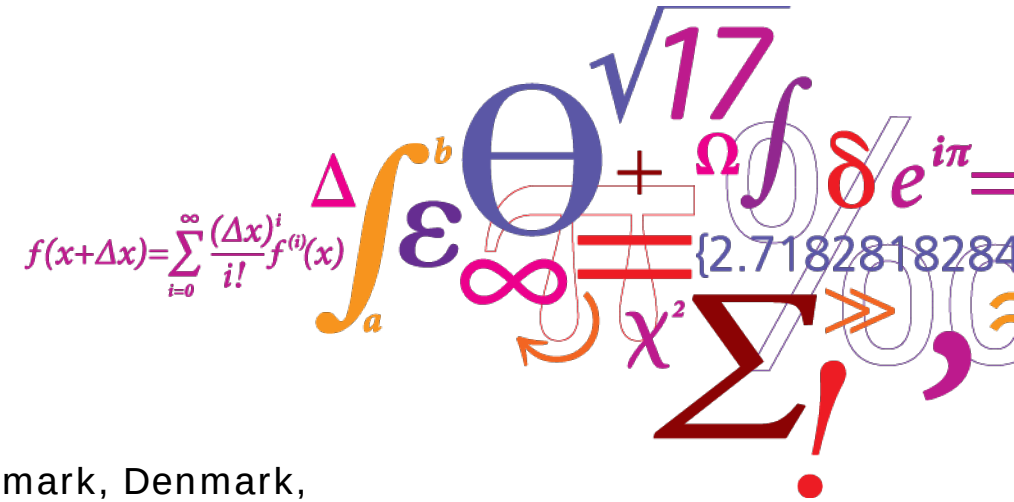
From Nanopore DNA Sequencing to Organic Ices for 3D electron beam patterning



Anpan Han, 韩安磐

Danchip/CEN, Technical University of Denmark, Denmark,

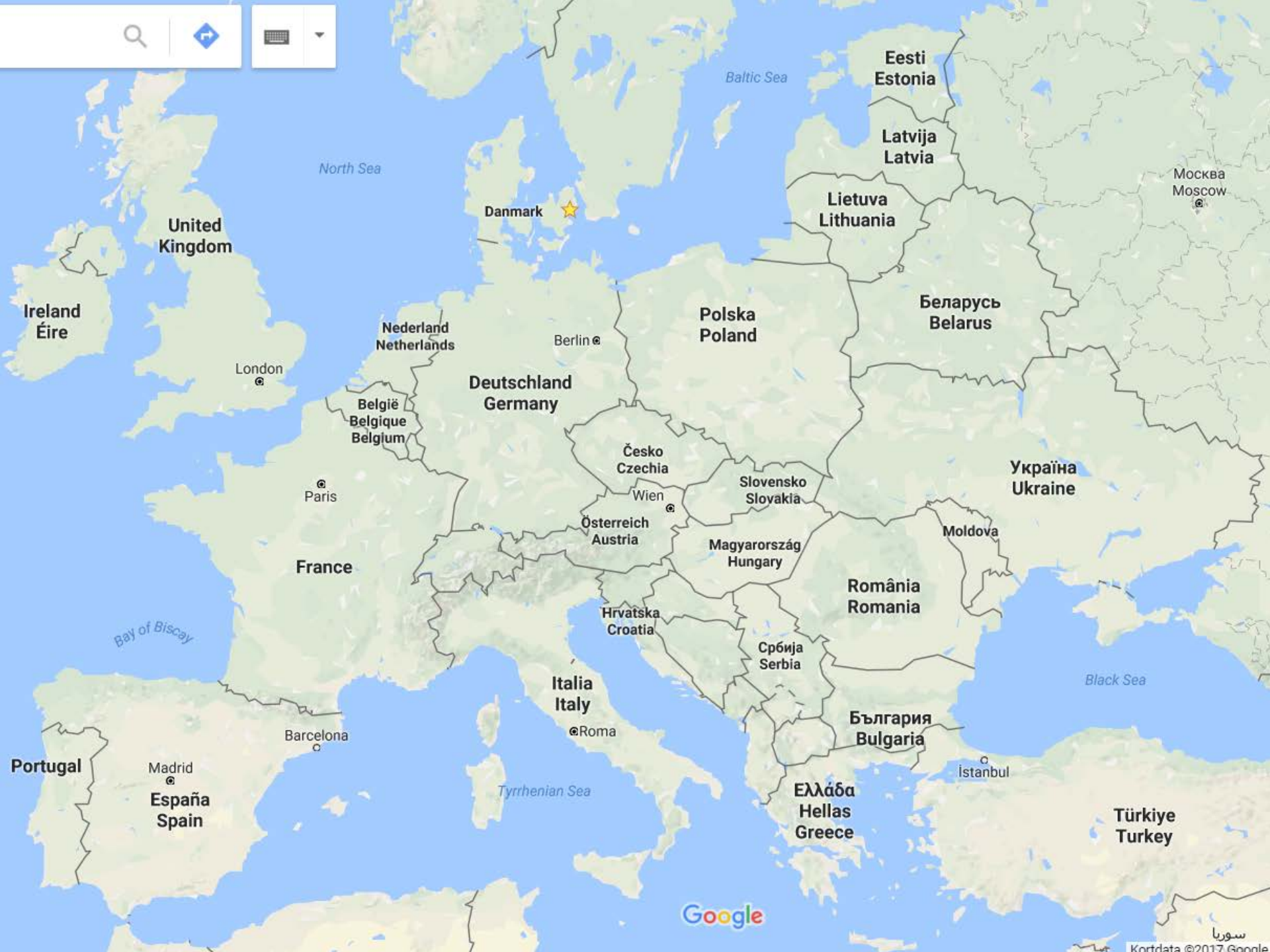
DTU Danchip
National Center for Micro- and Nanofabrication

DTU Cen
Center for Electron Nanoscopy

Content (75 +10 min)

- Denmark, DTU, Danchip and CEN (5 min)
- Nanopore DNA sequencing (6 min)
- Ice lithography (15 min)
- Quiz (5 min), Break (10 min)

- Organic ice resists for 3D nanolithography (15 min)
- Future research: 3D-OPE (5 min)
- Group work: 25 min: Please help me!


Baltic Sea
North Sea
Eesti
Estonia
Latvija
Latvia
Lietuva
Lithuania
Москва
Moscow
Danmark
United
Kingdom
Ireland
Éire
Беларусь
Belarus
Polska
Poland
Nederland
Netherlands
Berlin
London
Deutschland
Germany
België
Belgique
Belgium
Česko
Czechia
Slovensko
Slovakia
Україна
Ukraine
Paris
Wien
Österreich
Austria
Moldova
Magyarország
Hungary
France
România
Romania
Hrvatska
Croatia
Bay of Biscay
Србија
Serbia
Black Sea
Italia
Italy
Roma
България
Bulgaria
Barcelona
Portugal
Madrid
España
Spain
İstanbul
Tyrrhenian Sea
Ελλάδα
Hellas
Greece
Türkiye
Turkey
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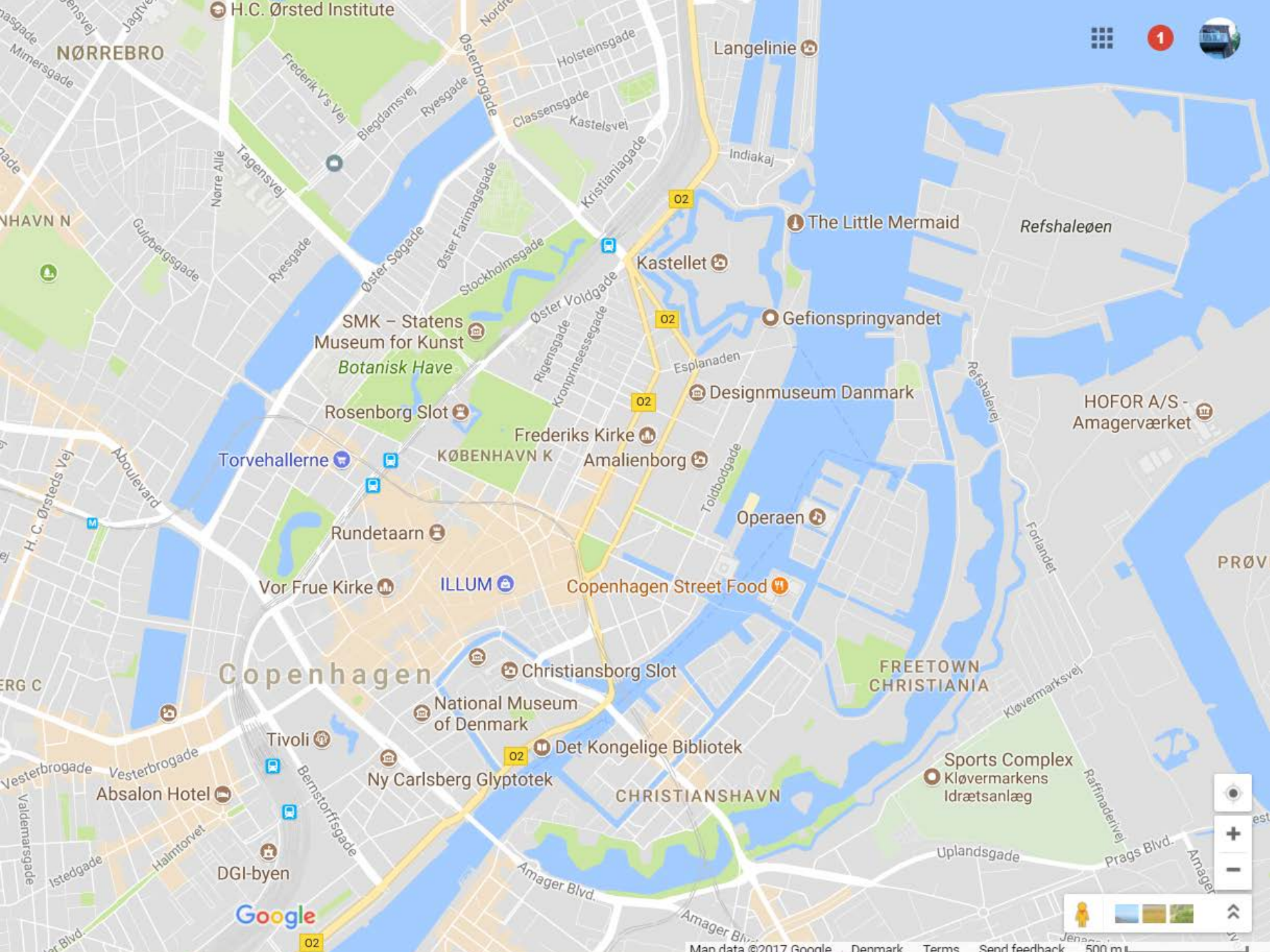
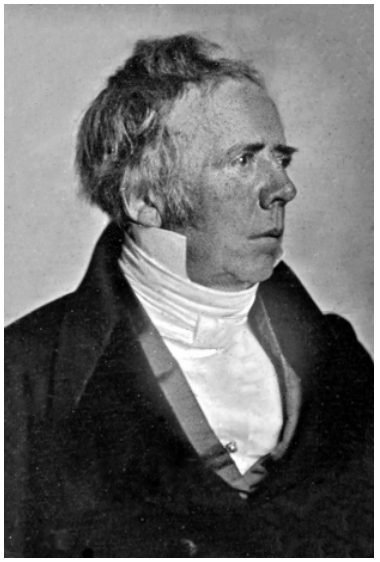
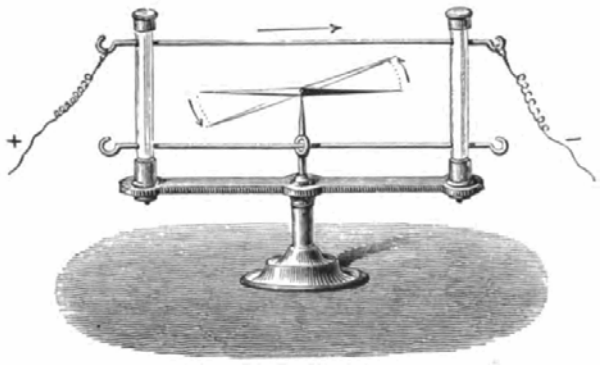
سوريا
Kortdata ©2017 Google

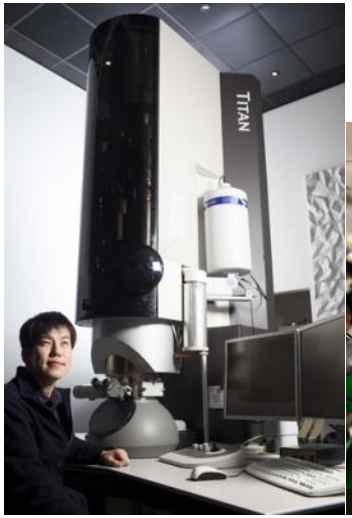

H.C. Ørsted Institute
NØRREBRO
Langelinie
The Little Mermaid
Refshaleøen
Kastellet
Gefionspringvandet
SMK – Statens Museum for Kunst
Botanisk Have
Designmuseum Danmark
Rosenborg Slot
Frederiks Kirke
Amalienborg
KØBENHAVN K
Torvehallerne
HOFOR A/S - Amagerværket
Operaen
Rundetaarn
Vor Frue Kirke
ILLUM
Copenhagen Street Food
Copenhagen
Christiansborg Slot
FREETOWN CHRISTIANIA
National Museum of Denmark
Tivoli
Det Kongelige Bibliotek
Ny Carlsberg Glyptotek
Absalon Hotel
CHRISTIANSHAVN
Sports Complex Kløvermarkens Idrætsanlæg
DGI-byen
Google
Indiakaj
Esplanaden
Toldbodgade
Refshalevej
Forlandet
Kløvermarksvej
Uplandsgade
Prags Blvd.
Amager Blvd.
Vesterbrogade
Halmtorvet
Istedgade
Bernstorffsgade
Raffinaderivej
Map data ©2017 Google
Denmark
Terms
Send feedback
500 m

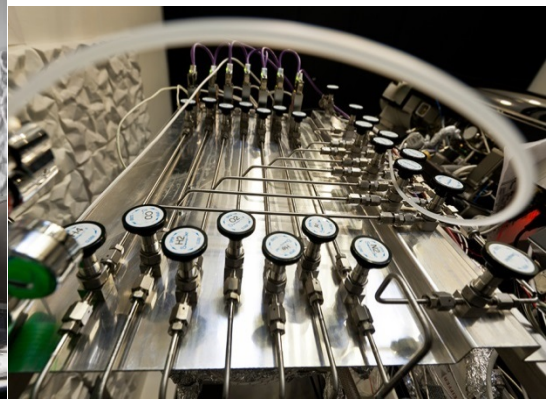
Technical University of Denmark

- Established 1829 by HC Oersted
- 1339 Faculty and Researcher
- 1540 Administrative and technical staff
- 6800 Undergraduate and Master student
- 1201 PhD students
- 26 Departments
- 30 min drive from Copenhagen Airport









300 kV aberration corrected environmental TEM

Cleanroom

150 nm half pitch
80 wafers/hour

Organic Ice

for 3D electron beam patterning ?!

How did you get this

“seemingly implausible (SI)”

idea?

HISTORICAL PERSPECTIVE

2016

nature biotechnology

Three decades of nanopore sequencing

David Deamer[1], Mark Akeson[1] & Daniel Branton[2]

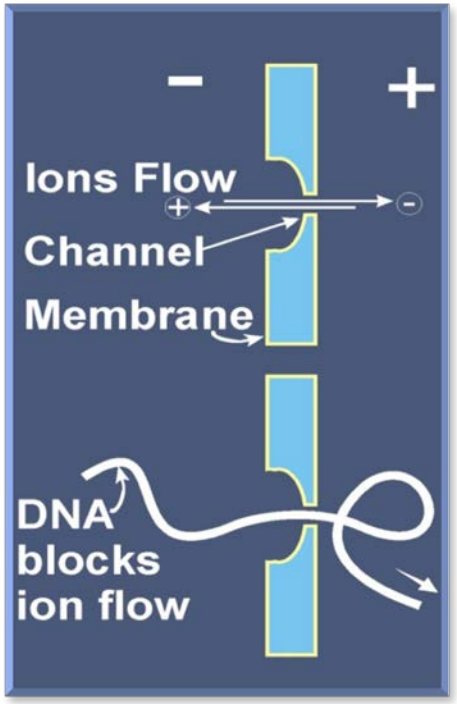
A long-held goal in sequencing has been to use a voltage-biased nanoscale pore in a membrane to measure the passage of a linear, single-stranded (ss) DNA or RNA molecule through that pore. With the development of enzyme-based methods that ratchet polynucleotides through the nanopore, nucleobase-by-nucleobase, measurements of changes in the current through the pore can now be decoded into a DNA sequence using an algorithm. In this Historical Perspective, we describe the key steps in nanopore strand-sequencing, from its earliest conceptualization more than 25 years ago to its recent commercialization and application.

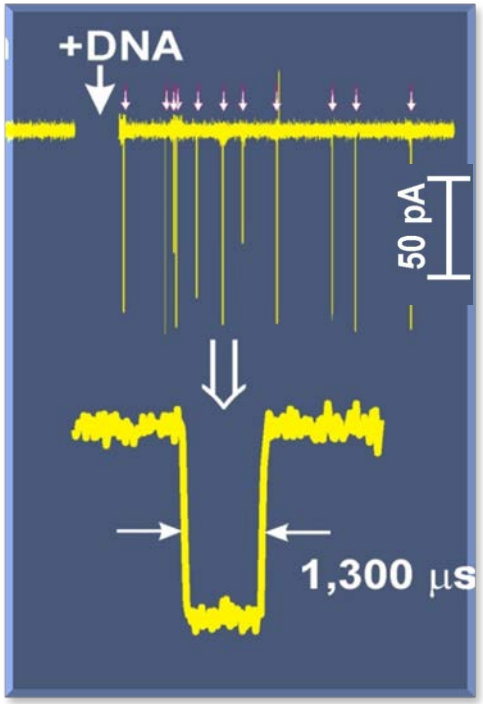
Nanopore sequencing (**Box 1** and **Fig. 1**) has its origins in several laboratories during the 1980s (**Fig. 2**). In 1989, one of us (D.D.) jotted a seemingly implausible idea in his notebook (**Fig. 3**), suggesting that it might be possible to sequence a single strand of DNA being drawn through a membrane's nanoscopic pore by electrophoresis. Around the same time, George Church's interest in scaling up DNA sequencing for

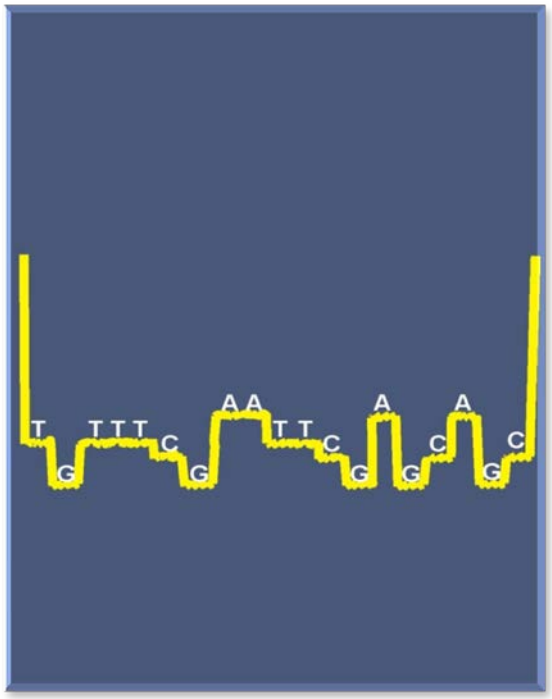
and together with Sergey Bezrukov at the US National Institutes of Health (NIH), had established the conditions that were necessary to avoid spontaneous gating (pore closure) of the α-hemolysin channel[3]. This was important, because spontaneous gating would have hindered, or confused, observations of nucleic acid translocation through the nanopore. Kasianowicz was also collaborating with Bezrukov to investigate the effect of polyethylene glycol on pore conductance and, consistent with earlier reports[2], found that a pore radius of ~1.1 nm accounted for their results[4].

In initial experiments, D.D. and Kasianowicz worked with a single α-hemolysin channel inserted into a lipid bilayer that separated two buffered KCl-filled compartments. RNA homopolymers (polyuridylic or polyadenylic acid) were then added to the *cis* side of the membrane. Immediately after addition of the polymers, the first encouraging results were observed. When a positive voltage bias greater than 80 mV was applied to the *trans* compartment, numerous blockades—transient, millisecond time-scale reductions of the ionic current through the α-hemolysin channel—appeared. No blockades were detected when the *trans* compartment was negatively biased. This was expected because the polyanionic RNA in the *cis* chamber would be inhibited from entering the nanopore when the *trans* side was negative. These preliminary results were consistent with the hypothesis that a voltage applied across the

Seemingly implausible Nanopore DNA sequencing idea (1989)







2018 Oxford Nanopore Technologies, 4th Gen DNA sequencing

nature biotechnology

OPEN

2018

Linear assembly of a human centromere on the Y chromosome

Miten Jain[1,5], Hugh E Olsen[1,5], Daniel J Turner[2], David Stoddart[2], Kira V Bulazel[3], Benedict Paten[1], David Haussler[1], Huntington F Willard[3,4], Mark Akeson[1] & Karen H Miga[1,3]

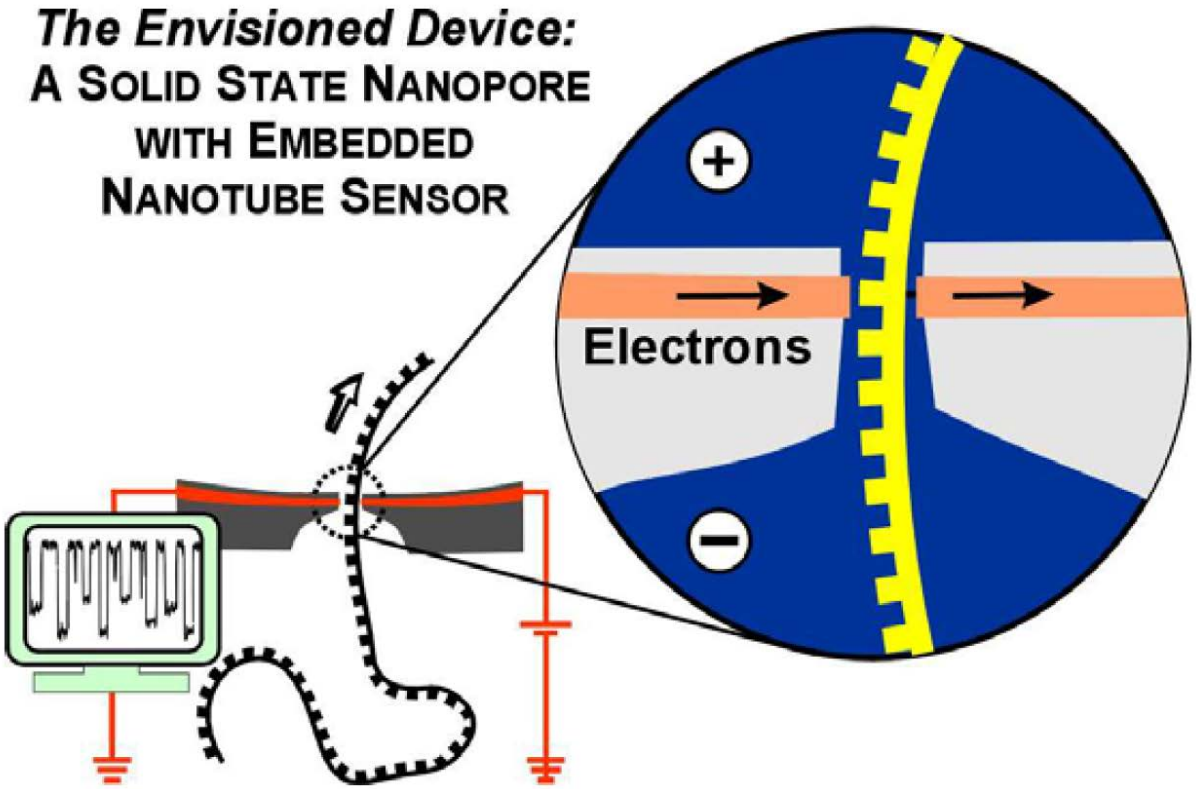
The human genome reference sequence remains incomplete owing to the challenge of assembling long tracts of near-identical tandem repeats in centromeres. We implemented a nanopore sequencing strategy to generate high-quality reads that span hundreds of kilobases of highly repetitive DNA in a human Y chromosome centromere. Combining these data with short-read variant validation, we assembled and characterized the centromeric region of a human Y chromosome.

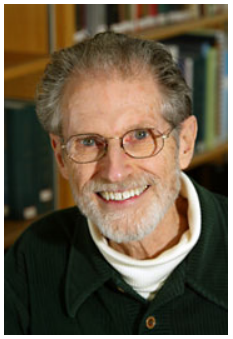
Centromeres facilitate spindle attachment and ensure proper chromosome segregation during cell division. Normal human centromeres are enriched with AT-rich ~171-bp tandem repeats known as alpha satellite DNA[1]. Most alpha satellite DNAs are organized into higher

longboard strategy, we linearize the circular BAC with a single cut site, then add sequencing adaptors (**Fig. 1a**). The BAC DNA passes through the pore, resulting in complete, end-to-end sequence coverage of the entire insert. Plots of read length versus megabase yield revealed an increase in megabase yield for full-length BAC DNA sequences (**Fig. 1b** and **Supplementary Fig. 2**). We present more than 3,500 full-length '1D' reads (that is, one strand of the DNA is sequenced) from ten BACs (two control BACs from Xq24 and Yp11.2; eight BACs in the DYZ3 locus[9]; **Supplementary Table 1**).

Correct assembly across the centromeric locus requires overlap among a few sequence variants, meaning that accuracy of base-calls is important. Individual reads (MinION R9.4 chemistry, Albacore v1.1.1) provide insufficient sequence identity (median alignment identity of 84.8% for control BAC, RP11-482A22 reads) to ensure correct repeat assembly[10]. To improve overall base quality, we produced a consensus sequence from 10 iterations of 60 randomly sampled alignments of full-length 1D reads that spanned the full insert length for each BAC (**Fig. 1c**). To polish sequences, we realigned full-length nanopore reads to each BAC-derived consensus (99.2% observed for control BAC, RP11-482A22; and an observed range of 99.4–99.8% for vector sequences in DYZ3-containing BACs). To provide a truth set of array sequence variants and to evaluate any inherent nanopore sequence biases, we used Illumina BAC resequencing (Online Methods). We used eight BAC-polished sequences (e.g., 209 kb for

Envisioned (SI) Nanopore DNA sequencing device 2005





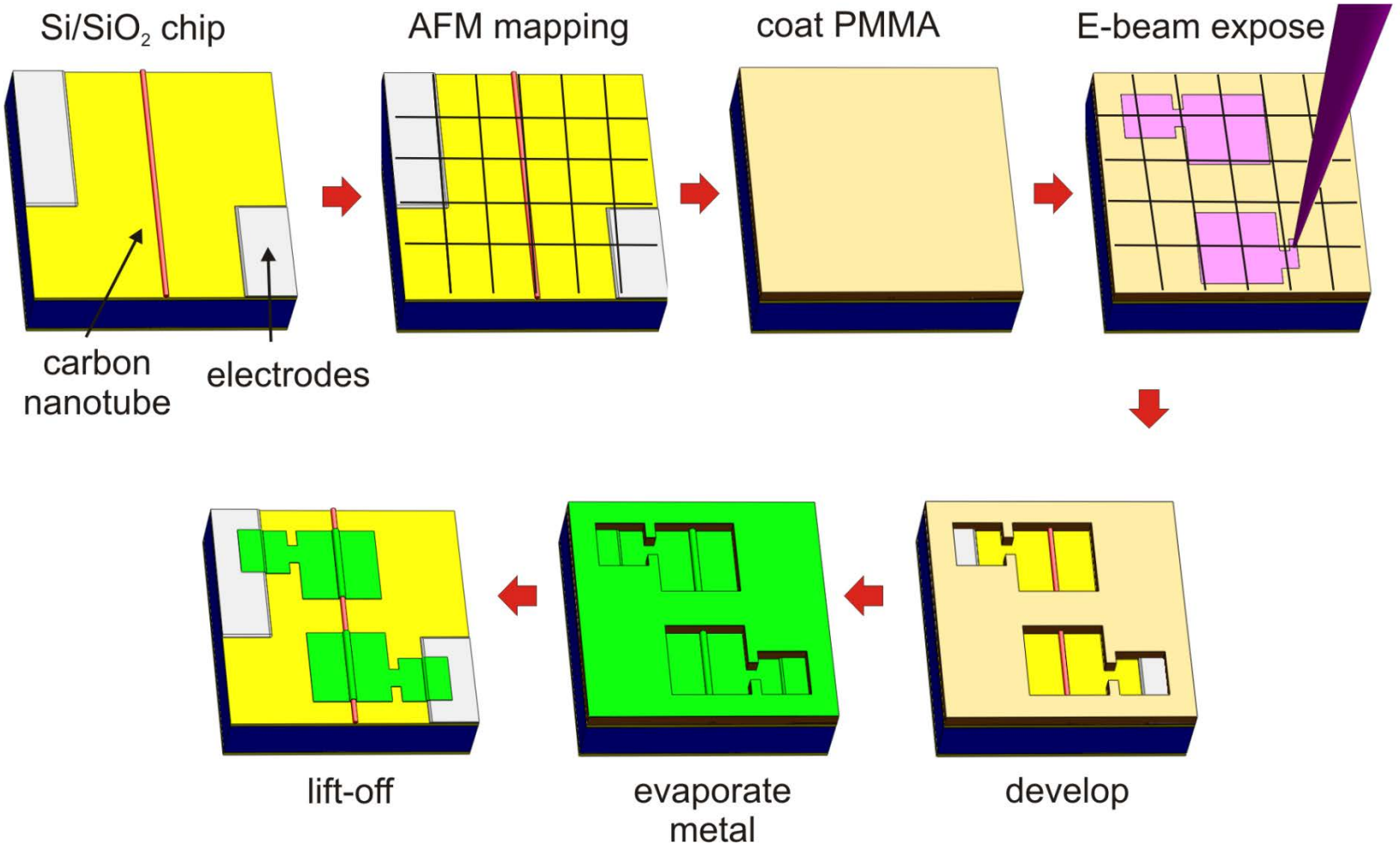
Dan Branton

Mole. Bio. Harvard

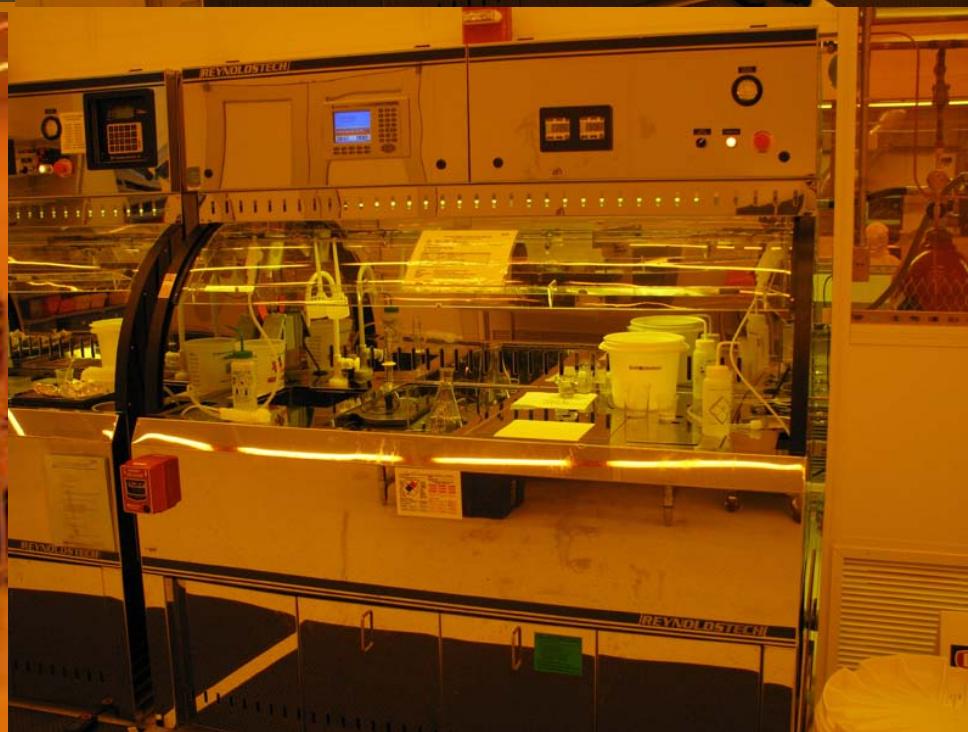
Jene Golovchenko

SEAS, Physics, Harvard

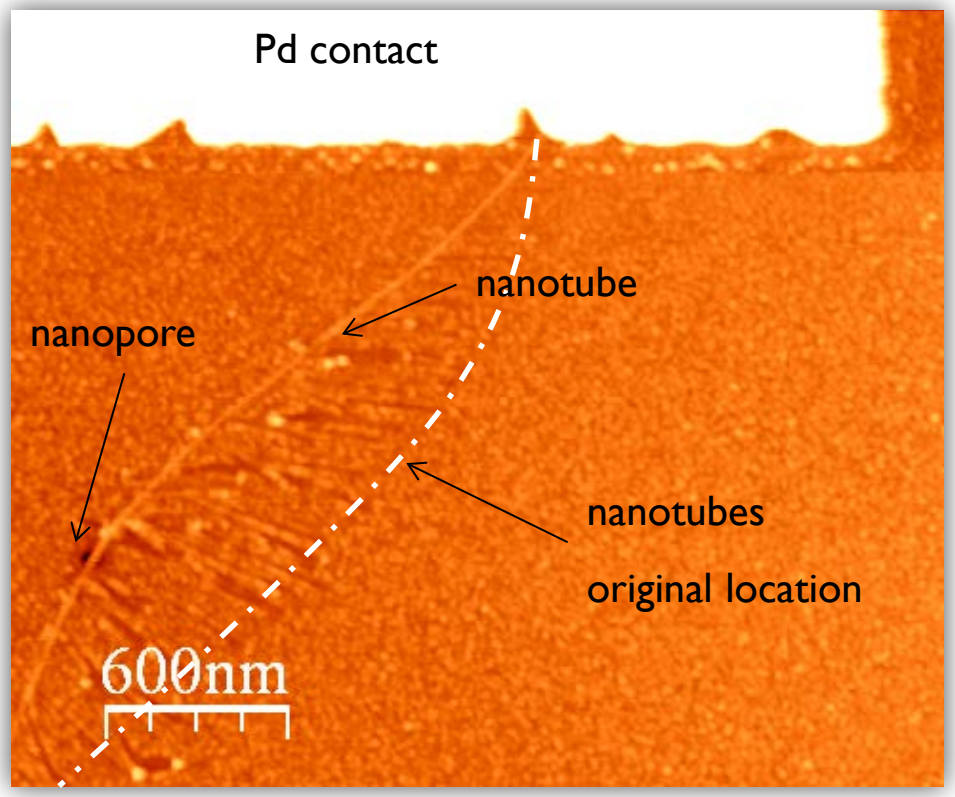
Electrical contacts to carbon nanotubes by electron beam lithography




REYNOLDSTECH


REYNOLDSTECH

Aligning nanotube with nanopore 2006-2007

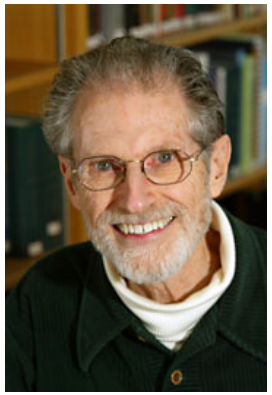


I have this great (SI) idea!

Lets use water ice as an e-beam resist!

Water is so much cleaner! I used to study proton - water ice interactions at Bell Labs.

I joined the group in 2008.



Dan Branton

Jene Golovchenko

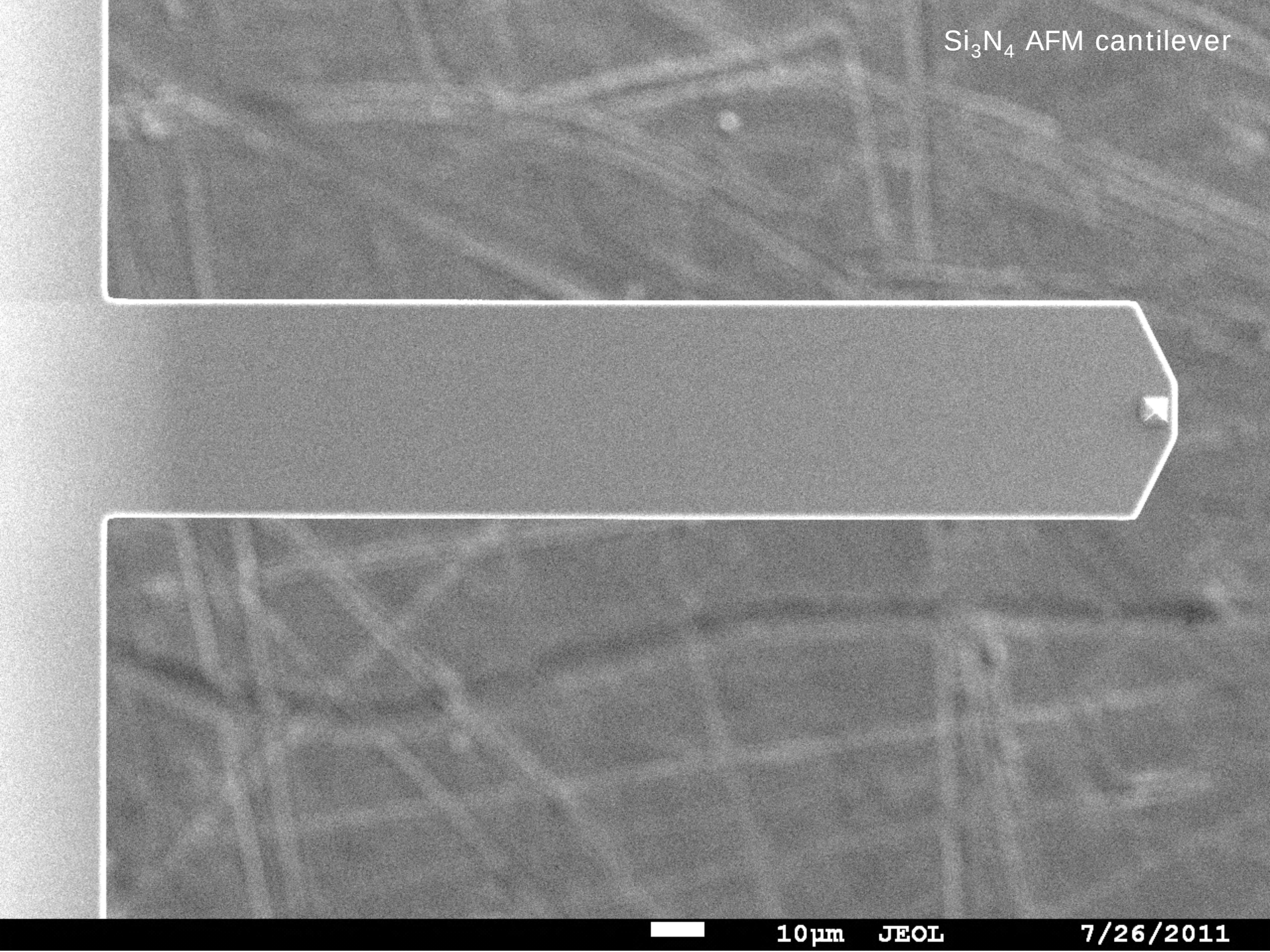
Ice Lithography for Nanodevices

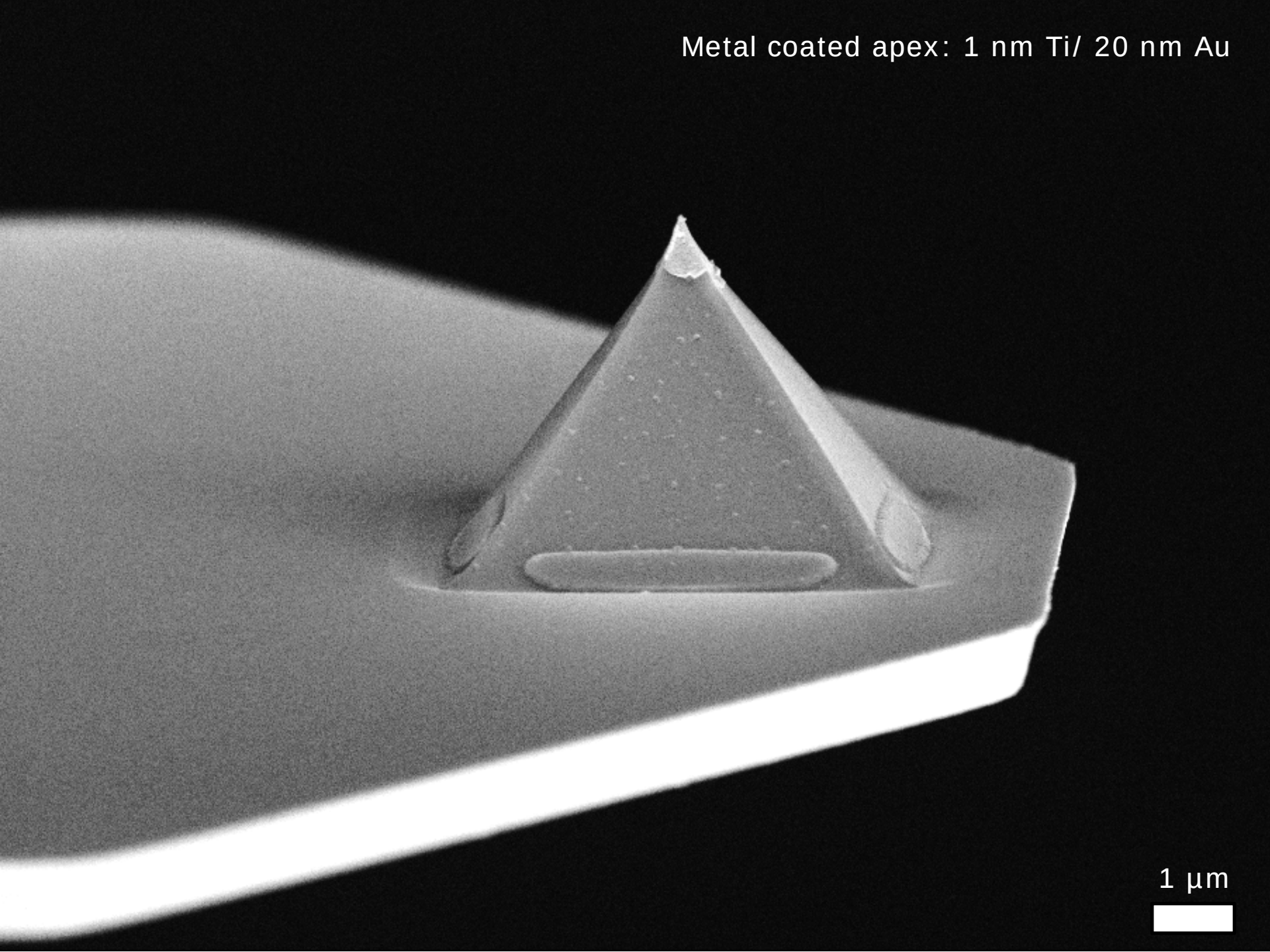
THE HARVARD NANOPORE GROUP

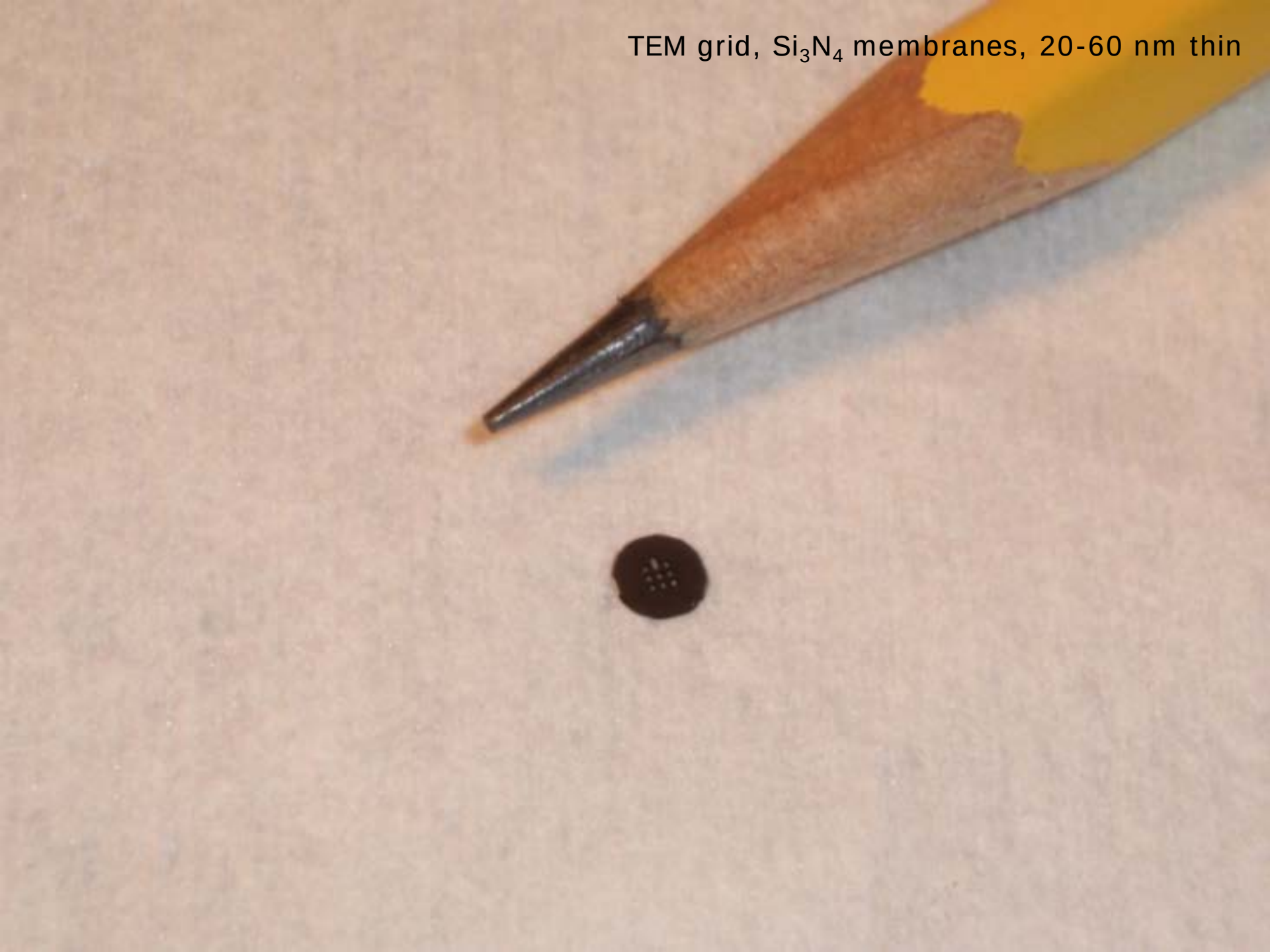
Anpan Han, Dimitar Vlassarev, Jenny Wang,
Jene A. Golovchenko, Daniel Branton

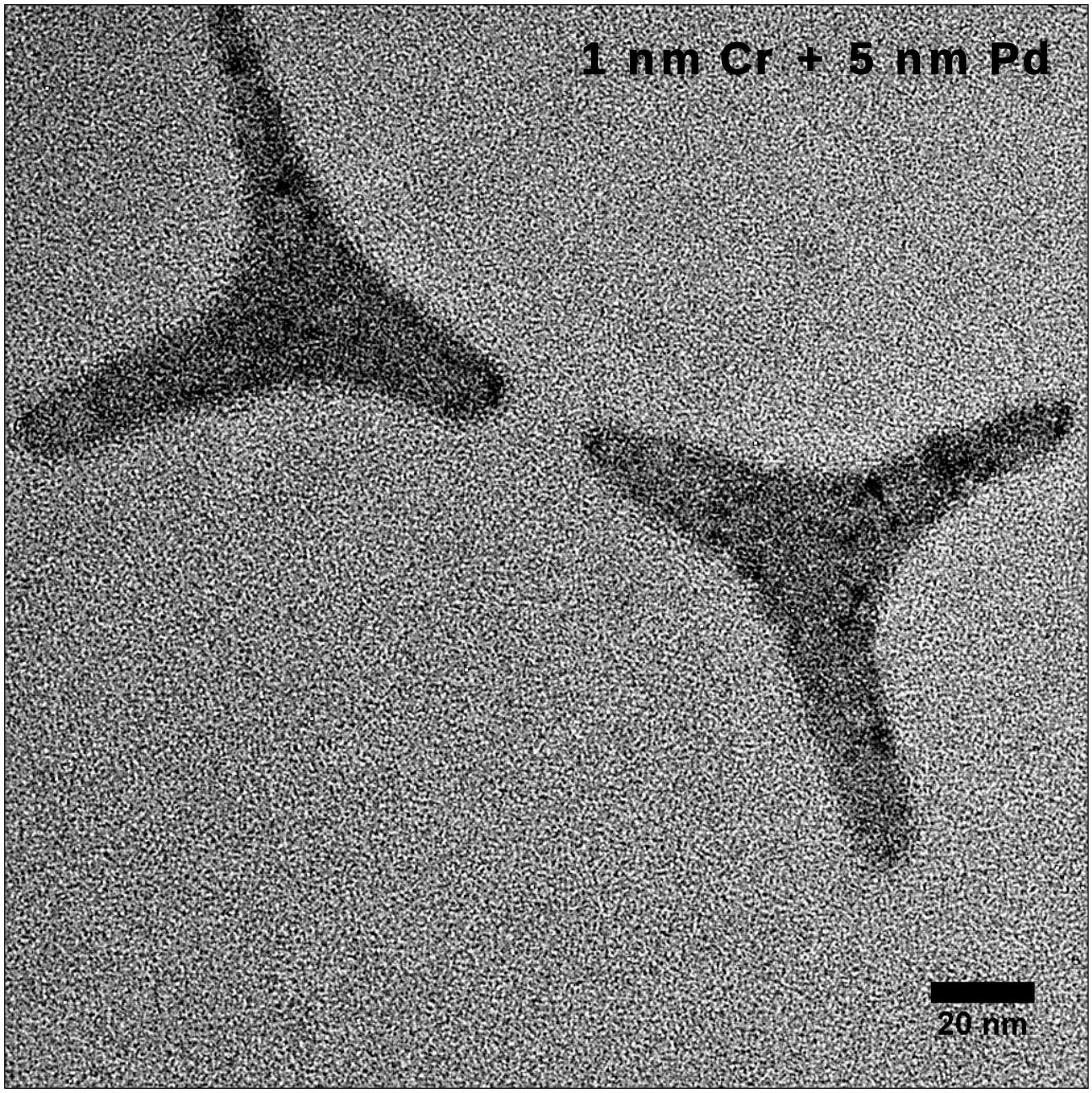
We can pattern on non-planar samples!

Han et al. 2012, Nano Letters

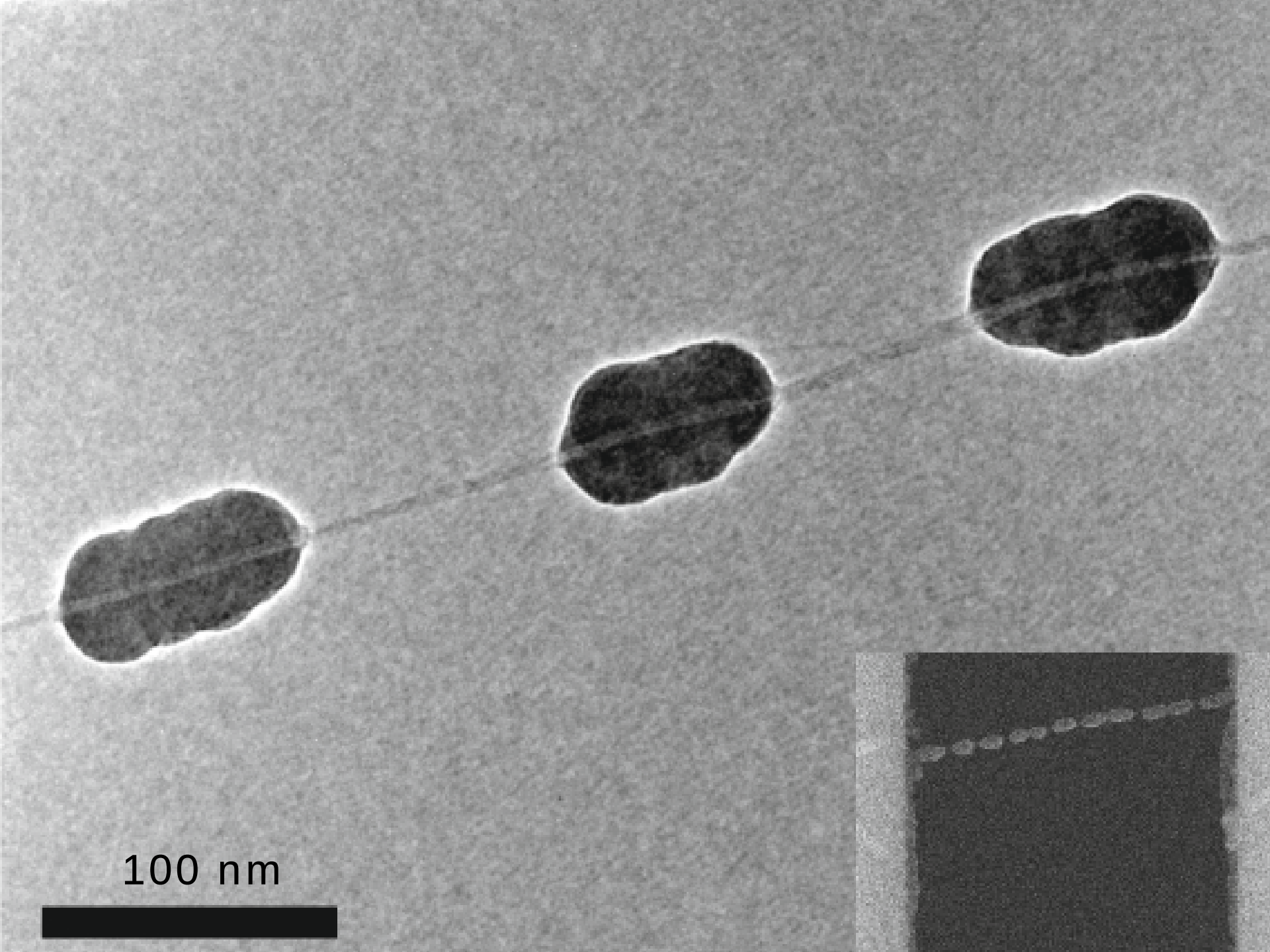

Si_3N_4 AFM cantilever
10µm JEOL 7/26/2011


Metal coated apex: 1 nm Ti/ 20 nm Au
1 µm

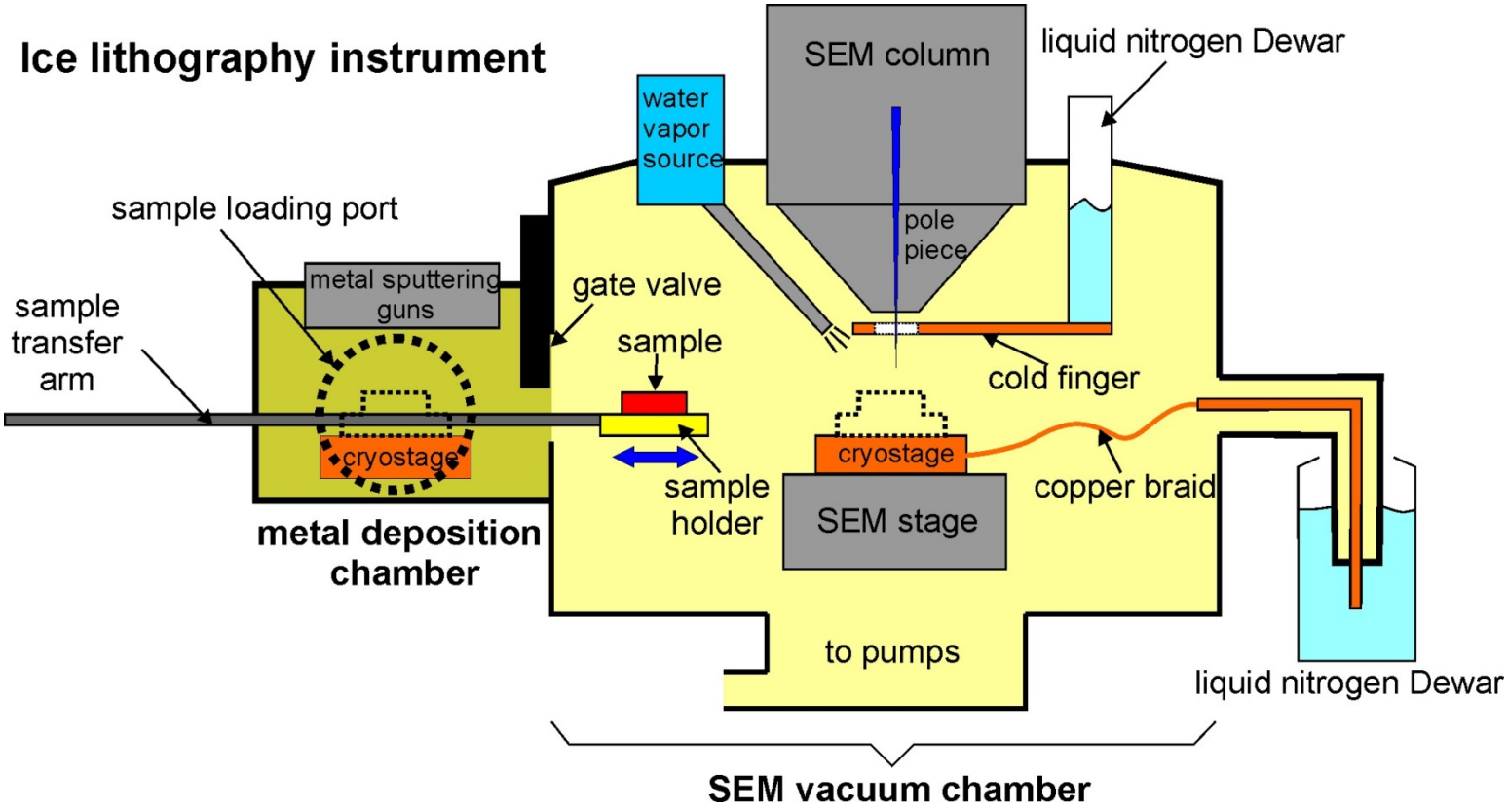

TEM grid, Si_3N_4 membranes, 20-60 nm thin


1 nm Cr + 5 nm Pd
20 nm

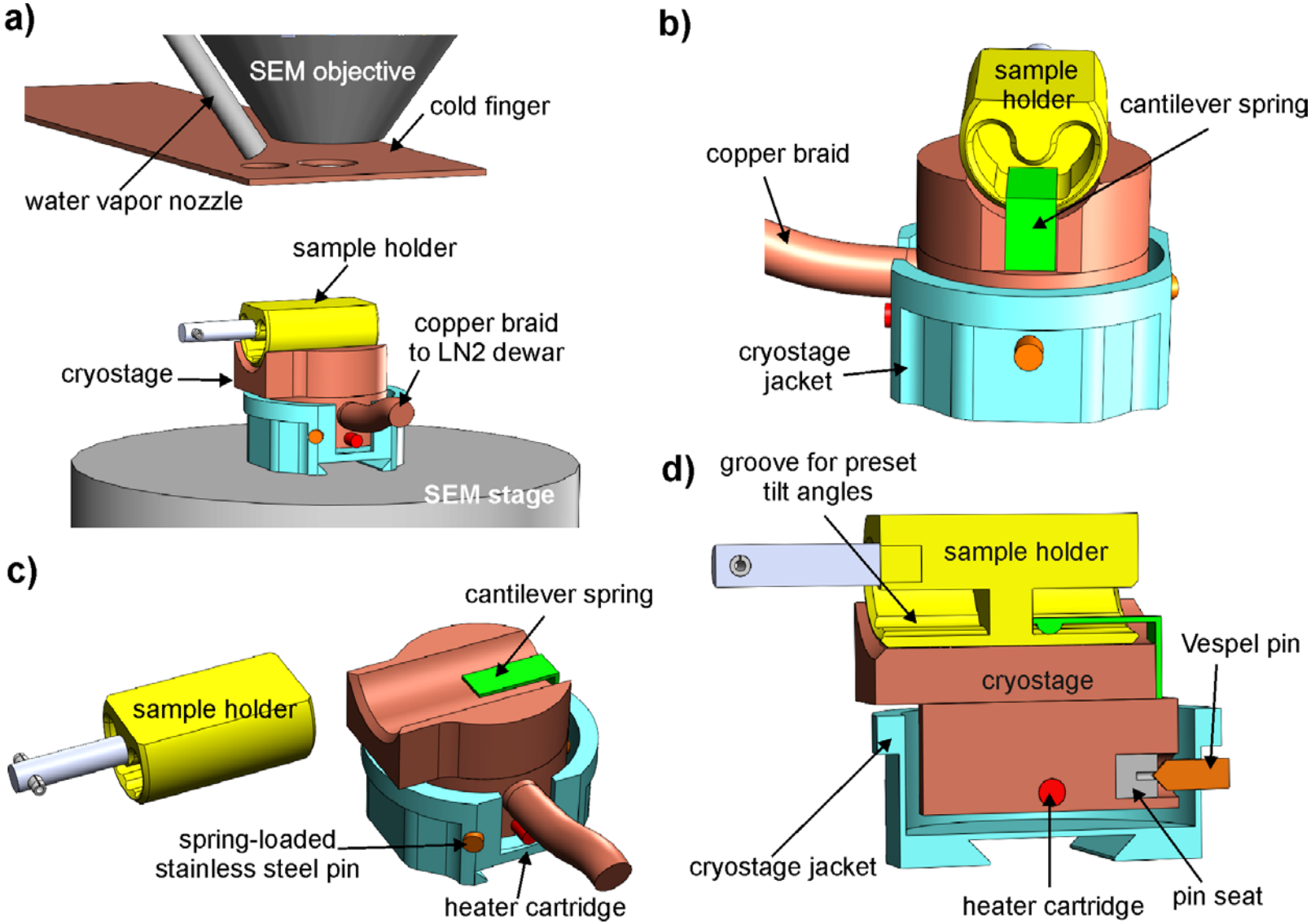

100 nm


100 nm

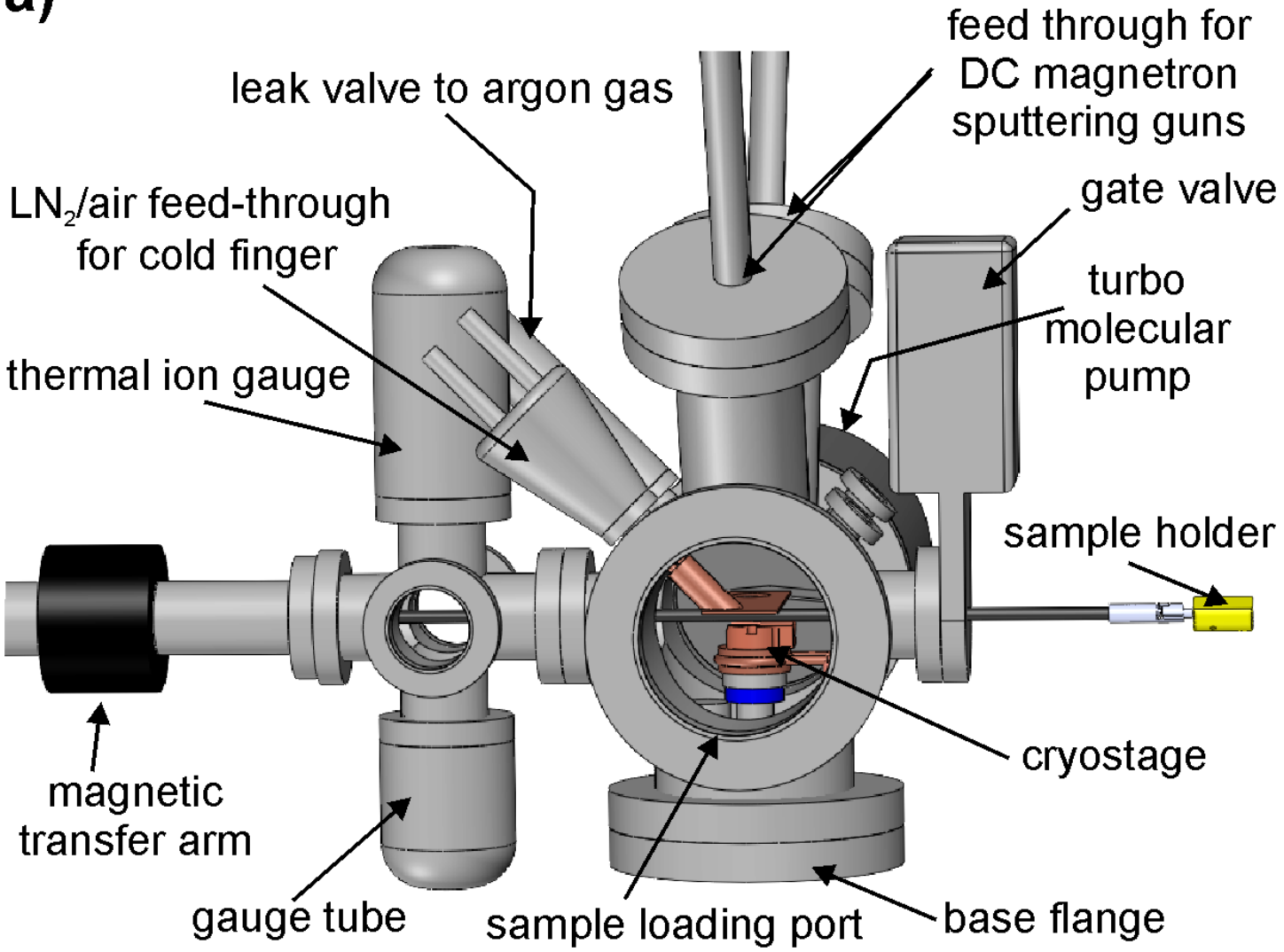
ice lithography instrument

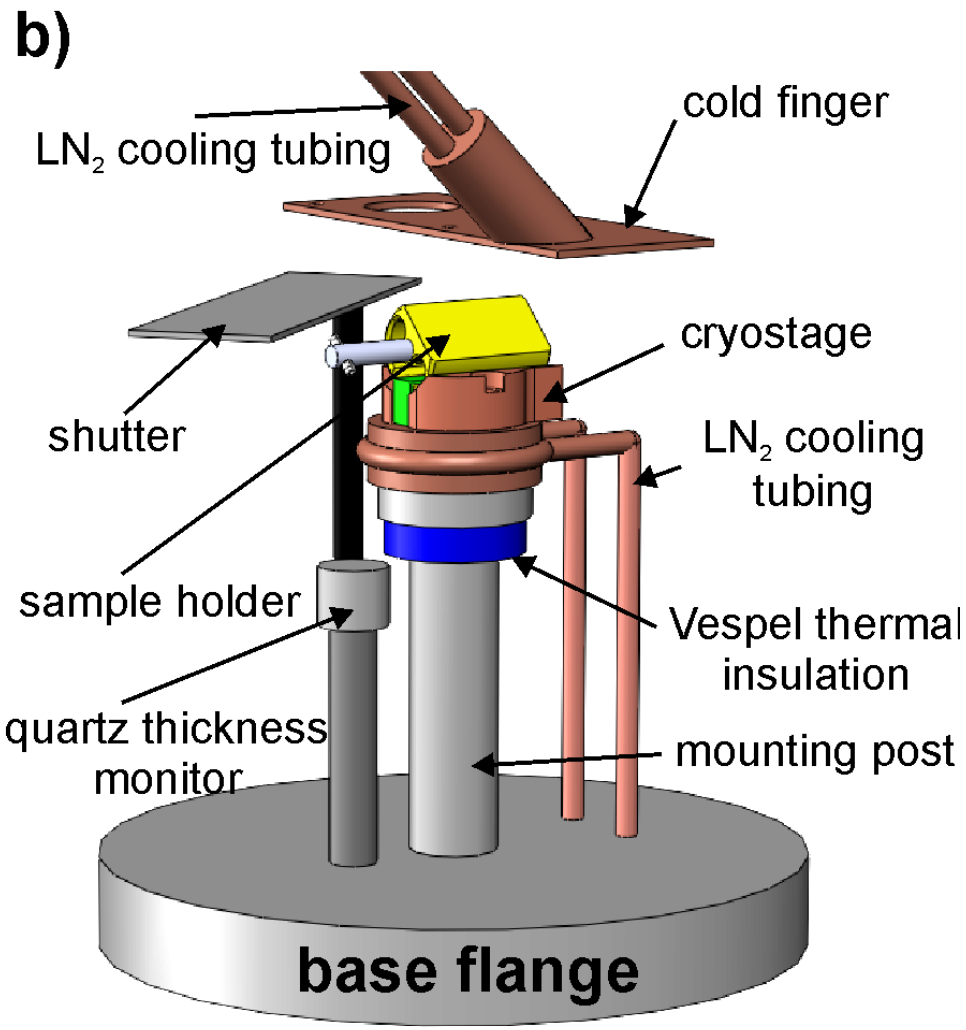


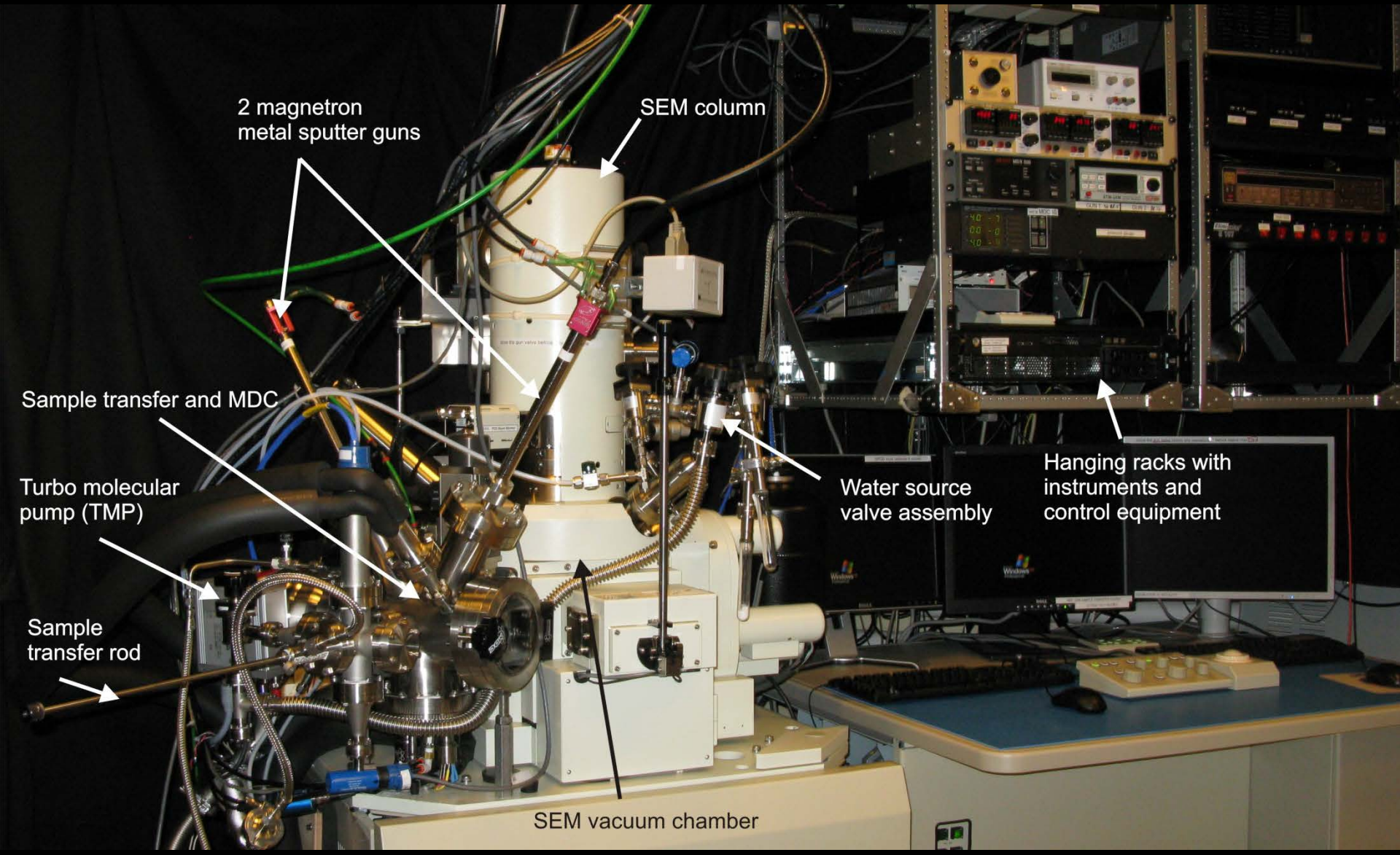
Han et al. 2011 Rev. Sci. Inst


a)
SEM objective
cold finger
water vapor nozzle
sample holder
copper braid
to LN2 dewar
cryostage
SEM stage
b)
sample
holder
cantilever spring
copper braid
cryostage
jacket
c)
cantilever spring
sample holder
spring-loaded
stainless steel pin
heater cartridge
d)
groove for preset
tilt angles
sample holder
Vespel pin
cryostage
cryostage jacket
heater cartridge
pin seat

a)


feed through for DC magnetron sputtering guns
leak valve to argon gas
gate valve
LN_2/air feed-through for cold finger
turbo molecular pump
thermal ion gauge
sample holder
cryostage
magnetic transfer arm
gauge tube
sample loading port
base flange


b)
cold finger
LN_2 cooling tubing
cryostage
shutter
LN_2 cooling tubing
sample holder
Vespel thermal insulation
quartz thickness monitor
mounting post
base flange

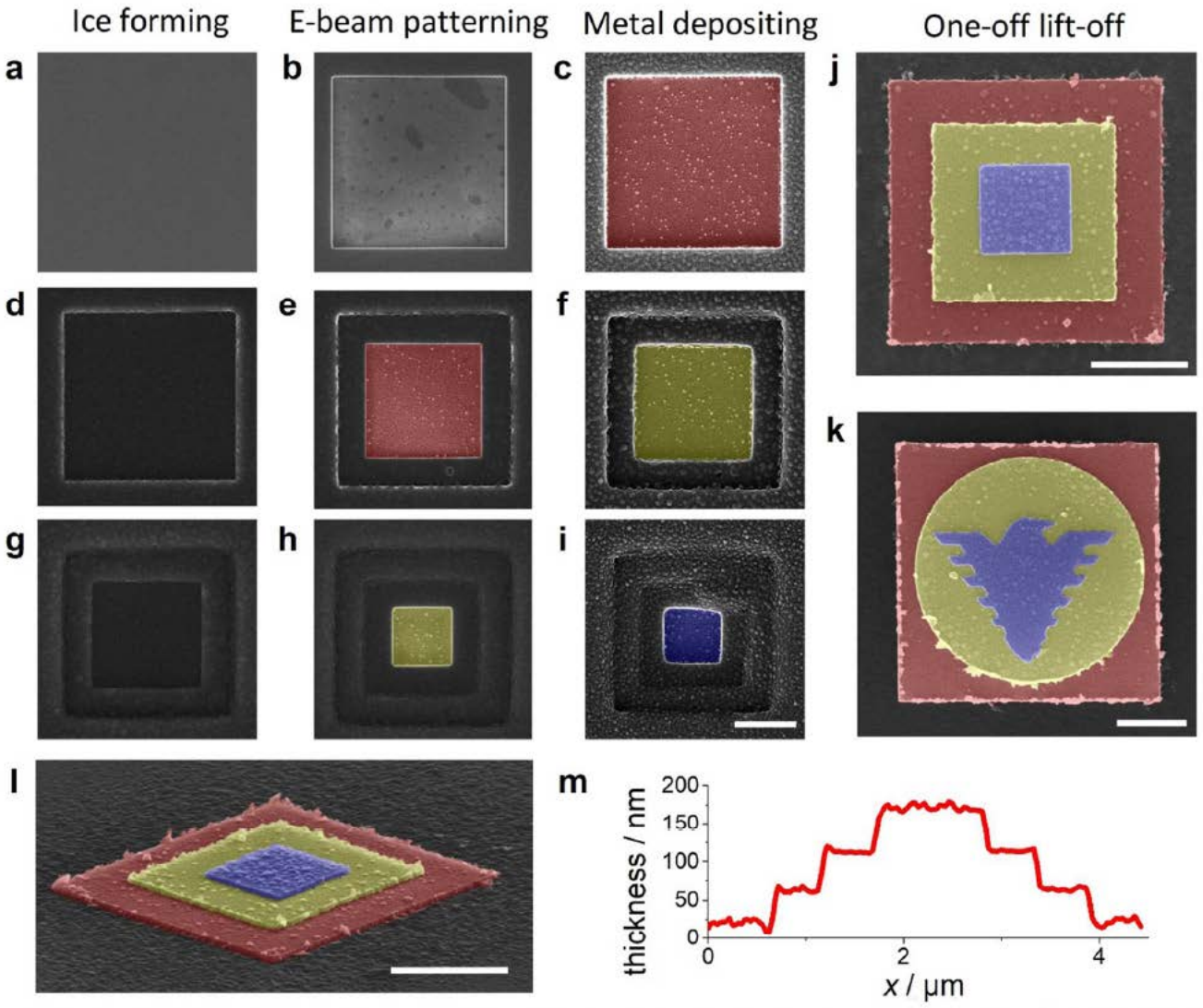

2 magnetron
metal sputter guns
SEM column
Sample transfer and MDC
Turbo molecular
pump (TMP)
Sample
transfer rod
Water source
valve assembly
Hanging racks with
instruments and
control equipment
SEM vacuum chamber

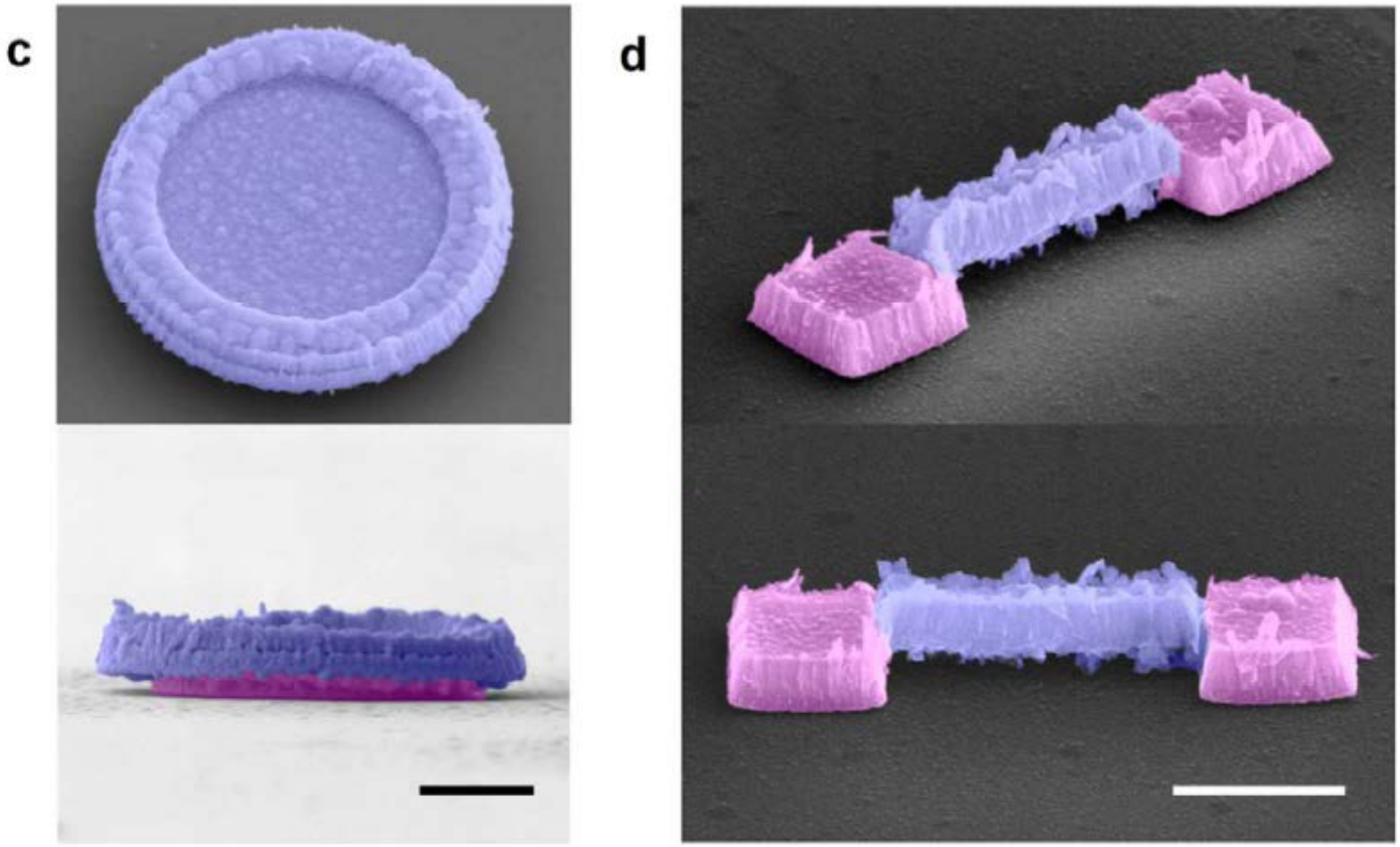
Communication

Three-dimensional *in situ* electron beam lithography using water ice

Yu Hong, Ding Zhao, Dongli Liu, Binze Ma, Guangnan Yao, Qiang Li, Anpan Han, and Min Qiu

Nano Lett., **Just Accepted Manuscript** • DOI: 10.1021/acs.nanolett.8b01857 • Publication Date (Web): 25 Jun 2018


Ice forming
E-beam patterning
Metal depositing
One-off lift-off
a
b
c
j
d
e
f
k
g
h
i
l
m
thickness / nm
200
150
100
50
0
0
2
4
x / µm


c
d

Advantages

- Non-planar and extremely fragile substrates
- High resolution: sub-10 nm
- Rapid and resource efficient: prototype in 3 h
- Clean: no resist contamination
- Fabrication of nanodevices

Draw backs

- All processes at cryogenic temperatures
- High critical dose: 1C/cm2
- Advanced instrument, high cost

Better ices?

Quiz

1. What does SI in the presentation stand for?
2. How many authors on the “3 decades of Nanopore DNA sequencing” paper?
3. How is “scum” removed?
4. How many nm are the smallest patterns made by ice lithography?
5. What is the critical dose for water ice resist?
6. Advantages of ice lithography? The more the better.

Answers

1. What does SI in the presentation stand for? Seemingly implausible
2. How many authors on the “3 decades of Nanopore DNA sequencing” paper? 3
3. How is “scum” removed? Oxygen plasma
4. How many nm are the smallest patterns made by ice lithography? 6 nm
5. What is the critical dose for water ice resist? 1 C/cm2
6. Advantages of ice lithography? The more the better.

10 min break, and bring your laptops

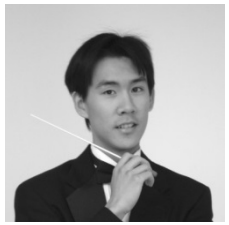
Seemingly implausible nanoscale 3D print of functional materials?

OVERVIEW OF THE 5 BRANCHES OF CHEMISTRY

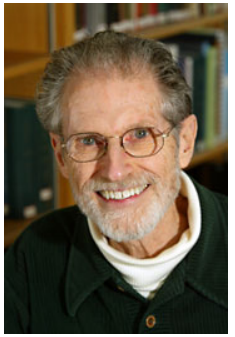
1. **Organic Chemistry** - The study of carbon and its compounds; the study of the chemistry of life.
2. **Inorganic Chemistry** - The study of compounds not-covered by organic chemistry; the study of inorganic compounds or compounds which do not contain a C-H bond. Many inorganic compounds are those which contain metals.
3. **Analytical Chemistry** - The study of the chemistry of matter and the development of tools used to measure properties of matter.
4. **Physical Chemistry** - The branch of chemistry that applies physics to the study of chemistry. Commonly this includes the applications of thermodynamics and quantum mechanics to chemistry.
5. **Biochemistry** - This is the study of chemical processes that occur inside of living organisms.

Branches of Organic Chemistry

- Metalorganic chemistry
- Polymer chemistry (dielectrics)
- Semiconducting organic chemistry
- and many more.



Acknowledgements at Harvard Uni.



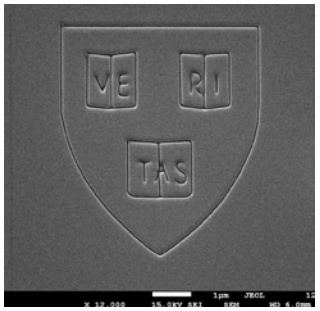
Dan Branton, Jene Golovchenko, Lene Hau.
Dimitar Vlassarev, Jenny Wang, Aaron Kuan.

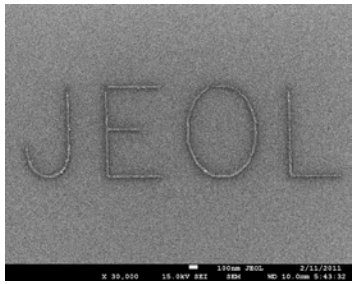
John Chervinski, Peter Frisella, Ray Aubut.

JEOL USA.

NIH National Human Genome Research Institute

CARLSBERGFONDET
CARLSBERG FOUNDATION

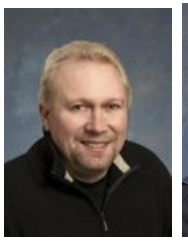




Acknowledgements for DTU ice activities


- Project: William Tiddi, Anna Elsukova, Liu Pei, Hoa Thanh Le, Marco Belegia


- Technical staff at Danchip CEN: Roy Cork, Jonas M. Lindhart, Anders Gregersen, Jakob B Wagner.




- Technical staff at DTU Risø: Michael Nimb; Troels Fel d, Johnny Egtved Jørgensen


- Management: Jörg Hübner, Flemming Jensen, Anders M. Jørgensen


- Zeiss R&D: Camille Stebler



THE VELUX FOUNDATIONS
VILLUM FONDEN ✕ VELUX FONDEN

Group work: 3-4 people in each group (25 min)

- Killer application? What would you 3D print? (15 min)

- Present your killer application to the class.
  - 2 min for each group,
  - 2-3 slides

- Vote for best killer application measured in impact:
  - New needs, saving lives, market size, live better and longer, solve big problems

- Winners shares a gift from Denmark.