

## Introduction to the Micro and Nano Fabrication

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## Fabrication:

## two principal approaches

#### **Bottom up**

Atomic, molecular and micro scale self assembly to generate regular or defined structures with engineered properties



#### Self Assembled

#### **Top Down:**

Combination of various techniques to create a final structure (pattern) with desired shape and size.



#### Machined

## **Bottom-up processes**

#### **Chemical synthesis**

- Nanotubes and nanowires
- Quantum dots and nanoparticles
- DNA
- ...

#### **Functional arrangement**

- Self assembly
  - Mono-layers, e.g. nano-sphere lithography
  - Block copolymers
  - Functionalized nanoscale structures
- Field assisted assembly
- Surface tension directed assembly
- Porous materials, e.g. anodized aluminum oxid



#### GaAs Nanowires (MBE growth)



Block copolymers assembly 3

## Nanosphere lithography (bottom up, self assembly)





#### Block Copolymers Patterning (bottom up, self assembly)



PMMA-c-PS



Top down fabrication: some example

## Micro and nano Lithography













#### Elettra Sincrotrone Trieste



Website: https://fnf.iom.cnr.it/

## Trieste – Friuli Venezia Giulia



## facility of nano **J**abrication

#### Patterning

•Growth

•Etching

Characterisation

- •Uv lithography
- •EBL
- •X-Rays litho
- •...
- •Sputtering
- •Evaporation
- •PECVD
- •...

•RIE

- •WET etching
- •...
- •SEM
- •AFM

•...

#### Clanroom

- Typical contaminants that must be removed prior to photoresist coating:
  - dust from scribing or cleaving (minimized by laser scribing)
  - atmospheric dust (minimized by good clean room practice)
  - abrasive particles (from lapping or CMP)
  - lint from wipers (minimized by using lint-free wipers)
  - photoresist residue from previous photolithography (minimized by performing oxygen plasma ashing)
  - bacteria (minimized by good DI water system)
  - films from other sources:
    - solvent residue
    - H<sub>2</sub>O residue
    - photoresist or developer residue
    - oil
    - silicone

#### ISO 14644-1 Cleanroom Standards

Class		FED STD 209E						
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1µm	≥5 µm	equivalent	
ISO 1	10	2.37	1.02	0.35	0.083	0.0029		
ISO 2	100	23.7	10.2	3.5	0.83	0.029		
ISO 3	1,000	237	102	35	8.3	0.29	Class 1	
ISO 4	10,000	2,370	1,020	352	83	2.9	Class 10	
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100	
ISO 6	1.0×10 <sup>6</sup>	237,000	102,000	35,200	8,320	293	Class 1,000	
ISO 7	1.0×107	2.37×10 <sup>6</sup>	1,020,000	352,000	83,200	2,930	Class 10,000	
ISO 8	1.0×10 <sup>8</sup>	2.37×107	1.02×107	3,520,000	832,000	29,300	Class 100,000	
ISO 9	1.0×10 <sup>9</sup>	2.37×10 <sup>8</sup>	1.02×10 <sup>8</sup>	35,200,000	8,320,000	293,000	Room air	



#### How to create micron-sized patterns

#### **Pattern generation**



#### How to create micron-sized patterns

### **Pattern transfer**

#### Wet etching



#### evaporation and lift-off



Crystallographic planes in Si







#### How to create micron-sized patterns





Photolithography  $\checkmark$ 

UV

EUV

- Particles Beam lithography E- $\checkmark$ beam/ion-beam/Neutral atomic beam lithography
- Interference lithography  $\checkmark$
- **Scanning Probe**  $\checkmark$
- Nanoimprinting  $\checkmark$
- Soft Lithography  $\checkmark$
- Shadow Mask  $\checkmark$











## Types of Lithography; LIGA



- 1. Exposure
- 2. Development



- 4. Stripping
- 5. Replication



X-rays (synchrotron radiation) mask resist substrate



polymer microstructure



mass production in polymers



#### XRL: advantages and disadvantages

#### **Advantages**

- Good resolution (down to 30 nm)
- No interference from dust
- Relatively fast
- Deep penetration to resist, high aspect ratio
- No depth of focus problem

#### High aspect ratio *micro*-structures by XRL



- X-ray masks are very difficult to make
- Conventional lenses cannot focus X-rays
- Expensive (synchrotron radiation source)



3.0 kV X1.70K 17.6 µm

80µm resist structure with aspect ratio > 10. White, APL, 66 (16) 1995.



#### Intersection of the three beams



Three-cylinder photonic crystal structure in ceramic. Exposed by repeated exposures at different tilt angles between the mask and synchrotron. Almost like mechanical drilling. G. Feiertag, APL, 71 (11) 1997.

Photolithography  $\checkmark$ E-beam EBL ion-beam FIB ✓ Particles Beam lithography Helium beam HIM Neutral ato Interference lithography  $\checkmark$ ✓ Scanning Probe Nanoimprintin<sub>{</sub>  $\checkmark$ Soft Lithograph  $\checkmark$ IELION Shadow Mask  $\checkmark$ RAITH

#### EBL



#### **Electron Beam Lithography**

#### System

- Electron source (gun)
- Electron column (forms beam)
- Mechanical stage
- Control Computer





https://smif.lab.duke.edu/pict.htm

#### Information from electron beam-specimen interactions

When electron beam strikes the sample, both photon and electron signals are emitted.



#### Interaction volume



Incident electrons penetrate surface layers to a depth dependent on beam energy and surface composition Carbon 16 µm @ 40 kV 80 µm @ 100 kV Gold 3.5 µm @ 40 kV Multiple elastic and inelastic scattering of electrons in arbitrary directions **Re-emission from a laterally** extended volume surrounding incident beam Minimise volume by heavy metal sputter coating 22

#### Forward/back scattering events



There is no clear-cut distinction of SE and BSE. Typically energy < ~50eV is "called" SE. SE with several eV are responsible for most (not all) resist exposure. Such SE diffuses laterally a few nm, which is one limiting factor for ultra-high resolution EBL.

### Forward/back scattering events

Scattering: spreading of the beam, lost of resolution



**Properties:** 

Very often

Small angle

Very inelastic (i.e. lose energy)

Generation of SE (secondary electrons) with low energy.



#### Properties:

Occasionally (collision with nucleus) Large angles, thus mainly elastic High energy, same range as primary electrons. Large travel length, cause proximity effect.

Backscattering is responsible for resist exposure far from incidence (proximity effect), as BSE can generate SE along its path to expose the resist there.

#### Monte-Carlo simulations of electron trajectory



Scattering probability varies as square of atomic number Z, and inversely as the incident kinetic energy.



Penetration depth decrease with Z.



Number of backscattered electrons is not so dependent on energy, but its spatial distribution is. Proximity effects are "diluted" (spread over larger area) at high energies.

### Effect of voltage on dose



- At small kV, penetration depth is low, so cannot expose thick resist. (e.g. at 0.8kV, penetration depth only ~40nm in PMMA).
- At >2kV, resist sensitivity is higher for lower kV, so faster writing.
- But lower kV has larger beam spot size due to aberrations, and more serious forward scattering, both of which reduces resolution.
- In addition, lower kV has lower attainable beam current that reduces writing speed.
- Therefore, typical EBL is done at >3kV.

#### **Proximity effect**

#### (similar to that in OPC – optical proximity correction)



- Proximity effect is negligible for isolated/sparse fine features.
- It is good for *areal* exposure (e.g. a big square >>1µm), since pixel can be much larger than beam spot size (right figure). E.g., beam step size (pixel) of 50nm is usually enough to give uniform areal exposure, even with a beam spot size only 5nm.
- Proximity effect is worst for dense and fine patterns, such as grating with sub-50nm pitch.

## **Resist profile**

A thin layer may be developed due to exposure by proximity effect

- Due to forward scattering and (to a less degree) proximity effect, positive resist has always an undercut profile, good for liftoff.
- Negative resist always has a tapered profile, bad for liftoff.
- For patterning dense fine features, an undercut profile often causes resist structure to collapse due to capillary force when developer is dried.
- That is, proximity effect makes patterning dense fine features difficult.



Dense AND fine structures

Resist (positive) profile, not mechanically stable





#### **Proximity effects causes underexposure in a test pattern**



- Exposure is affected by the shapes proximity to other nearby shapes in the pattern
- Edges, corners, and narrow, isolated lines are underexposed Retrieved from: Washington Nanofabrication Facility Electron Beam Lithography

# Proximity effect correction can be used to locally vary the dose to compensate for electron scattering



 Simply increasing the exposure dose will result in OVERexposure of the central regions and central lines will be much wider than the edge lines

# Proximity effect correction can be used to locally vary the dose to compensate for electron scattering



- Considers the effects of electron scattering
- Compensates for the effects by electron scattering

Retrieved from: Washington Nanofabrication Facility Electron Beam Lithography

#### Eliminate proximity effect using resist on membrane



(a)Standard PMMA-SiO<sub>2</sub>-Si<sup>+</sup> substrate. An incident electron beam "forward-scatters" slightly in the PMMA and SiO<sub>2</sub> layers. Strong scattering in the Si<sup>+</sup> results in broadly distributed "back-scattered" electrons which expose a wide region of the PMMA.
(b)PMMA-Si<sub>3</sub>N<sub>4</sub> substrate used to make nanogaps with EBL. Two nearby areas are shown being sequentially exposed to an electron

beam while the small "nanogap" region between them is left unexposed.

Fischbein, "Nanogaps by direct lithography for high-resolution imaging and electronic characterization of nanostructures", APL 88, 063116 (2006)



TEM image of nano-gaps with gaps 0.7-6nm

#### Contrast



High contrast:

- Steeper sidewalls
- Greater process latitude
- Better resolution
- Higher aspect ratio structure
- Less sensitive to proximity effect, higher density pattern. Low contrast: good only for 3D gray scale lithography



#### Sensitivity vs. contrast: a dilemma



L: resolution D: dose (sensitivity)

This dilemma is similar to that for EUV resist, where due to shot/statistically noise, higher sensitivity has higher LER (line-edge roughness).

In fact, shot noise is also important for EBL when using very sensitive resists.

Ocola LE and Stein A, JVST B, 24(6), 3061-3065 (2006).

#### No resist has both high sensitivity and high contrast/resolution.

This is not always that bad, because, anyway, even though such resist exist, it cannot be exposed using an inexpensive EBL system – too short dwell time (since high sensitivity) for exposing each tiny pixel (since high resolution), that beam blanker cannot follow. 34

#### Electron beam lithography (EBL)

- 1. Overview and resolution limit.
- 2. Electron source (thermionic and field emission).
- 3. Electron optics (electrostatic and magnetic lens).
- 4. Aberrations (spherical, chromatic, diffraction, astigmation).
- 5. EBL systems (raster/vector scan, round/shaped beam)
- 6. Interaction of electrons with matter (scattering, x-ray, Auger).
- 7. Proximity effect and how to reduce it.
- 8. Resist contrast and sensitivity.
- 9. Several popular resist materials.
- 10. High resolution EBL, resolution limit.
- 11. Grey-scale EBL for 3D structure fabrication.
- 12. Anti-charging techniques.

### Conventional and chemically amplified resists

	n an	resist tone	resolution / nm	sensitivity*	developer
They are	- PMMA	+	10	100	MIBK:IPA
more	ZEP-520	+	10	30	xylene : p-dioxane
popular	_ ma-N 2400	_	80	60	MIF726
	EBR-9	+	200	10	MIBK:IPA
	PBS	+	250	1	MIAK: 2-pentanone 3:1
	COP	_	1,000	0.3	MEK : ethanol 7:3

Table-3.8. Conventional e-beam resists and properties

\* sensitivity measured at 20 keV beam energy, unit:  $\mu$ C/cm<sup>2</sup>.

	resist	tone	prebake	exposure dose	postbake	development	minimum feature
	APEX-E	+	90° C	$3 \sim 6 \ \mu C/cm^2$	85° C	MF319 1 minute	150 nm
	AZPF514	+	120°C 2 minutes	$5 \sim 15 \ \mu C/cm^2$	60° C 1 minute	AZ518MIF 1 minute	100 nm
$\rightarrow$	UV3	+	150° C 1 minute	$20 \sim 30 \ \mu C/cm^2$	140° C 1 minute	CD26	<50 nm
	SAL601		90° C 10 minutes	$5 \sim 15 \ \mu C/cm^2$	115°C 1 minute	MF322 2 ~ 5 minutes	<100 nm
	SNR-200		120° C 2 minutes	>6.5 $\mu$ C/cm <sup>2</sup>	110°C 2 minutes	MF CD-14 20 seconds	<100 nm
	UVN30		140° C 90 seconds	$5 \sim 15 \ \mu C/cm^2$	130° C 40 seconds	MF702 30 seconds	<50 nm
	AZPN114		120° C 2 minutes	$5 \sim 15 \ \mu C/cm^2$	105° C 5 minutes	AZ518MIF 10 ~ 30 seconds	<50 nm
$\rightarrow$	NEB-22	_	110°C 2 minutes	$7 \sim 12 \ \mu C/cm^2$	95° C 2 minutes	MF321 2 ~ 5 minutes	<50 nm

 Table-3.10. Comparison of some commercial chemically amplified resists
#### The standard EBL resist: PMMA (positive)

- The most popular e-beam resist, very cheap and last forever, easy handling.
- Very high-resolution and contrast.
- Typical molecular weight is 950kg/mol. Lower M<sub>w</sub> (e.g. 15kg/mol) leads to higher sensitivity but lower contrast.
- Usually dissolved in a solvent: chlorobenzene, or anisole (less toxic, 2-4%).
- Developer mixtures can be adjusted to control contrast and sensitivity.
- The downside: low sensitivity, poor dry etch resistance (good for liftoff, not for direct etch pattern transfer).



#### **PMMA developer**

Table 3.3 Influence of developer con	centration on resist resolution
--------------------------------------	---------------------------------

Developer concentration MIBK:IPA)	Sensitivity	Resolution	
1:3	Low	Extremely high	
1:2 (MIRK: mothyl icohutyl kotono)	Medium	Very high	
1:1	High	High	
Pure MIBK	Very high	Low	

The dilemma again: higher sensitivity comes with lower contrast

- 1. MIBK : IPA (isopropanol)=1:3 for typically 60sec, most popular developer.
- 2. Cellosolve (2-ethoxyethanol): methanol=3:7 for 7-10sec, claimed by some to have slightly higher contrast than MIBK.
- 3. MEK (methyl ethyl ketone) : ethanol=26.5:73.5 for 2-5 second also works well.
- 4. IPA :  $H_2O=7:3$ , co-solvent system, i.e. neither IPA nor water alone dissolves exposed PMMA. Claimed by some to have better performance than MIBK.
- [1] "Enhanced sensitivity in the electron beam resist PMMA using improved solvent developer", Mohsin and Cowie, *Polymer*, 1988, page 2130.
- [2] "New high-contrast developers for PMMA", Bernstein and Hill and Liu, J Appl. Phys., 71(8), 1992, page 4066.
- [3] "Comparison of MIBK/IPA and water/IPA as PMMA developers...", Microelectronic Engineering, 61-62, 745-753 (2002).

#### PMMA dose table

	10kV	20kV	30kV
Area dose (µC/cm <sup>2</sup> )	100	180	250
Line dose (nC/cm)	0.5	0.9	1.3
Dot dose (fC)			1.5

Doses for MIBK:IPA=1:3 developer 60second.

All values are good starting points, need dose test before each writing. Use the above *area dose* only for large features (>proximity effect range). Otherwise, e.g. when writing  $1\mu m$  stripes,  $250\mu C/cm^2$  is not enough.

Proximity effect factor b can be estimated (very roughly) from the above table:

Real dose D=E(1+b) E is as-exposed dose b is due to proximity effect For line dose 1.3nC/cm, written line-width is ~15nm. So area dose 1.3nC/(15nm×1cm)=867 $\mu$ C/cm<sup>2</sup>. Therefore, 1+b=867/250, so b=2.5 (not small)

b=1.7 if estimated from the dot dose ((15nm)<sup>2</sup> dot).

#### The most popular commercial resist: Zep-520 (positive)

- Developed by ZEON in Japan to replace PMMA.
- Higher sensitivity (3-5x faster), and higher etch resistance (3x)
- For ultrahigh resolution (sub-10nm), PMMA *might* still be better.
- Expensive: \$1000/100ml.
- One-year shelf time, making it more expensive compared to PMMA.
- Composition: methyl styrene/chloromethyl acrylate copolymer.

#### Developer:

- ZED-N50 (100% n-Amyl Acetate)
- Xylene (o-,m-,p- mixed)

#### Solvent:

Anisole, for liftoff or diluting the resist for thinner film.



#### HSQ: hydrogen silsesquioxane (negative)

- Silicon dioxide based inorganic material (not polymer).
- Sensitivity and contrast similar to that of PMMA (depends on developer strength).
- Very high resolution and very dense pattern when using <25nm-thick film.
- Exposed HSQ is in the form of amorphous oxide, good etching mask.
- It is an unusual resist: development by chemical reaction (un-exposed HSQ reacts with diluted NH<sub>4</sub>OH or NaOH developer to produce H<sub>2</sub>), not by dissolution; and development "saturates" (i.e. no more reaction) after a certain time.
- Salty developer (add NaCl to NaOH solution) increases contrast.



HSQ structure, Product of Dow Corning under product code Fox12™

#### **Contrast curves of HSQ**



Fig. 3.32 Contrast curves of HSQ resist at different development temperatures

HSQ is not stable. So spin-coating, baking, writing and development must be done quickly.

E.g. 1 hour delay for development can increase feature size by 60%.

# It is worse for delay between sample preparation and e-beam writing.

Clark, "Time-dependent exposure dose of hydrogen silsesquioxane...", JVST B, 24(6), 3073-3076 (2006).

# Sub-10nm lines in HSQ 5.0kV 2.0mm x400k SE(U,LA0) 09/01/2008 13:09 ' ' ' 100mm

Fig. 3.25 Sub-10 nm lines exposed in HSQ resist (Reprint courtesy of NanoBeam Ltd. [52])

Contrast curves of HSQ at different exposure energies



#### SU-8 (very high sensitivity, but low contrast)

200 nm

- Chemically amplified negative tone resist
- Extremely high sensitivity over 100x that of PMMA
- Low contrast (0.9), unsuitable for dense patterning
- High resolution possible for *sparse* patterns at high kV
- Rough edges and "residues" due to random exposure from back scattering electrons and random photo-acid diffusion. (this is like shot noise for EUV resist that is also chemically amplified)
- Ideal for low resolution writing over large area (since it is fast).



10keV

Rough edge

#### 24nm line at pitch 300nm in 100nm thick SU-8

High resolution *sparse* pattern

Kristensen A, "High resolution 100 kV electron beam lithography in SU-8", Microelectronic Engineering, 83, 1609-1612(2006)

 "residues"
 50keV

 Odd
 Odd

 Odd

Fig. 3.26 SU-8 resist patterns exposed by e-beam at 50-keV energy

#### SU-8 resist formulation and process

#### Epoxy-based, mostly used as photo-resist



+Rubrene (Photoinitiator) +OPPI (PAGs) +Base

Glass transition temperature of SU-8 Un-cross-linked: 50°C. Fully cross-linked: 230°C

- Like all chemically amplified resist, post exposure baking temperature and time is very critical. Typically 90°C for 2-3min on hotplate.
- Spin-coating, bake, exposure, post-bake and development need to be done quickly without much delay.
- As it is so sensitive, don't let it exposed to room light for long (will be exposed by UV light, even though room light has very little UV component in it).

#### Undercut profile for liftoff



Fig.3.33. Process of double layers resists for lift-off

# High-Res. Positive Inorganic Resists



(a) BEGINNING OF EXPOSURE



(b) AFTER SOME IRRADIATION



(c) MORE IRRADIATION



Resist	Minimum linewidth	Typical aspect ratio	Deposition	expose 500-nm- thick layer (C/cm <sup>2</sup> )	Mechanism of exposure
PMMA	8–10 nm	45	Spinning	$5 \times 10^{-4}$	Bond breaking
NaCl	1.5 nm	>40	Sublimation 40-Å grain	10 <sup>2</sup> -10 <sup>3</sup>	Dissociation of Cl <sub>2</sub> Diffusion of Na
LiF	1.5 nm	>40	Sublimation 50-Å grain	$10^{-1} - 10^{-2}$	Dissociation of F <sub>2</sub> Diffusion of Li
$MgF_2$	1.5 nm	>40	Sublimation 50-Å grain	$1 - 10^{-1}$	Dissociation of $F_2$
AlF <sub>2</sub>	1.5 nm	>40	Amorphous	1–10	Dissociation of $F_2$ Diffusion of Al
KC1	1.5 nm	>40	Deposition 50-Å grain	1–10	Dissociation of Cl <sub>2</sub> Diffusion of K
Metal-	1.5 nm	>40	Cut thin-film	$1 \times 10^{7}$ (2000 Å thick)	

+ 100 L . V +-

Extremely high resolution, since only primary (not secondary) high energy beam is responsible for exposure. However, probably HSQ is the only useful inorganic resist. Inorganic resists listed here: very thin film, making liftoff difficult; very low sensitivity, need long writing time.

Self-development of metal halides by e-beam

#### Gray-scale electron beam lithography



#### AFM of a grating in resist



SEM of an 8-level FZL transfered into Si by analog RIE focal length f= 62  $\mu$ m @ 1,55  $\mu$ m; d = 610 nm<sup>7</sup>

#### Which resist is best for gray-scale EBL?





Contrast curve of PES with 10keV electron beam. Sensitivity was found to be  $\sim 200 \mu C/cm^2$ , with contrast only  $\gamma \sim 0.8$ .

- Ideal resist has positive tone with very low contrast (ideally  $\gamma$ <1) and high sensitivity.
- High contrast leads to very narrow process/dose window (tiny dose change → large pattern depth change).
- When using negative resist, make sure that the electrons can reach resist bottom (otherwise, resist at bottom will be dissolved by developer, lifting off all the resist above).

# Micro and nano optical elements in silicon and silica by regular and gray scale e-beam lithography



#### Focused Ion Beam FIB





#### Focused Ion Beam FIB



#### Helium ion Microscopy HIM







## FIB – dual beam





Cross section of a mouse bone. Courtesy of Baumman, et al.

Usually a FIB column is coupled with a SEM column. In this way the FIB column can e used to dig the sample and the SEM to image in cross section...



Figure 2 Cross-section view of the edit shown in figure 1 on a two metal layer IC



# **FIB induced deposition**



if a gas is introduced in the vicinity of the ion beam impact point: FIB induced deposition (FID) occurs.

The gas is introduced by a nozzle which is positioned a few hundreds of microns above the area of interest. The gas is then adsorbed on the surface of the material. When the FIB beam hits the surface, secondary electrons with energy ranging from a few eV to a few hundreds of eV are generated. These secondary electrons will break chemical bounds of the adsorbed gas molecules which will separate into different components: some of which remains volatile, others will form a deposition on the surface



# **FIB induced deposition**

#### Common precursors are:

- •W -Tungsten Carboxyl, W(CO)<sub>6</sub>
- Al -Trimethyl Al (TMA) Al(CH<sub>3</sub>)<sub>3</sub>
- C Naphtalene  $(C_{10}H_8)$
- Fe Iron pentacabonyl Fe(CO)<sub>5</sub>
- $Pt C_6H_{16}Pt$

(methyl cyclopetandienyl) trimethyl Pt







# FIB – cross section on fixed cells











4. Resin Infiltration



- 5. Wash 6. P

6. Polymerization





7. Cross Sectioning



Cell membrane readily deforms inward and wraps around protruding structures, but hardly deforms outward to contour invaginating structures.

A positive membrane curvatures with a radius <200 nm trigger Clathrinmediated endocytosis (CME).

SEQUENTIAL MILLING



Also the nuclear envelope is deformed upward by a nanopillar

The interface between cells and nonbiological surfaces that regulates cell attachment, chronic tissue responses, and ultimately the success of medical implants or biosensors is strongly influence by nanotopography



## Cryo - FIB

#### **Features:**

- sample preparation and transfer at cryotemperature (LN2 -193C)
- •Cooled sample holder and cold shield (to minimiza sample contamination)
- •No need for drying process
- •e-beam damage reduction
- •Freeze fracture
- Sectioning
- •TEM slice preparation



# Cryo – FIB - example

It has been known for several decades that fixing a biological sample in vitreous ice preserves it in a near-native state83. Still, there are limits on the thickness of a sample to be imaged by TEM, and this has restricted microscopy at cryogenic temperatures to studies



FIB operations, however, perform well under cryogenic conditions, and different groups have exploited this to generate TEM-ready lamellae from thick biological samples using various approaches



3D structure determination of native mammalian cells using cryo-FIB and cryo-electron tomography

Ke Wang<sup>a,1</sup>, Korrinn Strunk<sup>b,1</sup>, Gongpu Zhao<sup>a</sup>, Jennifer L. Gray<sup>b</sup>, Peijun Zhang<sup>a,\*</sup>

They show a simple and robust method for creating in situ, frozenhydrated cell lamellas using a cryo-FIB, allowing *in-situ* access to any interior cellular regions of interest.



Cryo-FIB milling and cryo-ET of frozen-hydrated HeLa cells

# Focused ion beam micromachining of eukaryotic cells for cryoelectron tomography

Alexander Rigort<sup>1</sup>, Felix J. B. Bäuerlein<sup>1</sup>, Elizabeth Villa, Matthias Eibauer, Tim Laugks, Wolfgang Baumeister<sup>2</sup>, and Jürgen M. Plitzko<sup>2</sup>



FIB is used for the micromachining of cells embedded in vitreous ice.

Thin lamellae are cut out of cellular volumes with geometries suitable for electron tomography. The lamellae are left in situ during transfer to the EM supported only by the surrounding bulk ice.



#### Cryoelectron tomograms of D. discoideum cells.

(A) Slice through thebx; y-plane of a tomographic reconstruction showing the nuclear envelope (black arrowhead) with nuclear pore complexes (white arrowheads) separating cytoplasm from nucleoplasm

Endoplasmic reticulum (white stars), tubular mitochondria (asterisks) and microtubules (white arrows)

(B and C) x; z and y, z-planes.

The thickness of the lamella is approximately 300 nm.

(D) Surface rendered visualization, displaying nuclear envelope, endoplasmic reticulum, mitochondria, microtubules, vacuolar compartment, and ribosomes

#### **Types of Lithography**

- ✓ Photolithography
- ✓ Particles Beam lithography
- ✓ Interference lithography
- ✓ Scanning Probe
- ✓ Nanoimprinting
- ✓ Soft Lithography
- ✓ Shadow Mask





#### **Interference Lithography**



## Types of Lithography

- Photolithography
- ✓ Particles Beam lithography
- ✓ Interference lithography
   ✓ AFM
   ✓ Scanning Probe
   ✓ STM
- Nanoimprinting
- ✓ Soft Lithography

e) macroscopic scale:



- Mechanical patterning: scratching, nano-indentation
- Chemical and molecular patterning (dip-pen nanolithography, DPN)
- Voltage bias application
  - Field enhanced oxidation (of silicon or metals)
  - $\circ~$  Electron exposure of resist materials
- Manipulation of atoms/molecules by STM, or nanostructures by AFM

#### AFM lithography – scratching (simplest, mechanical lithography)

Material is removed from the substrate leaving deep trenches with the characteristic shape of the tip used.



## Dip-pen nanolithography (DPN)



- AFM tip is "inked" with material to be deposited
- Material is adsorbed on target
- <15nm features</p>
- Multiple DPN tip arrays for higher throughput production



# AFM lithography: oxidation (local electrochemical anodization)



- Resulting oxide affected by experimental parameters
  - Voltage (typically from 5-10V)
  - $\odot$  Tip scan speed (stationary to tens of  $\mu m/s)$
  - Humidity (20% to 80%)
- Detected current can be used for process control
- Changes in translational velocity influence current flow



### STM lithography (STM: scanning tunneling microscopy)

By applying a voltage between tip and substrate it is possible to deposit or remove atoms or molecules.

Van der Waals force used to drag atoms/molecules.

#### Advantages of STM Lithography

- Information storage devices (one atom per bit, highest storage density).
- Nanometer patterning technique (highest resolution, ~Å).
- Manipulations of big molecules and individual atoms.





Iron on copper (111)

#### Scanning probe lithography (STM)









#### STM manipulation of atoms/molecules

- ✓ Photolithography
- ✓ Particles Beam lithography
- ✓ Interference lithography
- ✓ Scanning Probe

✓ Nanoimprinting

- ✓ Soft Lithography
- ✓ Shadow Mask



Nanoimprint lithography: patterning by mechanical replication


#### **Two NIL approaches**



#### Key advantage of NIL: highest resolution





10 nm dia pillar mold





Lift-Off



10 nm dia metal dots by imprint and lift-off

NanoStructure Laboratory



#### Another key advantage: 3D imprinting



- Patterning of the via and interconnect layers simultaneously, in CMOS BEOL .
- Potentially reduces the number of masking levels needed in BEOL.
  (BEOL: back end of line)



2 tier, using oxide/ITO

3 tier using oxide/ITO

Wikipedia: **Back end of line (BEOL)** is the portion of integrated circuit fabrication line where the active components (transistors, resistors, etc.) are interconnected with wiring on the wafer. BEOL generally begins when the first layer of metal is deposited on the wafer. It includes contacts, insulator, metal levels, and bonding sites for chip-to-package connections.

#### "Standard" resist for NIL: PMMA

Glass transition and flow temperature of PMMA



However, PMMA is far away from being an ideal NIL resist. It is popular simply because people are familiar with it (since it is resist for many other lithographies).

# Functional resist: nano-crystal(NC)/polymer based materials

Synthesis and functionalisation of colloidal nano-particles for incorporation into thermoplastic or thermal-curing (i.e. thermal-set) polymers.

Tuning of functional properties:

- Optical absorption and emission
- Mechanical Stability
- Conductivity
- Processability...



Size dependent luminescent CdSe NCs (quantum dot)

# Imprinting on luminescent nano-crystal/PMMA based co-polymer composites



CdSe@ZnS nano-crystals (NC) in PMMA modified co-polymer. Homogeneous distribution of NCs inside the polymer matrix.

### Functional "resist": semiconducting polymer

#### SEM image of 200nm period MEH-PPV grating



MEH-PPV T<sub>g</sub>=65°C. Hot embossing at 120°C and 20bar. MEH-PPV spun on a PEDOT/ITO/glass.





#### R-P3HT grating with 200nm period



R-P3HT 200nm period grating. NIL at 160°C and 35 bar. Strong physical bond, high transition temperature.

# NIL for large features (>100 $\mu$ m) - simultaneous pattern duplication of large and small features

- Application: large features are needed to connect small ones to the outside world (electrodes...).
- Challenge: more polymer must be displaced over longer distances.
- A popular approach: two-step process small features by NIL, large ones by photolithography with alignment.



Problems when both small and large features are present

Schematics of pattern failure mechanisms in NIL as a result of: (a) non-uniform pattern height; (b) non-uniform residual layer thickness; (c) incomplete nano-pattern replication.

Cheng, "One-step lithography for various size patterns with a hybrid mask-mold", Microelectronic Engineering 71, 288–293 (2004).

#### NIL pattern uniformity



Etch some dummy holes/trenches here

- The fill factor should be kept constant: better flow and shorter imprint time.
- Different fill factor across mold leads to different sinking rates.
- Mold bending leads to non-uniform residual layer on substrate.
- One solution: fabricate dummy cavities/protrusions to create constant fill factor.

#### Hot embossing pellets





V. Studer, A. Pépin, Y.Chen, Appl. Phys. Lett. 80, 3614 (2002)

#### NIL at 180°C, 50bar pressure for ~10 min



For fabricating micro- and nano-fluidic channels in thermoplastic polymers.

#### Hot embossing polystyrene pellets

Polystyrene is bio-compatible (cell culturing Petri-dish is made of polystyrene perhaps plus some additives).



#### Application: contact guidance of cell growth

- Definition: anisotropic topographic features induce cells to align along the direction of the anisotropy.
- Importance: in tissue engineering, if tissue is to be repaired, the new cells must be aligned and positioned correctly.

#### grating substrate



Journal of Cell Science 116 (10)

#### **MIcrofluidics**







- ✓ Photolithography
- ✓ Particles Beam lithography
- ✓ Interference lithography
- ✓ Scanning Probe
- ✓ Nanoimprinting

Soft Lithography

✓ Shadow Mask



# Soft lithography



# PDMS: poly(dimethyl-siloxane)

PDMS properties:

- Silicone elastomer with a range of viscosities
- Flexible (1 MPa Young's modulus, typical polymer 1 GPa) and easy to mold.
- Elastomer, conforms to surface over large areas.
- Chemically inert, optically transparent
- Low surface energy: bonds reversibly (or permanent).
- Seals to flat and clean surfaces for micro-fluidic channels
- Durable (reusable), low thermal expansion
- Biocompatible (even used for food additive)
- Environmentally safe
- Best Resolution: 2-10 nm (for hard PMDS)





#### **PDMS** fabrication



Master pattern (red color) can be in: photoresist (SU-8), silicon, glass... Silanization of master mold needed to obtain low surface energy for easy separation.

#### **Master Fabrication**



#### Substrate Preparation



Article	Biotechnology Bioengineering
Acceleration of Neuronal Precursors Differentiation Induced by Substrate Nanotopography	
Elisa Migliorini, <sup>1,2</sup> Gianluca Grenci, <sup>1</sup> Ielena Ban, <sup>3</sup> Alessandro Po	zzato, <sup>1</sup>



#### Hard PDMS (h-PDMS) ("Filler" added for more cross-linking)

- More cross-linked polymer, so harder.
- Less flexible than regular (soft) PDMS, more brittle.
- Must have a support in order to not crack the stamp, use thick layer PDMS or glass as support.



# Traction force microscopy – nano-pillars



from Fu et al., Nat Methods, 2010

- traction force microscopy is used to determine how much force is exerted by the cell onto an extracellular substrate
- nano-pillars of known size and elasticity can be used to determine traction forces microscopically

#### **PDMS surface treatment**



#### PDMS surface treatment

- PDMS has a low interfacial free energy such that molecules of most polymers won't stick on or react with its surface.
- The interfacial free energy can be manipulated with plasma treatment.
- For nano-imprint or soft lithography mold, plasma can make PMDS surface like SiO<sub>2</sub>, easy for mold release agent coating using silane chemistry.



Figure 6. Schematic procedure for the modification of the PDMS surface. a) Treatment with an  $O_2$  plasma; b) reaction with silvl chloride vapor. Different terminal groups X of the SAMs give different interfacial properties.<sup>[122]</sup>

#### Micro - contact printing (µCP)



# Self - assembling, classical –SH and Au bonding

- Definition: spontaneous organization of molecules (objects) into stable, welldefined structures by non-covalent forces.
- Driving force: thermodynamic equilibrium.
- Final structure: determined by the subunits.
- Biological 3D self assembly: folding of proteins, formation of DNA helix...



Chemi-sorption and selforganization of long-chain organic molecules on flat substrates.

Alkanethiolates CH<sub>3</sub>(CH<sub>2</sub>)<sub>n</sub>S-Au(111)

-SH also binds to Ag, but Ag surface not as stable as Au.

# Popular "ink" molecules

Substrate	Molecules
Au Ag Cu Pd GaAs InP	Alkanethiols (RSH) and Alkyldisulfides (RS-SR')
Glass, Mica, Si/SiO2 HO-Terminated Polymer	Alkylsilanes, RSiCl3 or RSi(OEt)3
Ag2O, Al2O3	Alkylcarboxylic Acids (RCOOH)
ZrO2	Alkylphosphates (RPO3)
Pt	Alkylamines, Alkylisonitriles

#### Micro - contact printing (µCP)



5 µm



Kumar & Whitesides et al, Langmuir, 1995, 11, 825

#### Micro-contact printing on curved substrates



Whitesides, "Fabrication of submicrometer features on curved substrates by microcontact printing", Science, 269, 664 (1995); Rogers and Whitesides, "Microcontact Printing and Electroplating on Curved Substrates: Production of Free-Standing Three-Dimensional Metallic Microstructures", Adv. Mater. 9, 475 (1997).

#### Microcontact printing of proteins



BSA: bovine serum albumin, bovine albumin

#### Microcontact printing of proteins

#### Fluorescent image





#### (anti-Goat IgG – Alexa 488 and 594)

#### Micro-contact printing of DNA



# Replica molding (REM)

#### It is similar to UV-curing nanoimprint lithography



PU = polyurethane







# Replica molding (REM)



Figure 23. An SEM image of a dome-shaped object in polyurethane with patterned microstructures (corner cubes ca. 100 µm) on its surface that was formed by replica molding against a stretched PDMS mold.<sup>[35]</sup> 104

# Micro-molding in capillary (MIMIC)

Uses capillary forces to fill the gaps between substrate and PDMS master.

- 1. The PDMS master is pressed tightly on a planar substrate.
- 2. Elastic PDMS seals off walls and creates capillary channels.
- 3. A drop of liquid prepolymer is placed at the ends of these channels and fills them automatically due to capillary force.
- 4. PDMS can absorb the solvent, which creates a partial vacuum inside the PDMS cavity and helps to draw in liquid polymer.
- 5. Cure and peel of the PDMS master.



# Micro-molding in capillary (MIMIC)



Xia, Y.; Whitesides, G. M. Ann. Rev. Mater. Sci 1998, 28, 153.



a: PU (polyurethane) on Si d: polystryene colloids b: polyaniline c: ZrO<sub>2</sub> e+f: free standing PU

# Micro - transfer molding (µTM)



# Micro - transfer molding (µTM)

#### 1-layer microstructures



#### 2-layer microstructures



#### **3-layer microstructures**





- a) An SEM image of a fractured sample showing a pattern of isolated stars of UV-cured polyurethane (NOA 73) on Ag.
- b) An array of parallel lines of spin-on glass on Si with an aspect ratio (height/width) of ~8.
- c) A two-layer structure: isolated micro-cylinders (1.5μm in diameter) on 5μm-wide lines, supported on a glass cover slide.
- d) A two-layer structure: a continuous web over a layer of 5µm-wide lines, supported on a glass cover slide.
- e) A three-layer structure on a glass cover slide. The layers of 4  $\mu$ m-wide lines are oriented at ~60° from each other.

Structures in c-e were made of heat-cured epoxy (F109CLR).
## Metal transfer assisted nanolithography



## Lithography – general distinction

# Lithography with particles or waves

- Photons: photolithography
- X-rays: from synchrotron, xray lithography
- Electrons: electron beam lithography (EBL)
- Ions: focused ion beam (FIB) lithography

## Imprint lithography (molding)

- Soft Lithography: microcontact-printing...
- Hot embossing
- UV-curable imprinting

### **SPM-lithography**

- AFM
- STM
- DPN (dip-pen nanolithography)

Pattern replication: parallel (masks/molds necessary) High throughput, but not easy to change pattern

- Optical lithography
- X-ray lithography
- Imprint lithography
- Stencil mask lithography

## Pattern generation: serial

### (Slow, for mask/mold making)

- E-beam lithography (EBL)
- Ion beam lithography (FIB)
- SPM-lithography

   AFM, STM, DPN

## Multiple serial (array)

- Electron-beam micro-column array (arrayed EBL)
- Zone plate array lithography
- Scanning probe array

### Lithography on surfaces

- Optical/UV lithography
- E-beam lithography
- FIB lithography
- X-ray lithography
- SPM-lithography
  - AFM
  - o STM
  - o DPN (dip-pen
    - nanolithography)
- Imprint lithography
  - Soft lithography
  - $\circ$  Hot embossing
  - UV imprinting
- Stencil mask lithography

### Lithography in volume

- Two photon absorption
- Stereo-lithography

### • Critical dimension (CD) control

Size of features must be controlled within wafer and wafer-to-wafer

### Overlay (alignment between different layers)

For high yield, alignment must be precisely controlled

Defect control

Other than designed pattern, no additional patterns must be imaged

### • Low cost

Tool, resist, mask; fast step-and-repeat

30-40% of total semiconductor manufacturing cost is due to lithography (masks, resists, metrology)

## Pattern transfer (next step after lithography)



- Lithography create patterns generally in a resist (polymer) layer.
- For device application, pattern needs to be transferred to another layer (metal, semiconductors...).

- ✓ Photolithography
- ✓ Particles Beam lithography
- ✓ Interference lithography
- ✓ Scanning Probe
- ✓ Nanoimprinting
- ✓ Soft Lithography

✓ Shadow Mask



## Controlled shadow evaporation (large undercut for liftoff)



ZEP is an EBL resist PMGI is an EBL resist AND liftoff layer

## Irradiate with electron beam



Develop the two layers selectively Top layer: Bottom Layer:

(PMGI is developed by diluted PR developer) PR=photoresist





## Controlled shadow evaporation (for tunnel junction)



## Controlled shadow evaporation (for spin-valve)



Evaporation of material B

- Useful for lateral devices (tunnel junctions, superconductor circuitry...)
- Lateral overlap determined by resist thickness and angle
- In-situ interface

Ex : lateral spin-valve T. Kimura et al., PRL 100, 66602 (2008)



Py = permalloy, NiFe alloy

le

## Applications: dry eching



Fields of 1  $\mu$ m high pillars separated by smooth regions were fabricated into silicon wafers using standard photolithography

- Pillars were 0.5 μm and 2 μm wide.
- The inter-pillar gap, varies from 0.5 to 5.0  $\mu m$  in 0.5  $\mu m$  steps

.Smooth-unetched (su) were chosen as control.

INSTITUTE OF PHYSICS PUBLISHING J. Neural Eng. 1 (2004) 78–90 JOURNAL OF NEURAL ENGINEERIN PII: S1741-2560(04)78717-

Topographically modified surfaces affect orientation and growth of hippocampal neurons

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#### Effects of surface topography on the polarity of cultured hippocampal neurons



Physical cues affect neuron growth, extracellular matrix topography may contribute to cell growth and differentiation. new strategies for directing and promoting neuronal growth will facilitate studies of synapse formation and function andprovide methods to establish defined neural networks



The vapor-Liquid-Solid mechanism allows the growth od semiconductor nanowires, at a random position. Depending on the growth condition wires can be: long or short, thick or thin,

dense or sparse





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 High aspect ratio silicon nanowires control fibroblast adhesion and cytoskeleton organization
 organization

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#### 48h recording of living MEF



## **Semiconductor Nanowires**

By localizing the position of the catalysis by lithographic means is possible to localize also the growth of the nanowires

#### Axonal guidance on patterned free-standing nanowire surfaces

14mm

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b







NW growth seeds are formed by different patterning, approaches. 20 nm Au film deposition, and pattern lift-off



SEM image of axons growing along rows of NWs. -Scale bars 1 µm

- The axons follow a line of NW internalizing (a) some wires
- The axons grow in the middle of two rows (b) of NW and internalized NW from both rows.



Nanowires can also be transferred to lithografically defined devices and act as a sensing element



Detection, Stimulation, and Inhibition of Neurona Signals with High-Density Nanowire Transistor Arrays Fernándo Patolsky, et al. Science 313, 1100 (2006); DOI: 10.1126/science.1128640

1000

13mm

NANOTECHNOLOG<sup>2</sup>

doi:10.1088/0957.4484/19/34/34510

Traces recorded on individual NWs separated by 10µm each (total 500µm) are delayed by 50µsec each for a total of 1msec



**MAAAS** 

#### IOP PUBLISHING Nanotechnology 19 (2008) 345101 (6pp)

## Graphene substrates



Graphene is patterned by standard UVL and plasma etching. Then is coated with a supporting matarial (Ti or PMMA) Cu is removed by chemical etching Graphene layer is transferred on a patterned substrated (here Ormocomp <sup>®</sup> by NIL) The supporting layer is finally dissolved Carbon 103 (2016) 355–310
Contents lists available at ScienceDirect
Carbon
ELSEVIER journal homepage: www.elsevier.com/locate/carbon

Contamination-free suspended graphene structures by a Ti-based transfer method



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## **Graphene substrates**



Neurons are suspended above the graphene layer ans show a preferential orientation along the underneath Ormocomp lines









Not topographical features nor mechanical features can explaine the graphene alignement. Only different conductivity of graphene supported vs suspended can explain it: neaurons follow the conducting lines?

— = 10 μm



#### **Master Fabrication**



#### Substrate Preparation



Article	Biotechnology Bioengineering
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#### Thin film deposition

- Physical vapor deposition (PVD): sputtering, e-beam or thermal evaporation
- Chemical vapor deposition (CVD): metal-organic CVD, plasma-enhanced CVD, low pressure CVD...
- Epitaxy: molecular beam epitaxy (MBE), liquid-phase epitaxy...
- Electrochemical deposition: electro- and electroless plating (of metals)
- Oxidation (growth of thermal SiO<sub>2</sub>)
- Spin-on and spray-on film coating (resist coating)

**Printing techniques**: ink-jet, micro-contact printing **Assembly**: wafer bonding, surface mount, wiring and bonding

#### Subtractive methods:

- Etching: wet chemical etching, reactive ion etching; ion beam sputter etching, focused ion beam etching.
- Tool-assisted material removal: chemical-mechanical polishing, chipping, drilling, milling, sand blasting.
- Radiative and thermal treatment: laser ablation, spark erosion.

### Modifying methods:

- Radiative treatment: resist exposure, polymer hardening
- Thermal annealing: crystallization, diffusion, change of phase
- Ion beam treatment: implantation, amorphization
- Mechanical modification: plastic forming and shaping, scanning probe manipulation