PAUL SCHERRER INSTITUT PSI

Wir schaffen Wissen – heute für morgen

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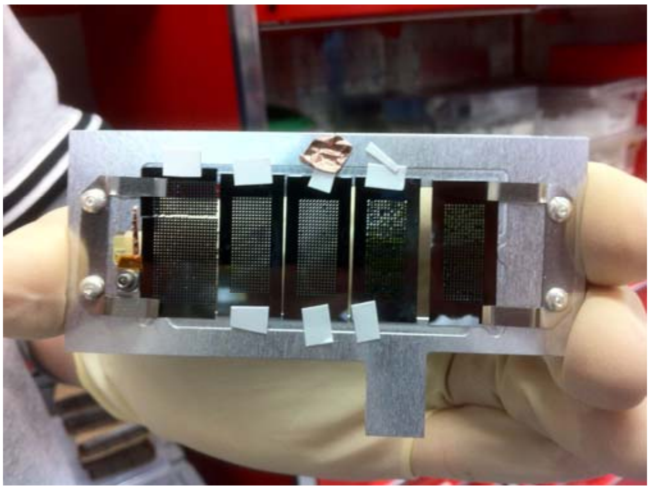
Membrane sample supports for XFEL-based protein crystallography

ISP 9, Lausanne, July 2012

The first successful experiments: what was done and what we learned

2-D crystals deposited on Silicon substrates with Silicon Nitride windows

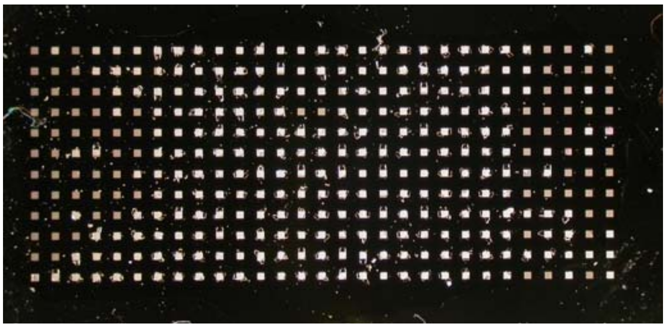


The first successful experiments: what was done and what we learned

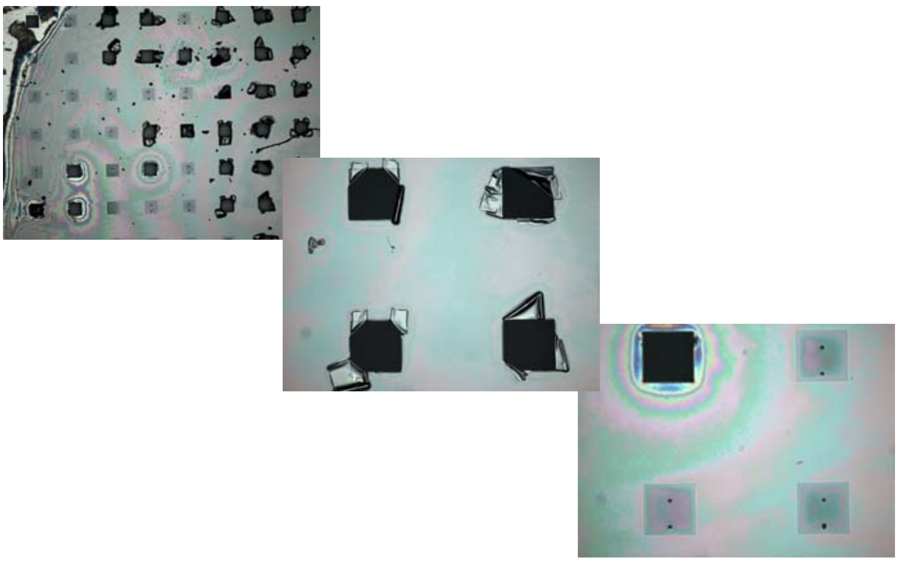
Substrates: 12 mm x 22 mm, 12 x 29 windows, 200 x 200 µm in size, 400 µm distance
Depostion of 2D-Crystal suspension, solution containg glucose, trehalose or paratone

Exposure strategy: two shots per membrane, distance: 100µm
Example: Run #0489 (bR), 553 pulses recorded, 405 show diffraction (spot/ring)

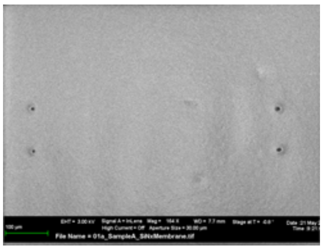
Survey sample A

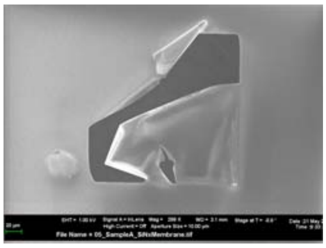


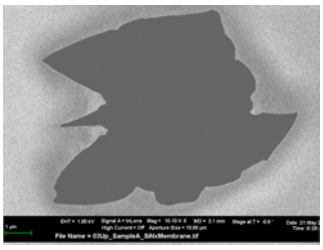
Sample A after experiments at LCLS: optical microscopy



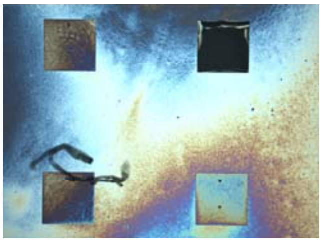
Sample A after experiments at LCLS: scanning electron microscopy

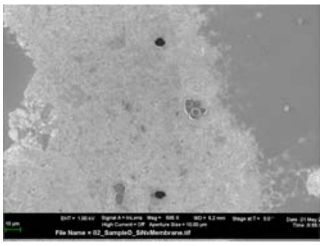


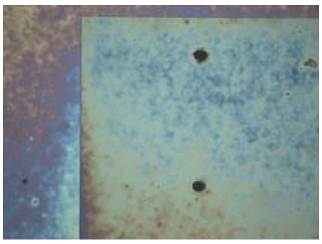


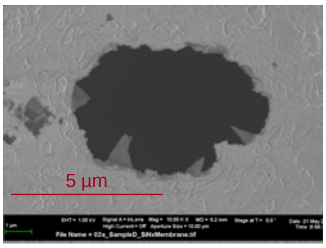


Sample D after experiments at LCLS: optical microscopy and scanning electron microscopy

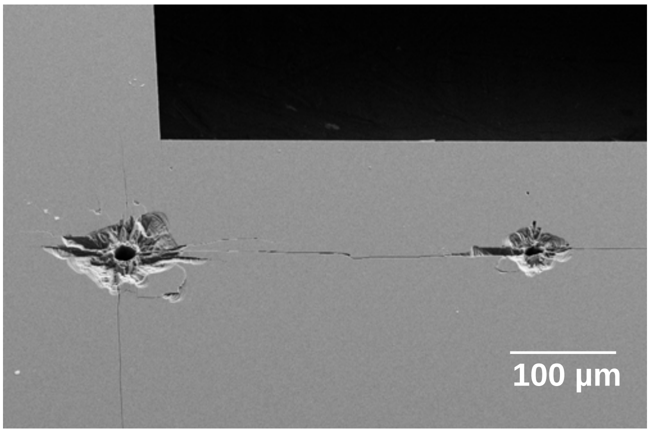




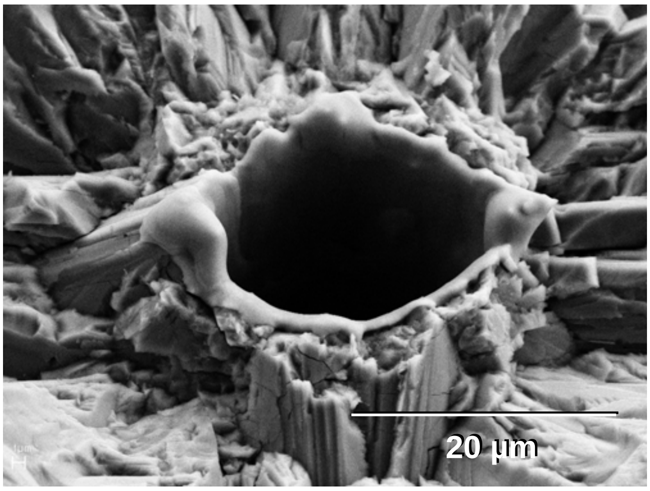




What happens when hitting the frame?



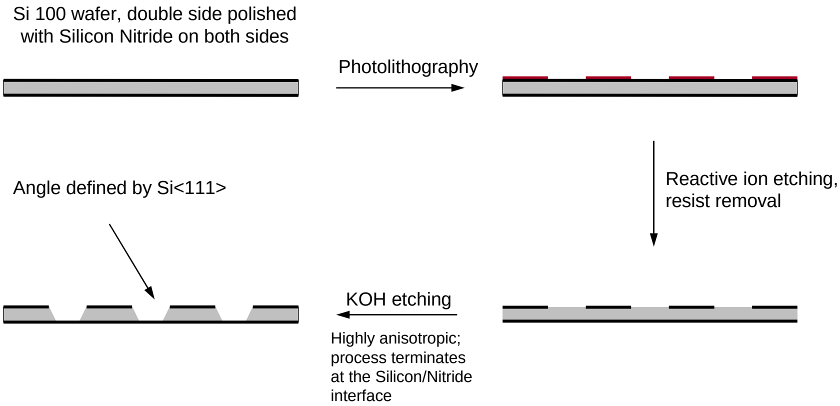
What happens when hitting the frame?



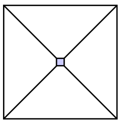
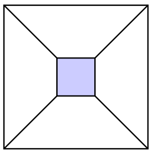
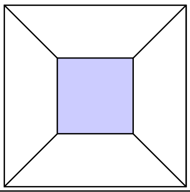
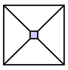
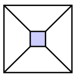
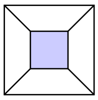
Silicon Nitride: the membrane material of choice ?

- High stability at low thickness
- Established processes for fabrication ← Geometrical Constraints
- Low absorption photons in the keV range ← Low enough, alternatives?
- Amorphous
- Functionalization simple ←Examples
- Structuring/Patterning ←Future Ideas

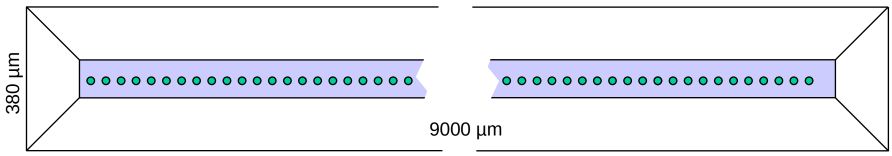
Silicon Nitride Membranes: The Basic Fabrication Process



Optimization of sample field density: square windows in silicon wafers

window size	20x20 µm	100x100 µm	200x200µm
200 µm thick wafer 100 µm wide bars			
space used	300x300 µm	380x380 µm	480x480 µm
Fields per cm^2	25 x 25 = 625	20 x 20 = 400	16 x 16 = 256
Membrane area (%)	0.25	4	10
window size	20x20 µm	40x40 µm	100x100µm
100 µm thick wafer 50 µm wide bars			
space used	160x160 µm	180x180 µm	240x240 µm
Fields per cm^2	47 x 47 = 2209	43 x 43 = 1849	34 x 34 = 1156
Membrane area (%)	0.9	2.9	11.6

Slit windows



200 µm thick wafer
100 µm x 9 mm sized windows

Windows per cm^2: 20
membrane area: 17.8%

Shots at 100 µm distance
➔88 shots per window
➔1760 shots

100 µm thick wafer
50 µm x 9 mm sized windows

Windows per cm^2: 23
membrane area: 10%

Shots at 100 µm distance
➔ 88 shots per window, 2024 shots per cm^2

Shots at 50 µm distance
➔ 178 shots per window, 4094 shots per cm^2

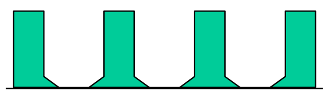
Densities of samples; possibilities and limitations

Summary

Wafer thickness	Window	windows / cm^2	Shots /window	Shots / cm^2	
200	20 x 20	625	1	625	
200	100 x 100	400	1 2	400 800	
200	**200 x 200**	**256**	1 **2** 4	256 **512** 1024	← as used
100	20 x 20	2209	1	2209	
100	100 x 100	1156	1 2	1156 2312	
200	100 x 9000	20	88 (100 µm distance) 178 (50 µm distance)	1760 3560	
100	100 x 9000	23	88 (100 µm distance) 178 (50 µm distance)	2024 4094	

Ways to still increase the density in Silicon/Nitride systems

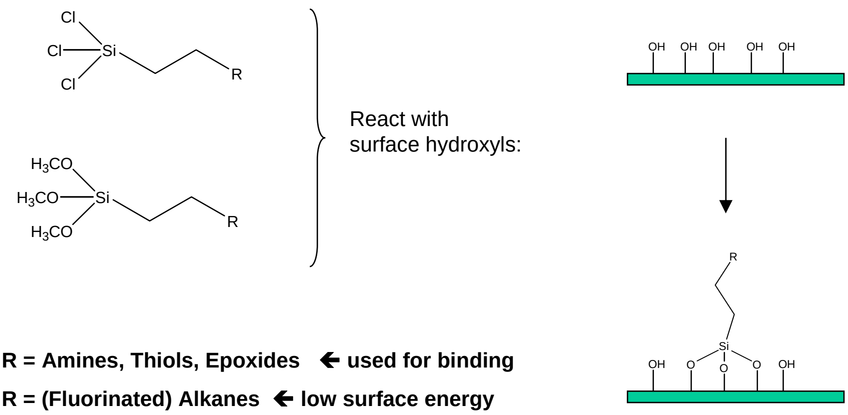
- Dry etching
  + Allows the formation of vertical side walls
  - Process not terminating at the Silicon/Nitride interface
    ➔ Process end / remaining membrane thickness very difficult to control
    ➔ diffraction if crystalline Si remains
- Wet etching in Silicon (110)
  + Process self-terminating
  - Possible geometries limited (line structures)
- Combinations of dry etching (for vertical side walls) and wet etching (self-terminating)



Surface Functionalization of Silicon Nitride

- Physical: Deposition of Materials (Evporation, Sputtering)
  ➔ Metals, Oxides, Carbon, etc.

- Chemical: Nitride is usually covered by Oxide
  ➔ Silanization ➔ many possibilities to define the surface chemistry

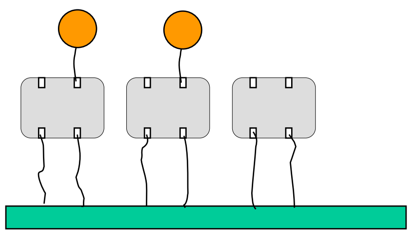


R = Amines, Thiols, Epoxides ← used for binding

R = (Fluorinated) Alkanes ← low surface energy

Affinity-Based Immobilization on Silicon Oxide.
Example: (strept)Avidin-Biotin Technology

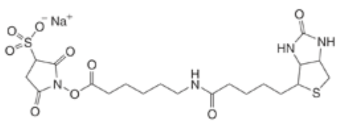
Typical architecture



Avidin:
- egg-white protein
- Ca. 60 KDa
- Tetramer with four binding sites for Biotin (Vitamin H)
- Very strong and quasi-irreversible binding

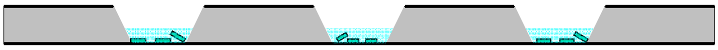
Streptavidin:
- analogon expressed from Streptomyces avidinii
- Advantage: much lower sticking (NSB)

Biotin Linker: bound to silanized SiO_2



Sulfo-NHS-LC-Biotin

Optimizing sample consumption: use the “back side”



Alternative Materials

Transmission of Membrane Materials at 8.5 KeV *

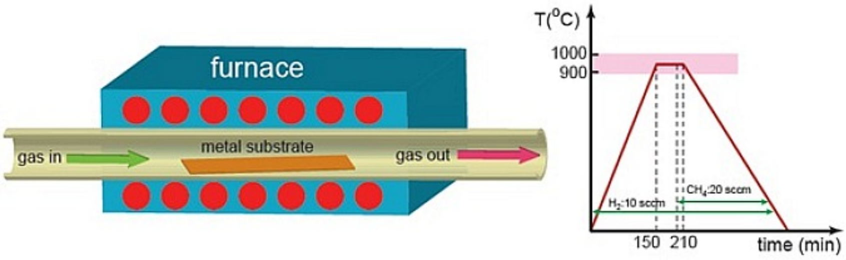
Thickness (nm)	Si_3N_4	C	Polyimide	Polycarbonate
10	99.988	99.999	99.999	
30	99.965	99.998	99.998	
100	99.884	99.992	99.993	99.995
300	99.635	99.976	99.979	99.984
1000	98.849	99.919	99.929	99.945
3000	96.585	99.758	99.797	99.835

* calculated using http://henke.lbl.gov/optical_constants

Main Questions:
- Stability
- Process compatibility
- Functionalization

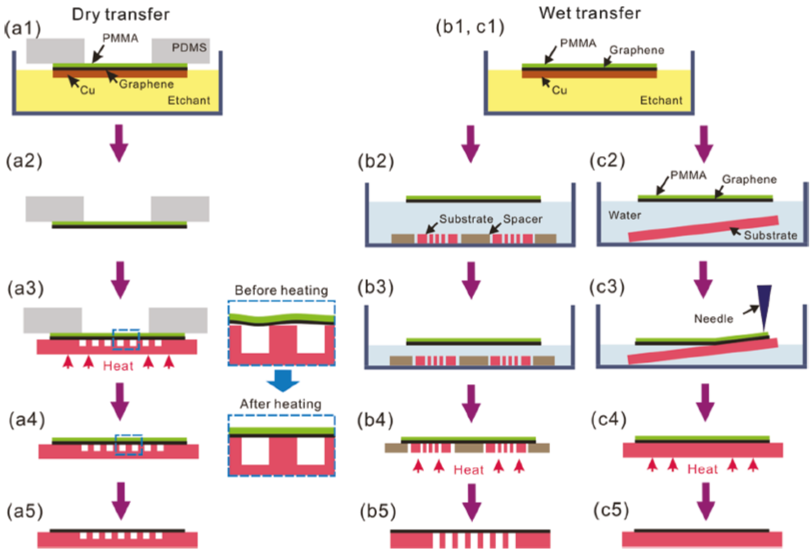
Alternative Membrane Materials: What about Graphene?

Production of large areas of graphene using CVD on metals



Source: http://emps.exeter.ac.uk/engineering/research/functional-materials/researchinterests/graphene/

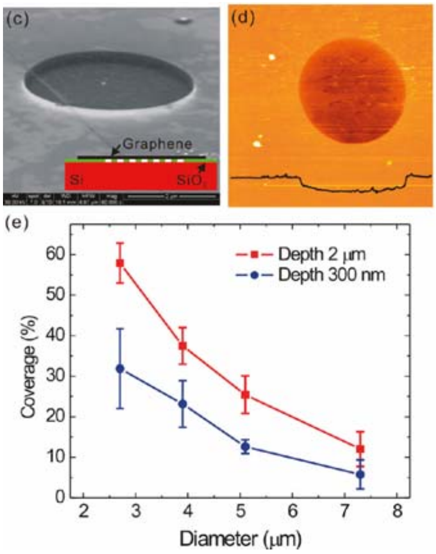
Transfer of Graphene to Grid substrates



J.W. Suk et al., ACS nano 5(9), (2011) 6916–6924.

Transfer of Graphene to Grid substrates

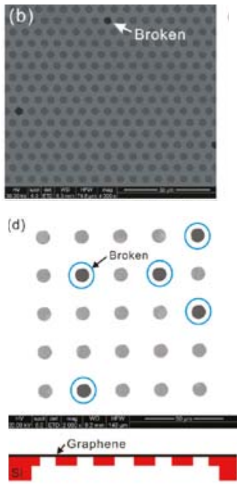
Graphene suspended over wells



Graphene suspended over holes

2.7 µm

10 µm



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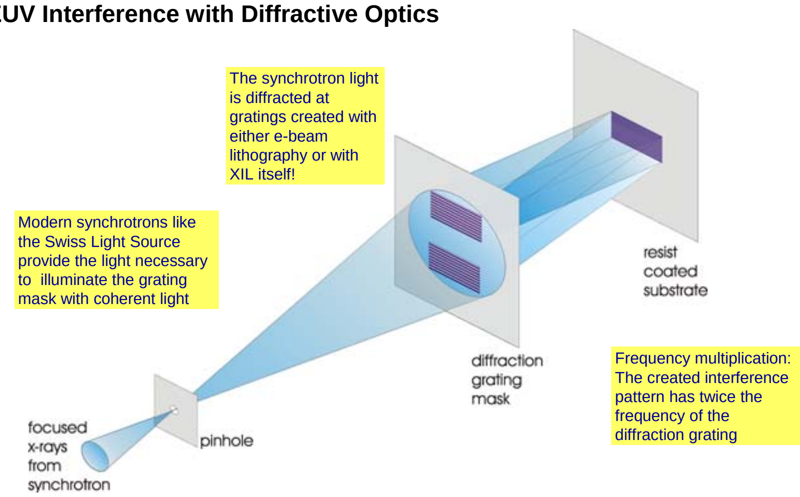
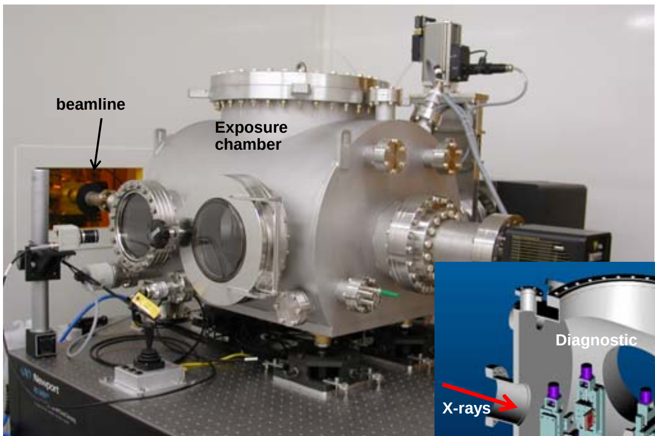
Further ideas: patterned and structured membranes

EUV-Interference Lithography with diffractive optics at the Swiss light source

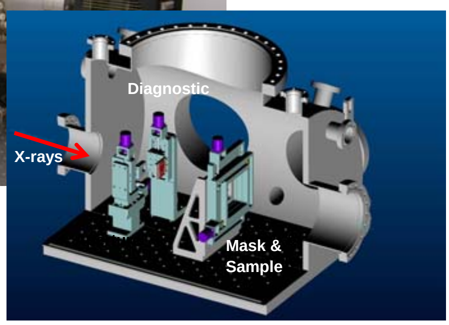
EUV Interference with Diffractive Optics



EUV - Interference Lithography at the SLS



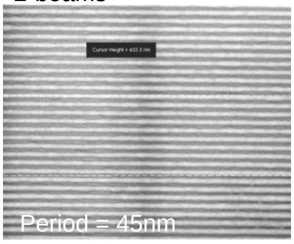
Targets:
10-50nm period
1-4 mm^2 area
10-30 sec exposure



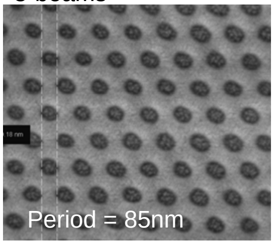
- Combination of high resolution and throughput
- SLS light coherence up to ~100eV
- Sample stage for 4, 5 & 6” wafers
- Mask stage with tilt and gap control
- Software for automatic exposure control

Examples of interference structures produced in PMMA

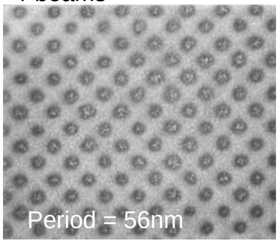
2-beams

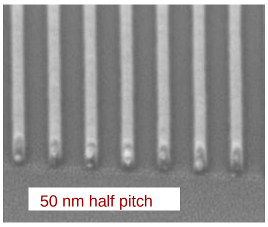


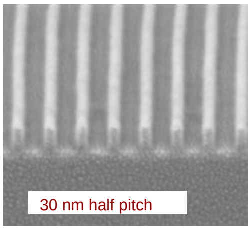
3-beams

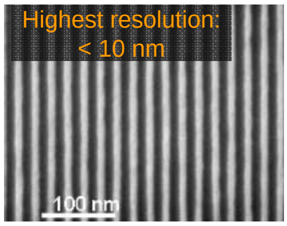


4-beams



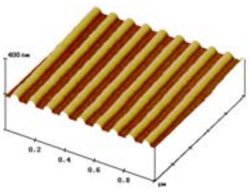






Protein Nanopatterns on XIL Structures on SiO_2

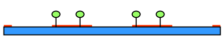
100 nm period PMMA-structures on SiO_2



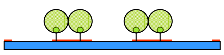
1) Amino-Silane deposition

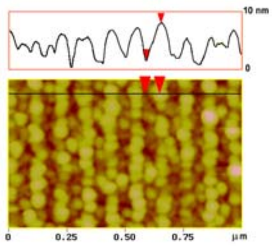
2) Lift-Off in acetone

3) Binding of NHS-LC-Biotin



4) Binding of Streptavidin

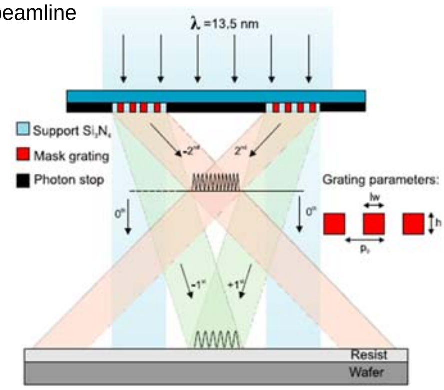


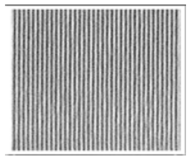


Further Ideas: Patterned and structured membranes

- Planned Project: Nanostructure induced Growth of Protein Crystals

Interference lithography at the XIL-II beamline

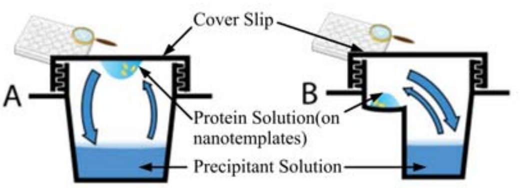




Large area gratings with 8 nm half pitch demonstrated at PSI/LMN

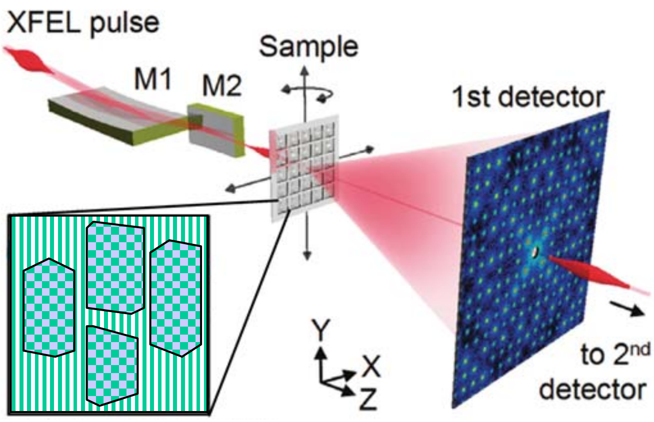
➔ test as templates for protein crystal growth

Protein crystal growth on nanopatterned substrates



Vapor diffusion methods for protein crystallization:
Hanging drop (A) and sitting drop (B)

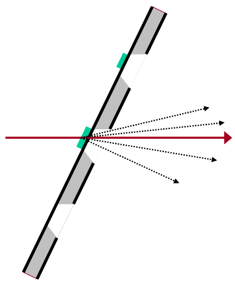
Nanostructured / Nanopatterned membranes



Ordered arrangement of
(2D) crystals on nanotemplates

Nanostructured / Nanopatterned membranes

- Topographical structure ➔ additional diffraction spots expected
  Might be used as internal reference
  (Indicate distortion of the membrane)
- Chemical Patterns ➔no additional diffraction expected
- Ordering of 2-d crystals
  ➔ facilitates indexing
  ➔ exposures at tilt angles
- Growth of 3-d crystals



Growth to sizes for conventional methods ⇔ Diffraction of nanocrystals at different angles.

Conclusions / Suggestions for the discussion

- Silicon Nitride: the membrane material of choice ? (!)
- Commercially available membranes may cover most of the needs
- Special developments are possible in collaboration with LMN

- Other materials: various options of materials with lower photon absorption than Si_3N_4
- Processes less standard
- Transfer of thin films onto grids
- Limited experience at PSI/LMN

- Patterned and structured membranes