Fixed-target serial X-ray crystallography in in-situ chips and on low-Z polymer sample supports

Michael Heymann,
old: Seth Fraden, Brandeis University
new: Henry Chapman, CFEL, DESY

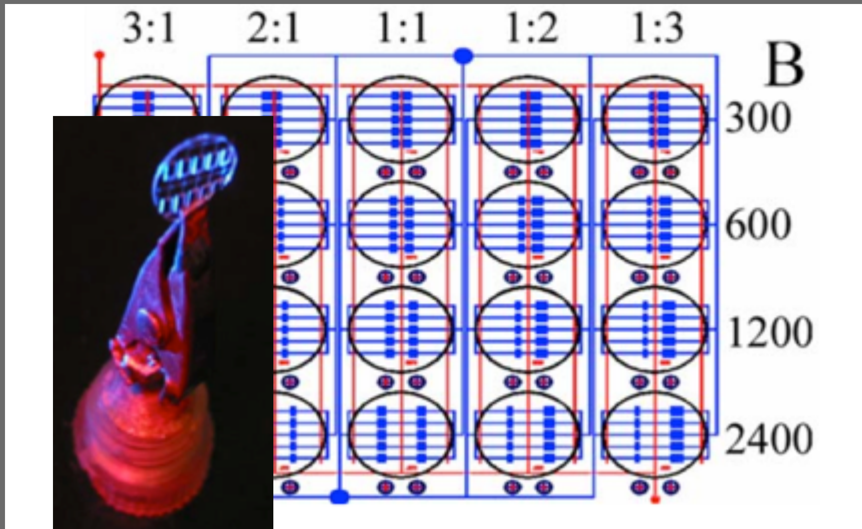
CFEL SCIENCE

Center for Free-Electron Laser Science

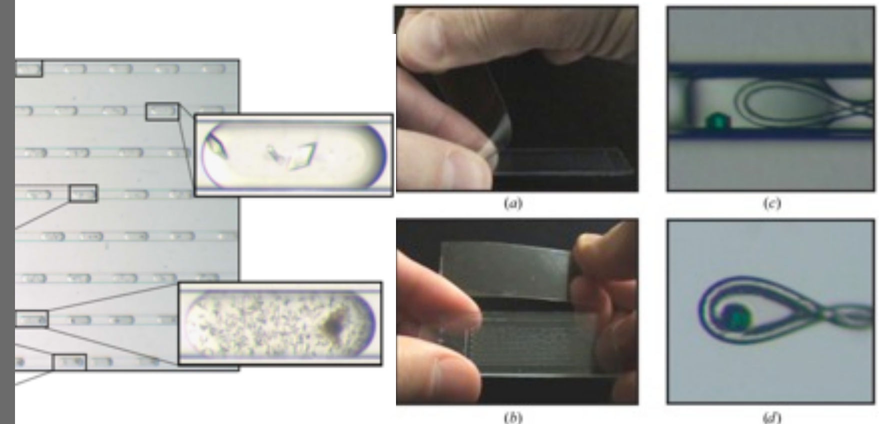
valves

drops

cryo

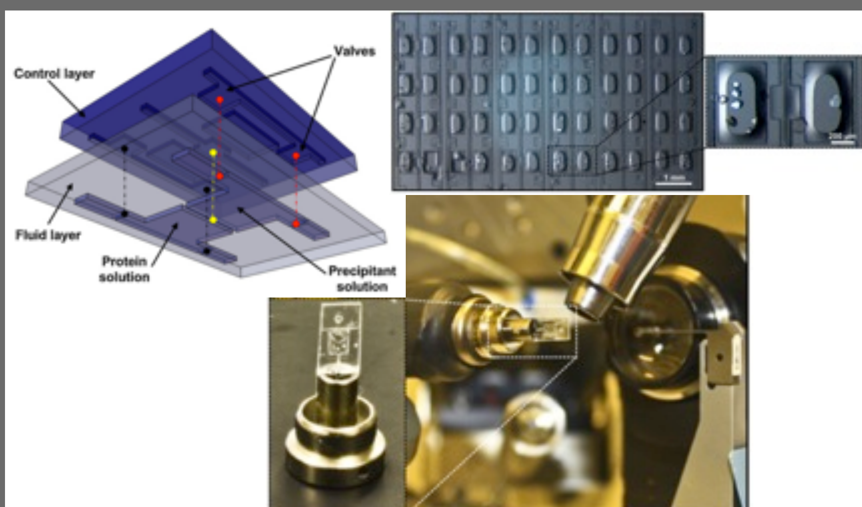


Hansen, C.L., Classen, S., Berger, J.M., Quake, S.R., A microfluidic device for kinetic optimization of protein crystallization and in situ structure determination, JACS, 2006 128:10, 3142-3

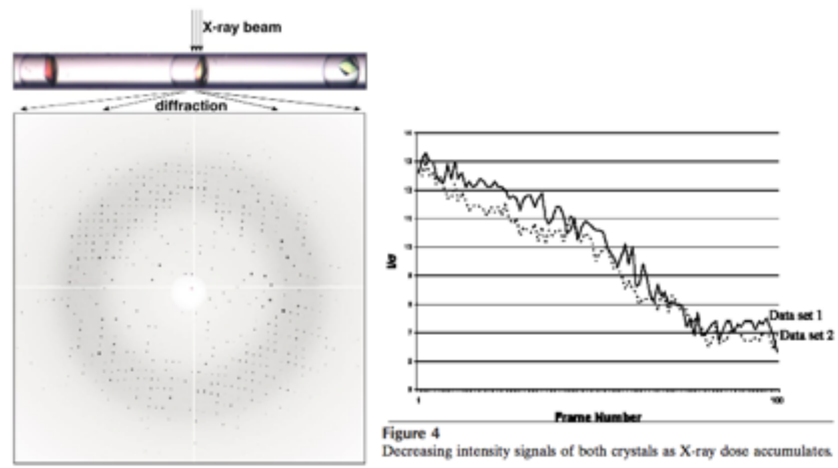


Gerdts, C.J., Elliott, M., Lovell, S., Mixon, M.B., Napuli, A.J., Staker, B.L., Nollert, P., Stewart, L.,The plug-based nanovolume Microcapillary Protein Crystallization System (MPCS), Acta Crystallography D, 2008, 64:11, 1116-22

room temperature

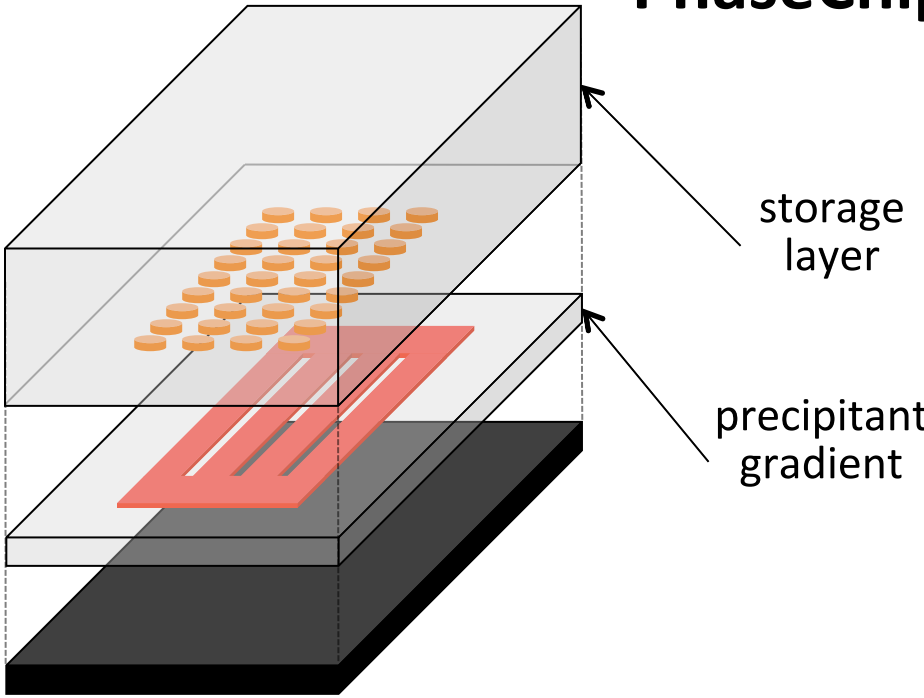


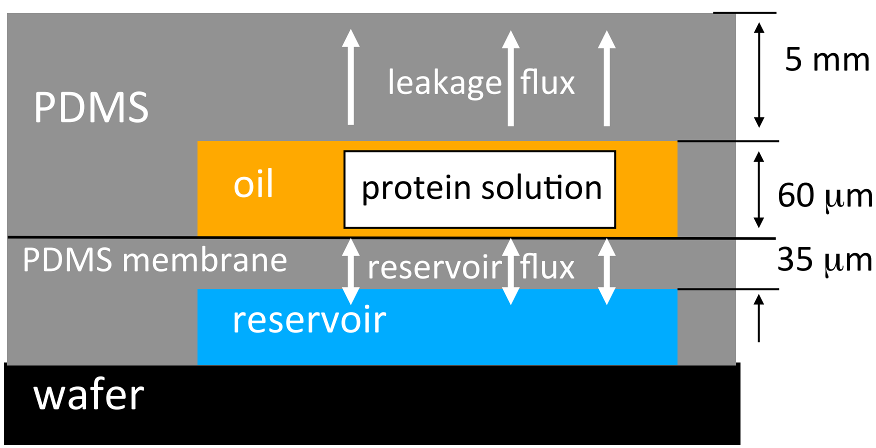
Guha, S., Perry, S.L., Pawate, A., Kenis, P.J.A., Fabrication of X-ray compatible microfluidic platforms for protein crystallization, Sensors and Actuators B, 2012, 174, 1-9

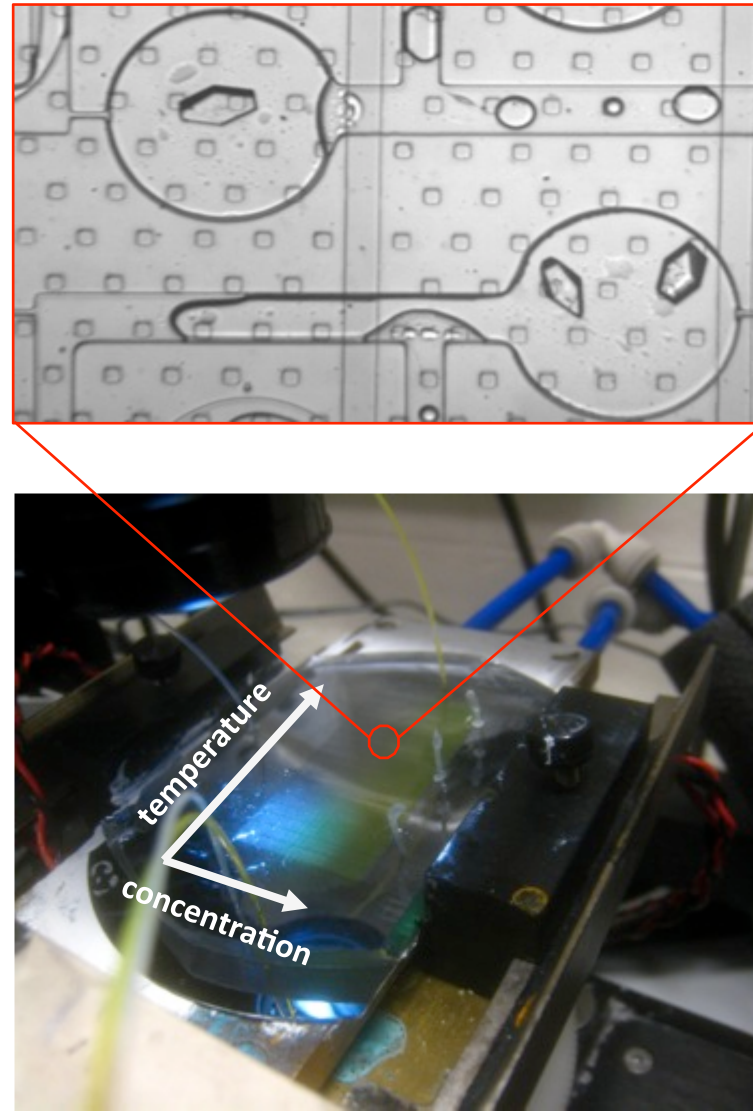


Zheng, B., Tice, J.D., Roach L.S., Ismagilov, R.F., A Droplet-Based, Composite PDMS/Glass Capillary Microfluidic System for Evaluating Protein Crystallization Conditions by Microbatch and Vapor-Diffusion Methods with On-Chip X-Ray Diffraction," Angew. Chem. Int. Edit. 2004 43: 2508-2511

PhaseChip



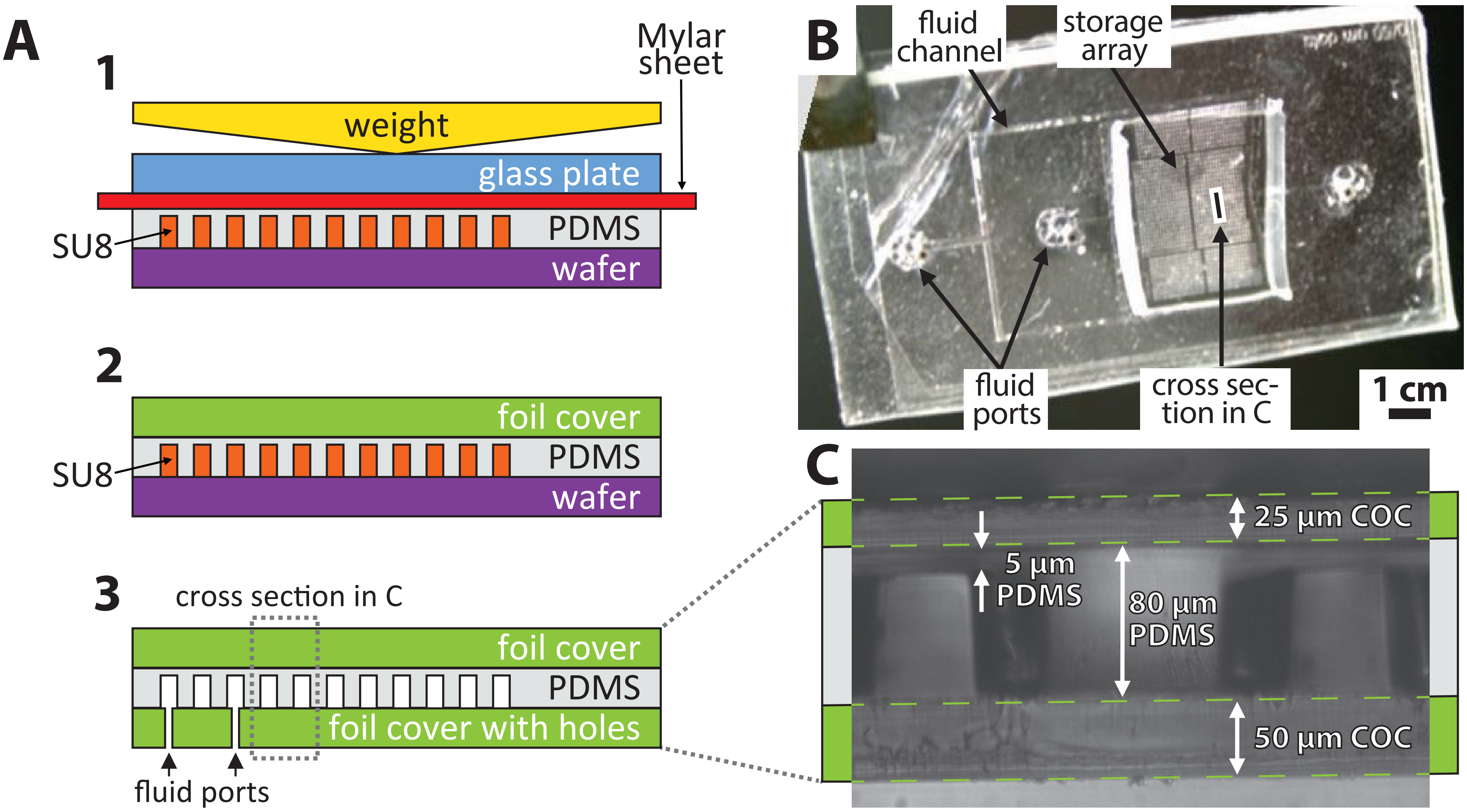




J.U. Shim, G. Cristobal, D.R. Link, T. Thorsen, Y. Jia, K. Piattelli, S. Fraden, *JACS.* 129, 8825 - 8835 (2007)

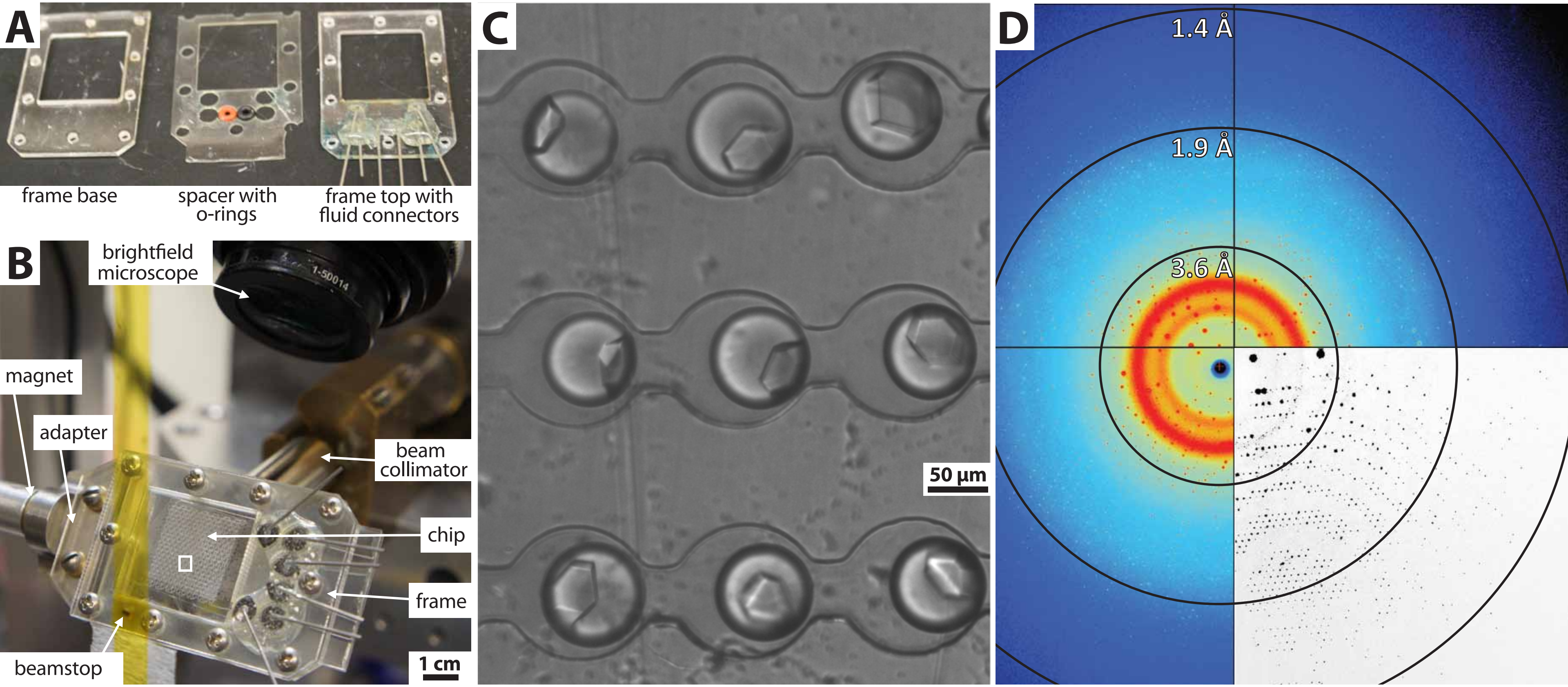
J.U. Shim, G. Cristobal G, D.R. Link, T. Thorsen, S. Fraden, *Crystal Growth* & Design, 7, 2192-2194 (2007).

Š. Selimovic, F. Gobeaux, S. Fraden Lab Chip, 10, 1696-1699 (2010)



fabrication:
Guha, S., Perry, S.L., Pawate, A., Kenis, P.J.A., Fabrication of X-ray compatible microfluidic platforms for protein crystallization, Sensors and Actuators B, 2012, 174, 1-9

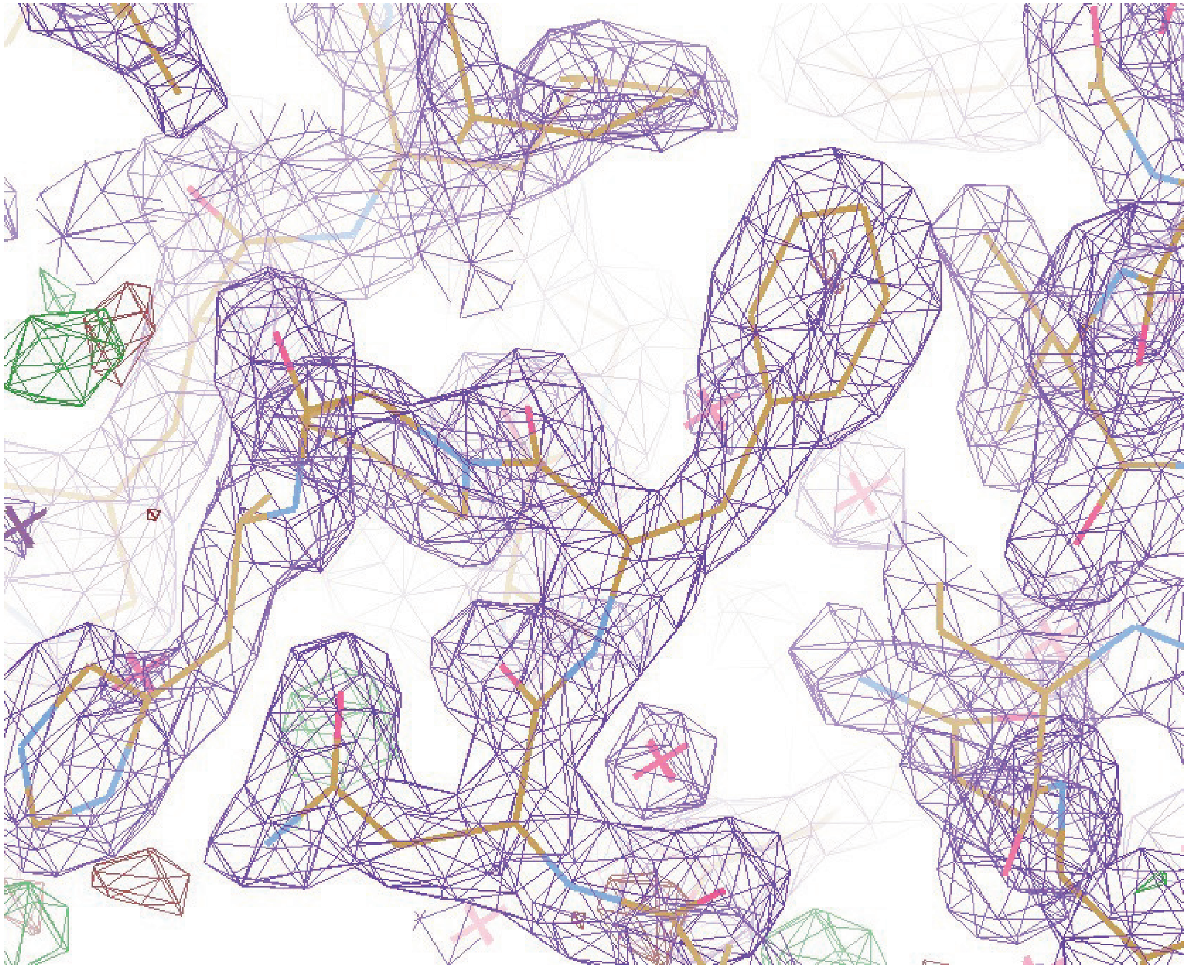
Cornell MacChess – F1



M. Heymann, A. Opthalage, J. L. Wierman, S. Akella, D. M. E. Szebenyi, S. M. Gruner, S. Fraden, IUCrJ (2014). 1, 349–360

Cornell MacChess – F1

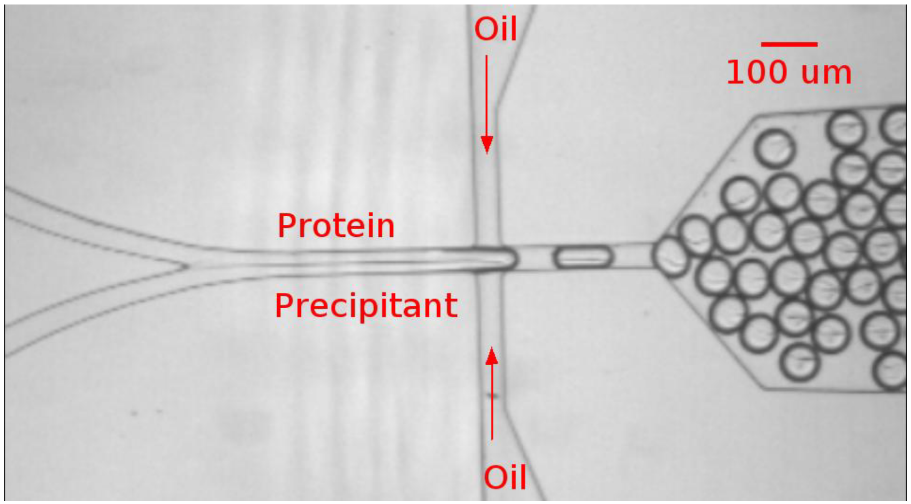
230 frames from 71 Glucose Isomerase crystals = 93% complete set, 2 A resolution



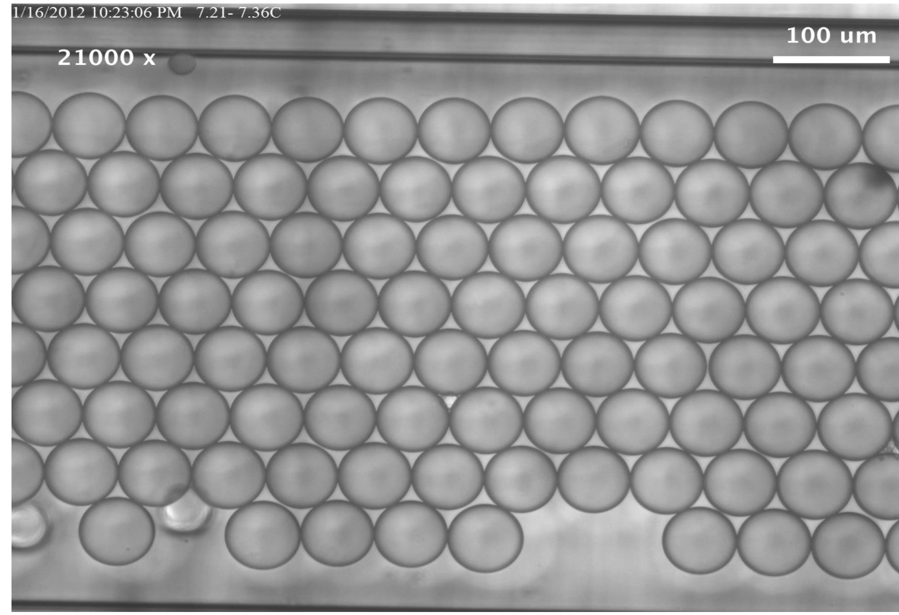
M. Heymann, A. Opthalage, J. L. Wierman, S. Akella, D. M. E. Szebenyi, S. M. Gruner, S. Fraden, IUCrJ (2014). 1, 349–360

refinement: M. Szerbeni

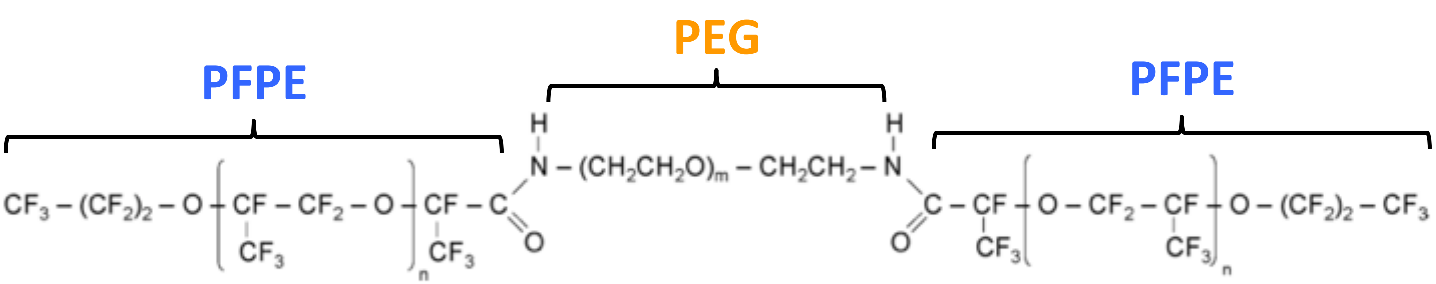
sample preparation

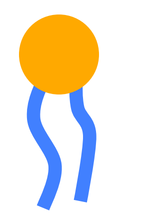


Lysozyme



movies: S. Akella

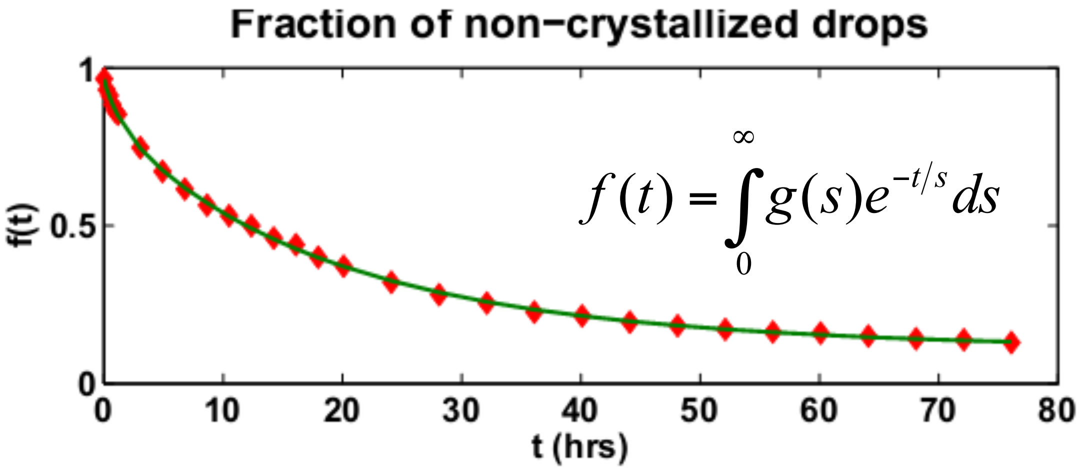


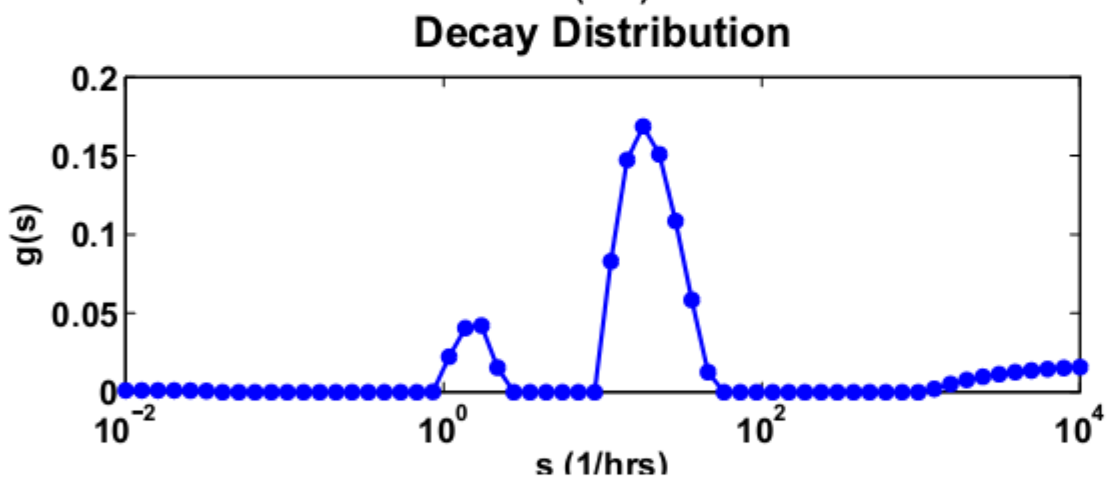


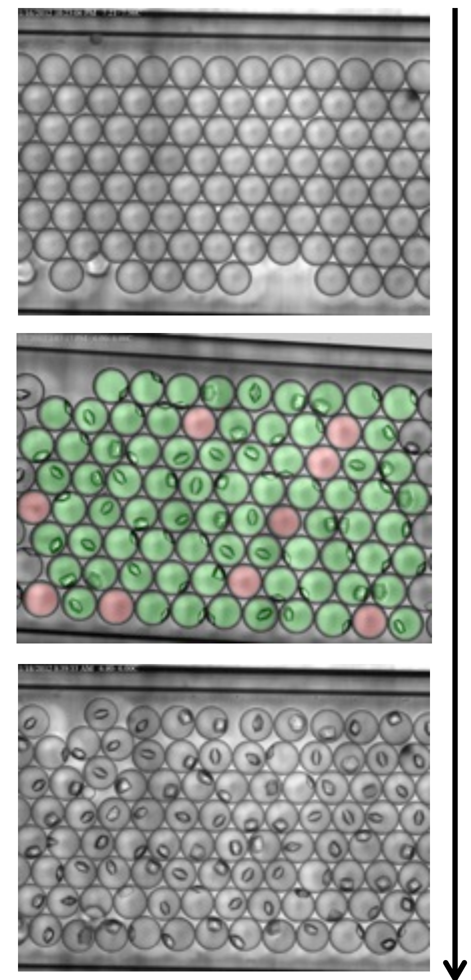
Holtze, C., Rowat, A.C., Agresti, J.J., Hutchison, J.B., Angilè, F.E., Schmitz, C.H.J., Köster, S., Duan, H., Humphry, K.J., Scanga, R.A., Johnson, J.S., Pisignano, D., Weitz, D.A., Biocompatible surfactants for water-in-fluorocarbon emulsions, LabChip, 2008, 8:10, 1632-9

Decay Statistics

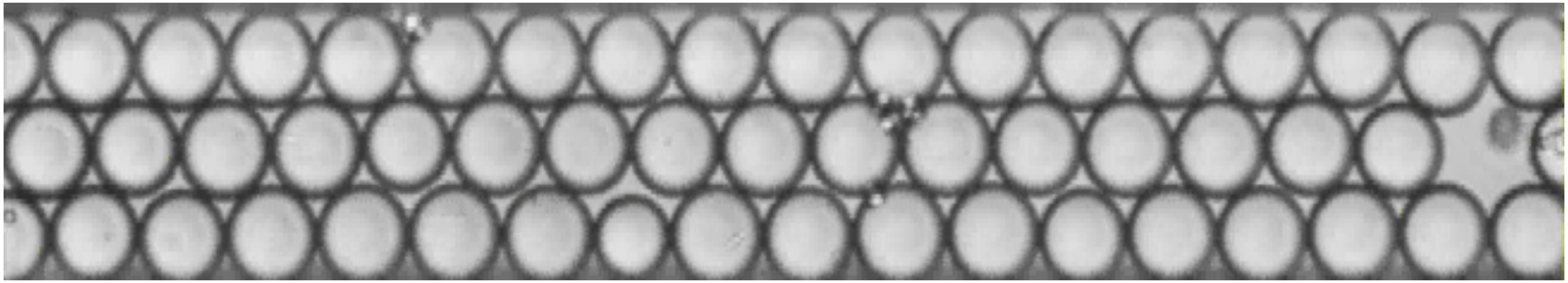
$$f(t) = \frac{N(t)}{N_0} = e^{-JVt}$$



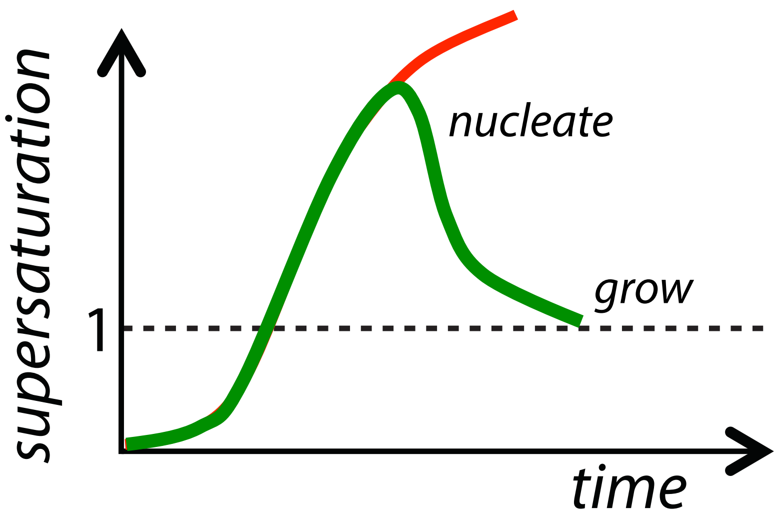


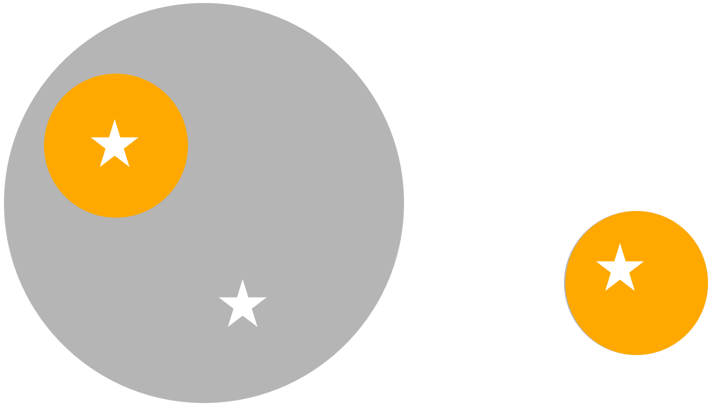


S. V. Akella, A. Mowitz, M. Heymann, S. Fraden
Crystal Growth & Design 14, 4487-4509 (2014)



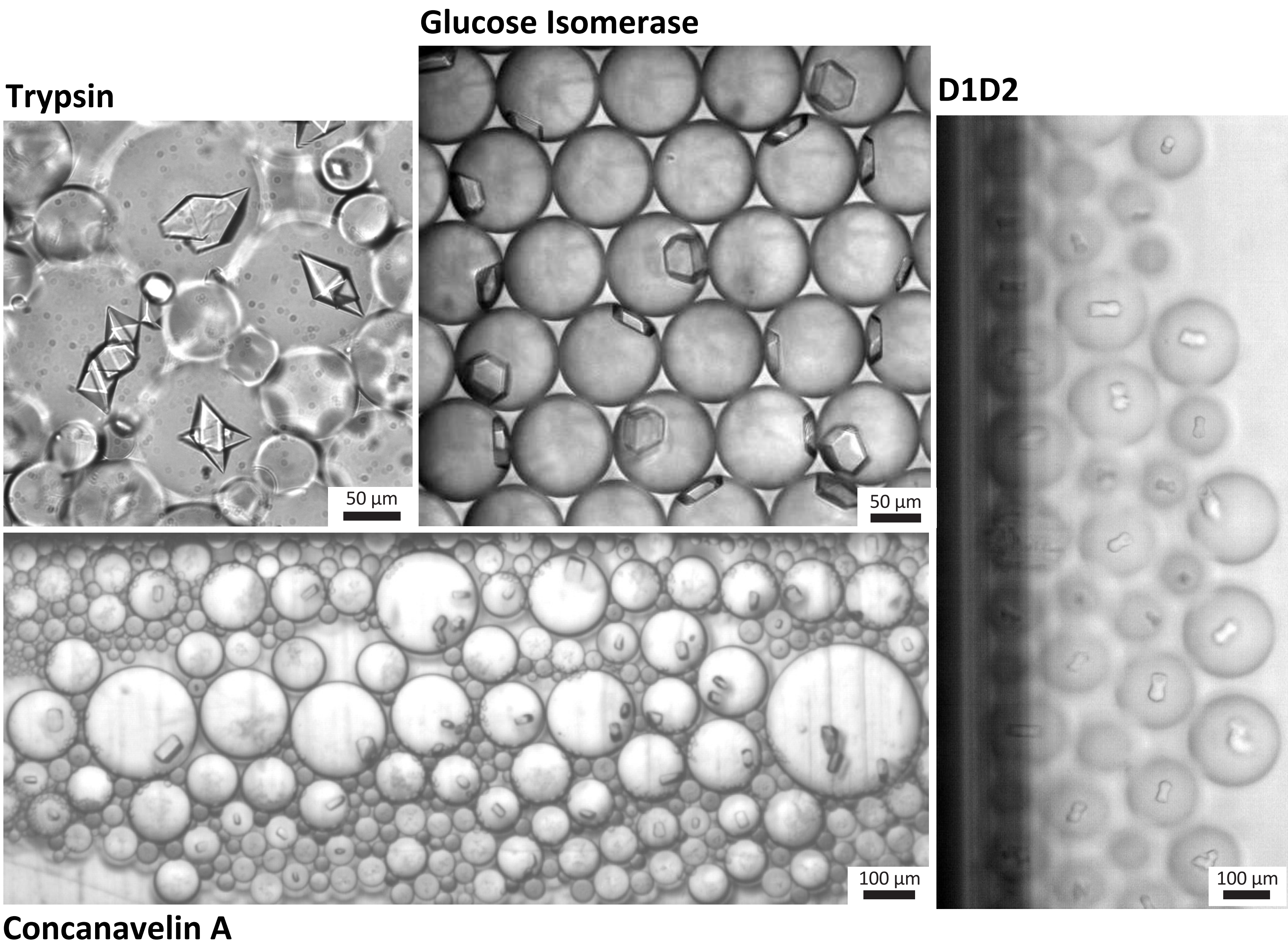
movie: F. Gobeaux

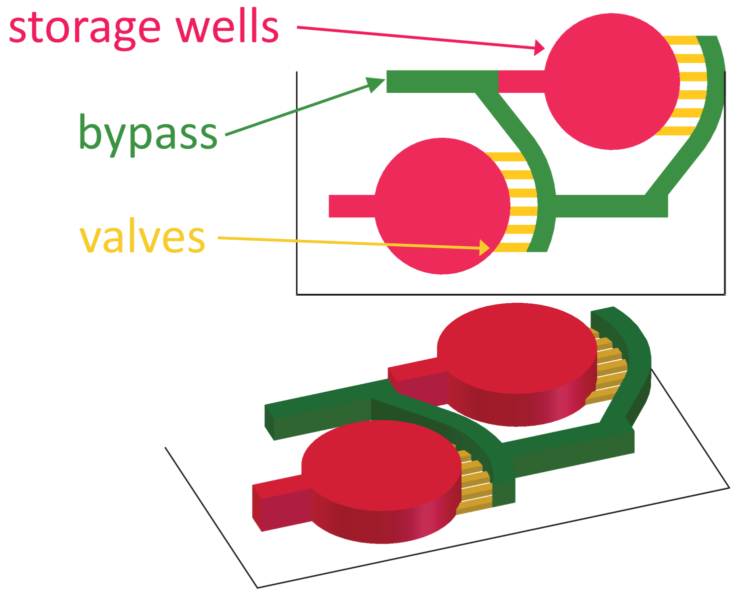




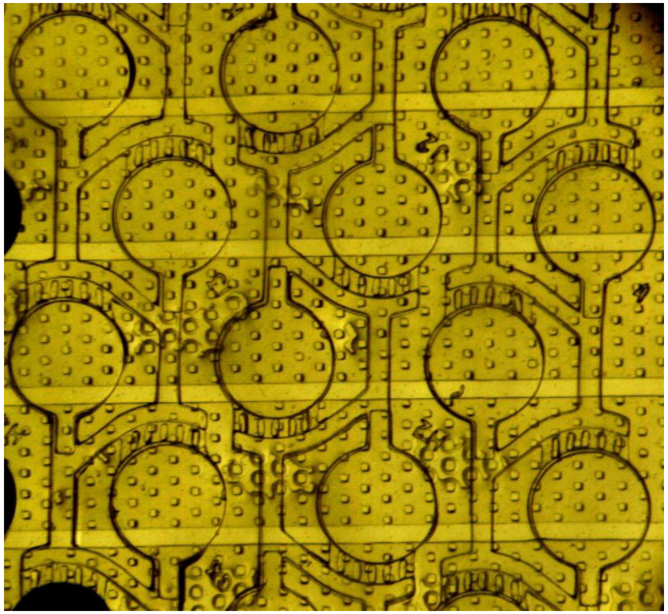
What is the right size of drop?

Dombrowski, R.D., Litster, J.D., Wagner, N.J., He, Y., Modeling the crystallization of proteins and small organic molecules in nanoliter drops, AiChE Journal, 2010, 56:1, 79-91

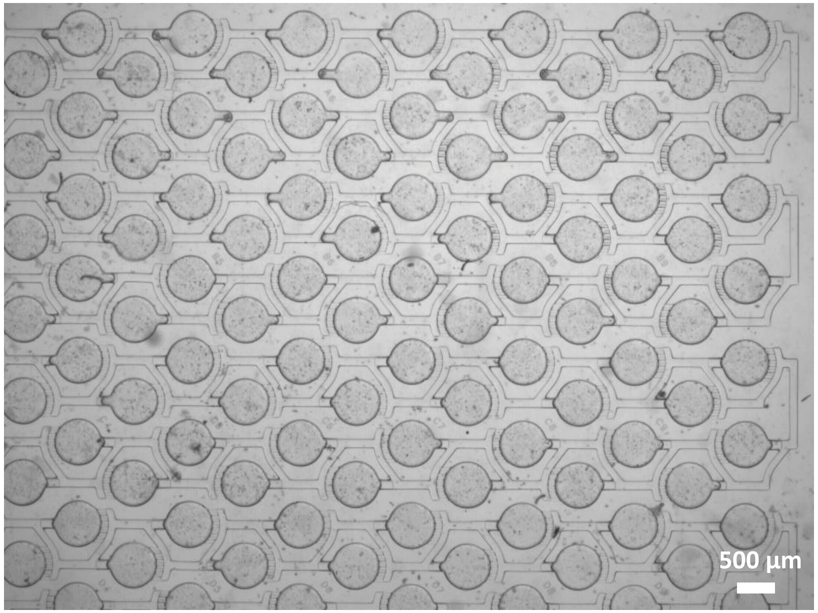

Glucose Isomerase
Trypsin
D1D2
50 µm
50 µm
100 µm
100 µm
Concanavelin A

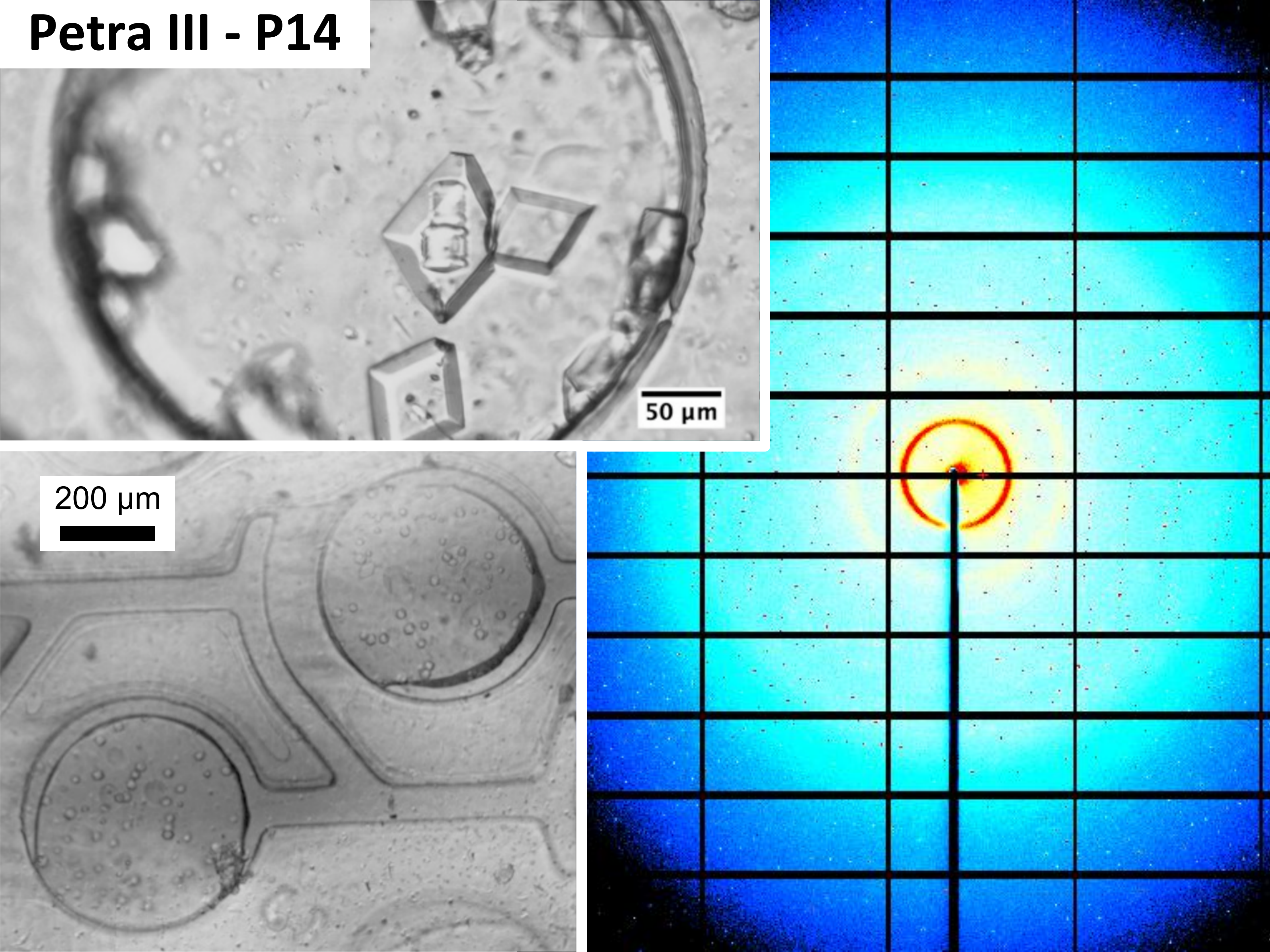


capilary valving to store drop:
H. Boukellal, S. Selimovic, Y. Jia, G. Cristobal, S. Fraden, *Lab on a Chip* 9, 331–338 (2009)



movie: A. Opthalage

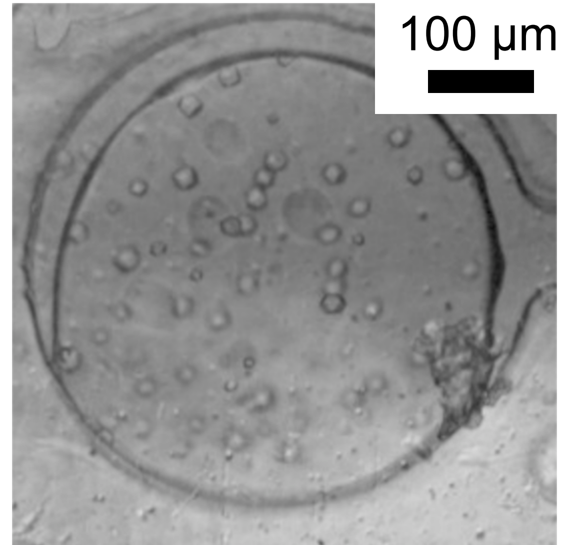


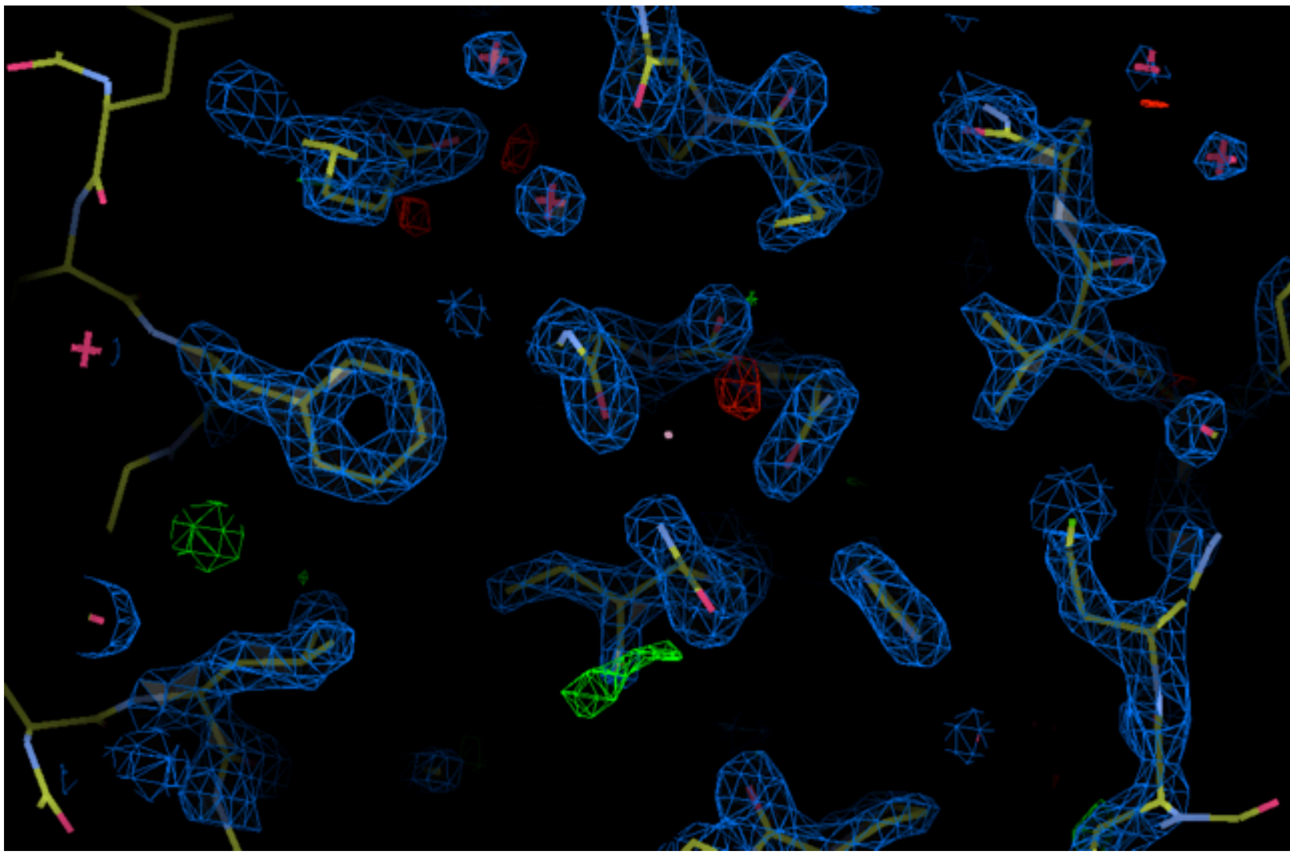

Petra III - P14
50 µm
200 µm

Petra III - P14

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

RESOLUTION	NUMBER OF REFLECTIONS			COMPLETENESS	R-FACTOR	R-FACTOR	COMPARED	I/SIGMA	R-meas	CC(1/2)	Anomal	SigAno	Nano
LIMIT	OBSERVED	UNIQUE	POSSIBLE	OF DATA	observed	expected					Corr		
7.83	5629	611	615	99.3%	18.6%	16.1%	5605	13.25	19.9%	94.6*	-3	0.847	373
5.53	11711	1067	1067	100.0%	17.8%	17.4%	11697	13.25	18.7%	93.9*	5	0.865	785
4.52	15740	1324	1328	99.7%	17.6%	17.1%	15733	14.19	18.4%	96.8*	-7	0.832	1050
3.91	18869	1560	1567	99.6%	18.1%	17.3%	18861	14.33	18.8%	97.4*	0	0.870	1271
3.50	21624	1769	1774	99.7%	17.9%	17.8%	21612	13.68	18.7%	97.3*	1	0.869	1473
3.20	24359	1952	1953	99.9%	18.5%	18.9%	24347	12.66	19.4%	96.2*	1	0.850	1628
2.96	26268	2090	2093	99.9%	20.0%	20.8%	26258	11.31	20.8%	97.3*	-1	0.832	1759
2.77	27935	2242	2246	99.8%	21.6%	22.5%	27929	10.25	22.5%	96.9*	-1	0.844	1880
2.61	28796	2405	2405	100.0%	23.6%	24.4%	28781	9.30	24.6%	95.8*	-2	0.858	2012
2.48	31290	2533	2534	100.0%	26.3%	27.1%	31273	8.48	27.4%	95.6*	1	0.885	2143
2.36	32420	2626	2627	100.0%	27.4%	28.1%	32407	8.07	28.6%	95.8*	-3	0.855	2252
2.26	34268	2776	2776	100.0%	30.2%	30.9%	34259	7.33	31.6%	94.9*	2	0.883	2435
2.17	35202	2878	2879	100.0%	32.7%	33.2%	35192	6.84	34.1%	92.5*	-2	0.856	2492
2.09	36851	2994	2999	99.8%	35.9%	37.2%	36840	6.08	37.4%	93.3*	-1	0.849	2615
2.02	37467	3095	3098	99.9%	41.2%	42.8%	37454	5.19	43.1%	91.4*	-3	0.849	2682
1.96	38131	3190	3195	99.8%	46.7%	48.6%	38111	4.51	48.8%	89.7*	-2	0.826	2749
1.90	38357	3277	3283	99.8%	53.5%	56.3%	38330	3.89	55.9%	86.6*	-2	0.828	2812
1.84	39650	3421	3422	100.0%	64.8%	69.7%	39632	3.07	67.8%	84.1*	-2	0.767	2922
1.80	33575	3465	3476	99.7%	72.3%	79.1%	33537	2.45	76.3%	82.2*	-1	0.739	2760
1.75	19109	3114	3562	87.4%	72.3%	79.2%	18760	1.86	78.2%	69.1*	4	0.765	1695
total	557251	48389	48899	99.0%	23.4%	23.6%	556618	7.24	24.5%	96.6*	-1	0.834	39788





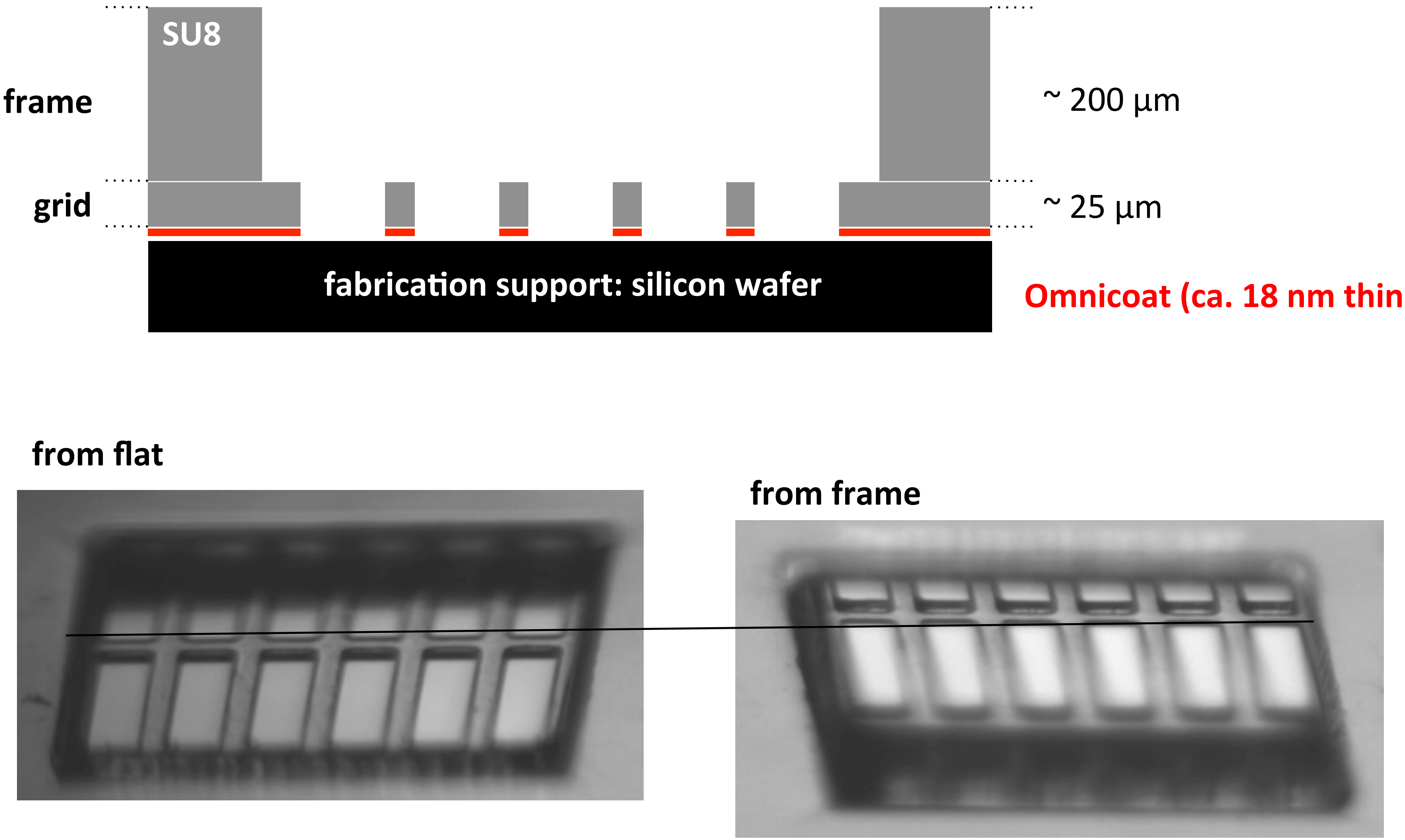
~15 um foil windows
~50 um channel

30 x 30 x 30um Xtals
20 x 20 um Beam

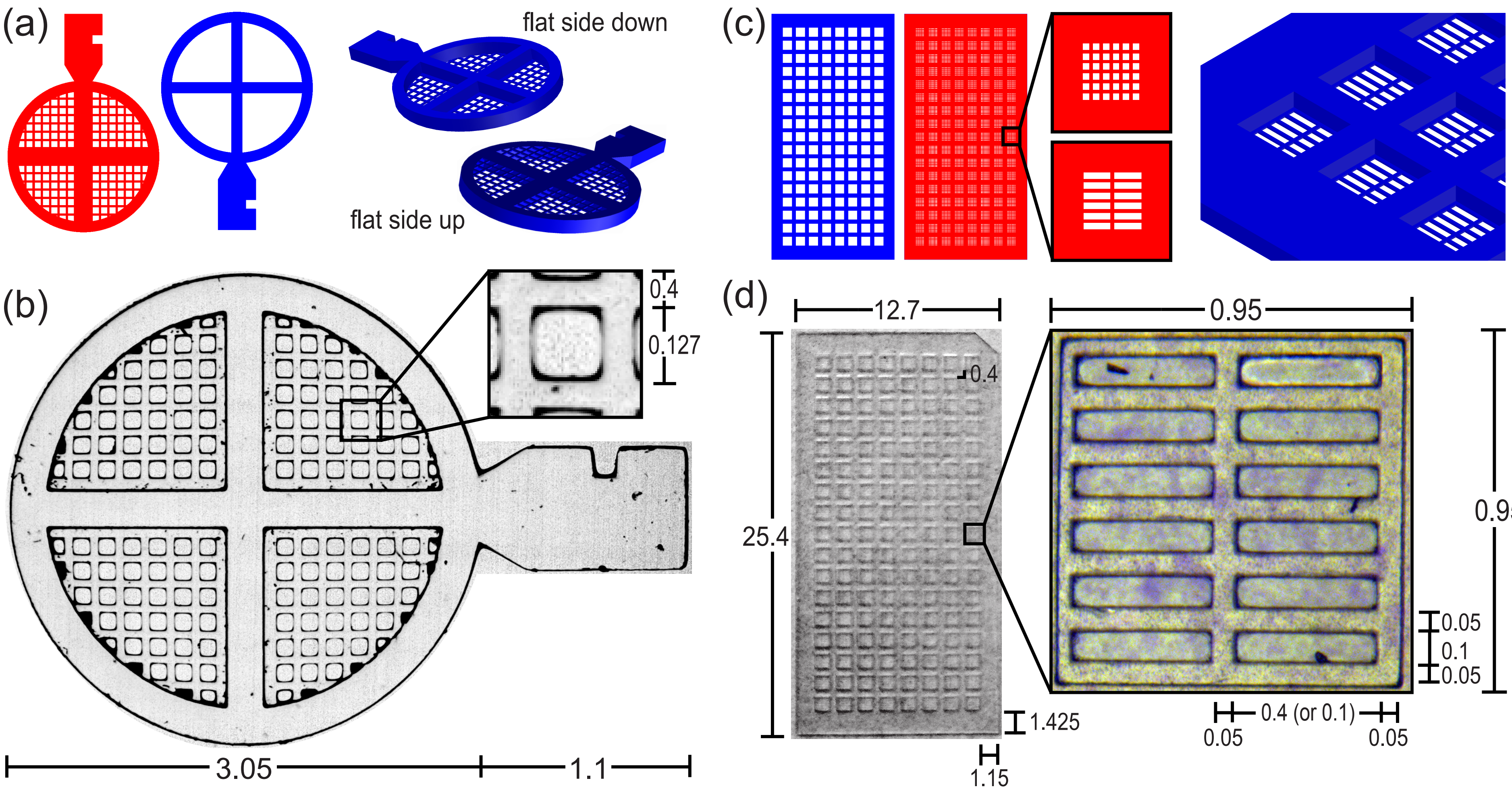
P14
30 ms exposures
100 frames per Xtal
0.1 degree oscillation

XDS
49 of 84 Xtals merged

Phenix
molecular replacement

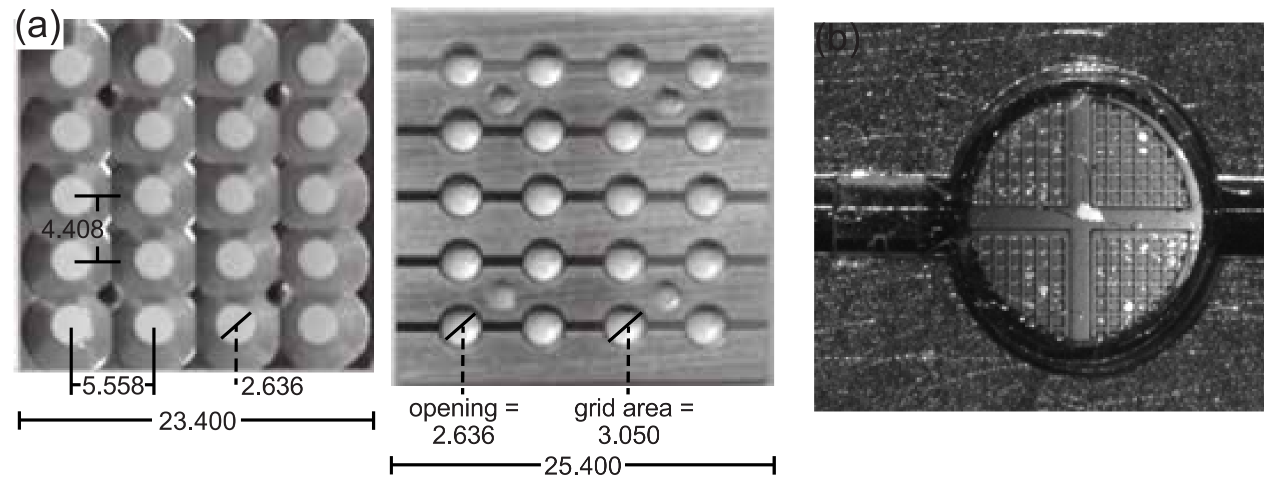

SU8
frame
grid
~ 200 µm
~ 25 µm
fabrication support: silicon wafer
Omnicoat (ca. 18 nm thin)
from flat
from frame

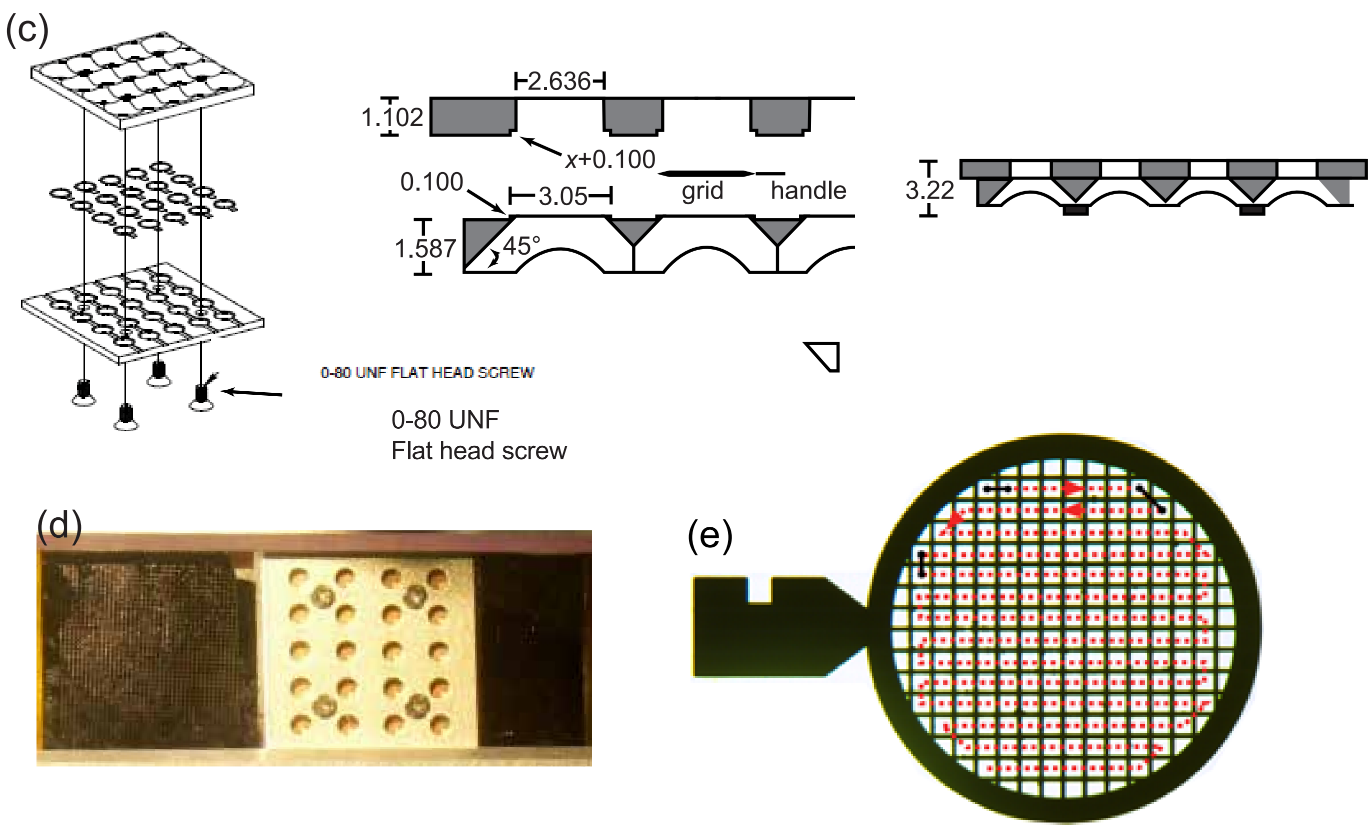
Use TEM grids for 2D/3D crystals?



LLNL style grid holder

(by W. Henry Benner)



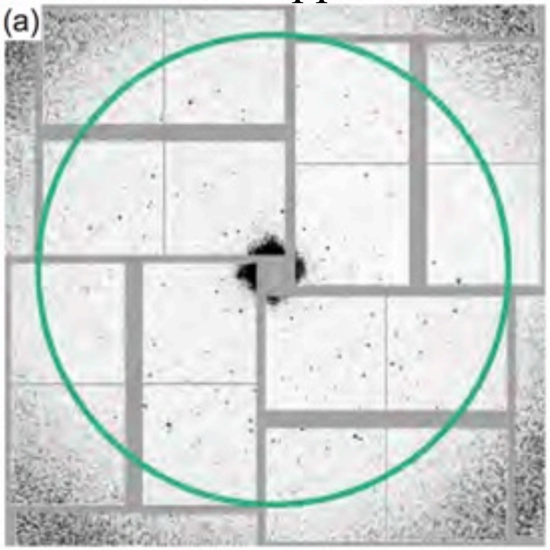


LCLS – CXI

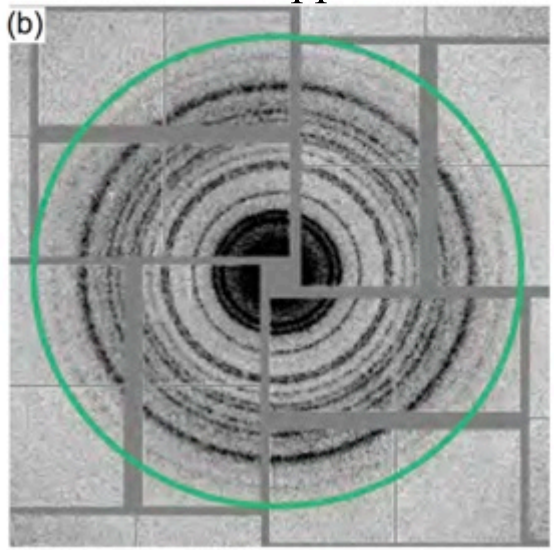
CSPAD detector
hdfsee in Crystfel

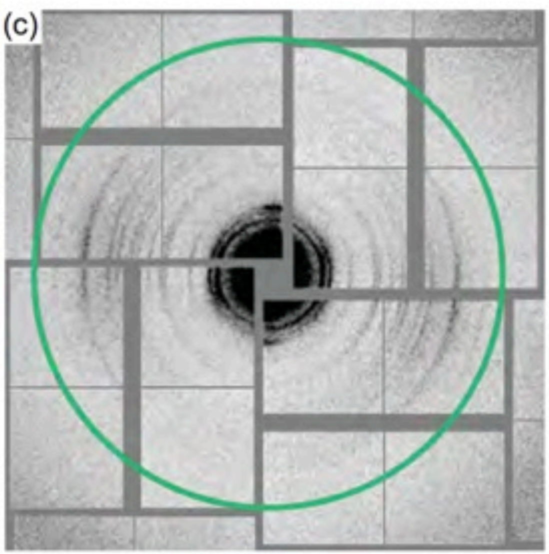
green ring = 7 Å

bR plastic grid, carbon support

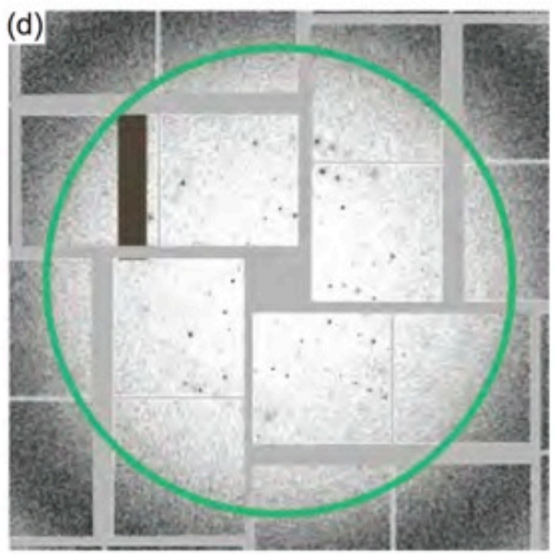


bR Cu metal TEM grid, carbon support





bR plastic wafer, carbon support



PA$^{\Delta MIL}$ plastic wafer, polyvinyl formal support

Efficiency comparisons for fixed-target support materials

Support material	Acquisition time (min / 1000 shots)	Window density (shots / cm^2)	Sample consumption^ (µL / 1000 shots)
Si_3N_4 wafer (100x100)$	16 ± 1	310	7.7
Metal grid (168)#	37 ± 4	538	11.9
Plastic grid (64)#	65 ± 4	205	31.3
Plastic grid (120)$	40.4 ± 0.7	384	16.7
Plastic grid (192)†	25	614	10.4
Plastic wafer (100x400)#	38 ± 2	1567	1.5
Plastic wafer (100x100)$	15 ± 4	522	4.6

#Data collected in May 2013; $Data collected in July 2014
†Numbers extrapolated from assuming the same s/shot rates achieved for 120 shot plastic grids
^Assume 2 µL per 3.05 mm diameter grid, 15 µL per 6.25 cm^2 wafer

Thank you!

Brandeis University
Seth Fraden
Achini Optalage
Sathish Akella
Frederic Gobeaux
Dongshin Kim

Daniel Pomeranz-Krummel
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Jenny Wierman
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Mike Cook
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National Laboratory
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Stefan Hau-Riege
W. Henry Benner
Tommaso Pardini
Matthew A. Coleman
Mark S. Hunter

Linac Coherent Light Source
Sébastien Boutet
Marc Messerschmidt
Garth J. Williams

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