



Celestino Padeste :: Lab. of Nanoscale Biology :: Paul Scherrer Institut :: 5232 Villigen PSI

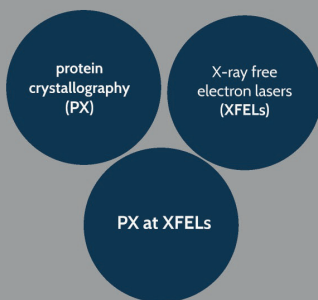
Polymer supports for serial protein crystallography at X-ray free electron lasers

prn2021-online, May 27, 2021

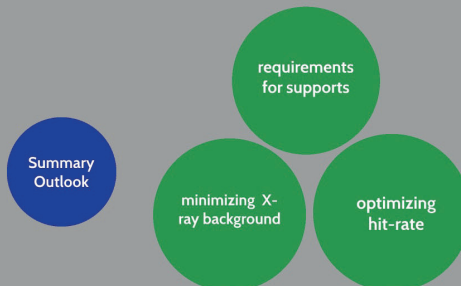


Polymer Supports for Serial Protein Crystallography

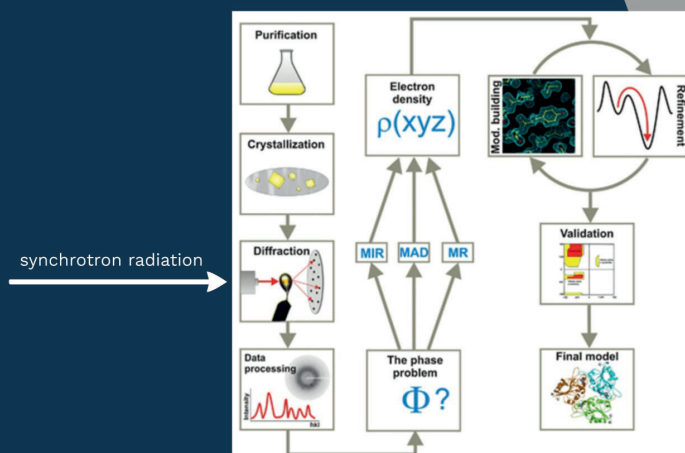
Background: A Very Special Application at Large Scale Facilities



Support Fabrication: Applied Polymer Technology



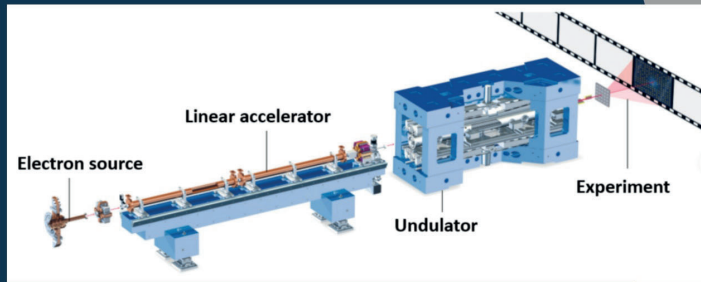
Synchrotron-based protein crystallography



source:
<https://www.xtal.iqfr.csic.es/Cristalografia/>

X-ray free electron lasers

working principle

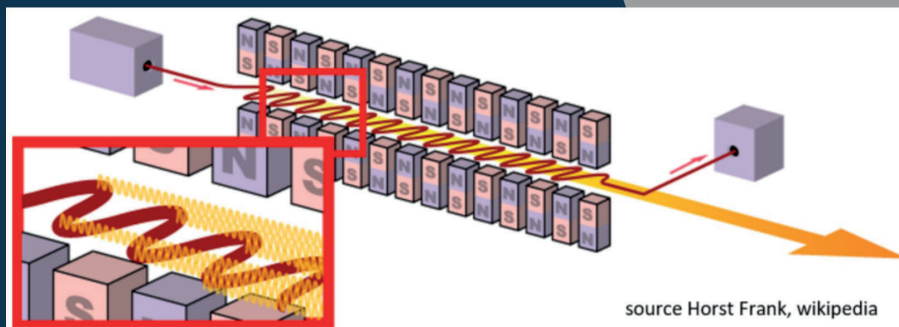


Undulator
scheme

SwissFEL

Undulators
at SwissFEL

Undulators at XFELs



SwissFEL: the Swiss XFEL at PSI



Undulators in the SwissFEL tunnel



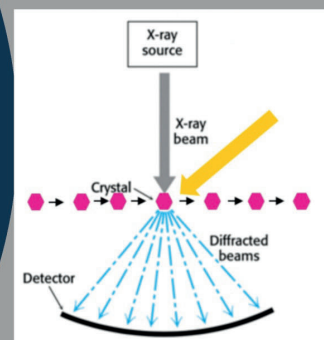
Protein Crystallography at XFELs

"Serial Femtosecond Crystallography" (SFX)

Takes advantage of the extreme pulse intensity
=> Smaller crystals, 2D crystals

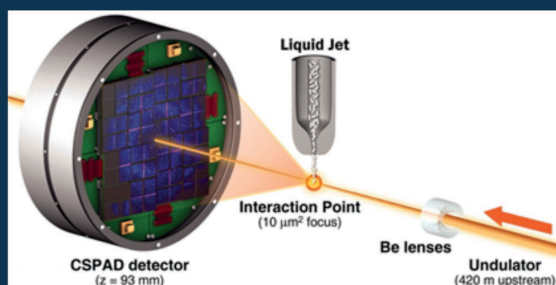
Takes advantage of the ultrashort pulse duration
=> Dynamic studies using pump-probe methods

Works on a "diffract-before-destroy" scheme
Minimized beam damage impact
=> Needs serial sample delivery schemes

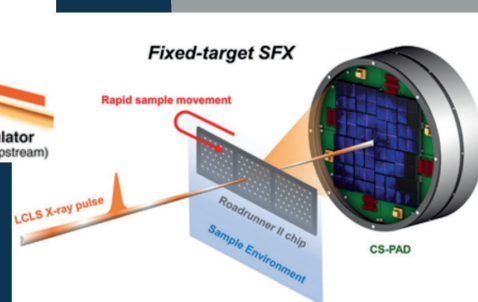


Sample delivery for serial crystallography

Jet methods



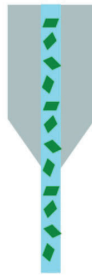
Fixed target methods



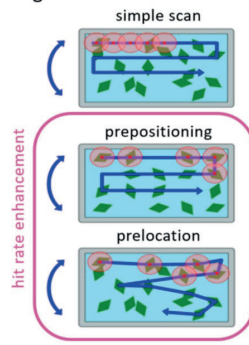
Hit-rate Matters

Crystals are precious => each crystal should be probed
Beamtime is precious => each laser pulse should hit a crystal
Challenge: SwissFEL repetition rate: 100 Hz!

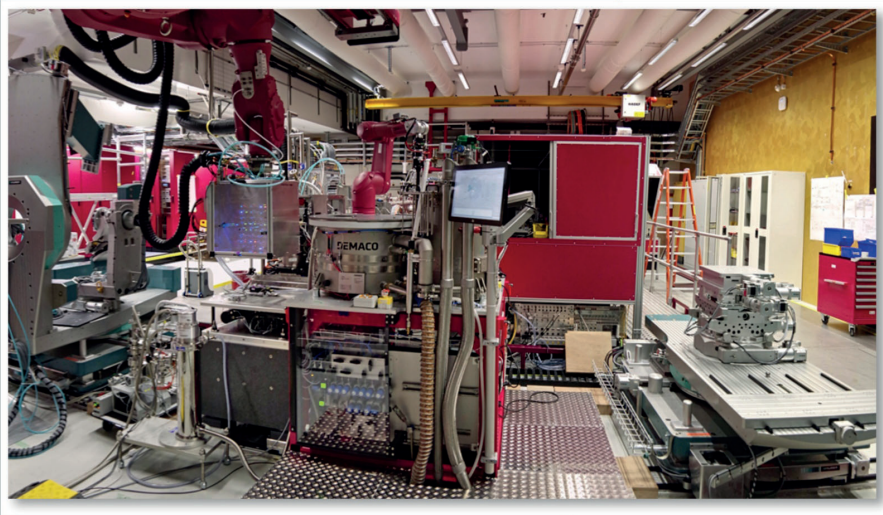
• Stream Jets



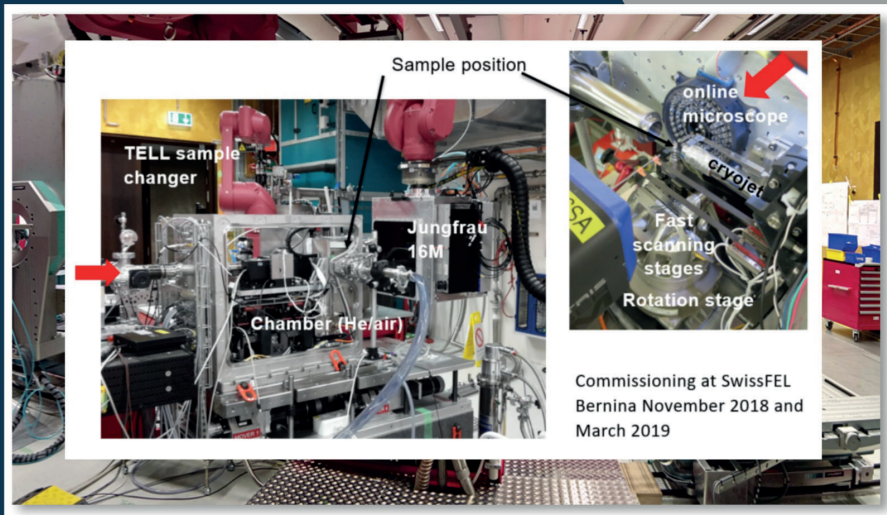
• Fixed-Targets



SwissMX: the fixed-target endstation at SwissFEL



SwissMX: the fixed-target endstation at SwissFEL

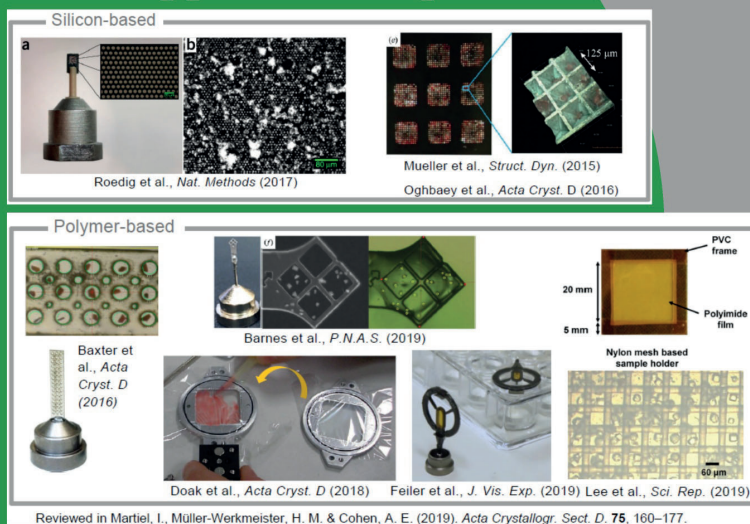


Requirements for Supports

- Low X-ray absorption
- Low X-ray scattering
- Simple sample deposition
- Efficient blotting of mother liquor
- Support shock-freezing (for cryo-measurements)
- Maintain humidity (for RT-measurements)

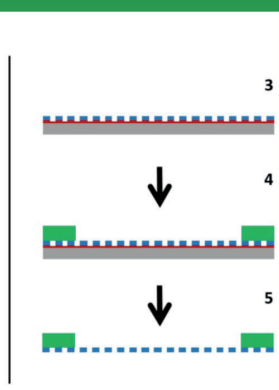
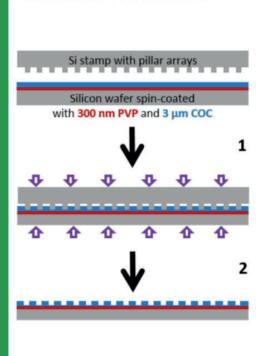
Existing solutions

Solid supports: Examples from Literature

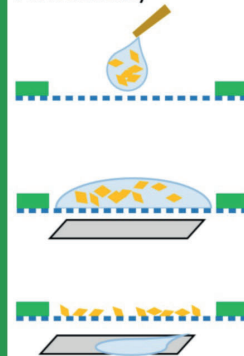


Ultra-thin supports with optimized blotting

Fabrication scheme



Functionality



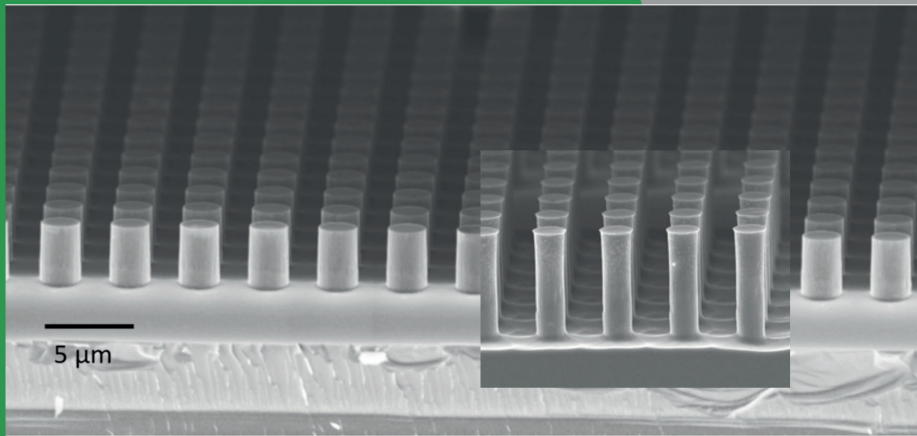
Masters

Membranes

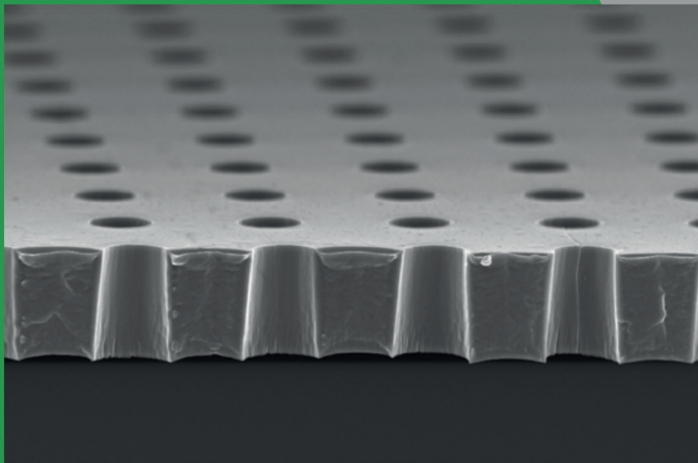
Stability

Products

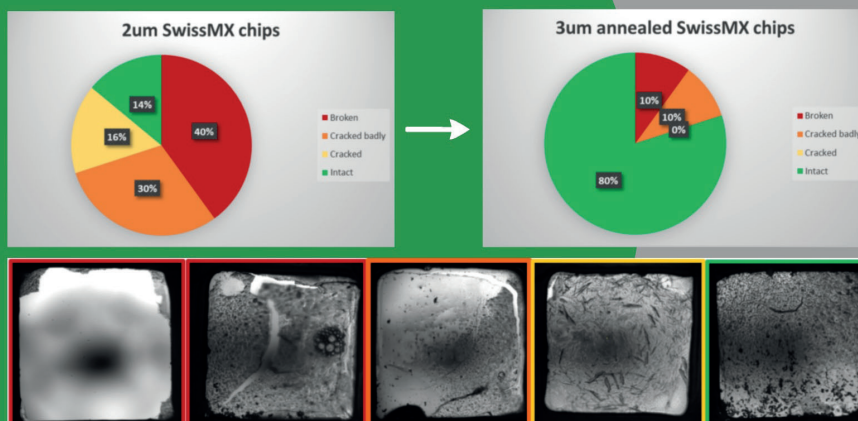
Master fabrication: photolithography and reactive ion etching on 4" silicon wafers



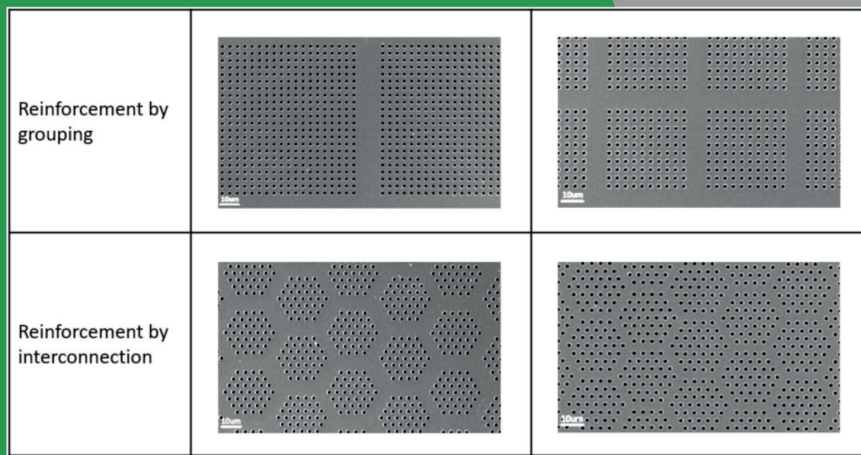
Replication into 3 μm thick COC



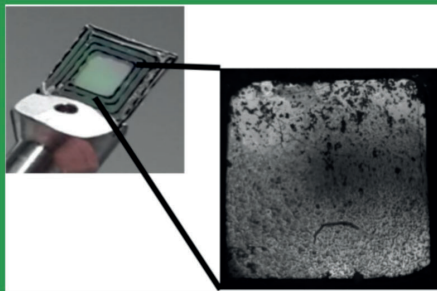
SwissMX cryomesh stability optimization



SwissMX cryomesh stability optimization



Polymer supports for cryo-conditions



Example from the SwissMX commissioning beamtime

- 5-20 μm thaumatin microcrystals with high concentration
- 70 chips collected with max 0.4 T
- 31'000 indexed patterns i.e. 18% overall indexing rate (46'000 using multiple pattern indexation)

Commercialized version

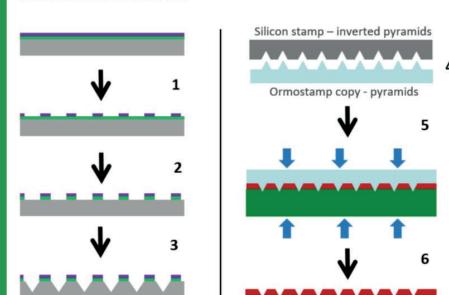


SWISSCI

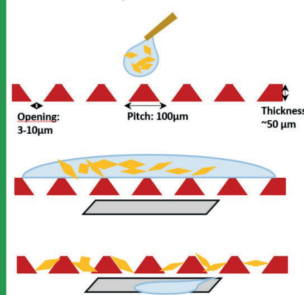
A. Karpik et al. Micro and Nano Engineering, 7, 2020, 100053

Supports with sorting function

Fabrication scheme



Functionality



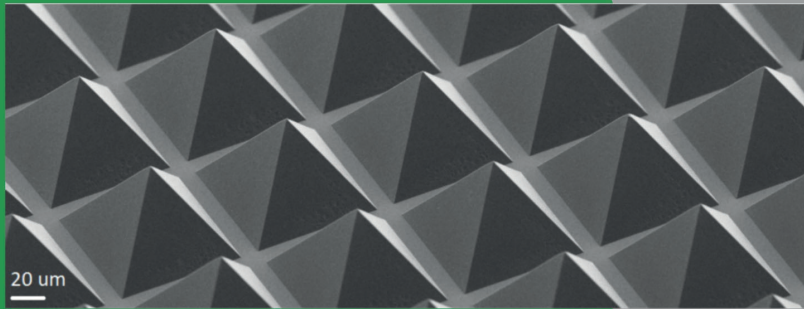
Master

Replication

Sample Deposition

Data collection

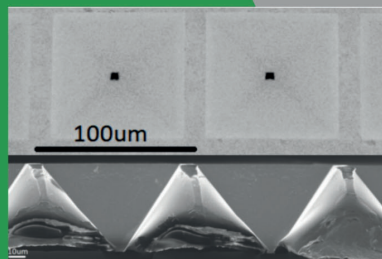
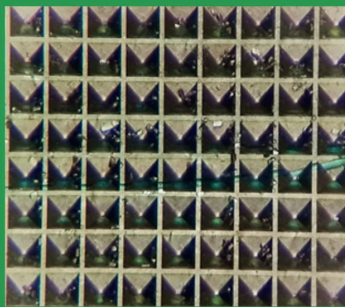
Imprint Master



Ormostamp copy of a structure wet-etched into a silicon wafer

Structures replicated by hot embossing

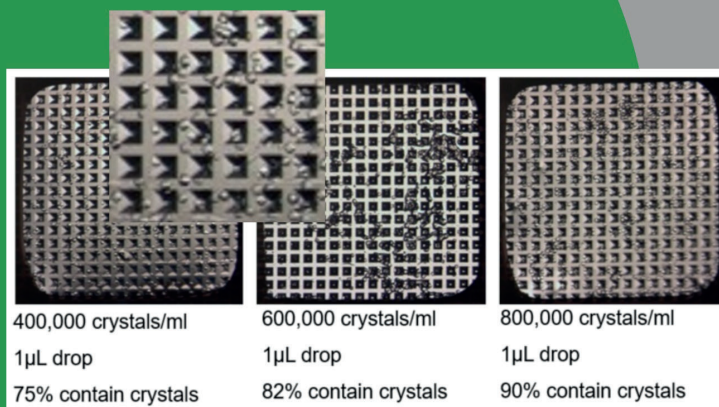
- 50 μm PC film
- 100 μm pitch
- 3-8 μm opening size



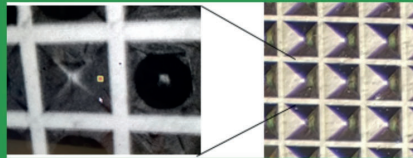
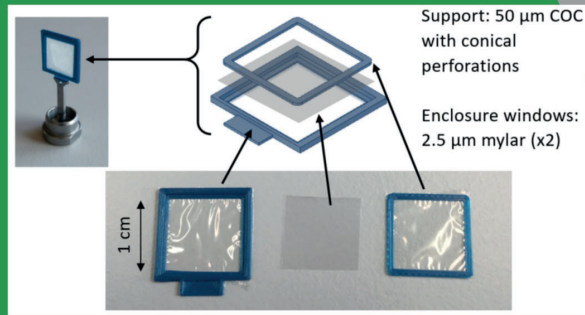
O₂ Plasma



Deposition of Insulin Crystals on Fixed-Targets



Room temperature supports and data collection



4000 indexed images measured on one single chip at RT

Summary and Outlook

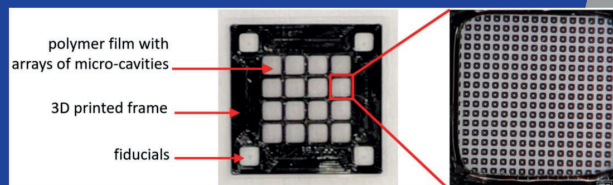
Micro- and nanofabrication using silicon and polymer technologies provide various options for the fabrication structured thin films.

Unique fabrication strategy for ultra-thin structured polymer films

- Explore limits
- Further applications

Films with sorting function

- Suitable for roll-to-roll processing?
- Combine with 3D printed frames
- Application in time-resolved studies



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