Microfluidics, crystallization and crystallography

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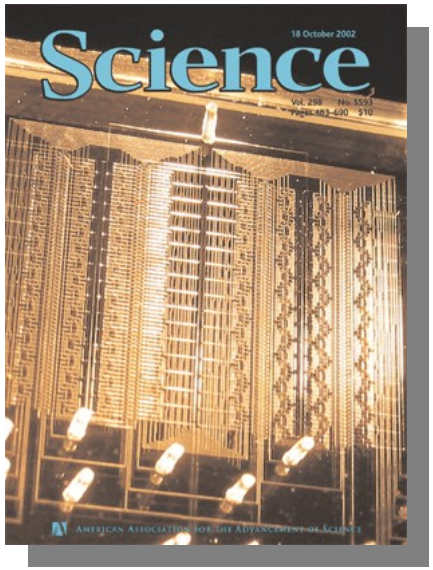
Microfluidic technology

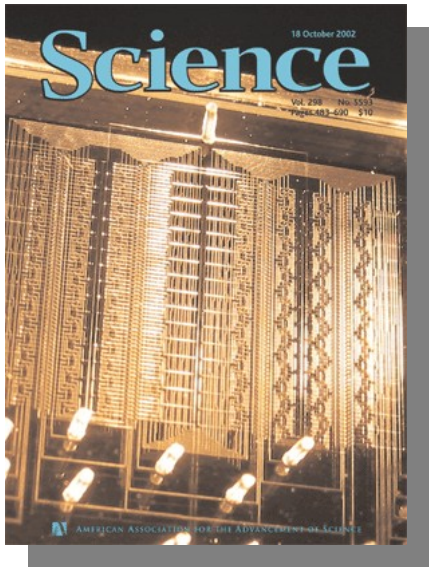


Thorsen et al.
Science (2002)

Integrated fluidic circuit

- ✔ handling small volumes of solution
- ✔ parallel processing of samples
- ✔ small – fast – low cost
- ✔ lab-on-a-chip concept
- ✔ many applications in biomedical diagnostic, chemistry, molecular biology...

Microfluidic technology



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Integrated fluidic circuit

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- ✔ many applications in biomedical diagnostic, chemistry, molecular biology...

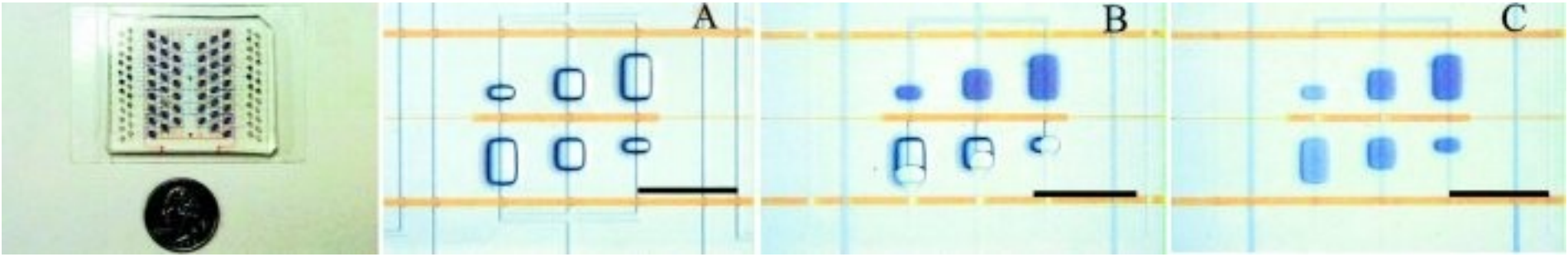
Advantages for crystal-growth

- ✔ limited samples
- ✔ high throughput screening
- ✔ convection-free environment

Free Interface Diffusion application

Hansen et al.
PNAS (2002)

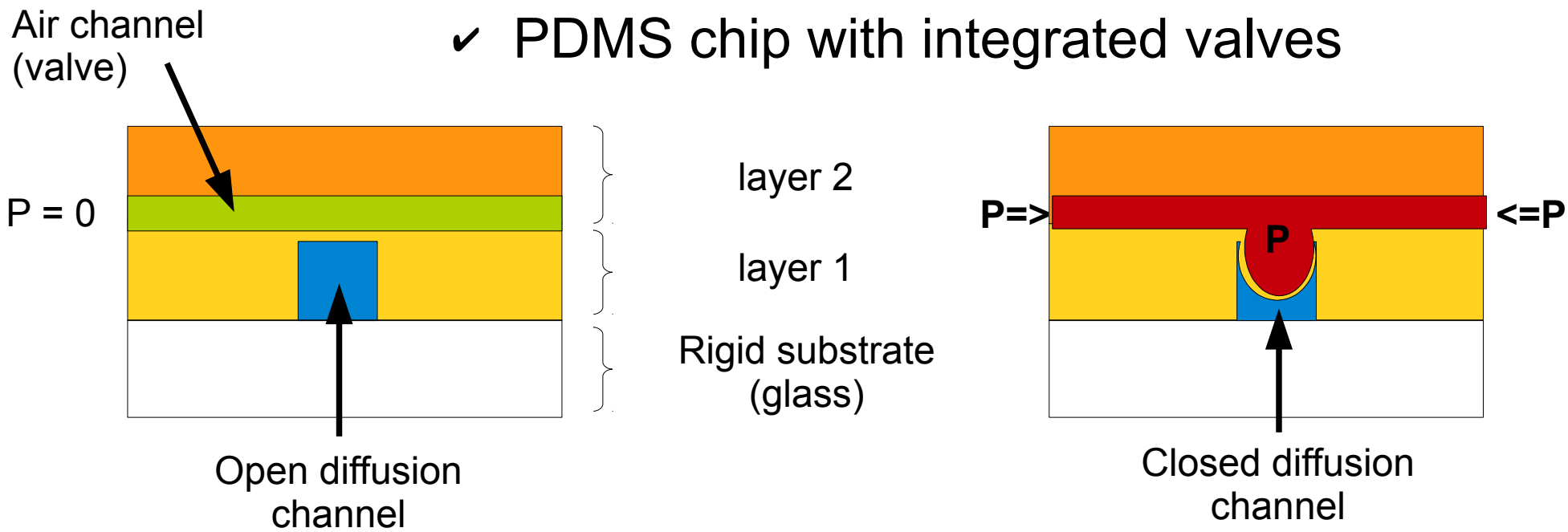
- ✔ multilayer soft lithography
- ✔ PDMS chip with integrated valves
- ✔ nanochambers of crystallization (5-20 nl) connected by a diffusion channel
- ✔ high throughput screening
- ✔ Free Interface Diffusion (FID)

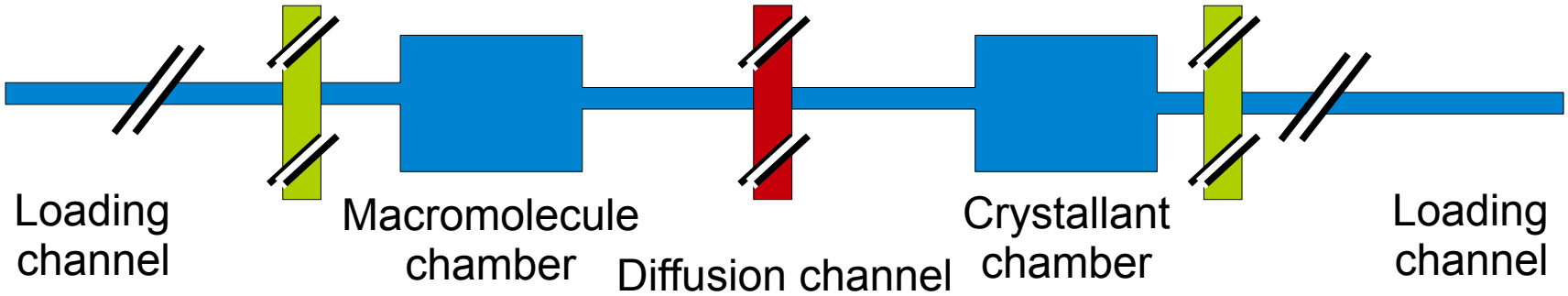


Quake group - Caltech

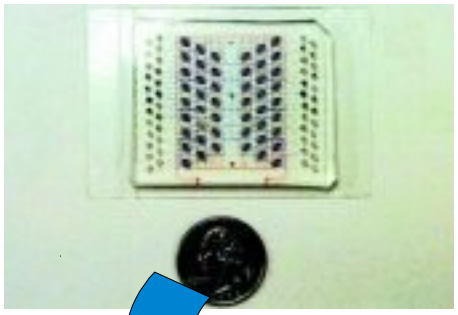
Free Interface Diffusion application

- ✔ multilayer soft lithography
- ✔ PDMS chip with integrated valves

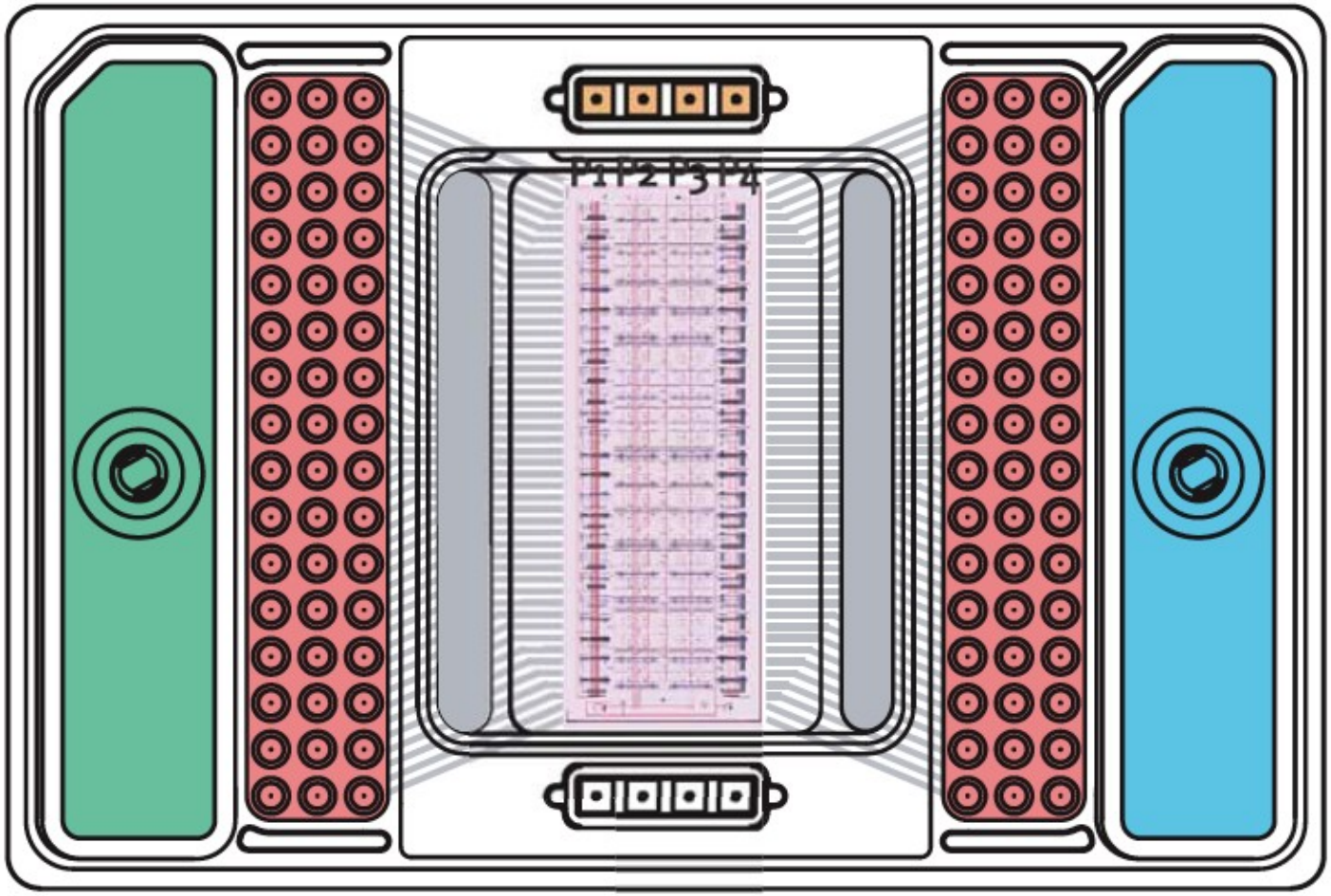




Topaz 1.46, 1.96, 4.96 by Fluidigm



SBS format



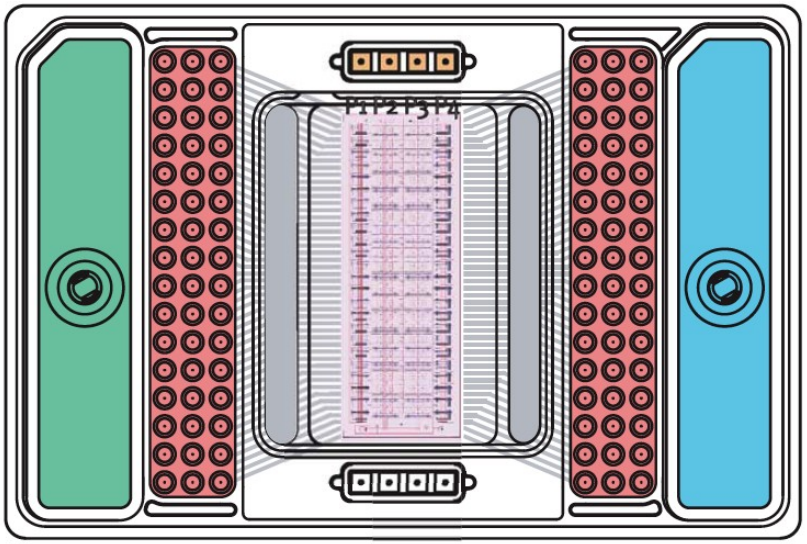
Protein Inlets
Reagent Inlets
Hydration Reservoirs
Integrated Fluidic Circuit (IFC)
Interface Accumulator
Containment Accumulator

Free Interface Diffusion application

Great ...but

- ✔ Extra equipment is required
- ✔ PDMS is gaz permeable...
- ✔ Scale-up can be an issue
- ✔ FID chips are still expensive

200 € / chip

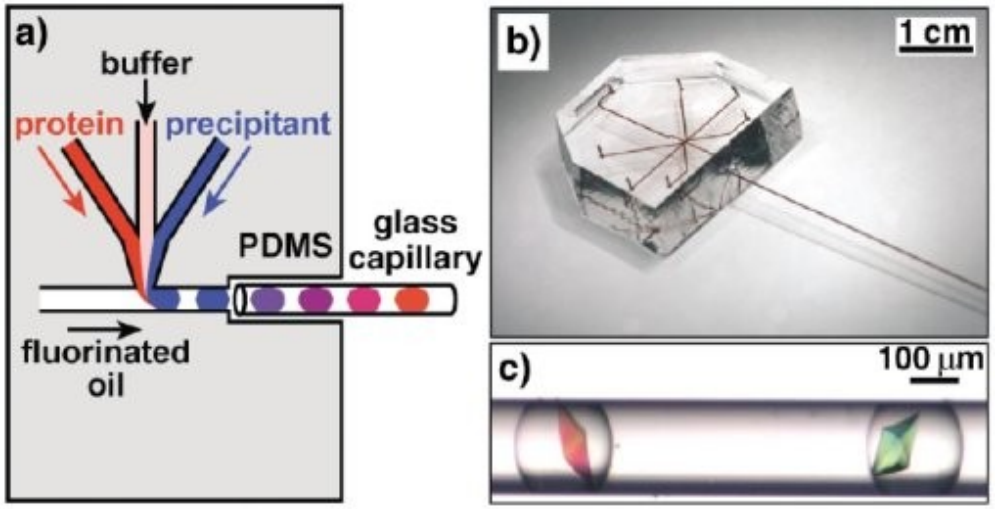


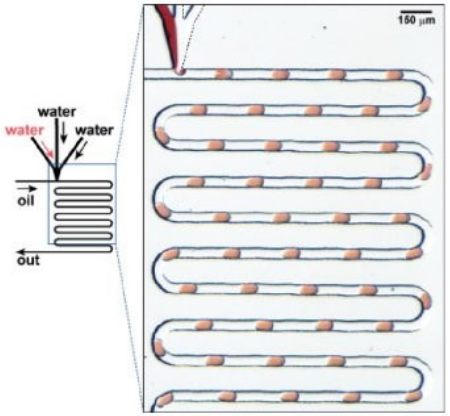
Nanobatch chip

Zheng et al.
Ang. Chem. (2004)

- ✔ PDMS chip with mixing channels
- ✔ production of nanodroplets (10-20 nl)
- ✔ high throughput screening
- ✔ X-ray analysis in capillary tubes
- ✔ Plugs with lipidic cubic phase

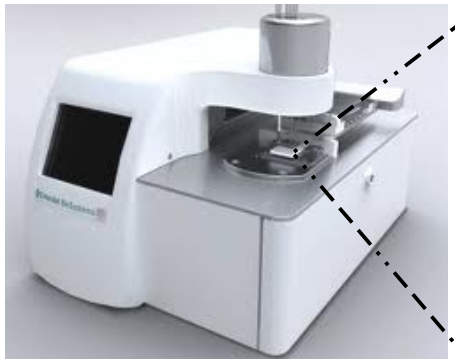
Li et al. Microfluid Nanofluid (2010)

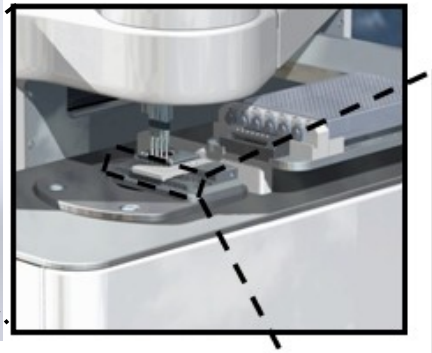


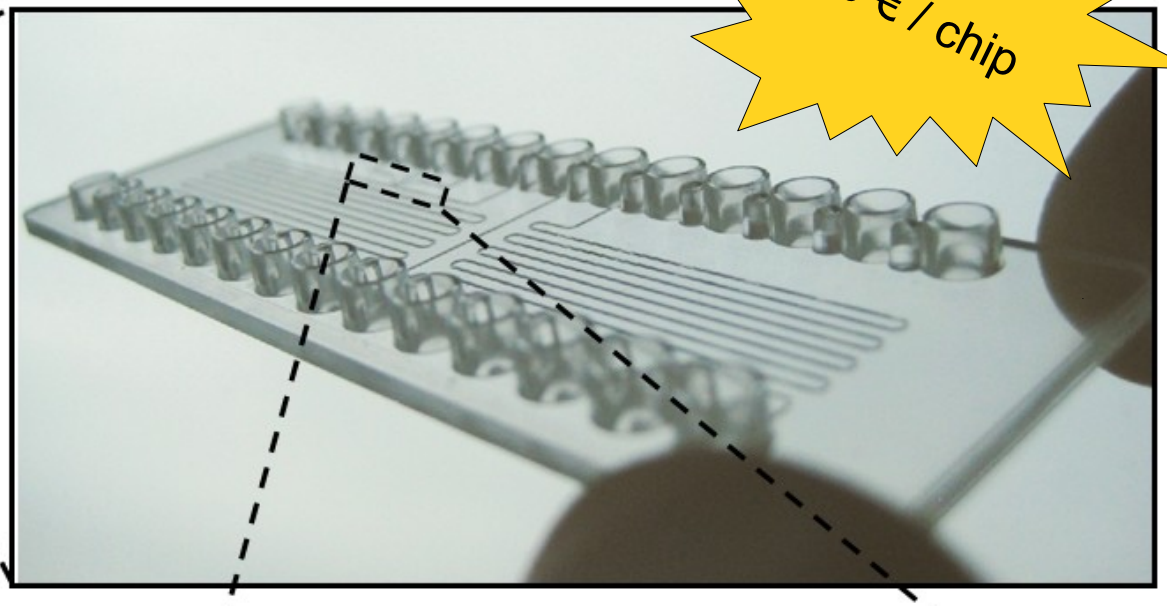


Ismagilov group
University of Chicago

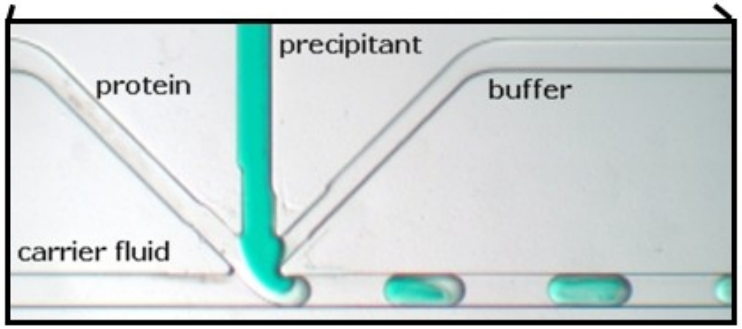
MPCS Plug Maker by Emerald BioScience







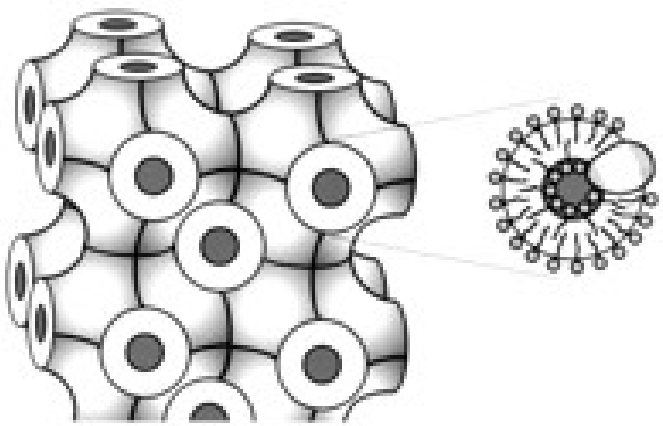
50 € / chip



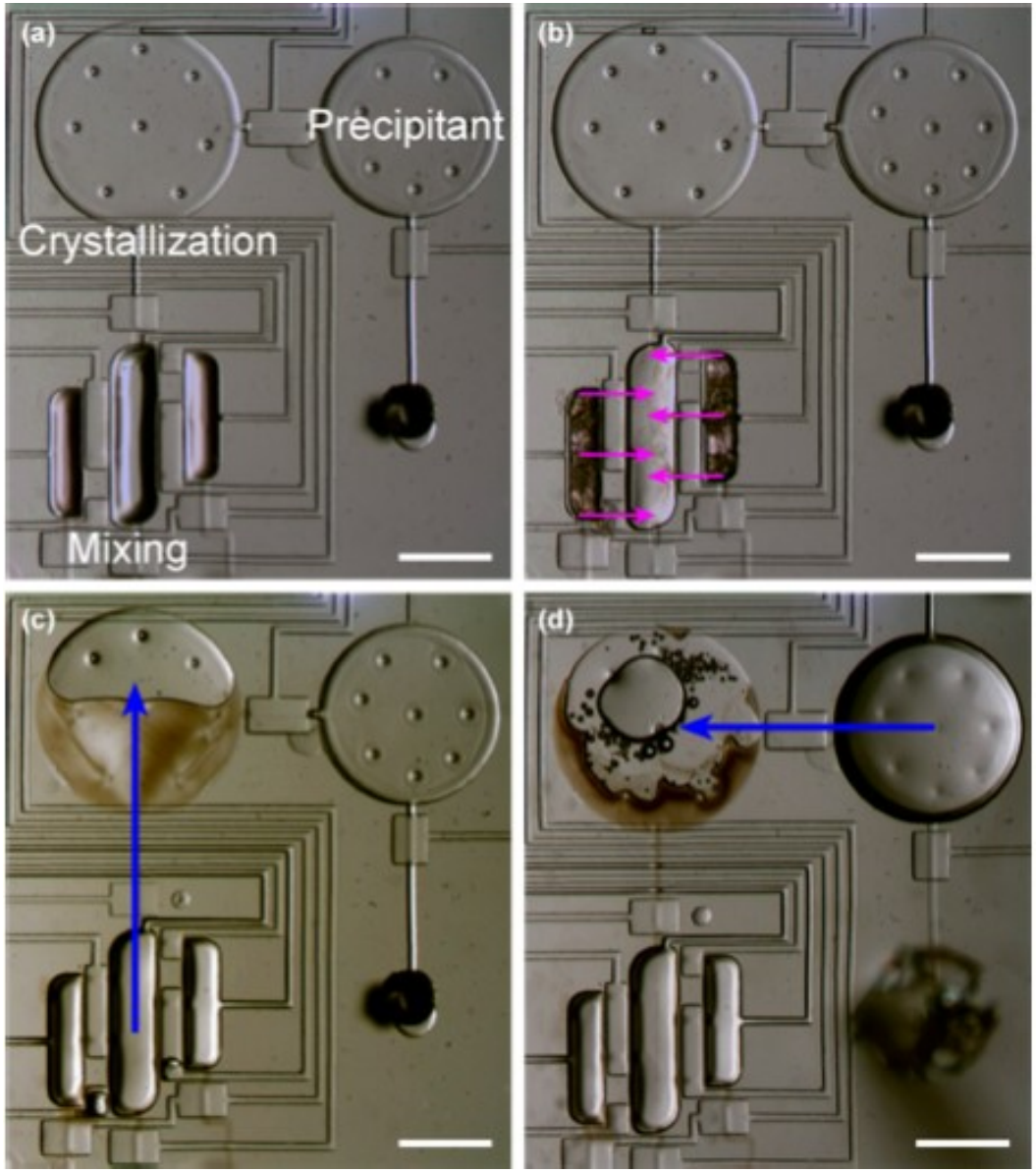
- ✔ Microcapillary Protein Crystallization System™
- ✔ 10 nl plugs
- ✔ 800 experiments / 4 µl sample
- ✔ Lipidic cubic phase friendly?

Christensen et al. Acta Cryst. F (2011)

Membrane protein crystallization



- ✔ Lipidic cubic phases
  = highly viscous
- ✔ Dedicated LCP mixer
- ✔ FID crystallization



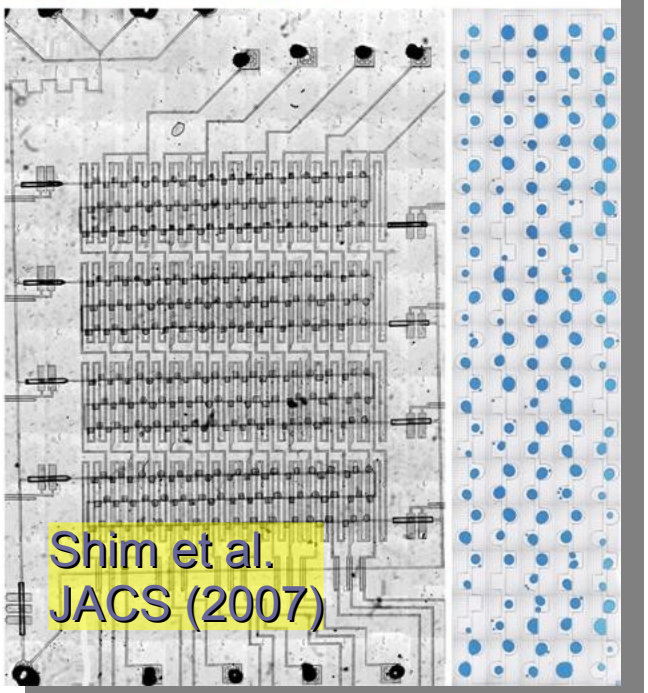
Perry et al. CGD (2009)
Kenis group
University of Illinois

In brief

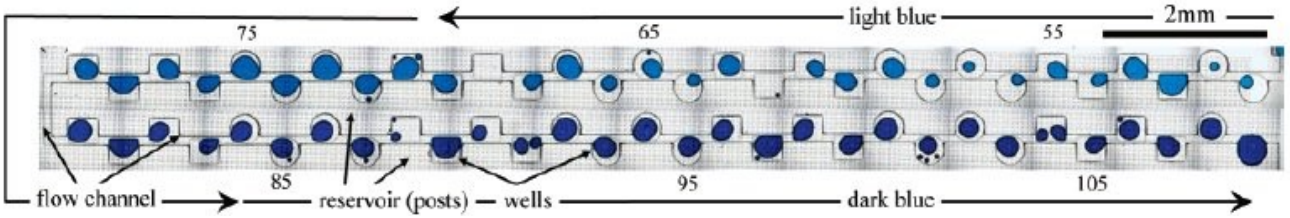
- ✔ High level of parallelization
- ✔ Ideal for HTP screening
- ✔ Nanovolumes of sample
- ✔ Two major issues:
  - ✔ scale-up?
  - ✔ cost?

Microfluidic tools for crystallogenesis

Phase chip

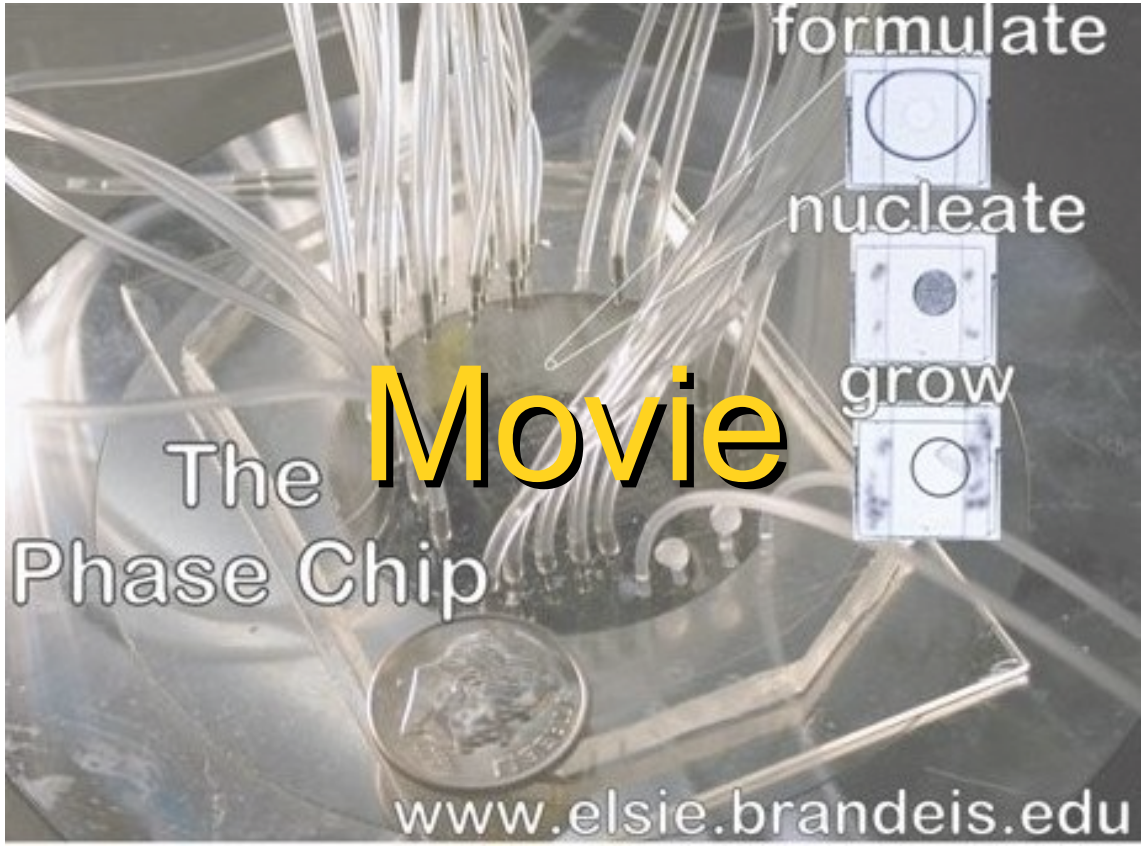


- ✔ multilayer PDMS microfluidic for batch experiments
- ✔ exploits the permeation of water through PDMS in order to vary the solutes' concentration
- ✔ determination of phase diagrams
- ✔ manipulation of the kinetics of nucleation and growth
- ✔ temperature control Selimovic et al LOC (2010)

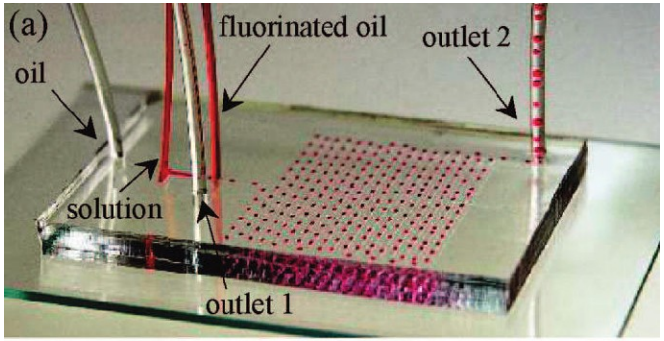


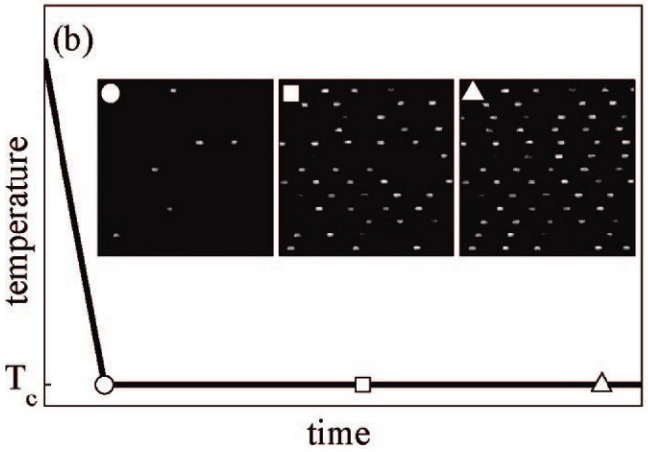
Fraden group - Brandeis University

Phase chip


formulate
nucleate
grow
Movie
The
Phase Chip
www.elsie.brandeis.edu

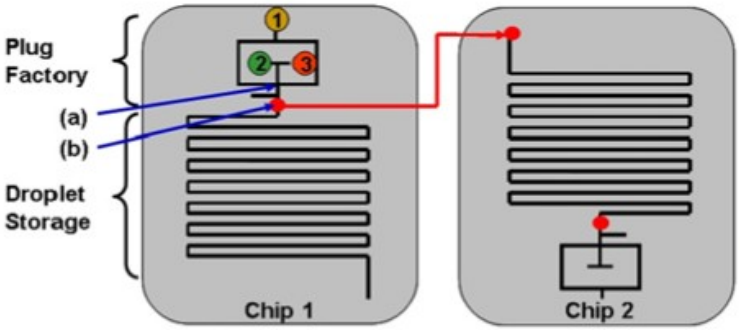
Studying nucleation kinetics, polymorphs...

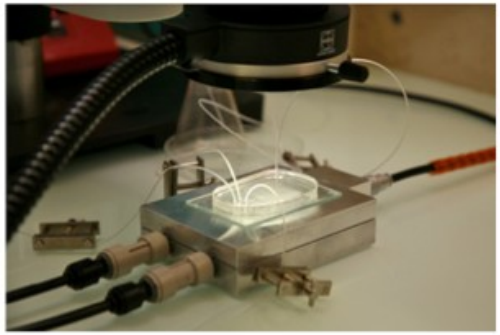




Laval et al. Langmuir (2009)

Salmon group – LOF Bordeaux





Ildefonso et al. JCG (2010)

Veesler group – CiNaM Marseille

In brief

- ✔ Nanodroplets = thousands of individual experiments
- ✔ Statistical quantification
- ✔ Spatial & temporal control
  - ✔ of nucleation
  - ✔ of crystal growth
- ✔ Monitoring of the phase diagram

ChipX project:
X-tallization and
on chip X-ray analysis

The CD crystallization chip

The project

- ✔ crystallization chip using counter-diffusion (CD)
- ✔ transparent material (observation, X-ray diffraction)
- ✔ easy to use => no valve, no pump
- ✔ simple design => *low cost chip*?!?
- ✔ lab-on-a-chip concept: from solution to diffraction

Rosaria Ferrigno

INL
Univ. Claude Bernard
Lyon I

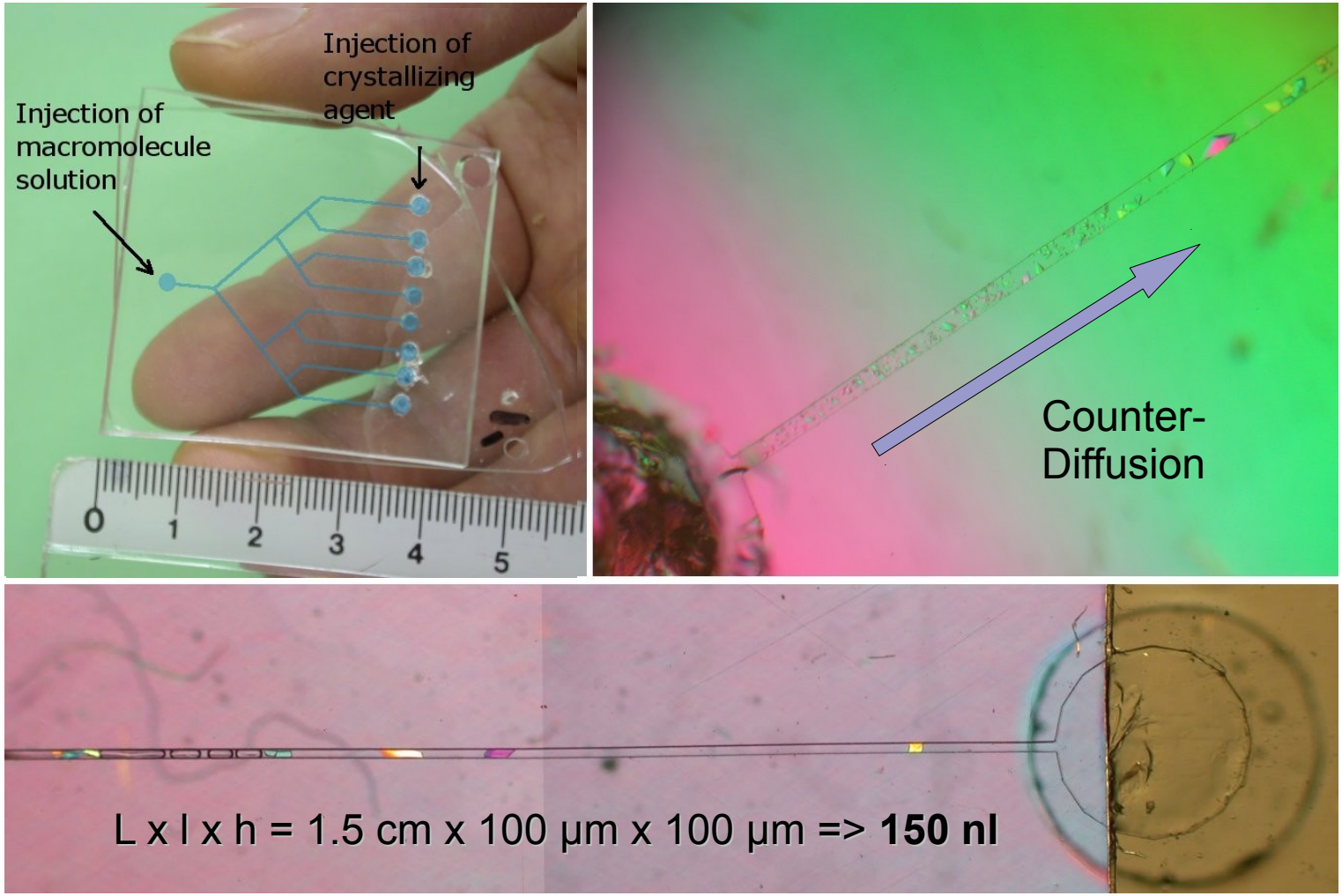
Chantal Khan-Malek
Bernard Gauthier-Manuel
Gaël Thuillier

FEMTO-ST – UMR 6174
Département LPMO
Besançon

Lilian Jacquamet
Jérémy Ohanna
Jean-Luc Ferrer

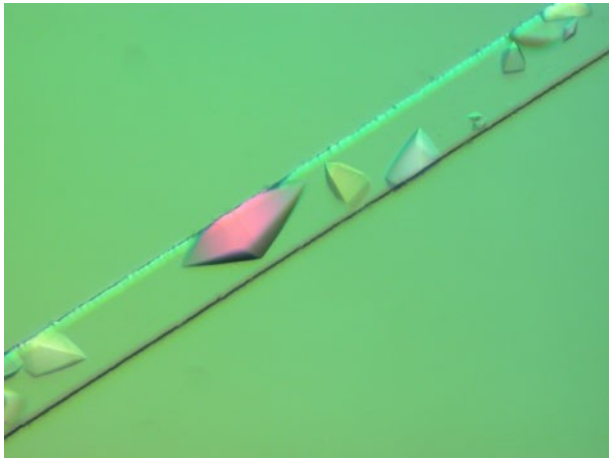
CRG – FIP
ESRF – IBS – CNRS
Grenoble

Crystallization assays

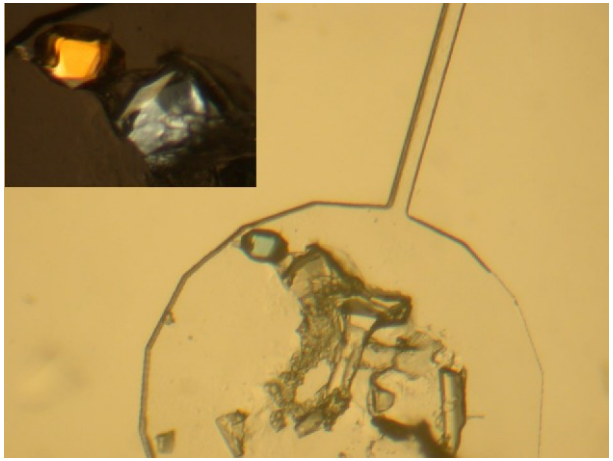


Crystallization assays

thaumatin (22 kDa)

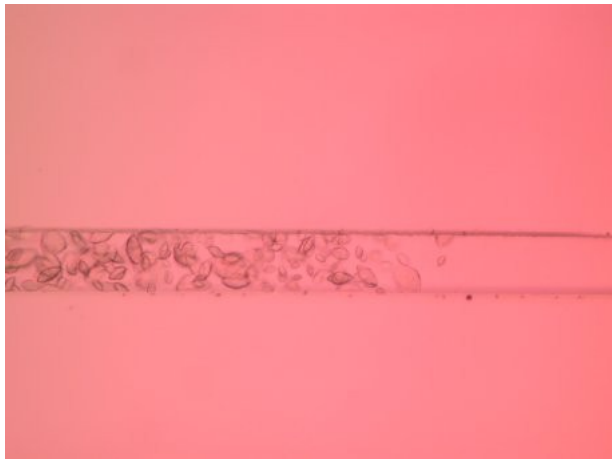


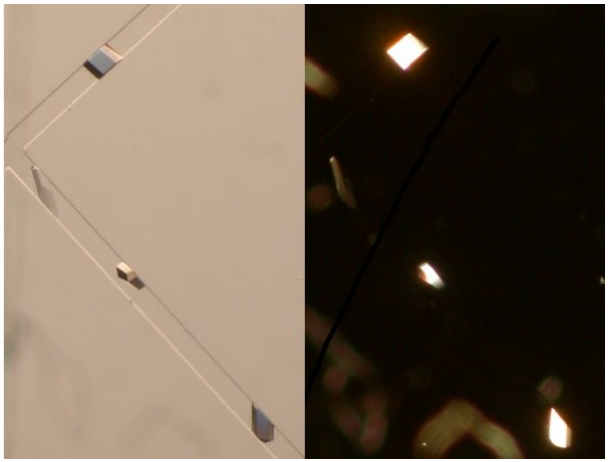
hen egg white lysozyme (HEWL – 14 kDa)



β clamp (75 kDa)

turnip yellow mosaic virus (TYMV – 5.5 10^6 kDa)

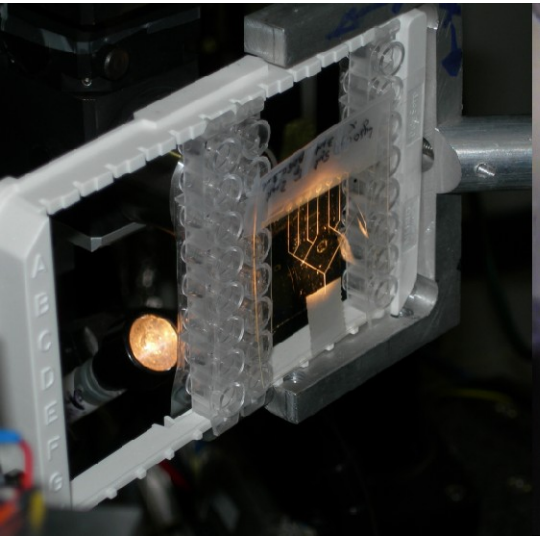




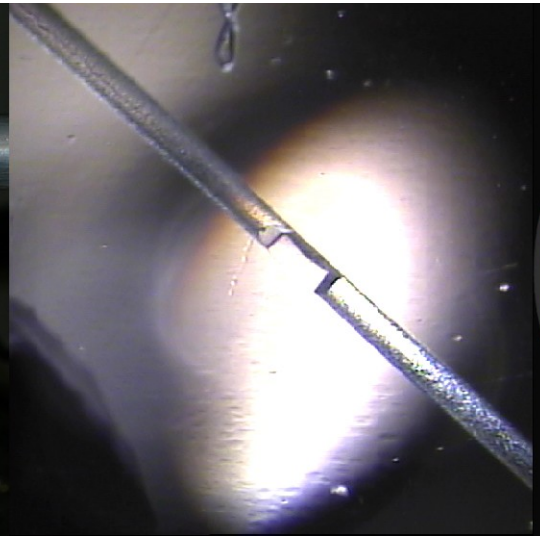
turkey egg white lysozyme (TEWL – 14 kDa)

Towards 'on chip' diffraction analysis

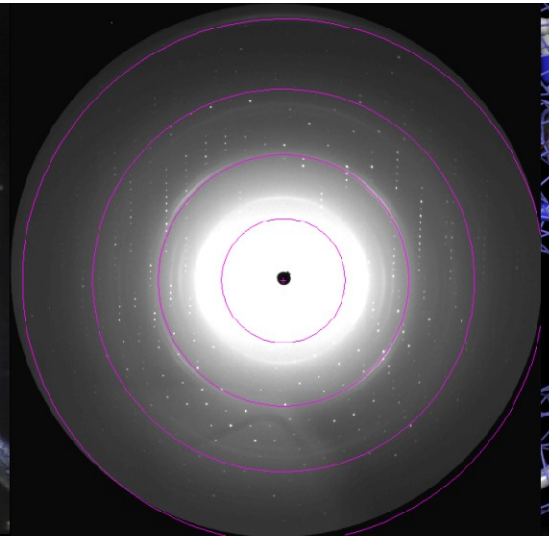
Analysis of hen egg white lysozyme crystals



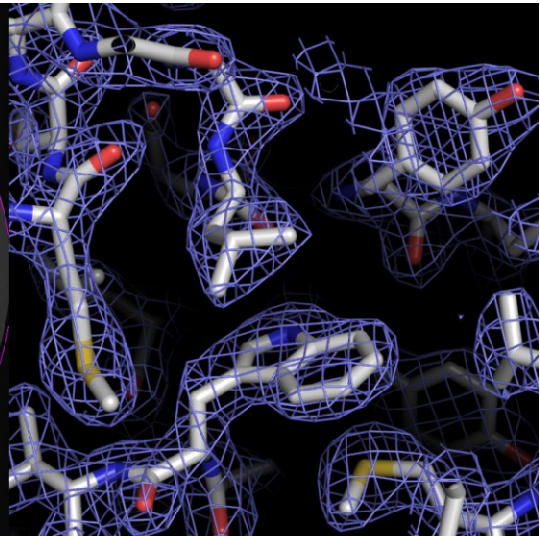
Chip held by robotic arm



Crystal in µfluidic channel

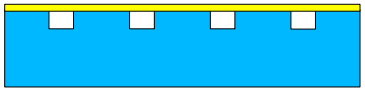


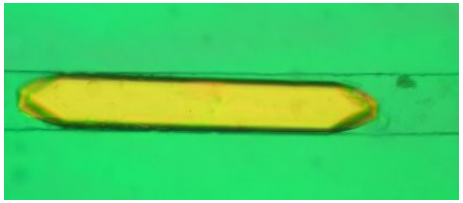
On chip diffraction

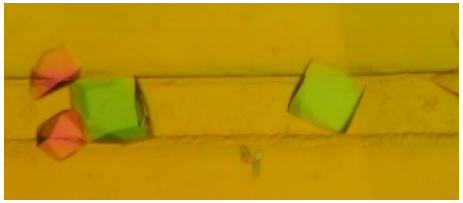


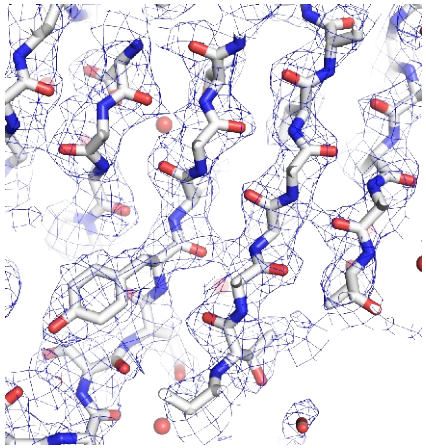
Electron density map (2.15Å)

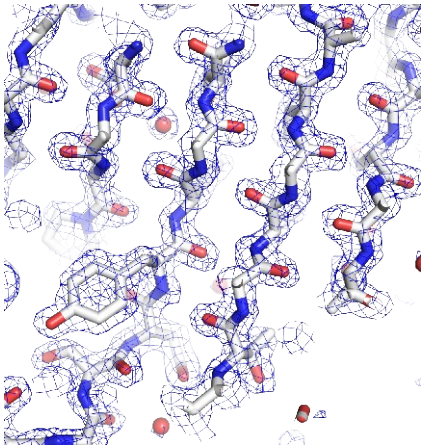
When matter matters...

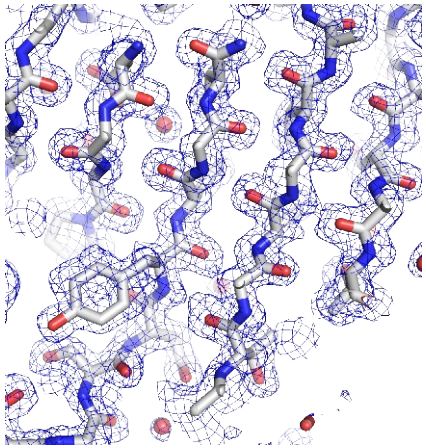












Data collection *(~25°C)*

Chip	PDMS	PMMA	COC
Nb of images	20	20 + 20	100
Distance	300 mm	300 mm	250 mm
Oscillation	2°, 180 sec	1°, 30 sec	0.5°, 20 sec
Resolution range	2.8 – 20 Å	1.85 – 20 Å	1.65 – 20 Å
Mosaicity	0.09°	0.07°	0.07°
Completeness	84.2 % (87.3 %)	95.9% (86.5%)	91.4% (88.5 %)
Rsym	12.9 % (22.6 %)	5.7% (15.1%)	10.2% (47.8 %)
I/σ	9.0 (5.3)	13.4 (4.9)	8.9 (2.2)

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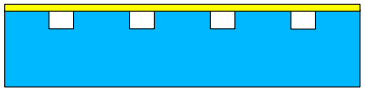
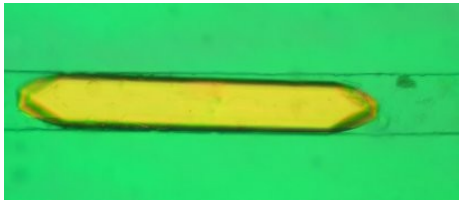
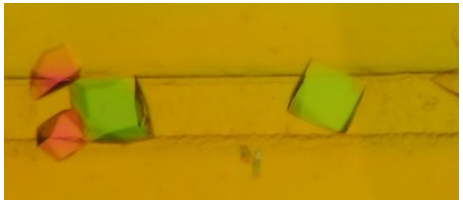


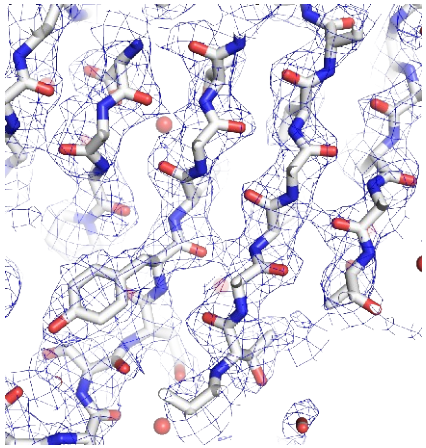
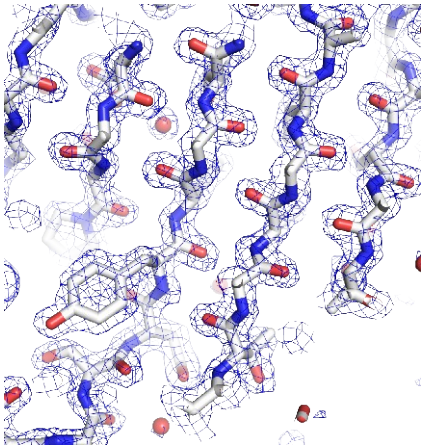
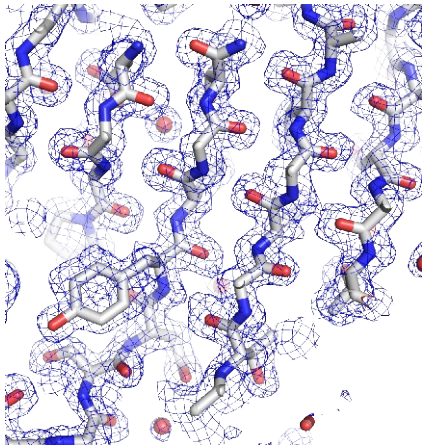

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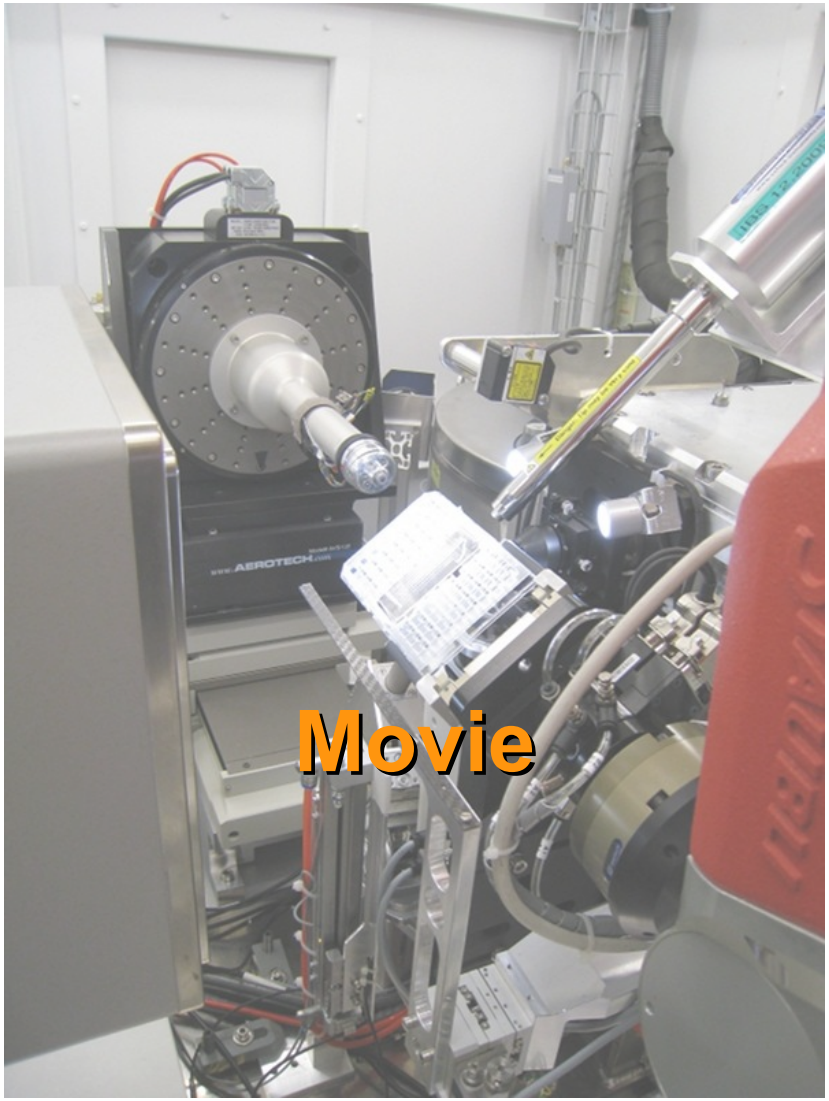
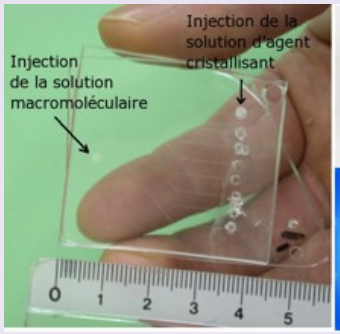
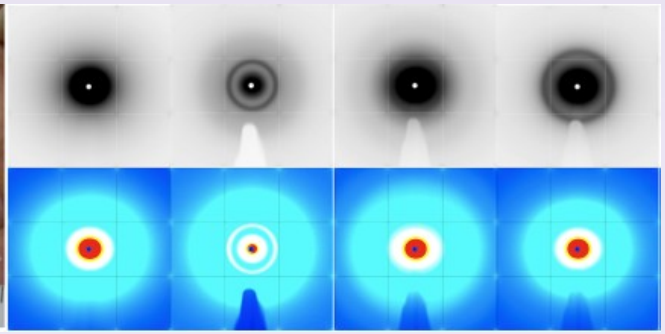
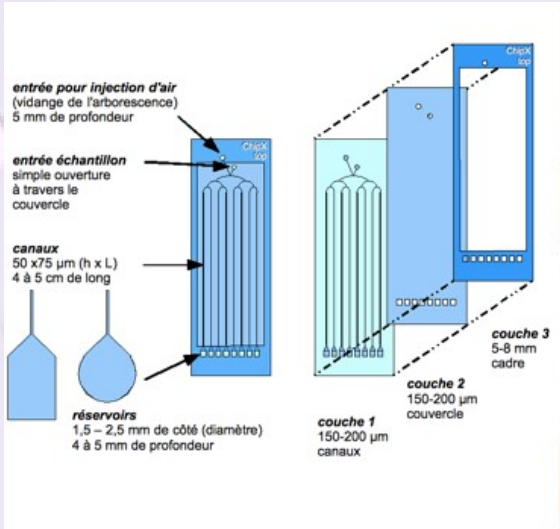
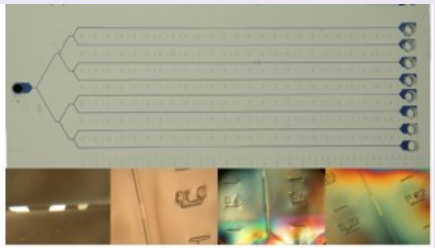


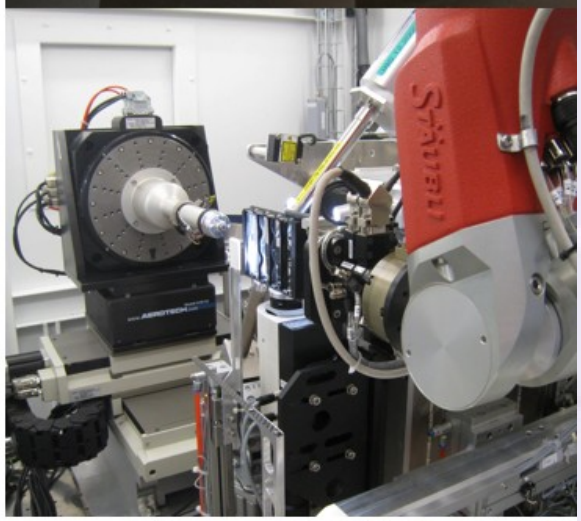
ChipX in brief

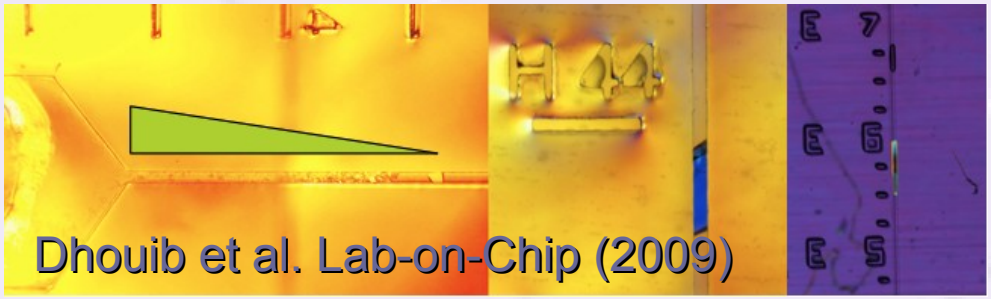




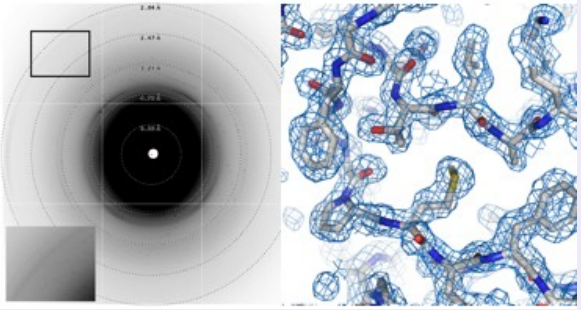








Dhouib et al. Lab-on-Chip (2009)

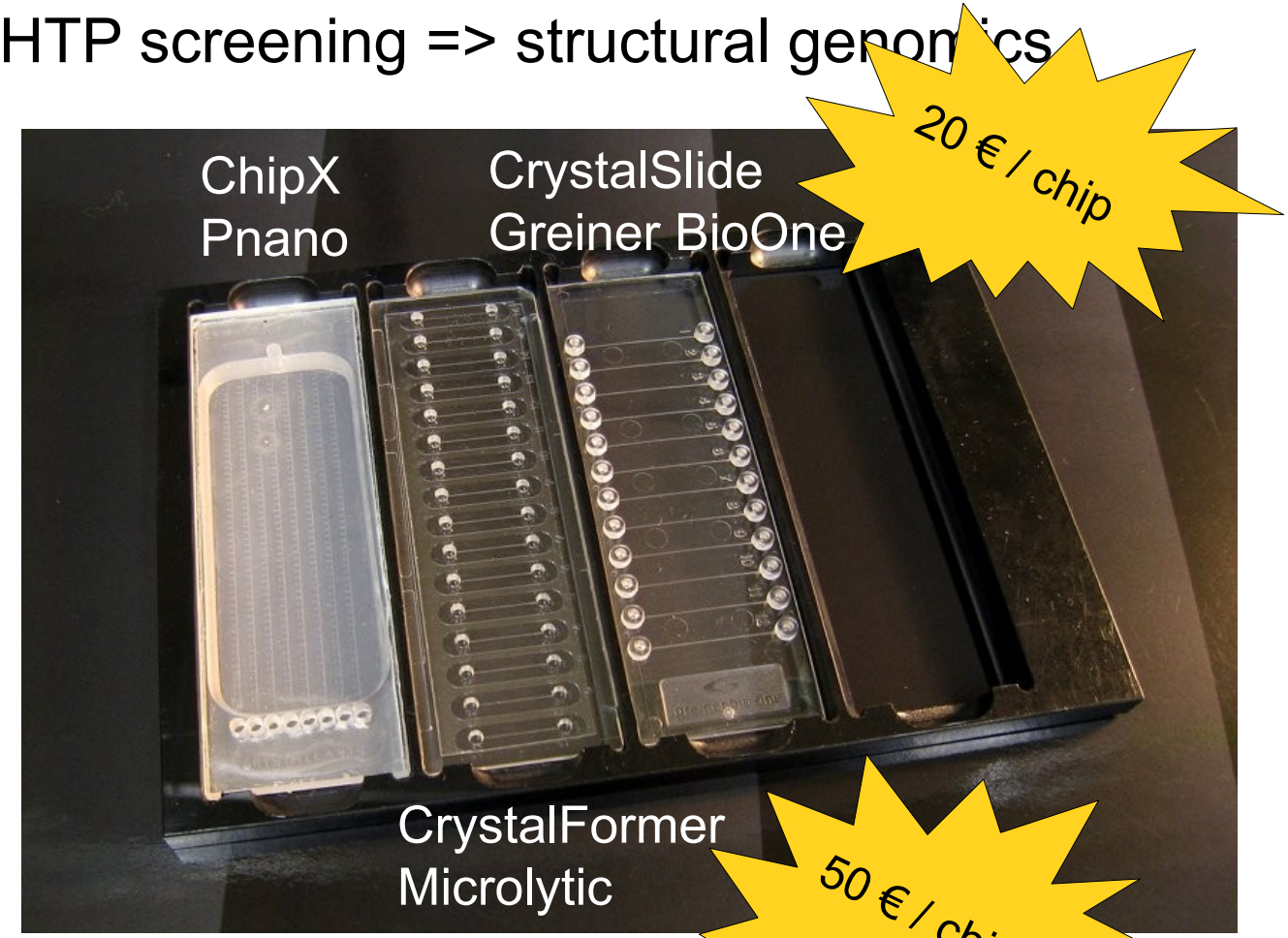


ChipX in brief

- ✔ Efficient miniaturization of counter-diffusion
- ✔ No scale-up issue
- ✔ Excellent optical properties
- ✔ X-ray analysis on chip
  = intrinsic crystal quality
- ✔ Low cost chip = no longer a dream!!!
- ✔ LOC concept
  = from the sample to its 3D structure

Perspectives for 'on chip' crystallography

- ✔ Automation of chip setup and of crystal analysis
- ✔ HTP screening => structural genomics



Ng et al. Acta Cryst D (2008)
Stojanoff et al. Acta Cryst. F (2011)

Dh[illegible] on-Chip (2009)

Microfluidics and crystallization...

- ✔ Microfluidic chips for
  batch, dialysis, CD, FID,
  vapor diffusion...
- ✔ HTP screening and crystallogenesis applications
- ✔ The field is moving fast, prices are decreasing...
- ✔ Possible coupling with diagnostic tools:
  video, DLS, interferometry, UV imaging...
- ✔ In a near future:
  from purification to X-ray diffraction?!?


Thank you !