Crystallography on a chip:
In the quest to improve sampling efficiencies for FEL sources

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and
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Revised Title(s)

One (good?) idea and way more challenges…Lessons learned…we now know what to do next…

VERY MUCH WORK IN PROGRESS

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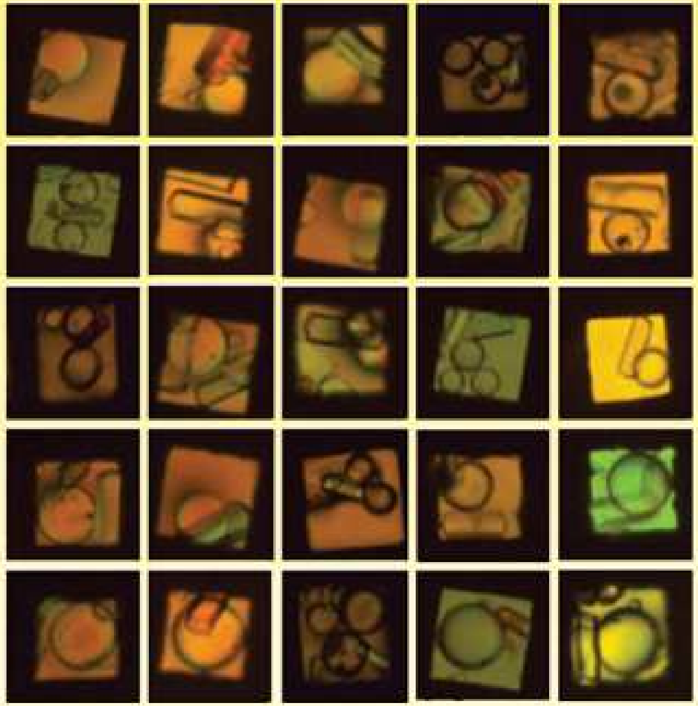
Acta Crystallographica Section D

Biological Crystallography

Editors: E. N. Baker and Z. Dauter



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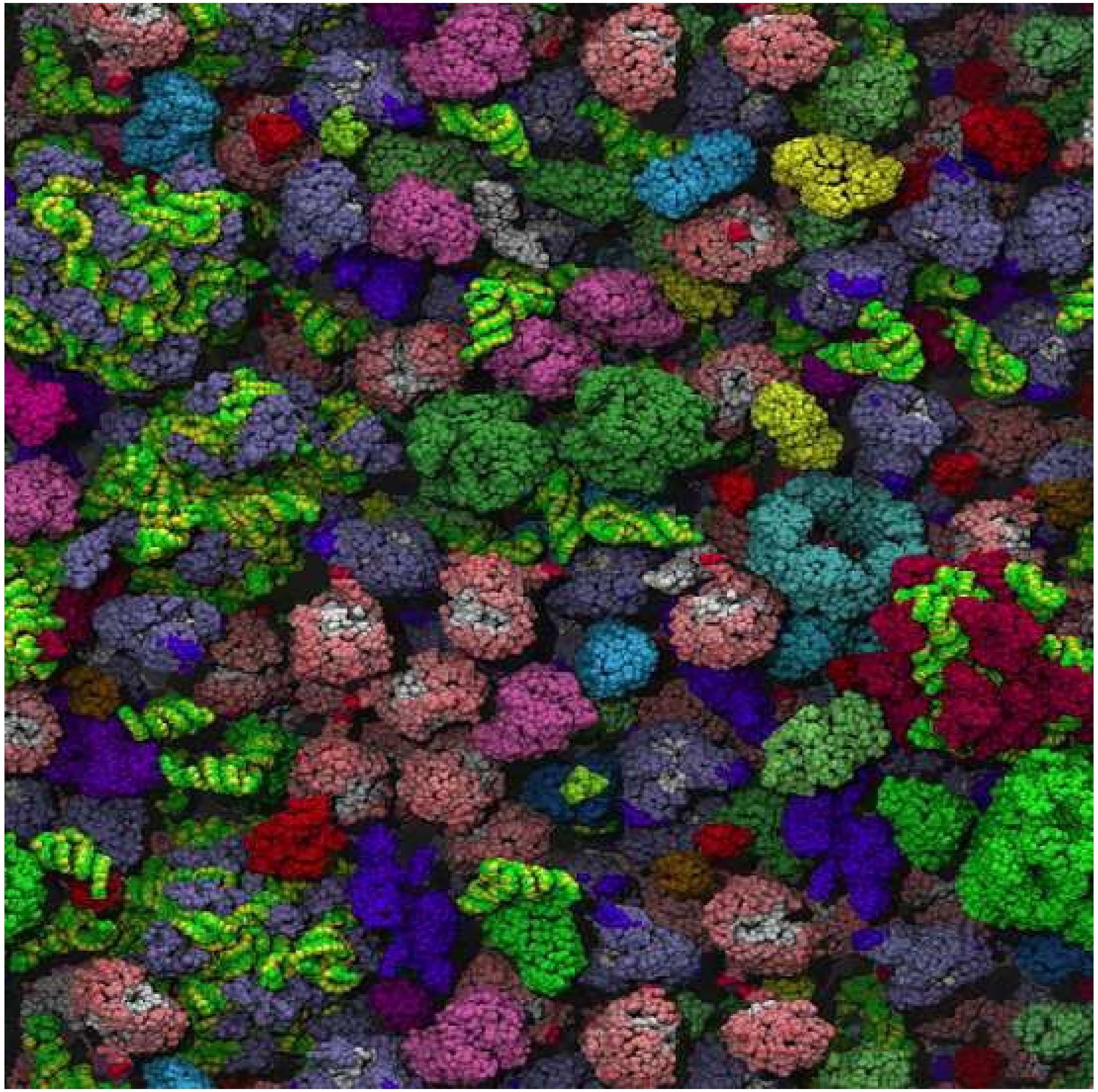
LCLS:XPP Beamtime in Dec 2010
L234: Moffat, Graber, Van Thor
L253: Miller, Neutze, Schlichting
L260: Anfinrud
hh-MbCO
Ime Schlicting
10 μm
X-ray 40 μm
laser
time resolution:
<20 fs

Dynamics in biological systems



Protein dynamics
(folding/unfolding)

Binding dynamics
(interaction proteomics)

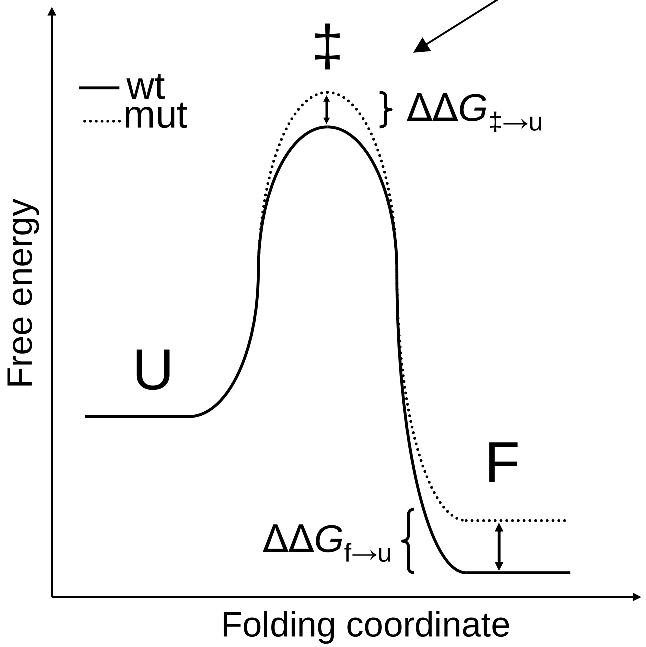
Proteome dynamics
(changes in disease state)
(changes with age!)

PLoS Computational Biology, Artist's rendition

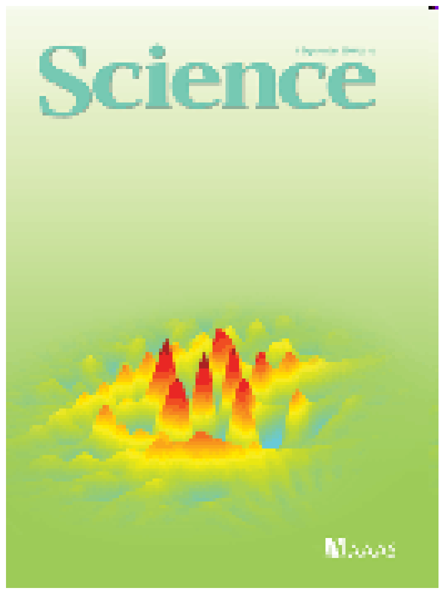
Time scales in protein and proteome dynamics

Protein dynamics generally ms
(folding/unfolding)

F⇆U



Barrier crossing where
ultra fast motions
ps-fs might be important
(quantum effects?)

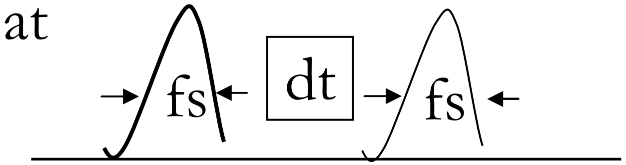


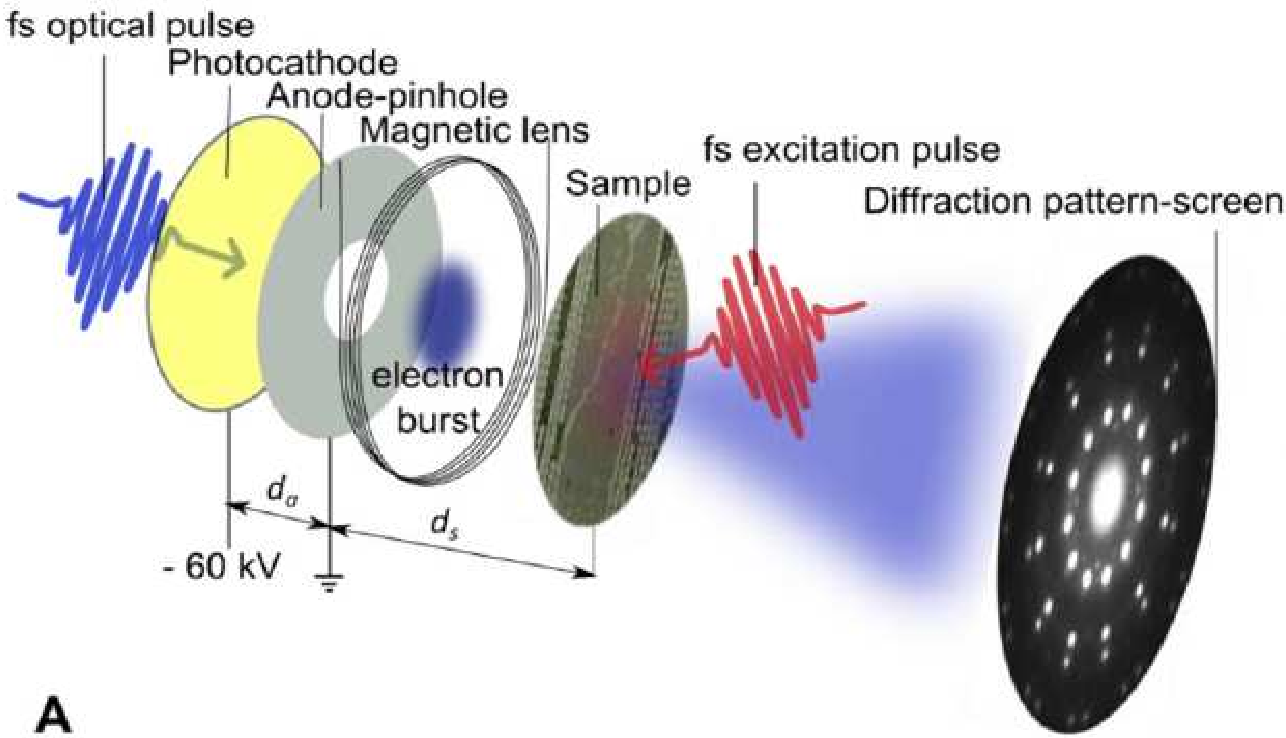
Coherent control of
Rhodopsin isomerization

Proteome dynamics
(hours to months)

Ultrafast dynamics

My introduction to ultrafast science
Complete sampling of structure state at time point





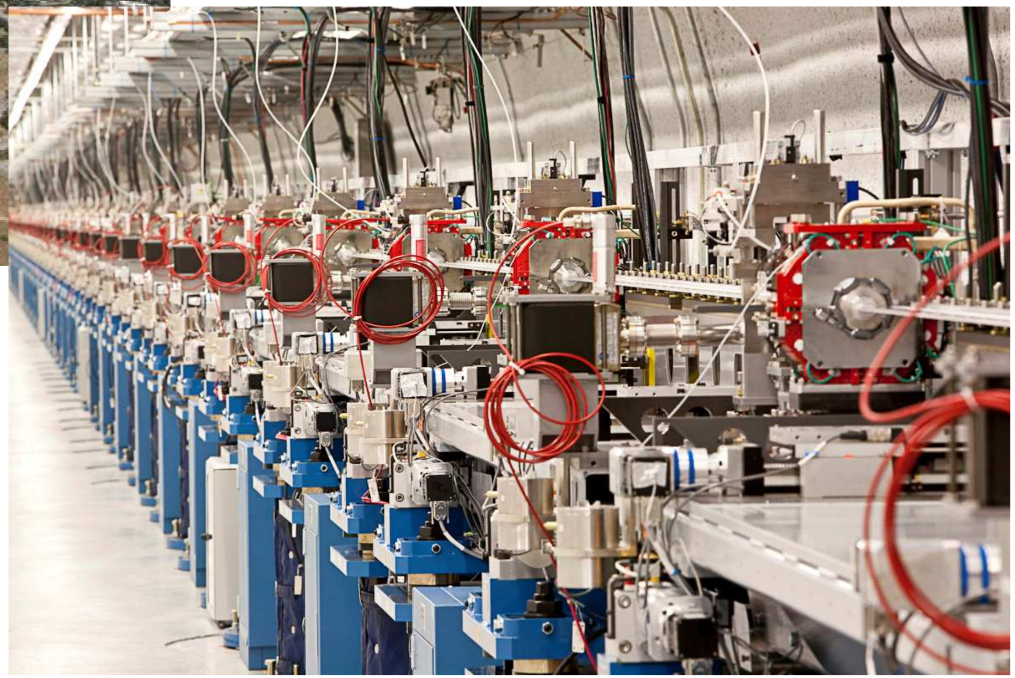
Can do pump probe for time resolved studies

Sciaini and Miller. (2011) *Rep. Peog. Phys*

Free Electron Lasers (FELs) produce fs pulses of coherent x-rays

LCLS-SLAC Stanford

Undulator hall

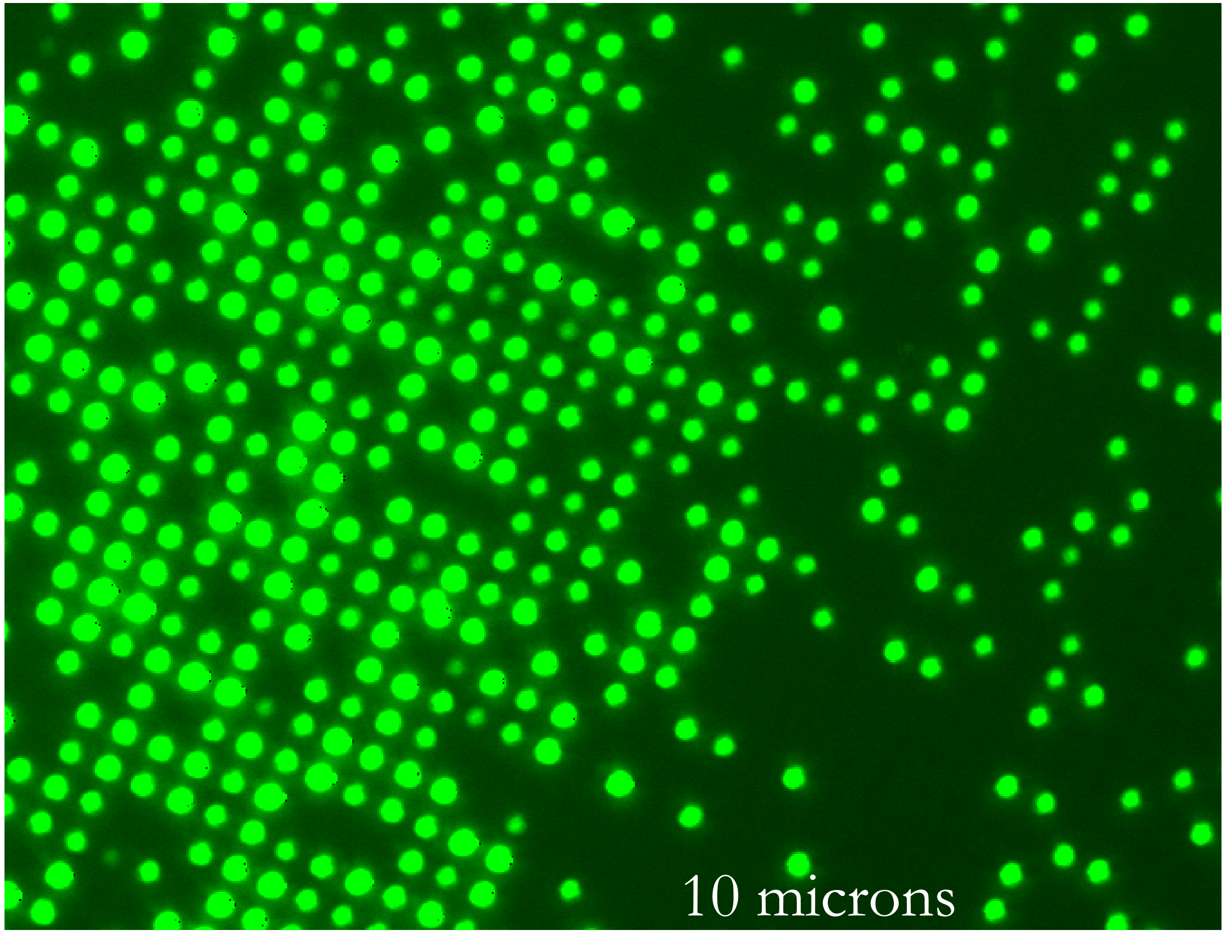


The problem

Ultrafast science generally requires irreversible sampling of the system dynamics

Develop skills for high throughput sampling of analytes (crystals) under study

Dynamics studies invoke another level of complexity;
t_0 background



Crystals in prescribed positions

Crystals are happy

Translation stage, detector read out are fast

Sampling efficiency is improved

This idea is suitable for dynamics because it allows t_0 diffraction orders to be recorded.

How to assemble this array?-what support material if recording in transmission

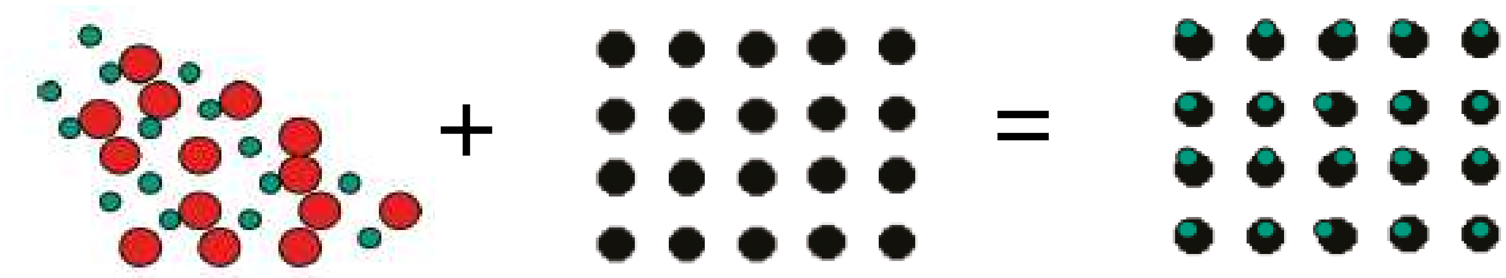
Assembly of the array

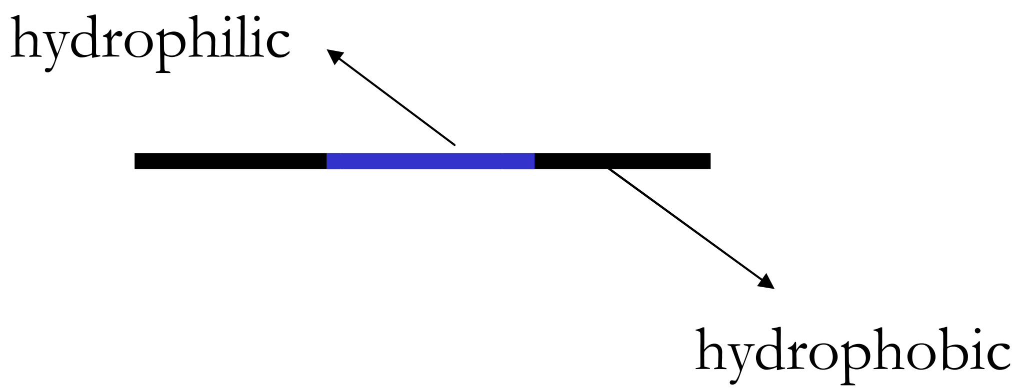
Thought experiments:

Grow crystals *in situ* and diffract

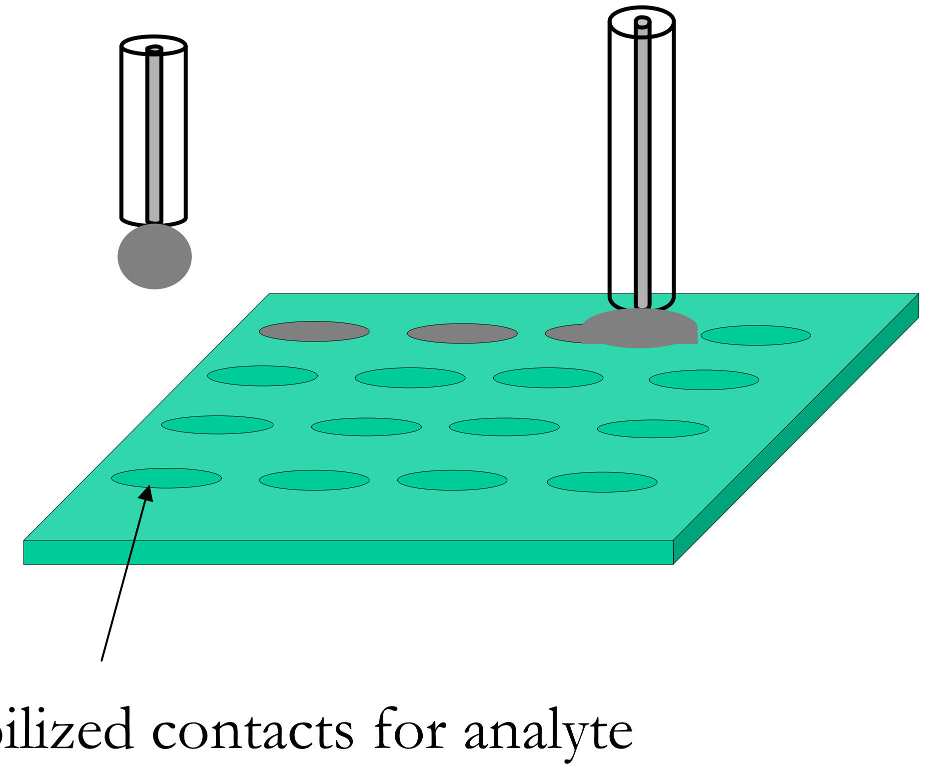
Position crystals in prescribed positions using robotics

Design a `smart`surface to fish out desired crystals from a suspension



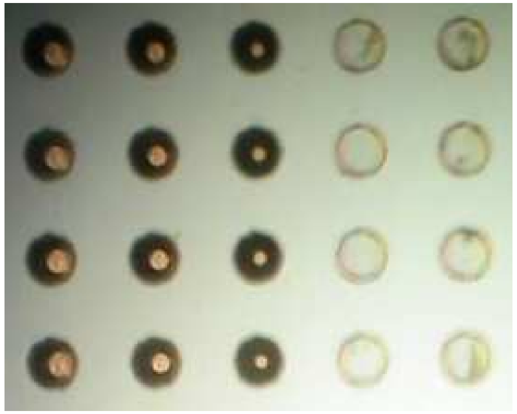


Enhancing the liquid pinning potential



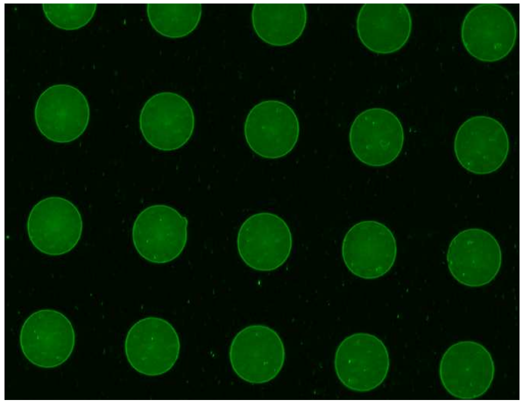
Wetting is very specific

A

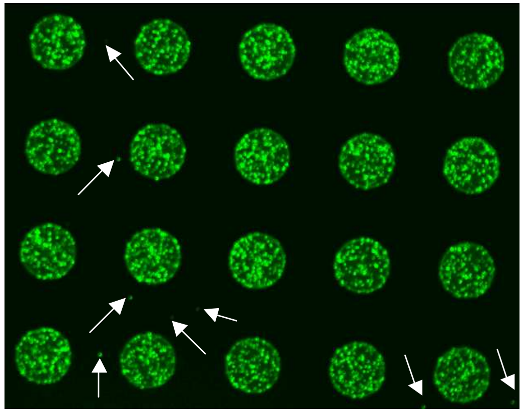


Empty wells

B



C



A: water

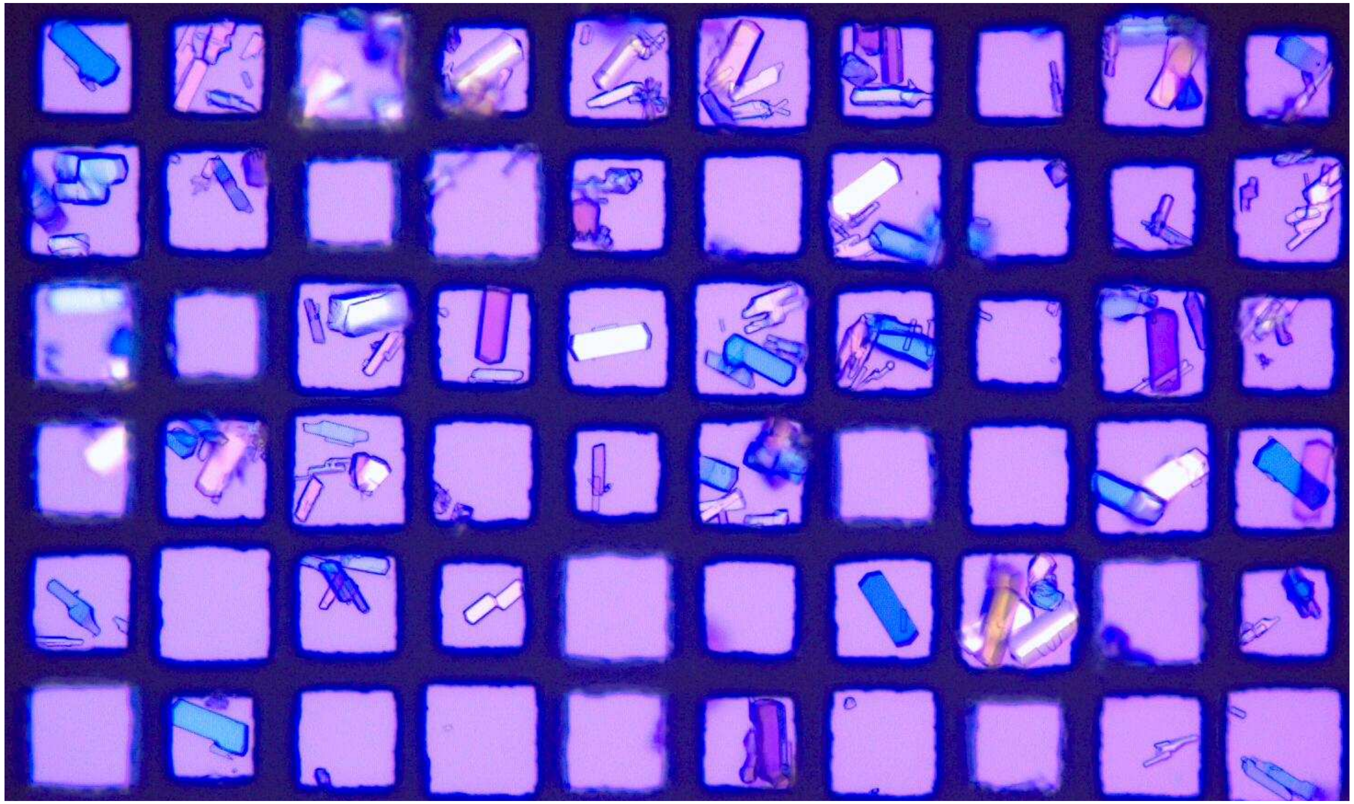
B: Qdot solution. Intensities are identical

C: ~3 μm beads suspension. 0.1% nonspecific wetting.

Aspect ratio of 20

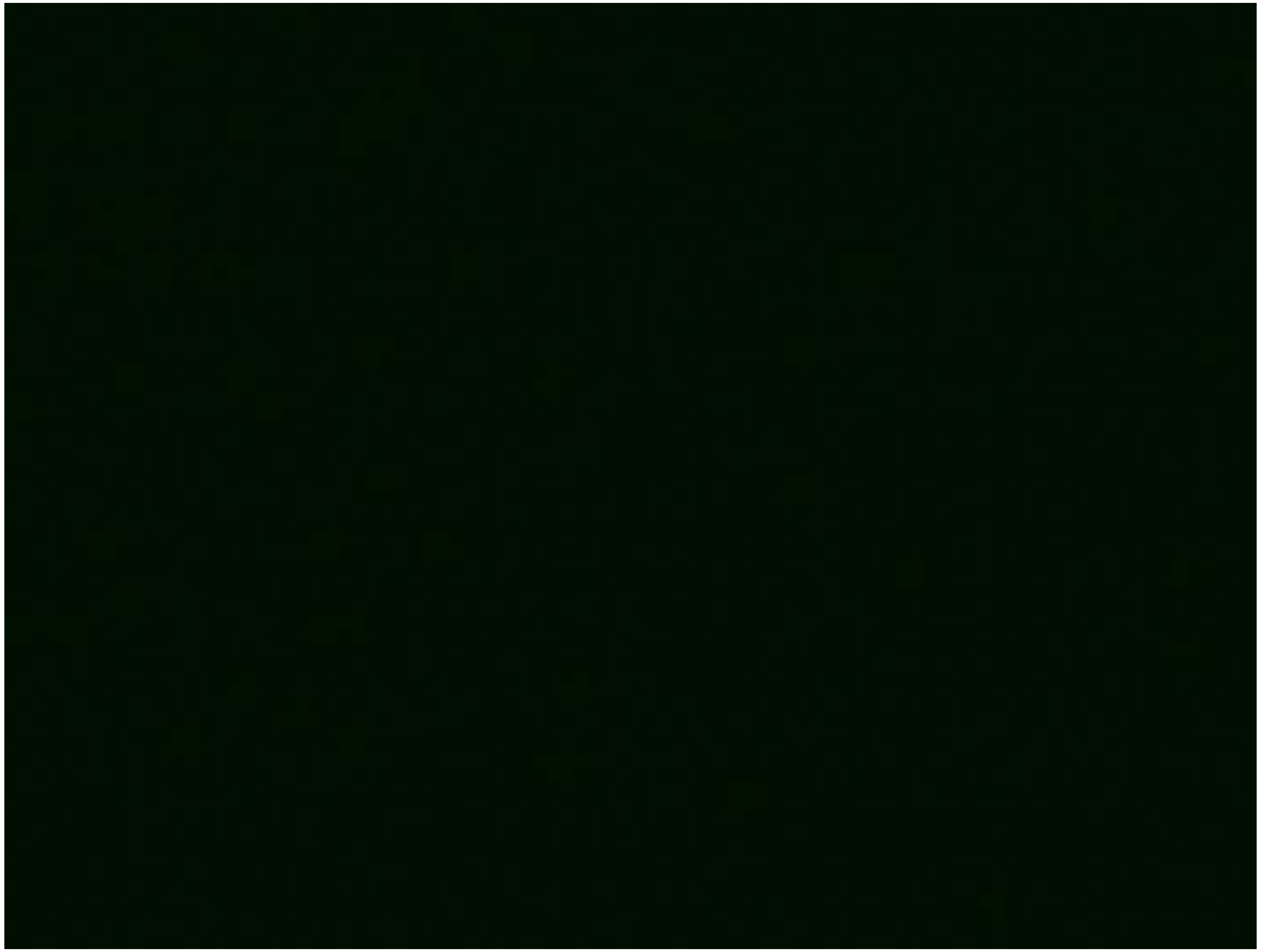
Substrate is silicon (etched by Reactive Ion Etching; F^- plasma)

The idea is to have the liquid localization self assemble the crystal array

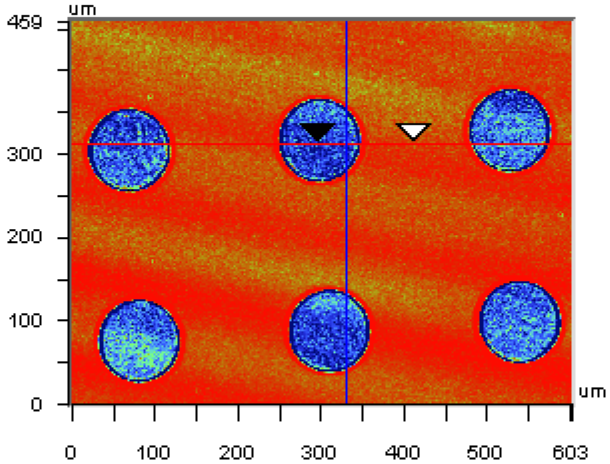


50 micron lysozyme crystals

Enhancing liquid pinning potential results in self assembly



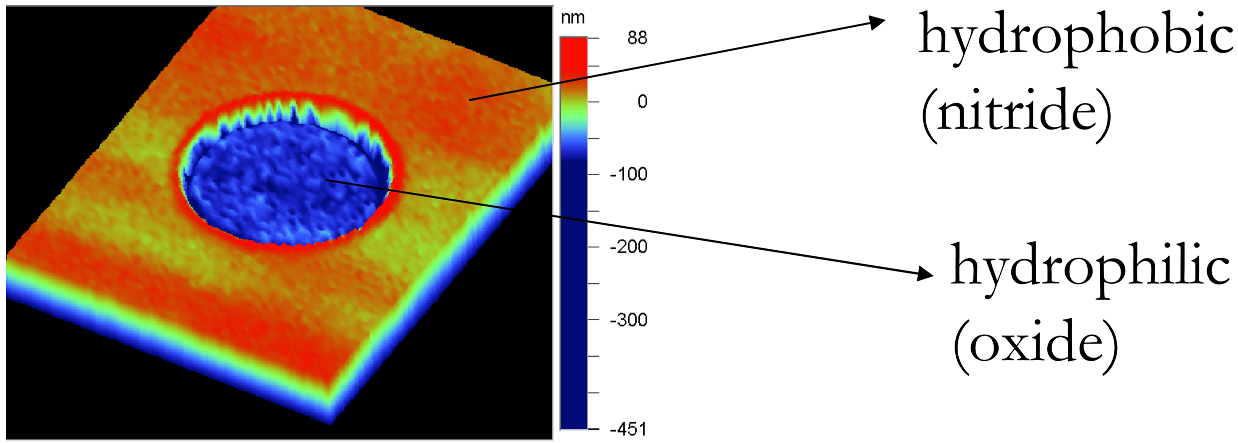
Veeco



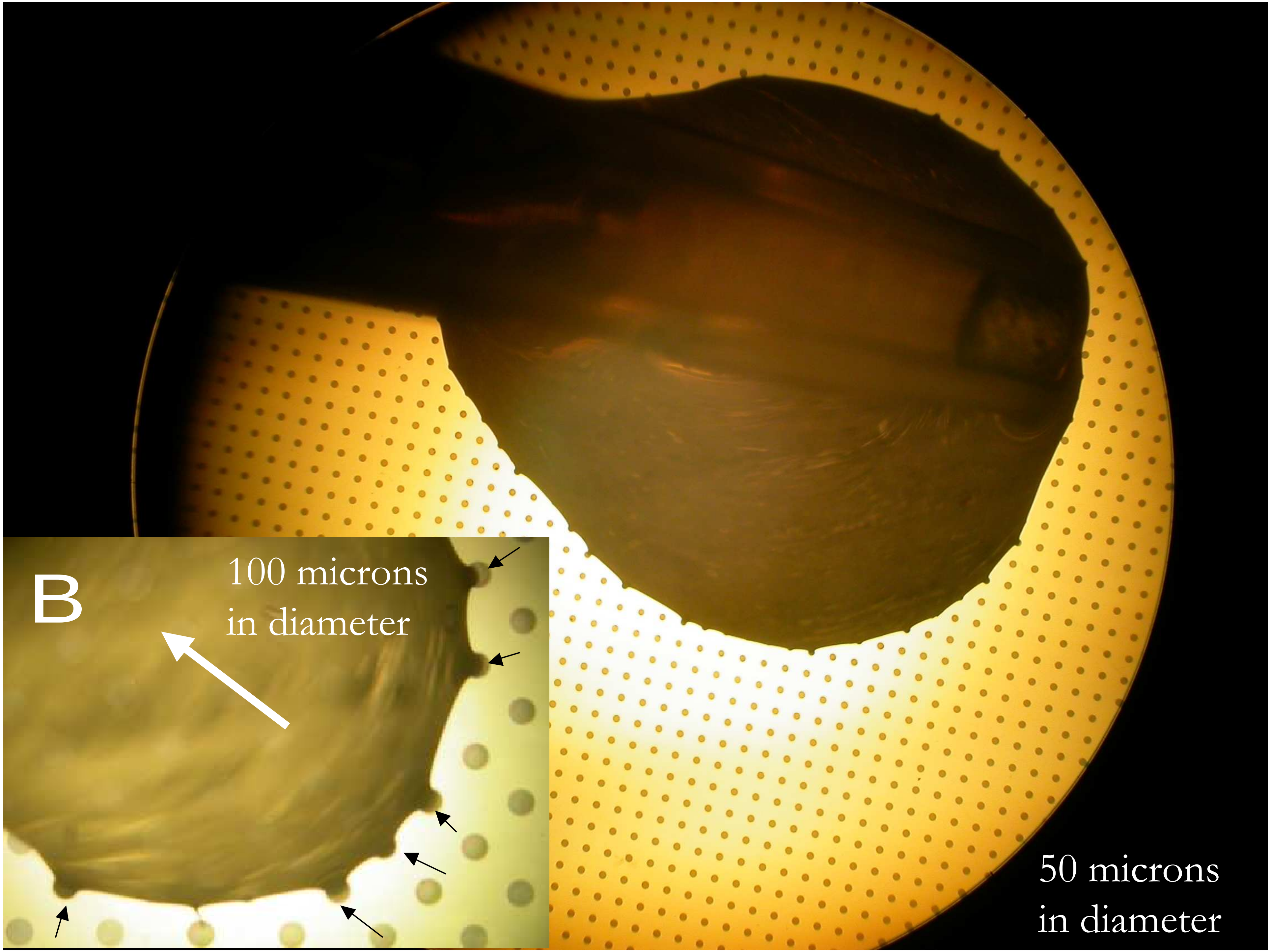
Y Profile

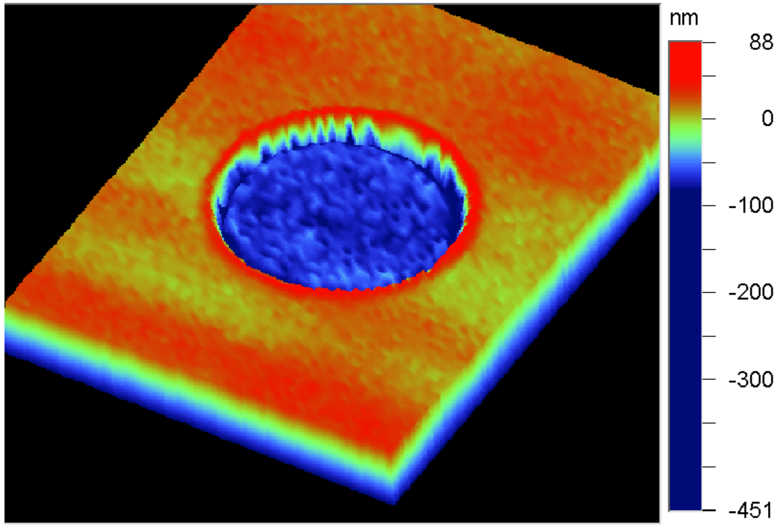
um

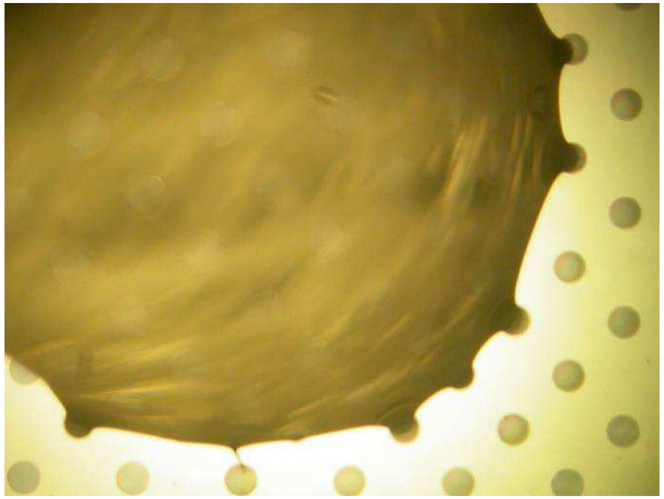
Silicon surface (native oxide) with 80nm silicon nitride

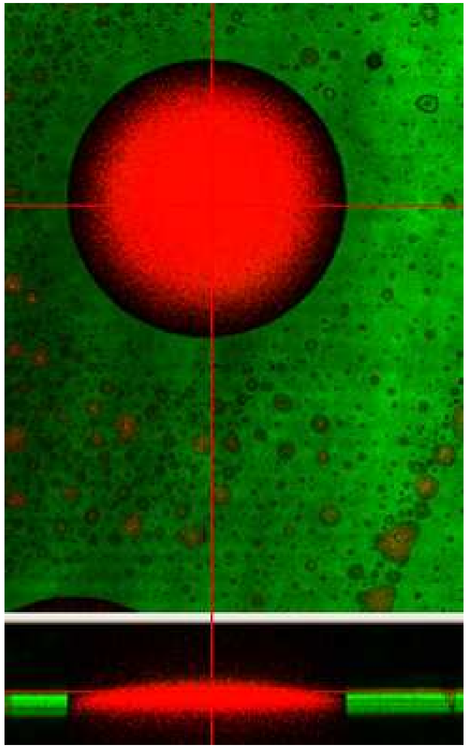


Plasma etched


B
100 microns
in diameter
50 microns
in diameter





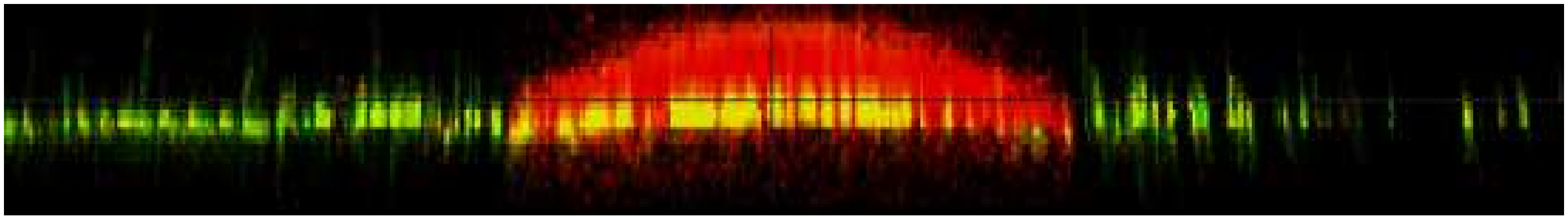


Laser scatter, 543

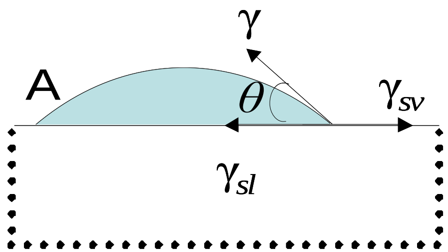
Rhodamine fluorescence, filter cube

Film thickness= 3 microns

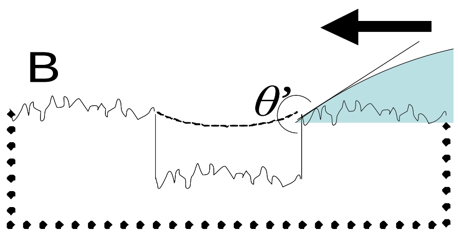
Well diameter=100 microns

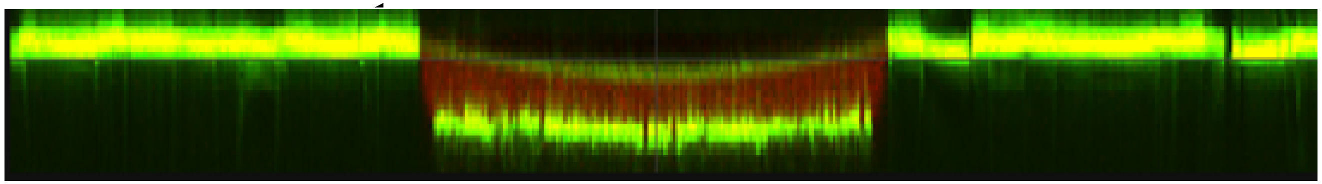


Can make the wells deeper (carrying larger crystals)



Contact line instability and wells with sharp walls allow consistent volumes to be captured



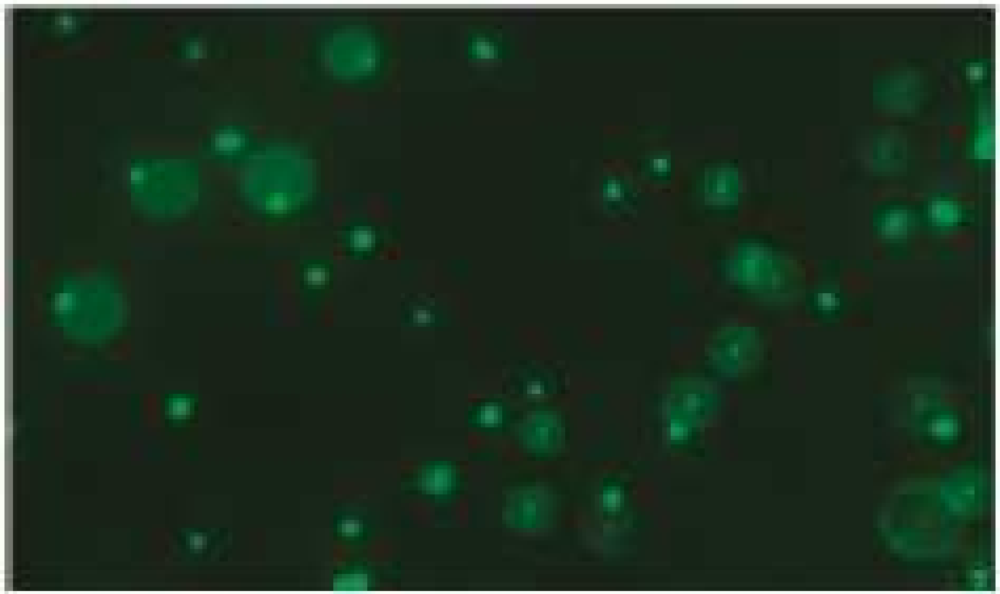


Aspect ratio 10
200 micron diameter

Laser scatter, 543
Rhodamine fluorescence, filter cube

Zarrine-afsar et al (2011) Anal Chem

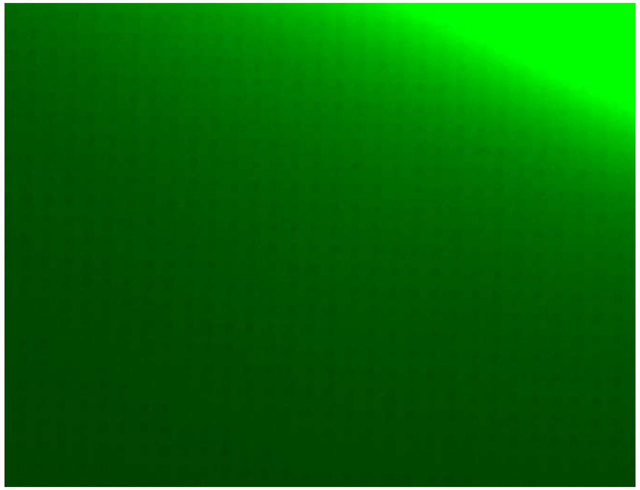
Characterization of capture by beads carrying FITC label



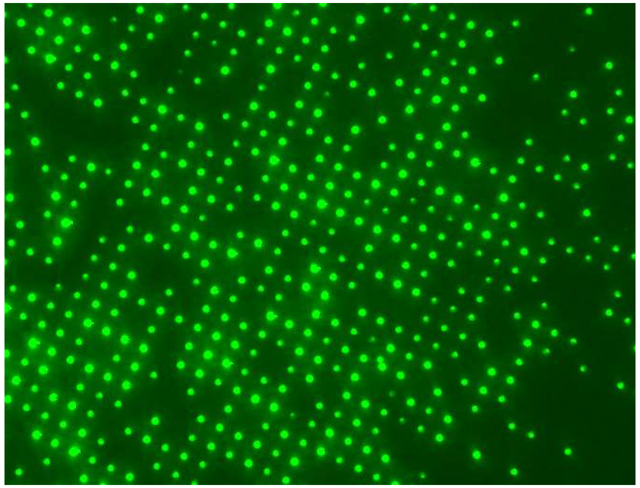
Mixture
Varying sizes, densities, available

Can make the wells smaller (carrying smaller crystals)

Chip with microwells

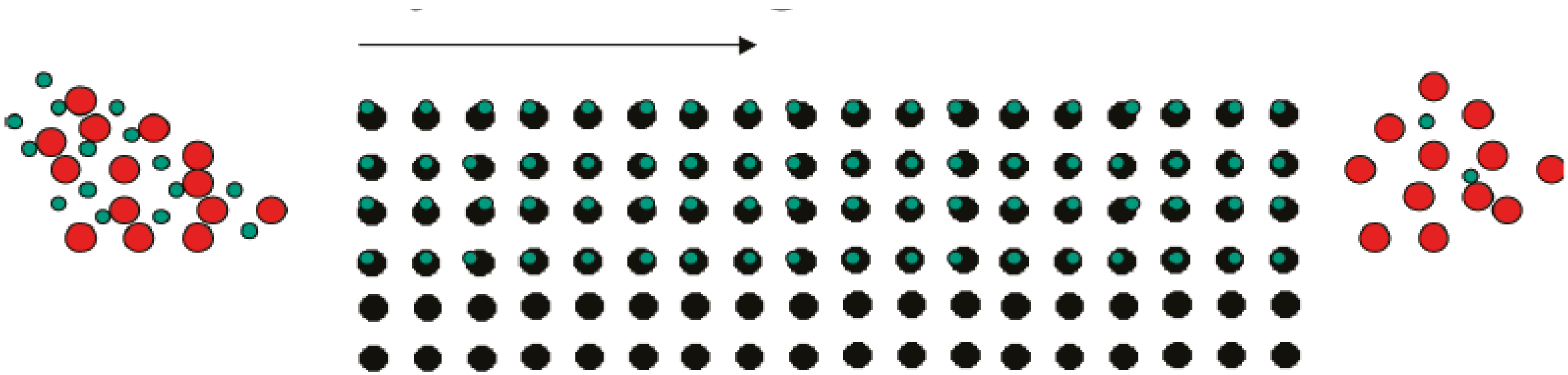


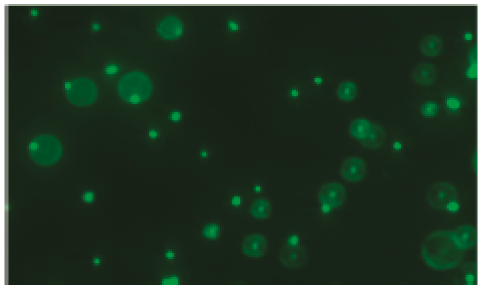
Chip filled with beads

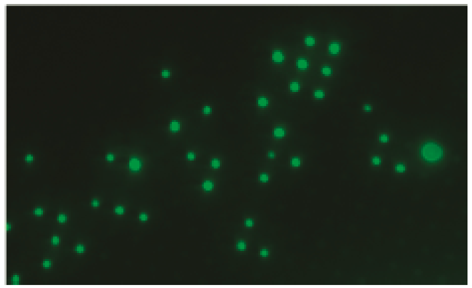


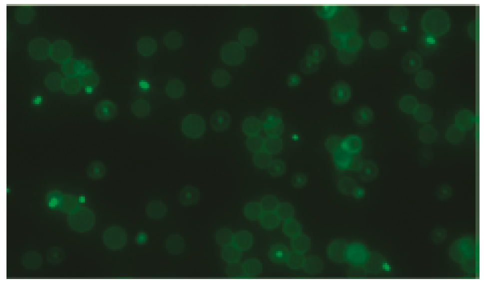
4 micron wells, beads are 2.9 micron

Size exclusion is available

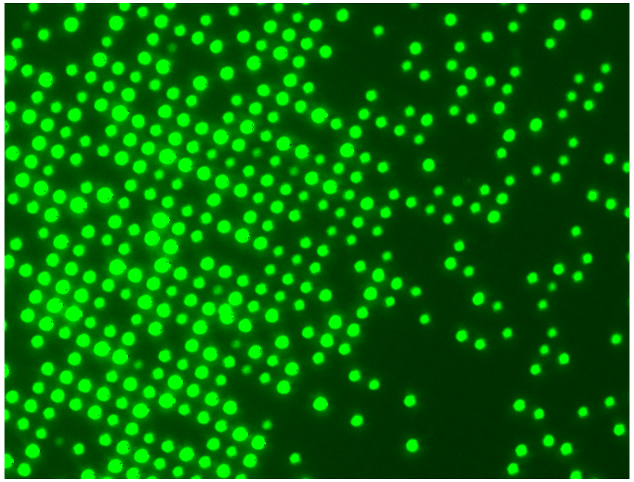


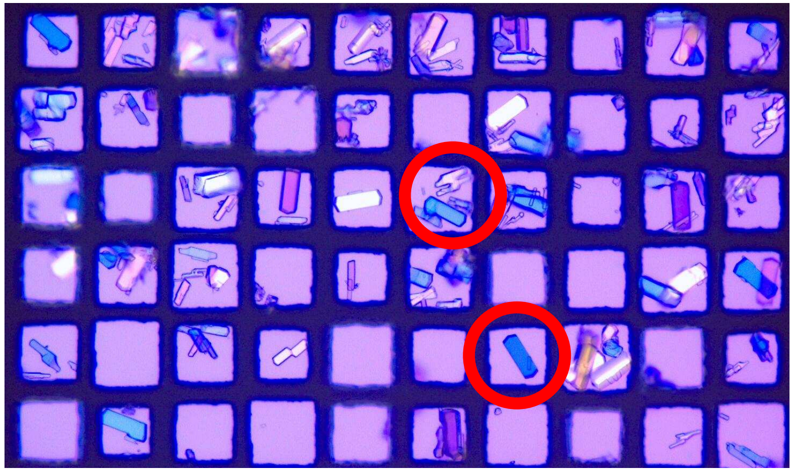






Can this be adapted to crystals?





well size ~ crystal size

In adapting the scheme to crystals

(I) Buoyancy is an issue

(II) Beads are homogeneous in size; crystals may not be

(III) Beads are suspended in low salt; evaporation forms salt crystals

(IV) Crystal morphologies could be complicating; do they ‘roll’ or stick to surface?

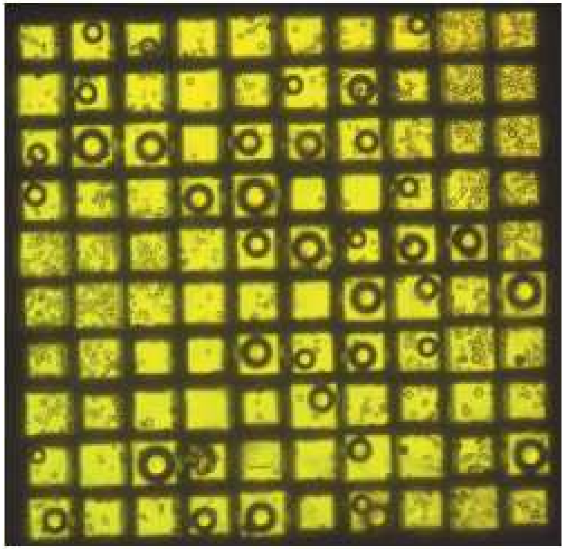
(V) Interactions with the surface may result in preferred orientations that can limit sampling of reciprocal space

(VI) Solid support should have small background (exposure in transmission)

What is needed

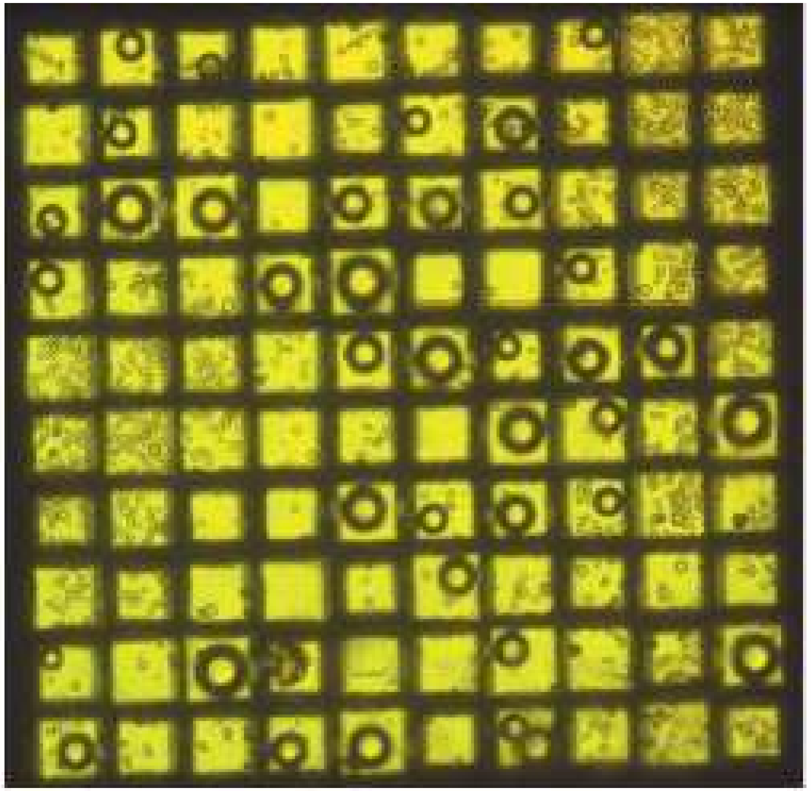
(I) A support transparent to x-rays (low background)
(II) A surface that collects little water (reduce absorption)
(III) A surface that has roughness to induce some randomness to flat crystals

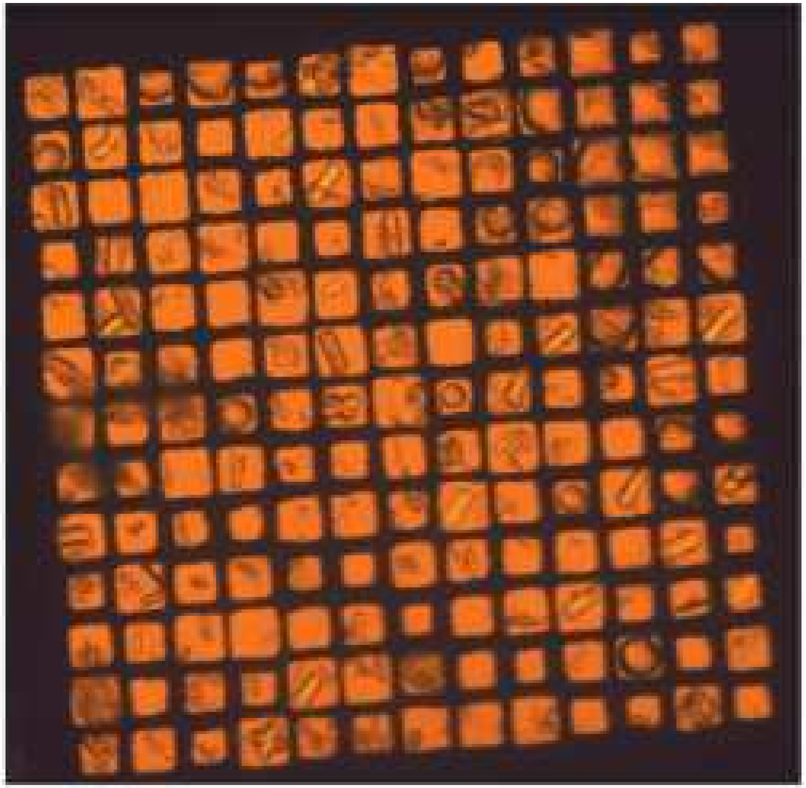
Multiple ways to satisfy these conditions with silicon technology yet as a proof-of-principle we followed another approach



Silicon mesh custom to size of crystals with **polyimide** support sprinkled with glass beads of varying sizes to create roughness

Role of hydrophilicity



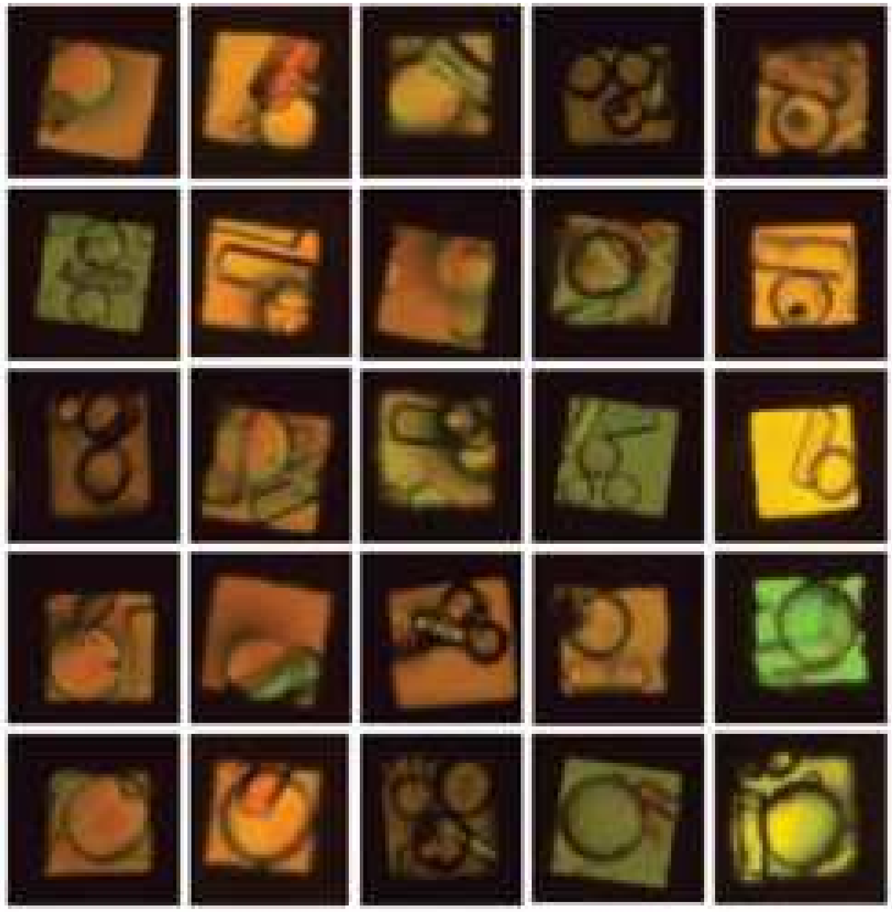


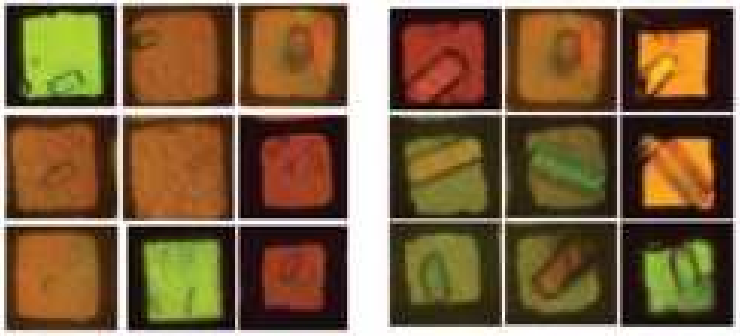
Hydrophilicity of glass on **hydrophobic** polyimide creates pinning for lysozyme storage solution that results in significant single crystals per well

Window size is matched to average crystal size in the batch

Role of surface roughness

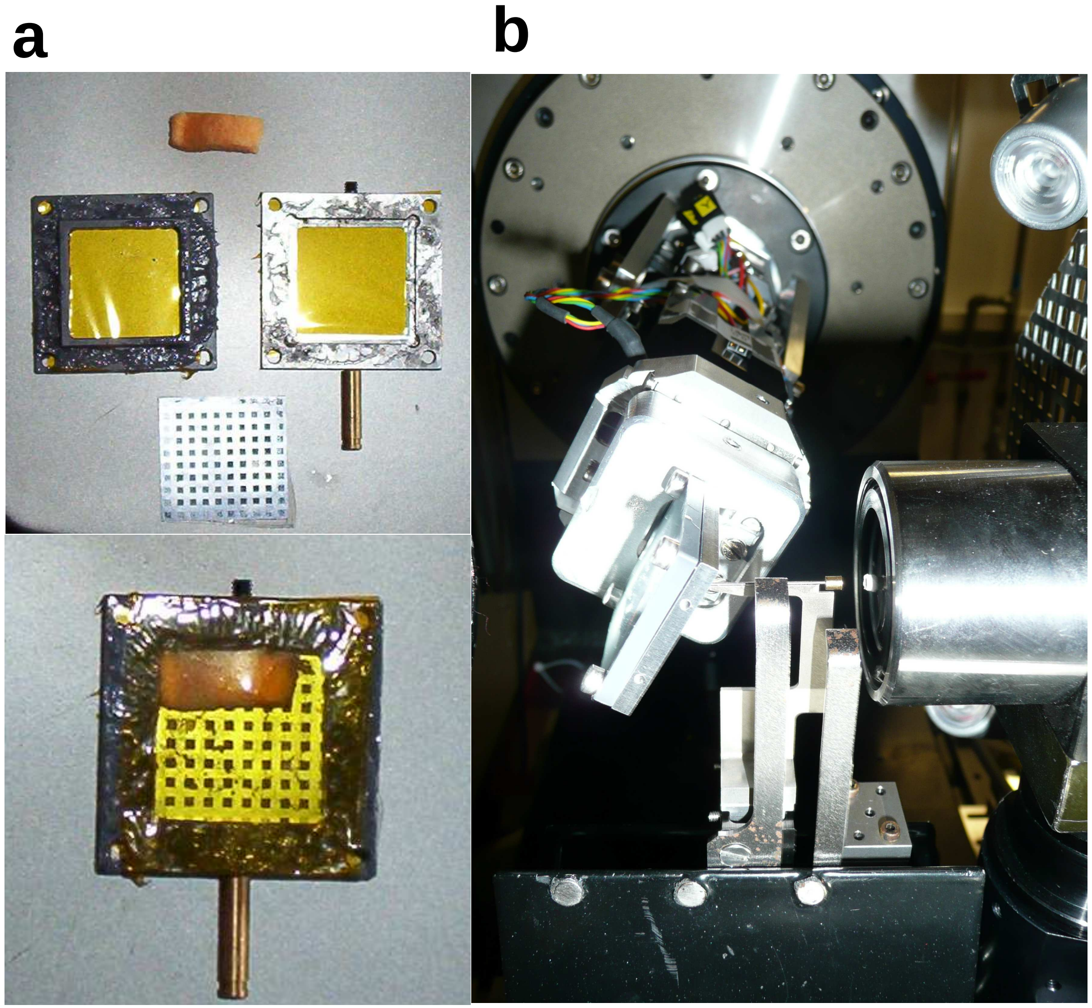
Induces some degree of randomness in crystal orientations





Bead dimensions versus crystal dimensions

Chip holder to satisfy evaporation issues and interfacing

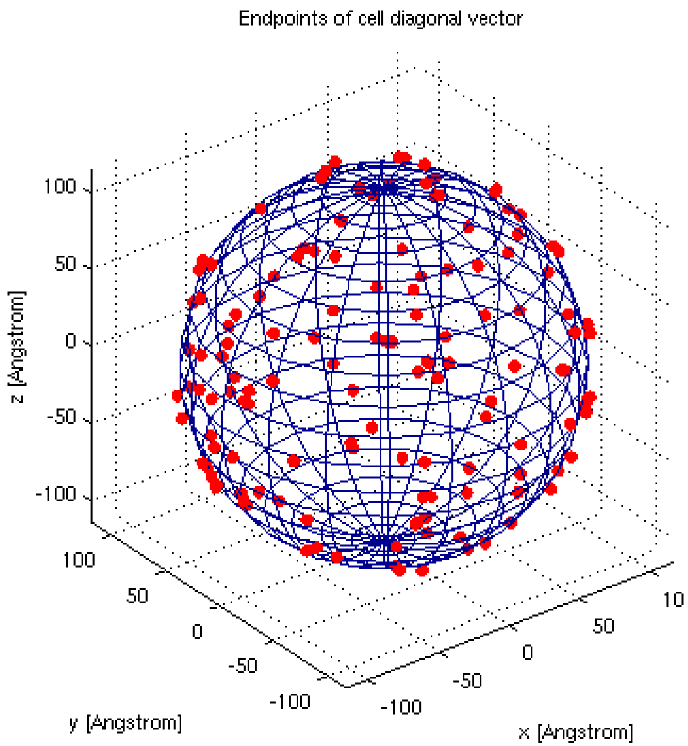


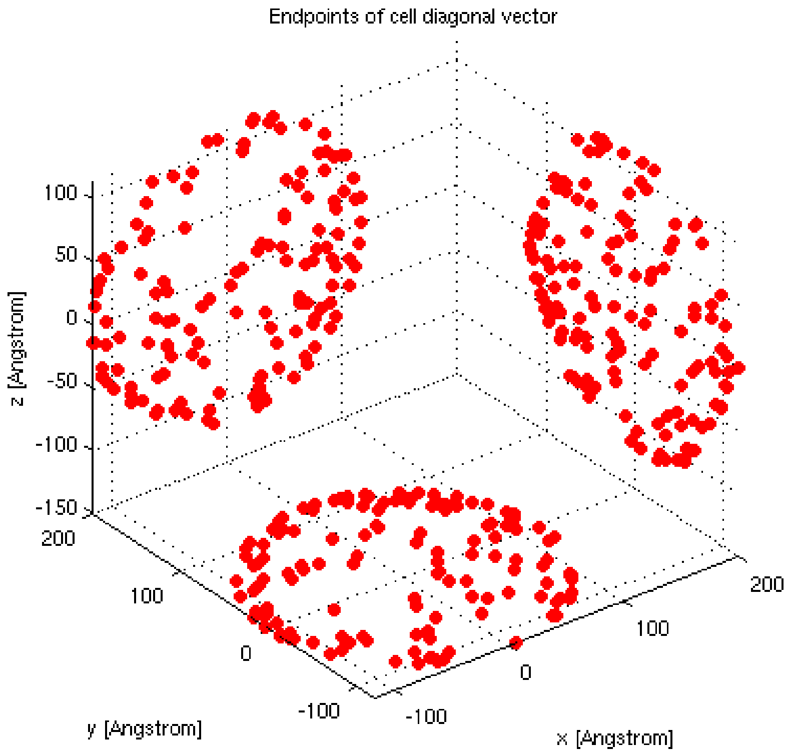
Saturate the environment
With storage solution
vapour pressure

Limited goniometer motion
also underscores the
significance of randomness

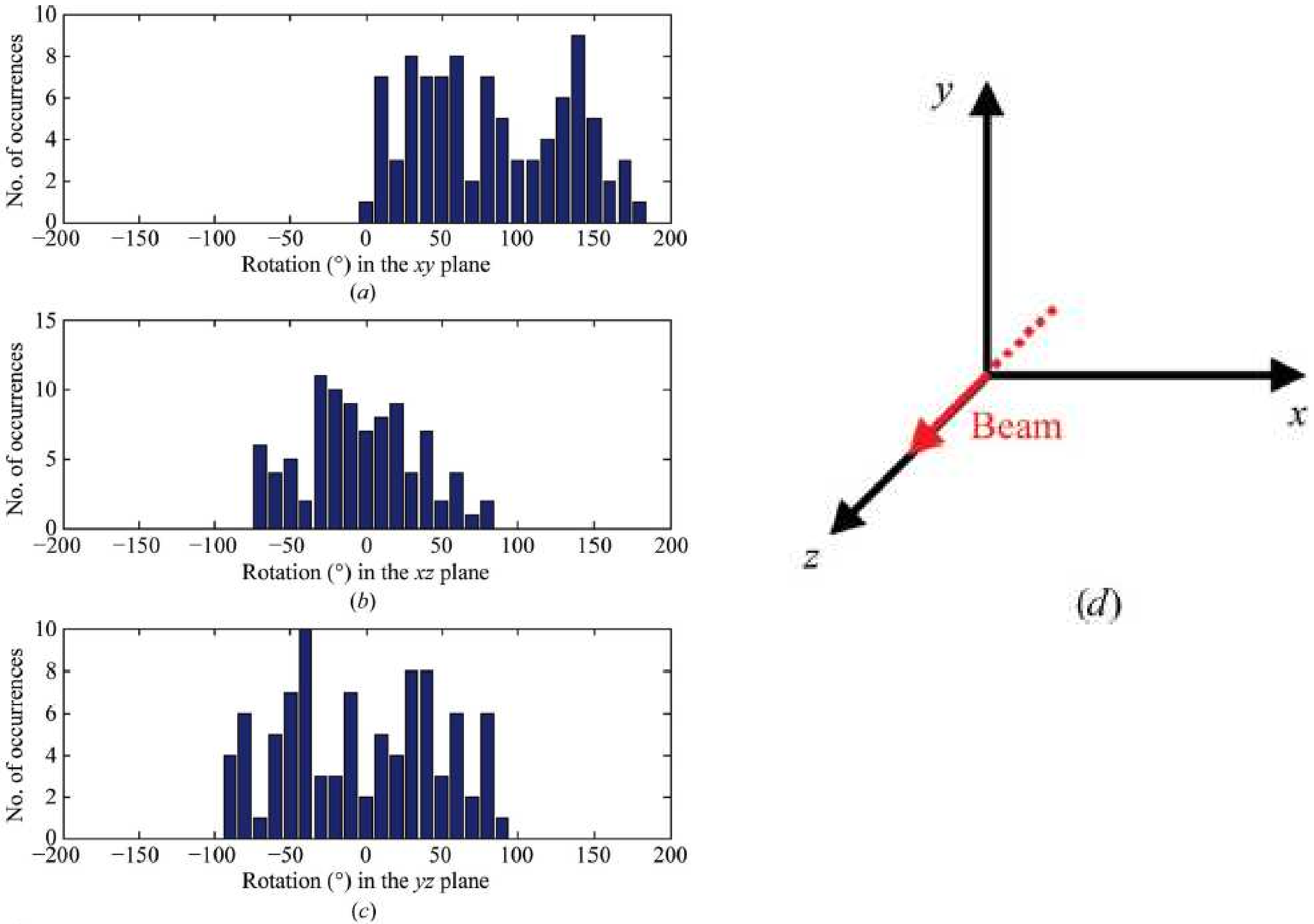
Proof-of-principle assessment of crystal orientations

End points of cell diagonal vector

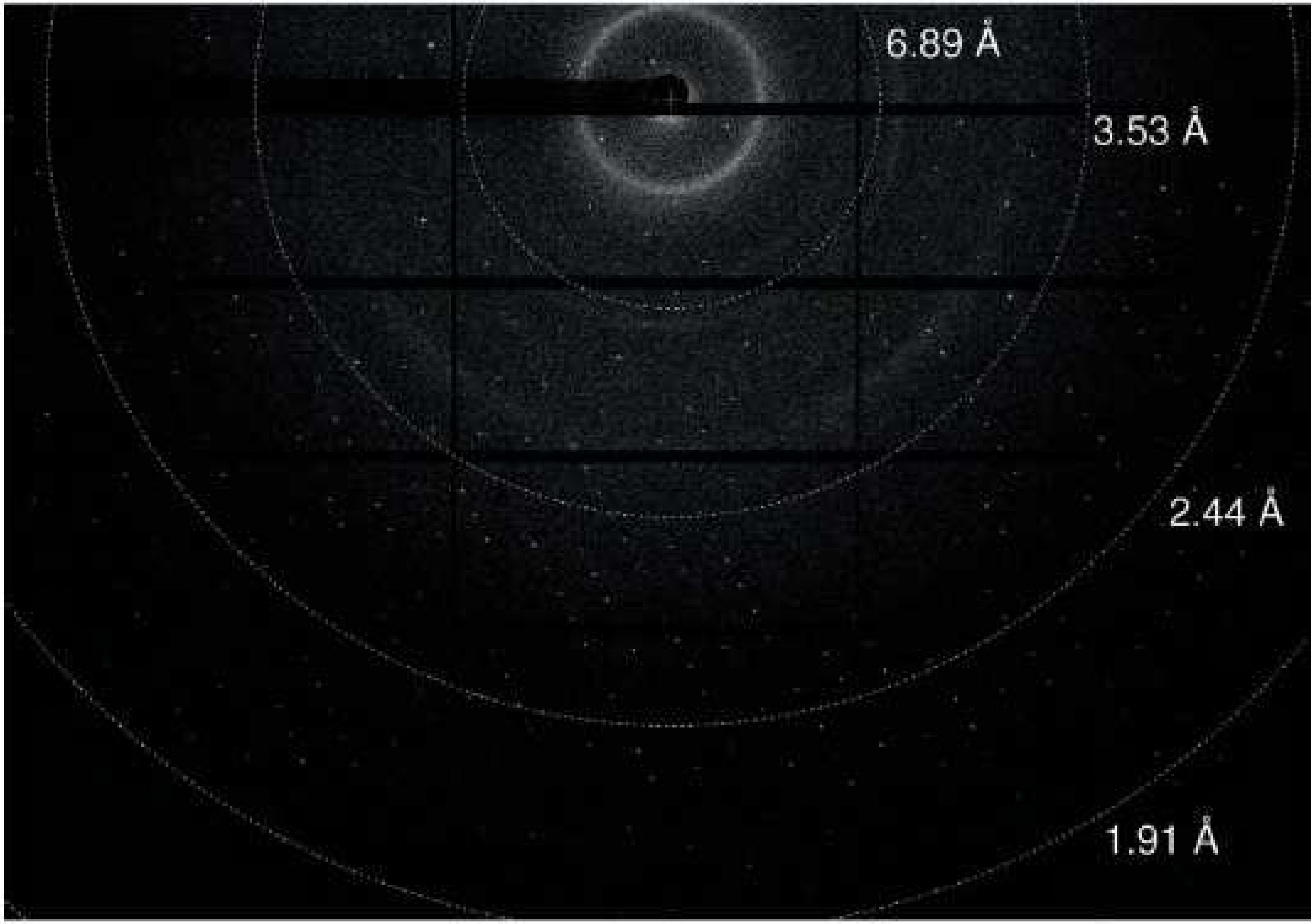




Proof-of-principle assessment of crystal orientations



Diffraction

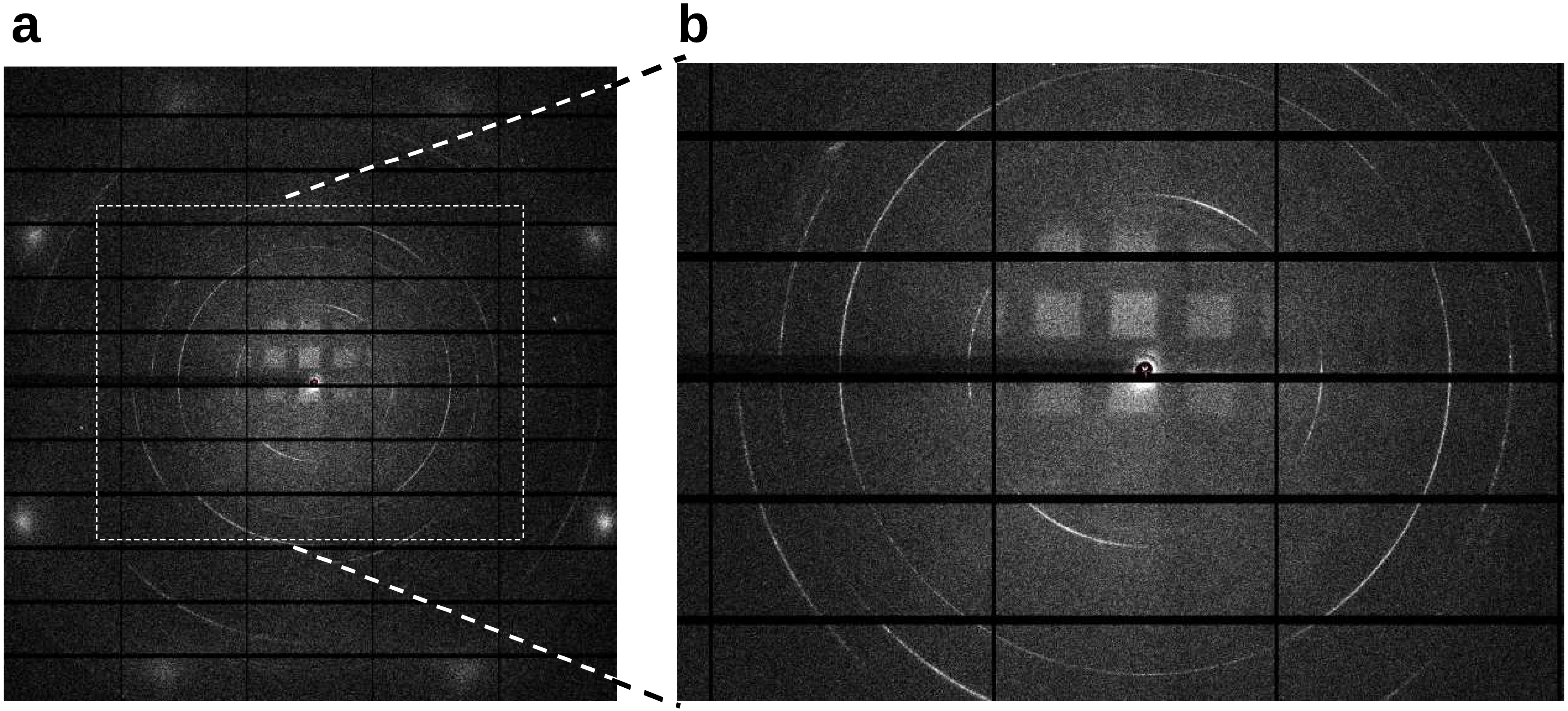


Diffraction statistics

	Ferritin	Lysozyme
Data collection		
Space group	$F432$	$P4_32_12$
Unit-cell parameters (Å)	$a = b = c = 182.5$	$a = b = 79.1$, $c = 38.5$
X-ray source	Swiss Light Source, beamline X10SA	Swiss Light Source, beamline X10SA
Wavelength (Å)	1.00	1.00
Resolution (Å)	10–2.5	10–2.3
R_{merge}†	0.13 (0.38)	0.19 (0.31)
$\langle I/\sigma(I)\rangle$	8.0 (3.3)	5.0 (2.8)
Completeness (%)	98.6 (98.8)	92.0 (91.1)
Multiplicity	4.2 (4.4)	3.4 (3.1)
Refinement		
Resolution (Å)	41.88–2.5	69–2.3
R_{work}/R_{free}	0.19/0.24	0.217/0.256
No. of atoms		
Protein	1377	1001
Ligand/ion	1 [Cd^{2+}]	1 [Cl^-]
Water	44	29
B factors (Å^2)		
Protein	23.9	29.2
Ligand/ion	22.4	29.4
Water	25.3	27.7
R.m.s. deviations		
Bond lengths (Å)	0.01	0.01
Bond angles (°)	1.12	0.94

† $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$.

Collimating is necessary



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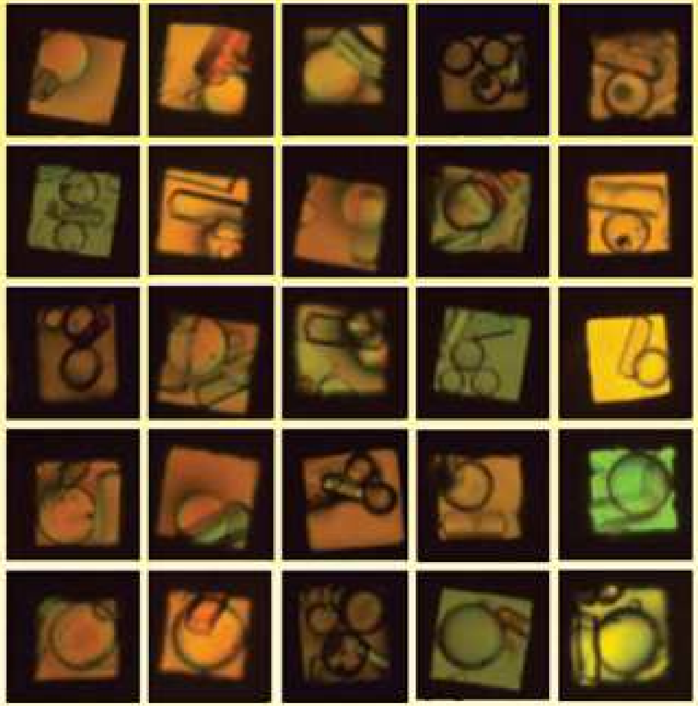

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Lessons learned

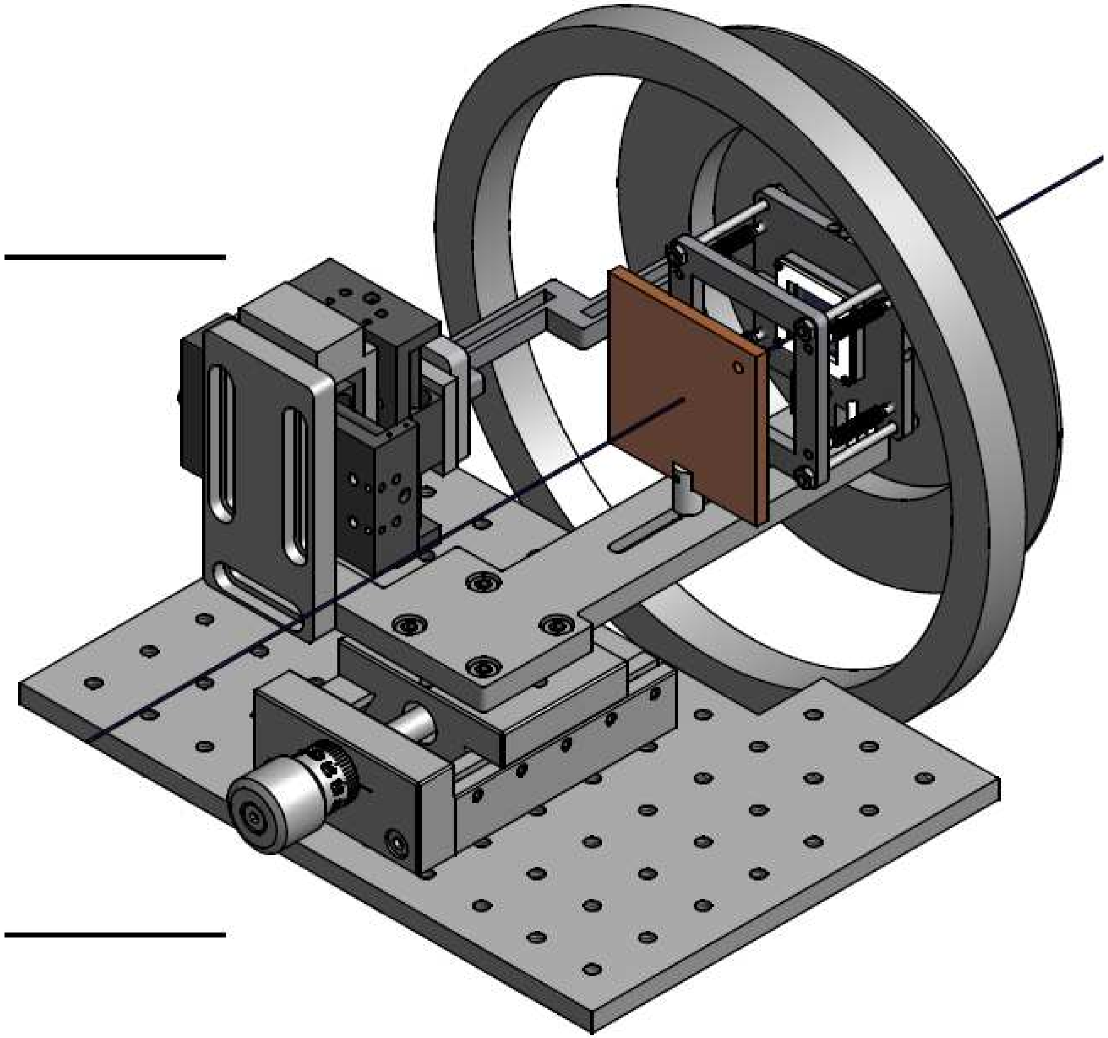
(I) Chip holder is too bulky
(II) Beads are strongly absorbing
(III) There is too much liquid that causes some crystals to swim!

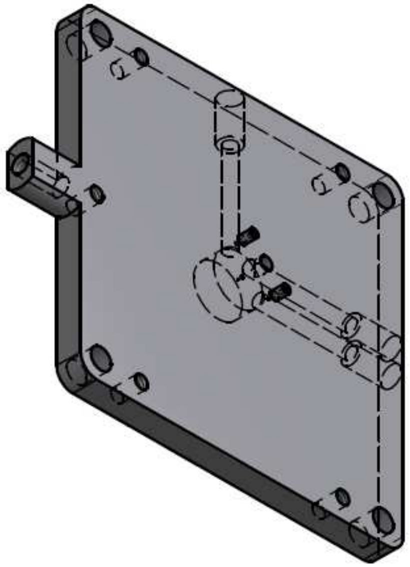
Solutions:

(I) Go all silicon, make the chip thin (60-80 nm silicon oxide support)
(II) Include drainage holes, and use centrifugation to get rid of excess liquid
(III) Pump in He
(IV) Enclose in He chamber
(V) Move away from beads; fuse small silicon, carbide chunks to surface

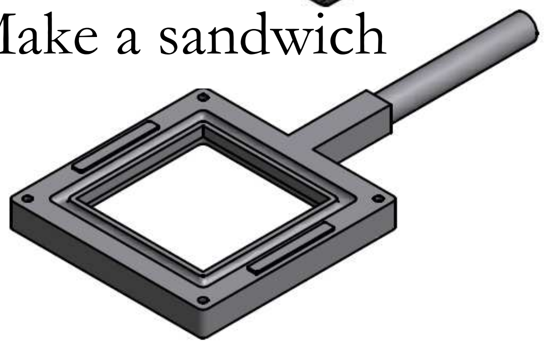
Moving forward

Online sample delivery system



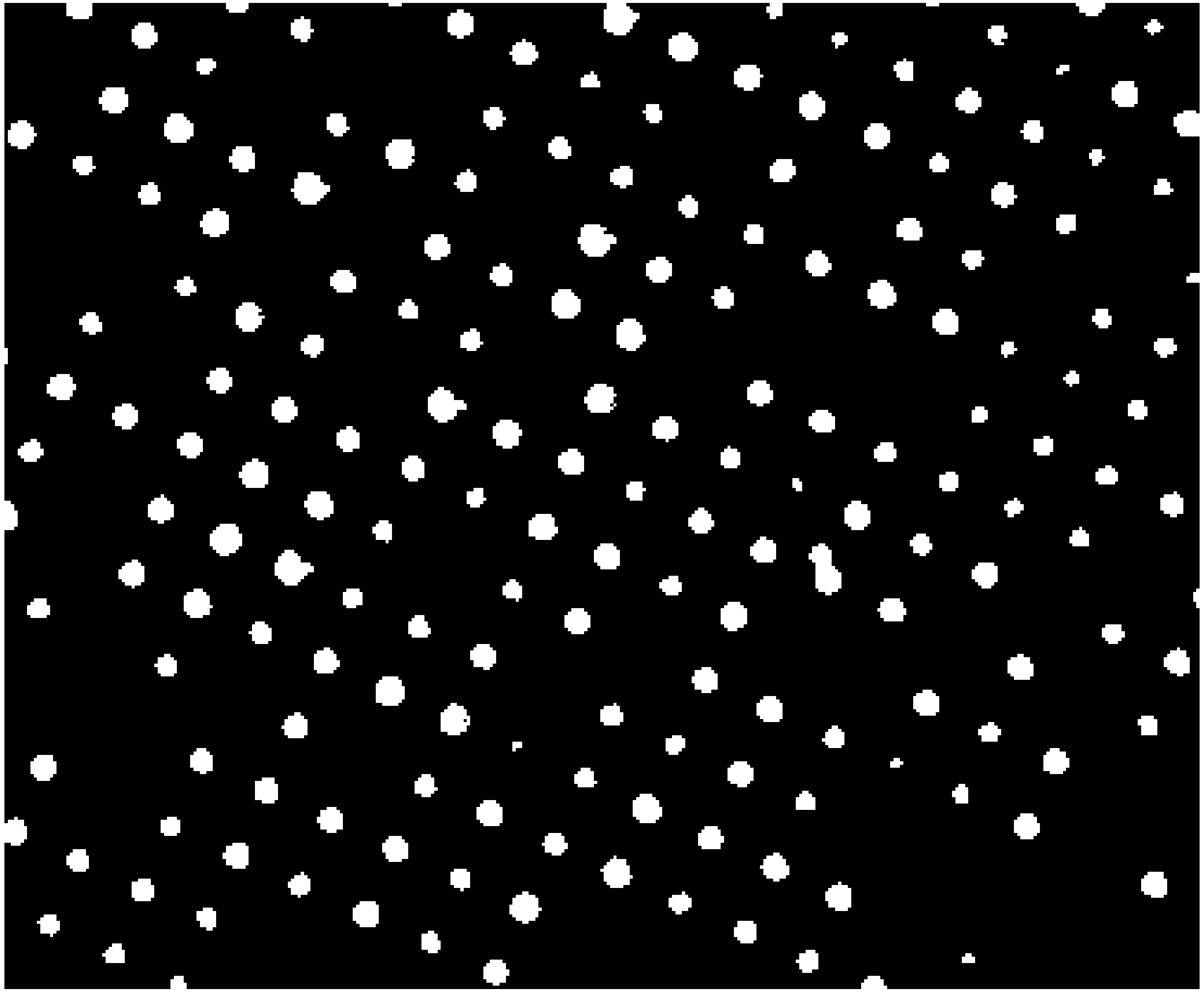


Make a sandwich



Online delivery

"Truly" nanocrystallography



2 micron wells and 900 nm beads

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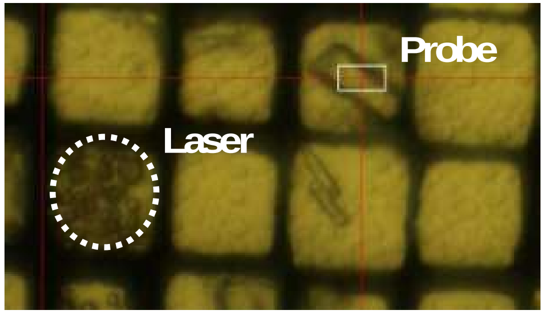
Max Planck Group for Structural Dynamics, DESY, Hamburg, Germany
Prof. Dr. RJ Dwayne Miller

The Swiss Light Source
Dr. Martin Fuchs

Chip diffuses the ‘shockwaves’ expected from FEL pulse

A



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