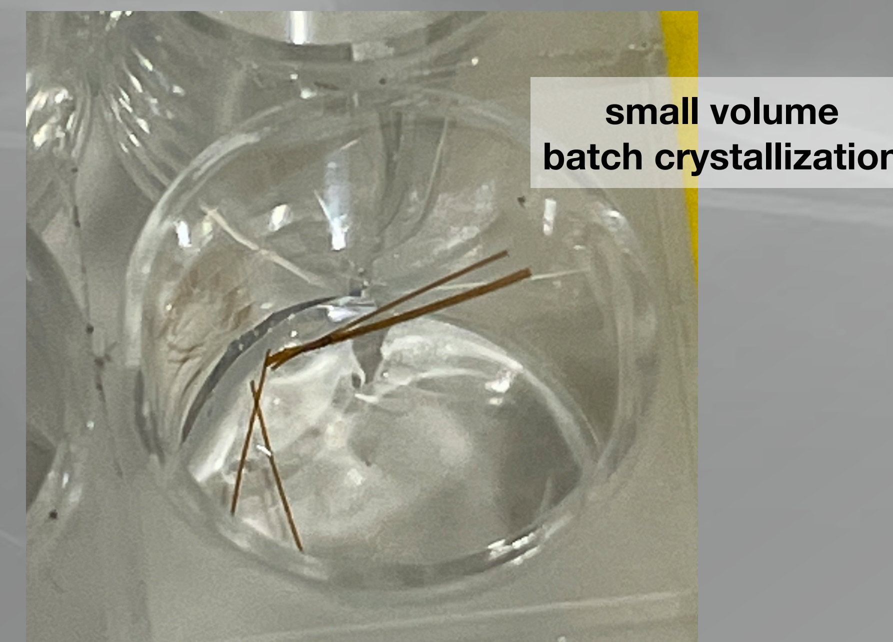


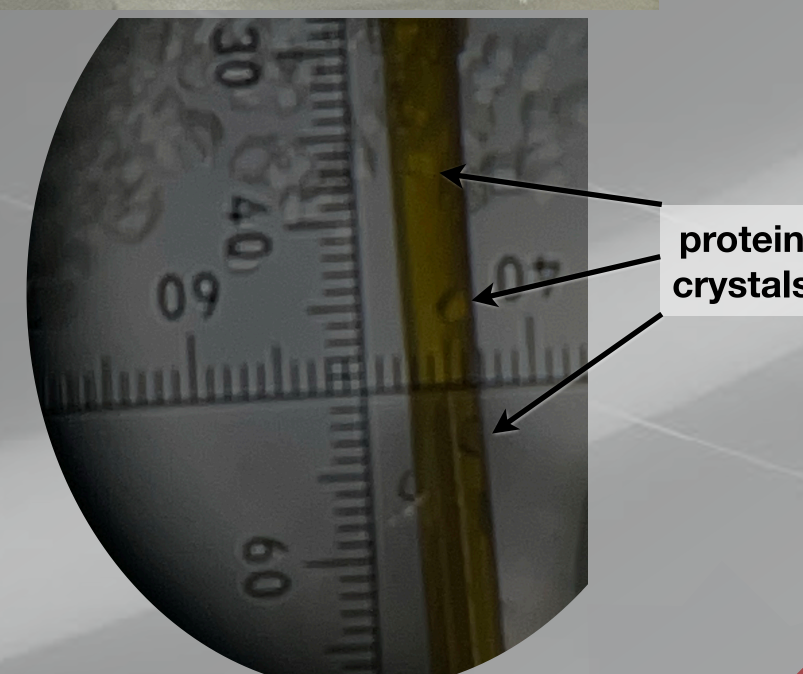
Passive fluid control

Quick experiment:

- choose a capillary with the proper inner diameter (~ beam size)
- setup crystallization conditions





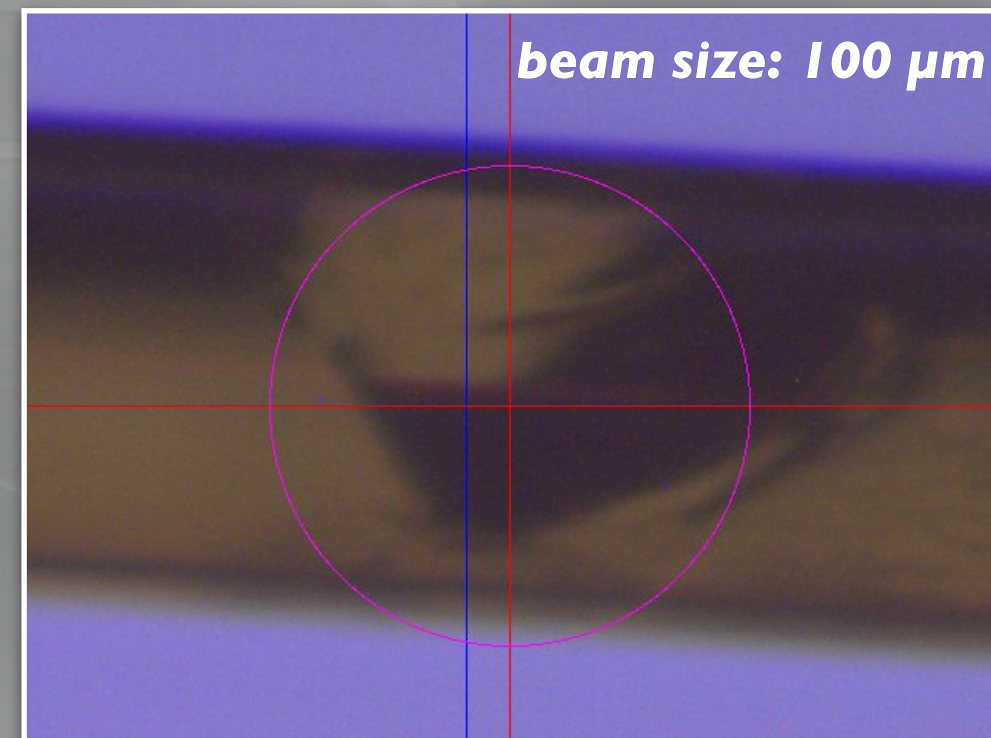


Chavas *et al.* (submitted)

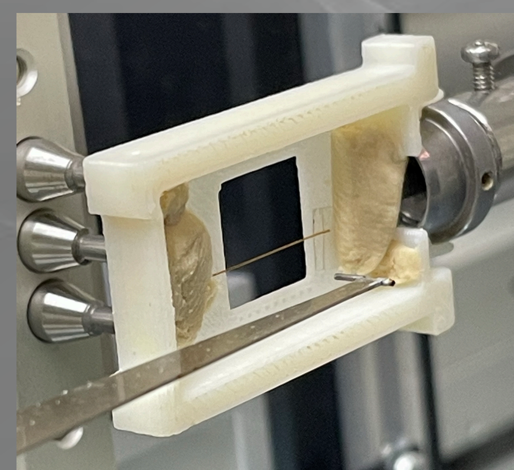
Passive fluid control

Quick experiment:

- choose a capillary with the proper inner diameter (~ beam size)
- setup crystallization conditions
- pick up and mount the capillary
- perform diffraction experiments



Chavas *et al.* (submitted)



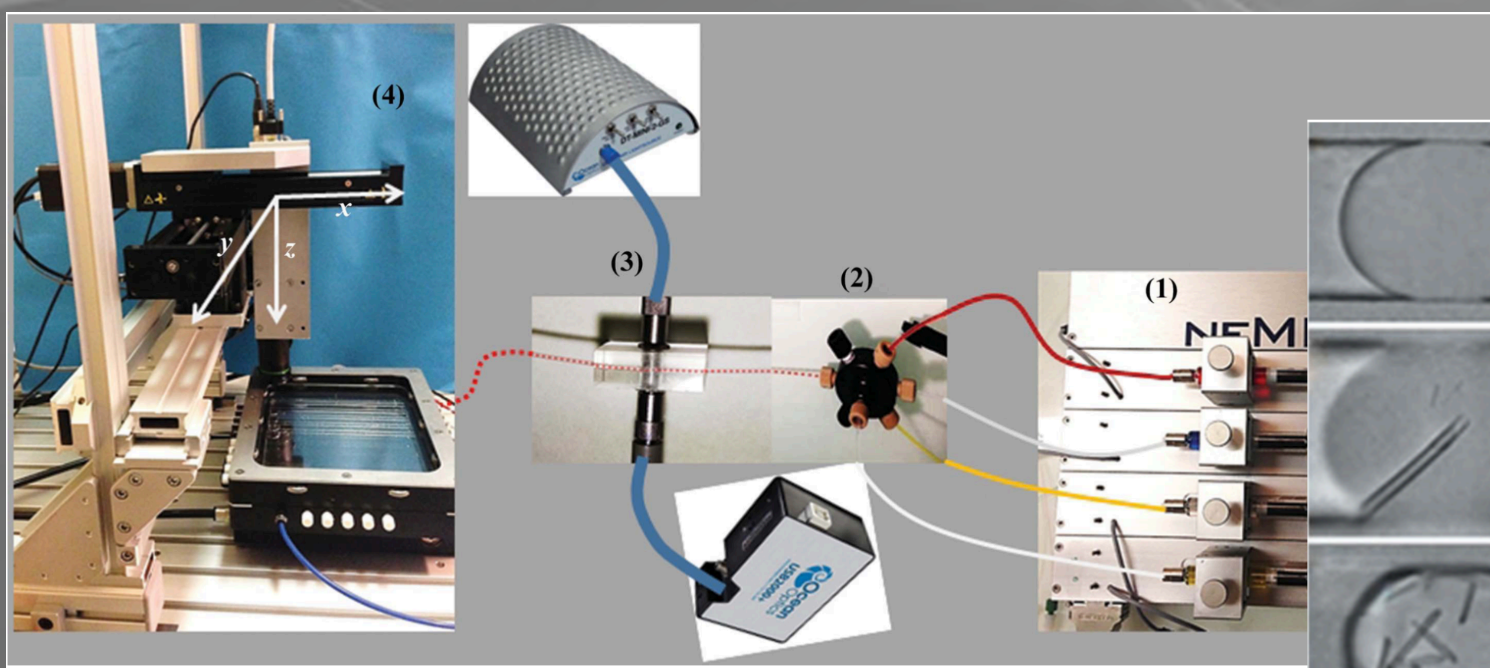
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOL

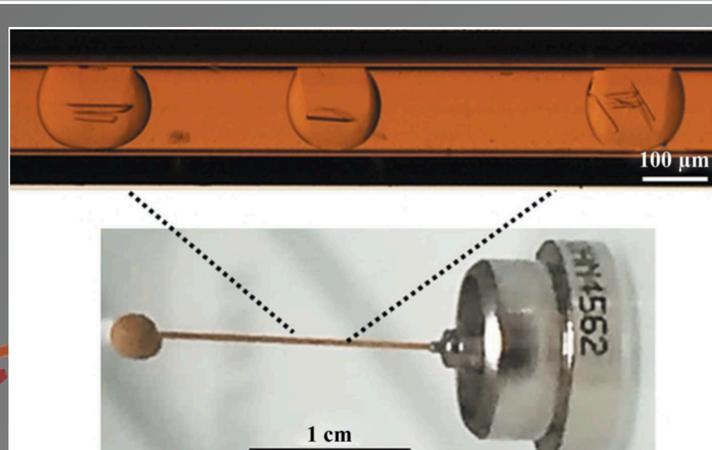
RESOLUTION LIMIT	I/SIGMA	R-meas	CC(1/2)	Anomal Corr	SigAno	Nano
4.71	8.19	18.6%	94.2*	43*	1.132	114
3.34	8.57	18.6%	94.9*	54*	1.473	235
2.73	7.01	25.9%	91.5*	41*	1.219	335
2.37	5.70	33.0%	85.3*	31*	1.103	413
2.12	4.81	37.7%	86.6*	28*	1.014	482
1.94	3.52	49.3%	83.2*	16*	0.900	539
1.79	2.08	79.6%	64.2*	7	0.695	598
1.68	1.36	112.2%	48.9*	-1	0.615	656
1.58	0.92	148.8%	31.9*	1	0.587	602
total	3.74	32.6%	95.3*	27*	0.877	3974

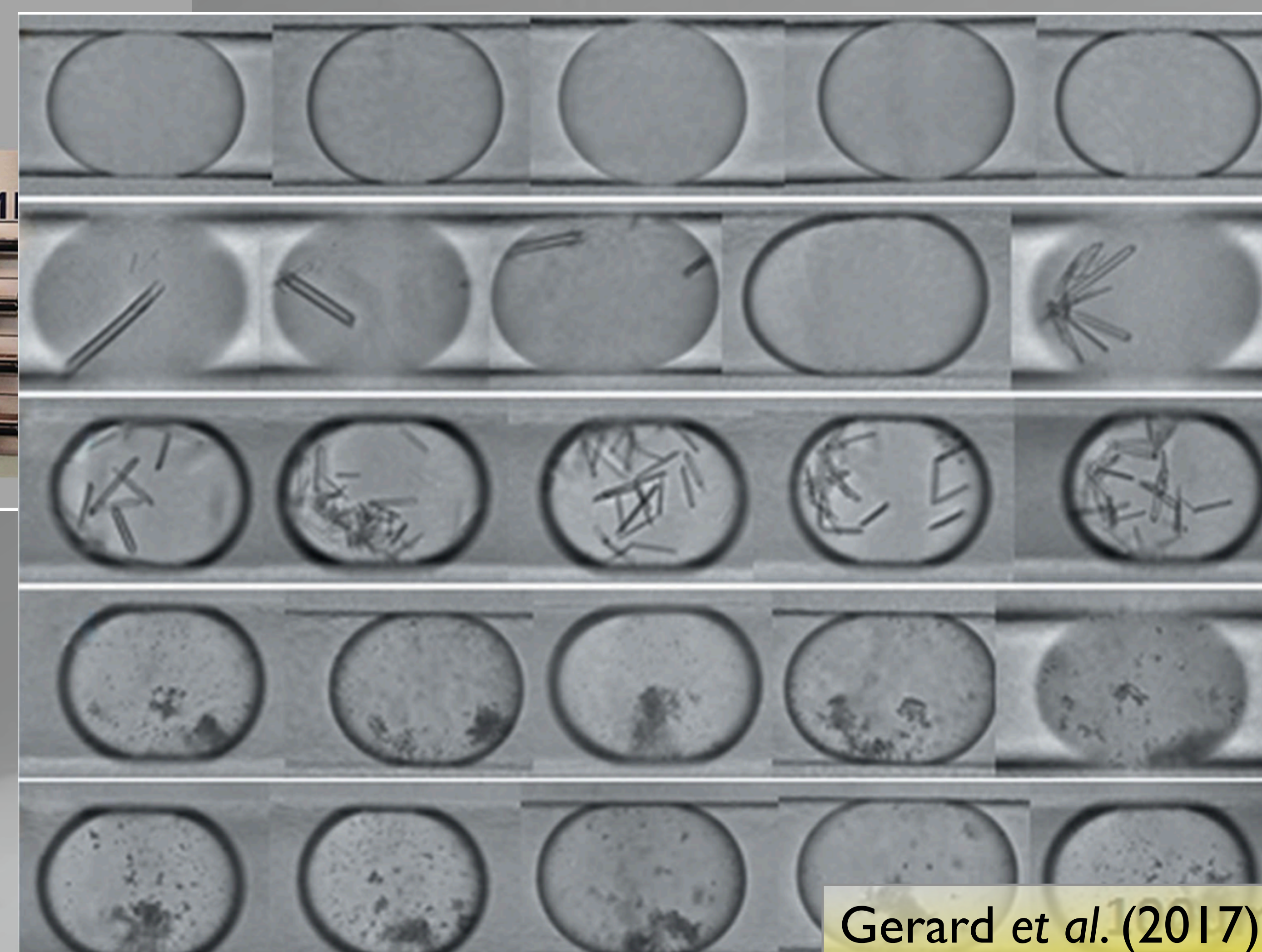
Active fluid control

Crystallization via tubing:

- *using syringes, micropumps, pressure controllers …*
- *much more sophisticated setup to permit matrix screening*
- *systematic and repeatable drop size, easily scalable*
- *room for improvement (addition of inlets for ligand screening)*







Gerard *et al.* (2017)

Active fluid control

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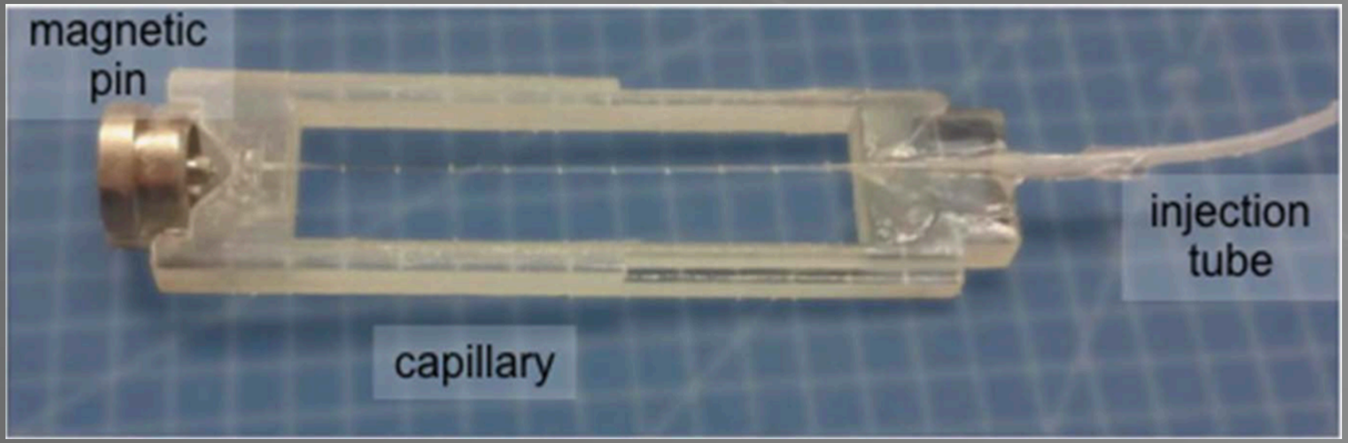
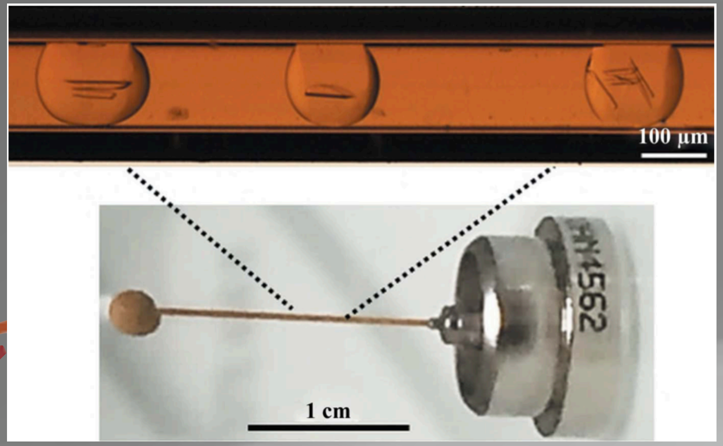
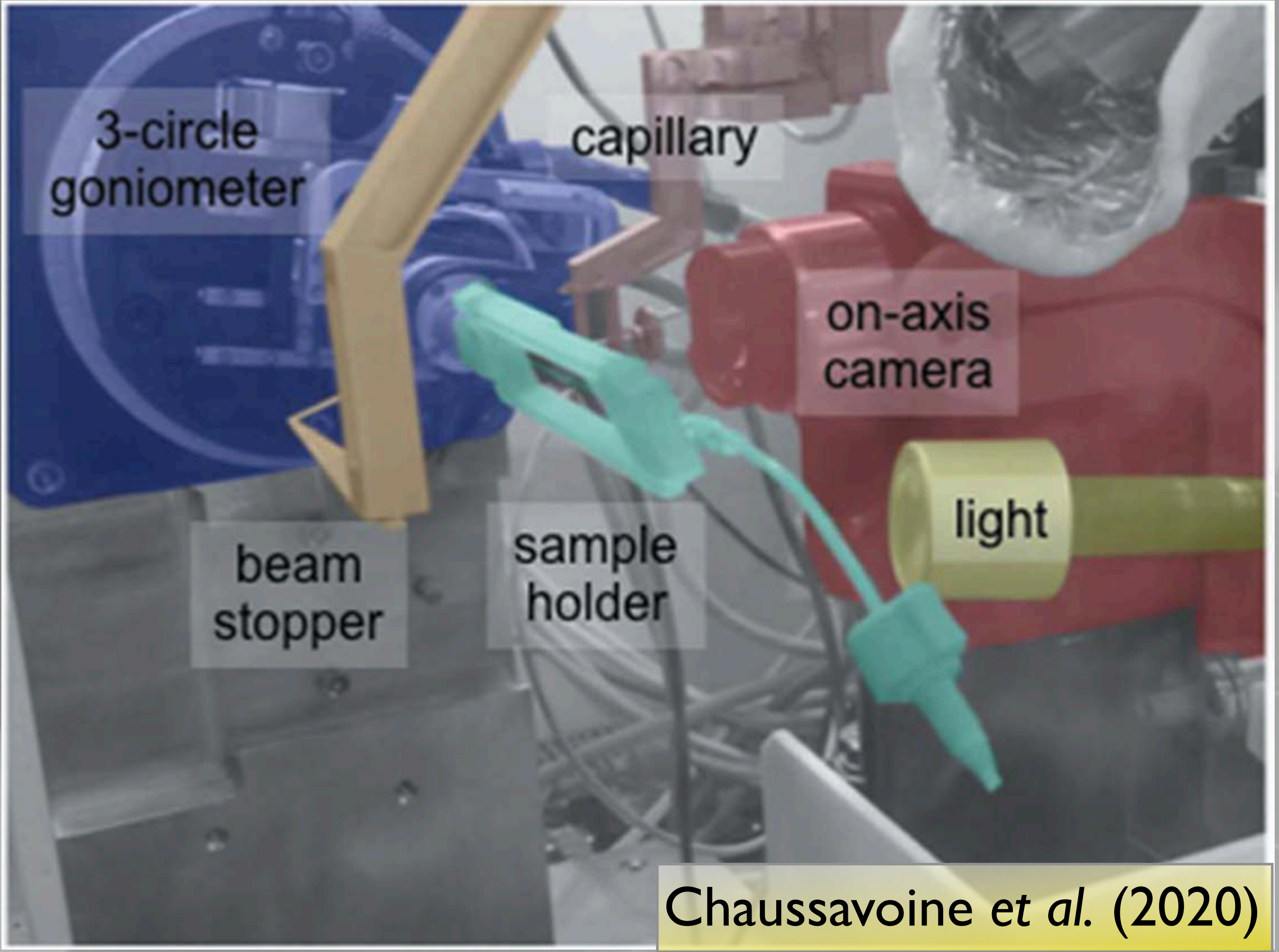
- *room for improvement (addition of inlets for ligand screening)*
- *the capillaries can be used mounted for in-flow Serial SX*







Chaussavoine *et al.* (2020)

Active fluid control

Crystallization via tubing:

- *using syringes, micropumps, pressure controllers …*
- *much more sophisticated setup to permit matrix screening*
- *systematic and repea*
- *room for improveme*
- *the capillaries can b*

- *the same capillaries can be used for harvesting the crystals for more classical cryo MX*

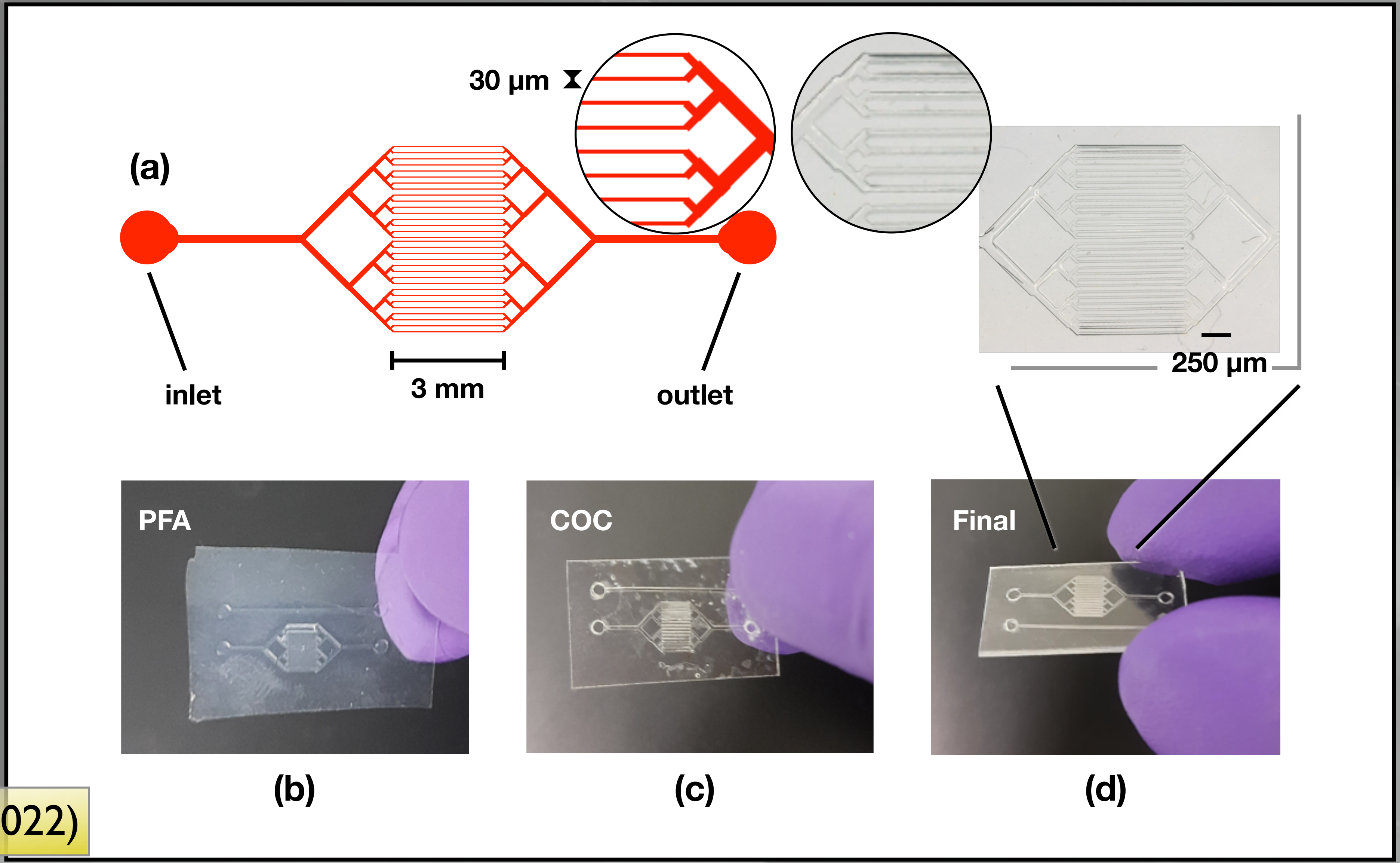
Gerard *et al.* (2017)

Chip devices - crystallization

If the end goal is to place the device in the X-ray beam, it needs to be X-ray transparent to avoid large diffusion background.

Classical compounds: Silica, PDMS, Kapton, COC…

Example of a COC device 100 μm thick (in the X-ray direction)



Vasireddi *et al.* (2022)

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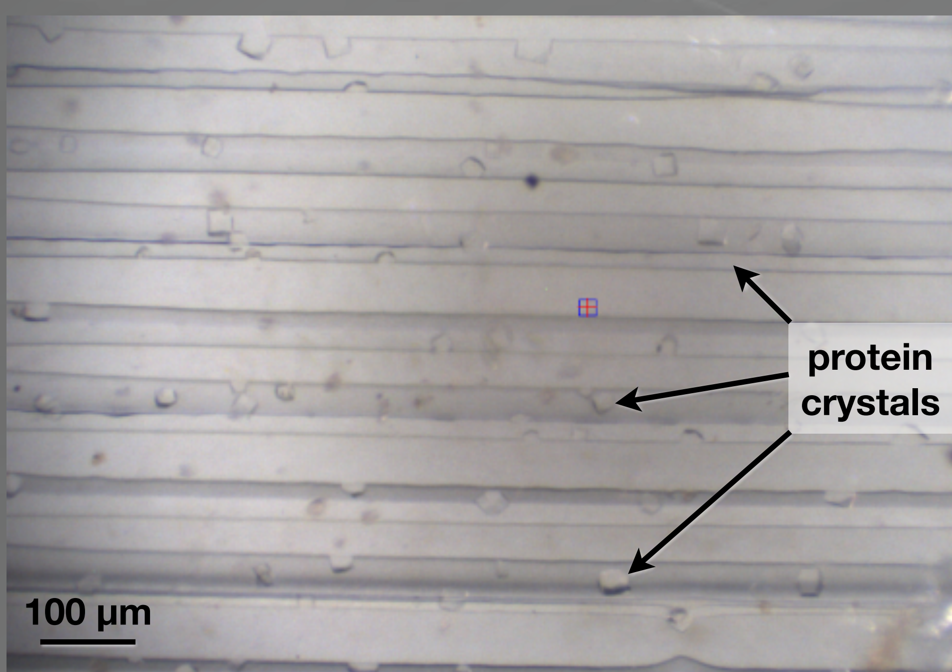

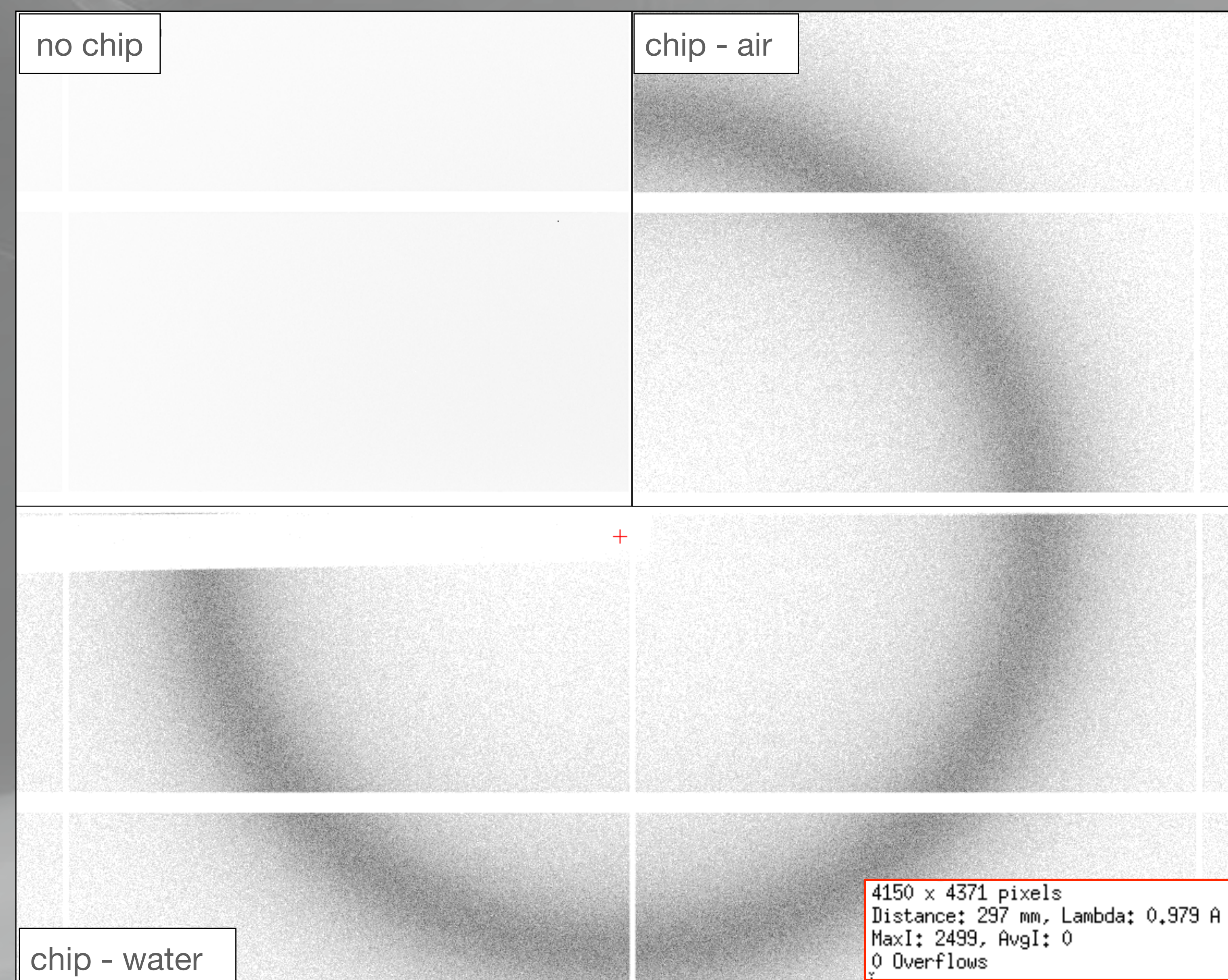

Vasireddi *et al.* (2022)

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Example of a COC device 100 µm thick (in the X-ray direction)

A total of 21 data sets of 20 degrees each were merged to improve the statistics at higher resolution and limit possible damages from X-ray radiation

DATA COLLECTION	
Space group	$P4_32_12$
Unit cell parameters (Å, °)	$a = b = 79.61$, $c = 37.88$, $\alpha = \beta = \gamma = 90$
Resolution (Å)	56.28–1.83 (1.86–1.83)
No. of observed reflections	306,732 (4445)
No. of unique reflections	11,146 (525)
Completeness (%)	100 (99.4)
R_{merge}	0.111 (0.578)
R_{meas}	0.113 (0.614)
R_{pim}	0.021 (0.2)
$\langle I/\sigma(I) \rangle$	22.2 (2.8)
$CC_{1/2}$	0.999 (0.884)
Multiplicity	27.5 (8.5)
Wilson B factor (Å^2)	28.68
REFINEMENT	
R_{free}	0.19
R_{work}	0.17
r.m.s.d., bond lengths/angles (Å, °)	0.008/0.92
Ramachandran (favored/allowed, %)	99.21/0.79
Average B factor (Å^2)	
Overall	29.18
For protein residues	27.73
For water	44.52

Vasireddi *et al.* (2022)

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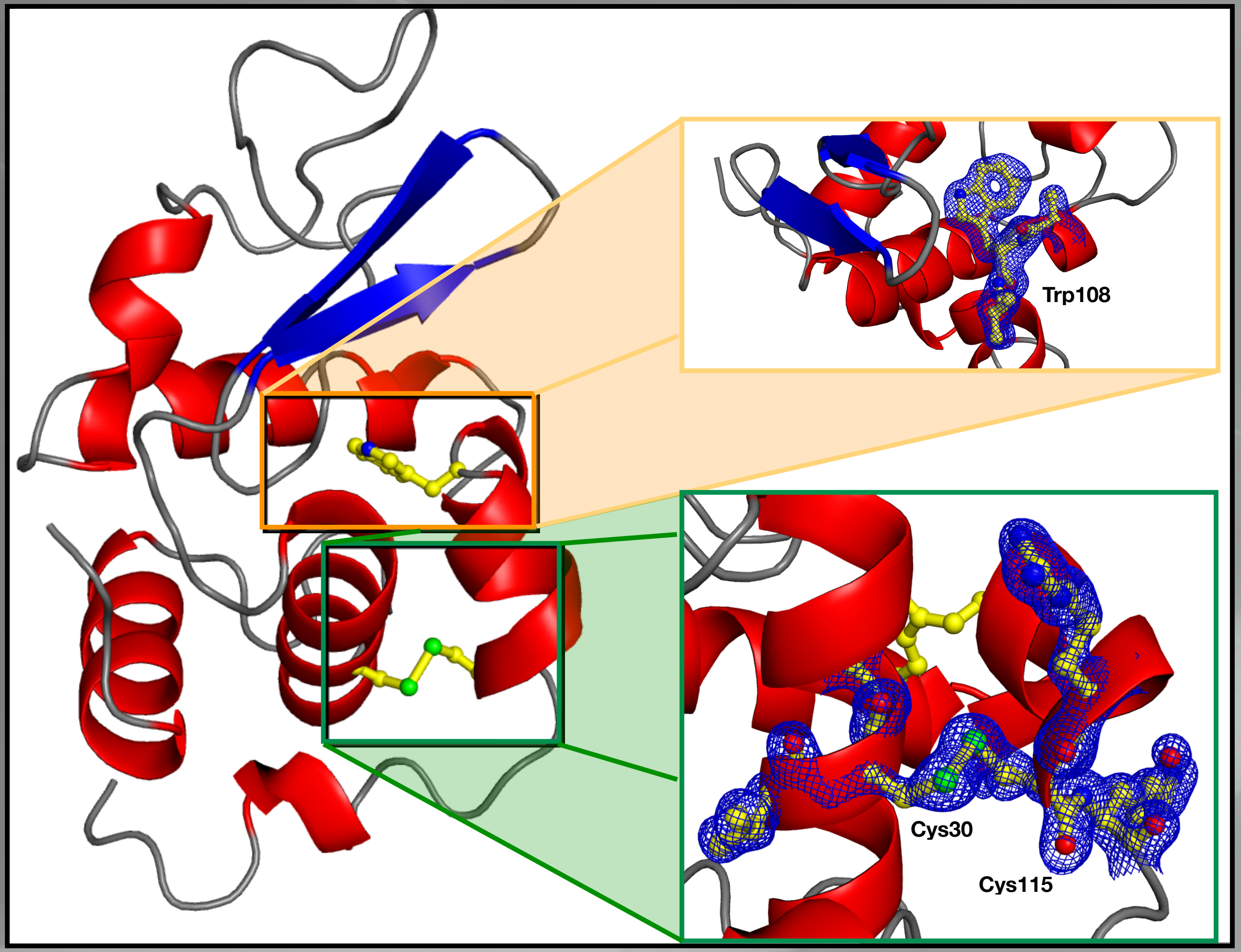
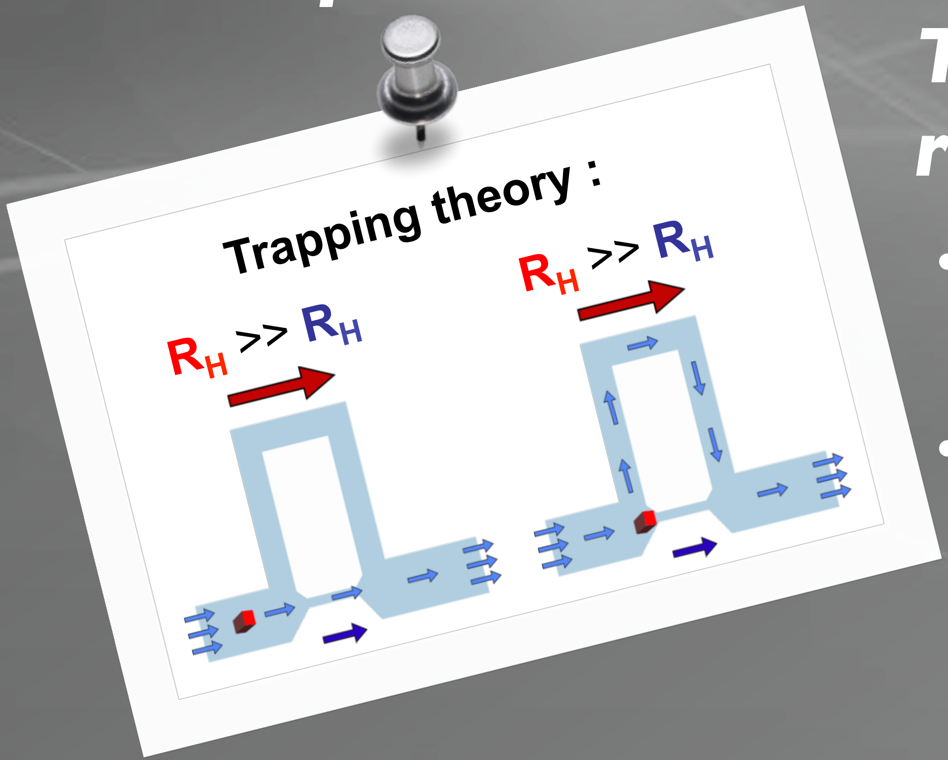


The electron density is neat and does not show strong signs of radiation damages.

Vasireddi *et al.* (2022)

Chip devices - crystal trapping

One can also take advantage of the fluidic properties to play with the samples.

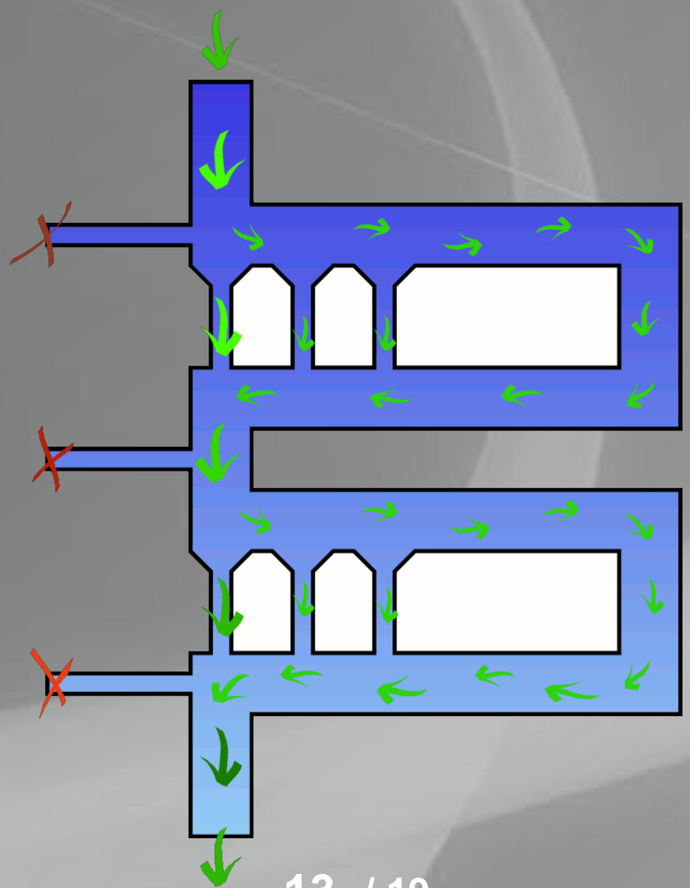


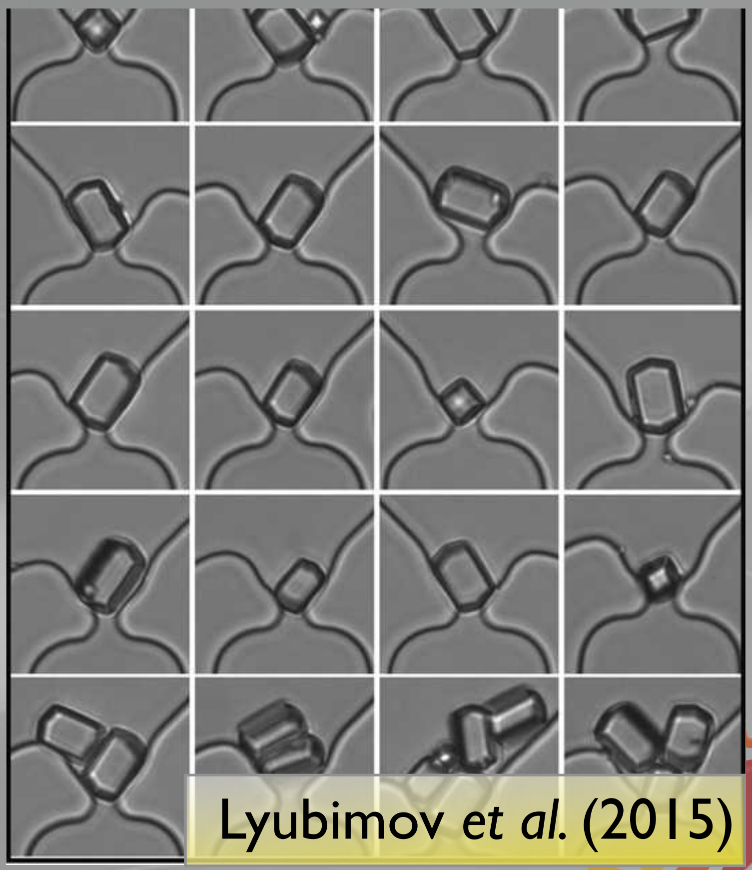
There is a similitude between fluidics and resistance:

. the fluid will go through the less resistive path

. if the path is blocked, the fluid will look for another path of smaller resistance

These properties can be applied to serial trapping of samples.



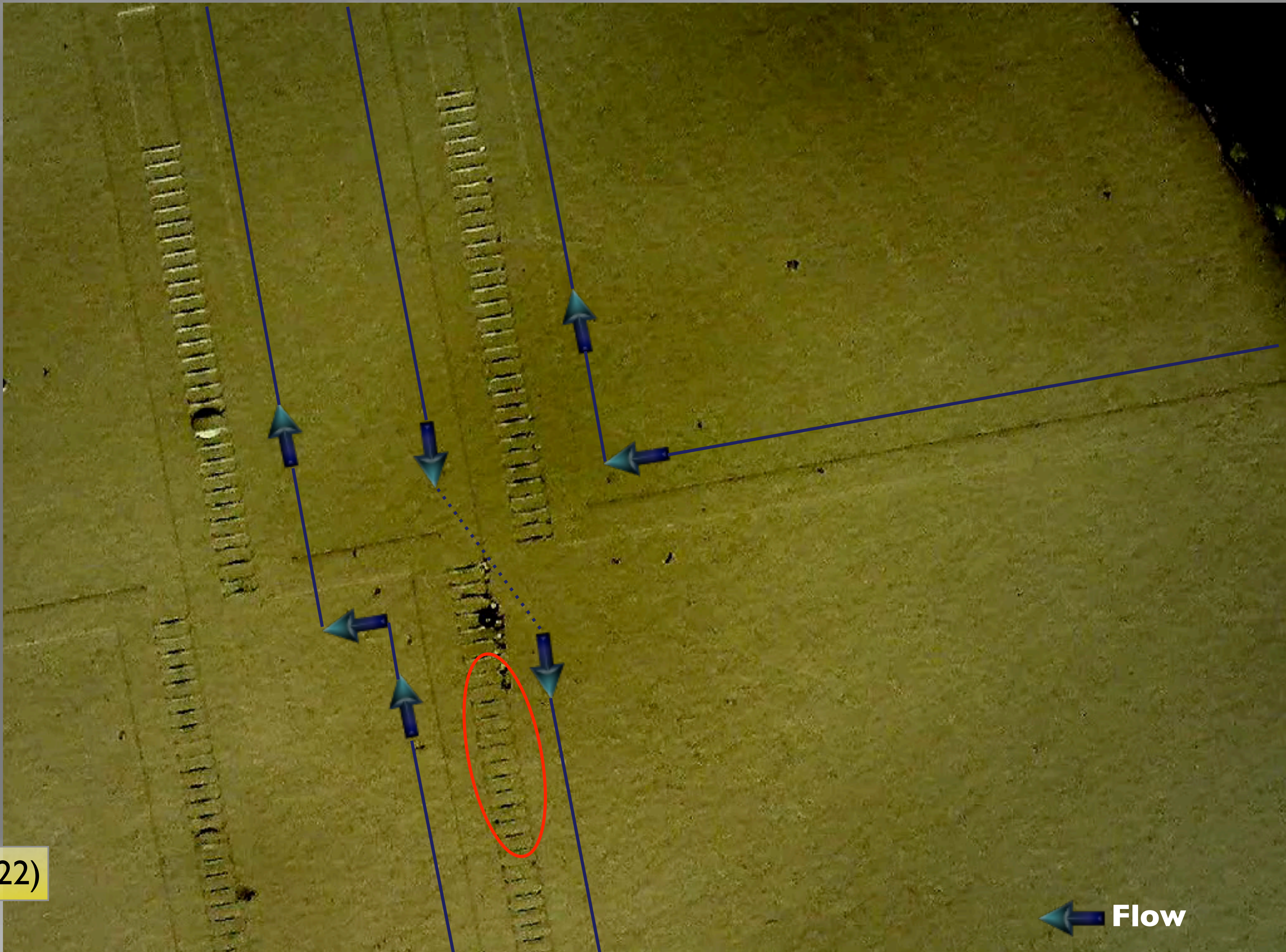


Lyubimov *et al.* (2015)

Chip devices - crystal trapping

One can also take advantage of the fluidic properties to play with the samples.

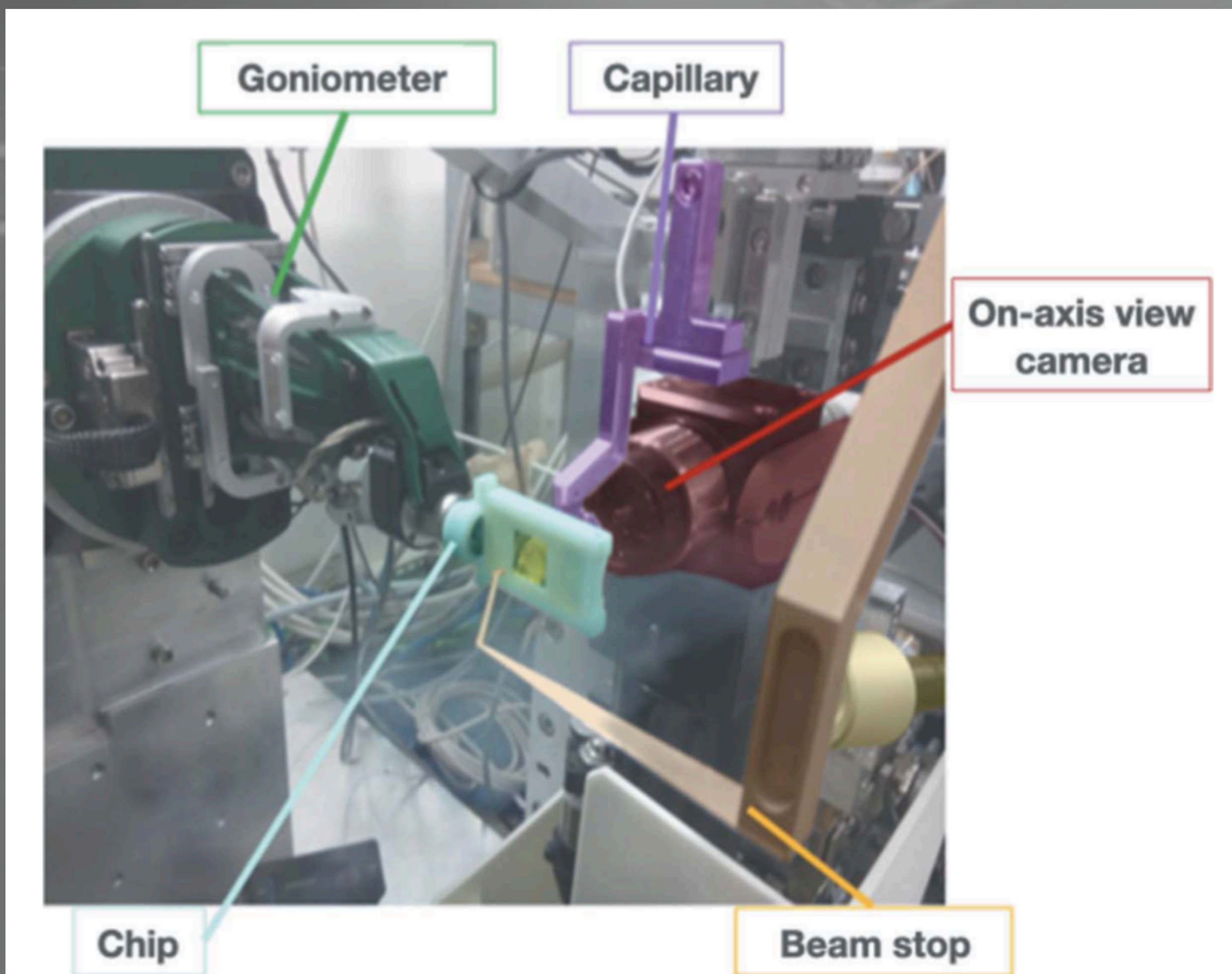
While increasing the number of traps, it is possible to multiply the number of samples that can be trapped



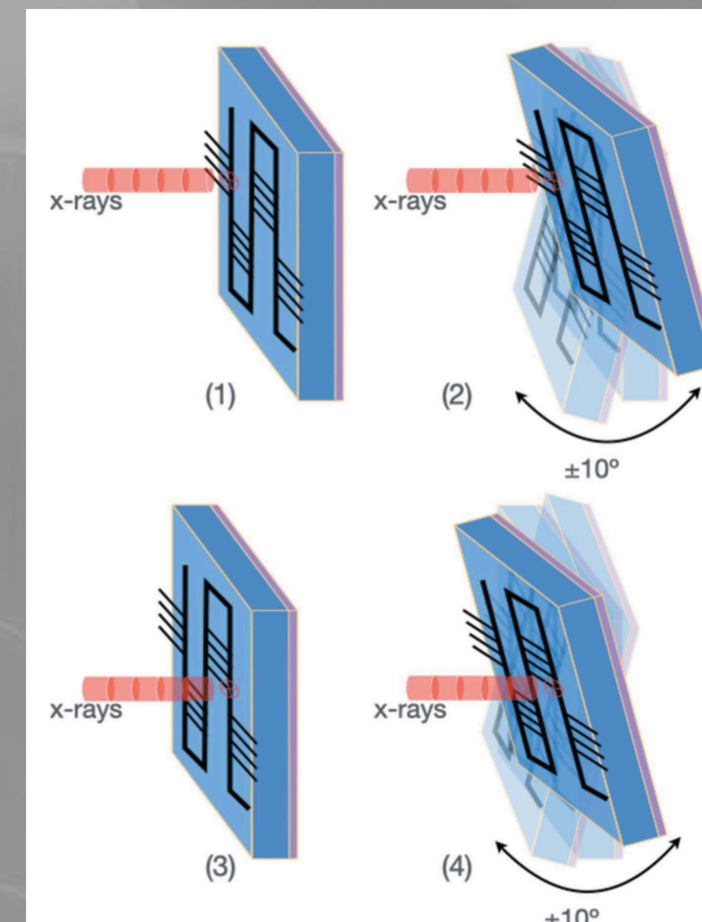
Chaussavoine *et al.* (2022)

Chip devices - crystal trapping

The main advantage of the trapping chips lies in their adequacy toward the full automation of data collection



The known location of the traps permits an automated data collection procedure of small wedges without prior manual sample centering

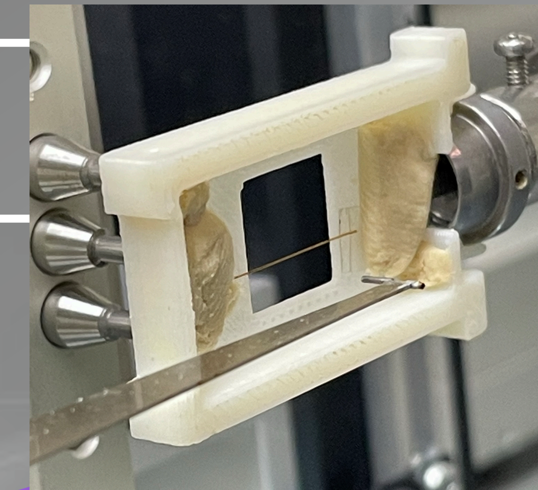


The frame around the chip is adequate for handling using a robot with adapted tongs

Data collection	Lysozyme	Insulin
Number of merged data	30	13
Space group	$P4_32_12$	$R3$
Unit-cell parameters (Å)	$a = b = 79.67$, $c = 37.90$	$a = b = 83.08$, $c = 34.39$
Resolution (Å)	35.63–1.60 (1.64–1.60)	41.54–2.33 (2.39–2.33)
No. of observed reflections	116929 (5636)	9615 (168)
No. of unique reflections	16516 (1196)	2464 (102)
Completeness (%)	99.3 (99.3)	65.1 (37.0)
Multiplicity	7.1 (4.7)	3.9 (1.6)
R_{merge}	0.064 (0.891)	0.295 (0.554)
$CC_{1/2}$	0.997 (0.658)	0.890 (0.560)

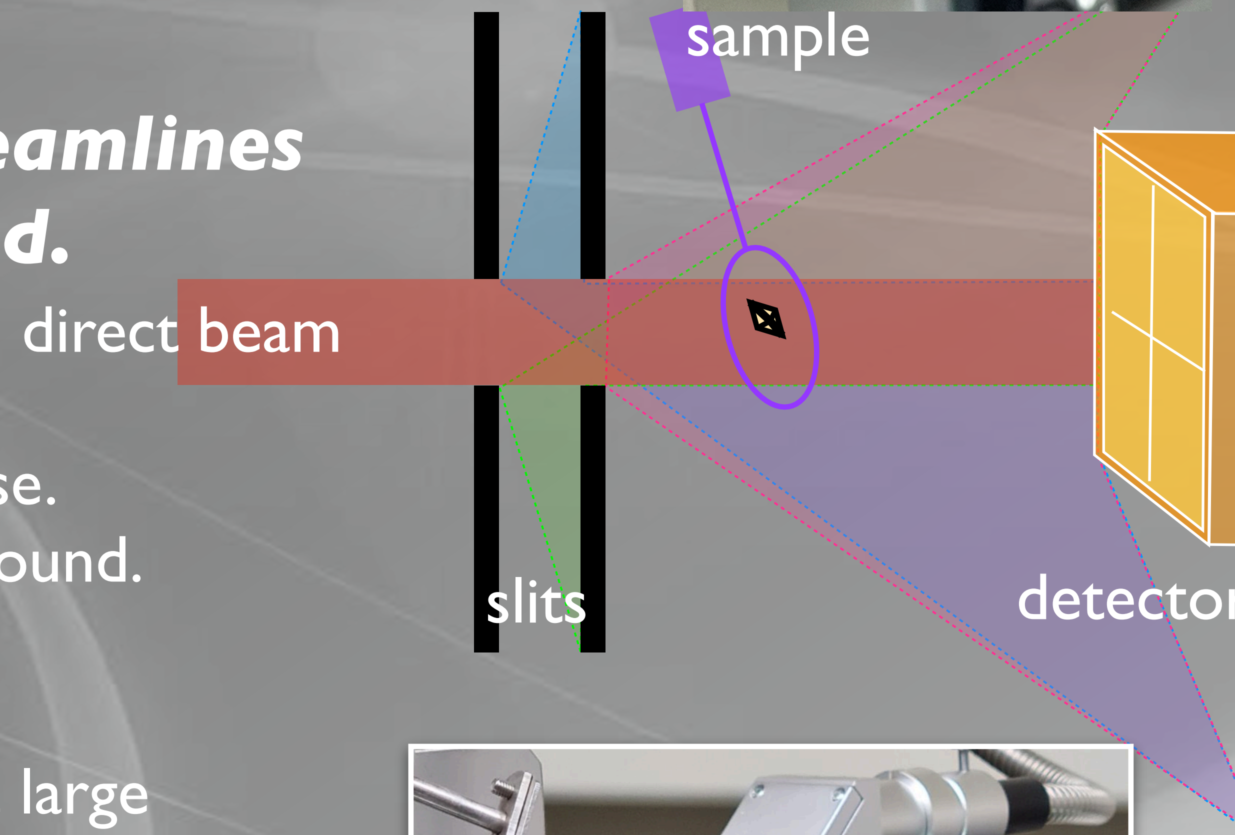
Chaussavoine *et al.* (2022)

Handling at the beamline



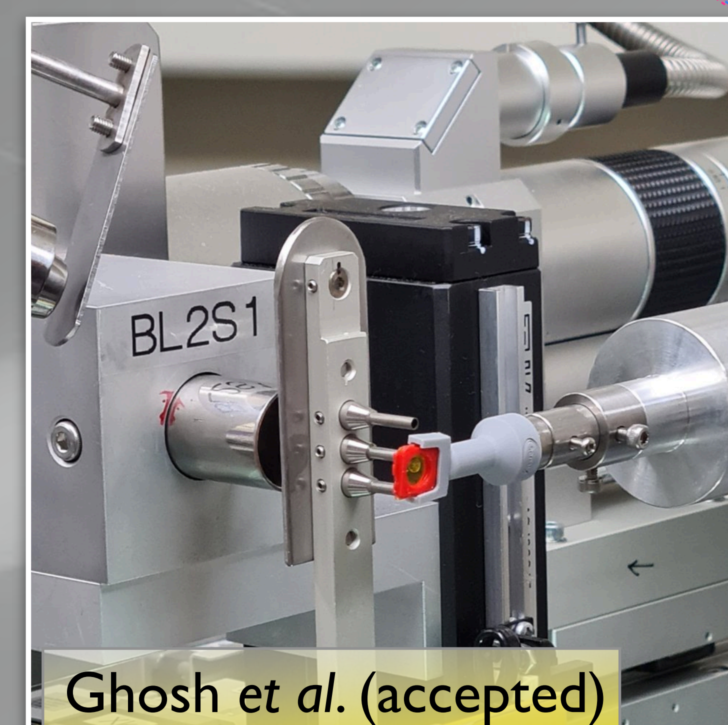
To optimize data quality, we aim at minimizing the background while preserving the capacity to rotate the sample

This is a major challenge at MX beamlines where the sample environment is fixed.



What are the possible causes of noise?

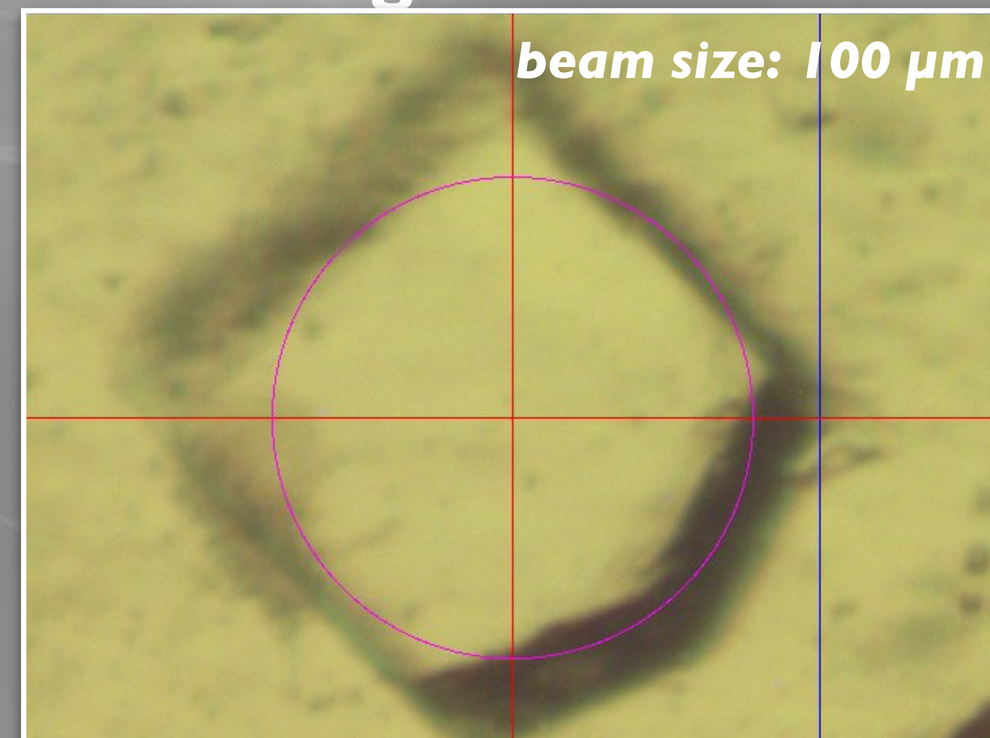
Every aperture is a new source of scattering and noise.
A long path through air results in a scattering background.
An X-ray beam larger than the crystal will increase the noise.
A crystal loss in a large quantity of buffer will show a large background.
The latest slits met by the X-ray beam should be brought as close as possible to the sample.
The direct-beam stopper should be brought as close as possible to the sample.
The detector distance should be kept short to avoid air scattering and allow reaching the highest resolution.

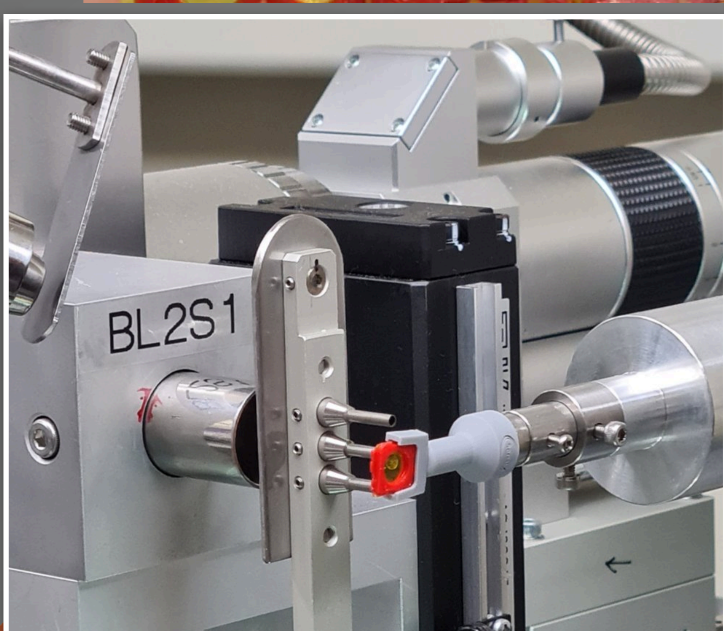


Ghosh *et al.* (accepted)

3D printed classic crystallization devices

Newly developed 3D-printed crystallization sheet
Ready for X-ray diffraction without crystal handling
Great signal/noise ratio!





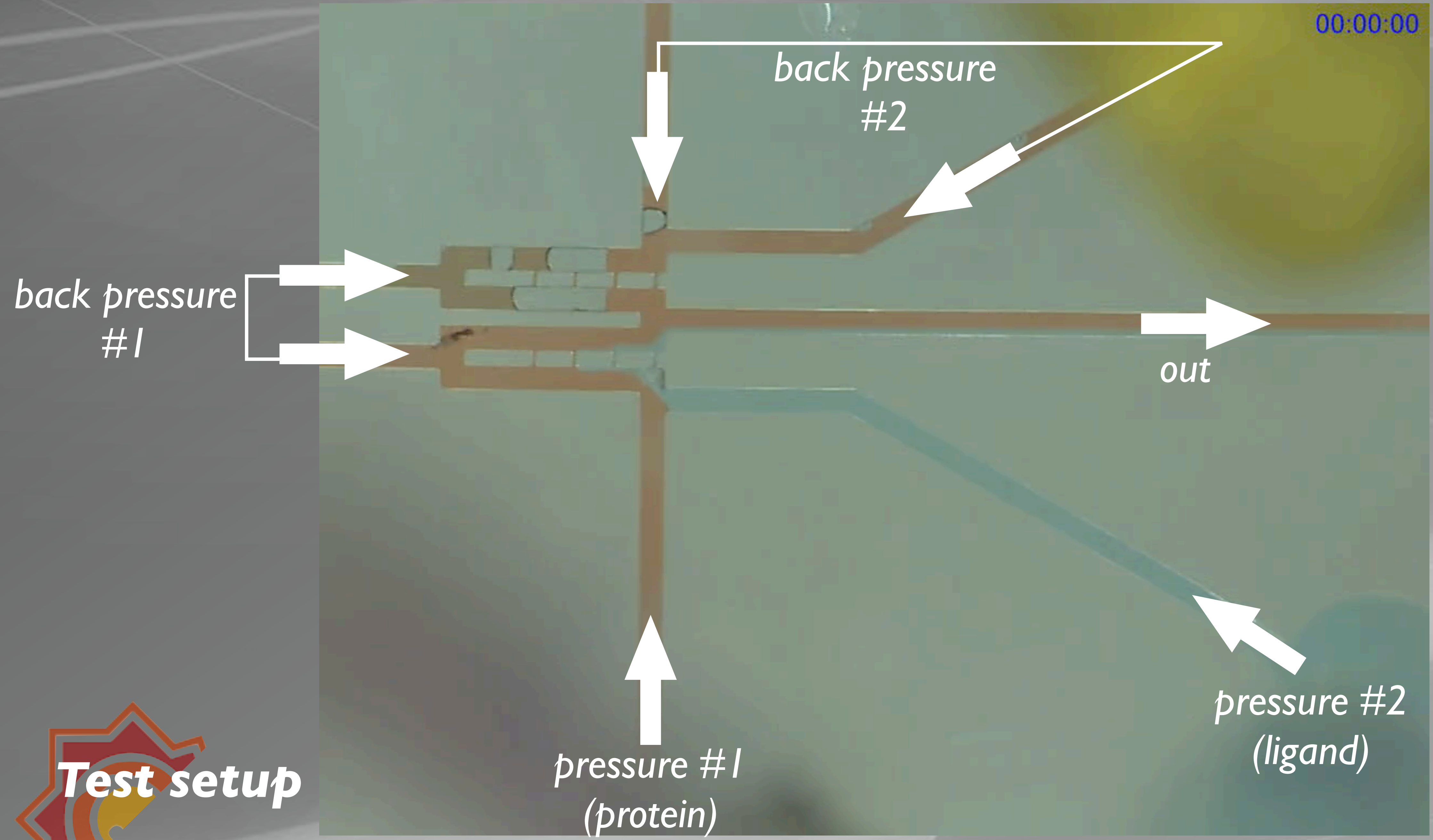
Ghosh *et al.* (accepted)

SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESO

RESOLUTION LIMIT	I/SIGMA	R-meas	CC(1/2)	Anomal Corr	SigAno	Nano
4.15	57.32	2.3%	99.9*	23*	1.051	621
2.94	50.05	2.4%	99.9*	12*	0.904	1293
2.41	34.99	3.8%	99.9*	10*	0.952	1775
2.08	26.08	5.2%	99.7*	10	0.954	2161
1.86	16.61	8.9%	99.3*	7	0.910	2500
1.70	8.99	17.0%	97.7*	5	0.851	2810
1.58	5.41	28.7%	93.6*	4	0.800	3037
1.47	2.92	52.4%	81.6*	0	0.729	3336
1.39	1.59	90.8%	61.4*	1	0.694	3304
total	15.85	5.1%	99.9*	5	0.835	20837

Chip devices - trap and dynamics

What's next? On the to-do list: dynamics
By plugging in a sophisticated pressure controller, we can study the dynamics of enzymes directly with the trapped crystals



Test setup

Acknowledgments

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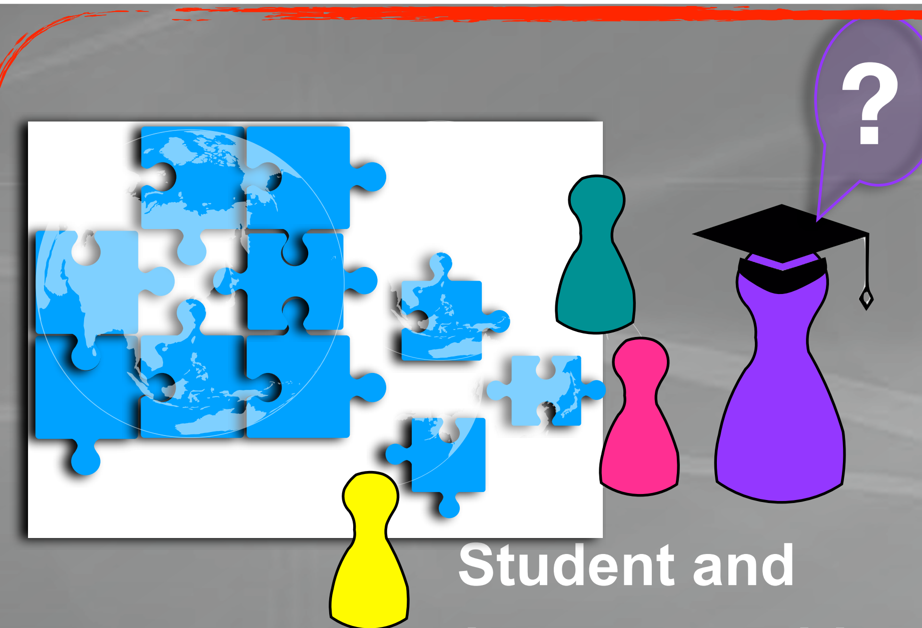
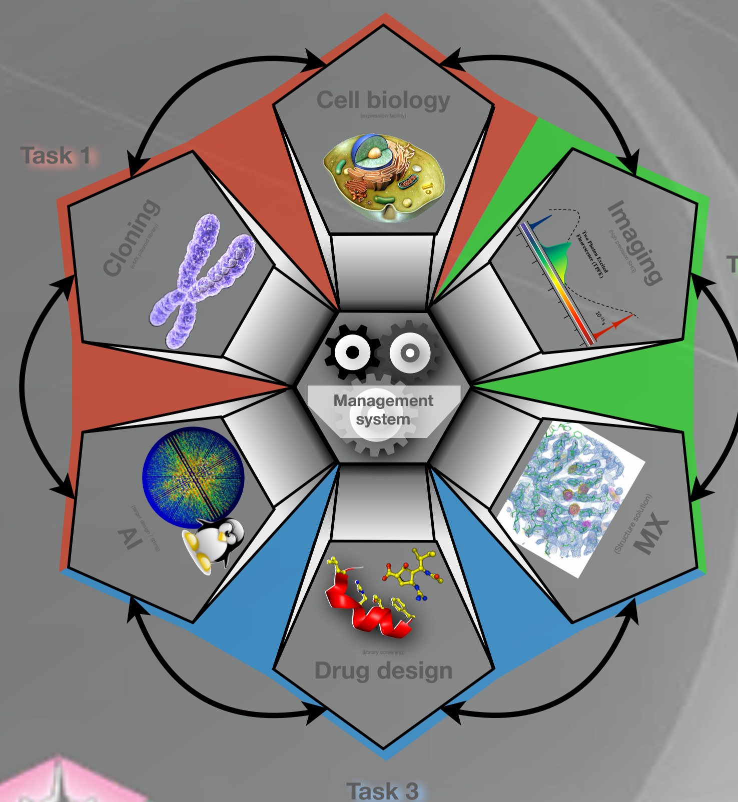
I. CHAUSSAVOINE
T. ISABET
C. BREWEE
A. GARDAIS
B. PINEAU



Student and
post-doctoral positions
available
l.chavas@nusr.nagoya-u.ac.jp

Platform for in vivo crystallization



SerialX

BINDS

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