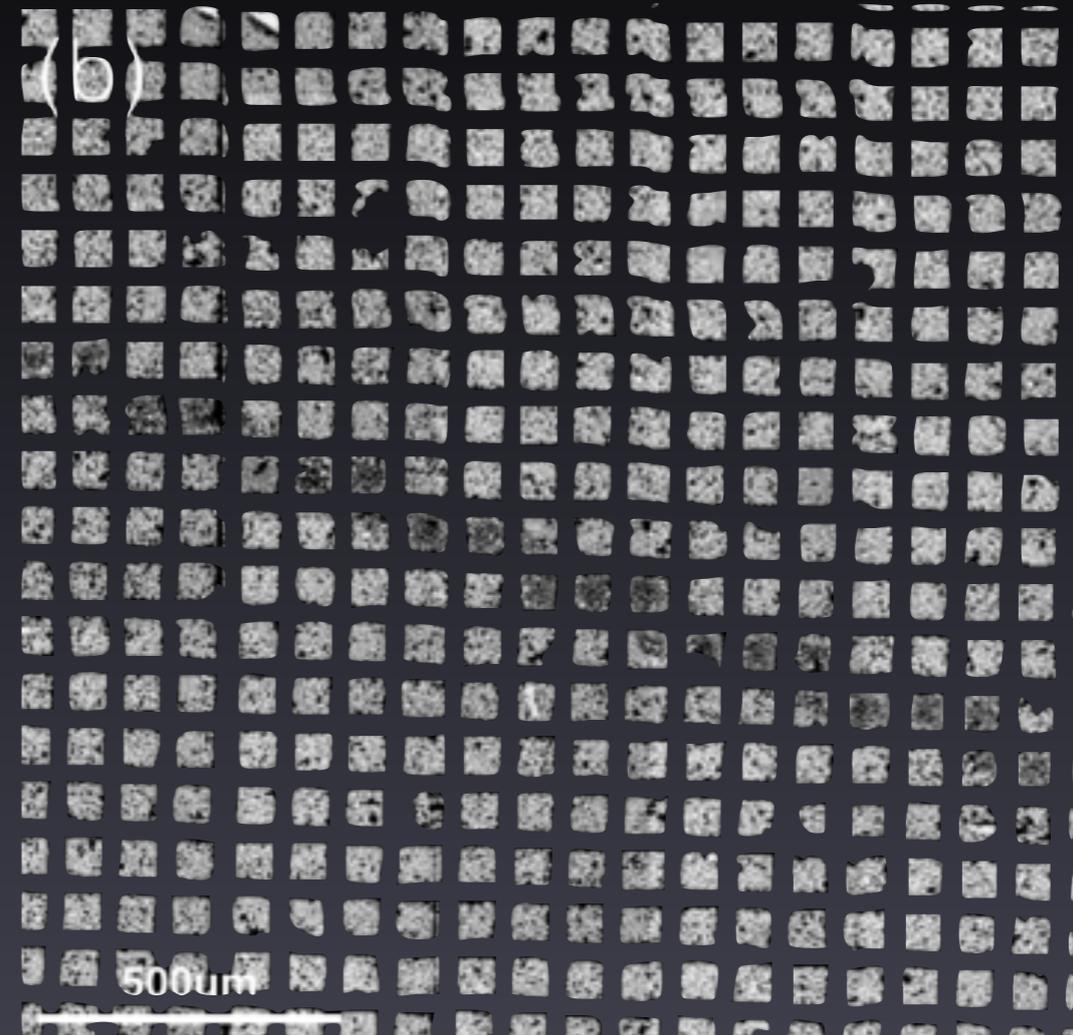
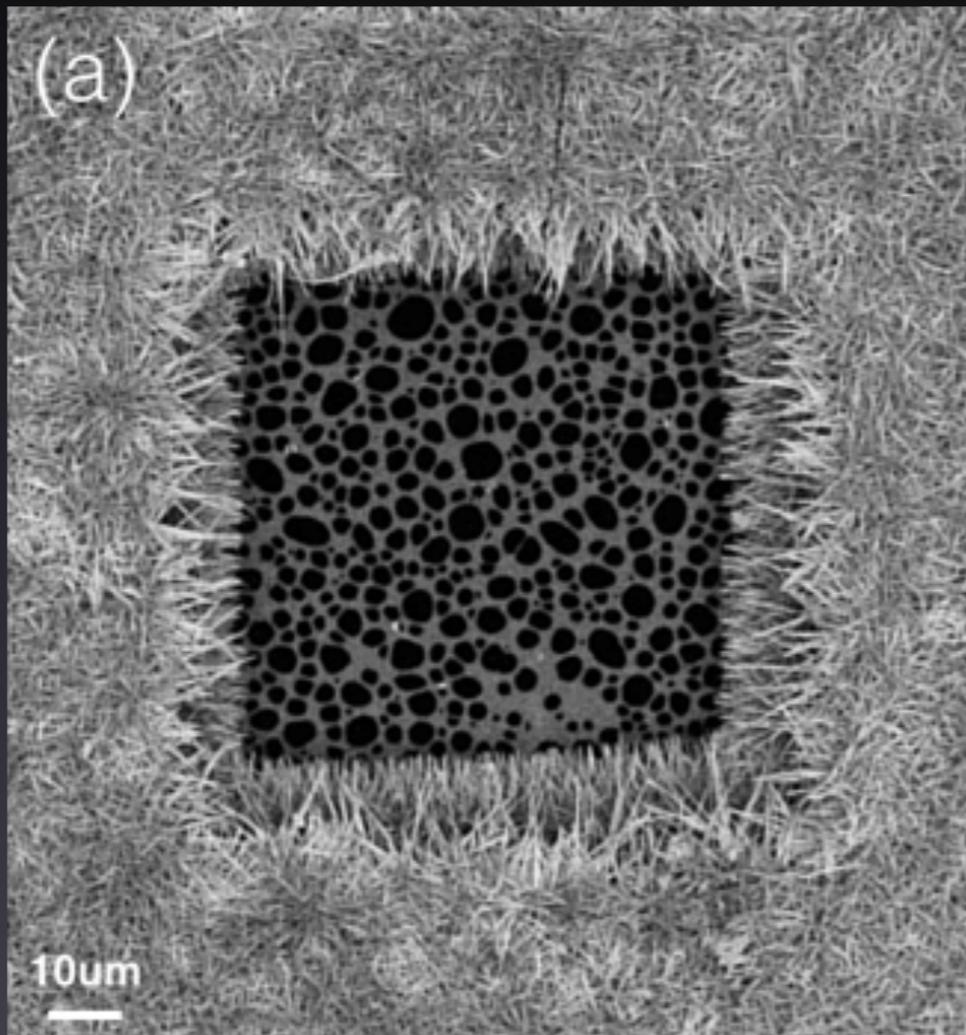




# A new method for cryoEM sample preparation

NEW YORK STRUCTURAL BIOLOGY CENTER  
Simons Electron Microscopy Center



Bridget Carragher



National Resource for Automated Molecular Microscopy  
<http://nramm.nysbc.org>

NRAMM Workshop  
20 October 2017



# NRAMM



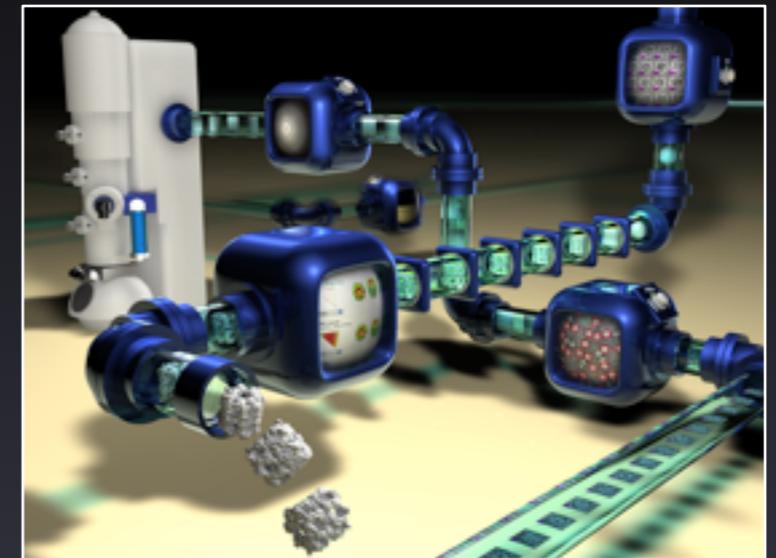
The overall mission of NRAMM is to develop, test and apply technology for automating and streamlining cryo-electron microscopy (cryoEM) for structural biology.



Specimen preparation



Image acquisition



Data processing

## Technology enables:

Accessibility

Higher throughputs

“High” resolution structures of “small” / asymmetric / heterogeneous particles  
(may need to analyze 1,000,000’s molecules)

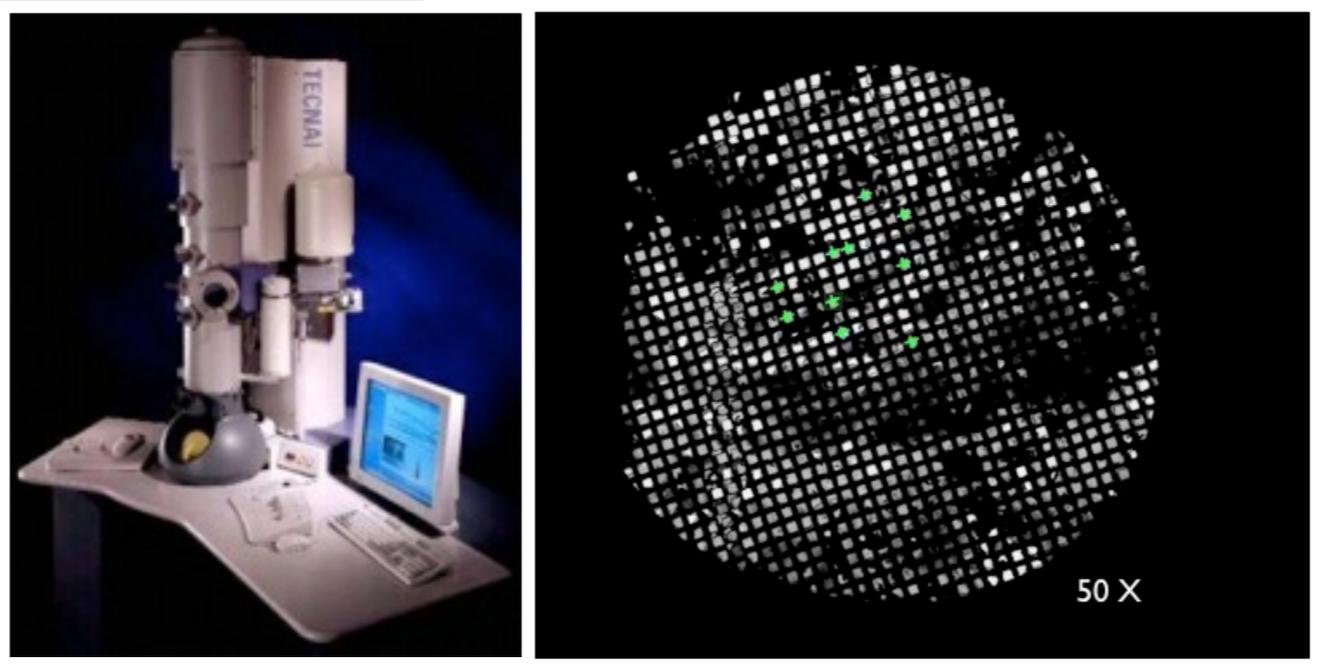
Determination of many 3D structures in different states  
(may need 100’s of maps)

**Investigation of the structure, function and dynamics of molecular machines**

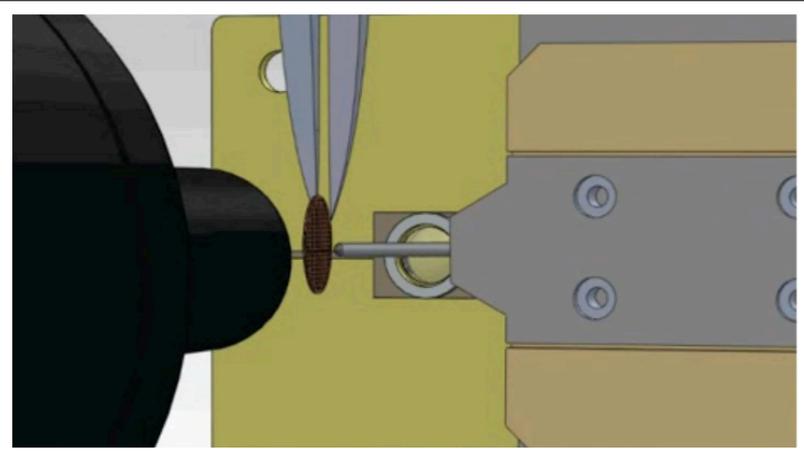
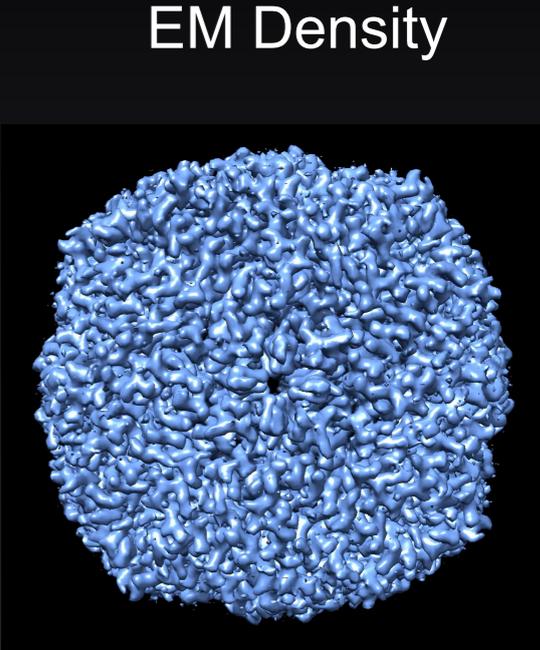
# Automation for Single Particle CryoEM



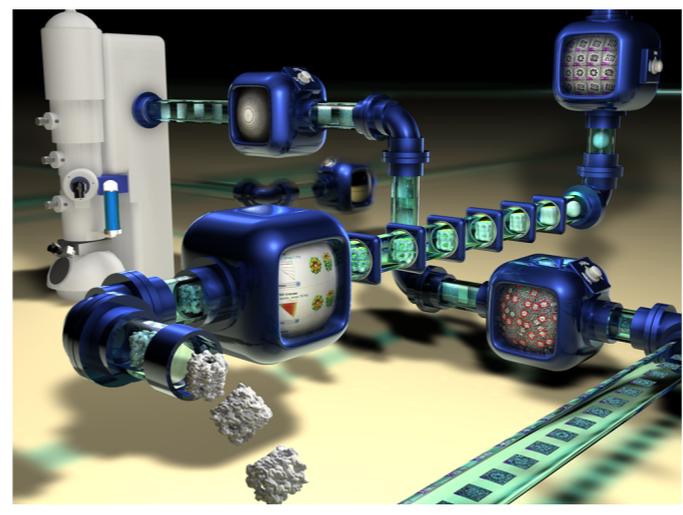
Sample



Automated Data Collection (Leginon)



Grid preparation (Spotiton)



Streamlined Processing (Appion)

Appion Data Processing

Project: NRAMM - GroEL 100K  
 Session: 06jul12a - GroEL 100K at 100K at 120KeV  
 Image Path: /amixdata08/legion/06jul12a/reldata

Exemplar Iteration ID: 4877

CTF info		name	nb	min	max	avg	stddev
nominal		en	1756	-5.330 $\mu\text{m}$	-0.801 $\mu\text{m}$	-1.392 $\mu\text{m}$	0.367 $\mu\text{m}$
ACE Estimation		en	1756	0.412 $\mu\text{m}$	5.594 $\mu\text{m}$	0.813 $\mu\text{m}$	1.872 $\mu\text{m}$
defocus1		fc	390	0.908 $\mu\text{m}$	10.000 $\mu\text{m}$	2.081 $\mu\text{m}$	0.422 $\mu\text{m}$
ACE 2 Estimation		en	1756	$6.385 \times 10^{-3}$	0.986	0.794	0.226
confidence		fc	390	$6.871 \times 10^{-3}$	0.889	0.761	$9.495 \times 10^{-2}$
CTF11 Estimation		en	1756	$1.639 \times 10^{-2}$	0.986	0.822	0.196
confidence_d		fc	390	$5.181 \times 10^{-2}$	0.863	0.764	$9.234 \times 10^{-2}$
min stack base		en	1756	$2.106 \times 10^{-9}$	$4.443 \times 10^{-6}$	$-5.737 \times 10^{-7}$	$1.719 \times 10^{-6}$
Particle Alignment		fc	390	$8.233 \times 10^{-11}$	$8.000 \times 10^{-6}$	$8.131 \times 10^{-8}$	$4.224 \times 10^{-7}$
Run Alignment		fc	390	$8.233 \times 10^{-11}$	$8.000 \times 10^{-6}$	$8.131 \times 10^{-8}$	$4.224 \times 10^{-7}$

Particle Selection info		name	dbid	totparticles	numimgs	min	max	avg	stddev
inspected images: 605, [inspected data]		test1	249	34082	561 (60.9 prt/img)	0.3500	0.5473	0.4023	$3.6425 \times 10^{-2}$

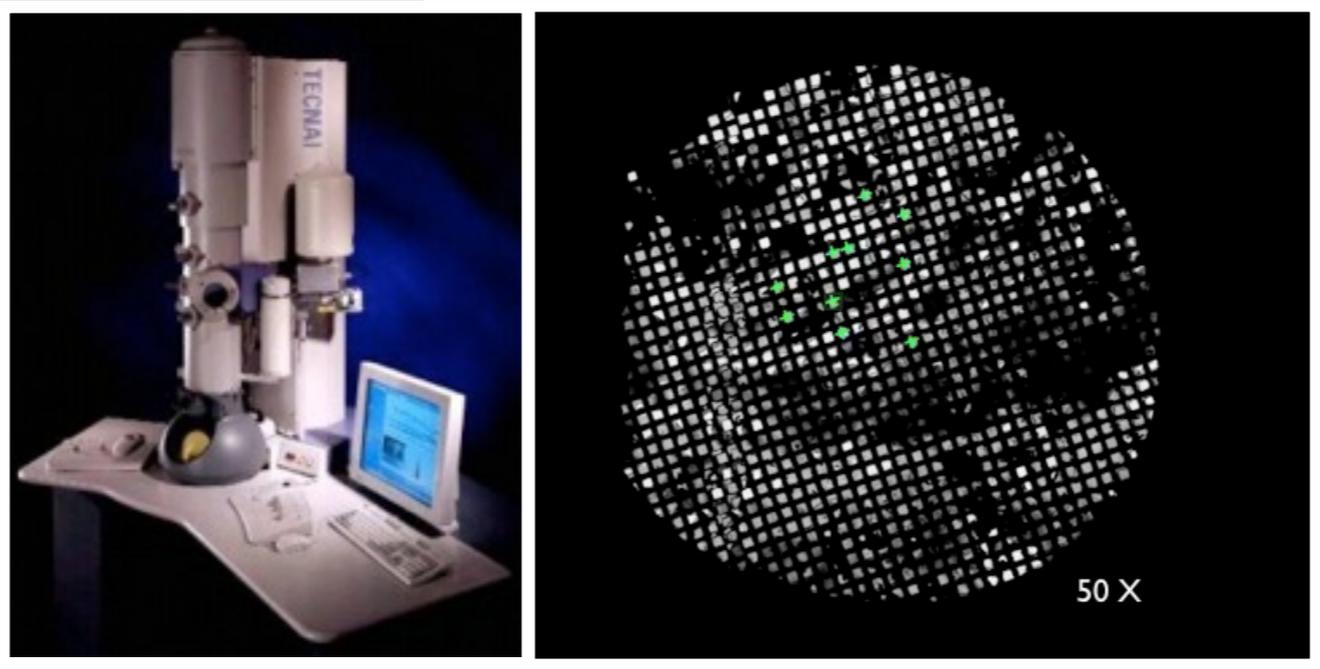
  

Reconstructions: 28		name	dbid	totparticles	numimgs	min	max	avg	stddev
EMAN Reconstruction		run4	279	79890	1703 (46.9 prt/img)	0.5000	0.7309	0.5736	$4.7857 \times 10^{-2}$

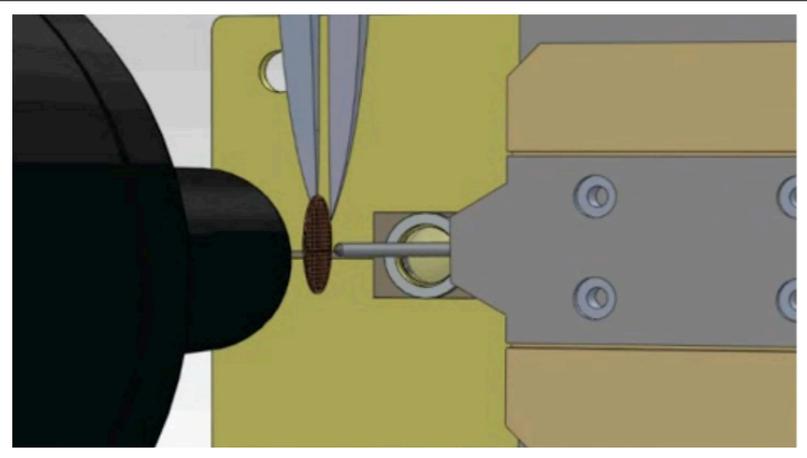
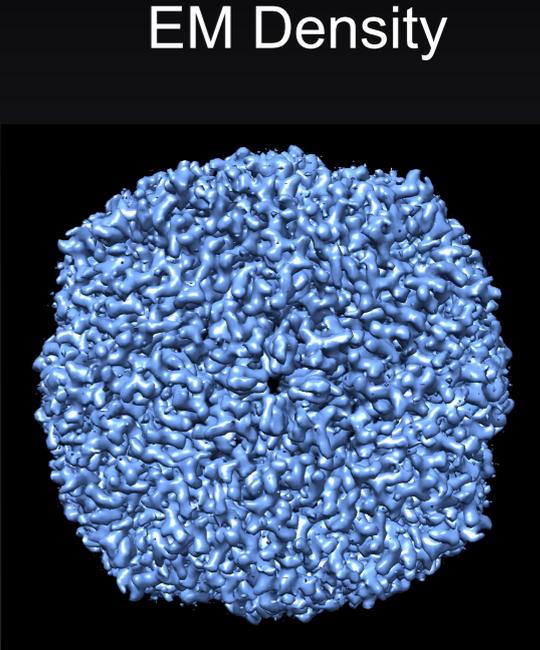
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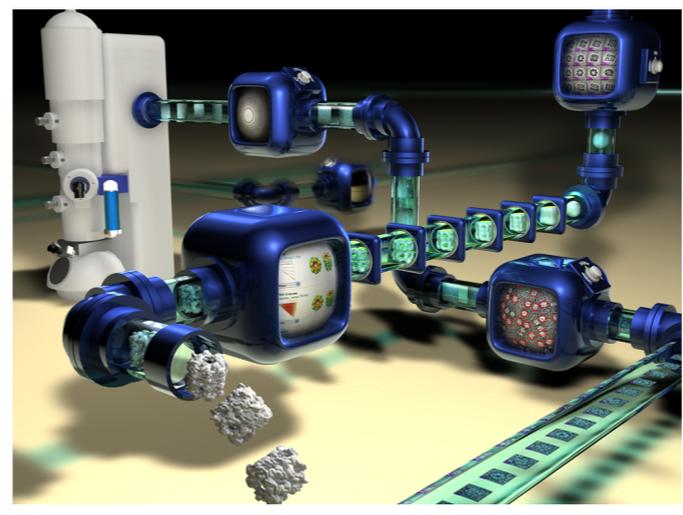
Sample



Automated Data Collection (Leginon)



Grid preparation (Spotiton)



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Streamlined Processing (Appion)

# Simons Electron Microscopy Center Electron Microscopes



**FEI Titan Krios#1  
Falcon3  
K2**



**FEI Titan Krios#2  
Falcon 3  
BioQuantum Energy Filter/K2  
Volta Phase Plate  
Cs Corrector**



**FEI Titan Krios#3  
Falcon 3,  
BioQuantum Energy Filter/K2  
Volta Phase Plate**



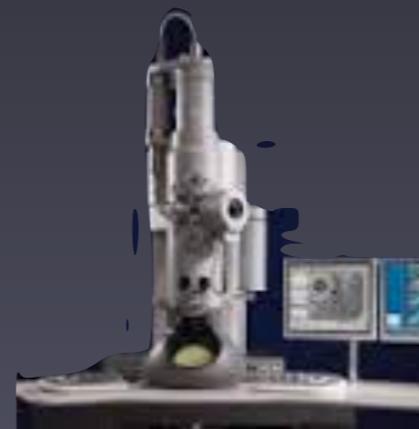
**FEI Helios 650  
Quorum cryotage**



**FEI Tecnai F20  
+  
DE20  
TVIPS 4K CMOS**



**JEOL 1230  
+  
Gatan US4000 CCD**



**FEI Tecnai Biotwin  
+  
TVIPS 4K CMOS**

# Simons Electron Microscopy Center Electron Microscopes



# Simons Electron Microscopy Center Electron Microscopes



~250 active users,  
~150 Krios users.

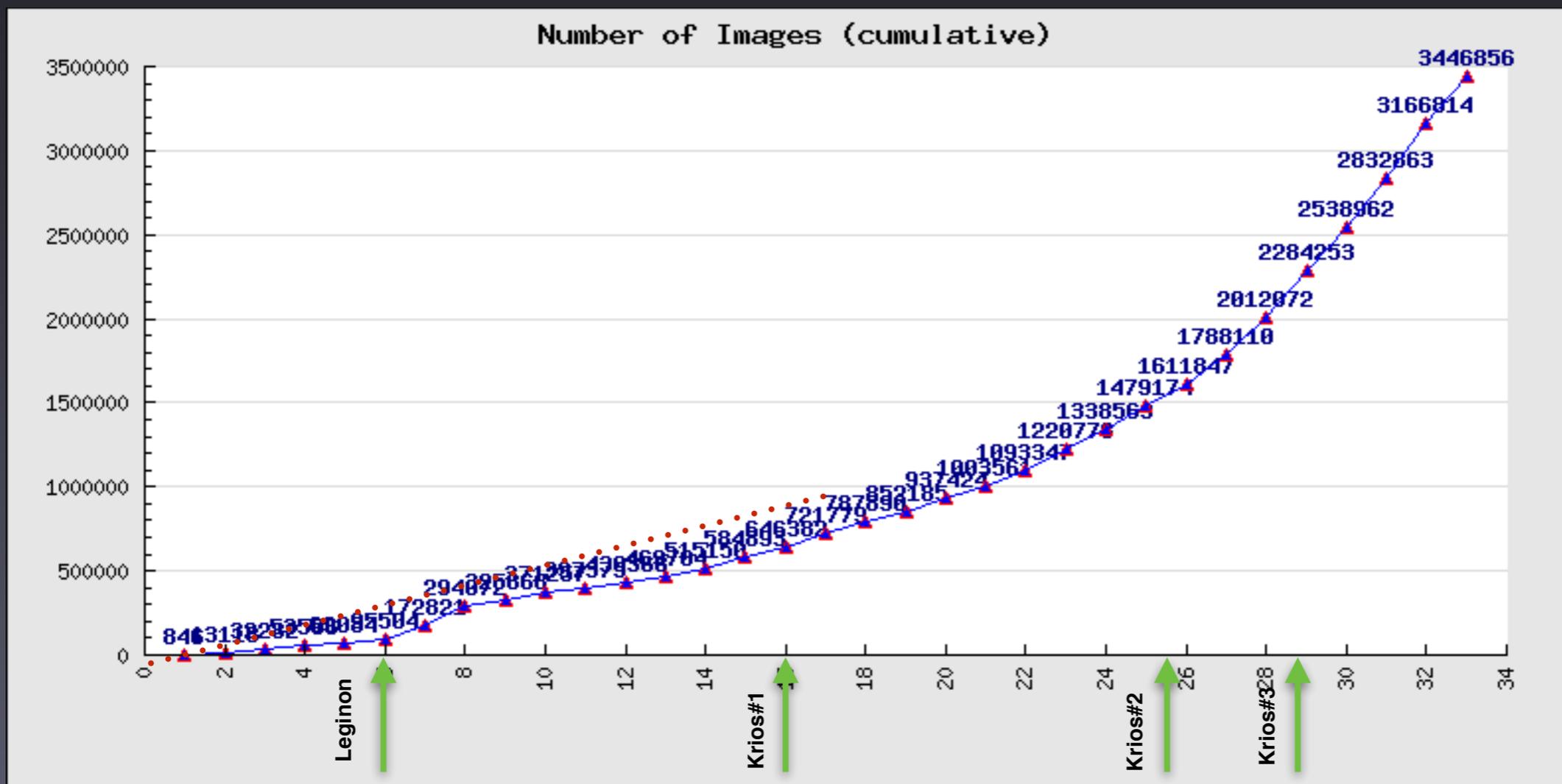
~175 Krios projects.

# Simons Electron Microscopy Center Electron Microscopes



~250 active users,  
~150 Krios users.

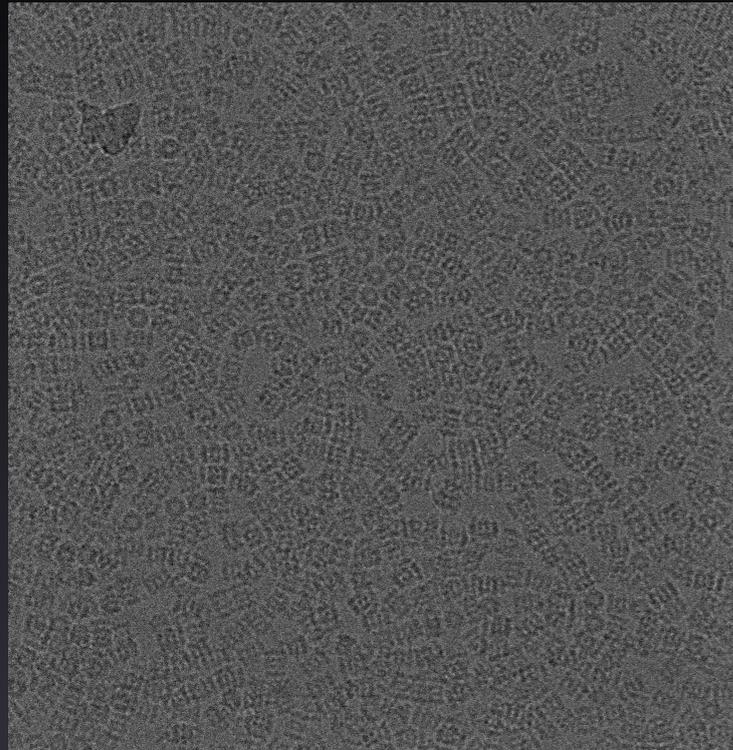
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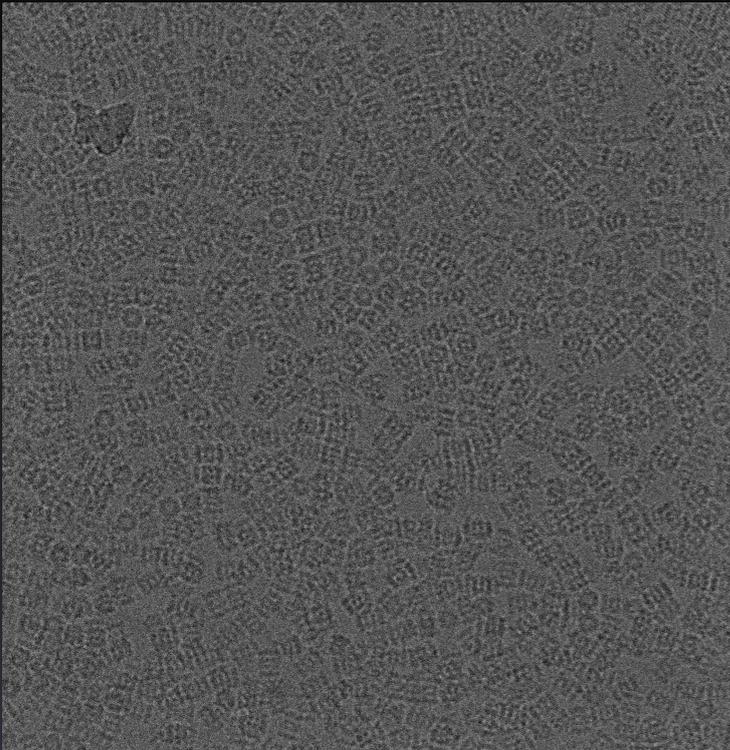
# A streamlined and automated pipeline for cryoEM

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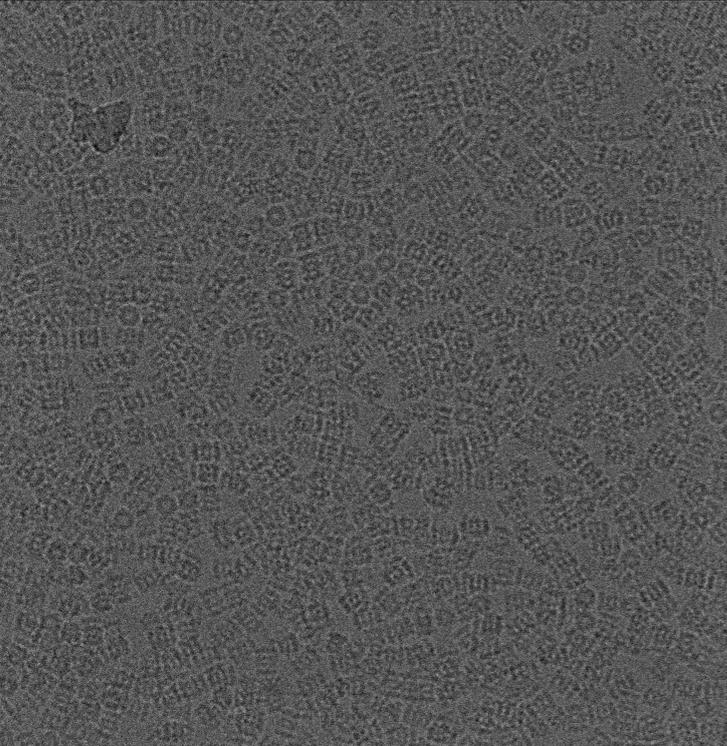
# A streamlined and automated pipeline for cryoEM



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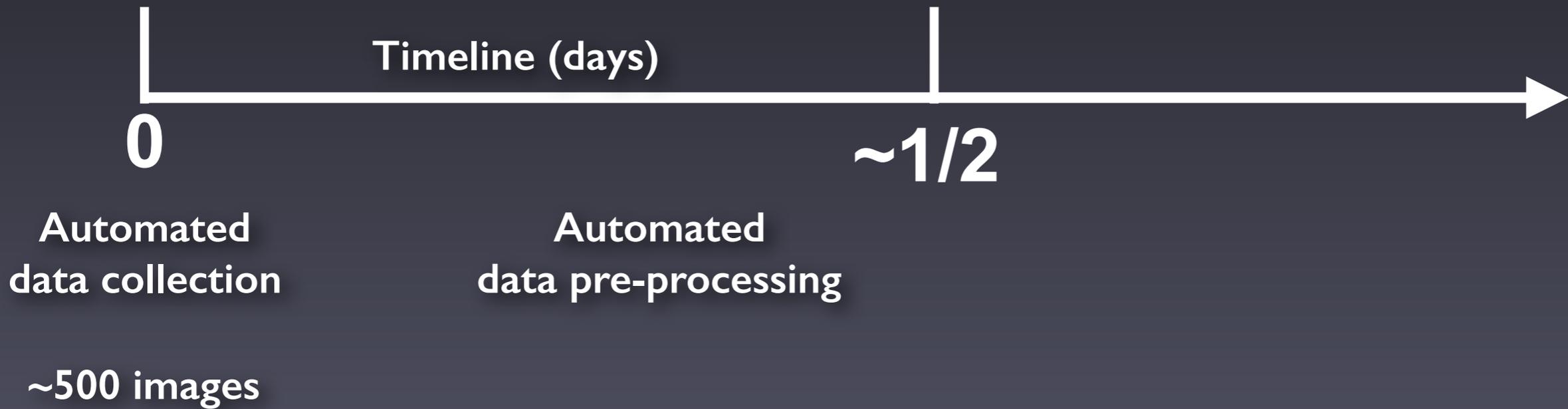
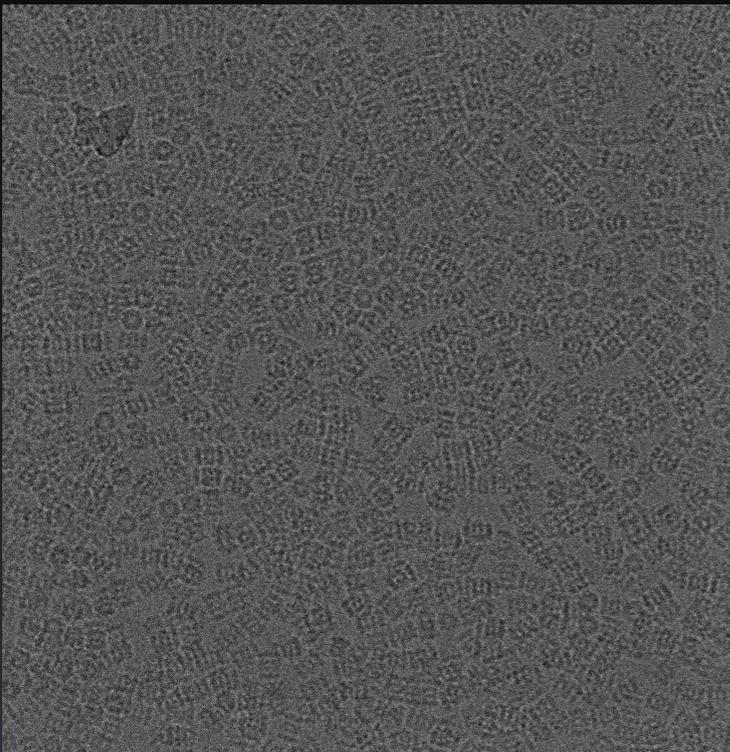
# A streamlined and automated pipeline for cryoEM



Automated  
data collection

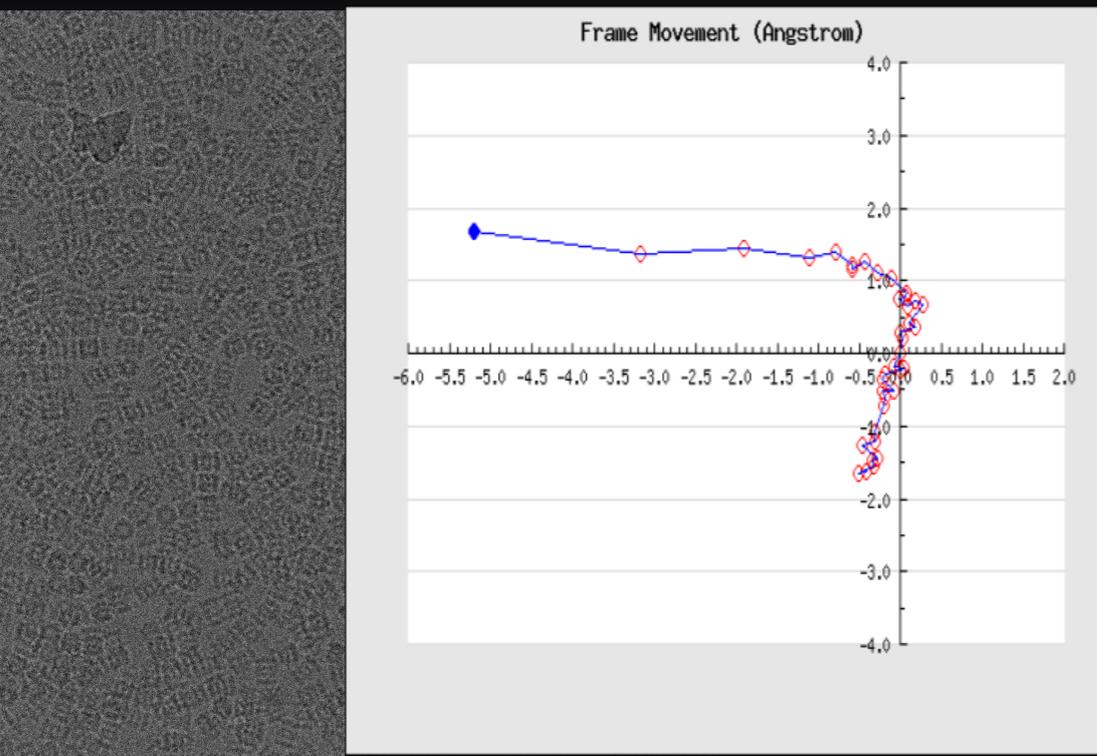
~500 images

# A streamlined and automated pipeline for cryoEM



Sample courtesy of Yifan Cheng, Kiyoshi Egami, Zanlin Yu

# A streamlined and automated pipeline for cryoEM

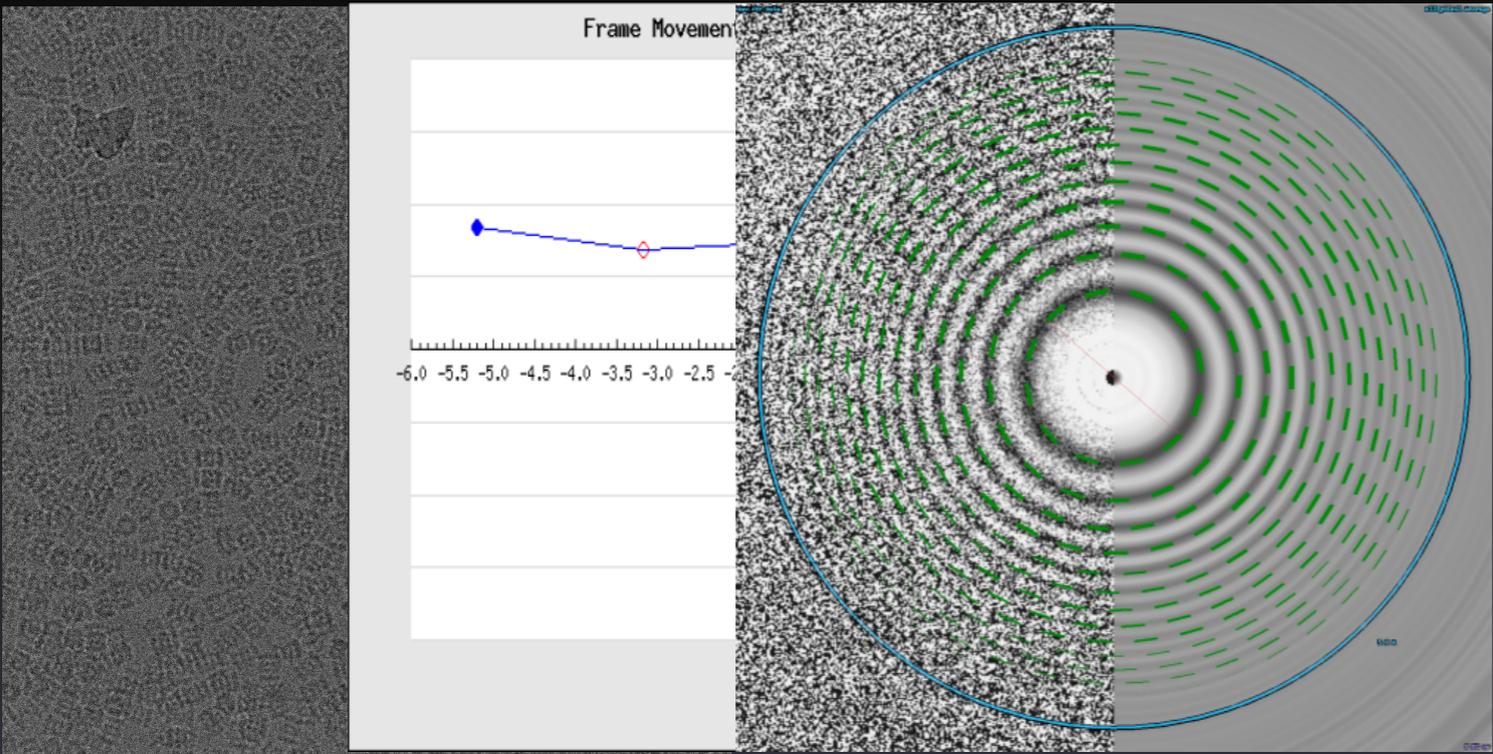


Automated data collection

~500 images

Automated data pre-processing

# A streamlined and automated pipeline for cryoEM

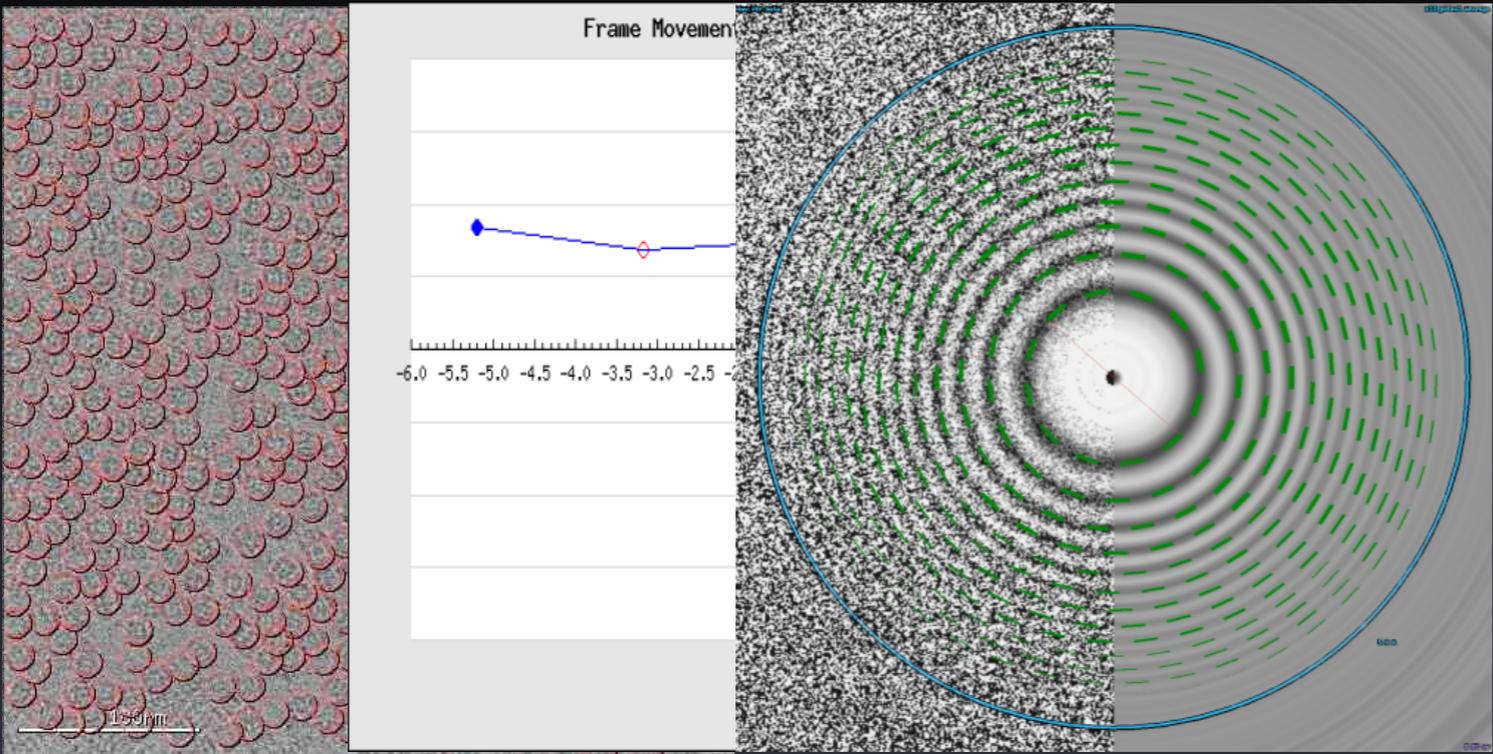


Automated data collection

~500 images

Automated data pre-processing

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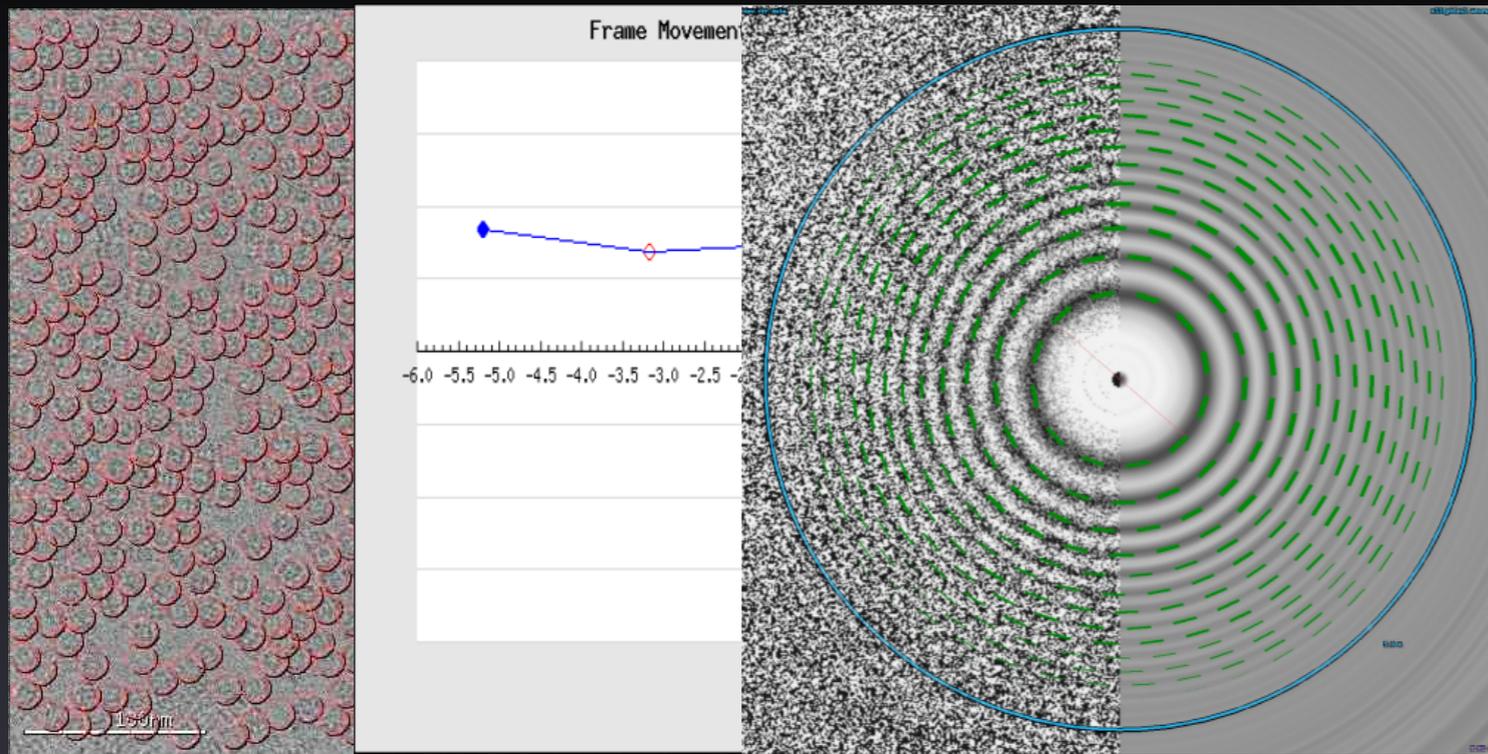


Automated data collection

~500 images

Automated data pre-processing

# A streamlined and automated pipeline for cryoEM



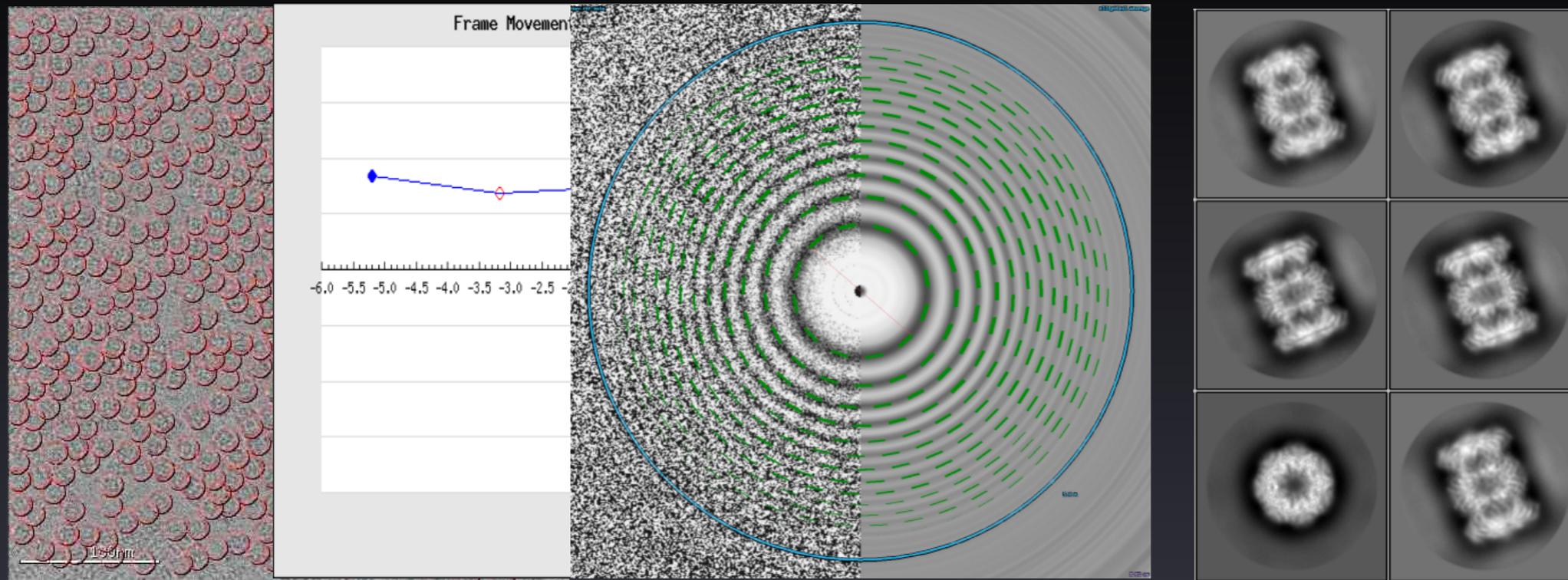
Automated  
data collection

~500 images

Automated  
data pre-processing

~100 “shiny” images  
~50,000 particles

# A streamlined and automated pipeline for cryoEM



Automated  
data collection

~500 images

Automated  
data pre-processing

~100 “shiny” images  
~50,000 particles

Processing

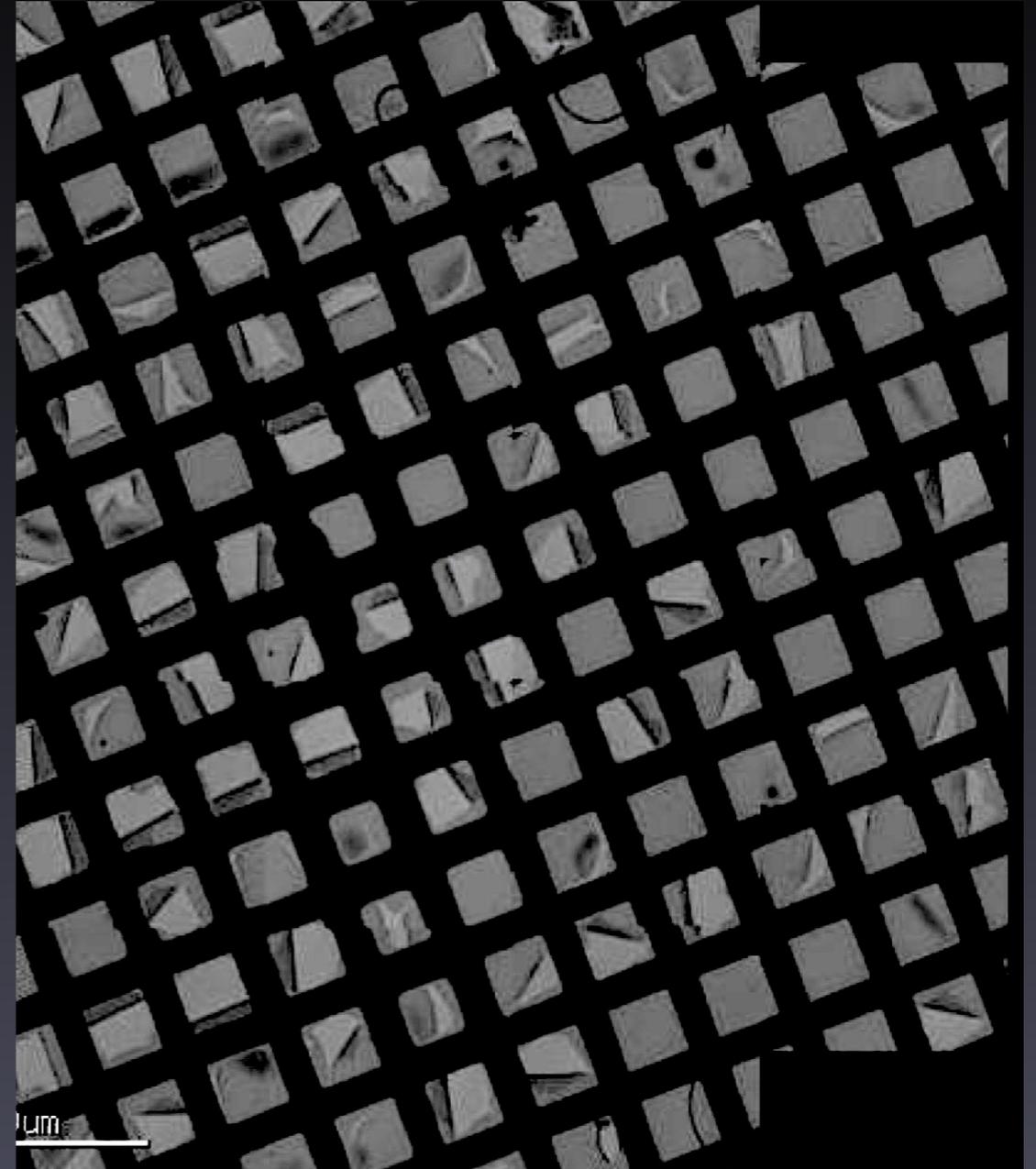
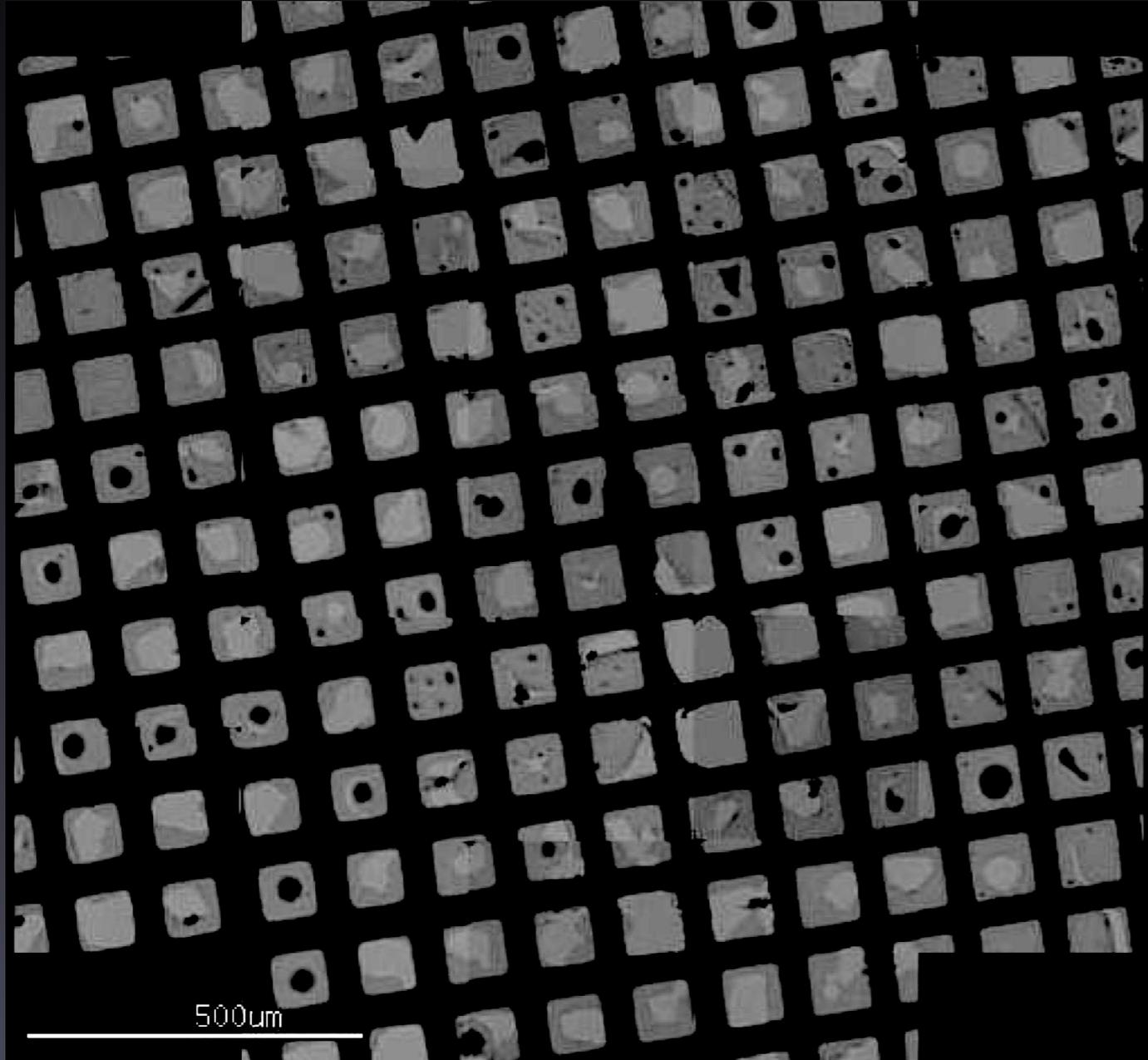
~40,000 particles



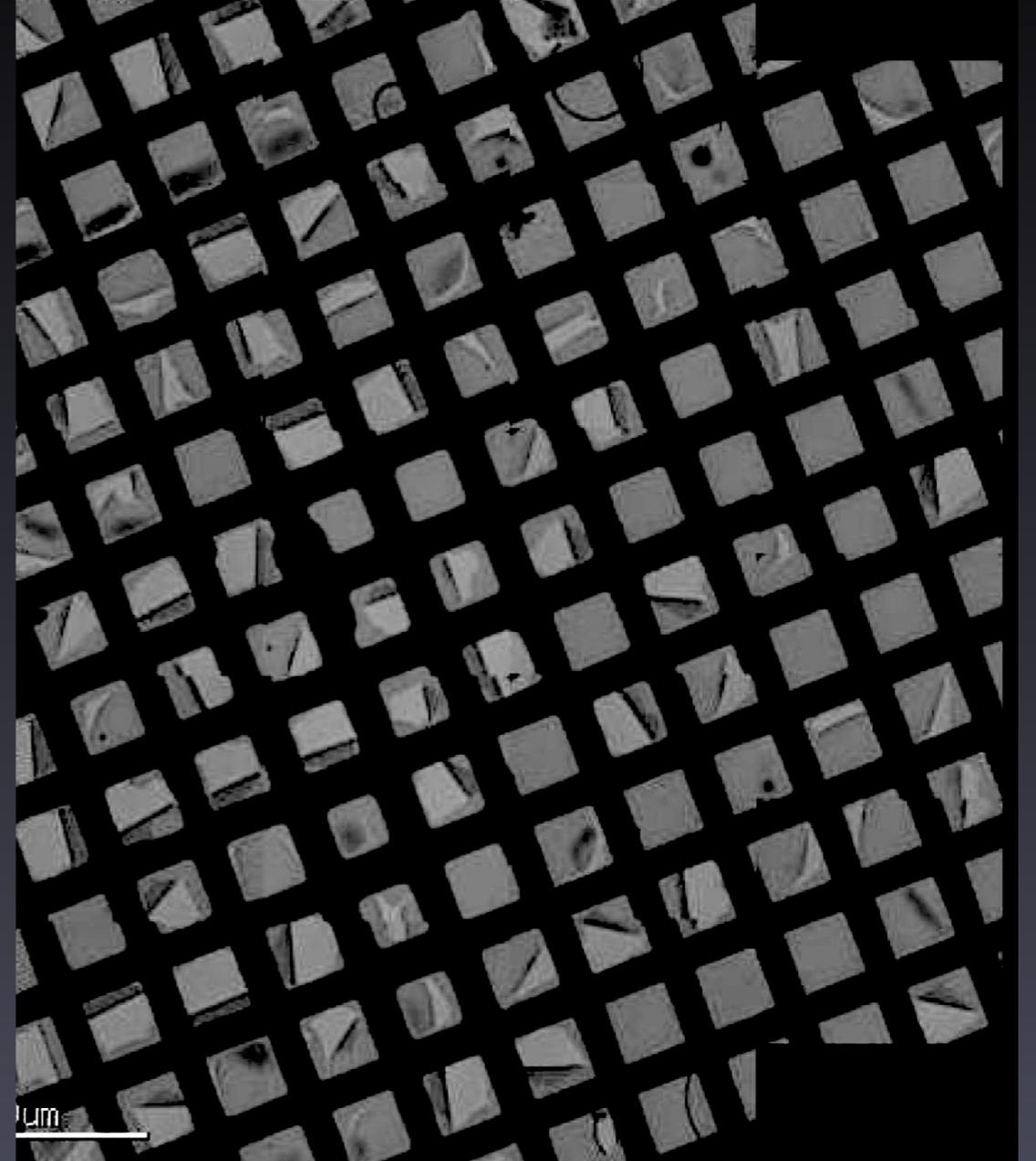




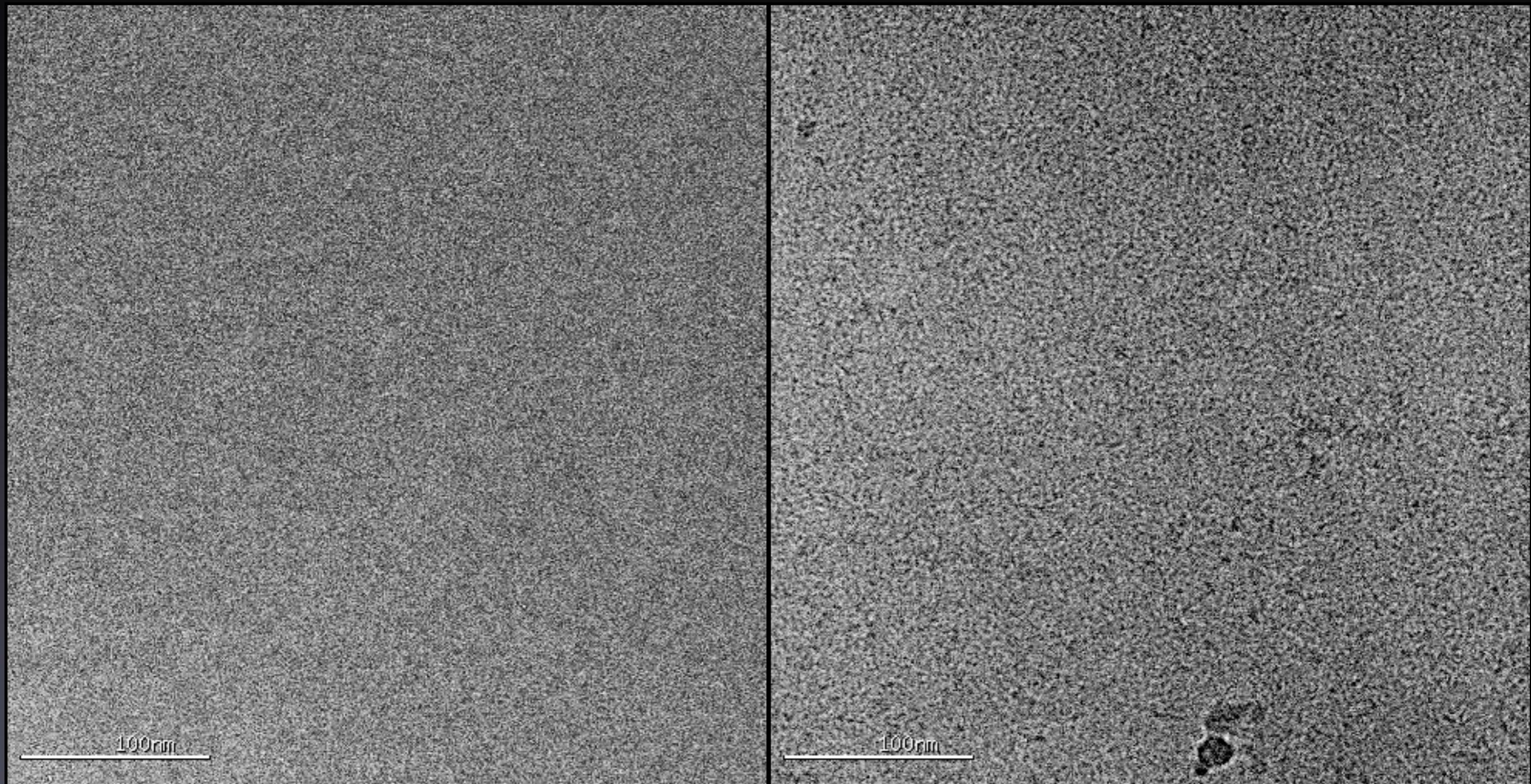
# Grid preparation is still a challenge



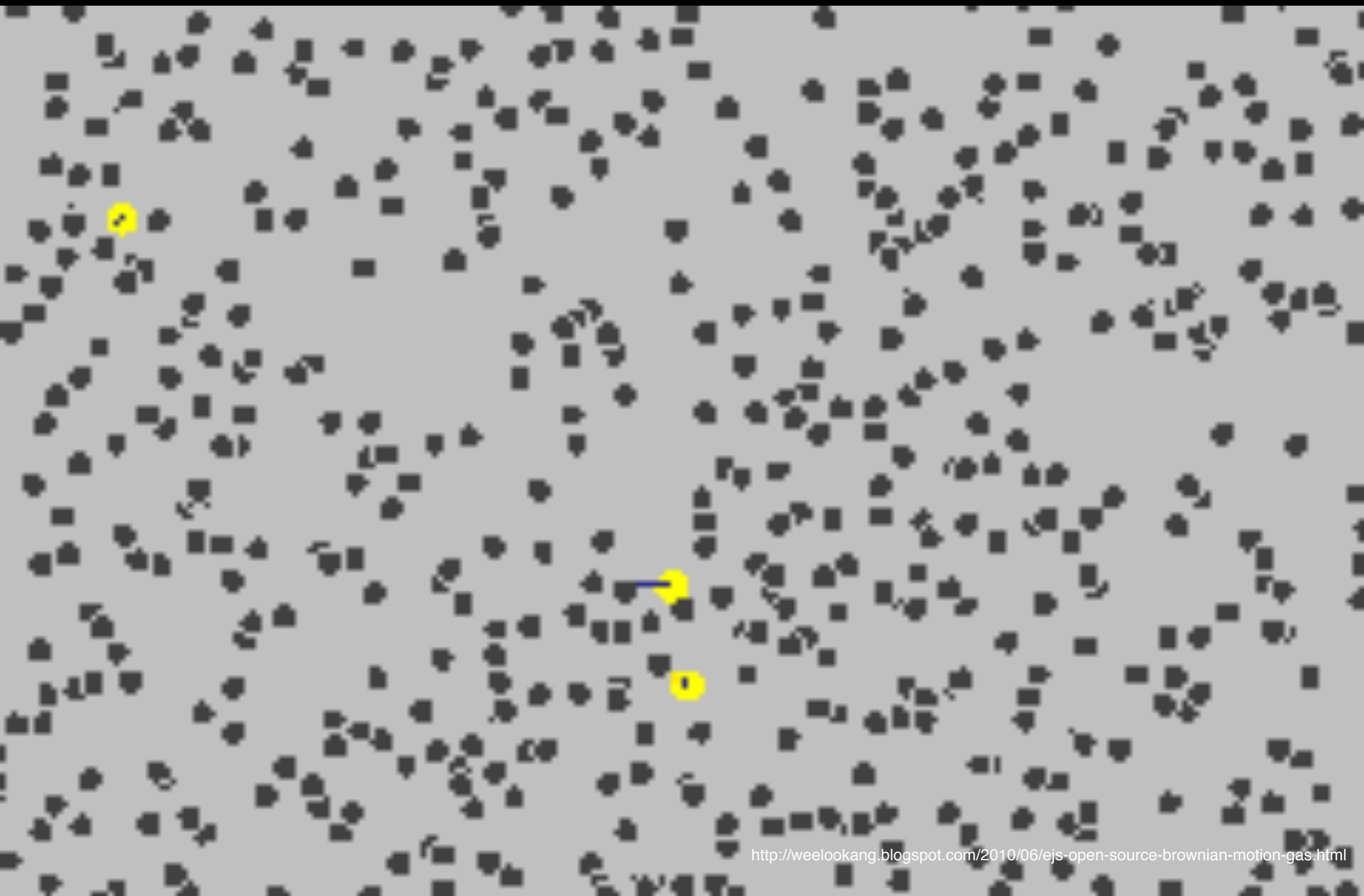
# Grid preparation is still a challenge



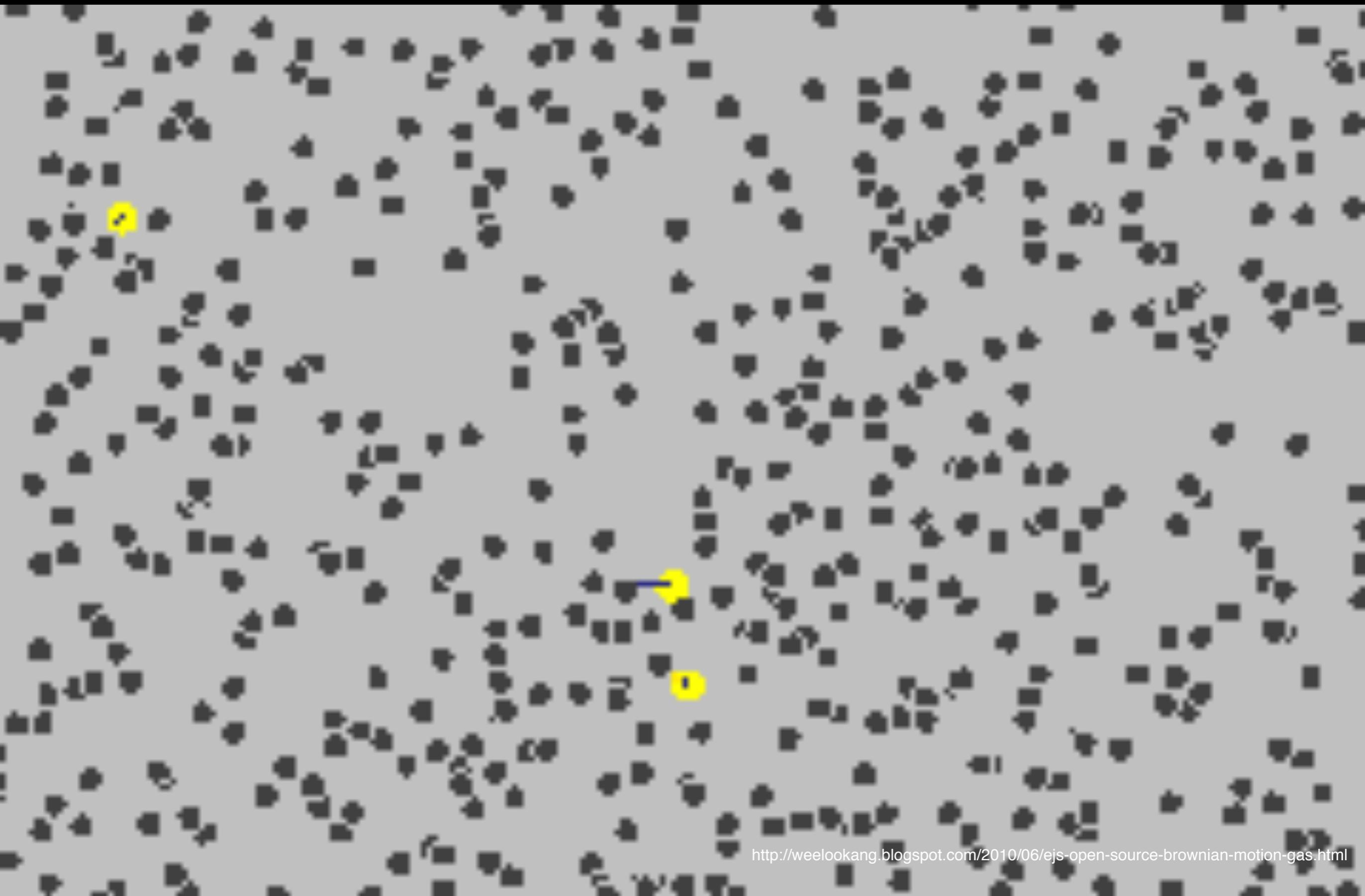
# Grid preparation is still a challenge



# What happens to samples during vitrification?

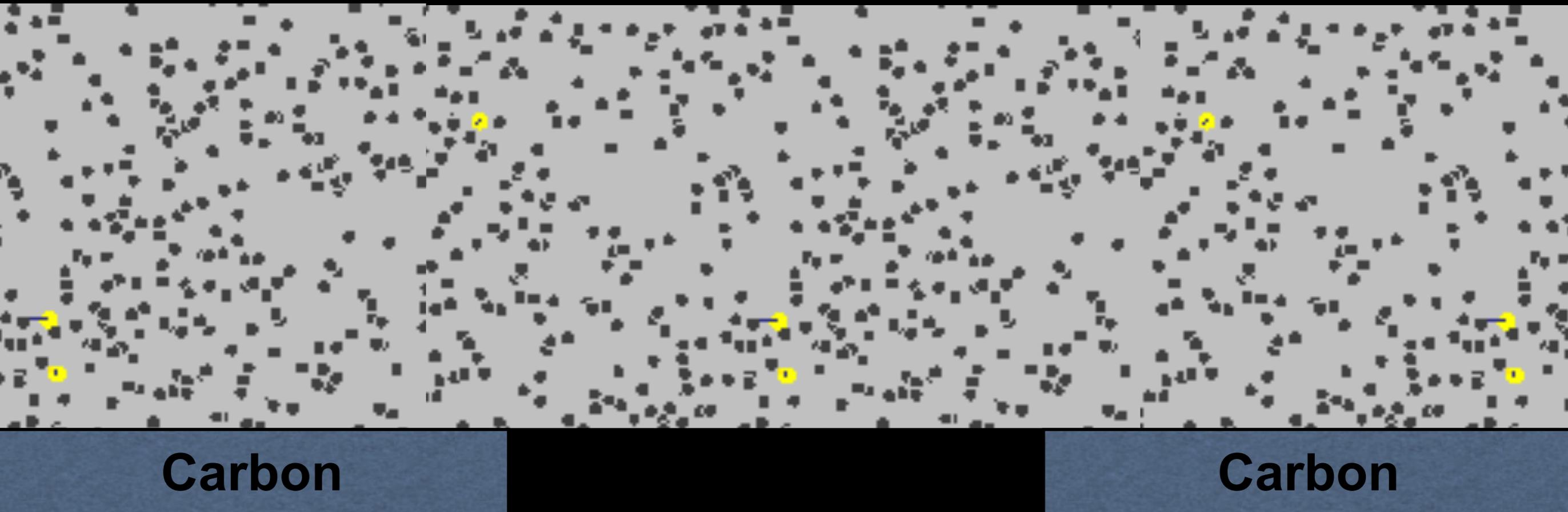


# What happens to samples during vitrification?



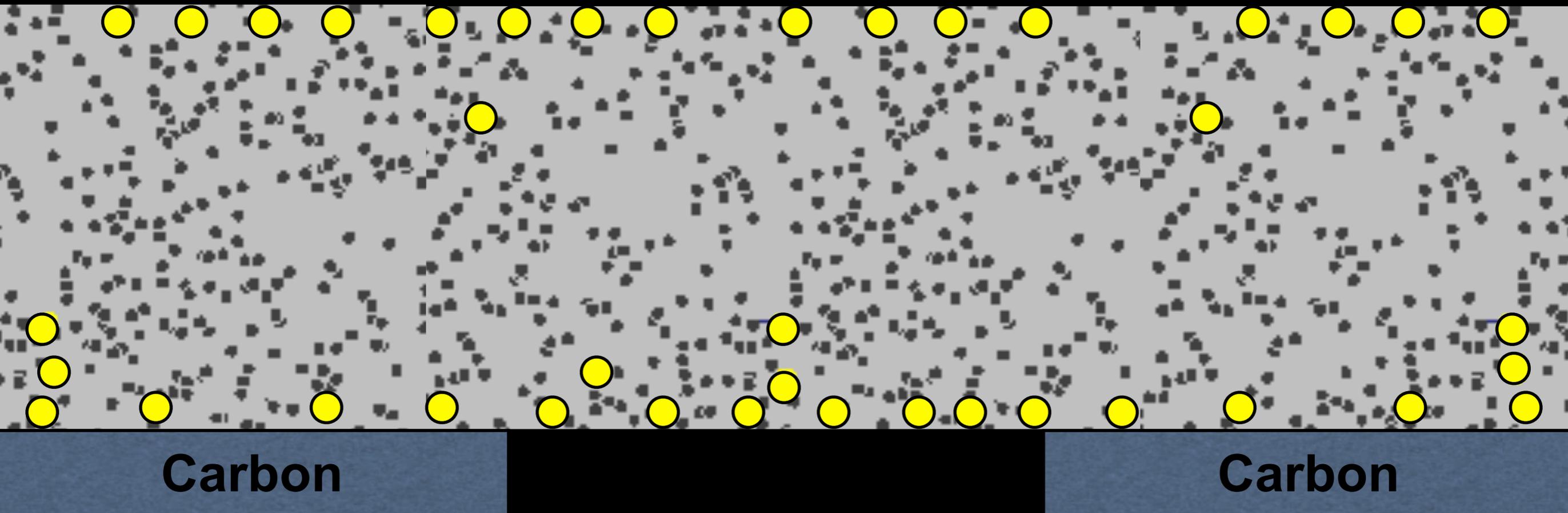
# What happens to samples during vitrification?

A hypothetical scenario during cryoEM grid preparation



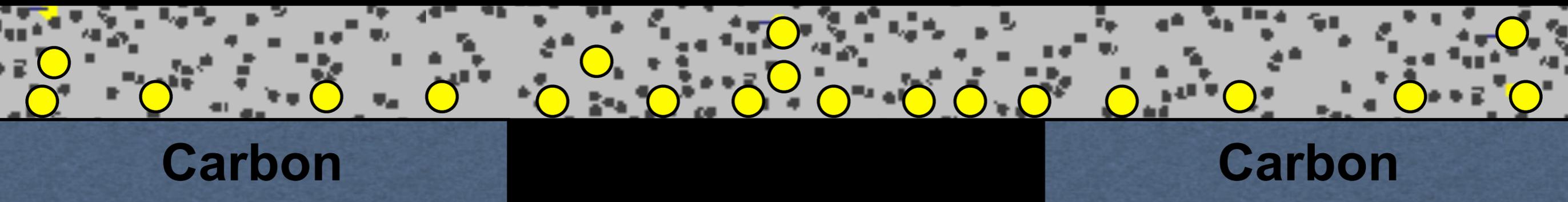
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A hypothetical scenario during cryoEM grid preparation



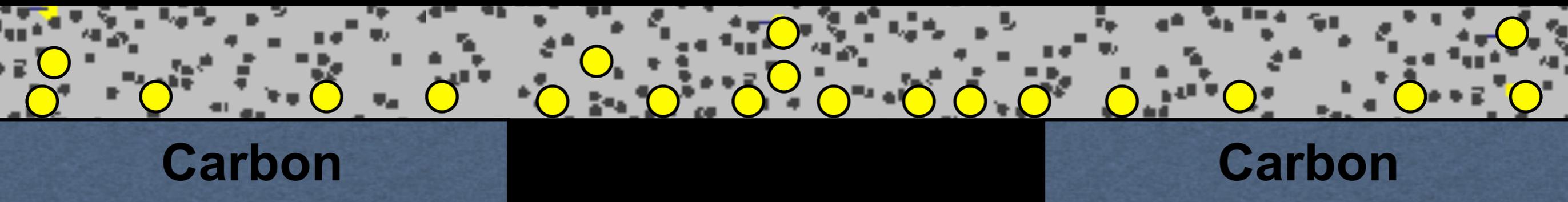
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A hypothetical scenario during cryoEM grid preparation



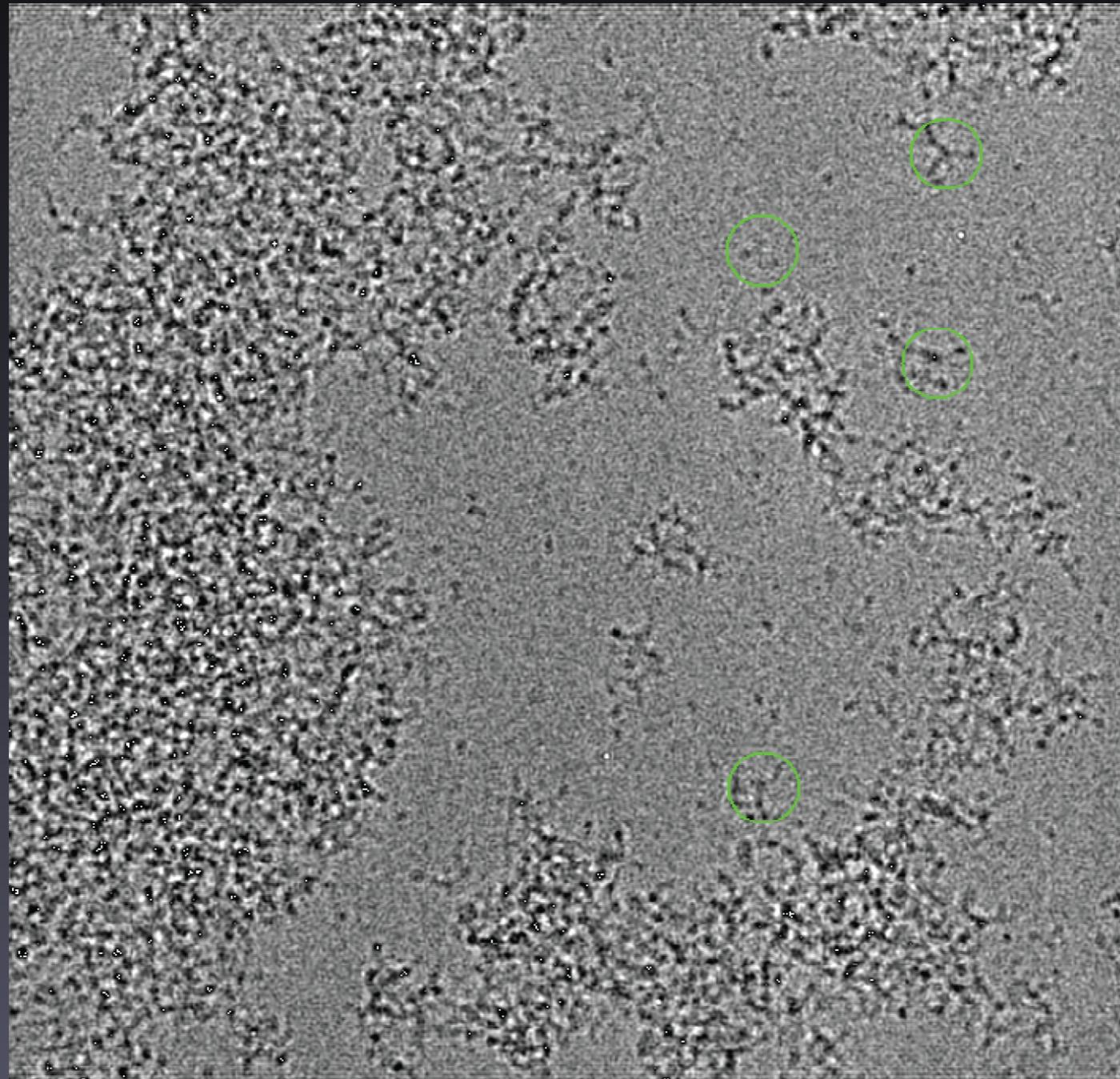
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A hypothetical scenario during cryoEM grid preparation



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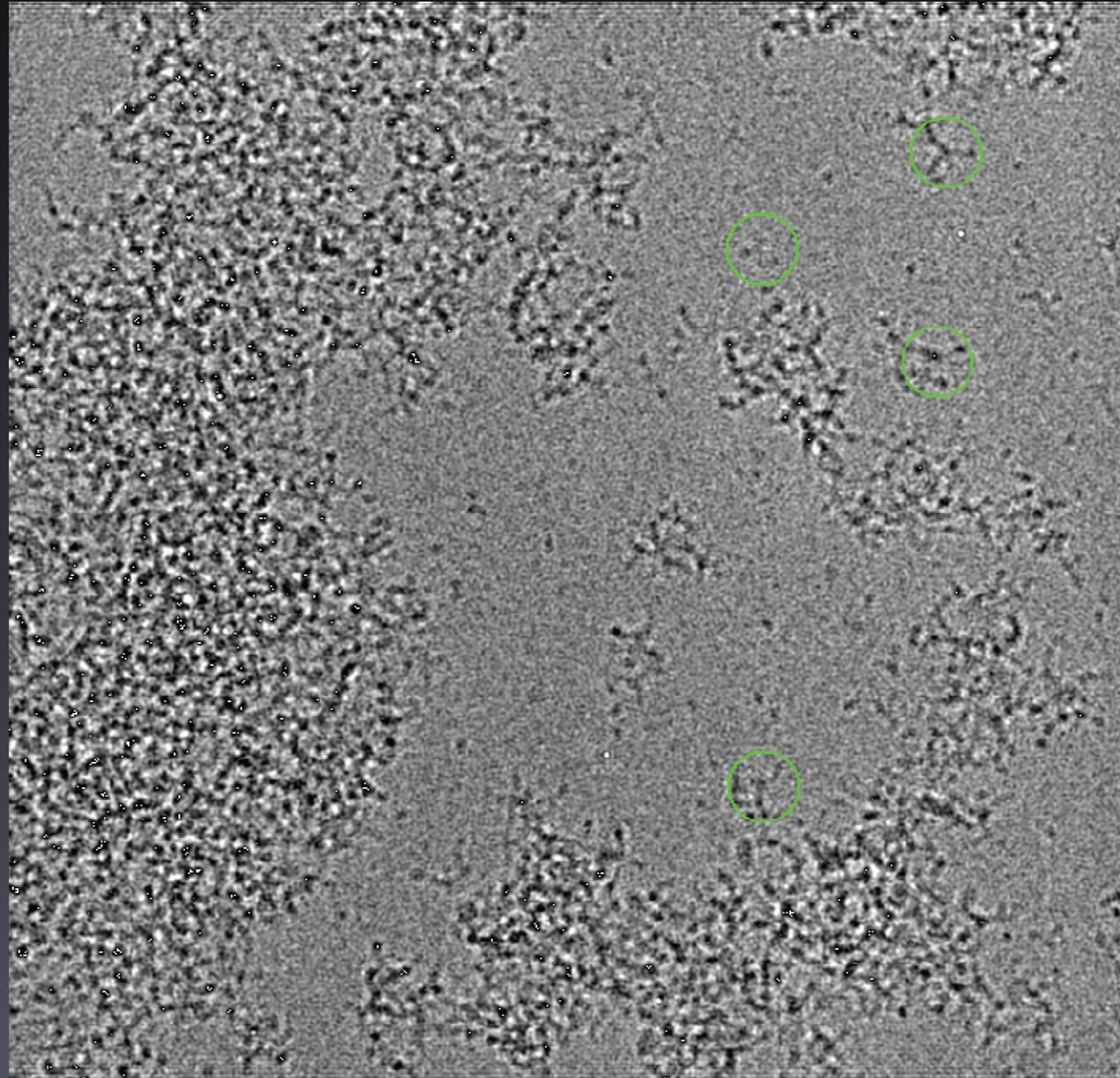
**Fusion protein(~300kDa)**



Luke Chao and Steve Harrison

# What happens to samples during vitrification?

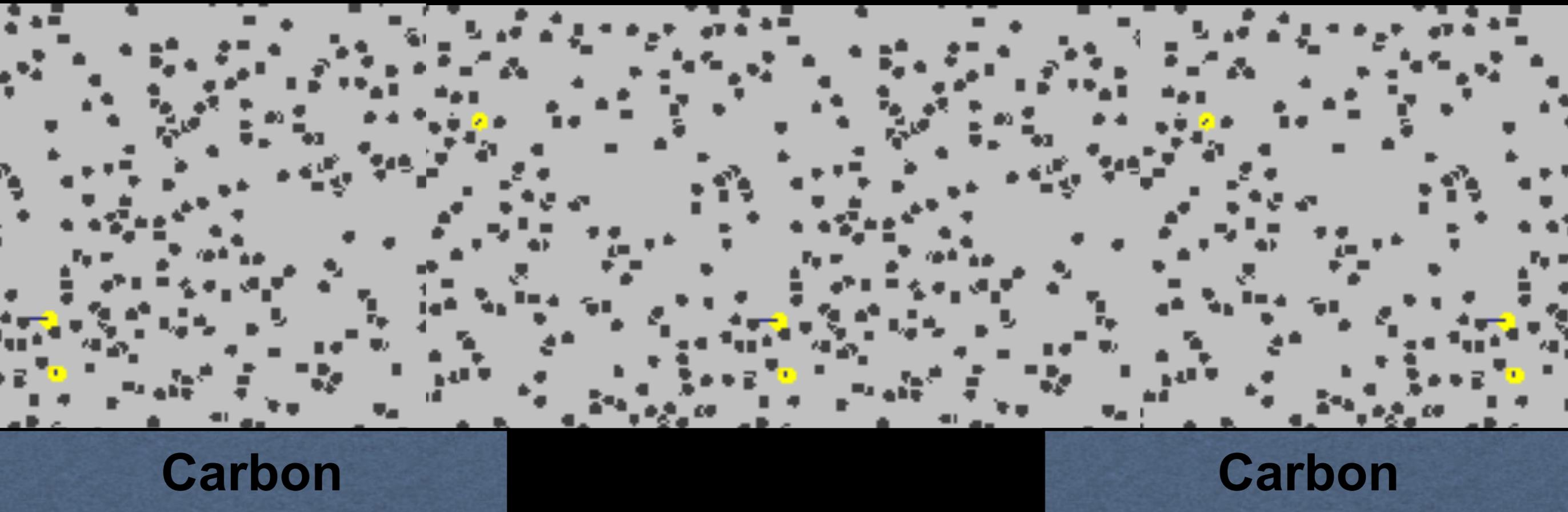
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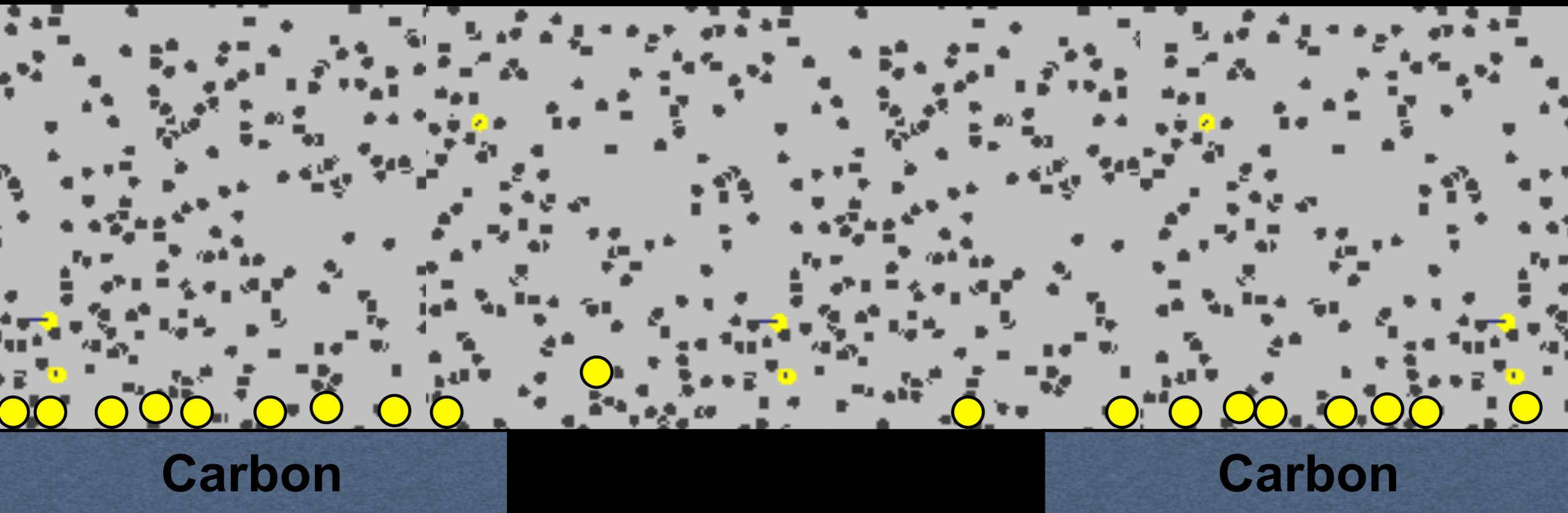
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A hypothetical scenario during cryoEM grid preparation



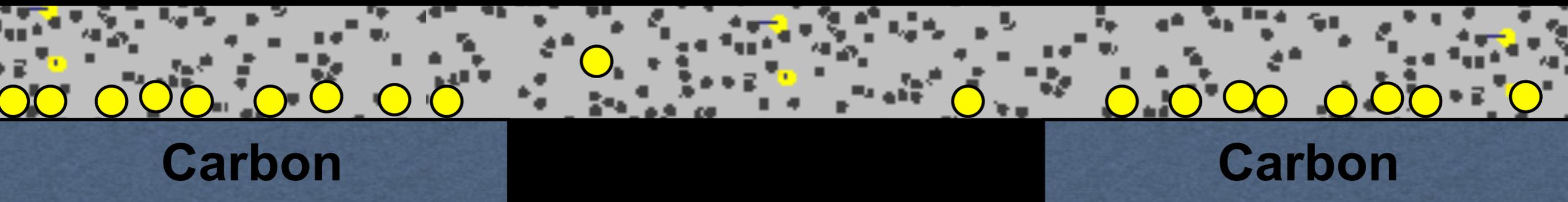
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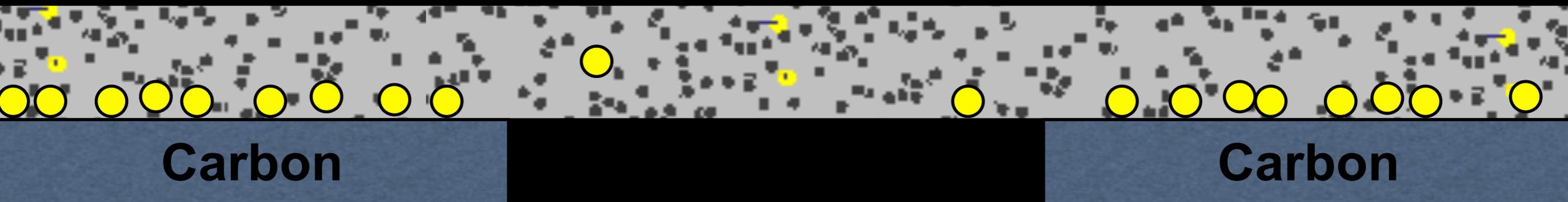
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A hypothetical scenario during cryoEM grid preparation



# What happens to samples during vitrification?

A hypothetical scenario during cryoEM grid preparation



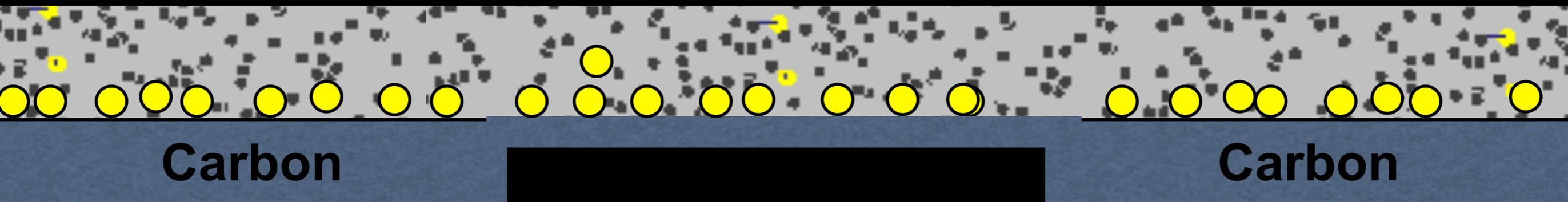
# What happens to samples during vitrification?

A hypothetical scenario during cryoEM grid preparation



# What happens to samples during vitrification?

A hypothetical scenario during cryoEM grid preparation



# What happens to samples during vitrification?

**Human protein complex(~300kDa)**



Vignesh Kasinath and Eva Nogales, UCB

# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

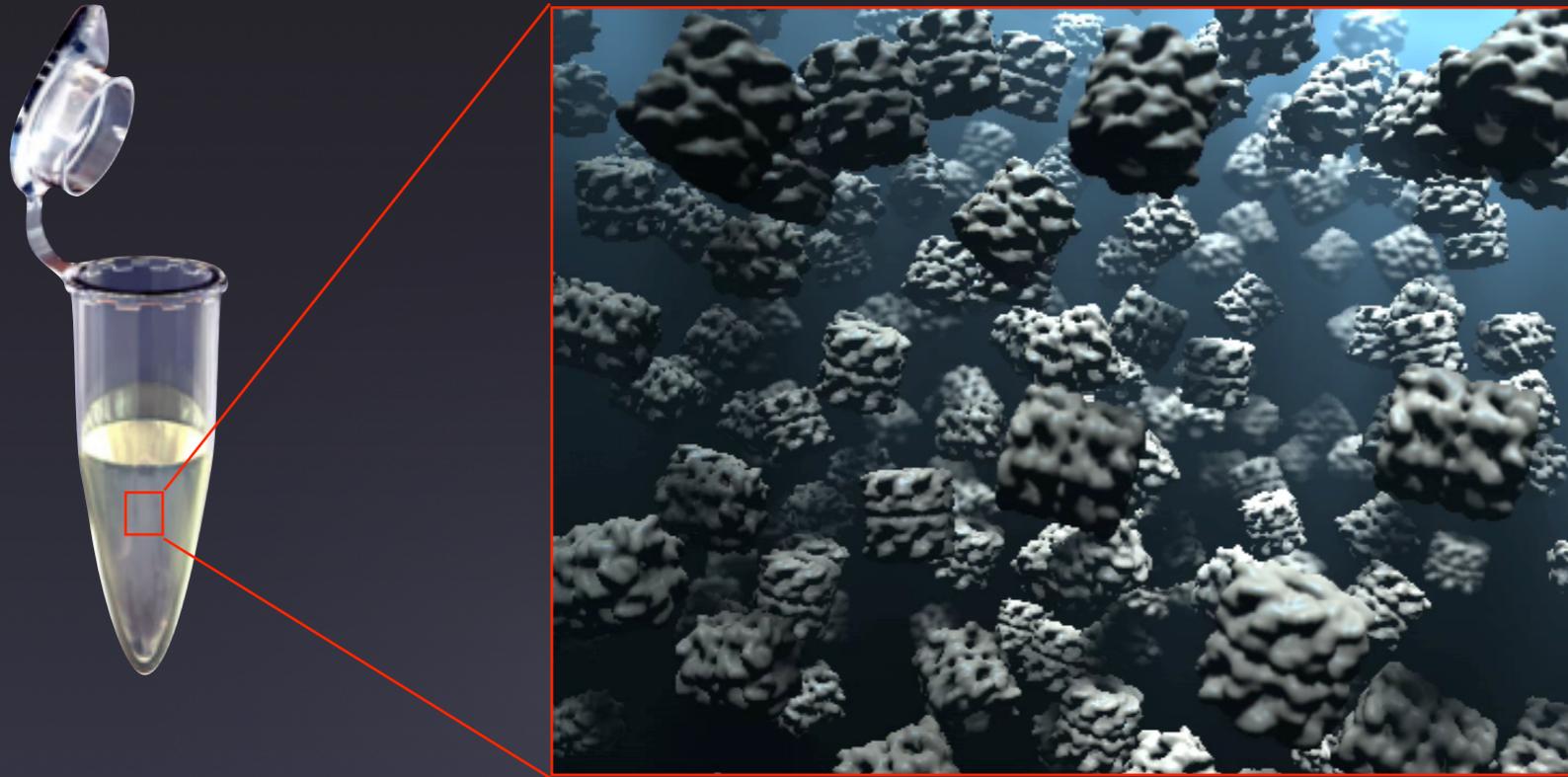
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# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

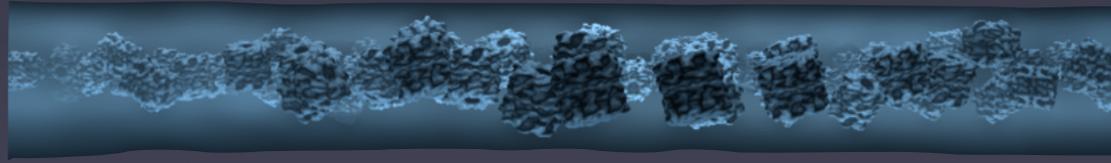
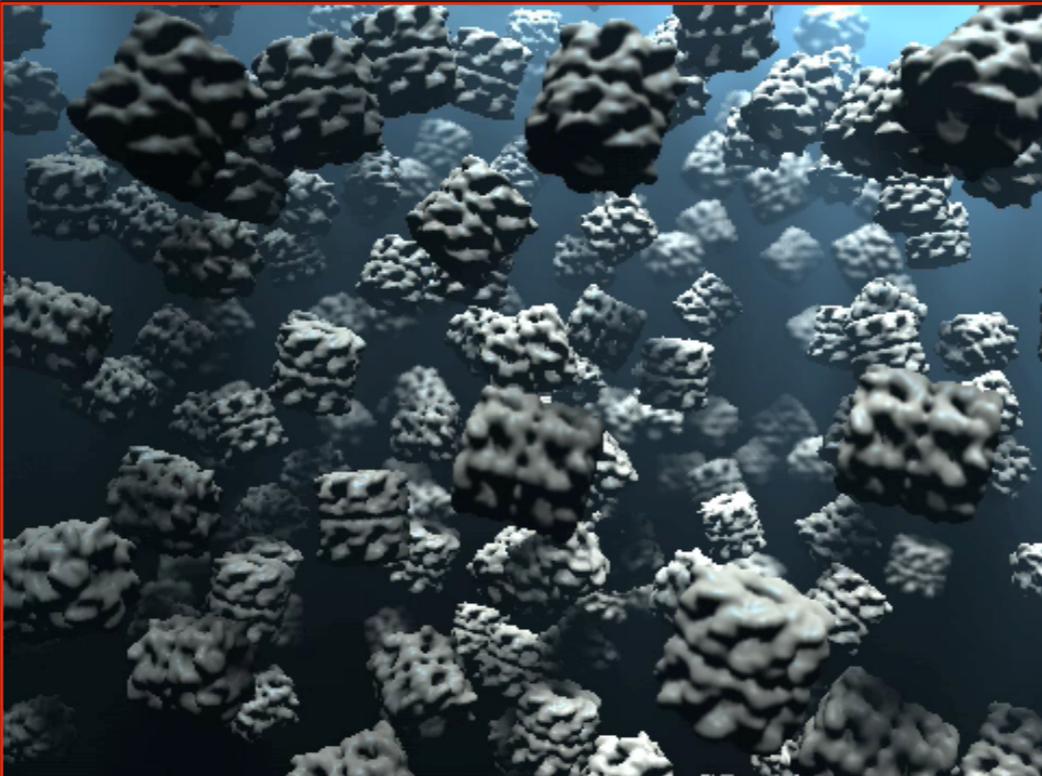
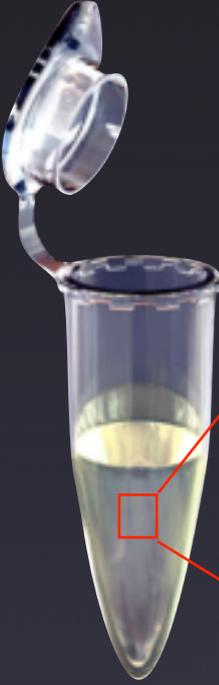
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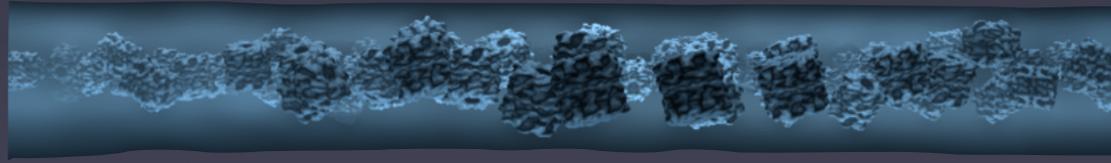
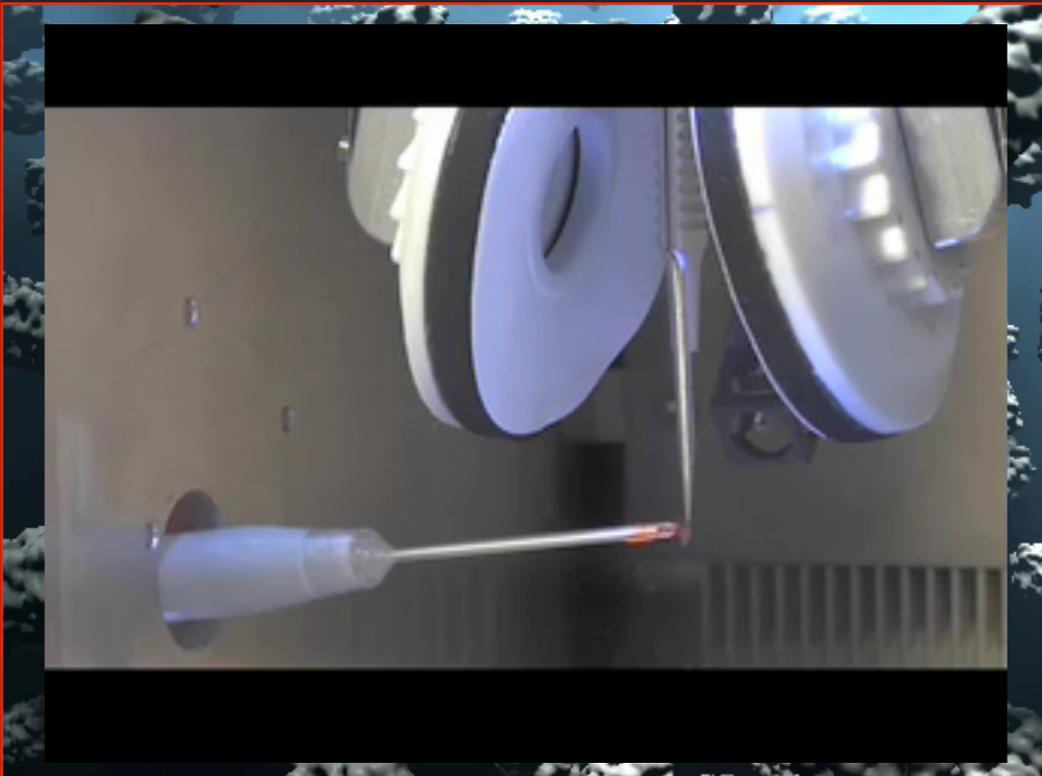
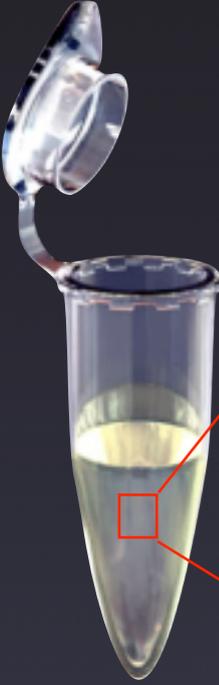
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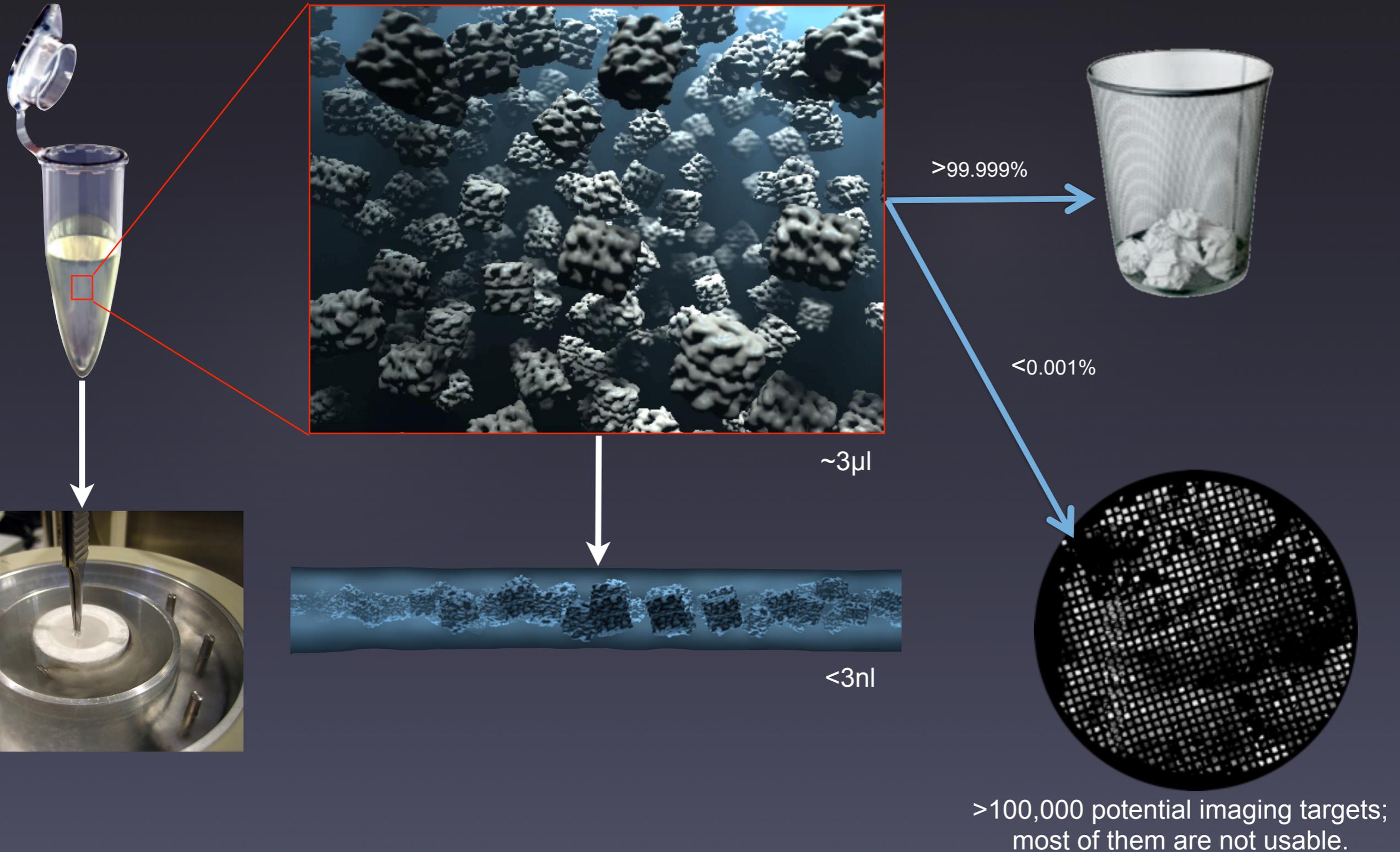
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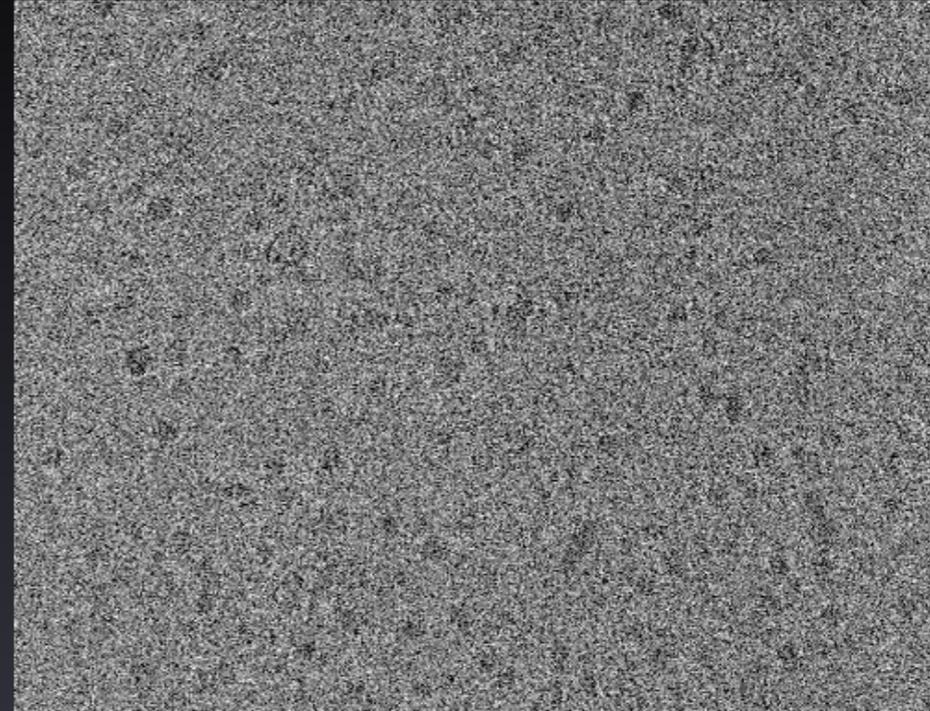
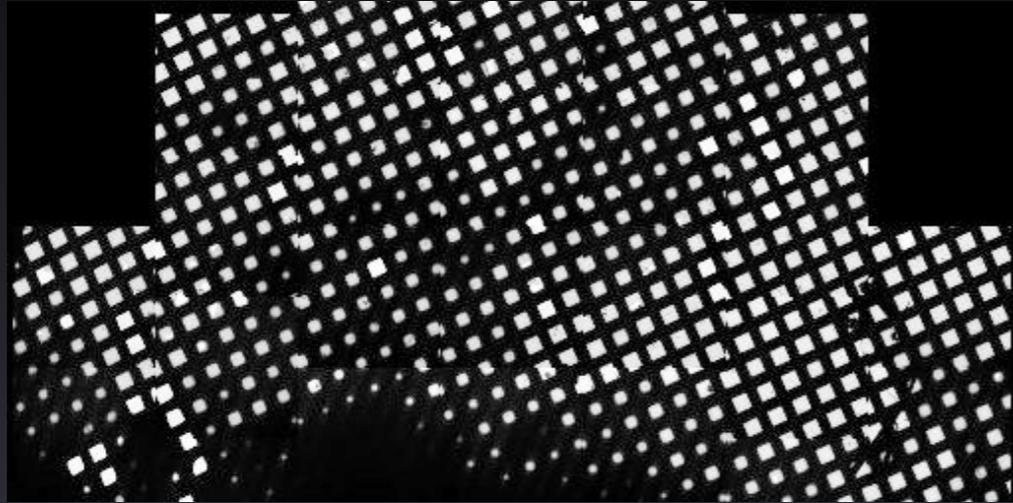


# **The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods**

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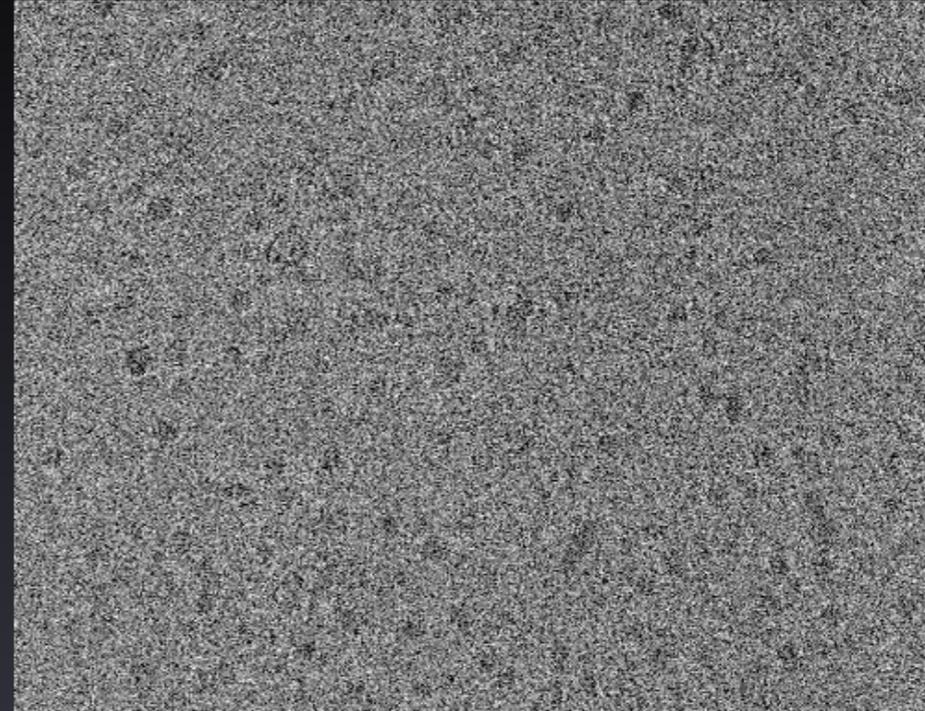
Vitrobot



# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

Vitrobot

3  $\mu\text{L}$  of sample required for each  
grid;  $\sim 2\text{nL}$  on grid



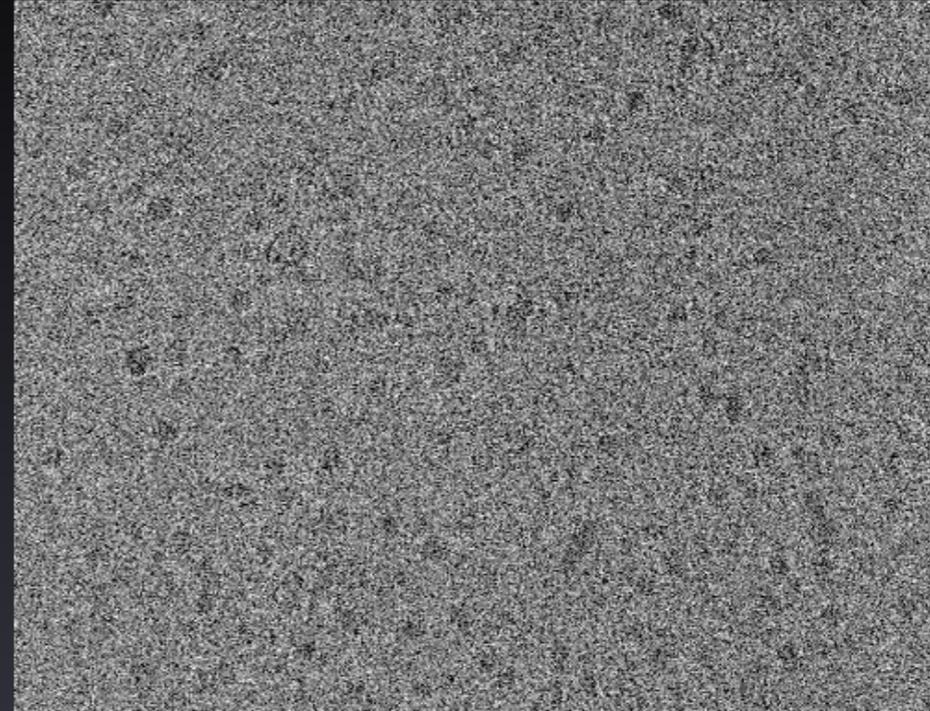
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Vitrobot

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Usable area:  $\sim 0-10\%$



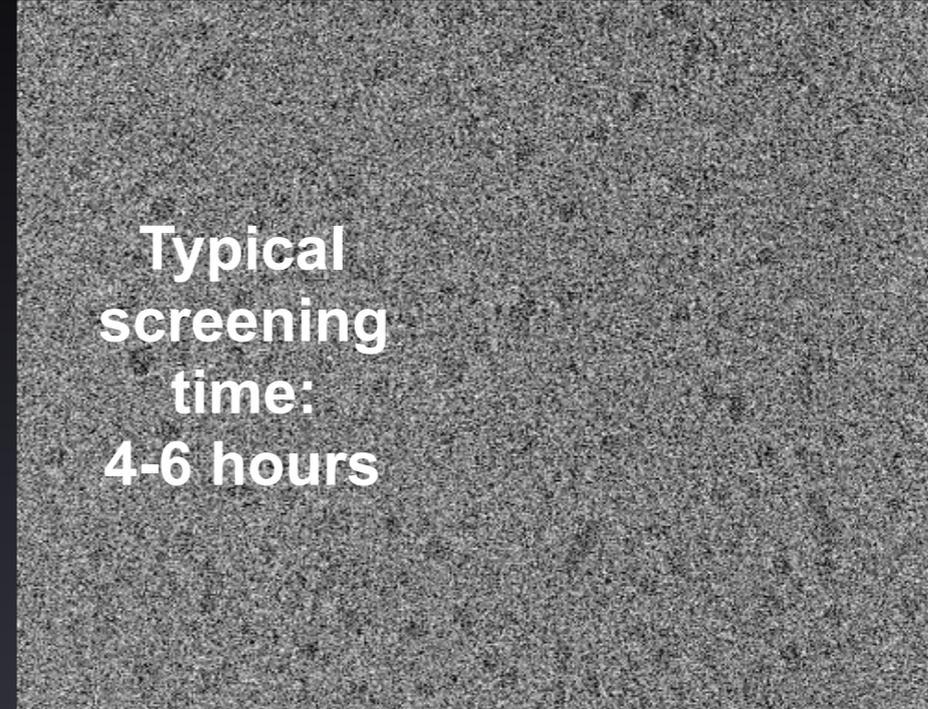
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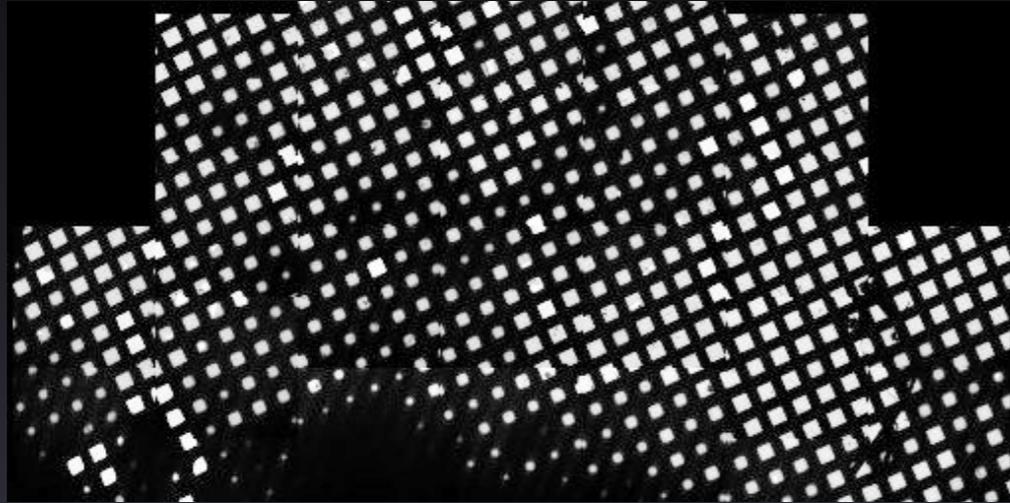


Typical  
screening  
time:  
4-6 hours

# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

Vitrobot

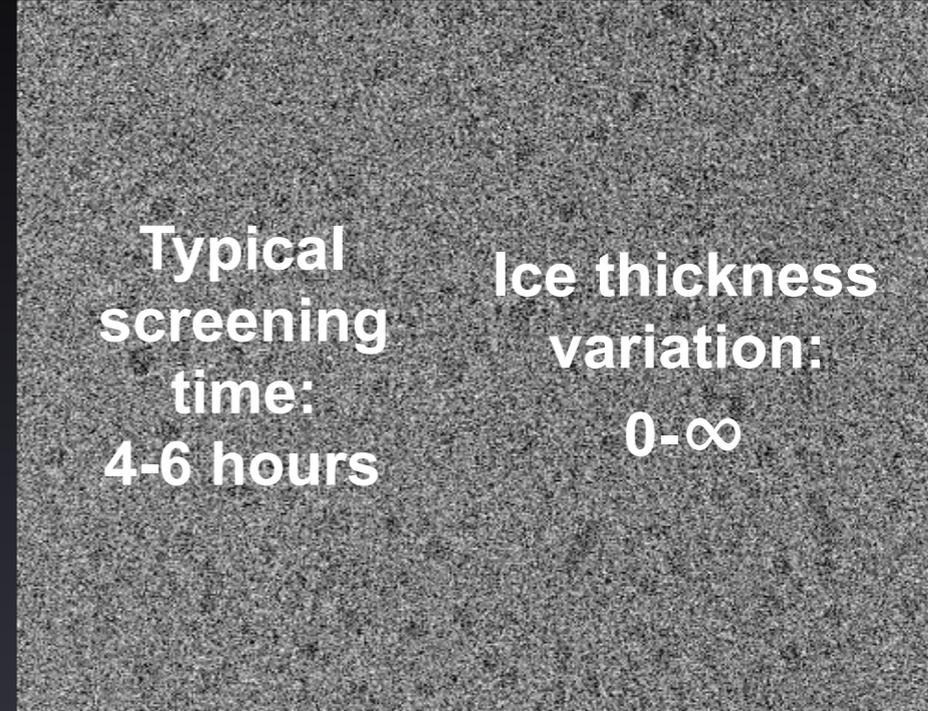
3 uL of sample required for each  
grid; ~2nL on grid



Usable area: ~0-10%

Typical  
screening  
time:  
4-6 hours

Ice thickness  
variation:  
0-∞



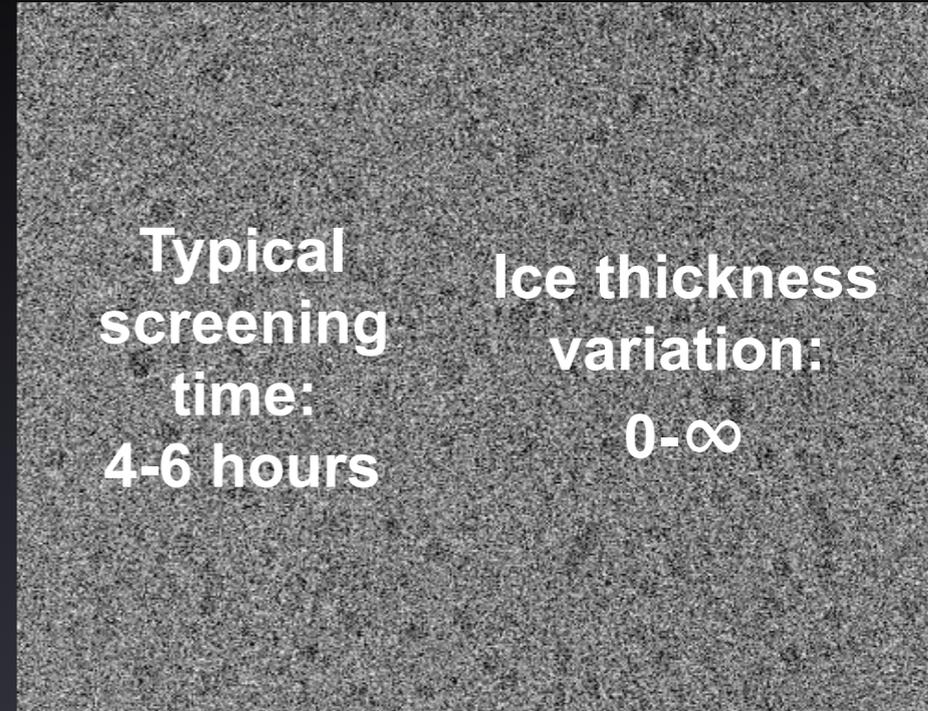
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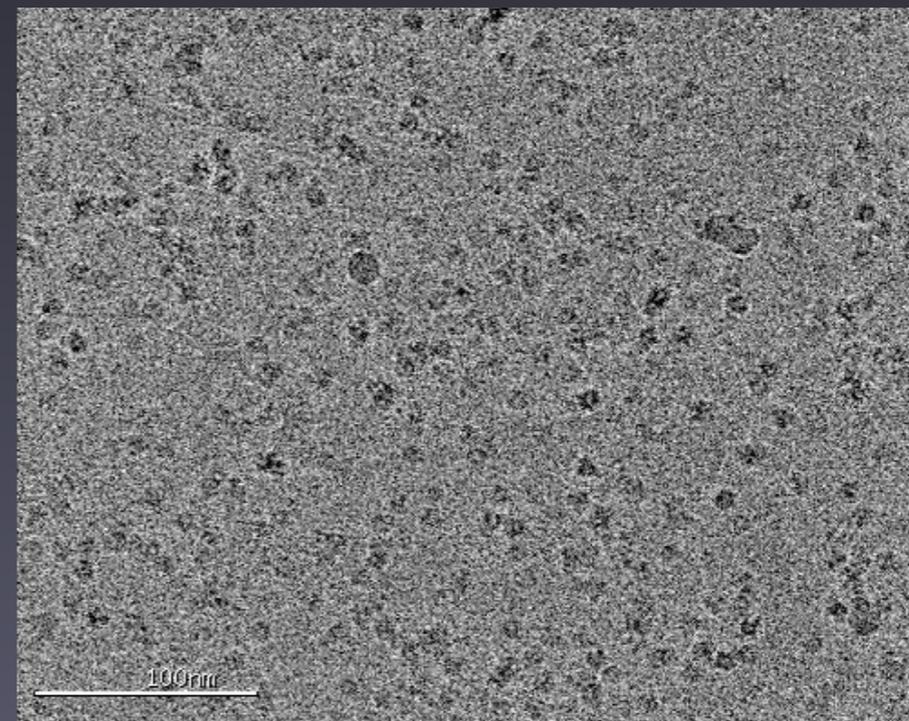
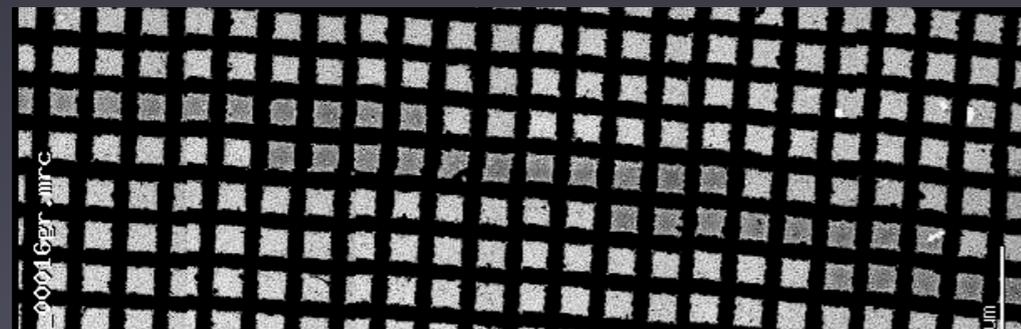
3  $\mu\text{L}$  of sample required for each grid;  $\sim 2\text{nL}$  on grid



Usable area:  $\sim 0-10\%$



Spotiton



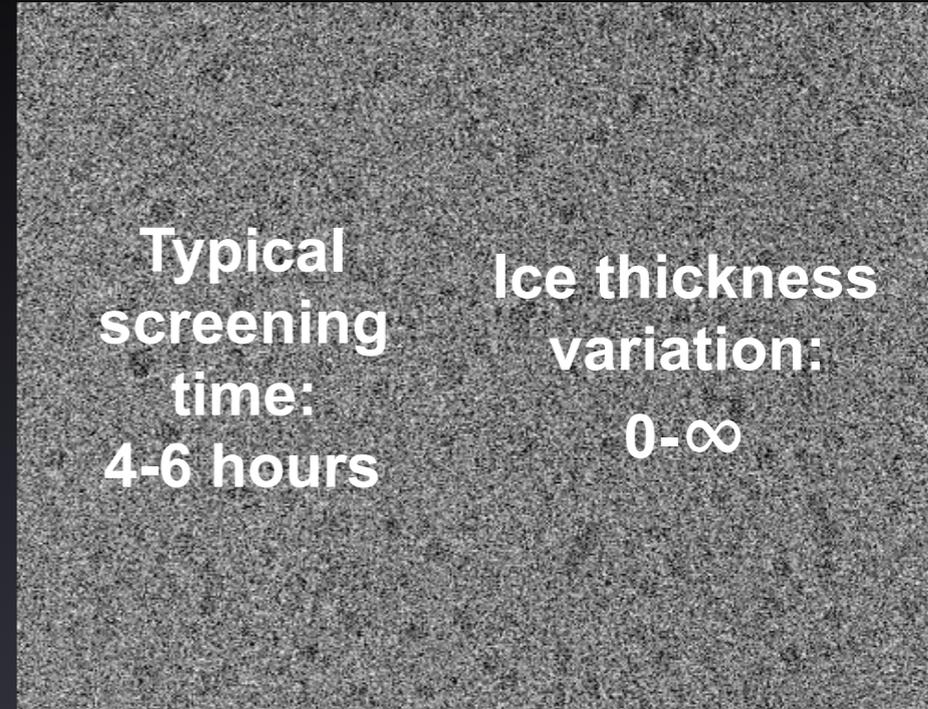
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3  $\mu\text{L}$  of sample required for each grid;  $\sim 2\text{nL}$  on grid



Usable area:  $\sim 0-10\%$

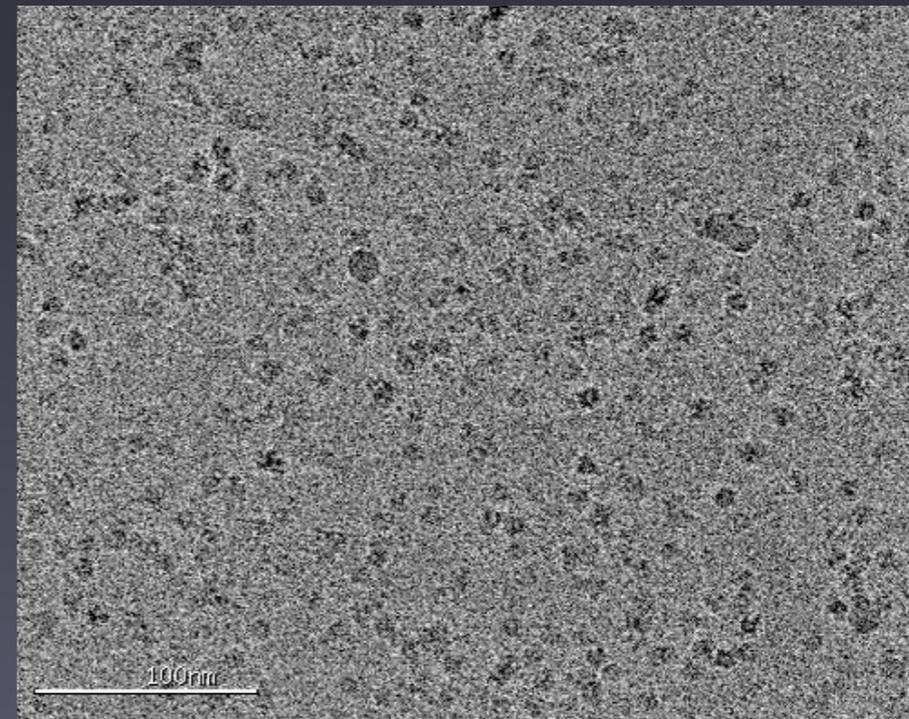
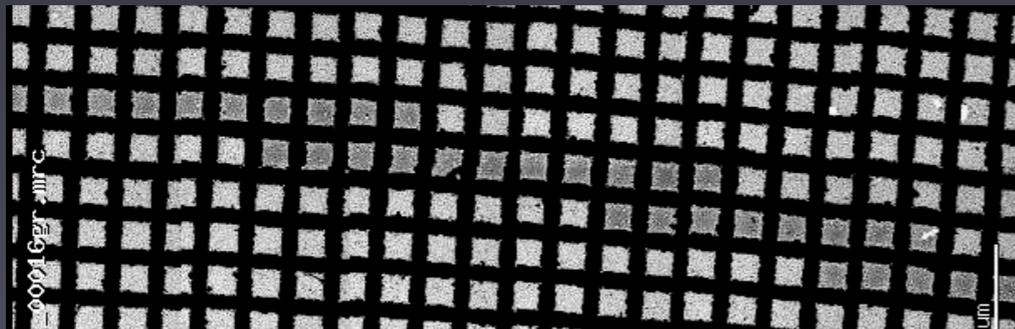


Typical screening time:  
4-6 hours

Ice thickness variation:  
 $0-\infty$

Spotiton

3  $\mu\text{L}$  of sample enough for  $\sim 1000$  grids;  $\sim 500\text{pL}$  on grid



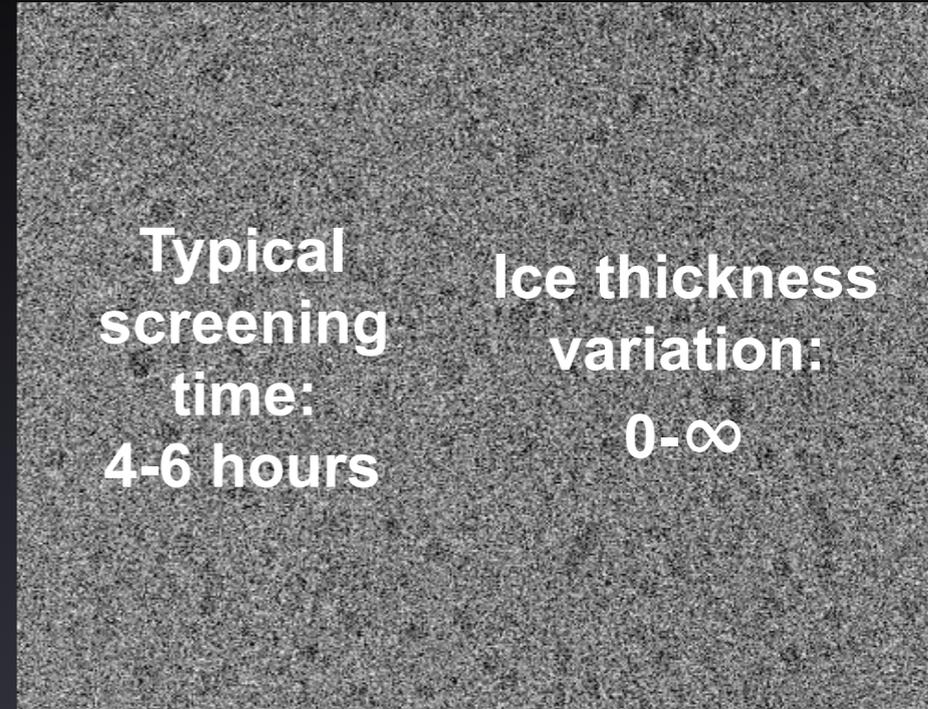
# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

Vitrobot

3  $\mu\text{L}$  of sample required for each grid;  $\sim 2\text{nL}$  on grid

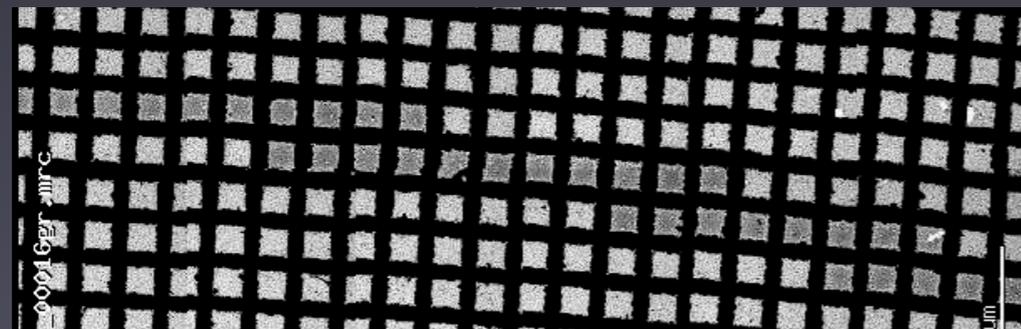


Usable area:  $\sim 0-10\%$

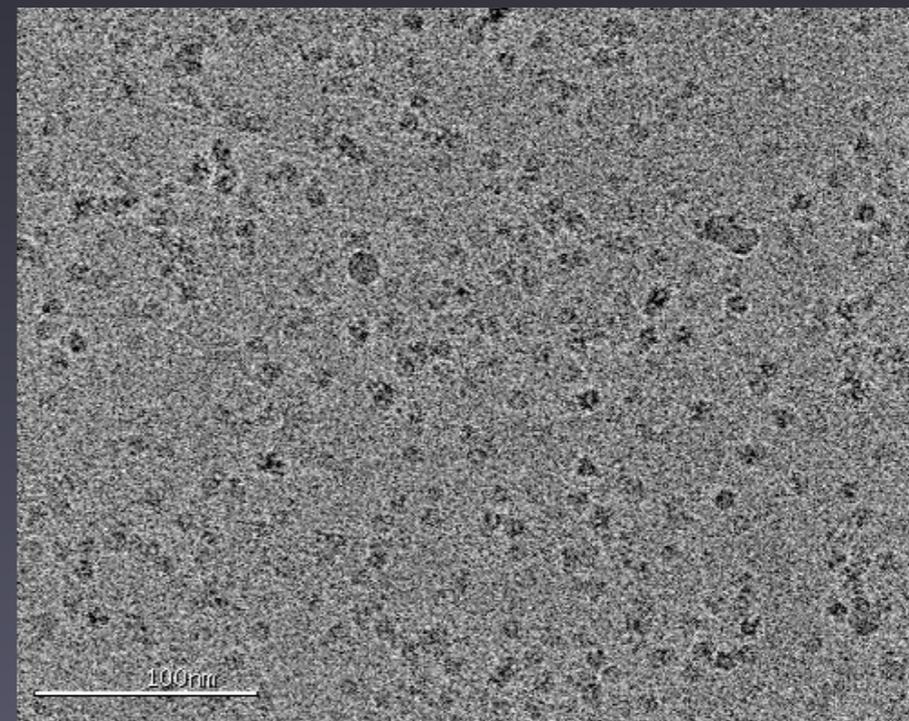


Spotiton

3  $\mu\text{L}$  of sample enough for  $\sim 1000$  grids;  $\sim 500\text{pL}$  on grid



Usable area:  $\sim 100\%$



# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

Vitrobot

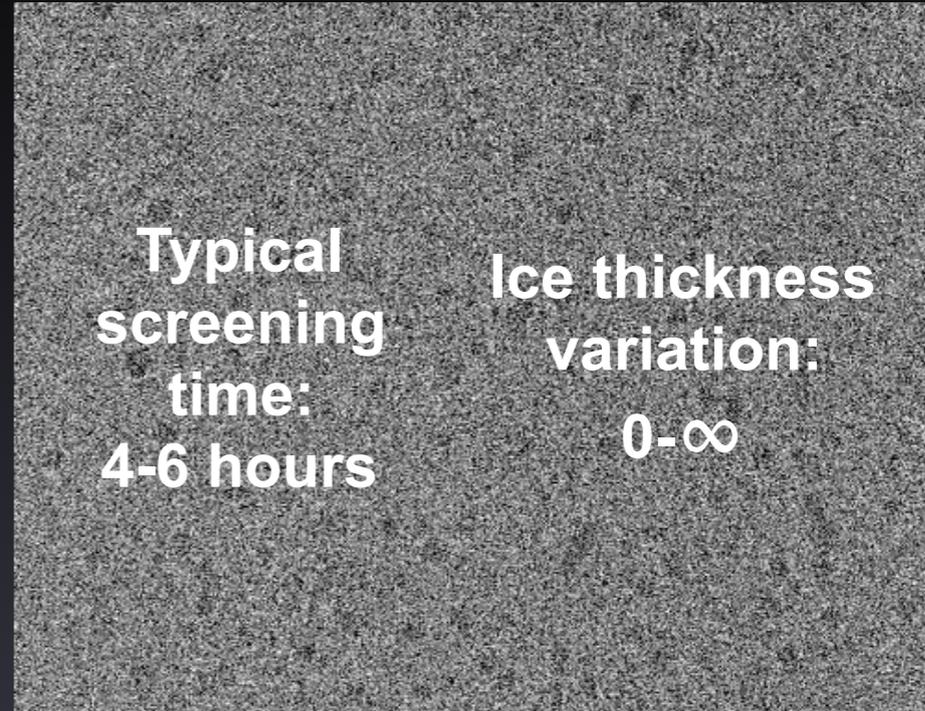
3  $\mu\text{L}$  of sample required for each grid;  $\sim 2\text{nL}$  on grid



Usable area:  $\sim 0-10\%$

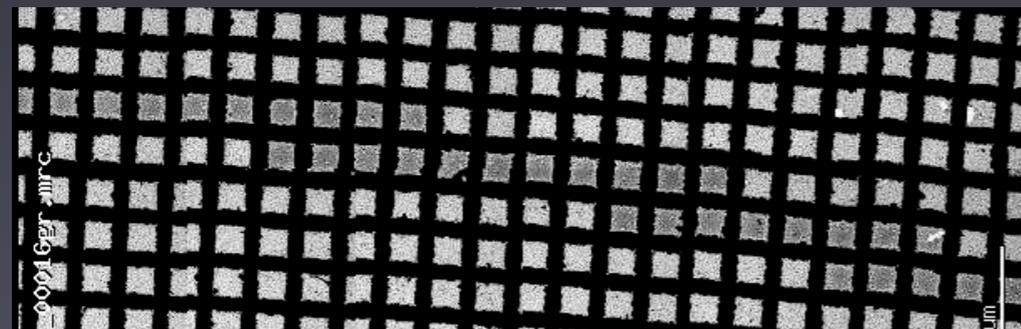
Typical screening time:  
4-6 hours

Ice thickness variation:  
 $0-\infty$



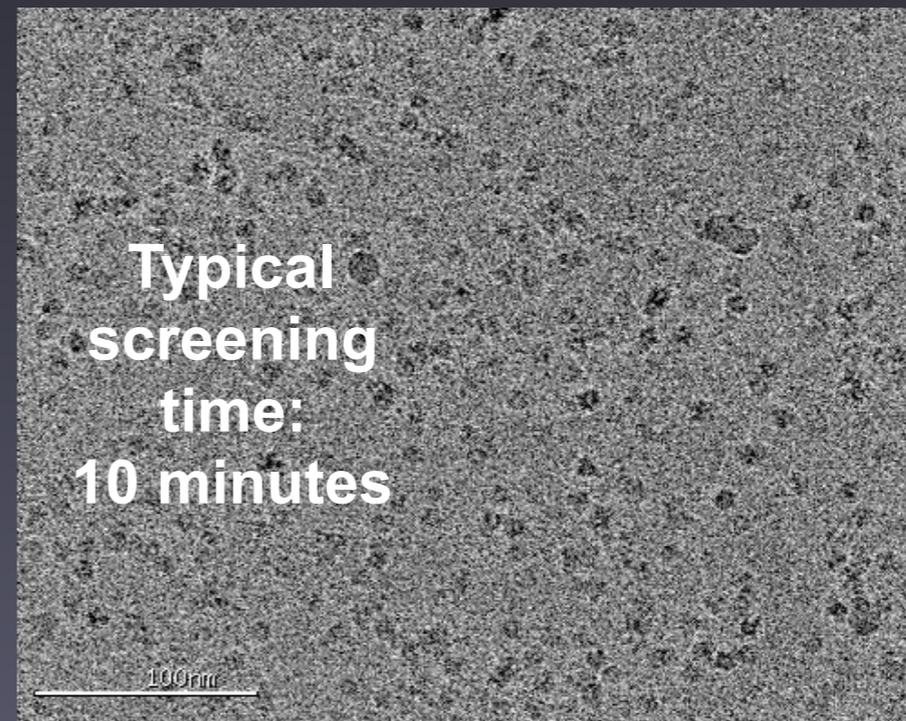
Spotiton

3  $\mu\text{L}$  of sample enough for  $\sim 1000$  grids;  $\sim 500\text{pL}$  on grid



Usable area:  $\sim 100\%$

Typical screening time:  
10 minutes



# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

Vitrobot

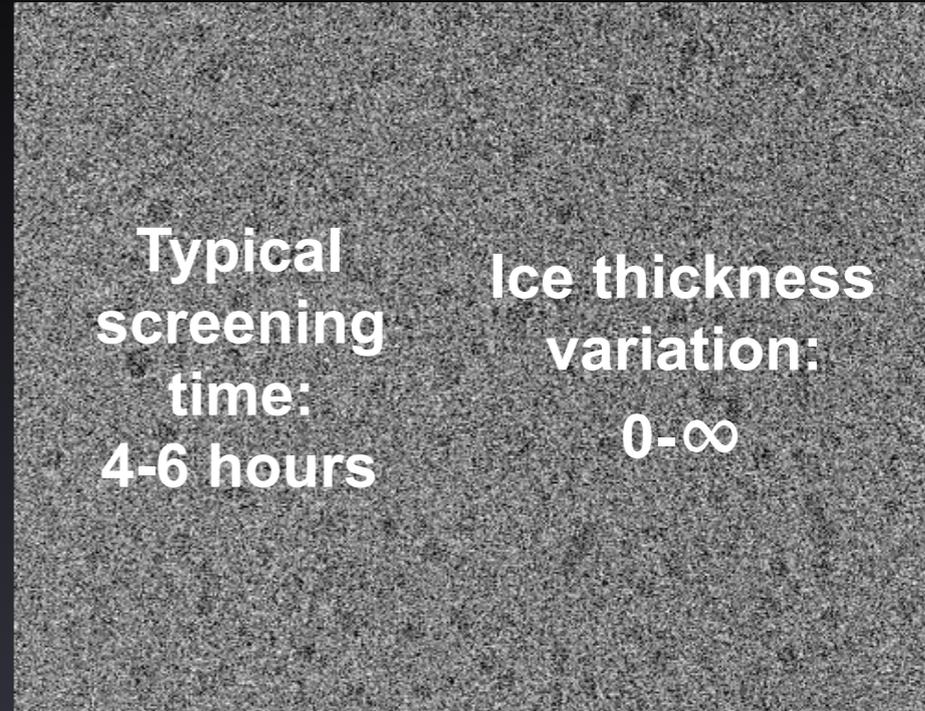
3  $\mu\text{L}$  of sample required for each grid;  $\sim 2\text{nL}$  on grid



Usable area:  $\sim 0-10\%$

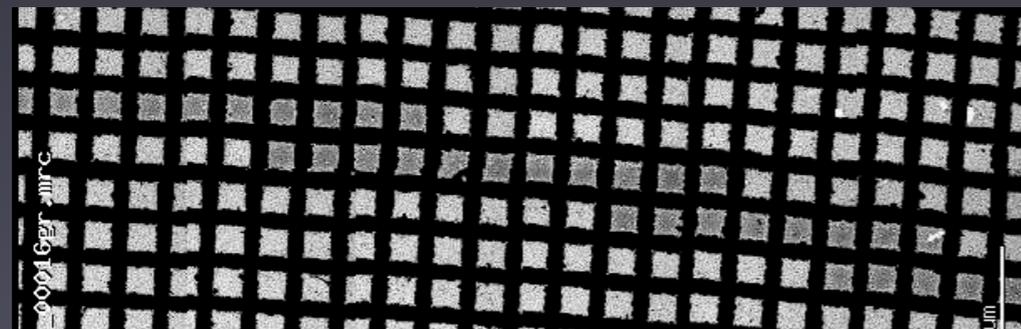
Typical screening time:  
4-6 hours

Ice thickness variation:  
 $0-\infty$



Spotiton

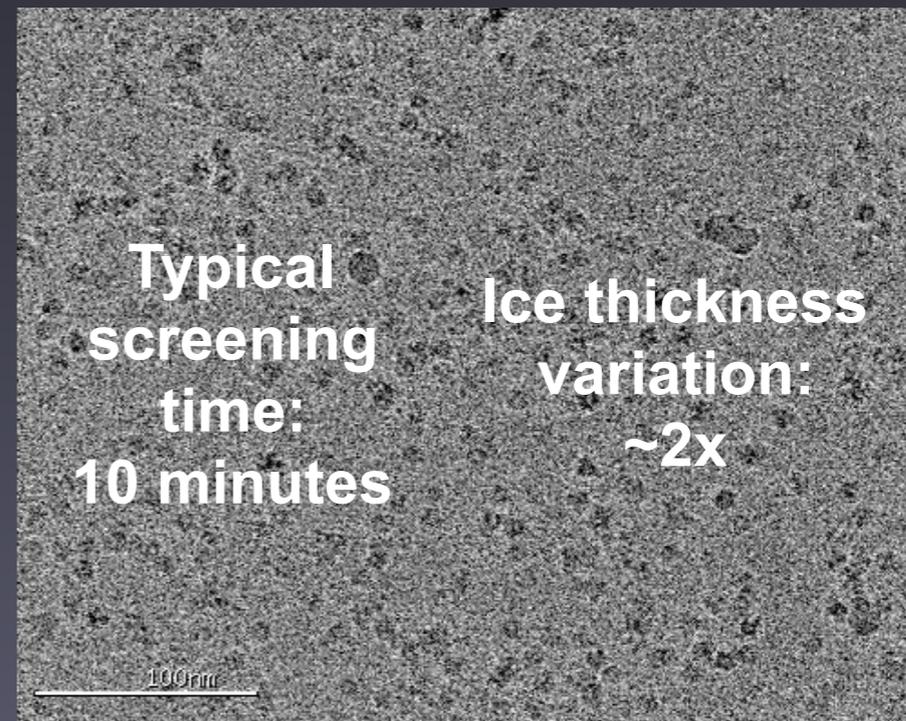
3  $\mu\text{L}$  of sample enough for  $\sim 1000$  grids;  $\sim 500\text{pL}$  on grid



Usable area:  $\sim 100\%$

Typical screening time:  
10 minutes

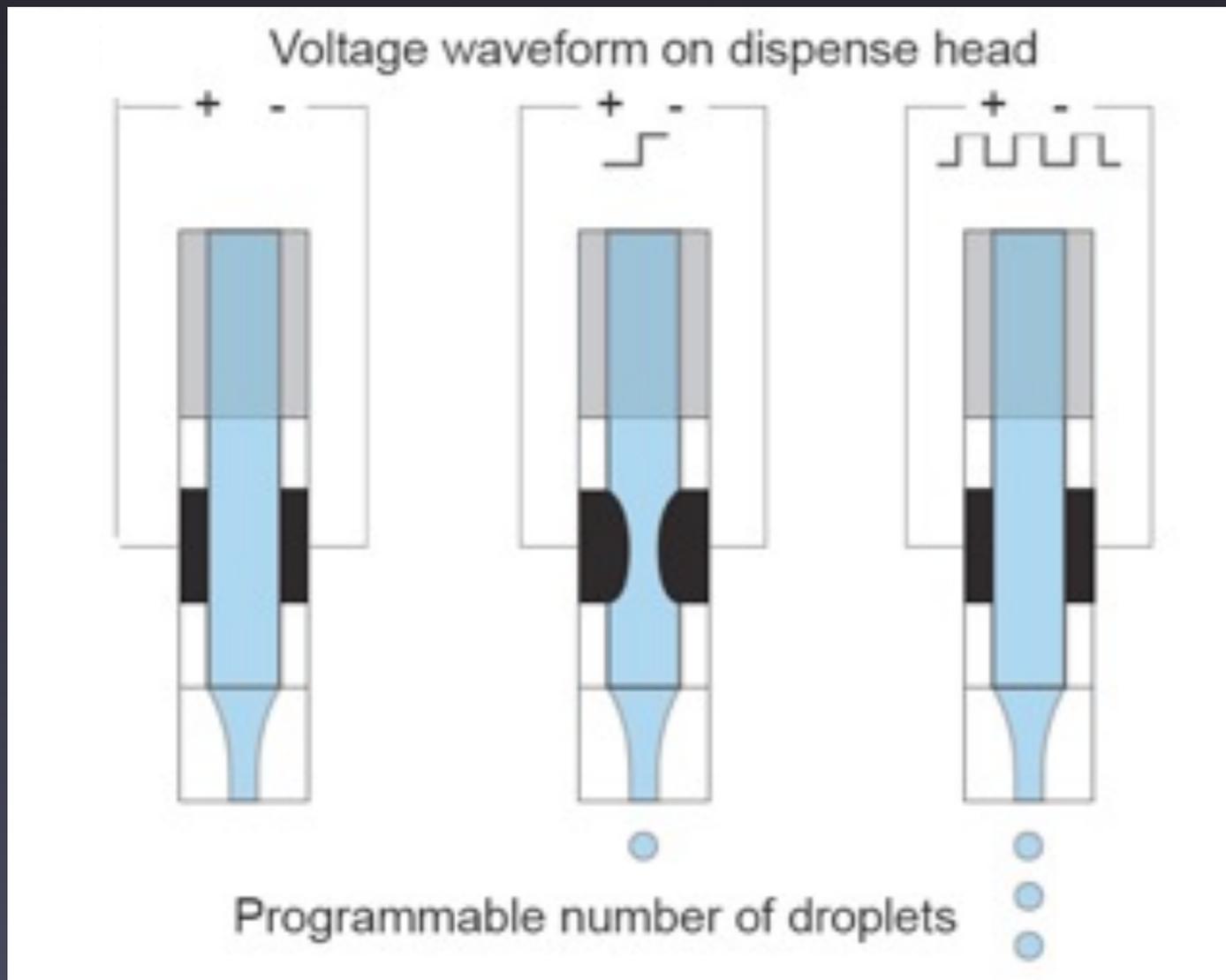
Ice thickness variation:  
 $\sim 2\text{x}$



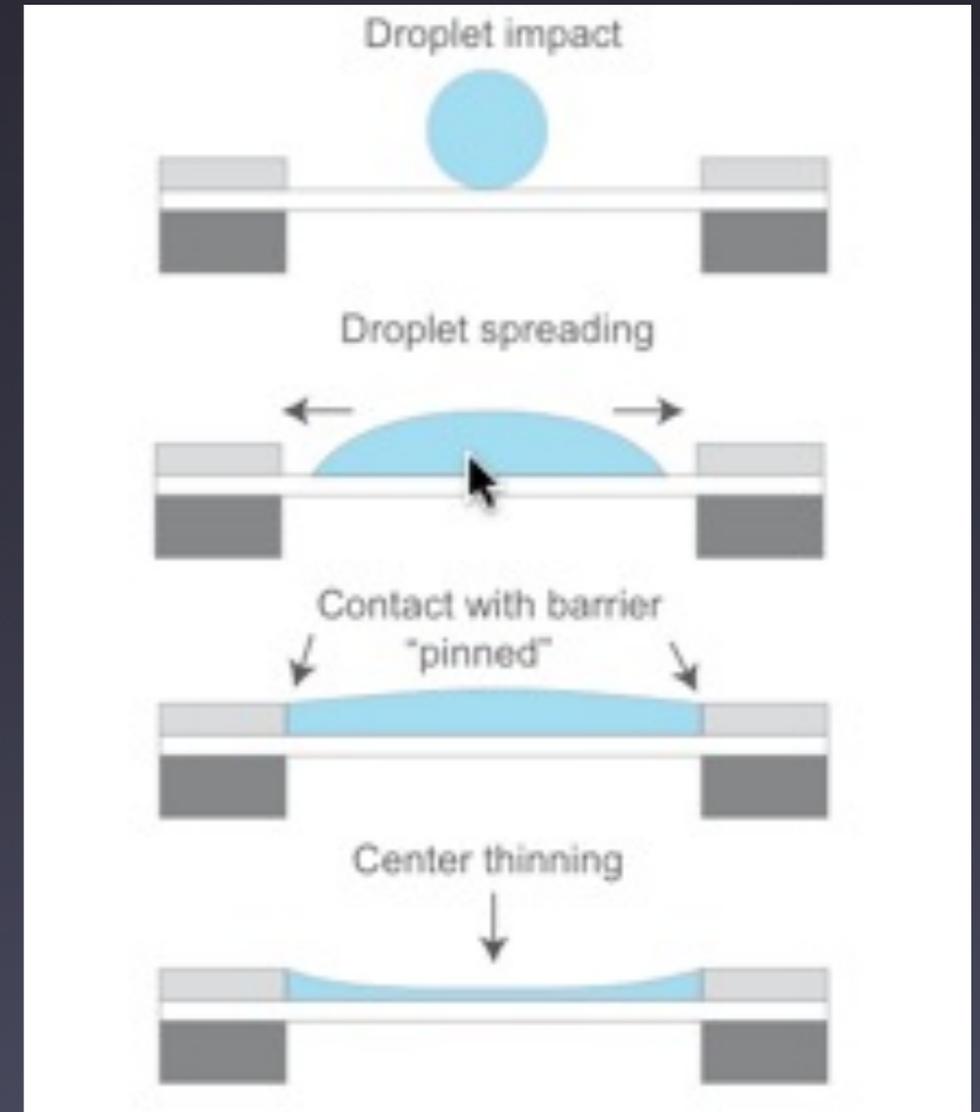
# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

## New Technologies

### Accurate pL dispensing

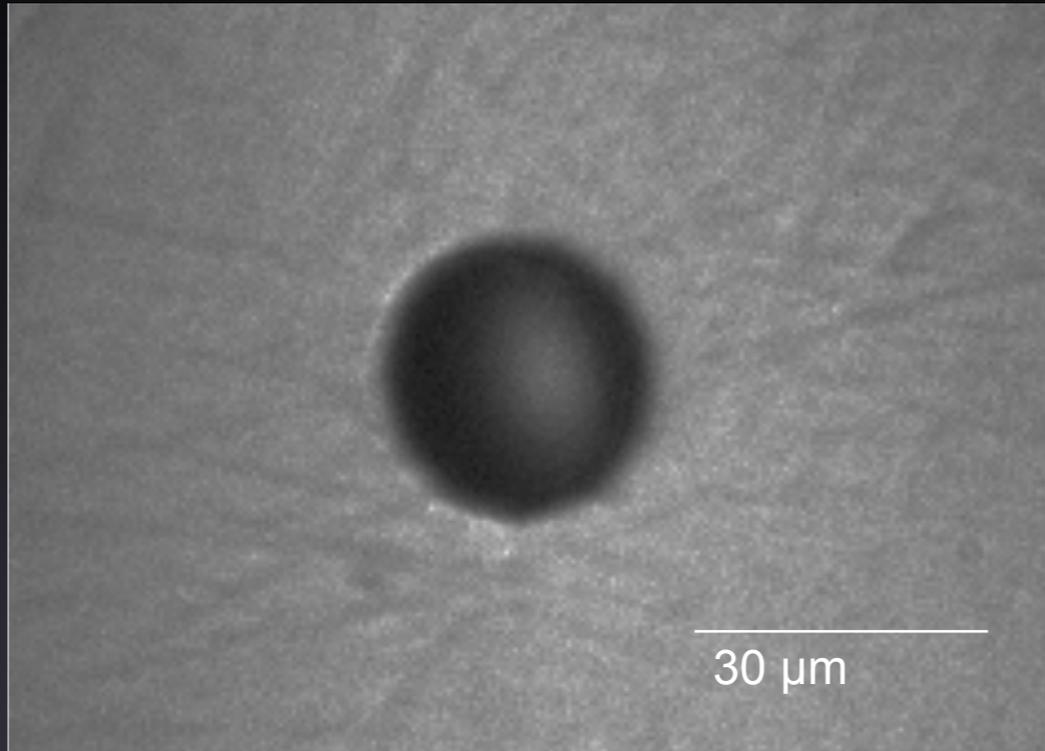


### Thin films without blotting



# Piezo Electric Dispensing

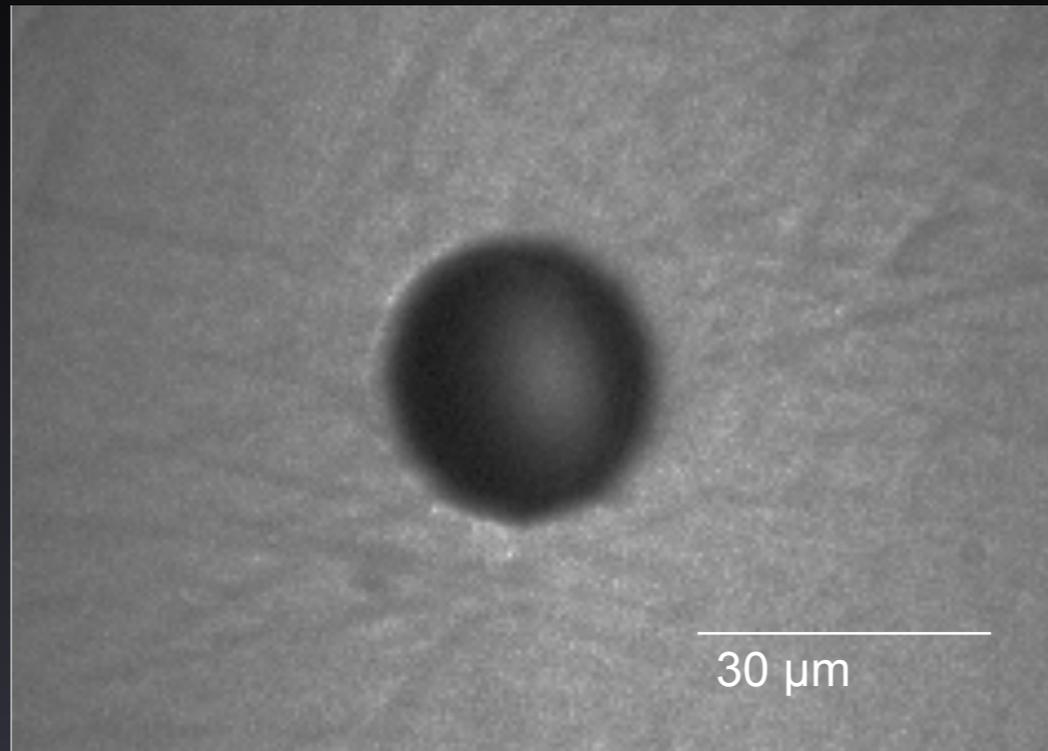
## Sample volume is precise and controllable



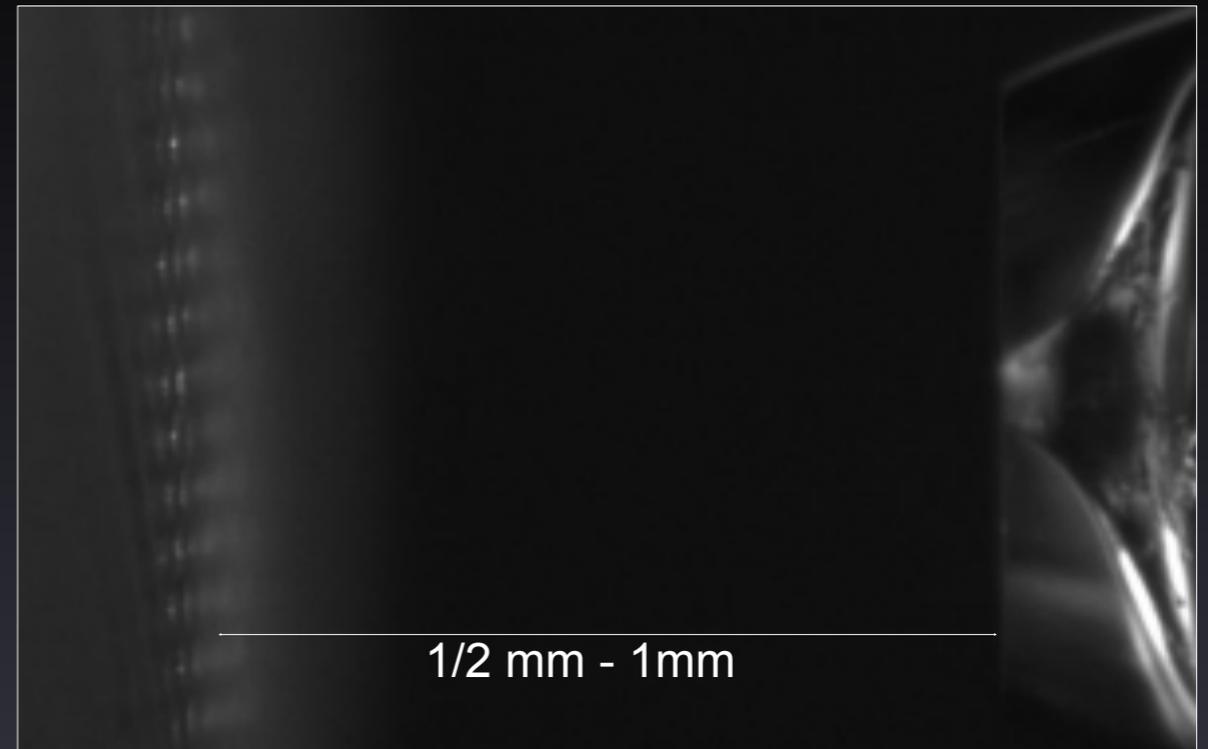
Dispense tip front view

# Piezo Electric Dispensing

## Sample volume is precise and controllable



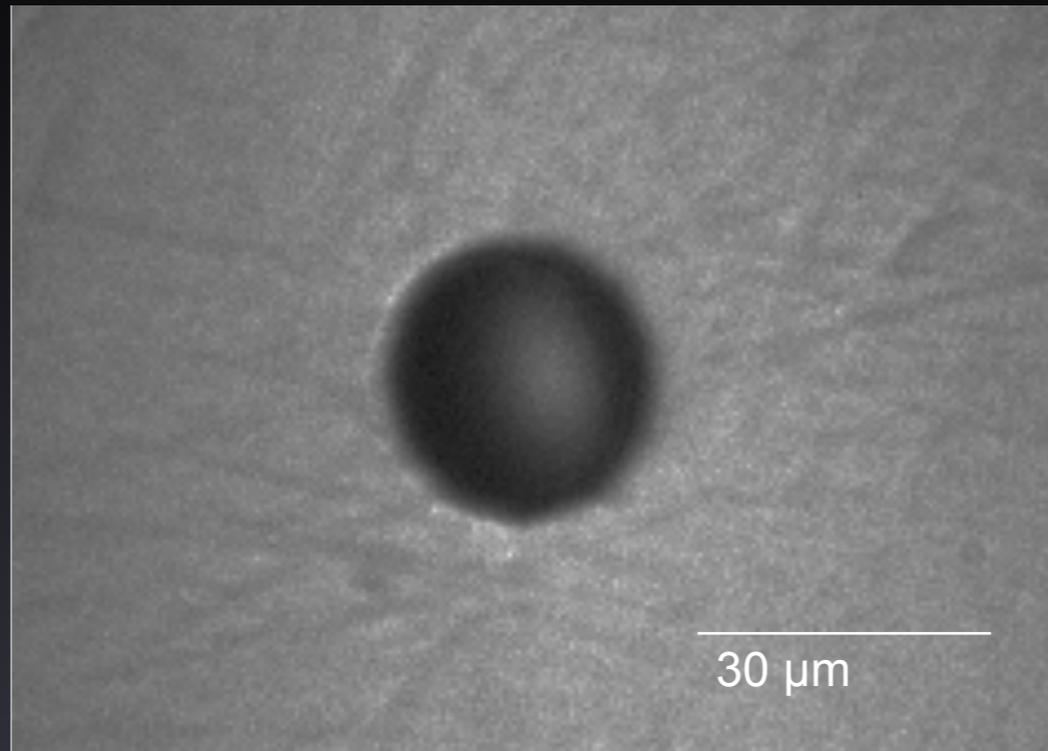
Dispense tip front view



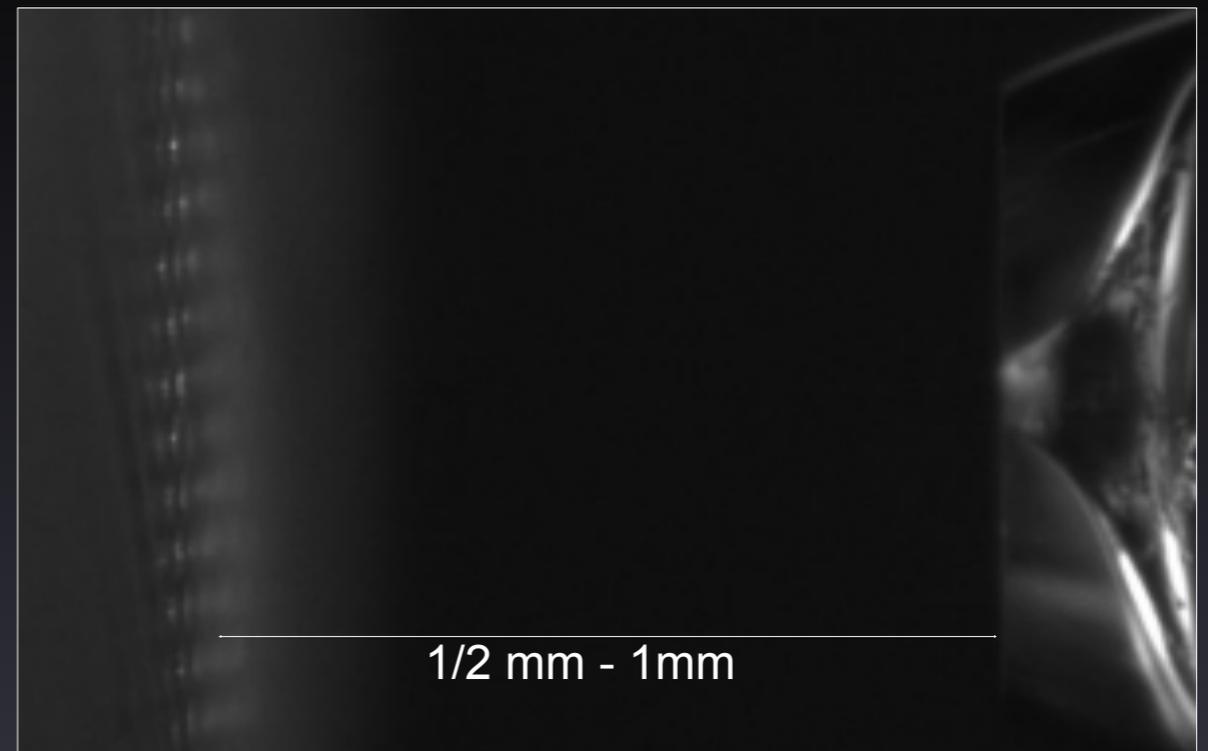
Grid-tip positioning

# Piezo Electric Dispensing

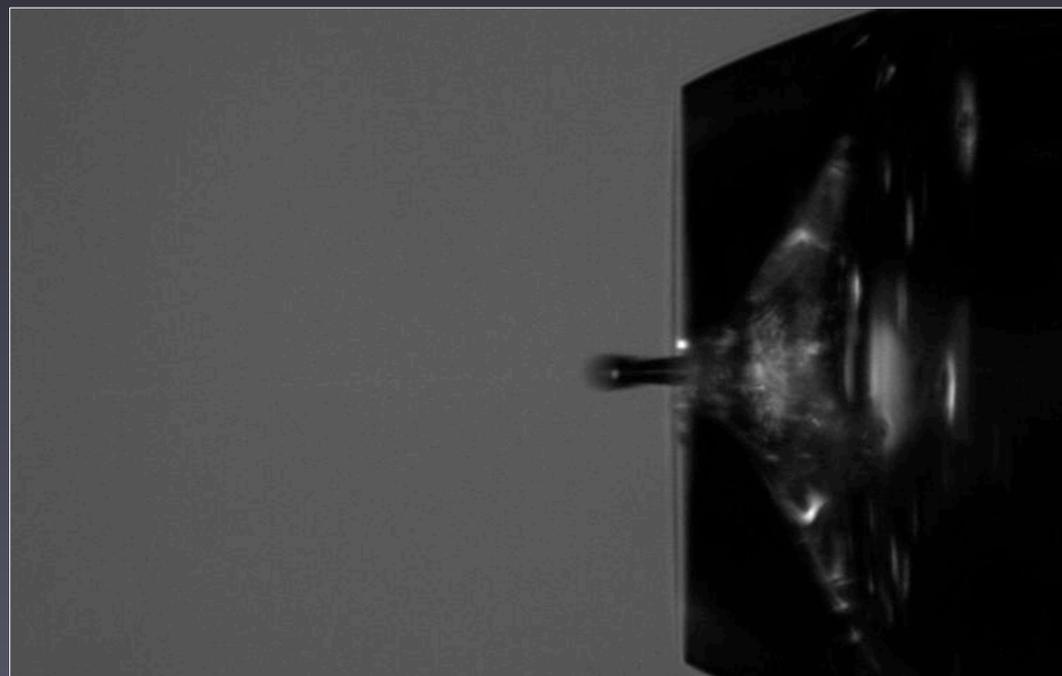
## Sample volume is precise and controllable



Dispense tip front view



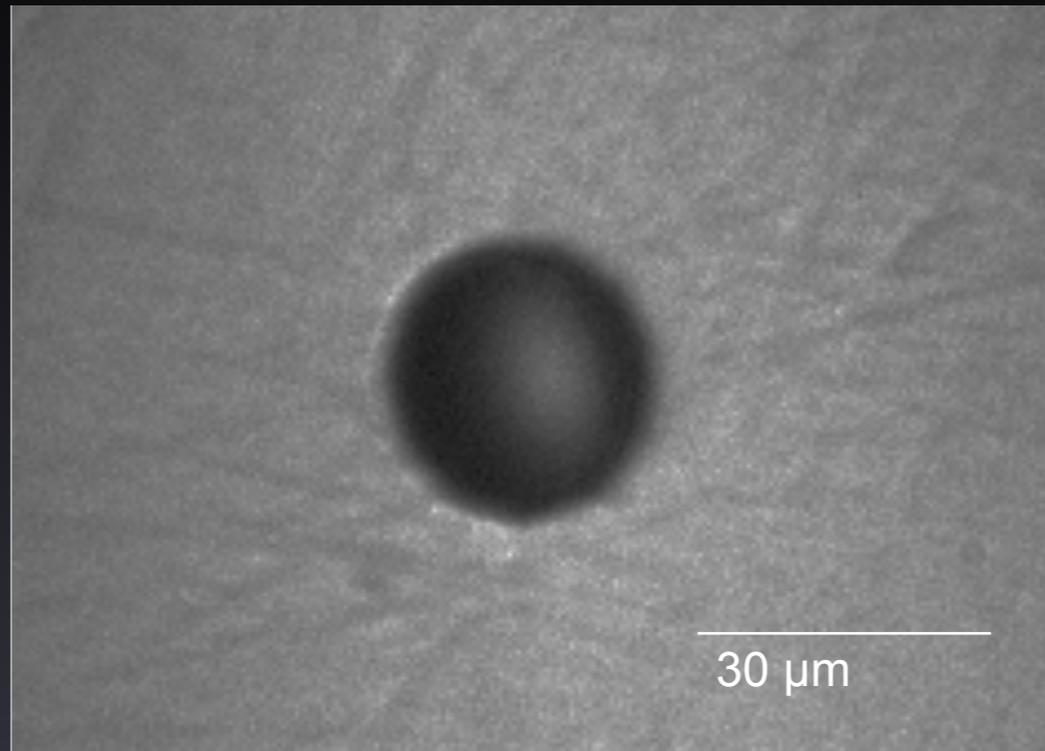
Grid-tip positioning



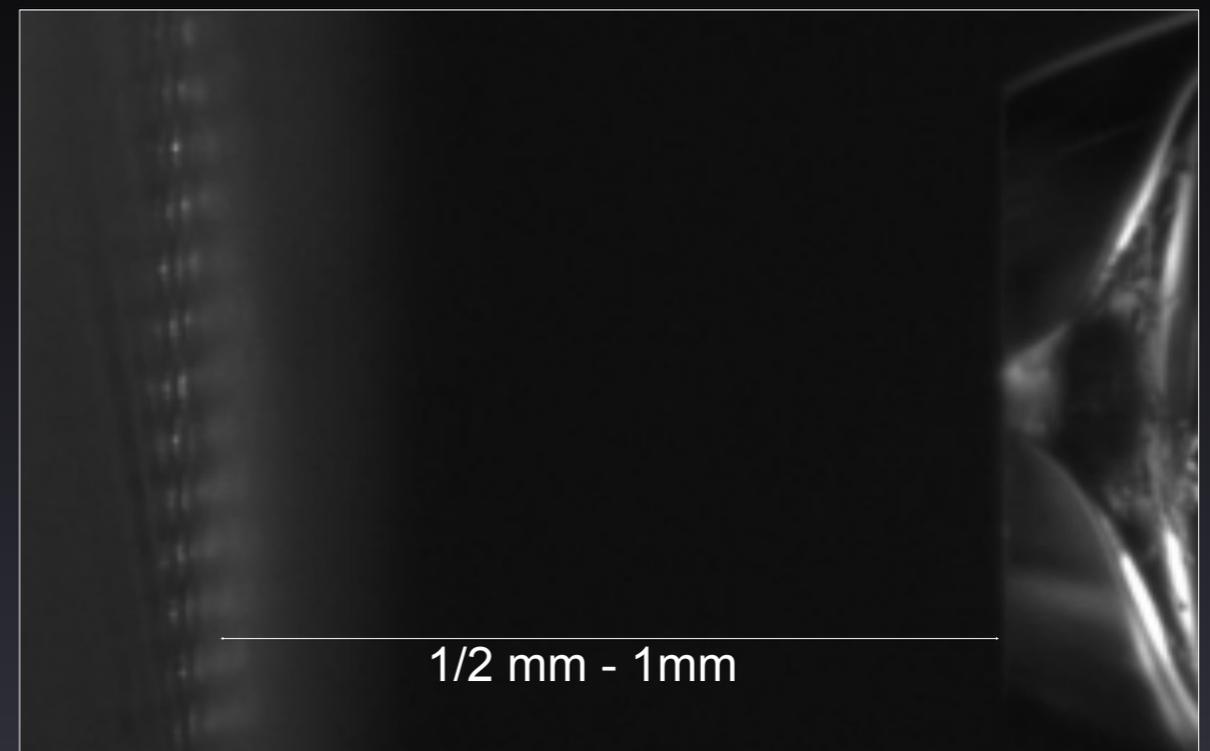
1 droplet (32 pl)

# Piezo Electric Dispensing

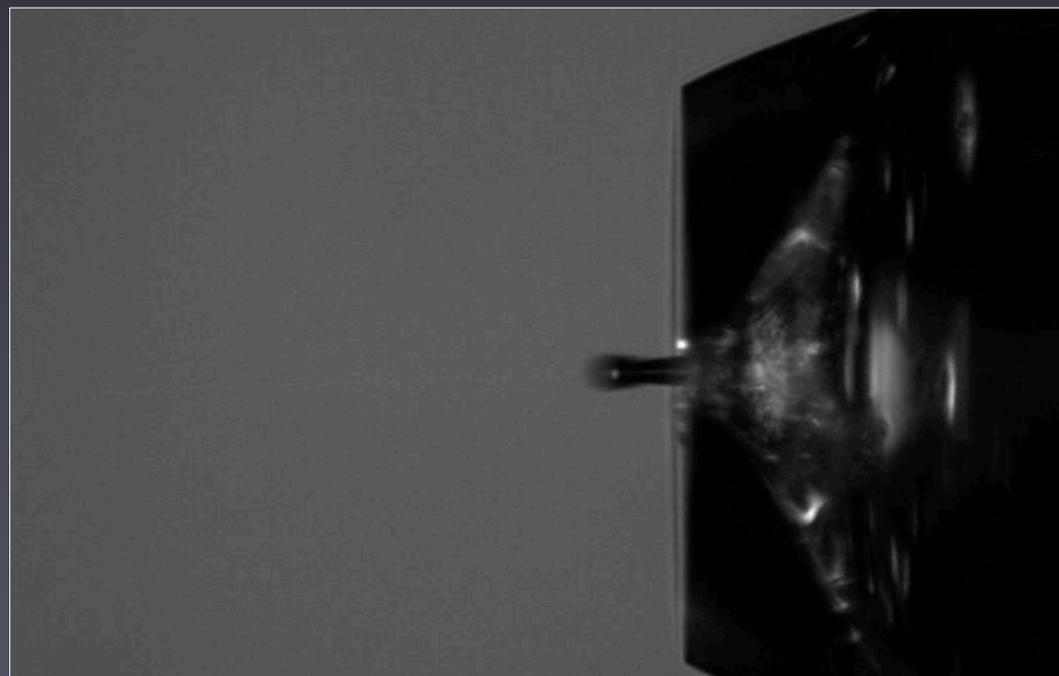
## Sample volume is precise and controllable



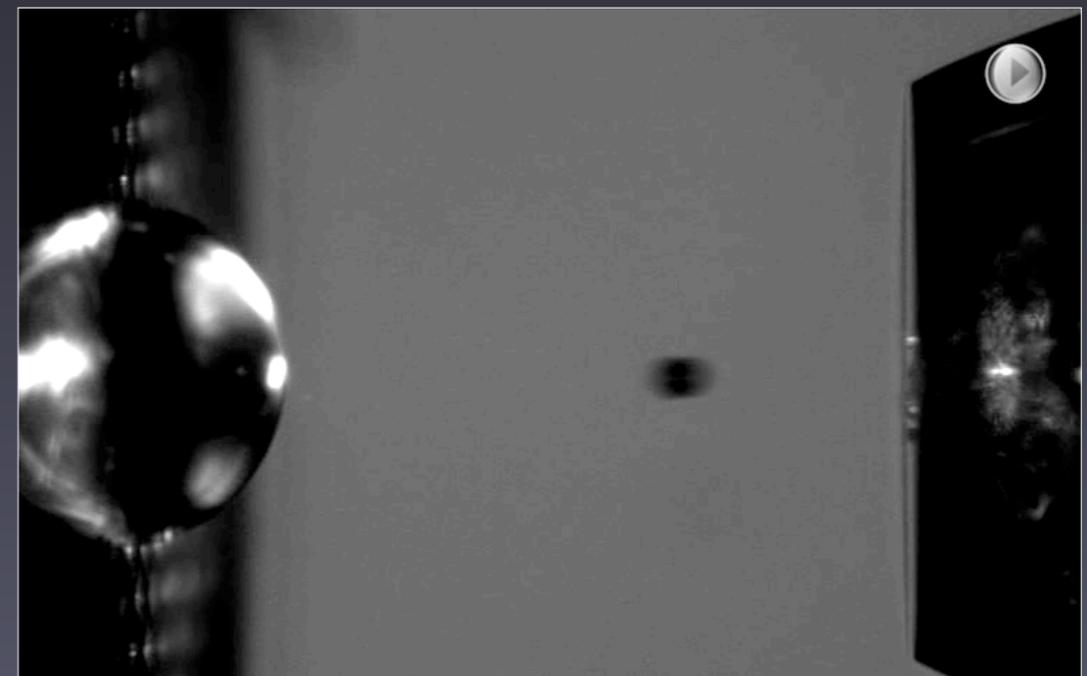
Dispense tip front view



Grid-tip positioning

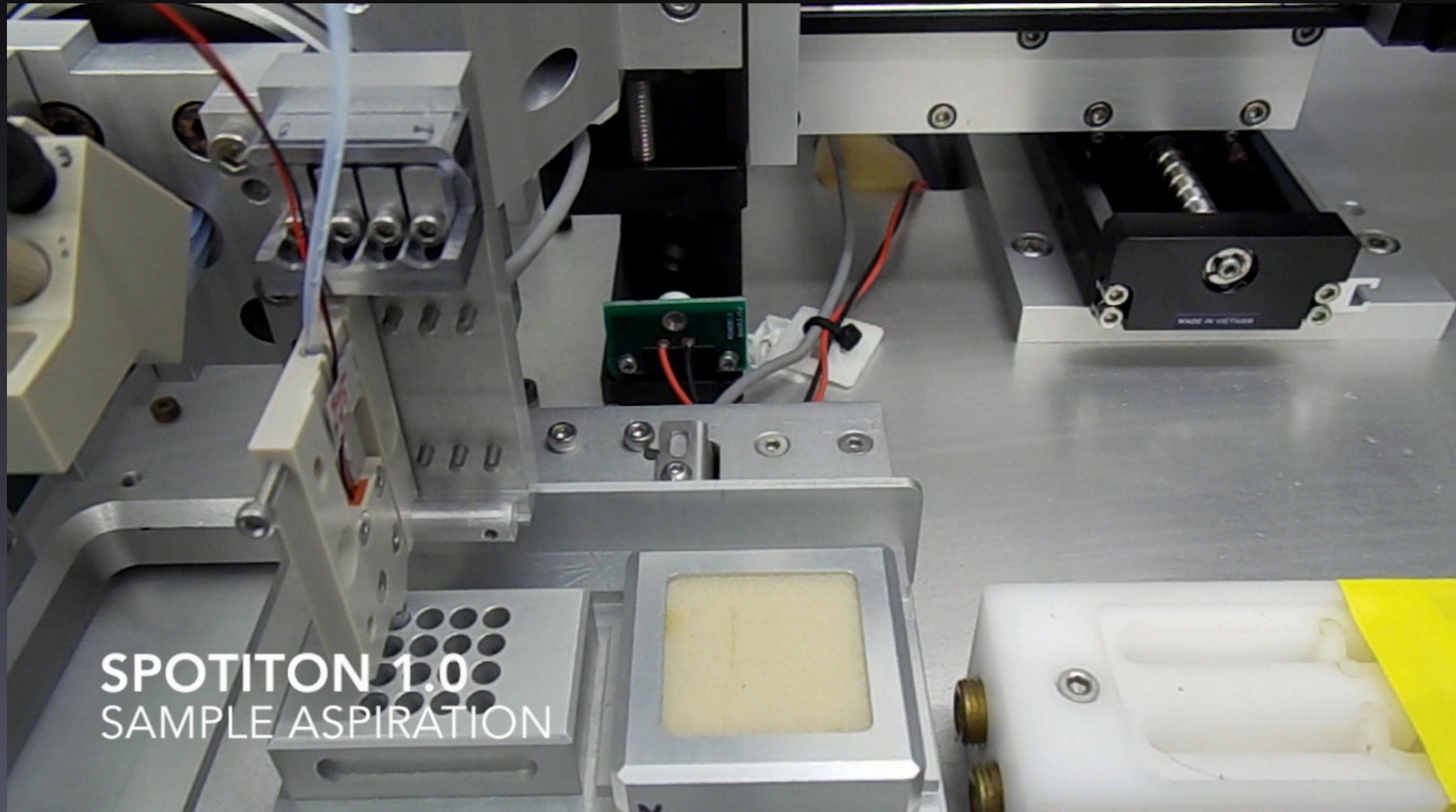


1 droplet (32 pl)



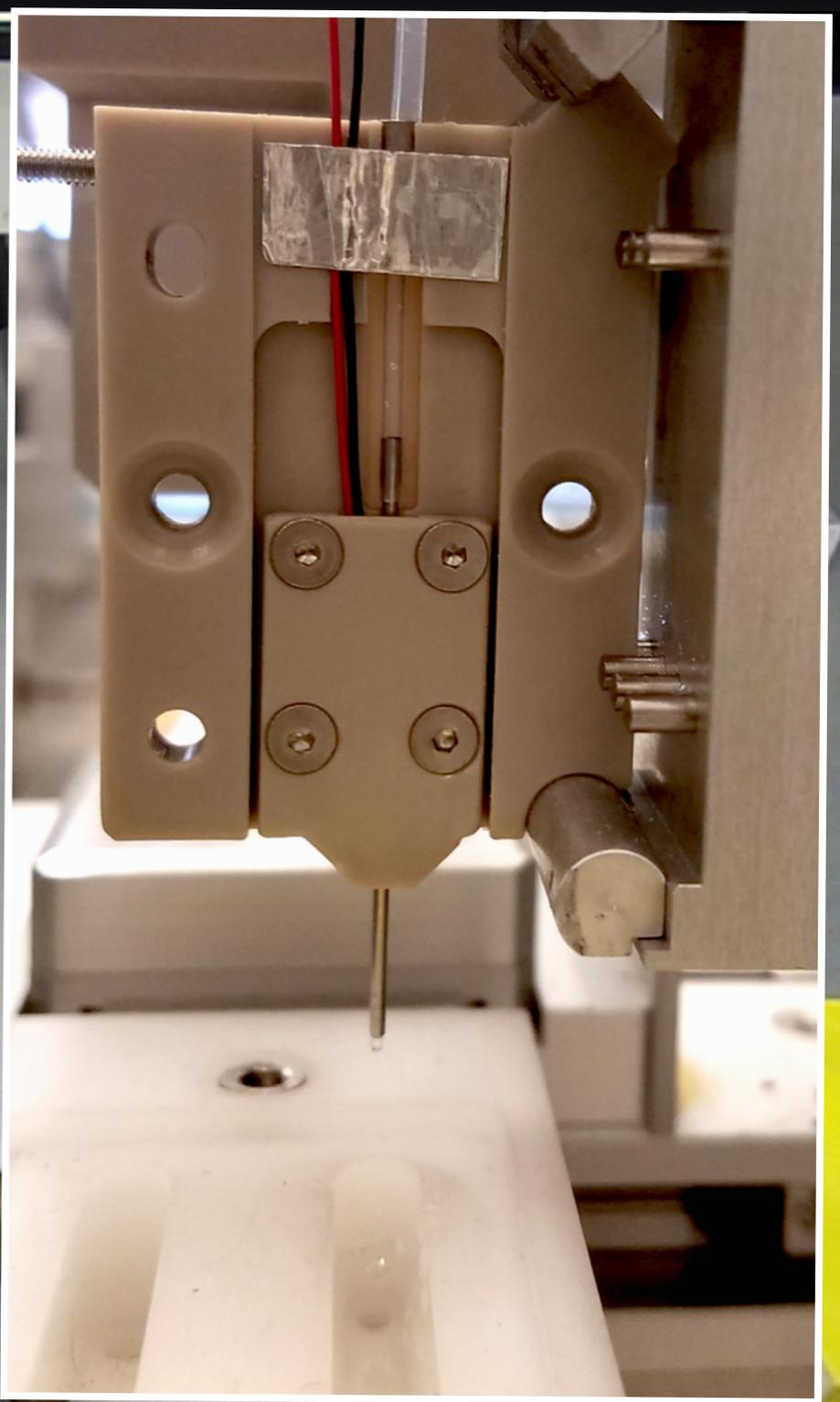
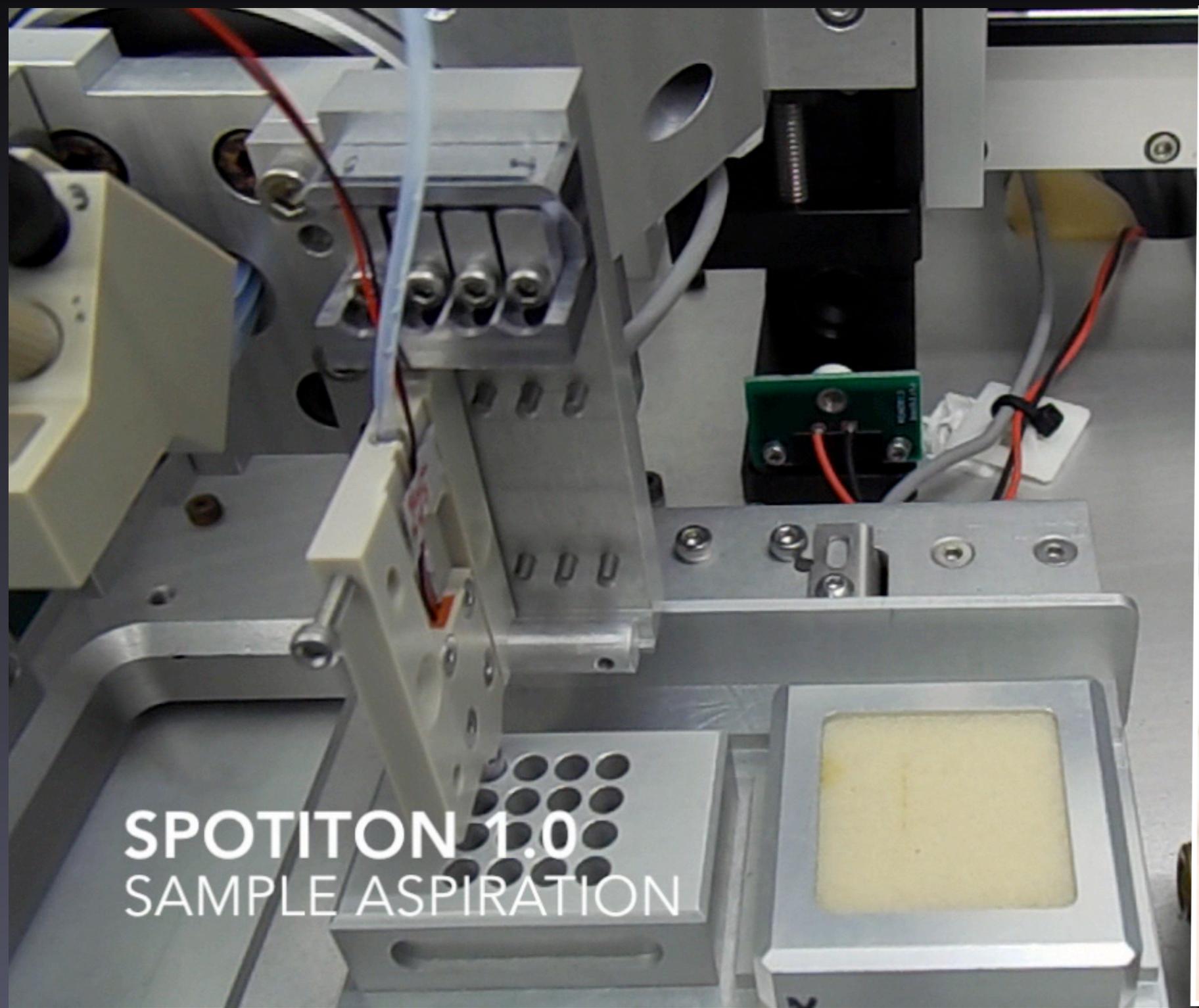
1000 droplets (32 nl)

# The Spotiton Project: Spotiton 1.0



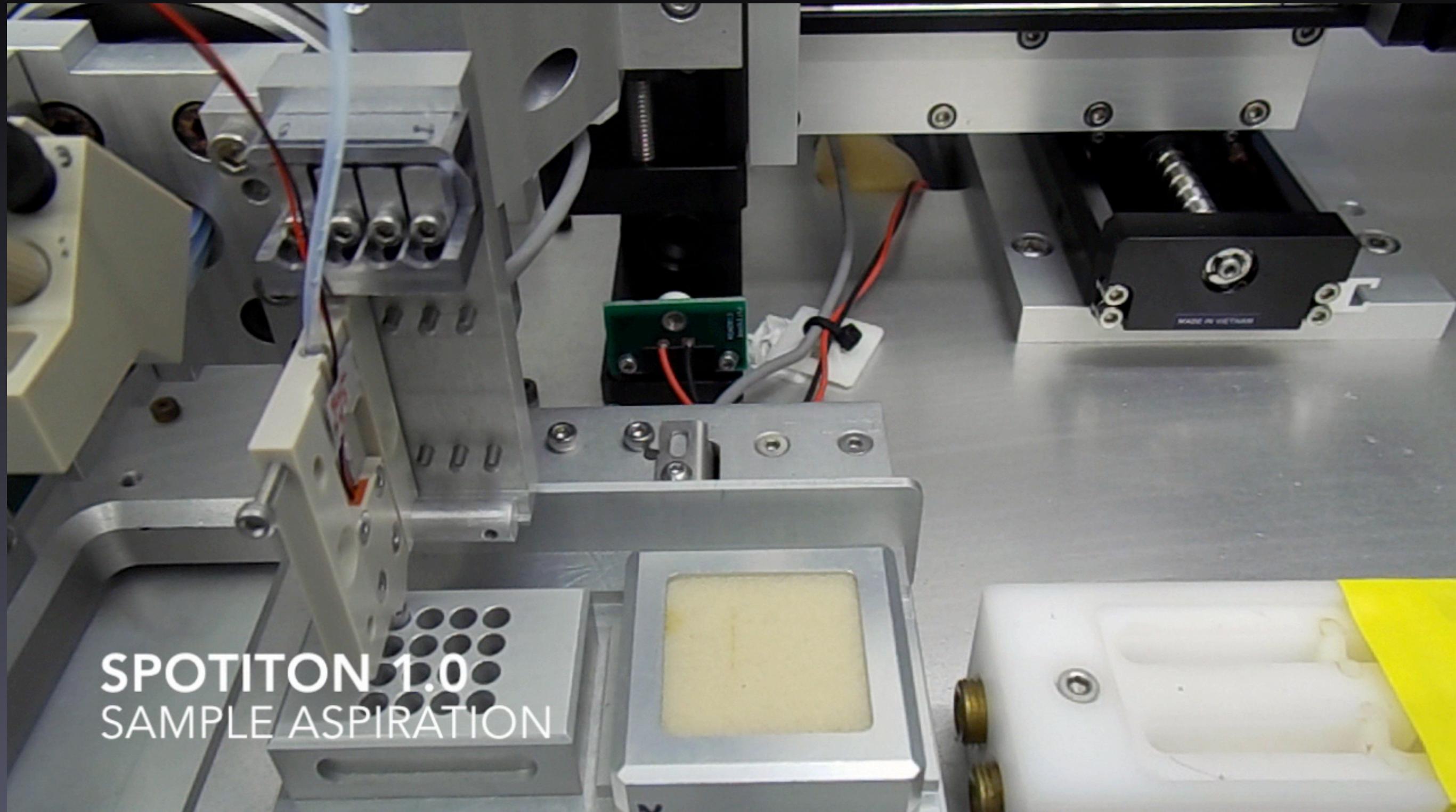
**SPOTITON 1.0**  
SAMPLE ASPIRATION

# The Spotiton Project: Spotiton 1.0



**SPOTITON 1.0**  
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# The Spotiton Project: Spotiton 1.0



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# The Spotiton Project: Spotiton 1.0



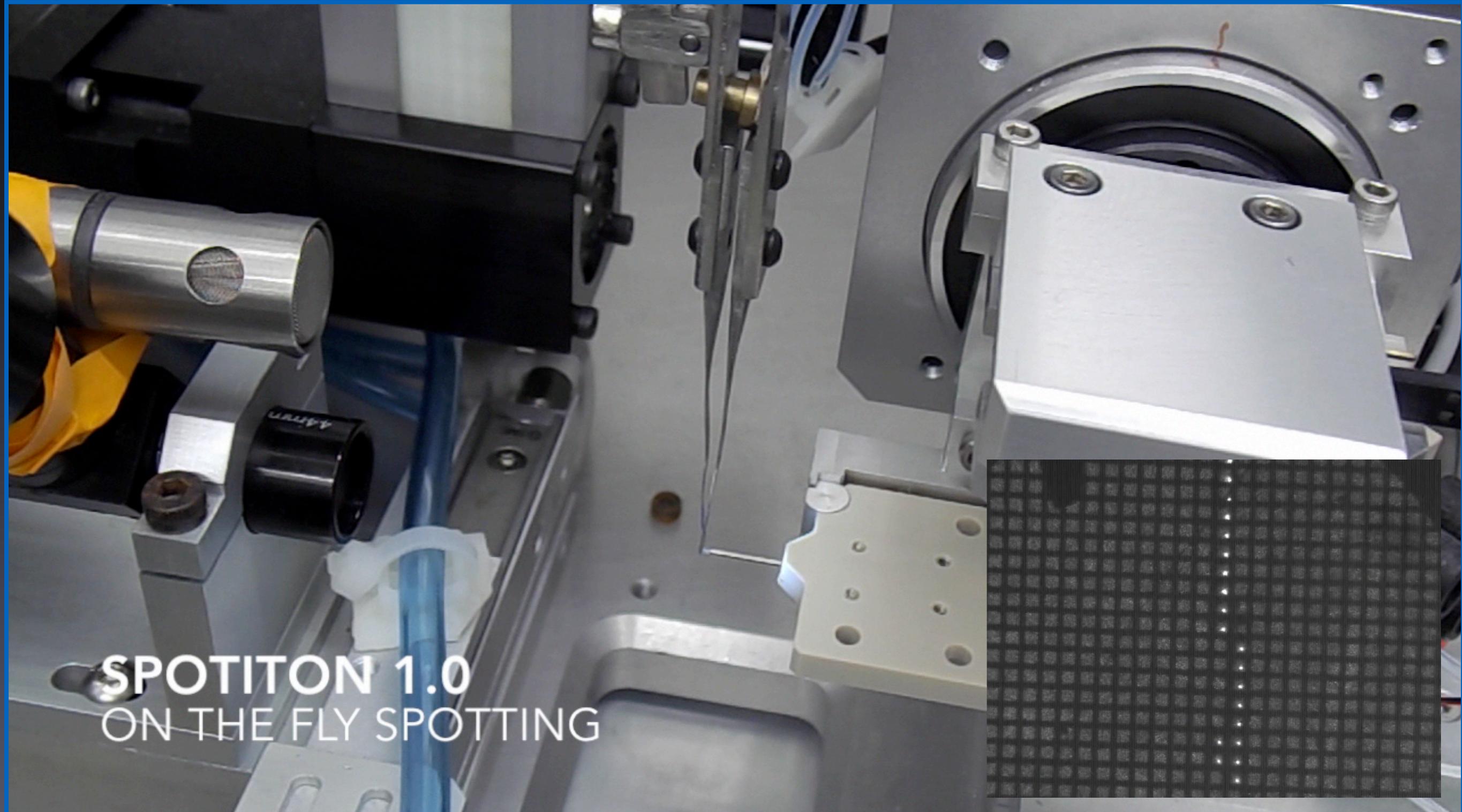
**SPOTITON 1.0**  
ON THE FLY SPOTTING

# The Spotiton Project: Spotiton 1.0



**SPOTITON 1.0**  
ON THE FLY SPOTTING

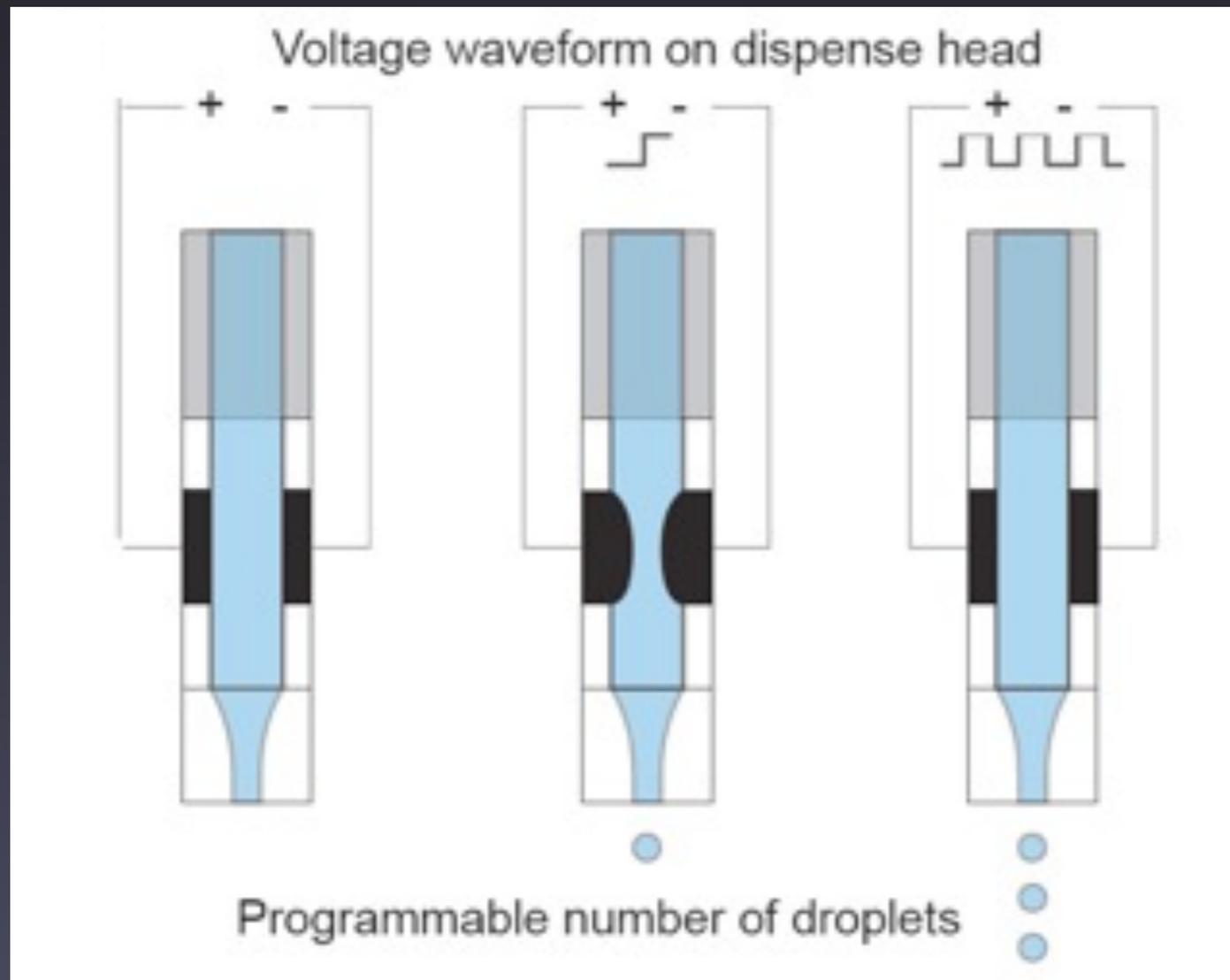
# The Spotiton Project: Spotiton 1.0



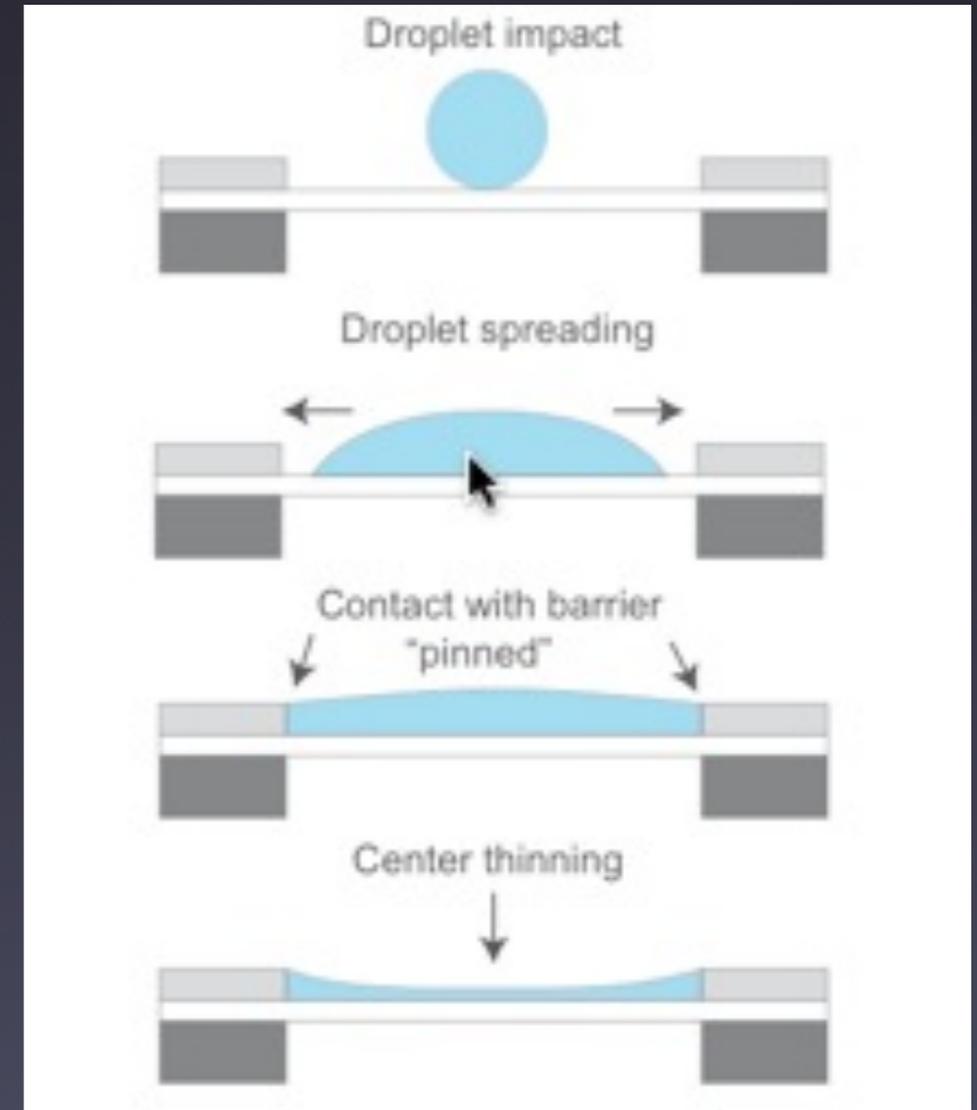
# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

## Required New Technologies

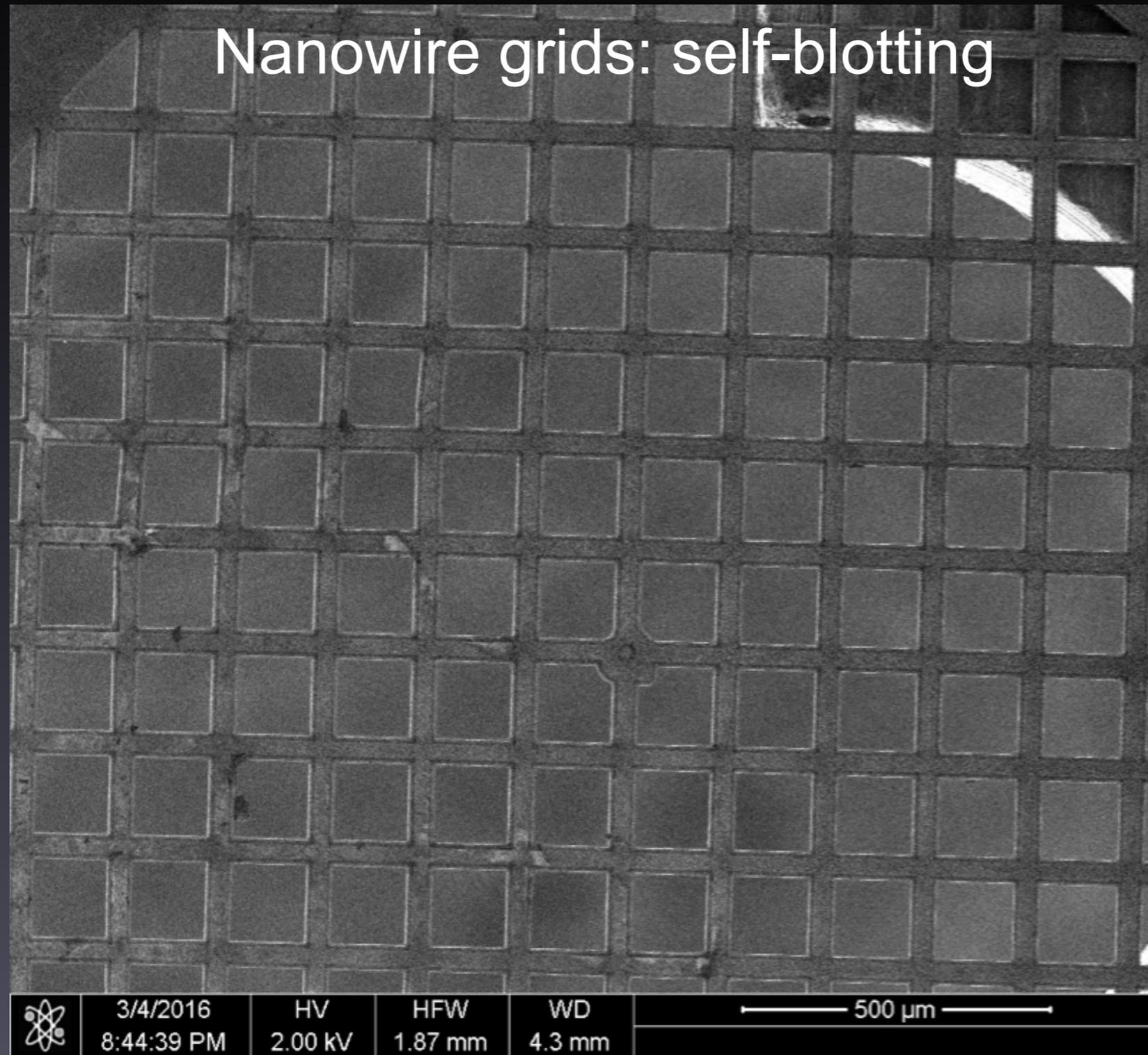
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Thin films without blotting



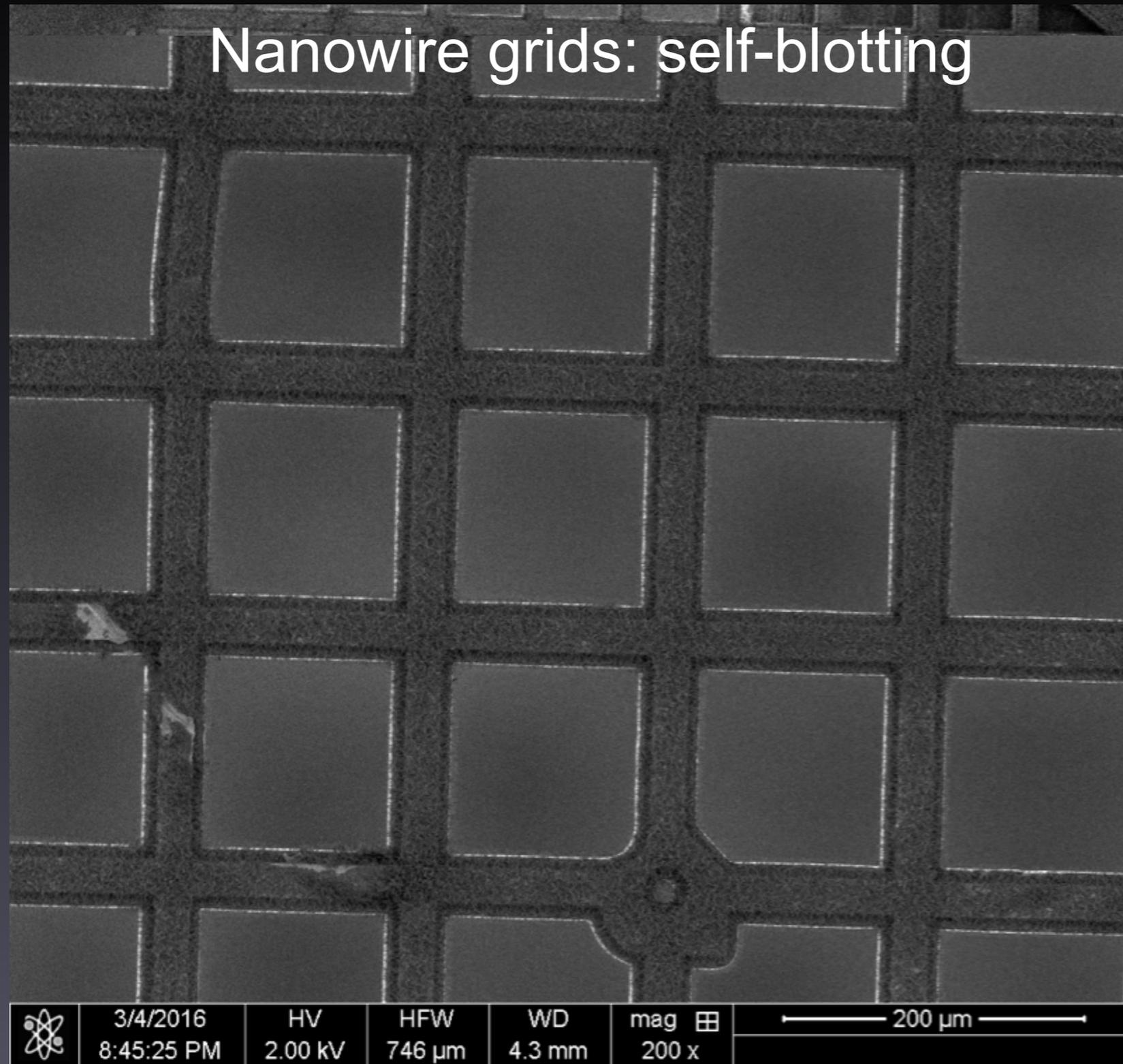
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**Nanowires can be grown on copper grids using a simple chemical treatment**

Ivan Razinkov<sup>1</sup>, Venkat Dandey<sup>1</sup>, Hui Wei, Zhening Zhang, David Melnekoff, William J. Rice, Christoph Wigge, Clinton S. Potter, Bridget Carragher. A new method for vitrifying samples for cryoEM. (2016). JSB (In press).

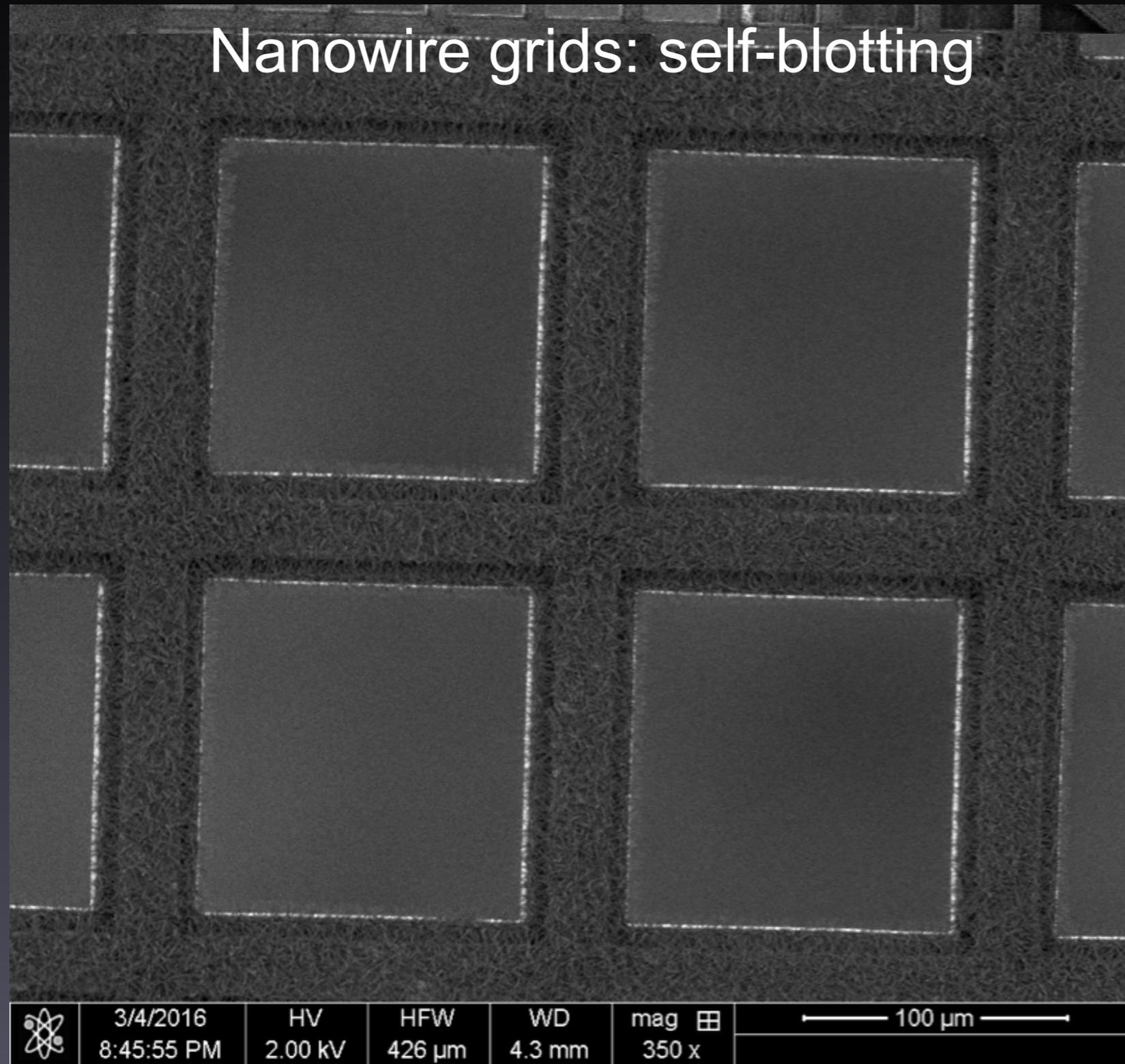
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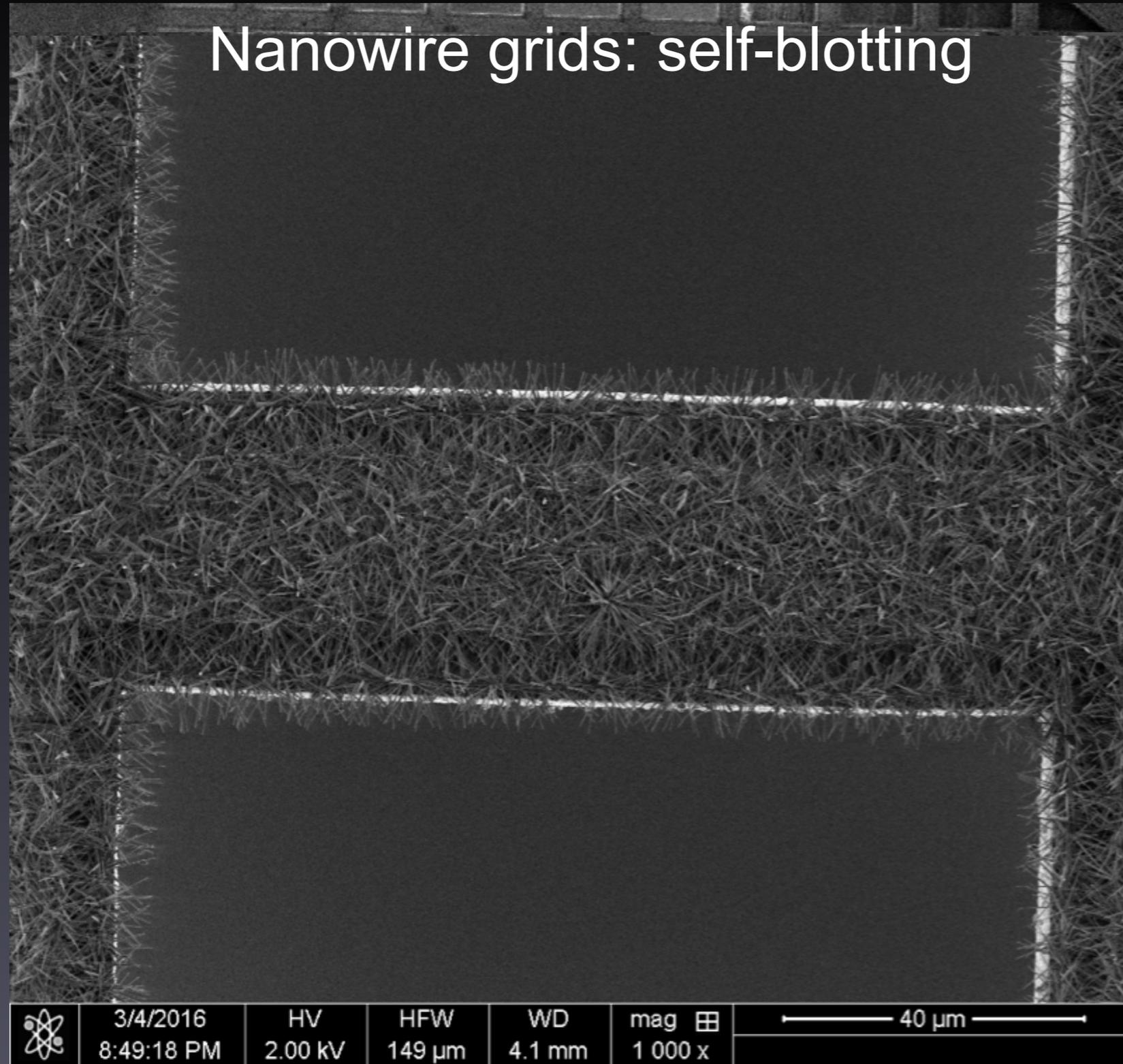
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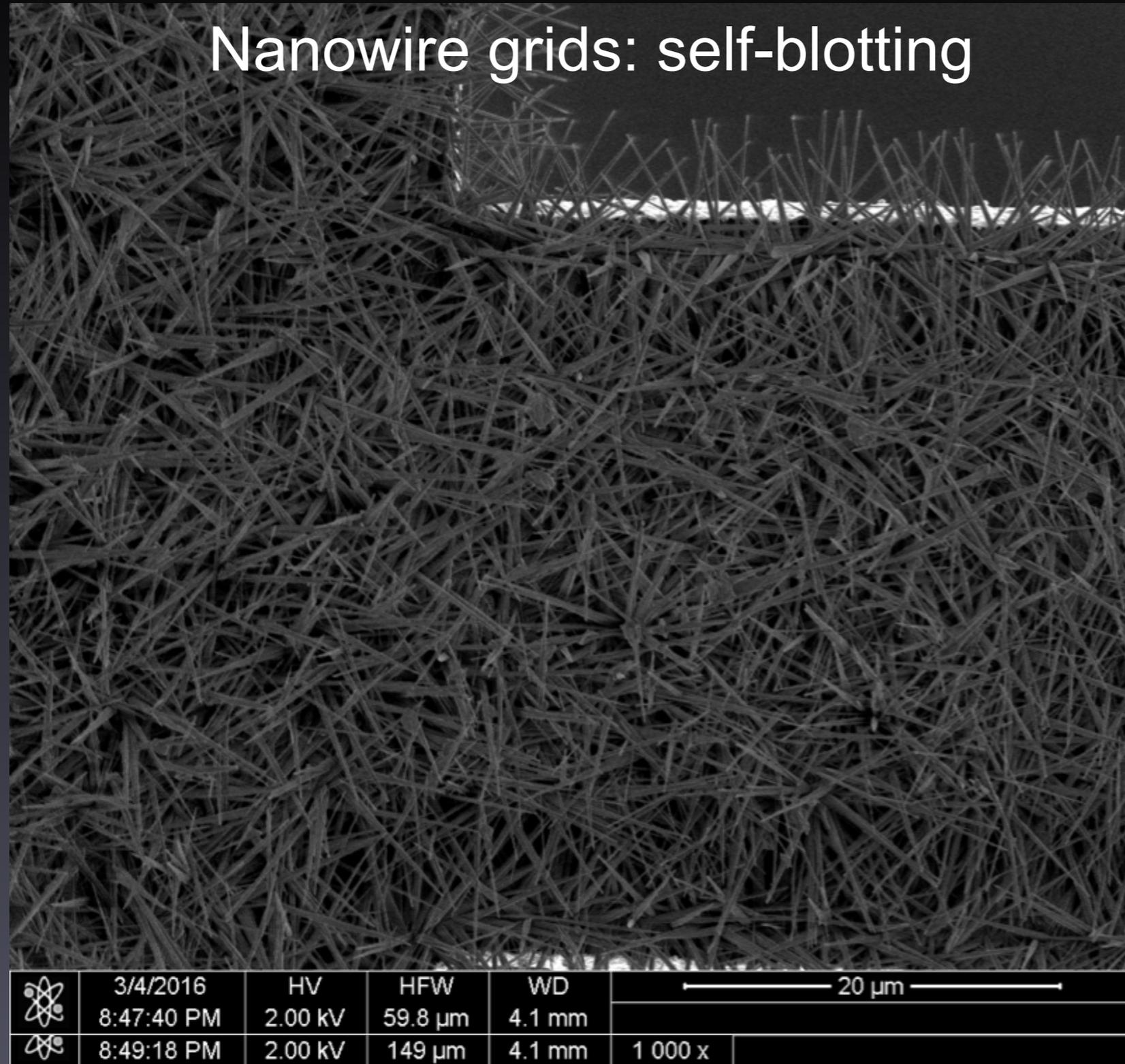
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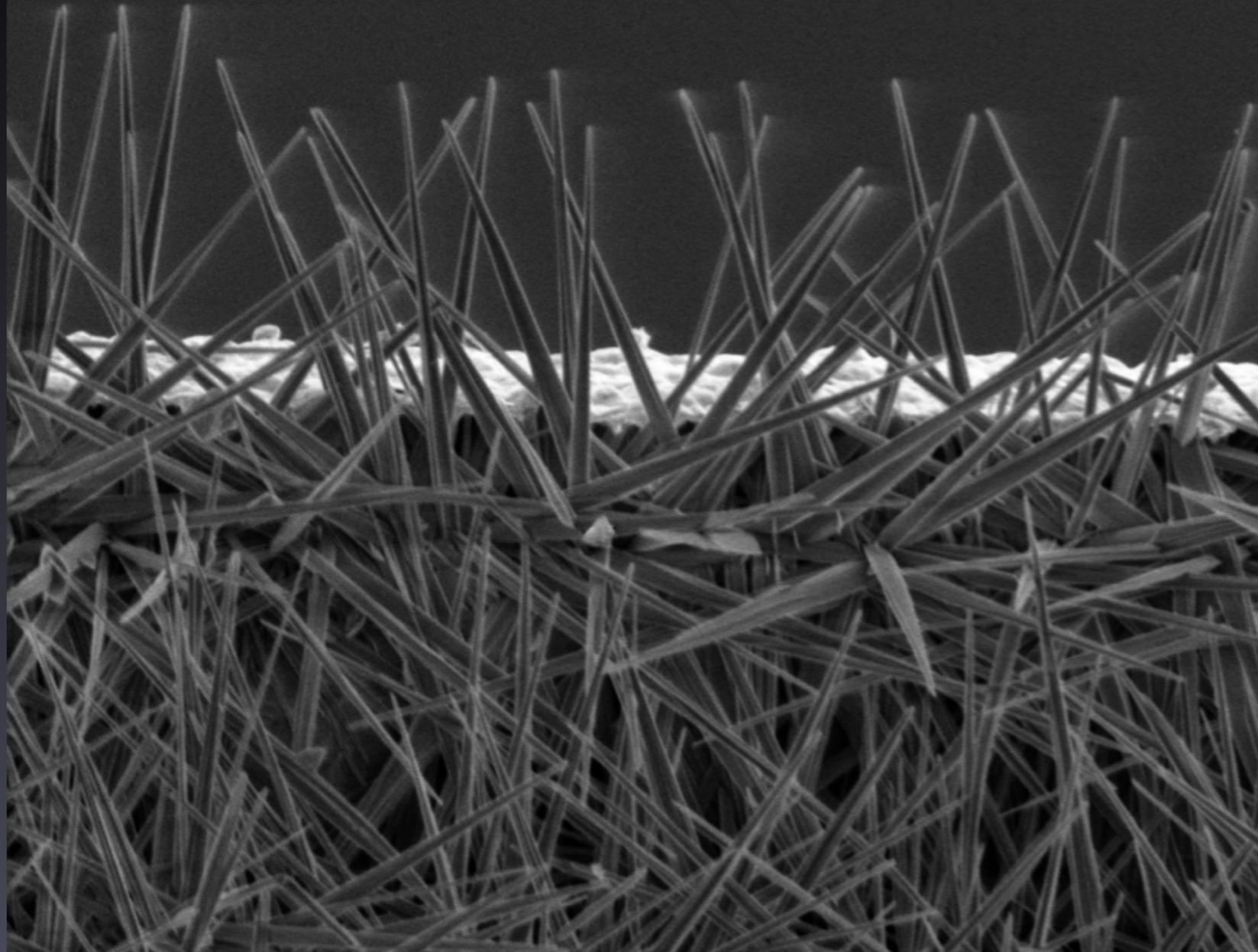


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# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

Nanowire grids: self-blotting

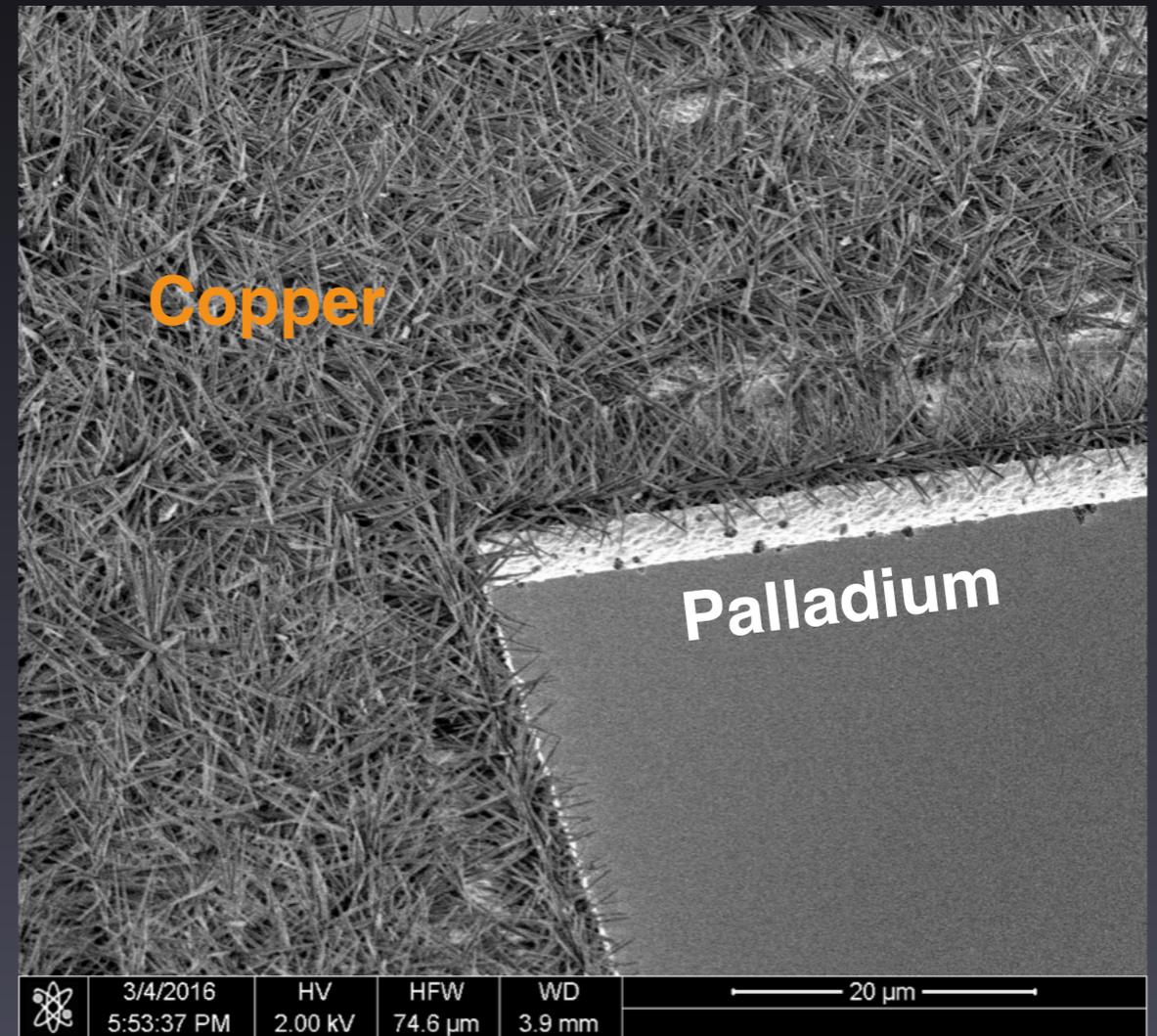
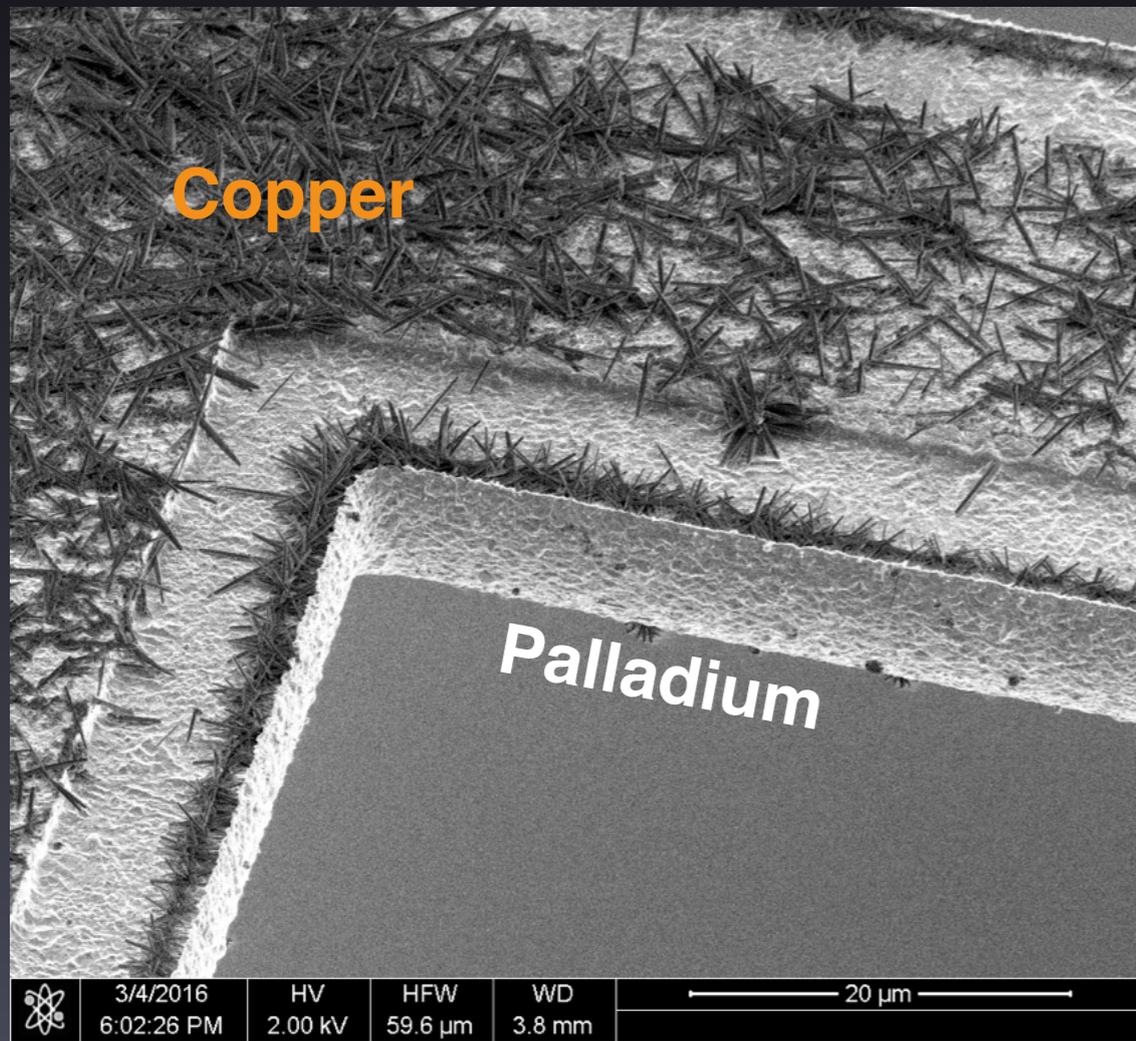


	3/4/2016	HV	HFW	WD	5 $\mu$ m	
	8:48:50 PM	2.00 kV	18.7 $\mu$ m	4.1 mm		
	8:49:18 PM	2.00 kV	149 $\mu$ m	4.1 mm	1 000 x	

**Nanowires can be grown on copper grids using a simple chemical treatment**

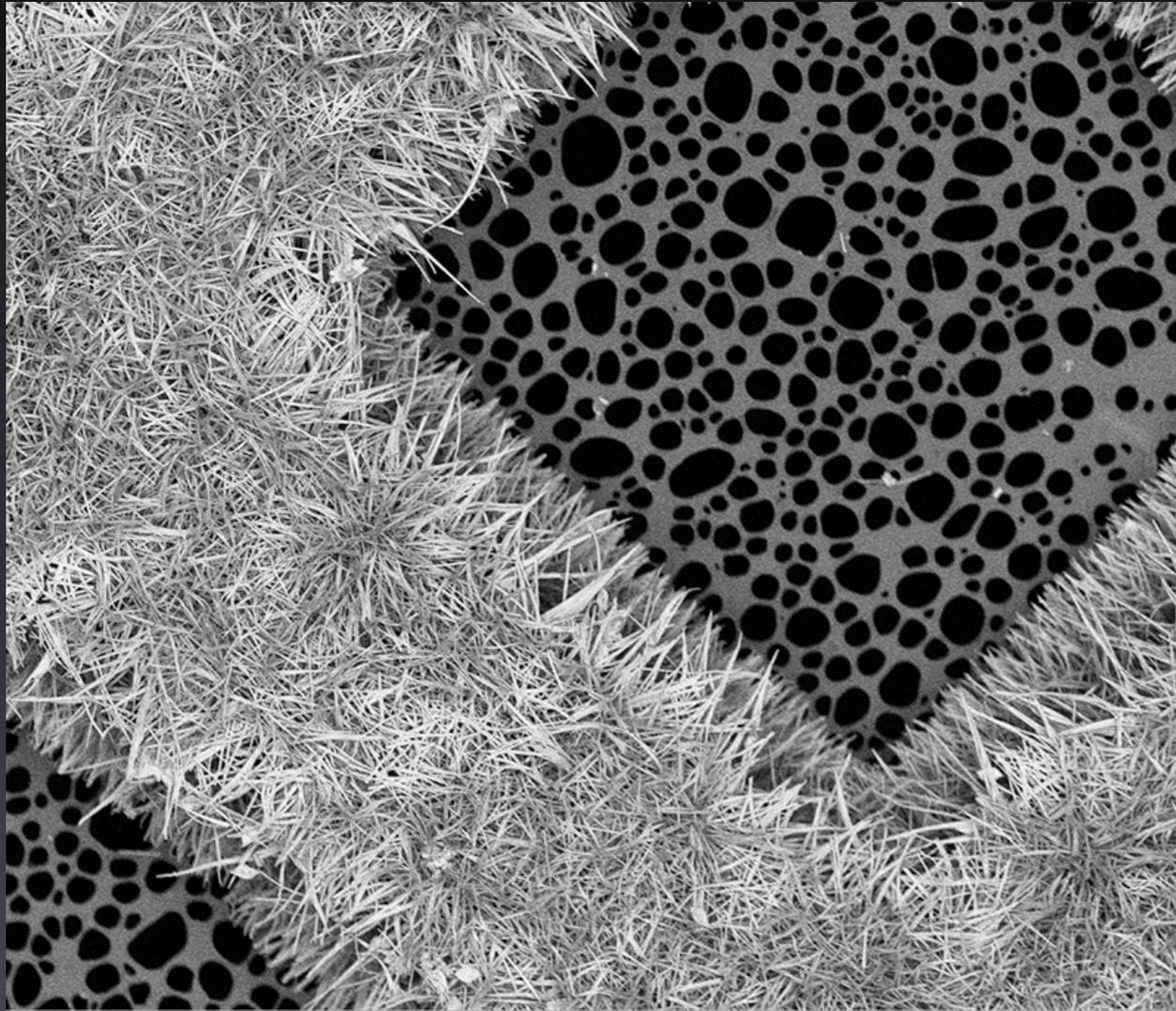
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# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

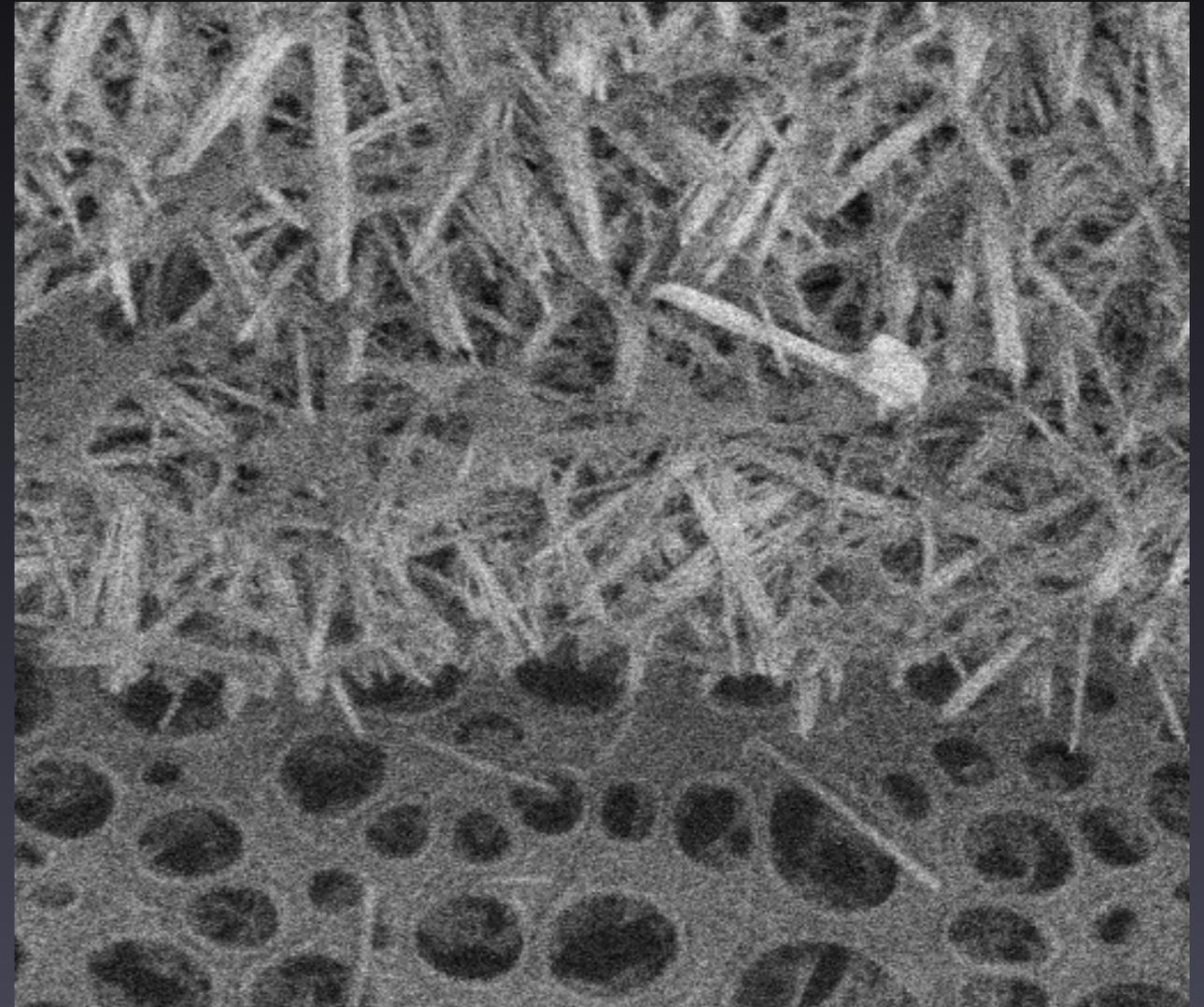


Copper palladium/rhodium/gold sandwich grids provide one smooth surface for attachment of the carbon substrate

# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods



6/13/2016	HV	HFW	WD	20 $\mu$ m
3:50:27 PM	2.00 kV	59.5 $\mu$ m	4.9 mm	NYSBC Helios 650

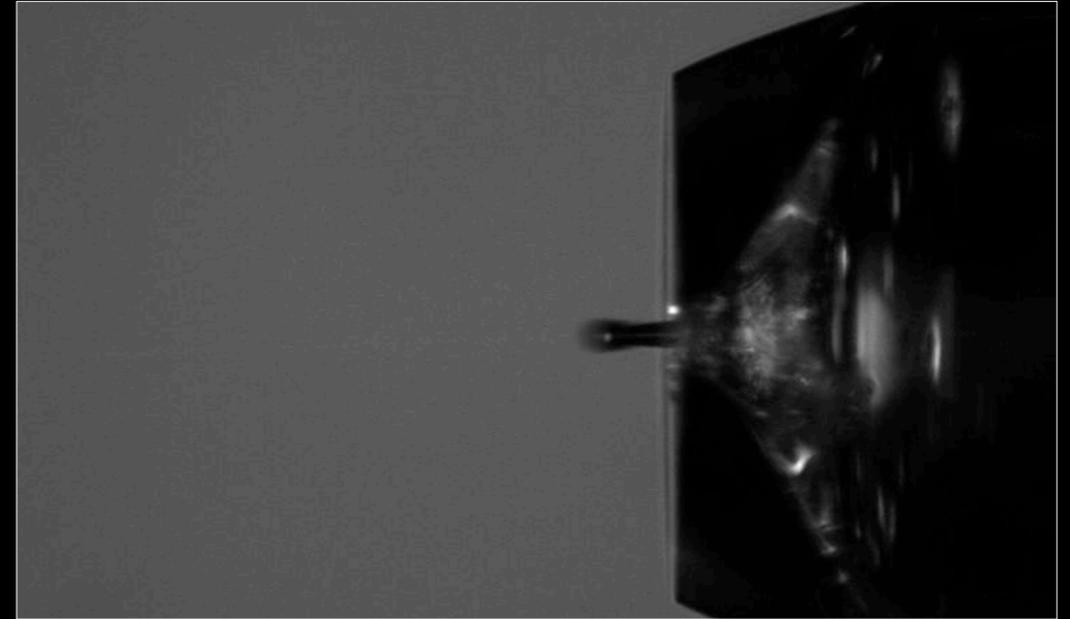
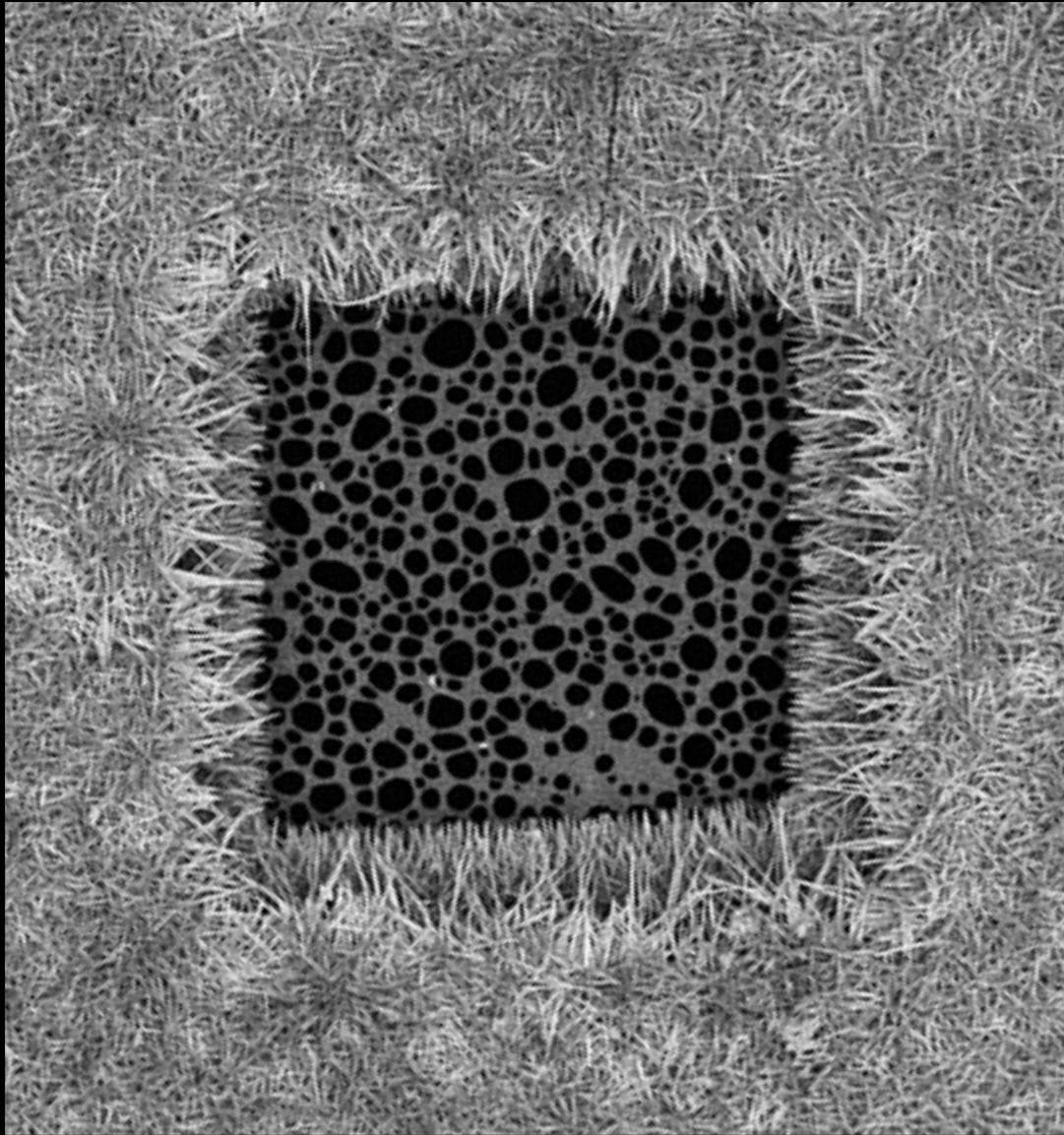


3/11/2016	HV	HFW	WD	5 $\mu$ m
9:00:33 PM	2.00 kV	14.9 $\mu$ m	4.2 mm	

**Copper palladium/rhodium/gold sandwich grids provide one smooth surface for attachment of the carbon/gold substrate**

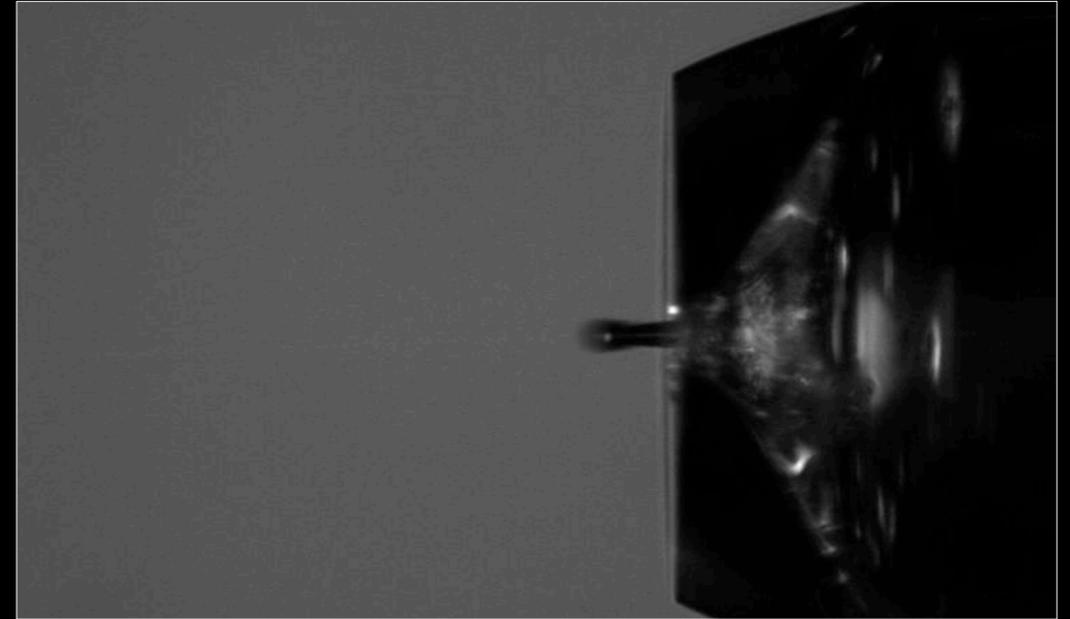
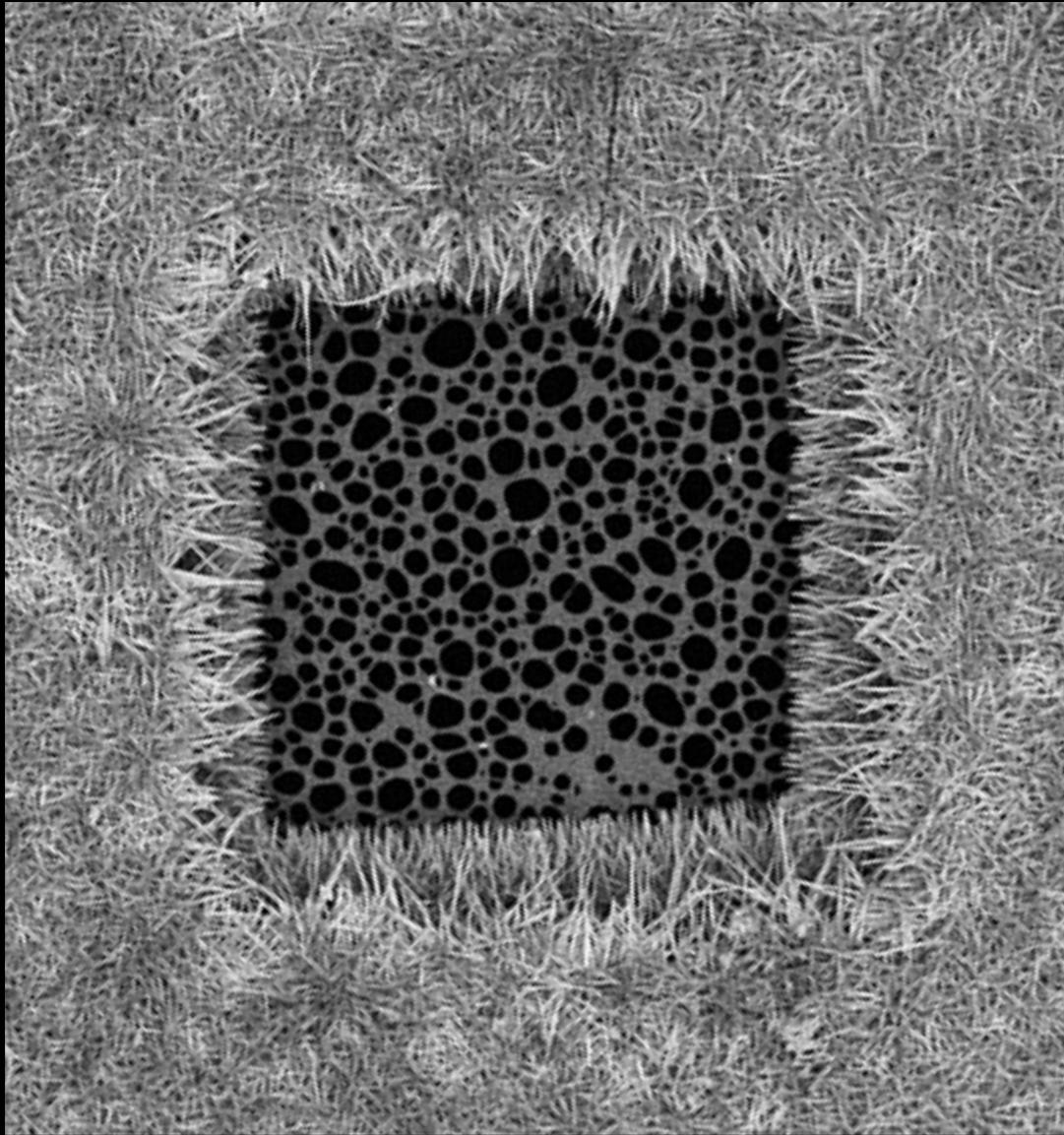
# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

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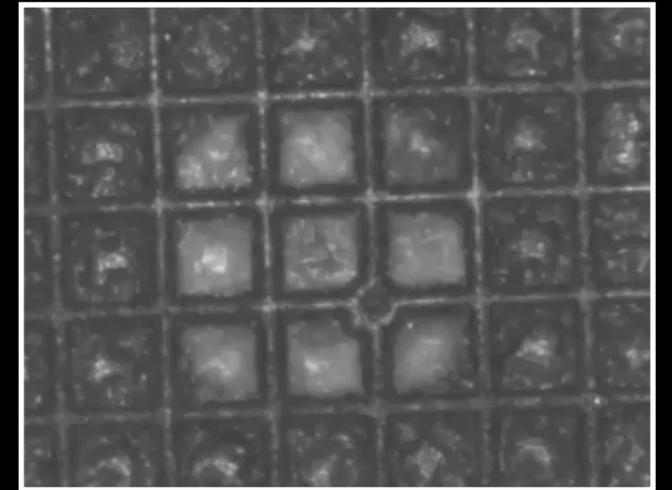
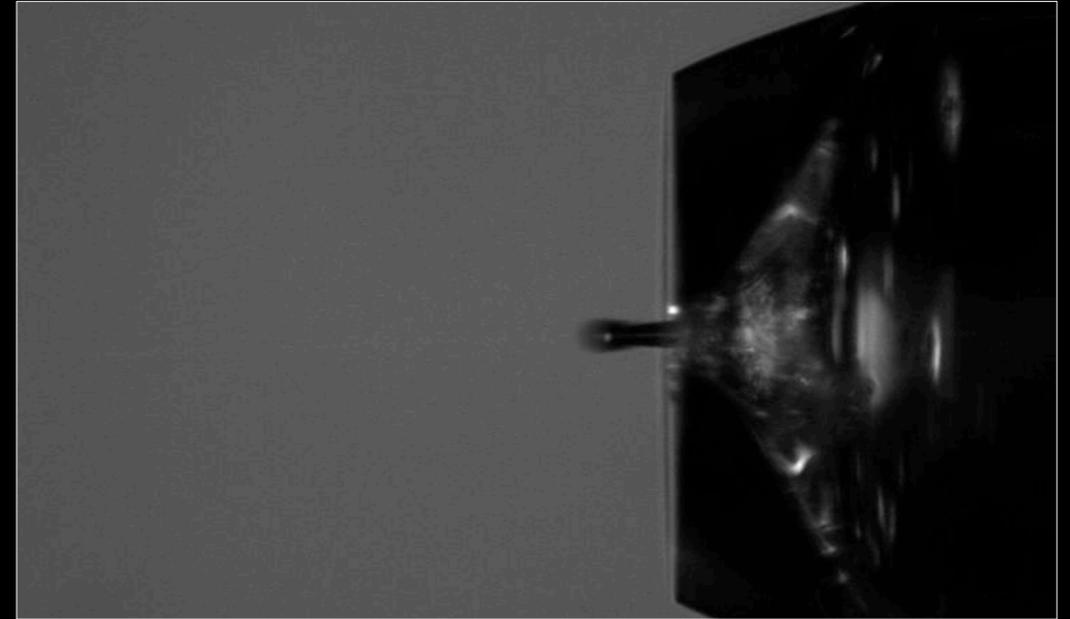
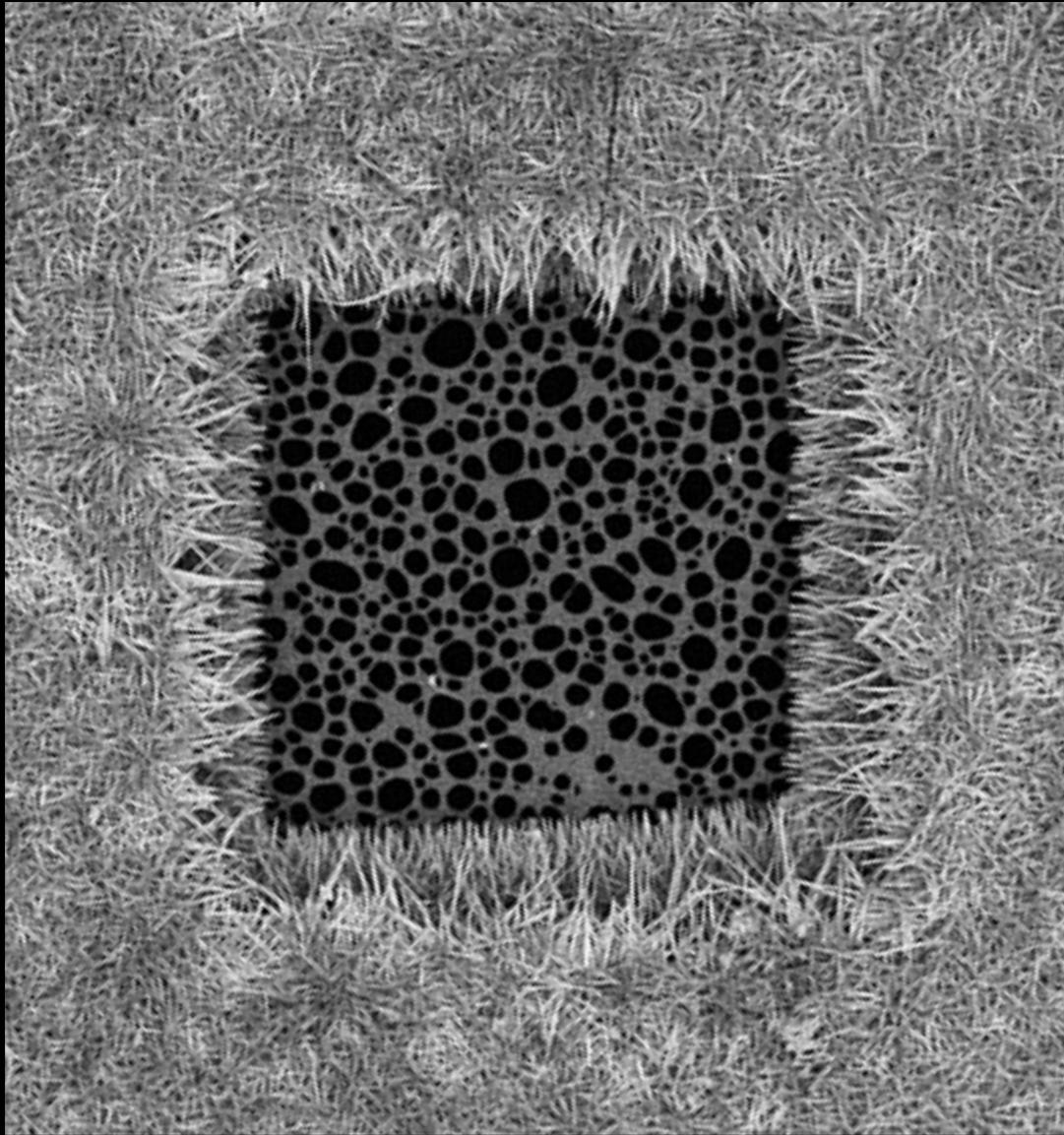


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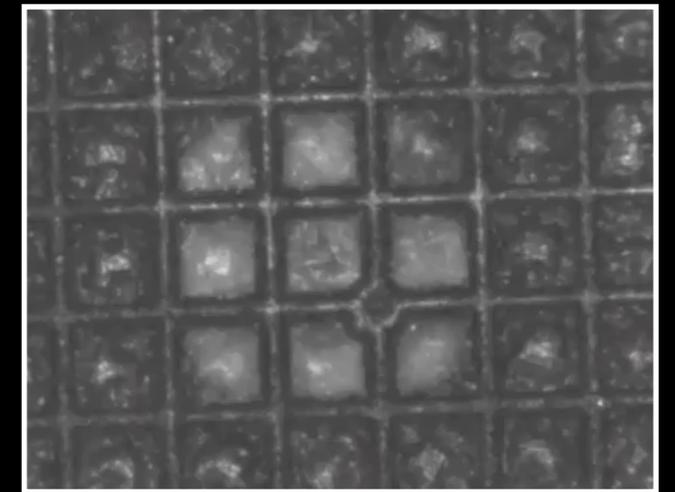
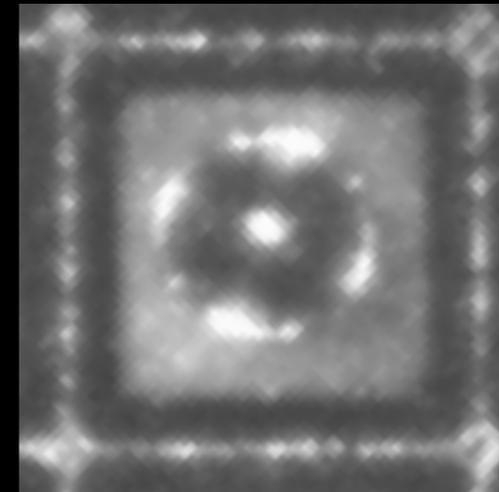
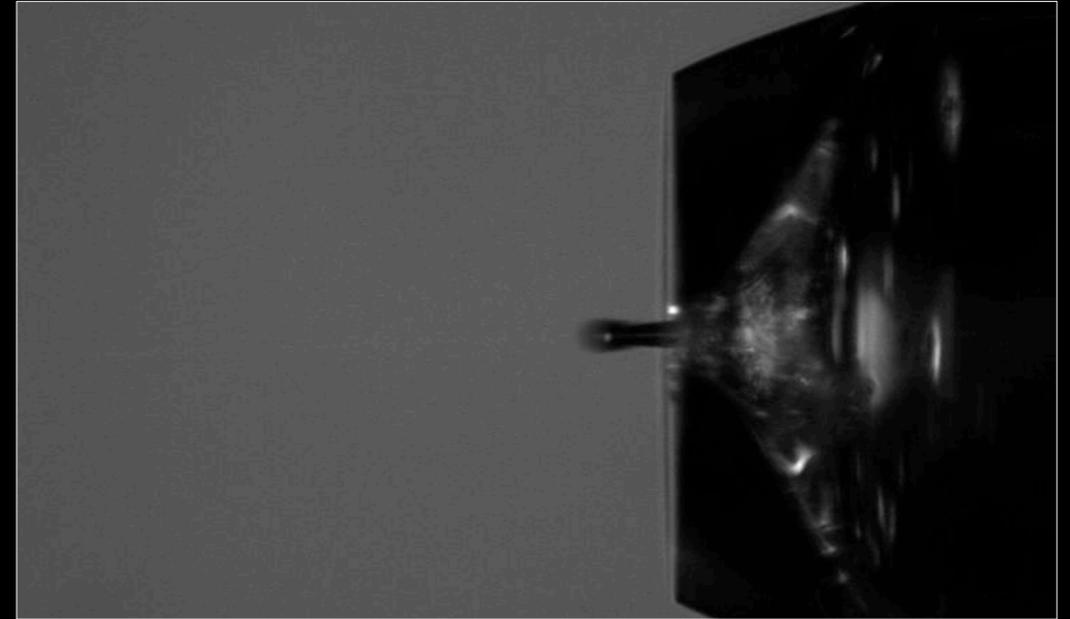
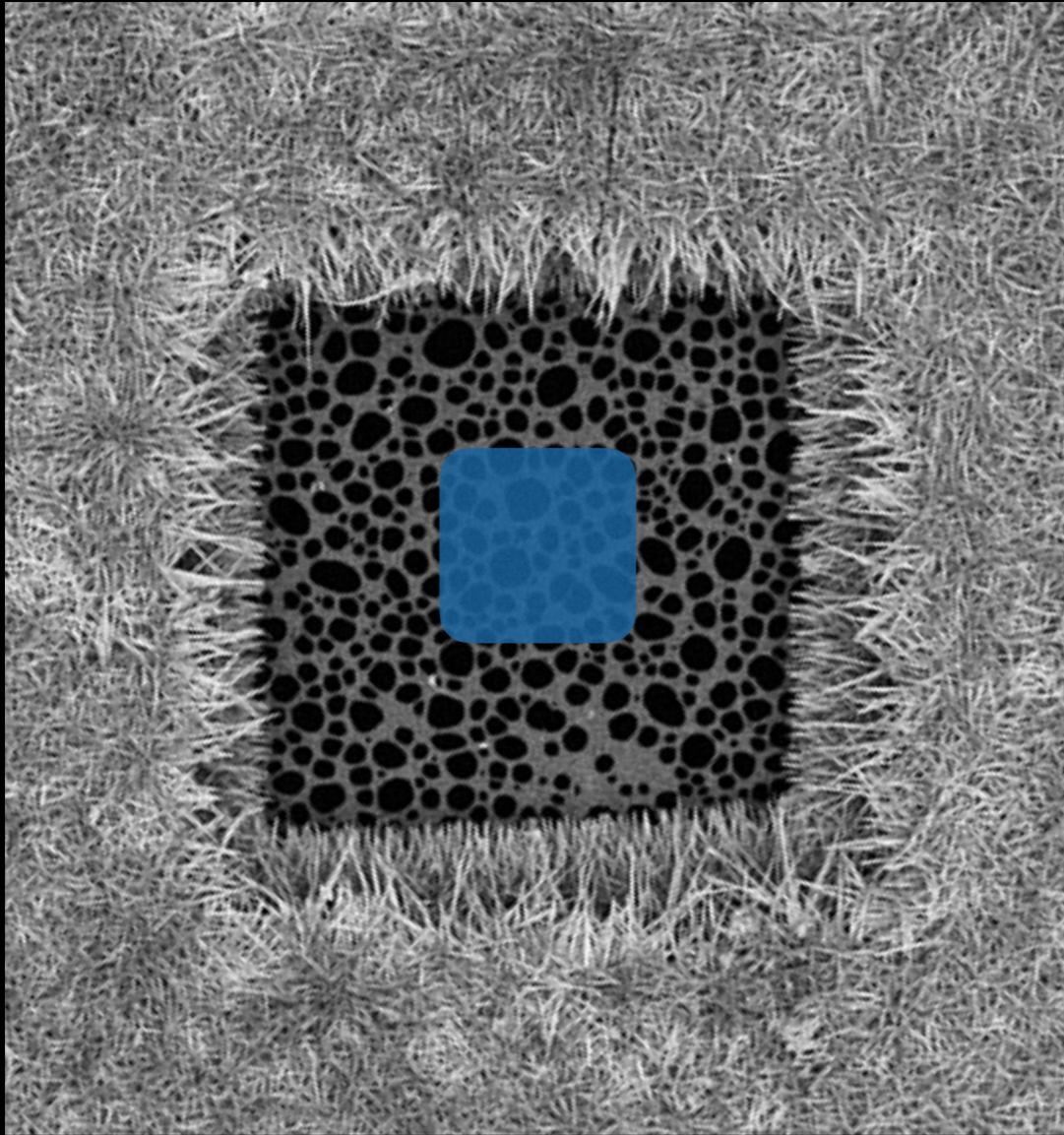
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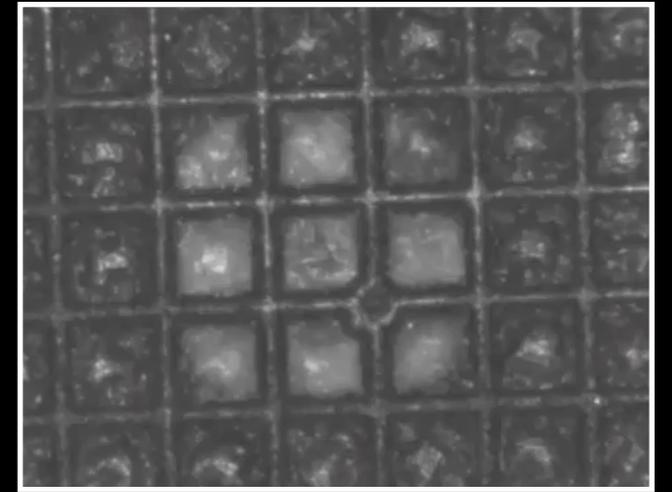
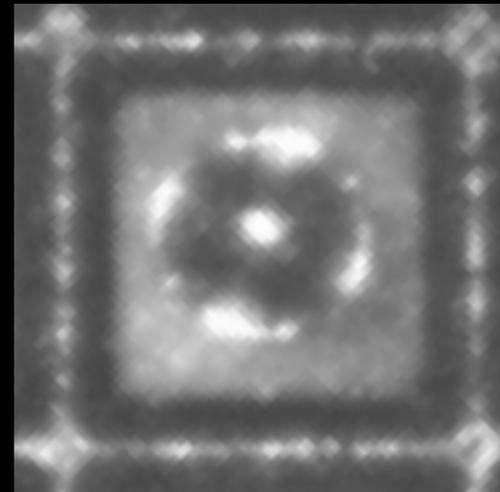
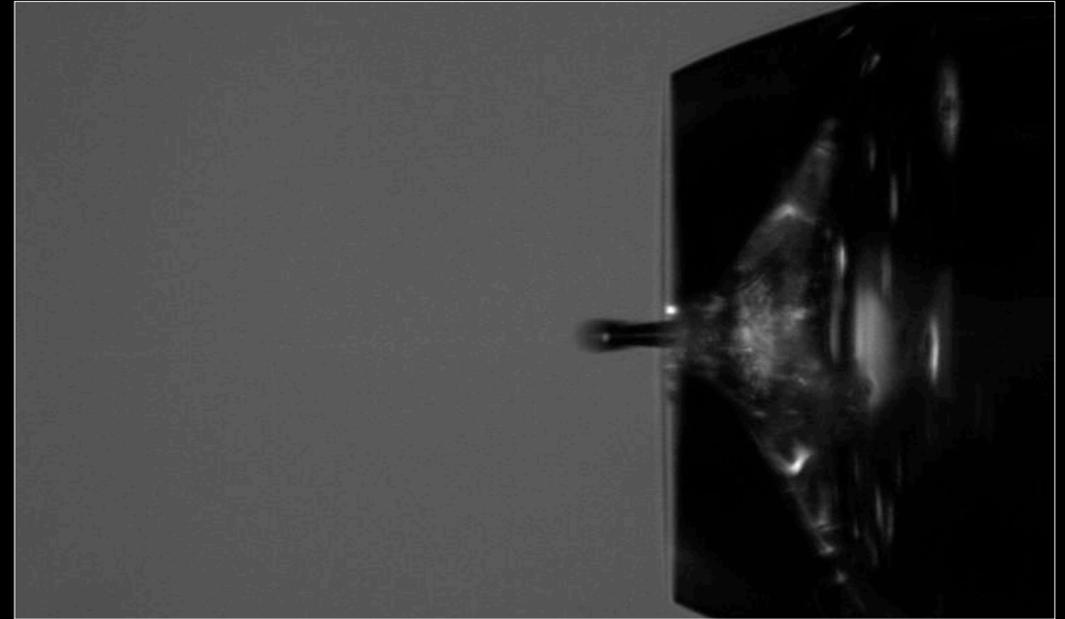
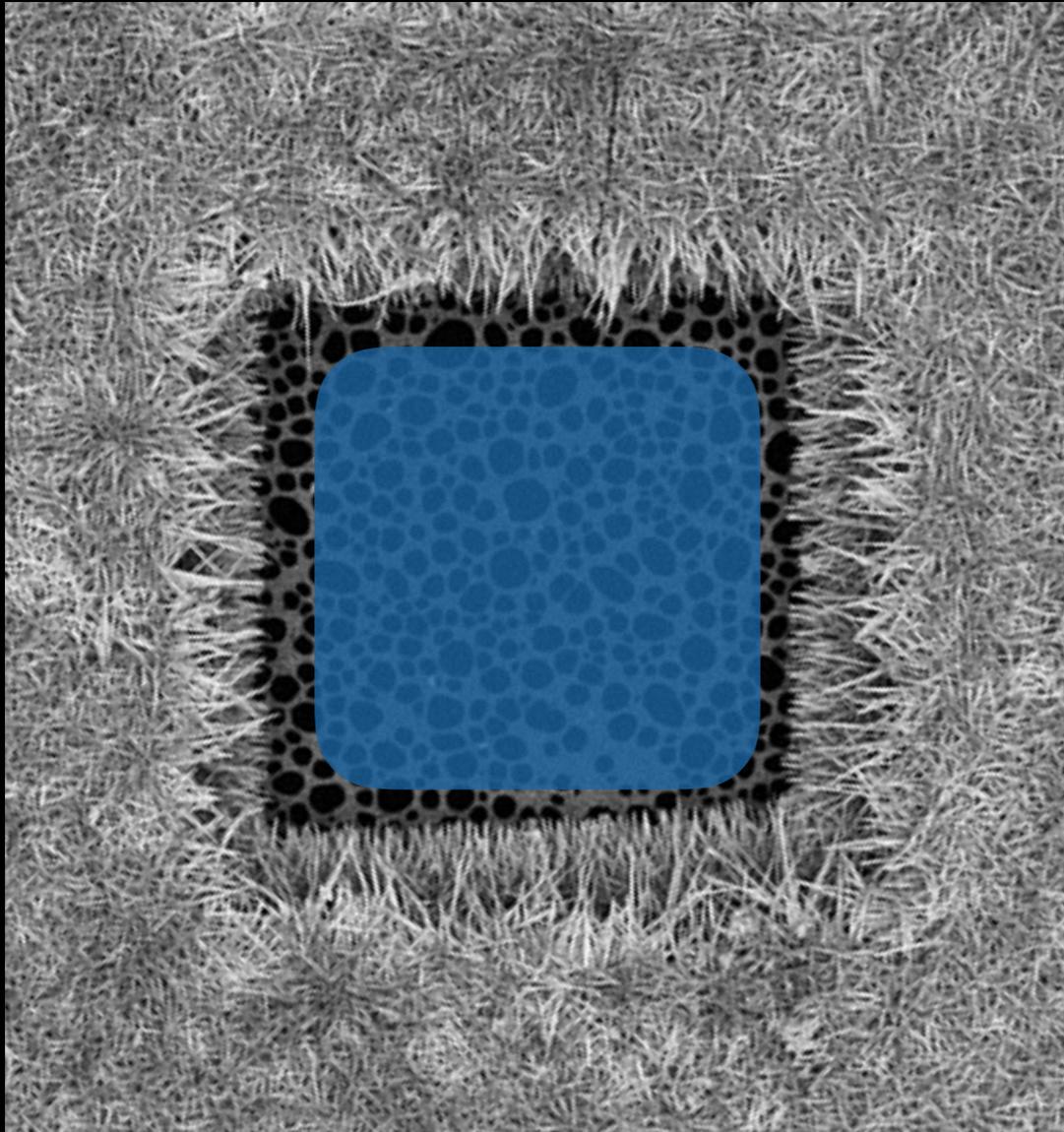
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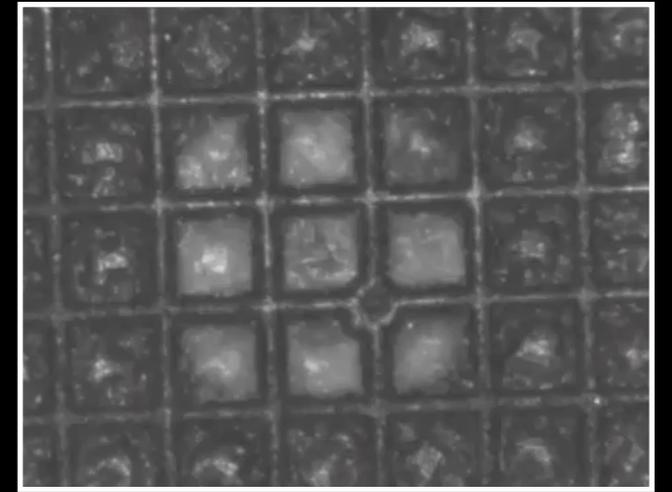
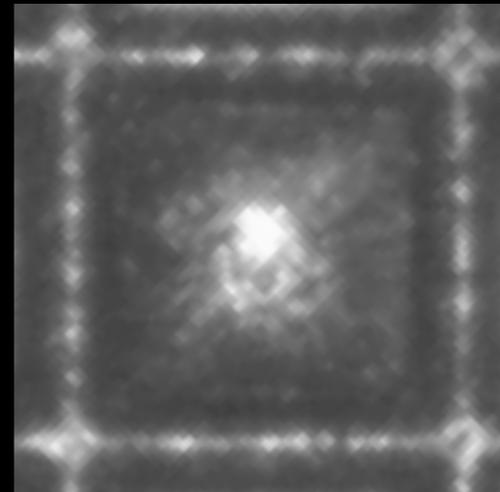
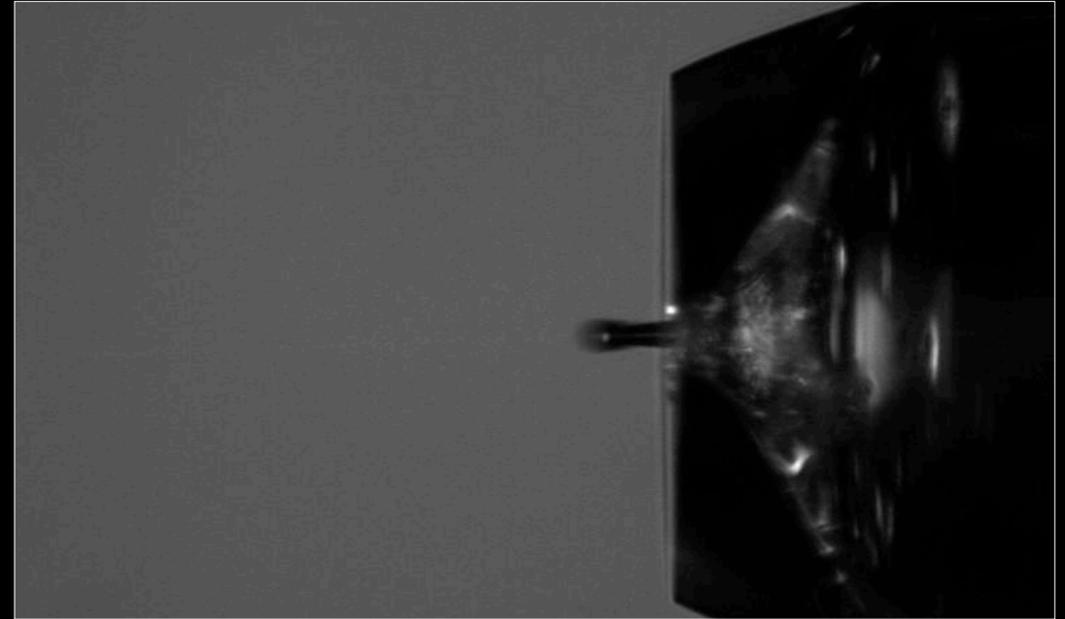
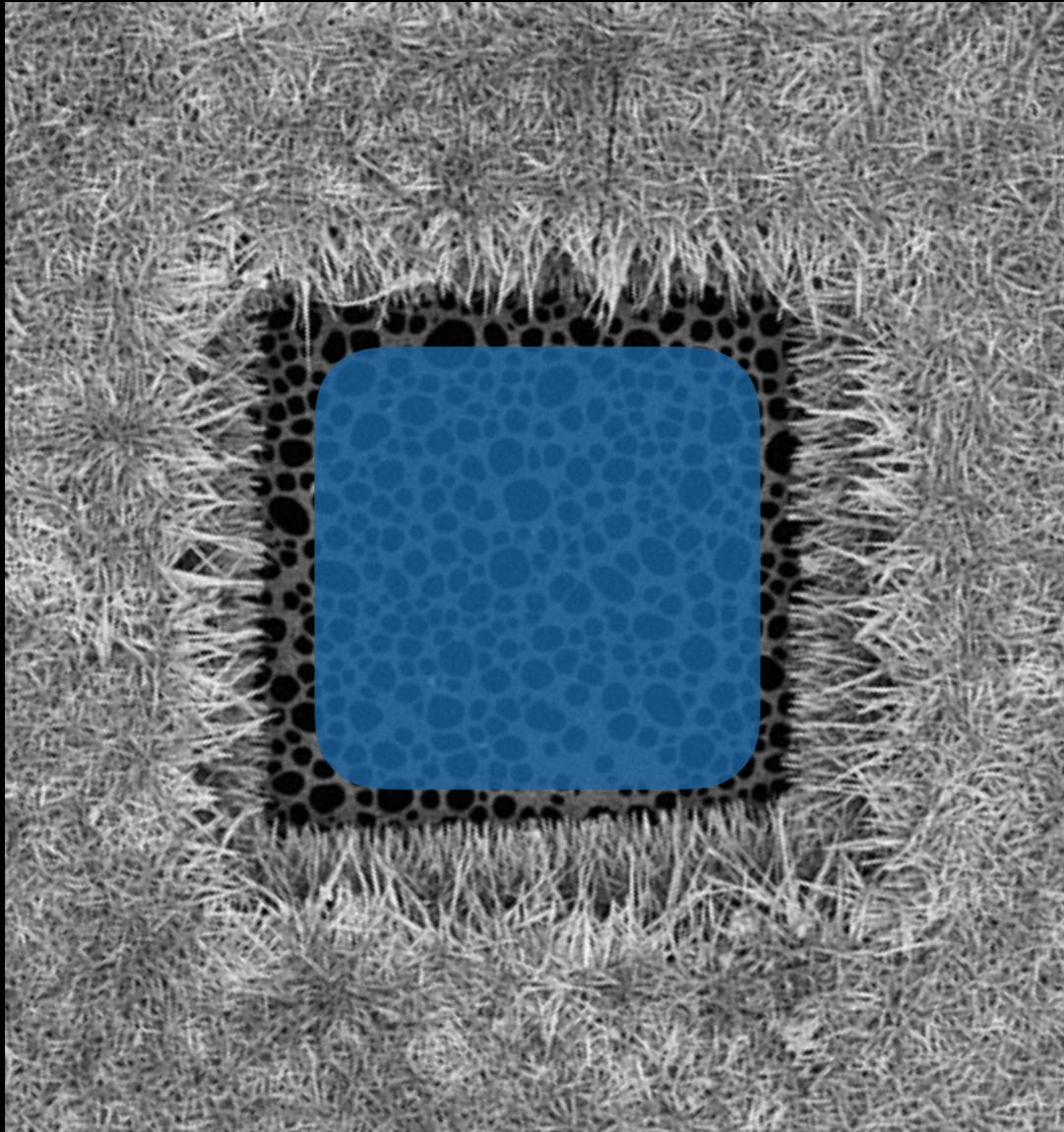
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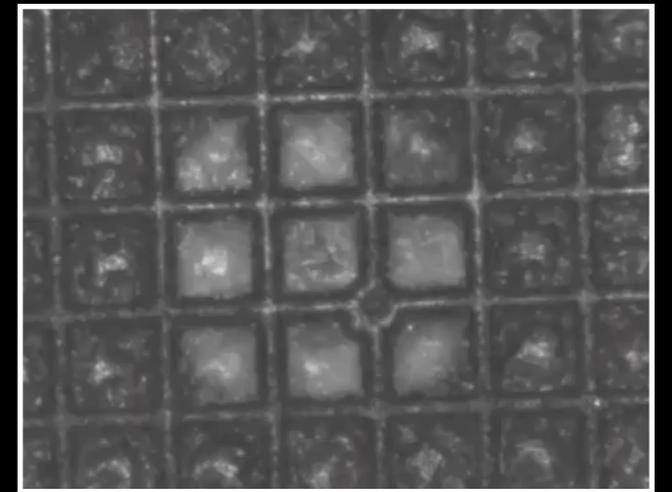
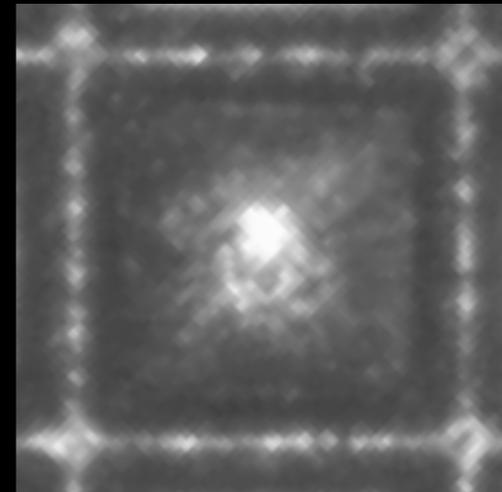
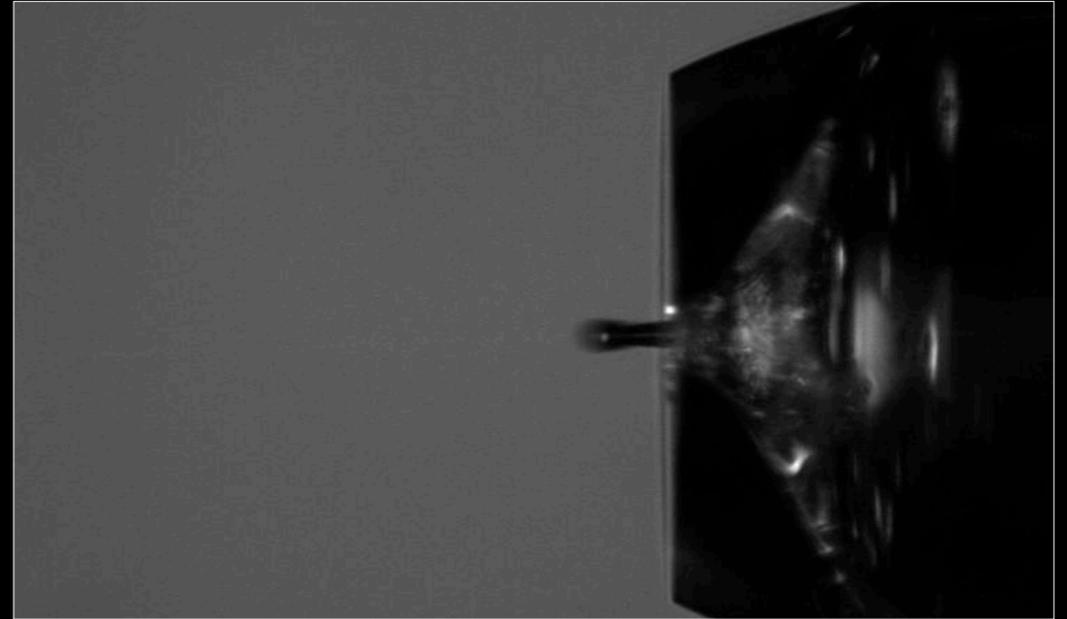
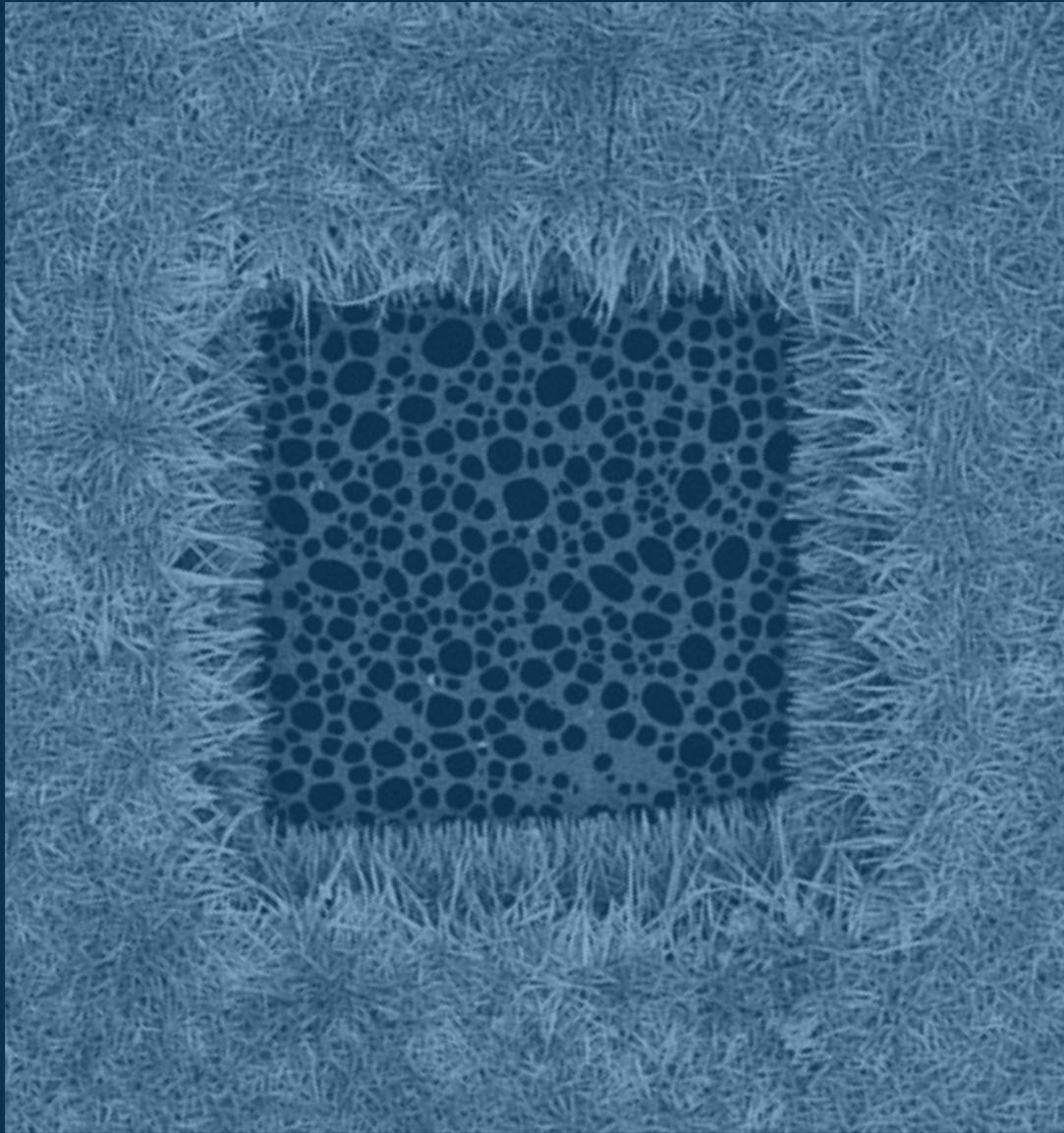
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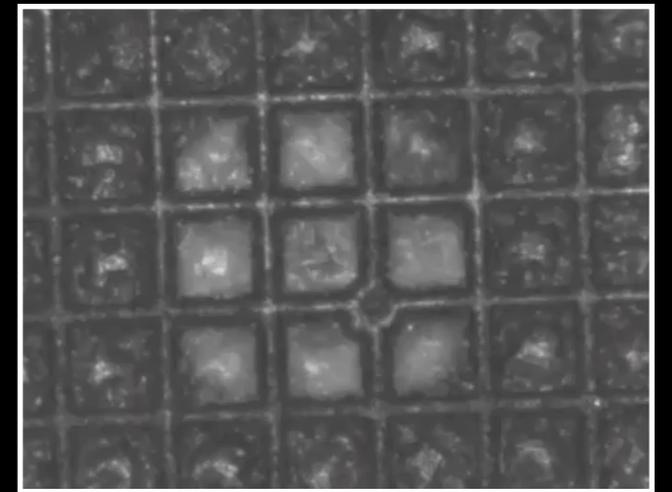
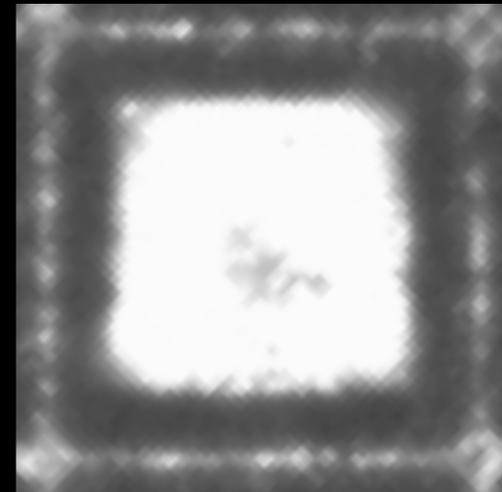
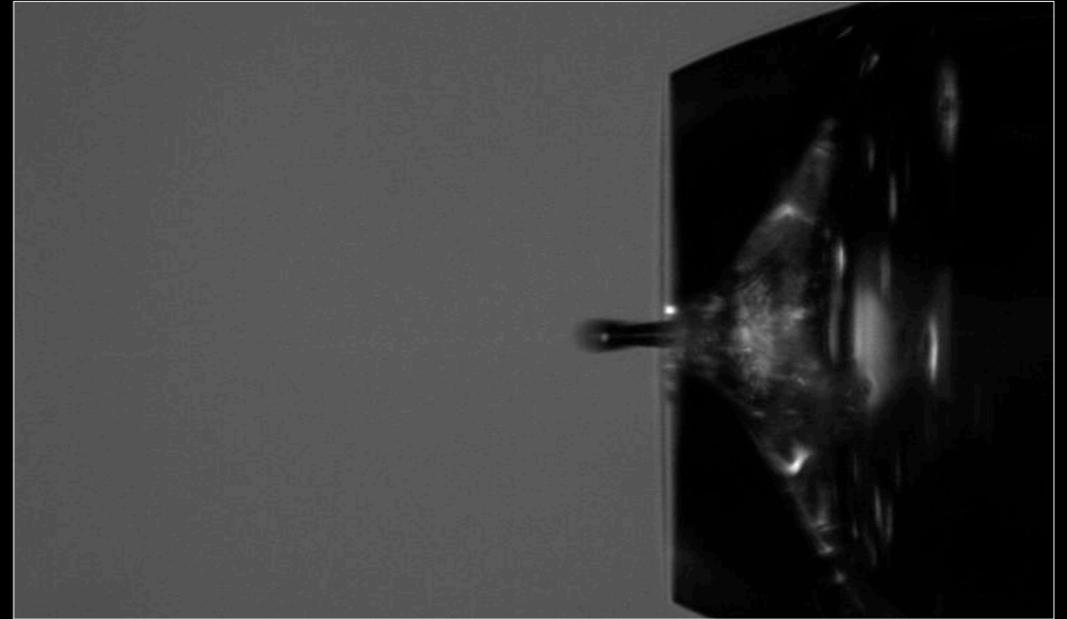
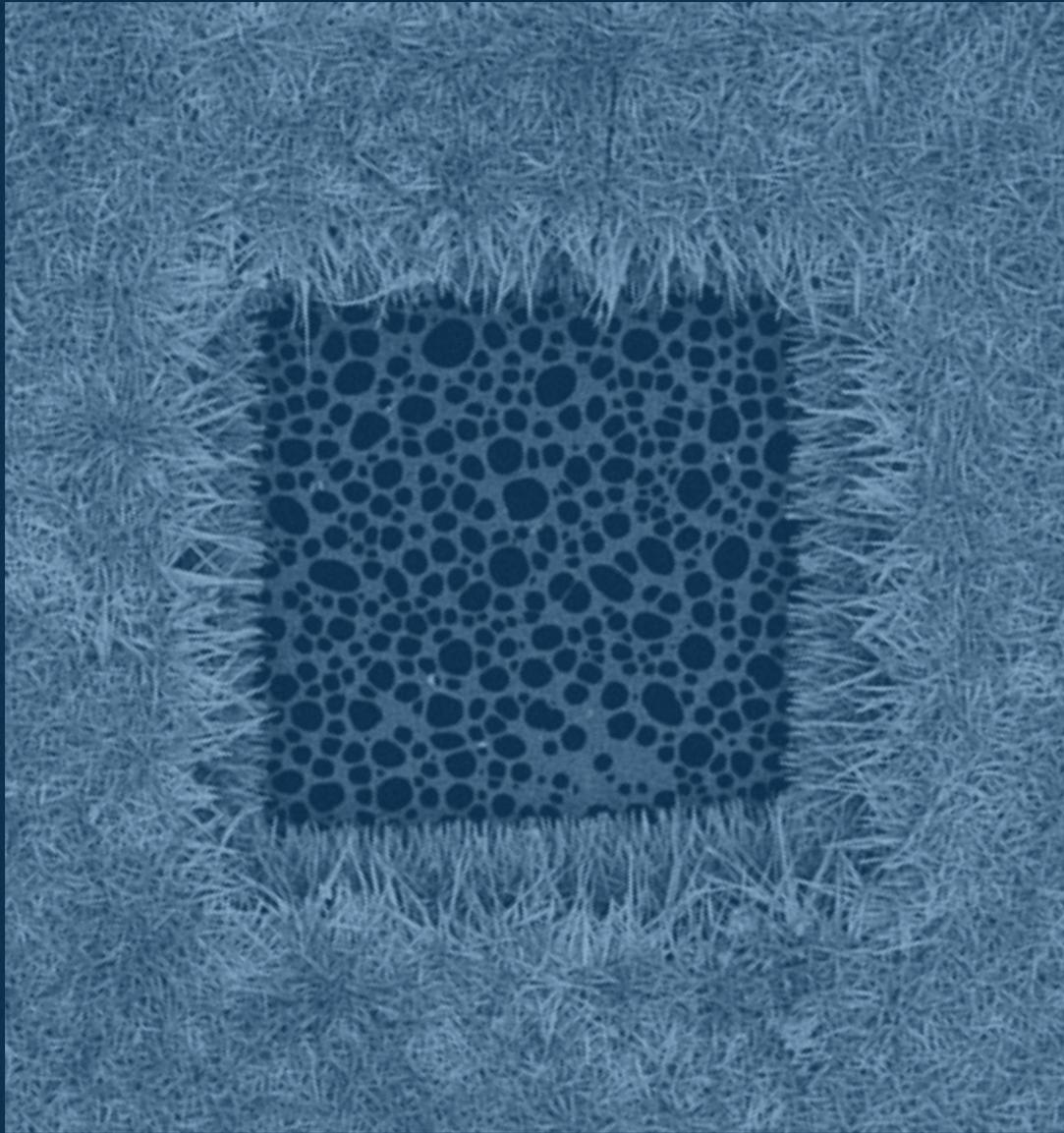
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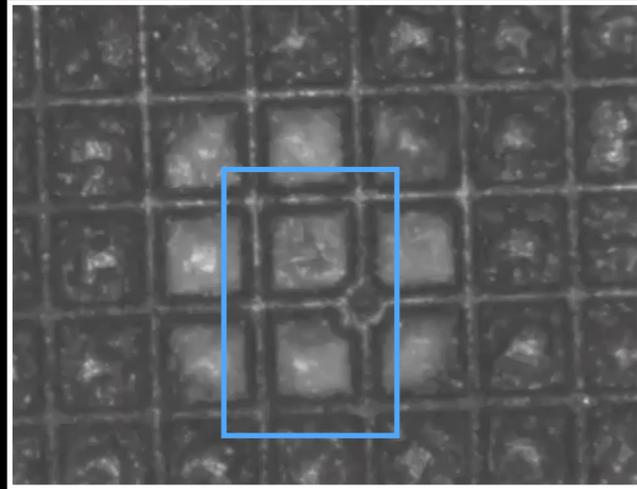
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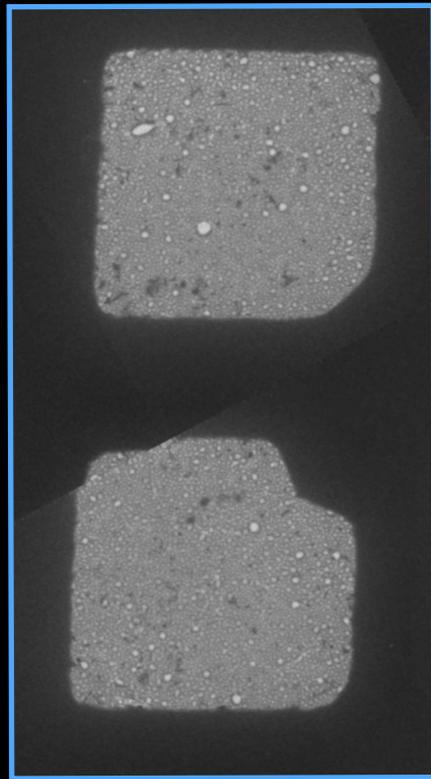
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LM

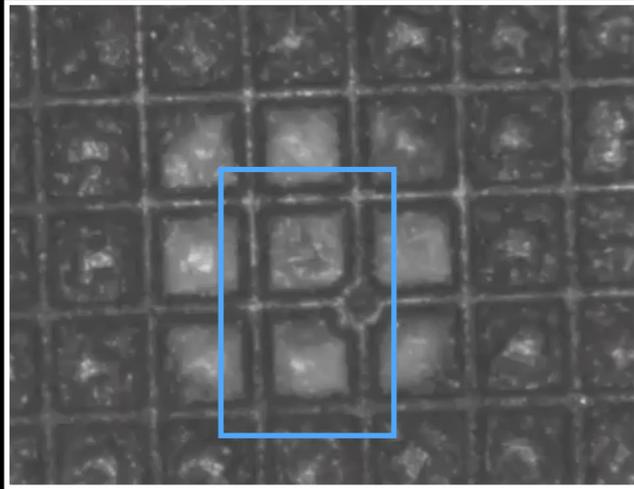


EM

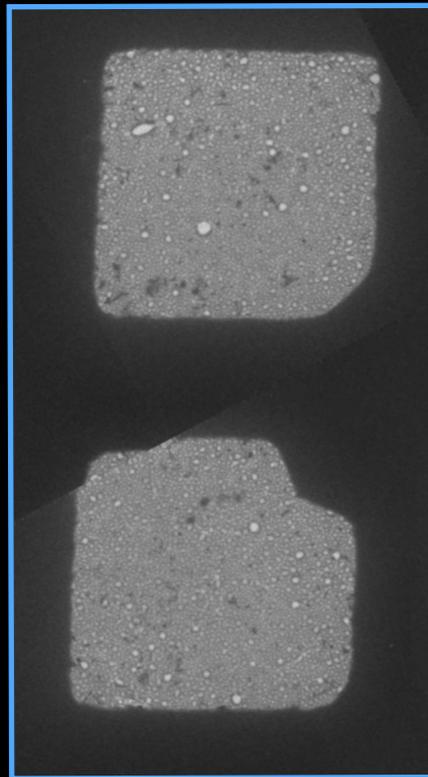


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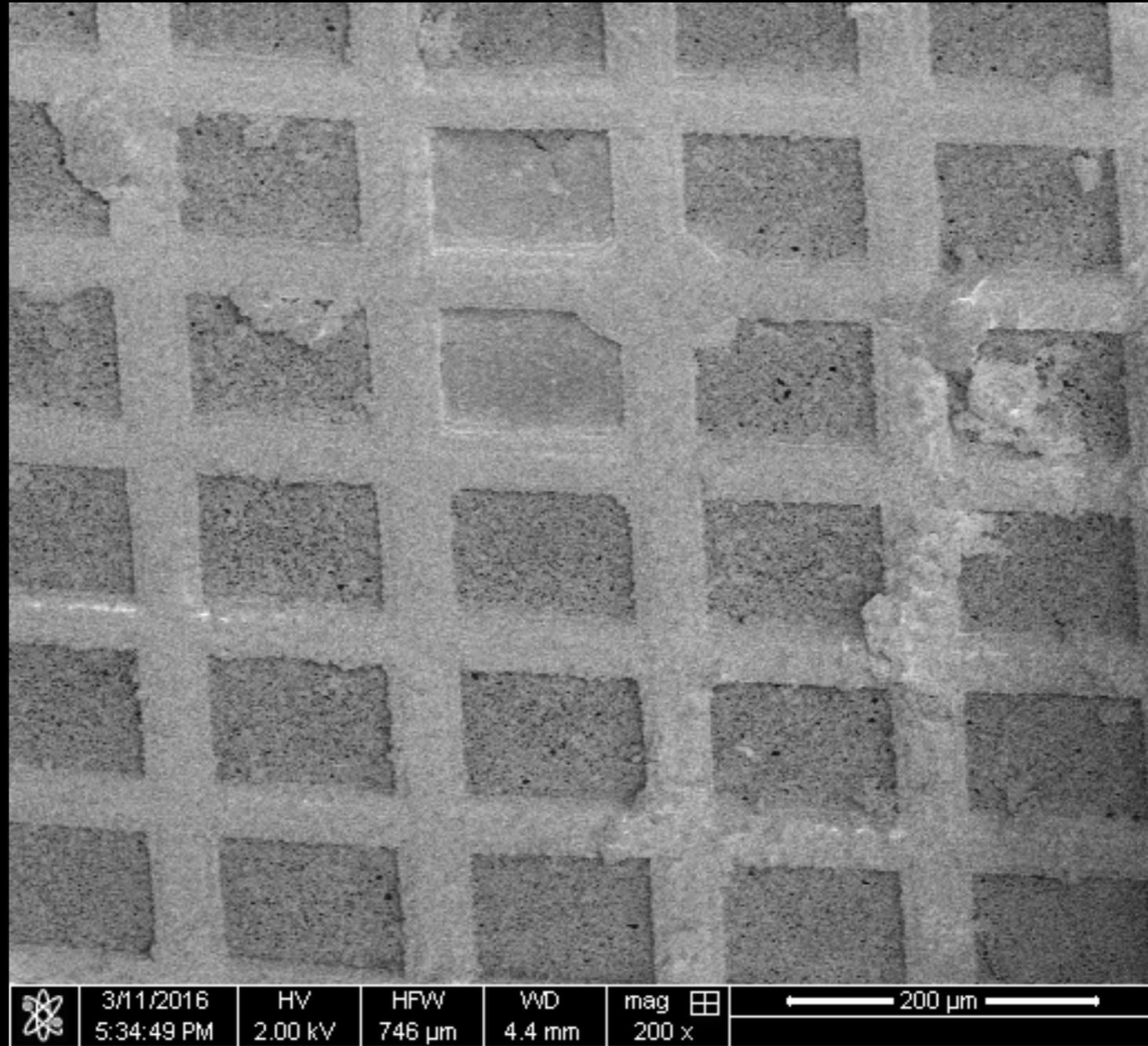
LM



EM

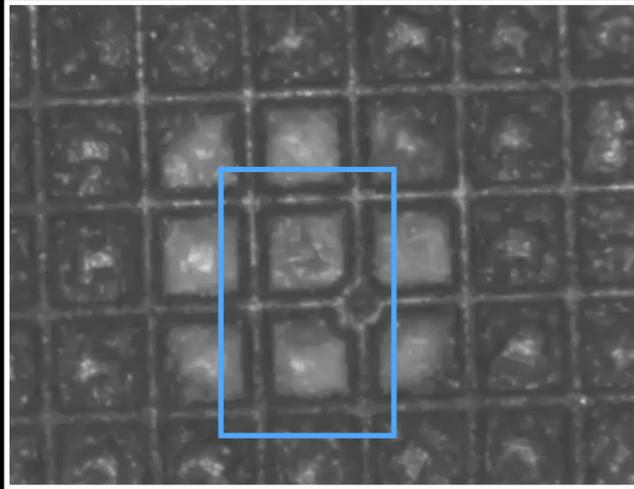


CryoSEM

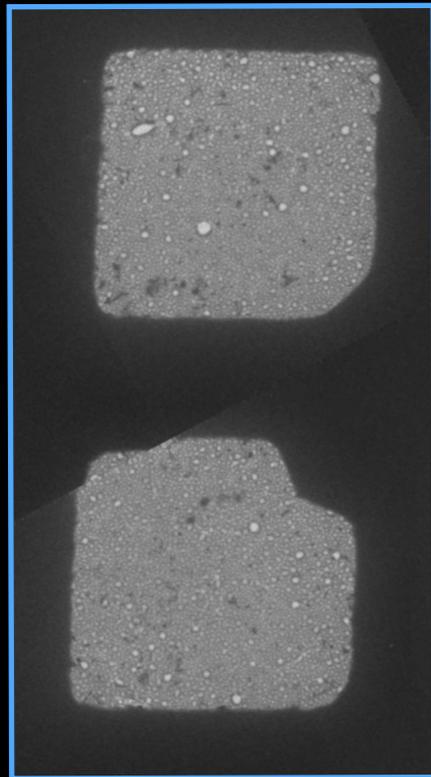


# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

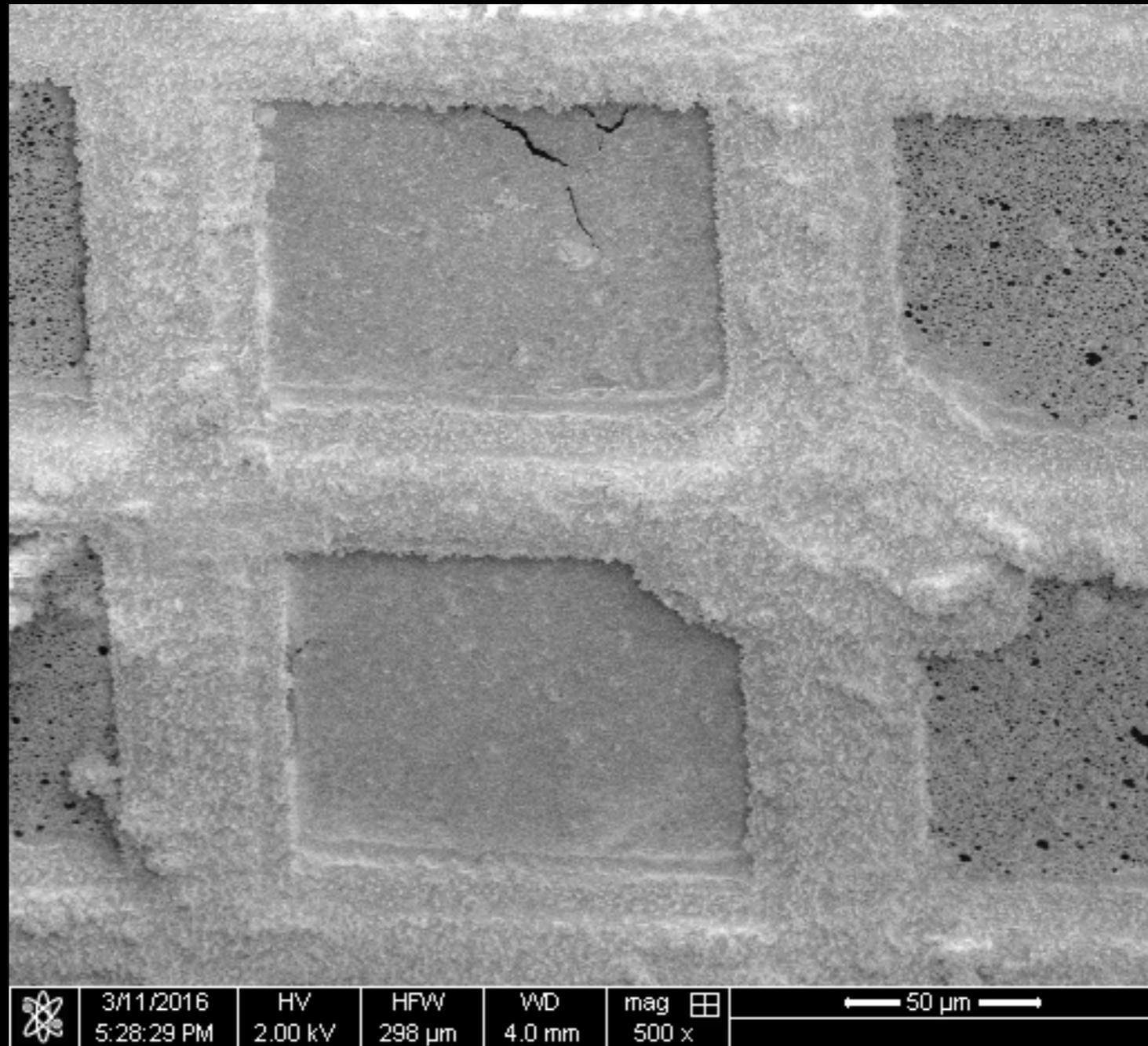
LM



EM

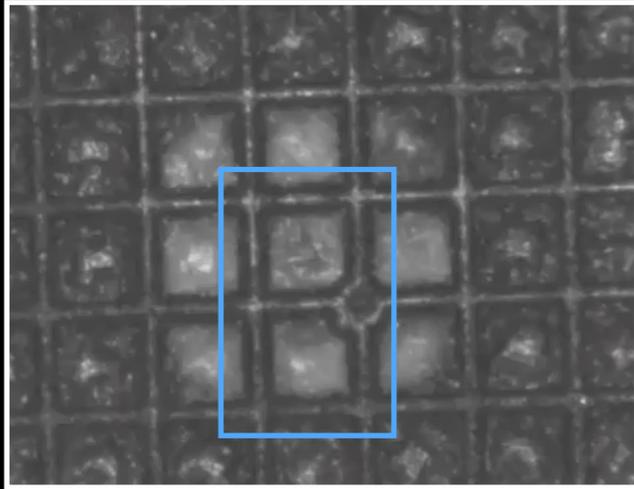


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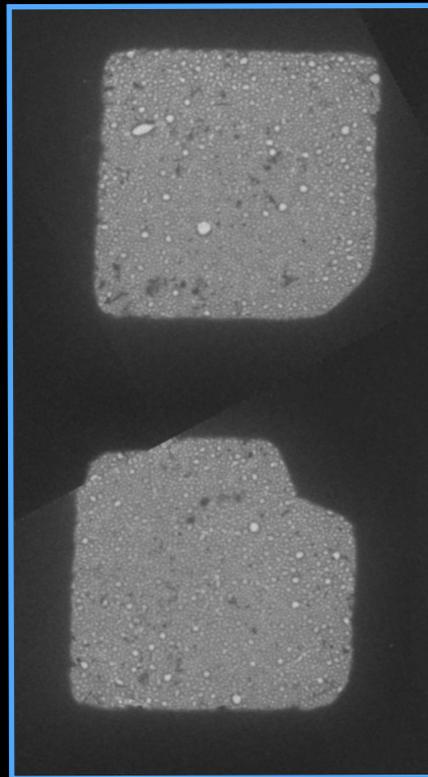


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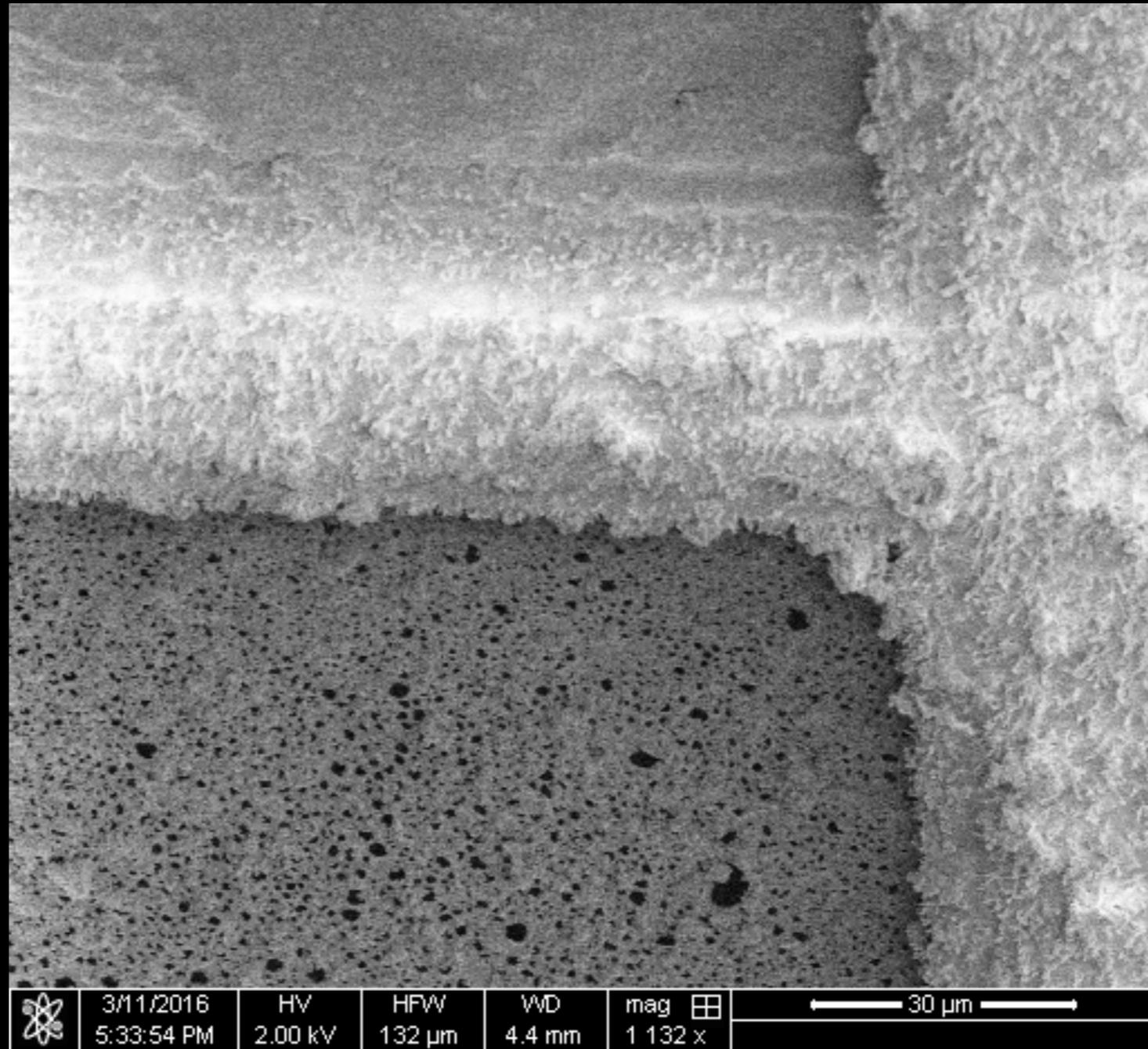
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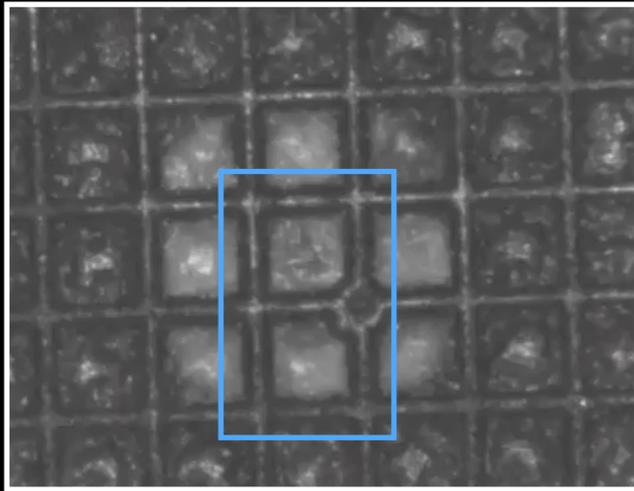


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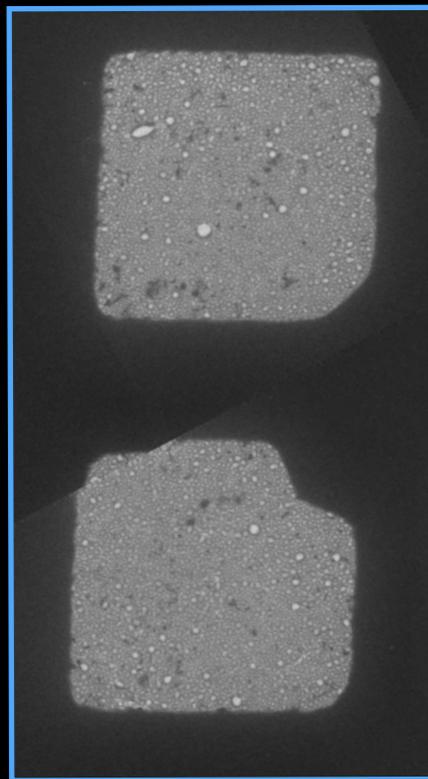


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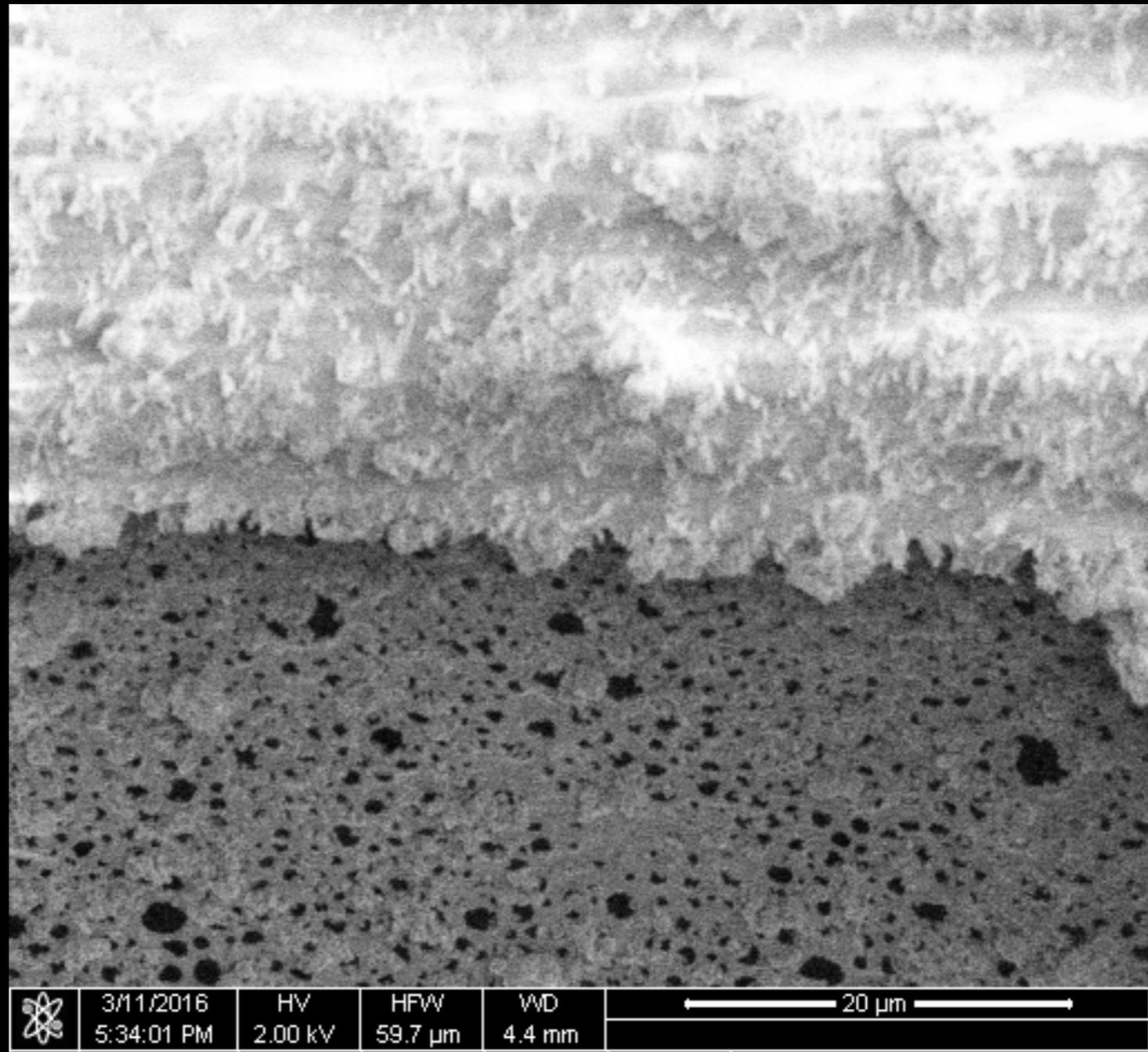
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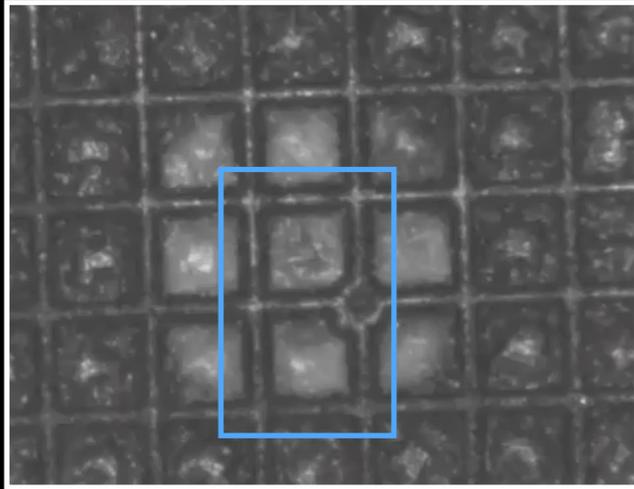


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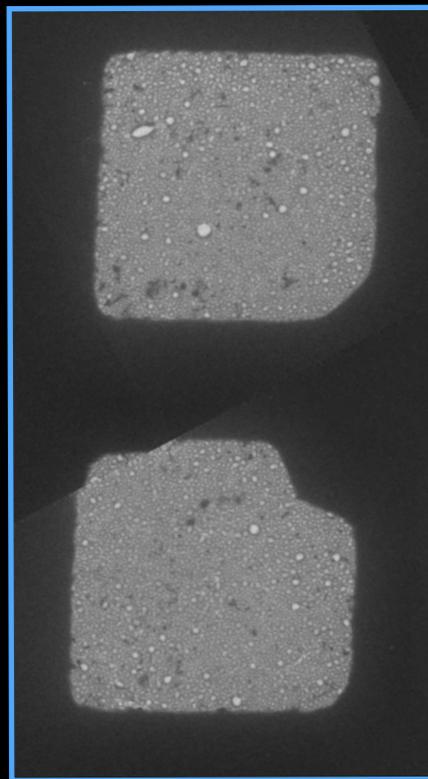


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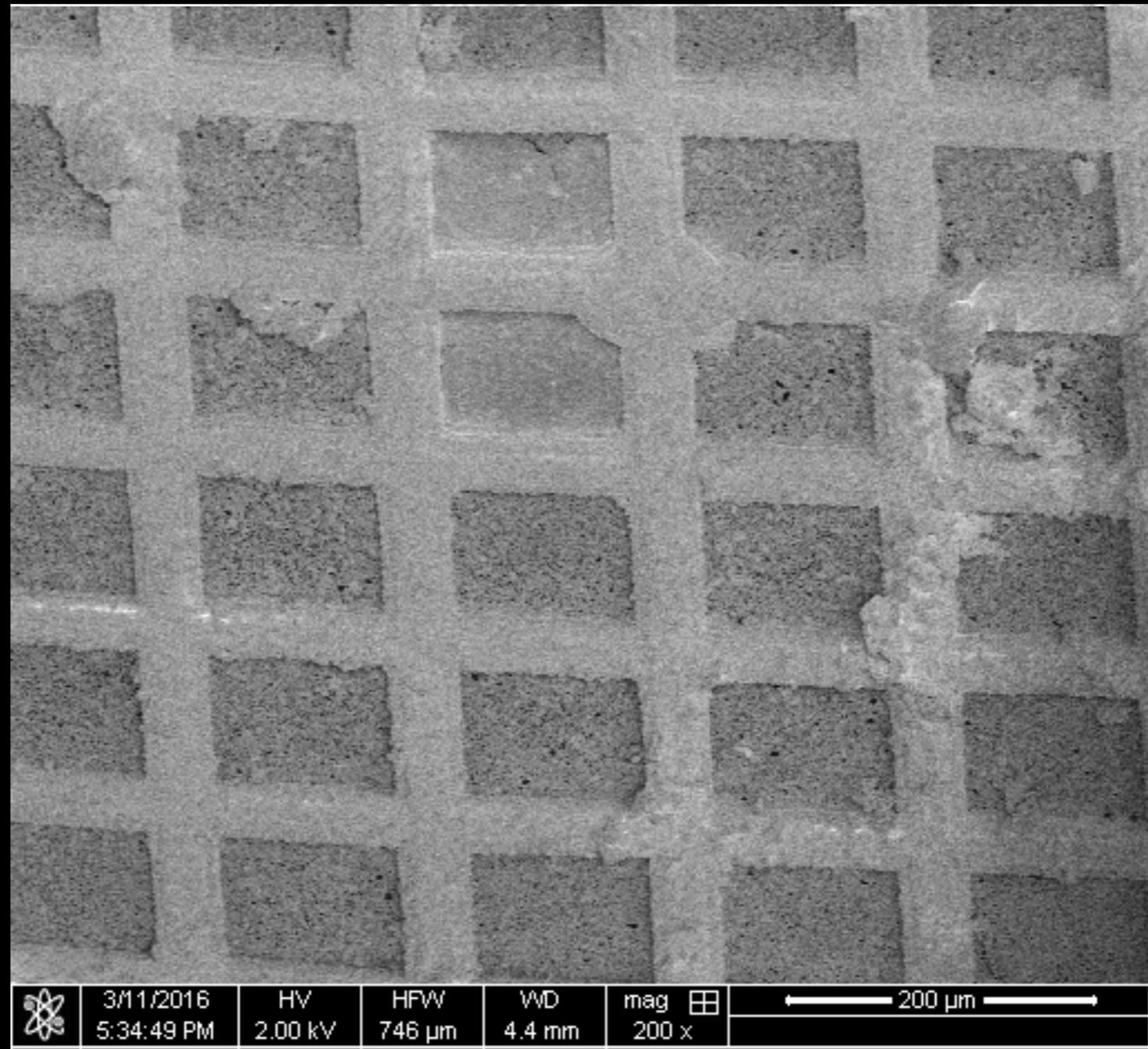
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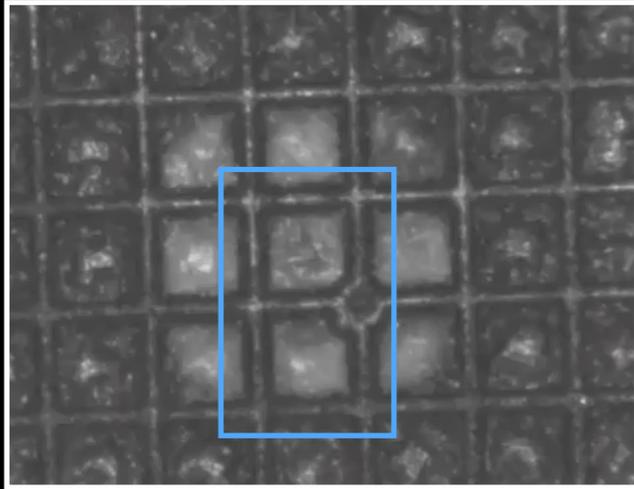


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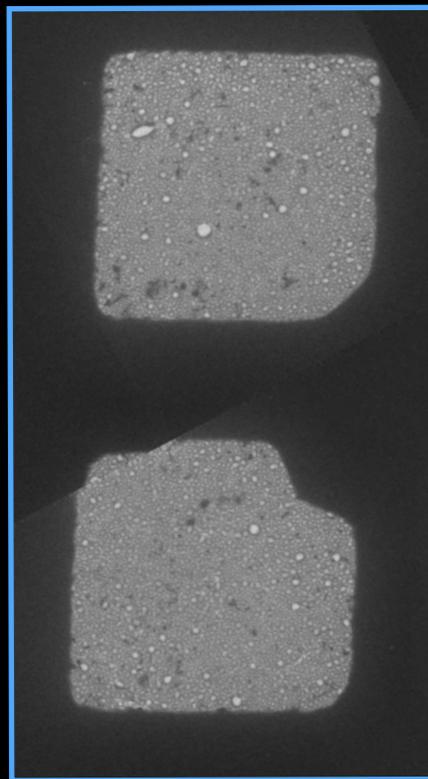


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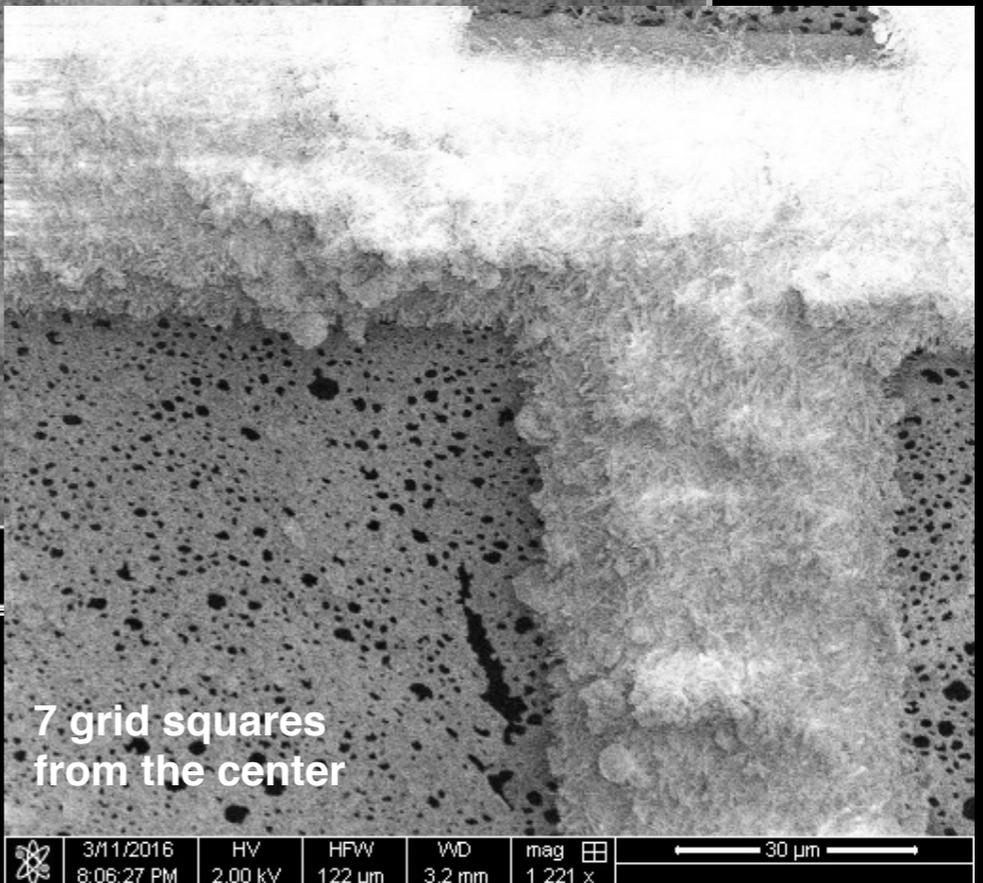
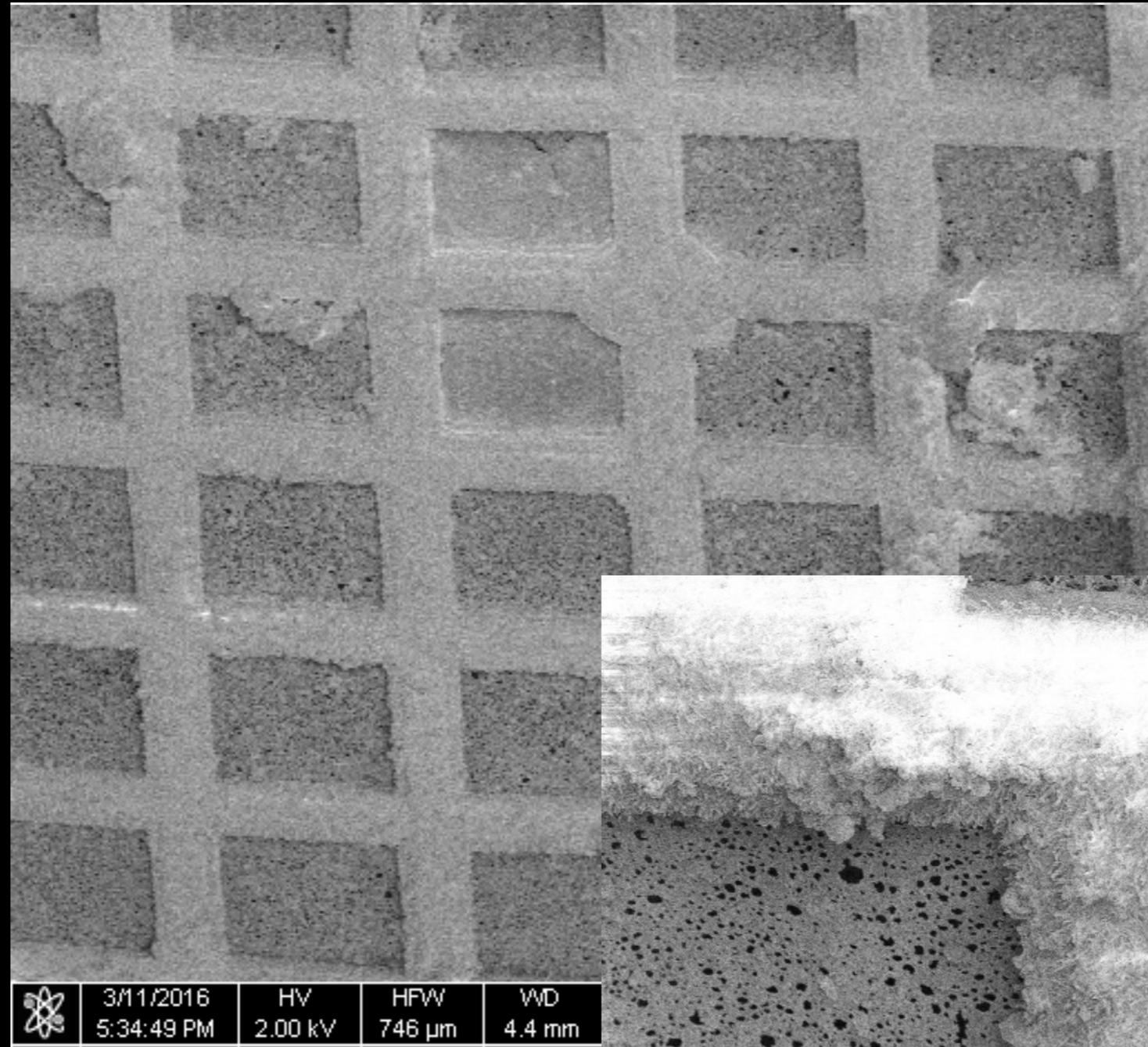
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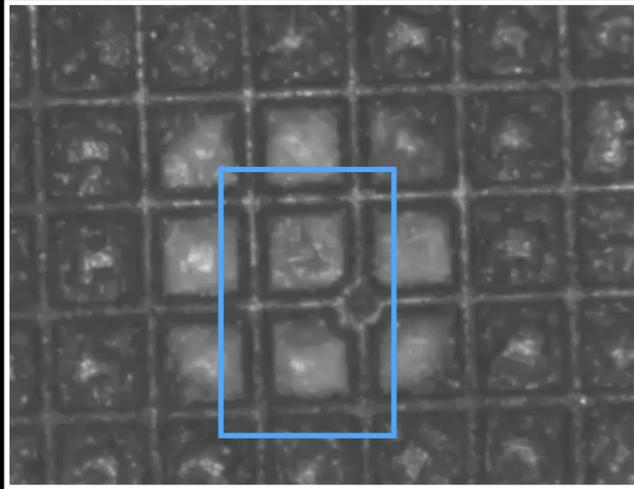


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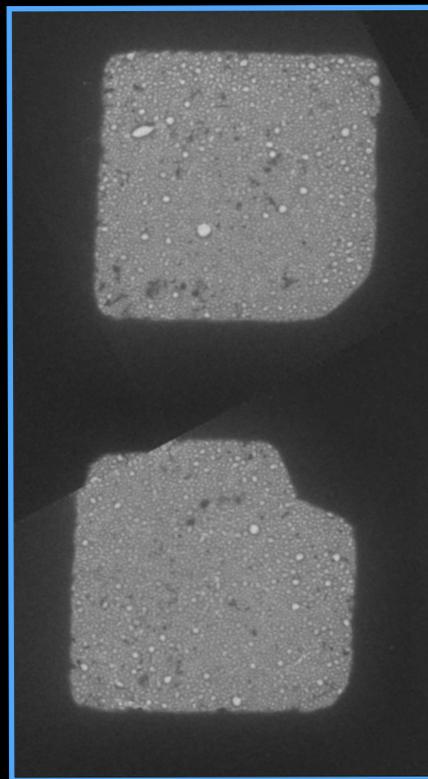


# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods

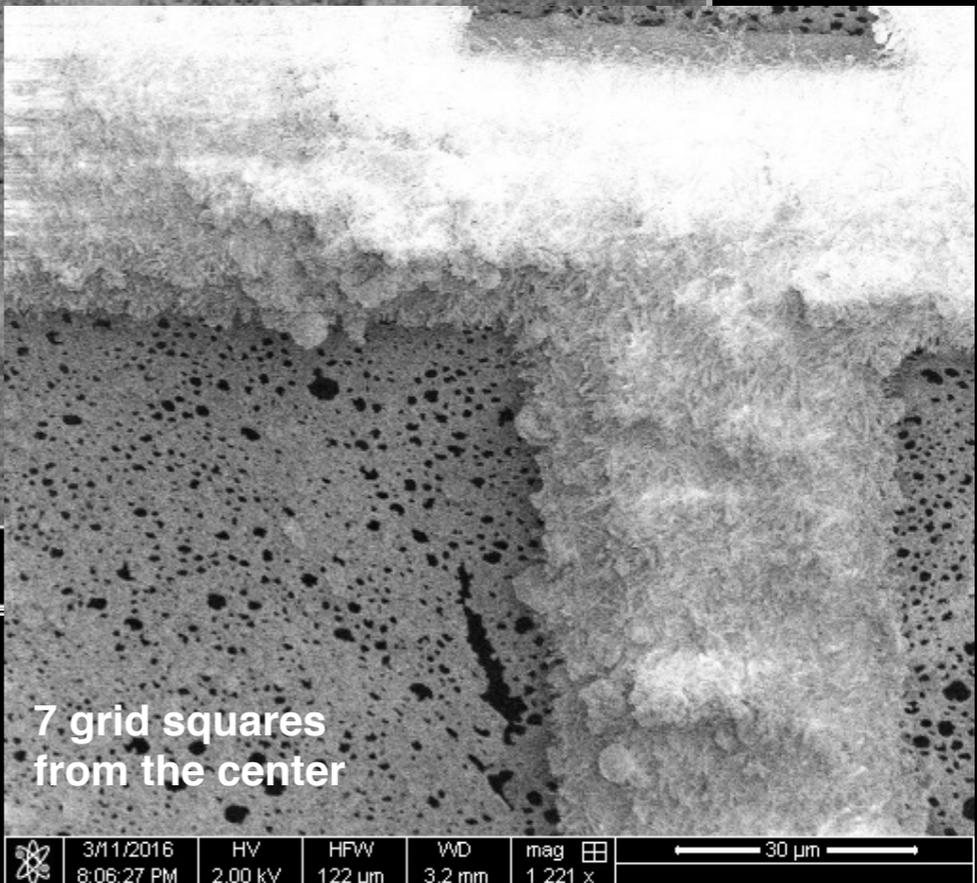
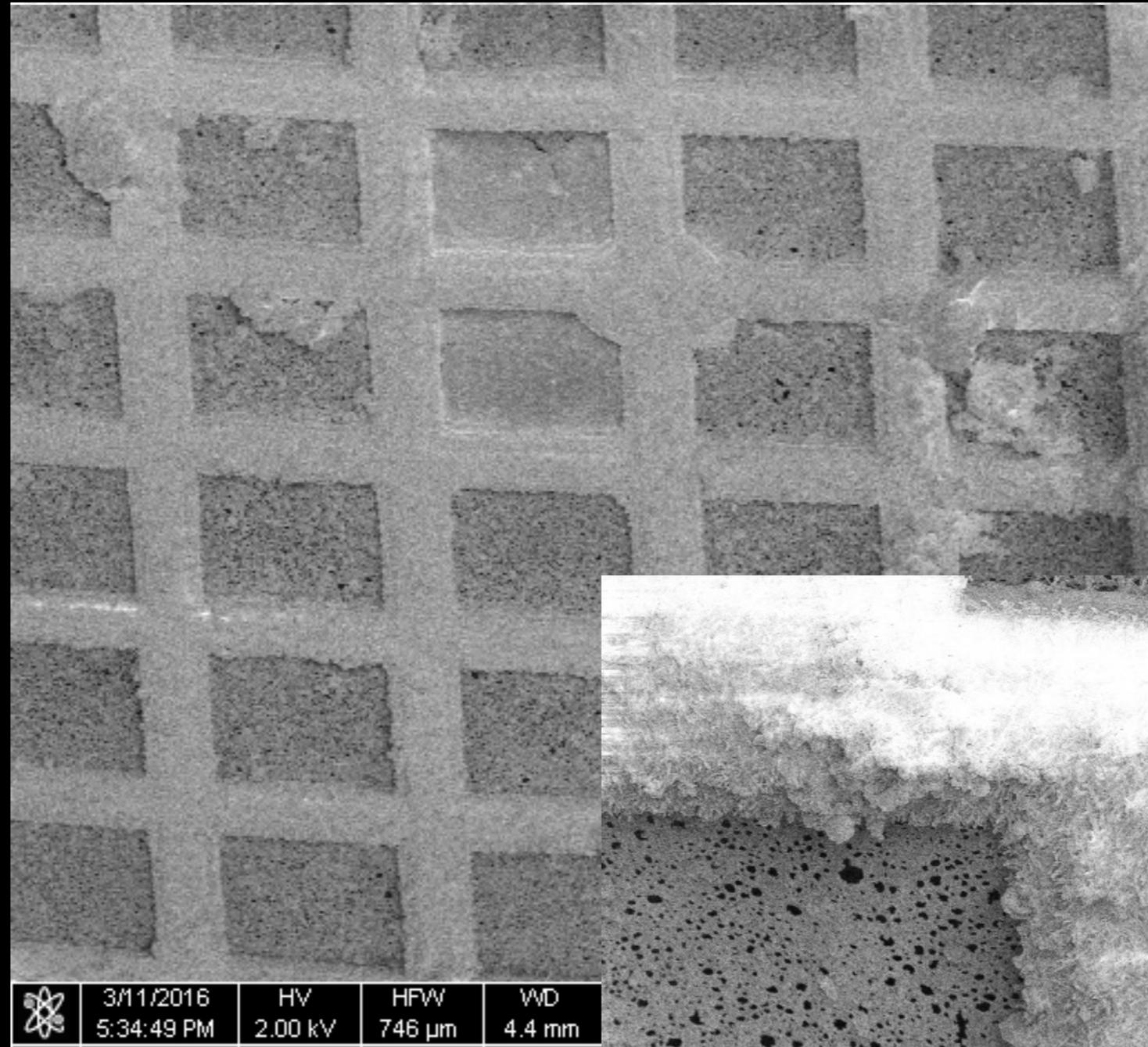
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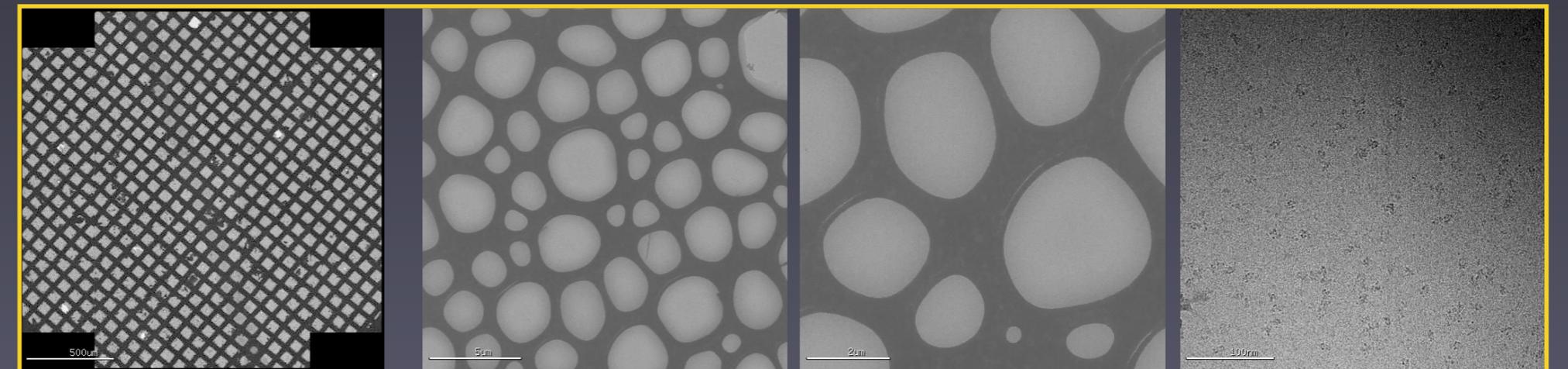
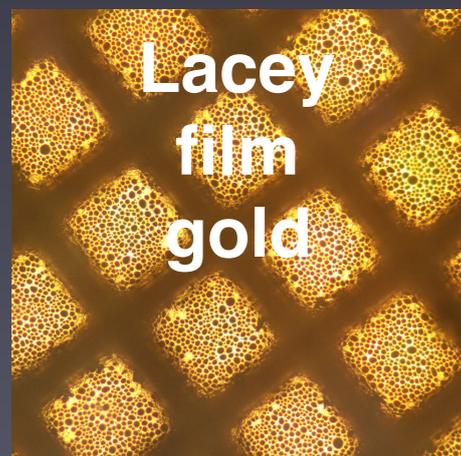
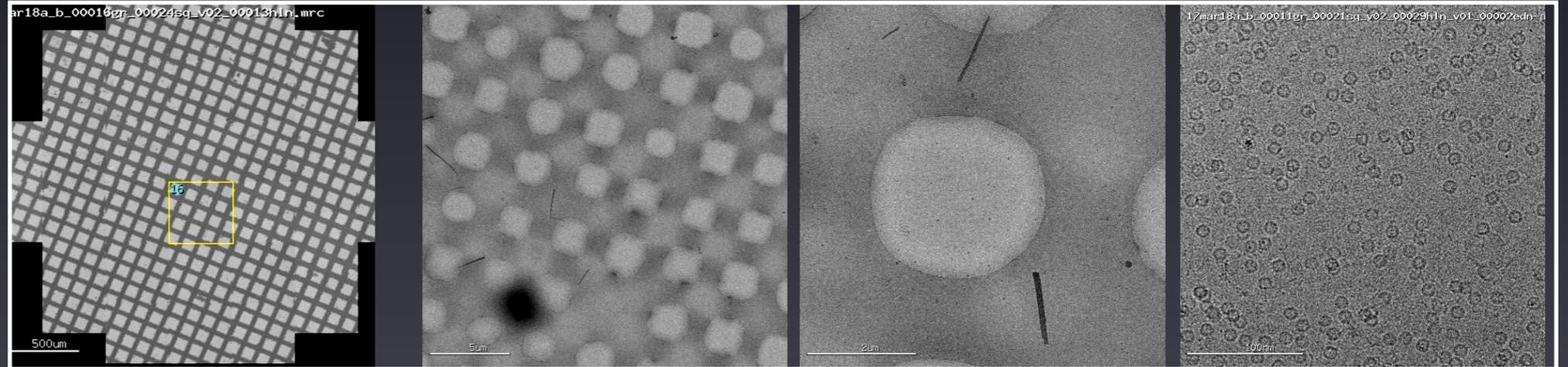
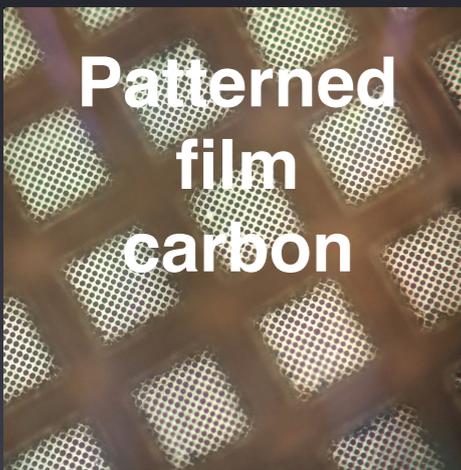
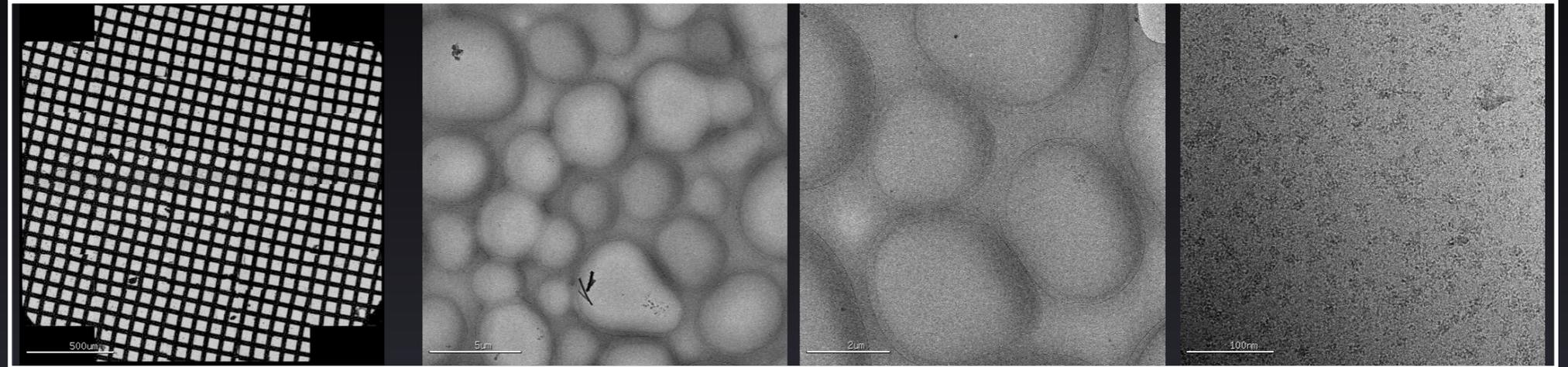
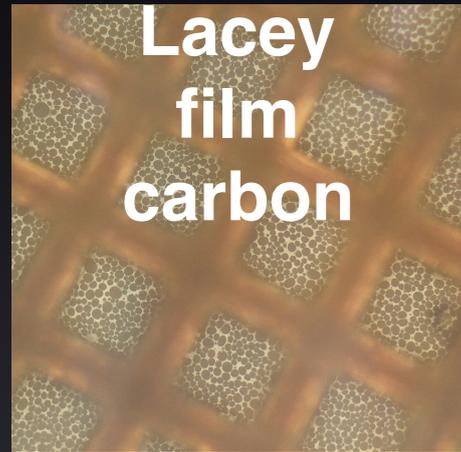
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CryoSEM



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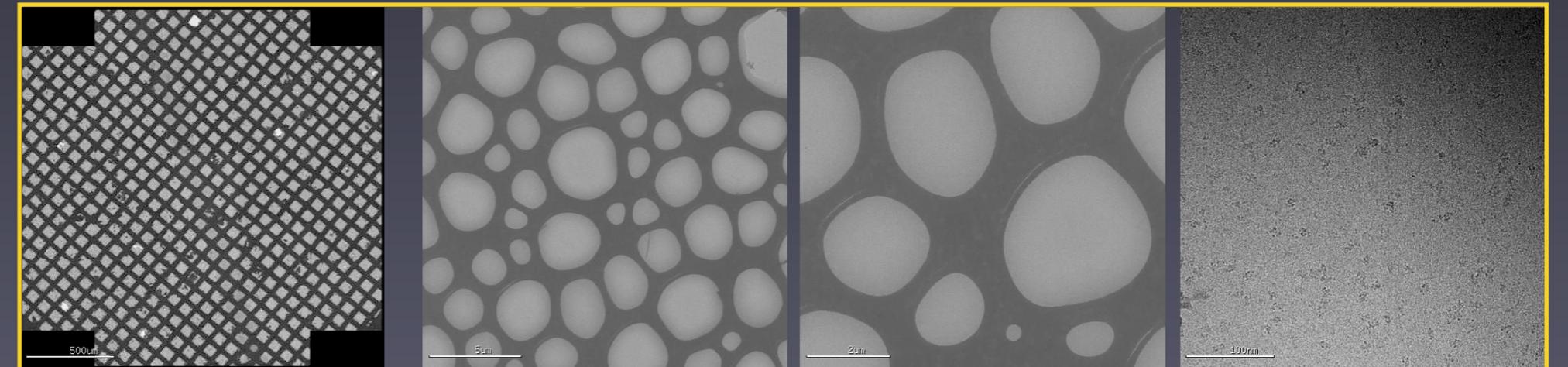
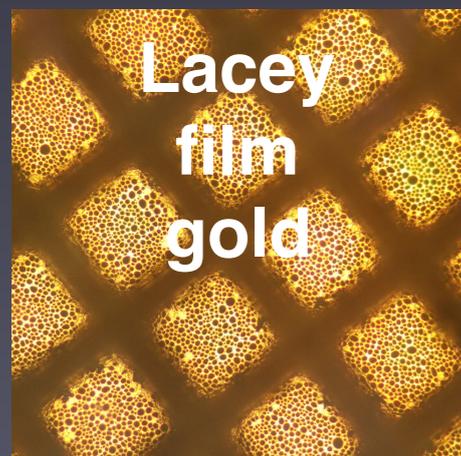
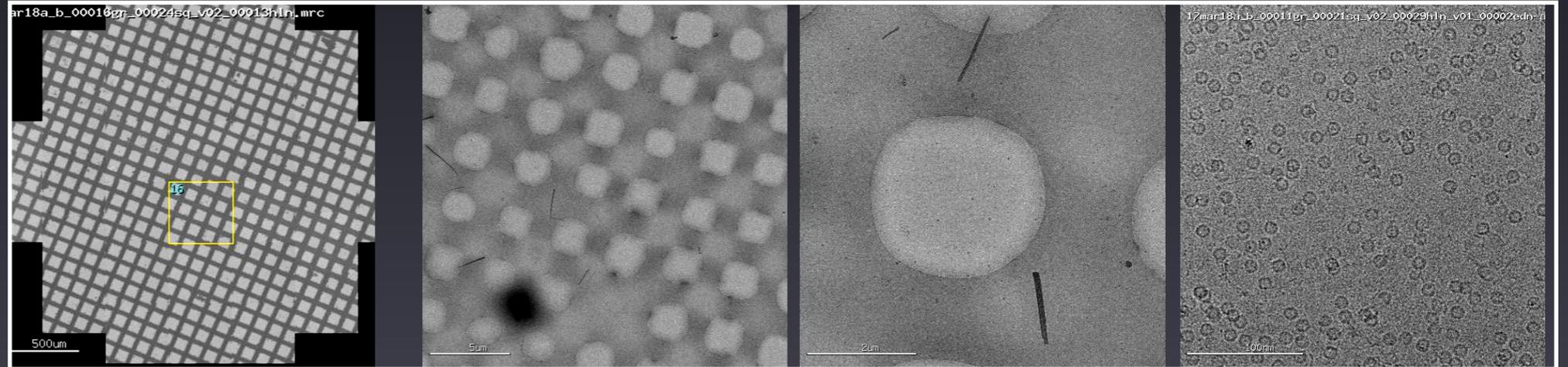
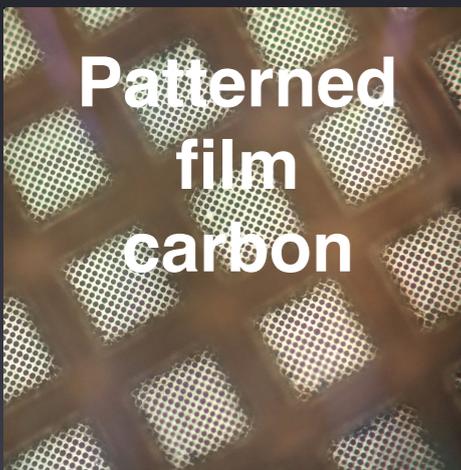
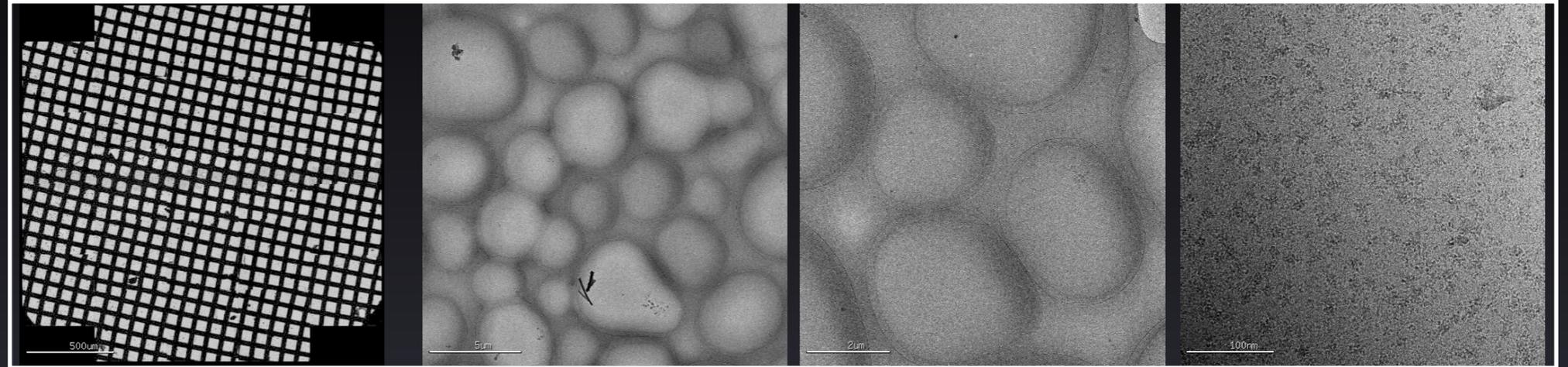
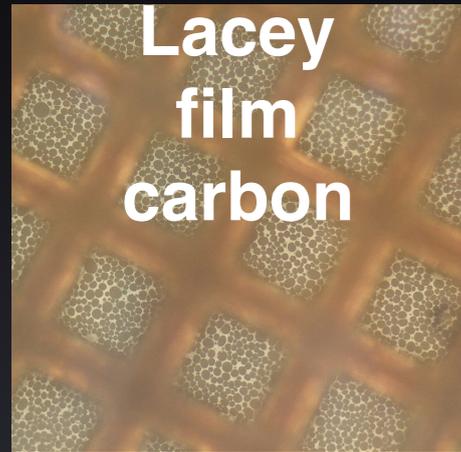


etc.

# The Spotiton Project: Improving Current CryoTEM Grid Preparation Methods



Hui Wei

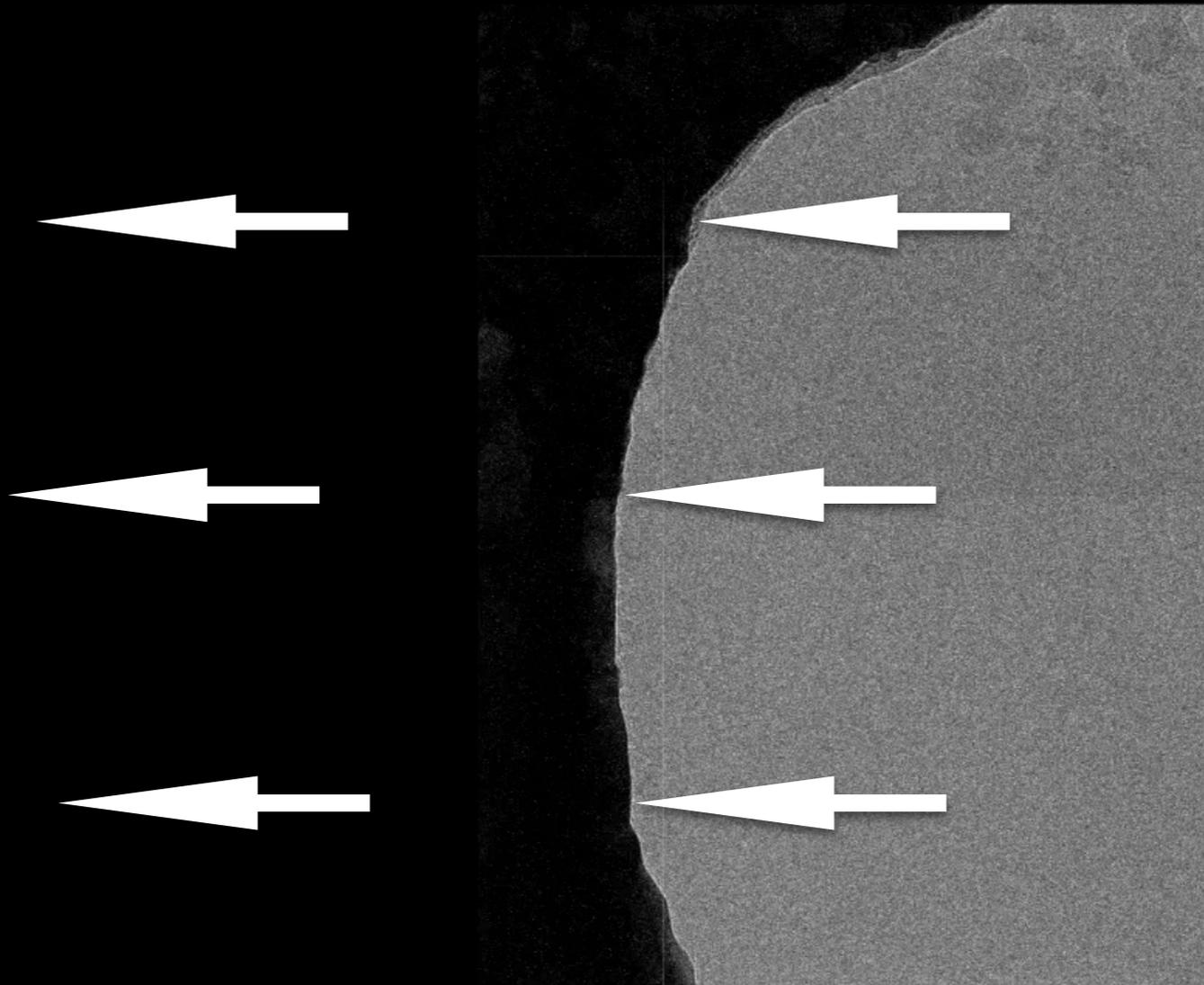


etc.

# Gold substrates and supports with nanowire grids

Holy carbon substrate  
on Rh and Cu nanowire  
45 degree tilt

Holy gold substrate  
on Au and Cu nanowire  
45 degree tilt



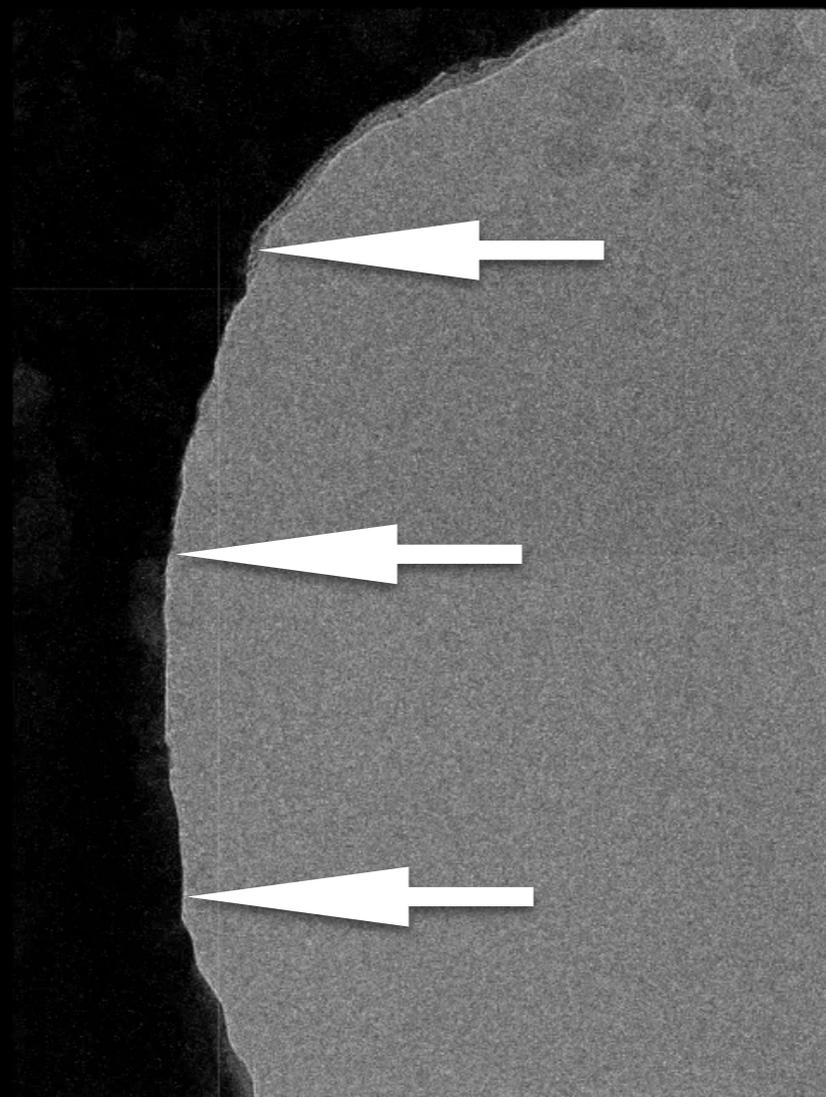
200 nm

# Gold substrates and supports with nanowire grids

Holy carbon substrate  
on Rh and Cu nanowire  
45 degree tilt



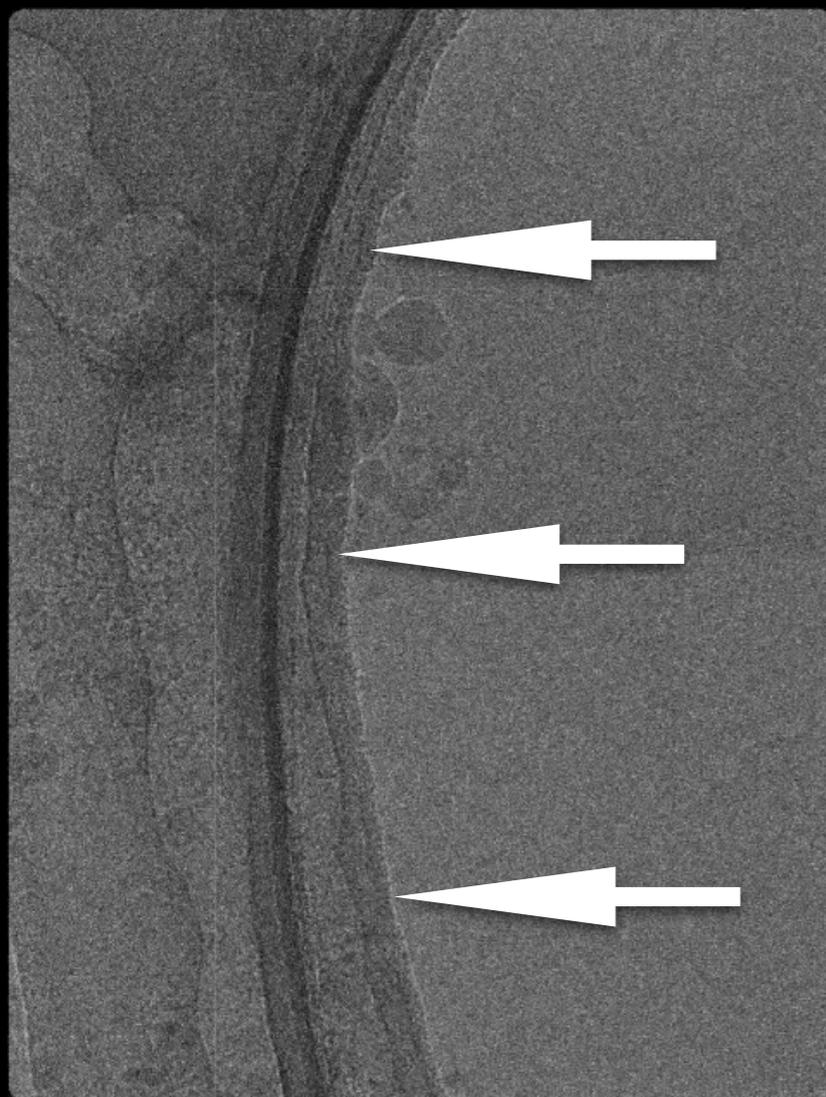
Holy gold substrate  
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45 degree tilt



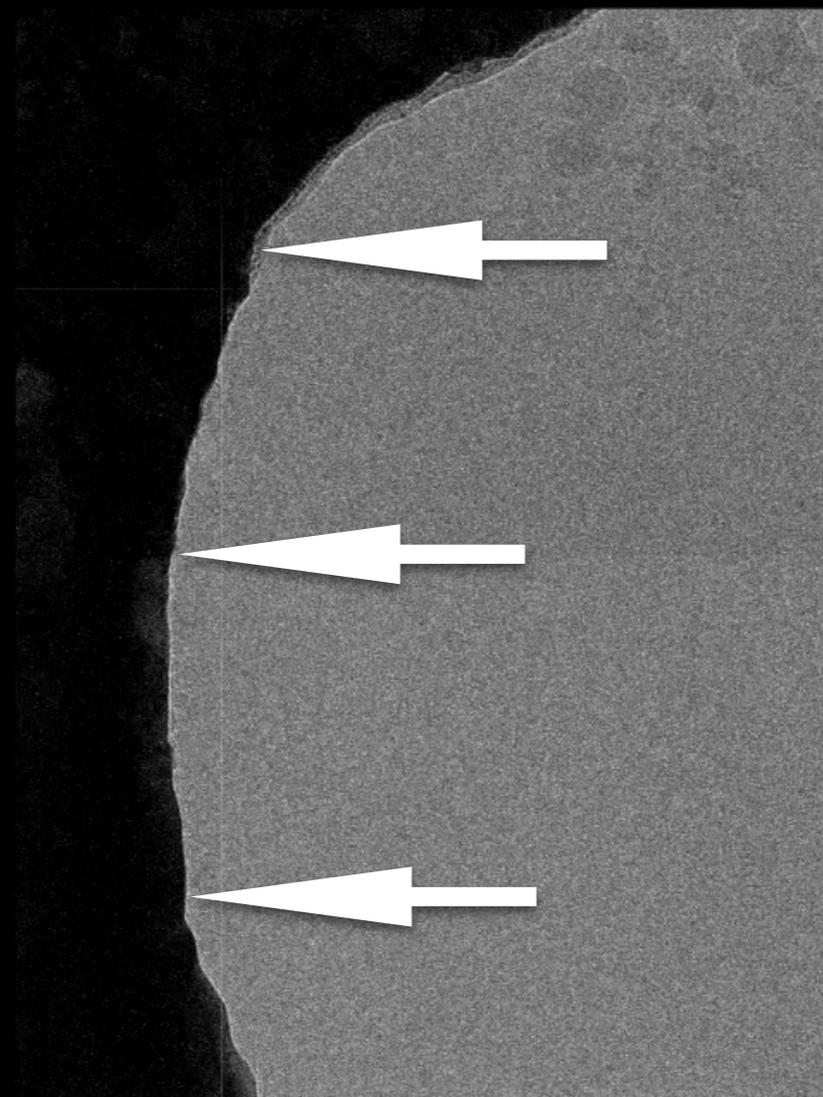
200 nm

# Gold substrates and supports with nanowire grids

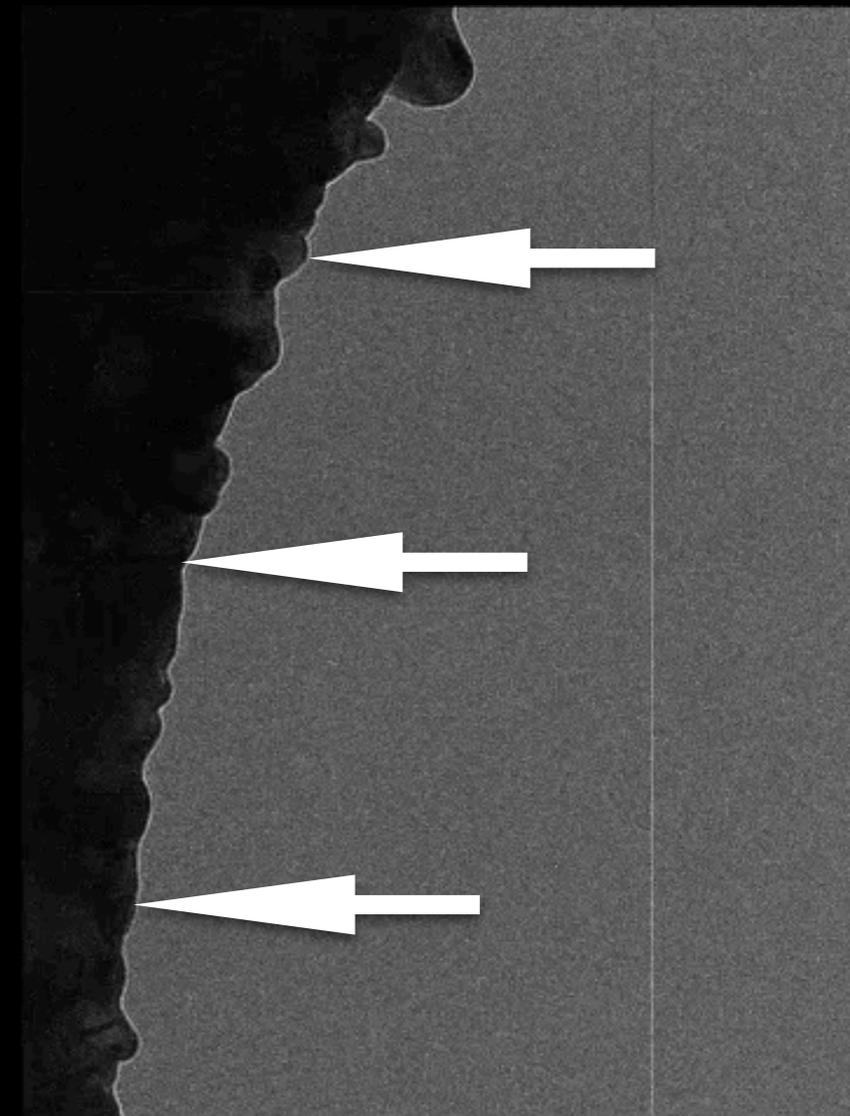
Holy carbon substrate  
on Rh and Cu nanowire  
45 degree tilt



Holy gold substrate  
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45 degree tilt



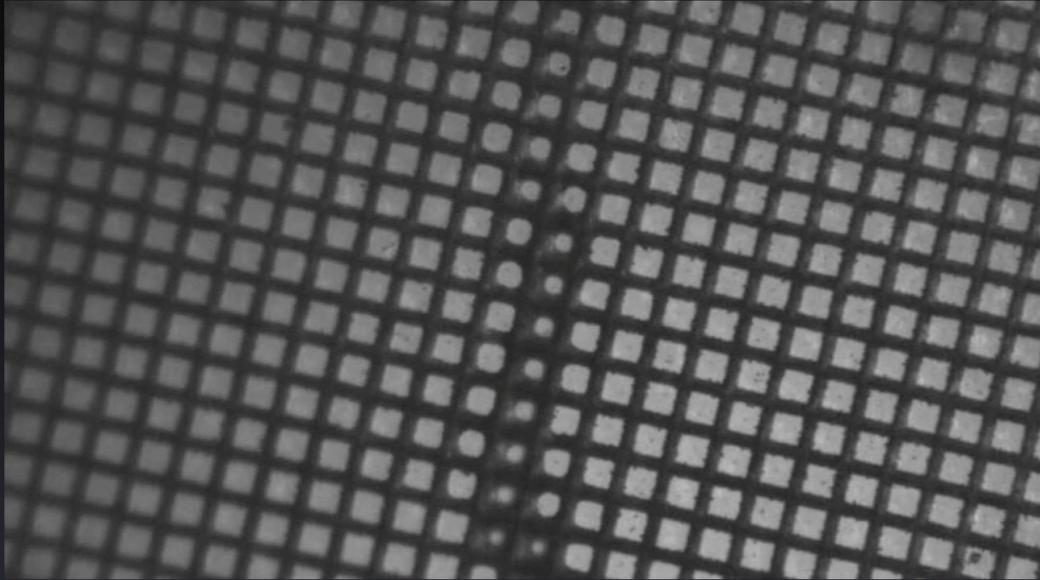
Holy gold substrate  
on Rh and Cu nanowire  
45 degree tilt



200 nm

# The Spotiton Project: Test sample: Apoferritin

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Zhening Zhang



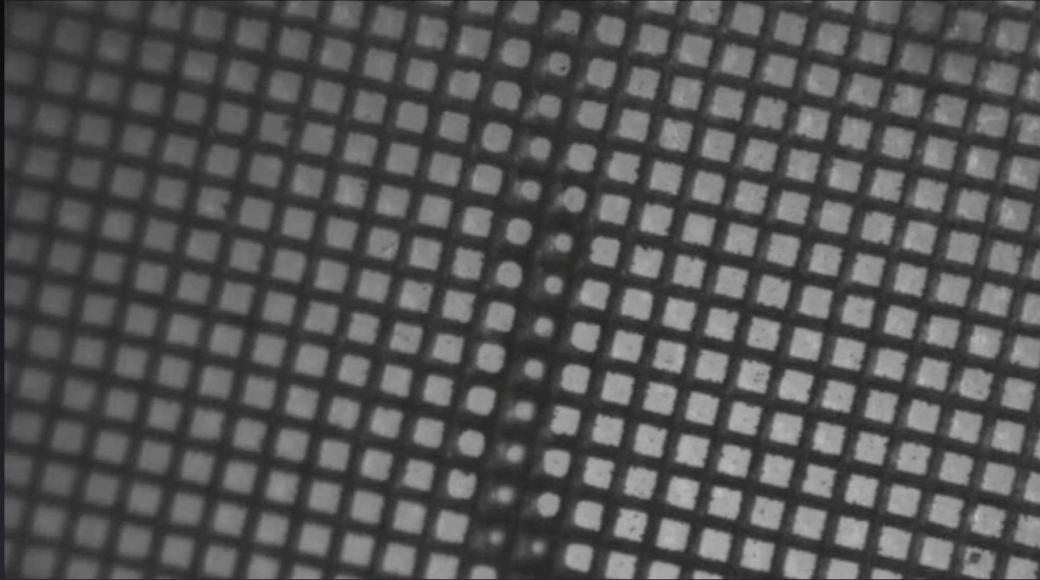
Hui Wei



Venkat Dandey

# The Spotiton Project: Test sample: Apoferritin

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Zhening Zhang

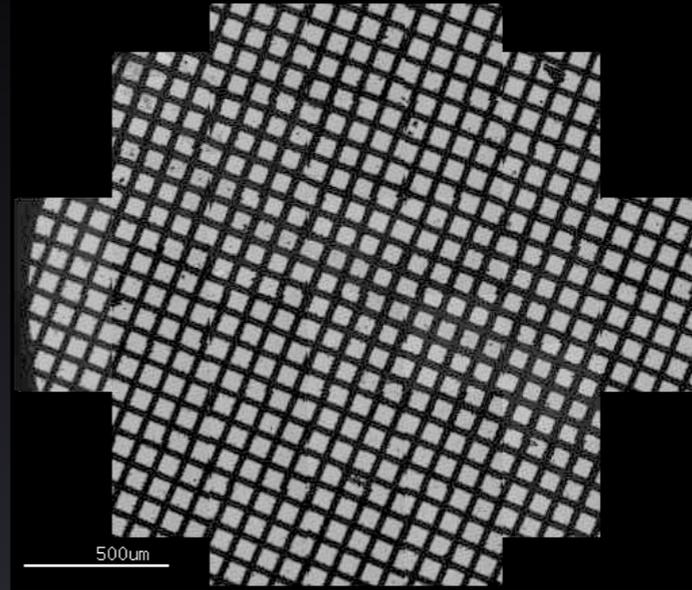
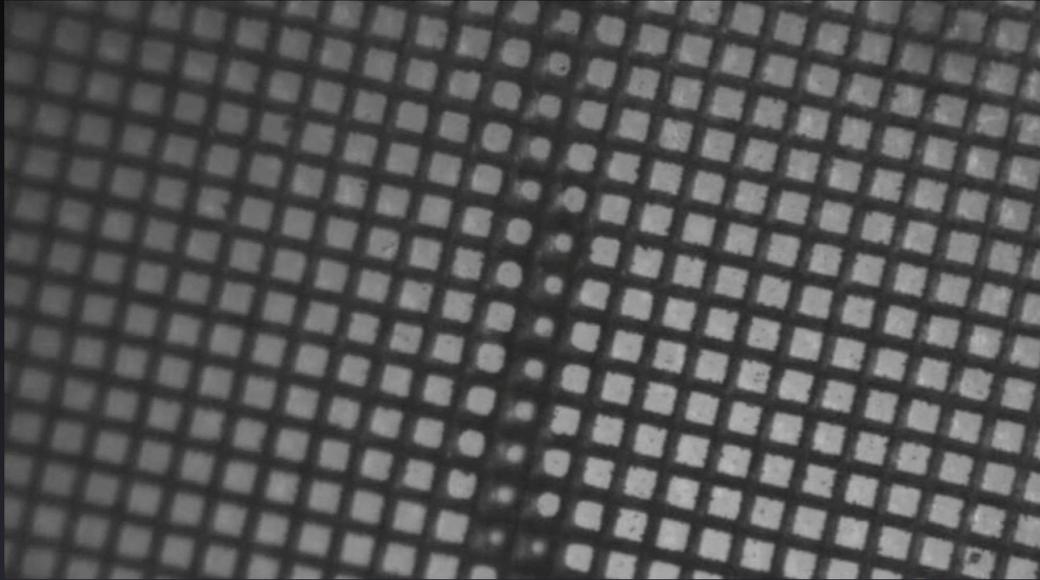


Hui Wei



Venkat Dandey

# The Spotiton Project: Test sample: Apoferritin



Zhening Zhang

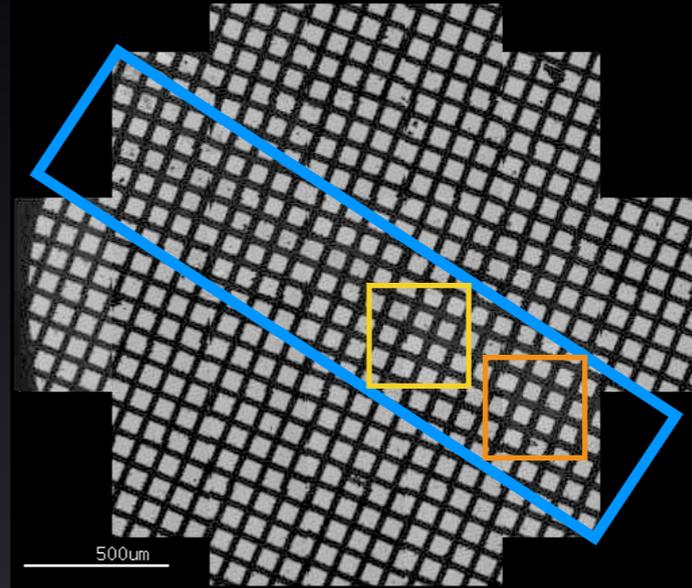
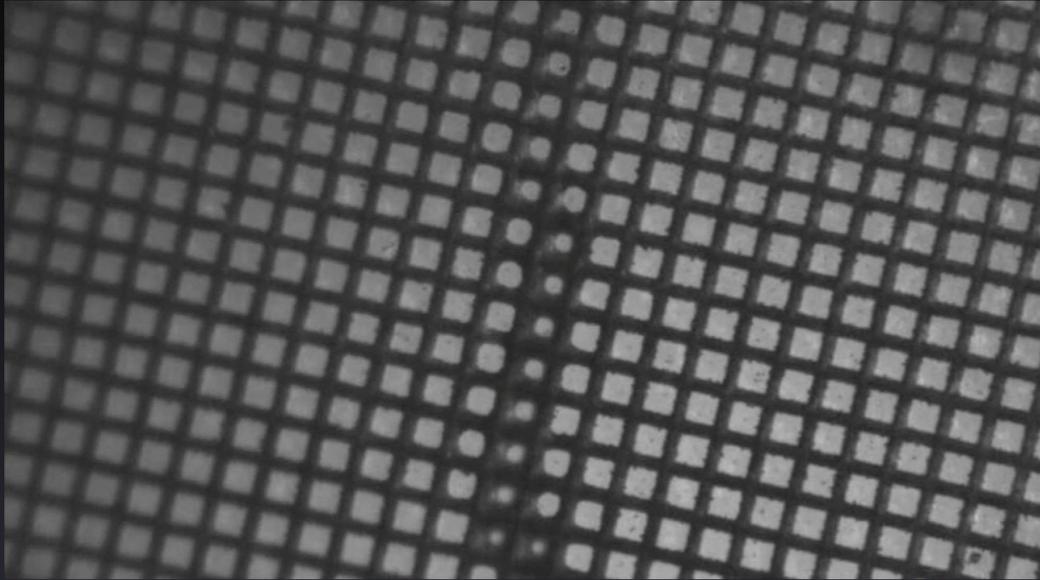


Hui Wei



Venkat Dandey

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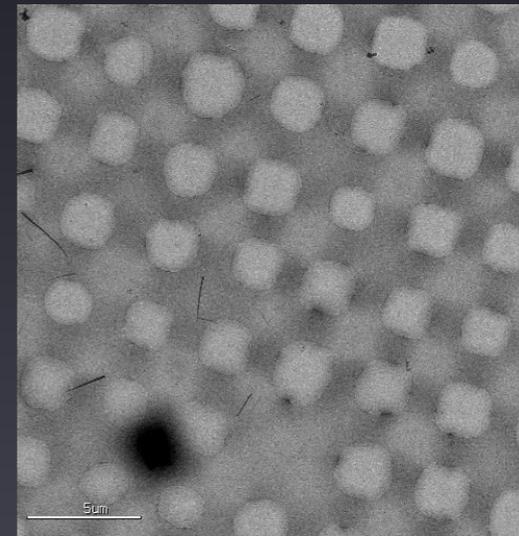
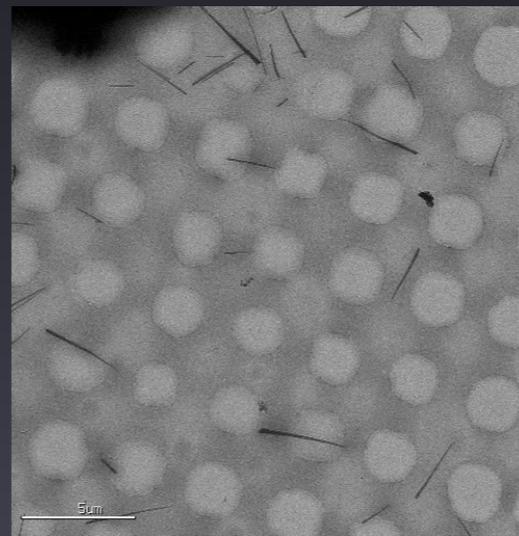
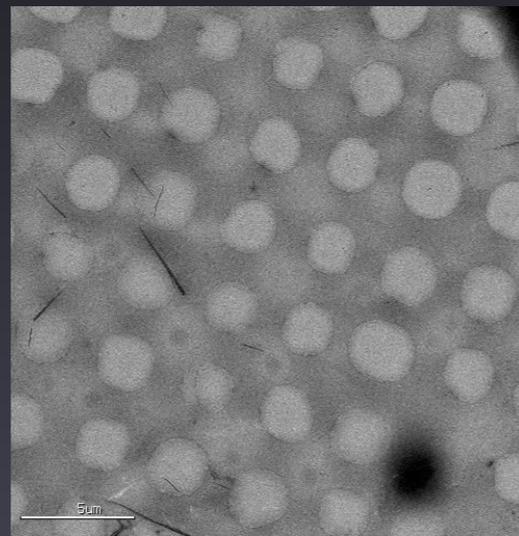
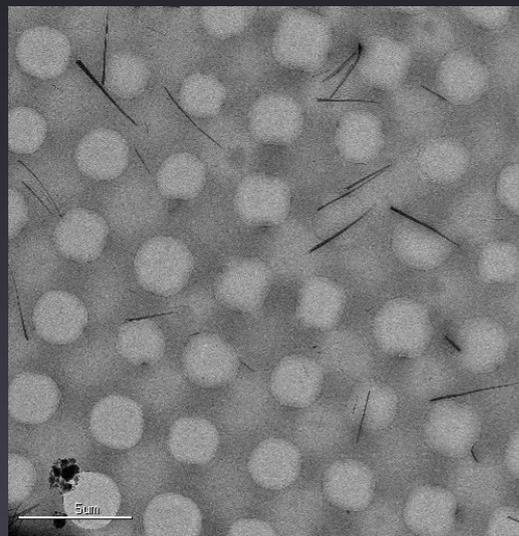
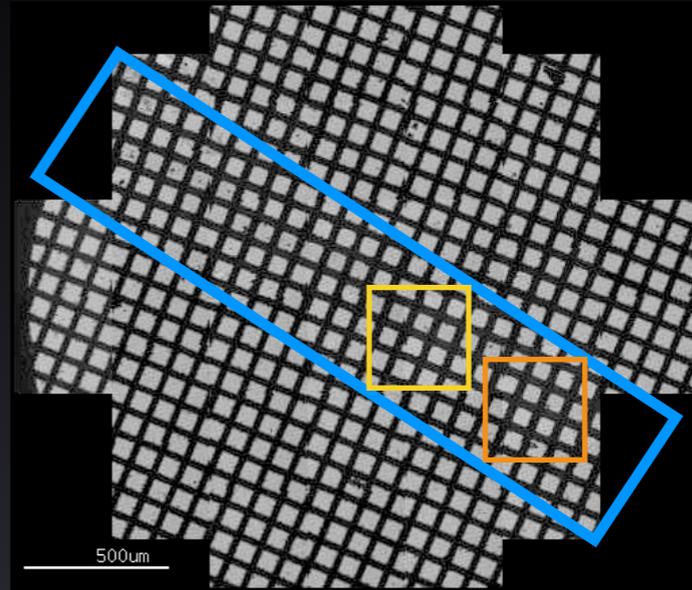
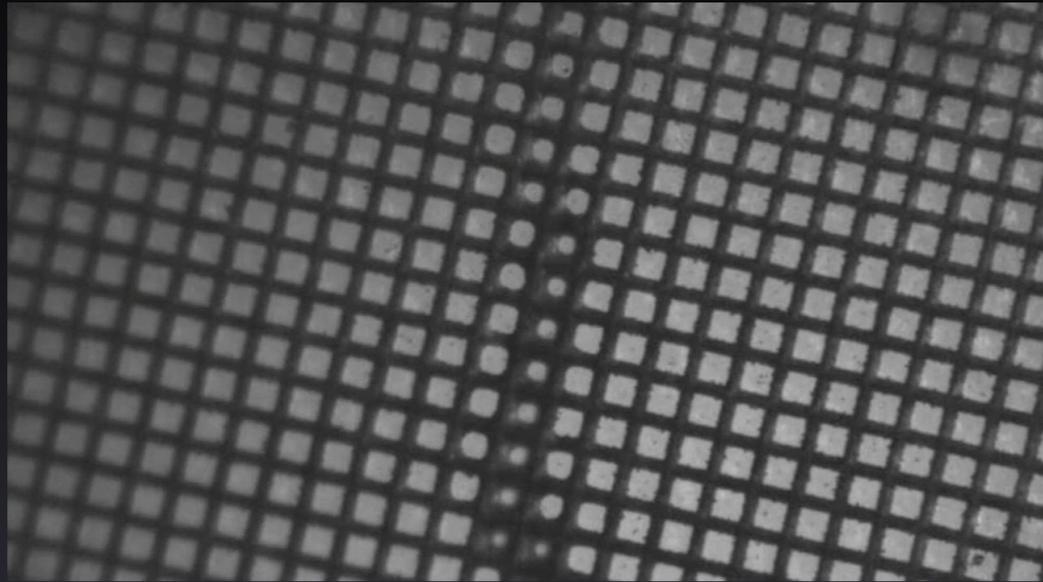


Hui Wei



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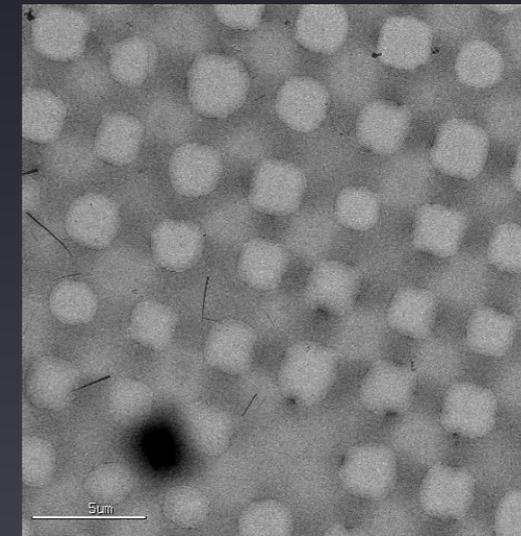
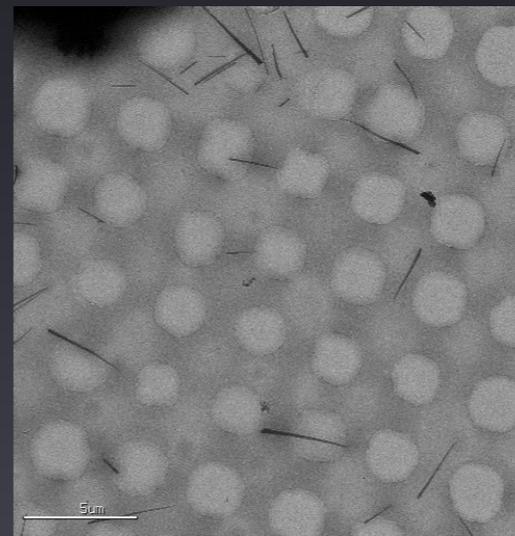
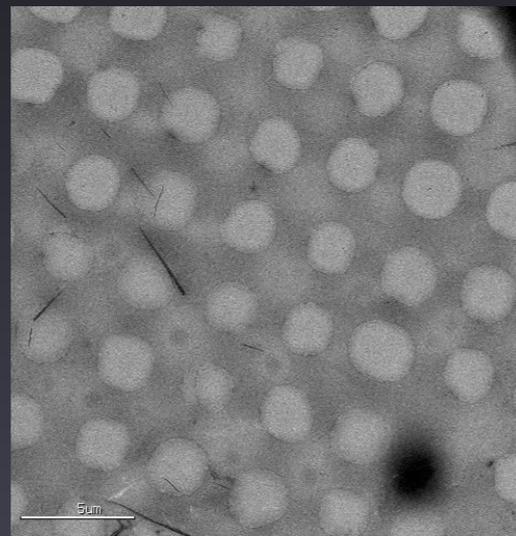
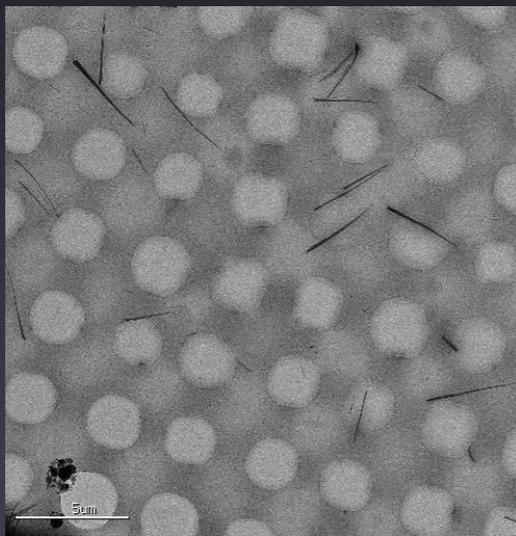
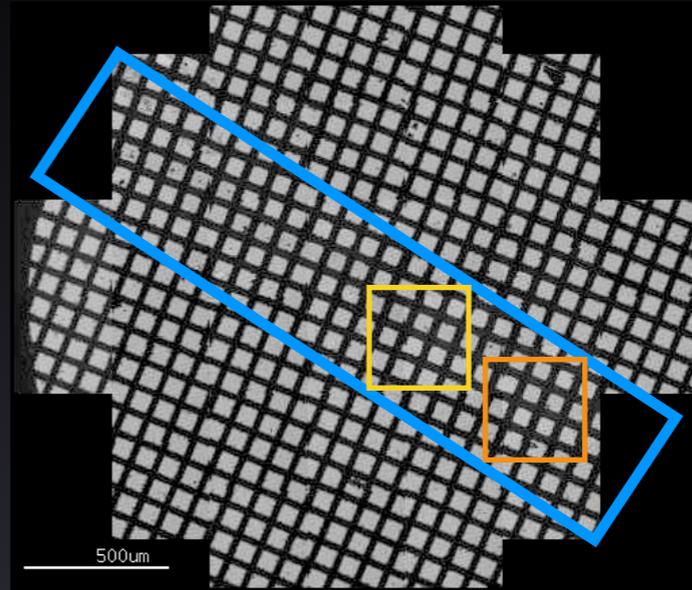
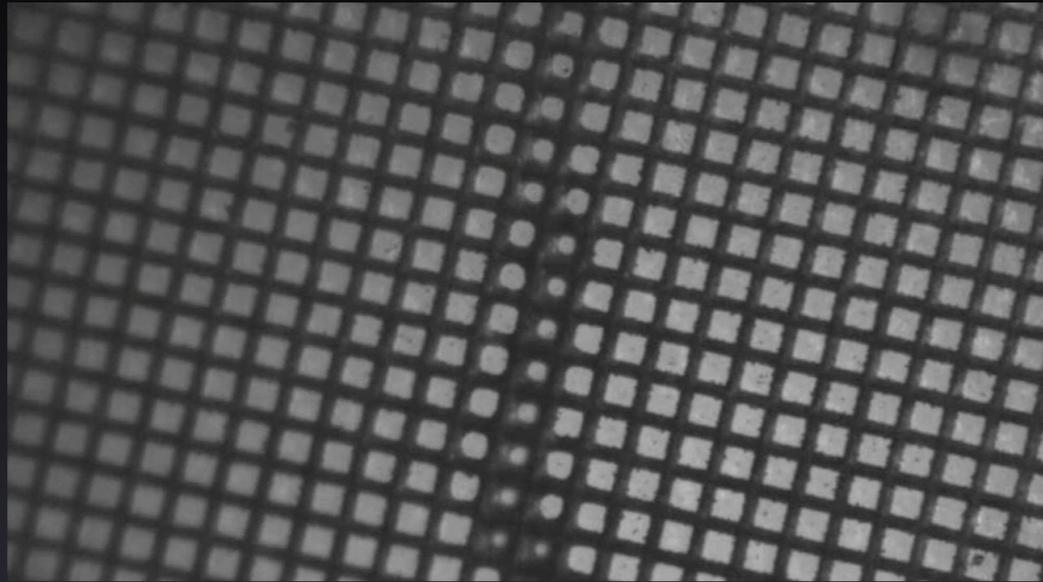


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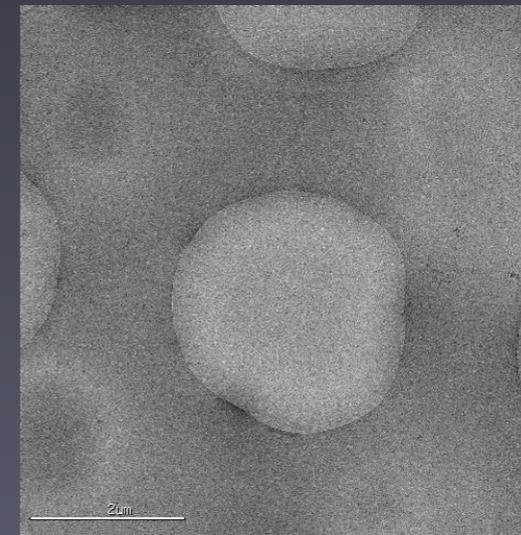
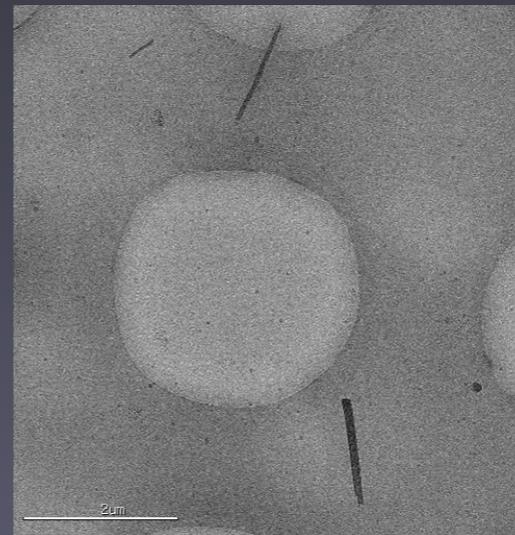
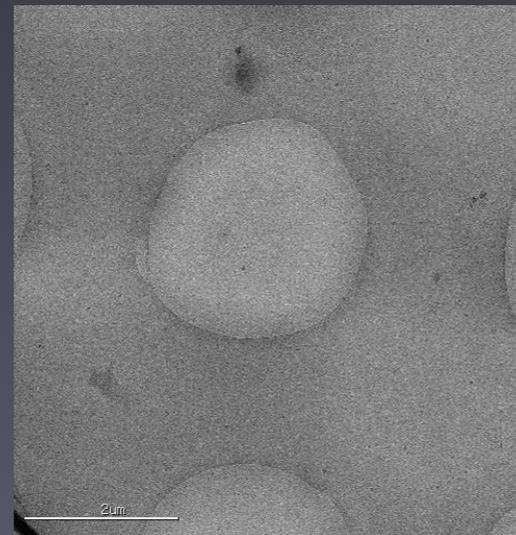
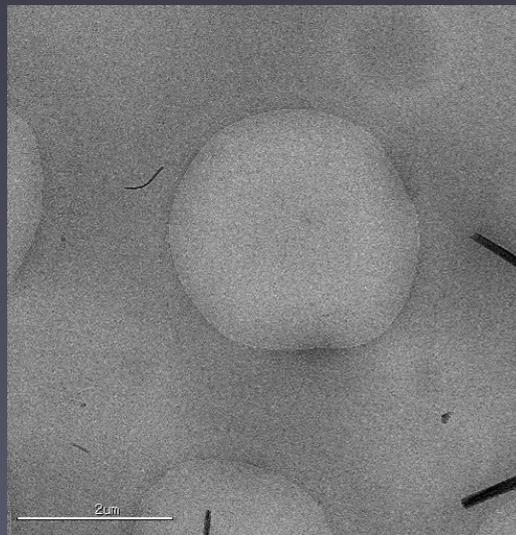
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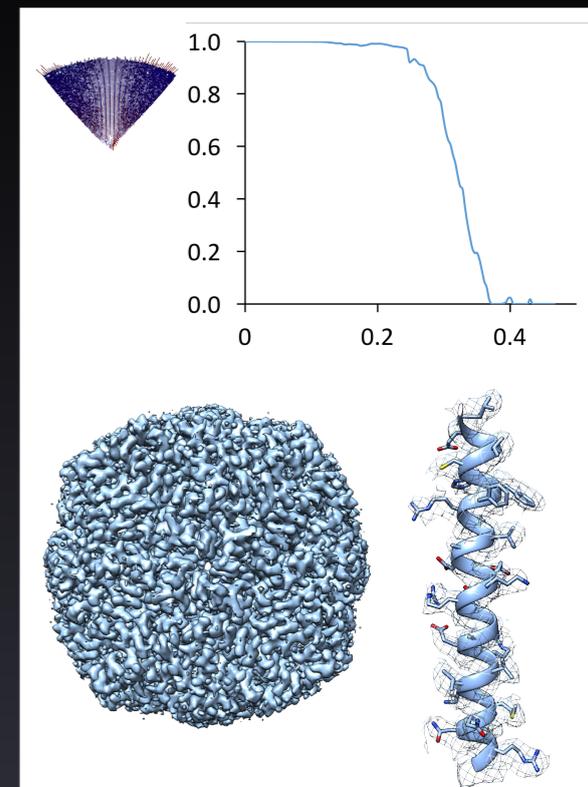
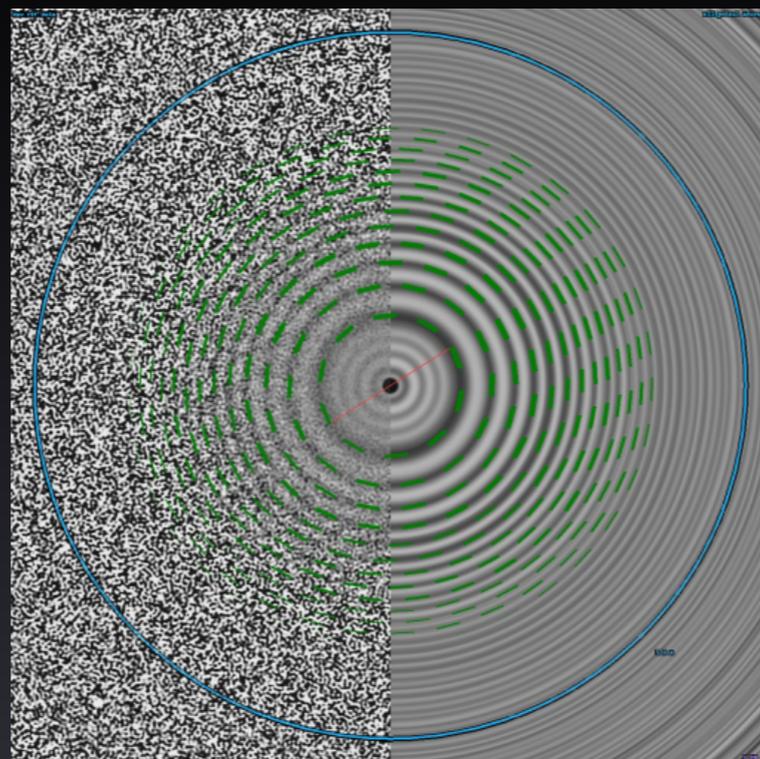
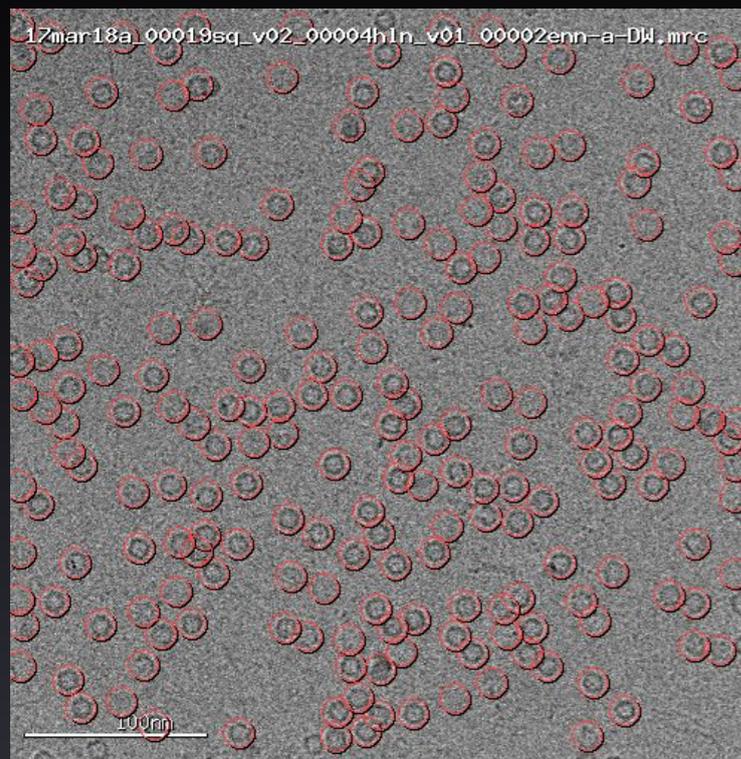


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# A Streamlined and Automated CryoEM Pipeline



**Automated data collection**  
(Patterned Spotiton grid)  
**133 images**

**Automated data pre-processing**  
**112 MotionCor2 images**  
**27,324 particles**

**Processing**  
**15,835 particles**

**Refinement**  
**2.8 Å (FSC<sub>0.143</sub>)**  
**9,721 particles**



Hui Wei

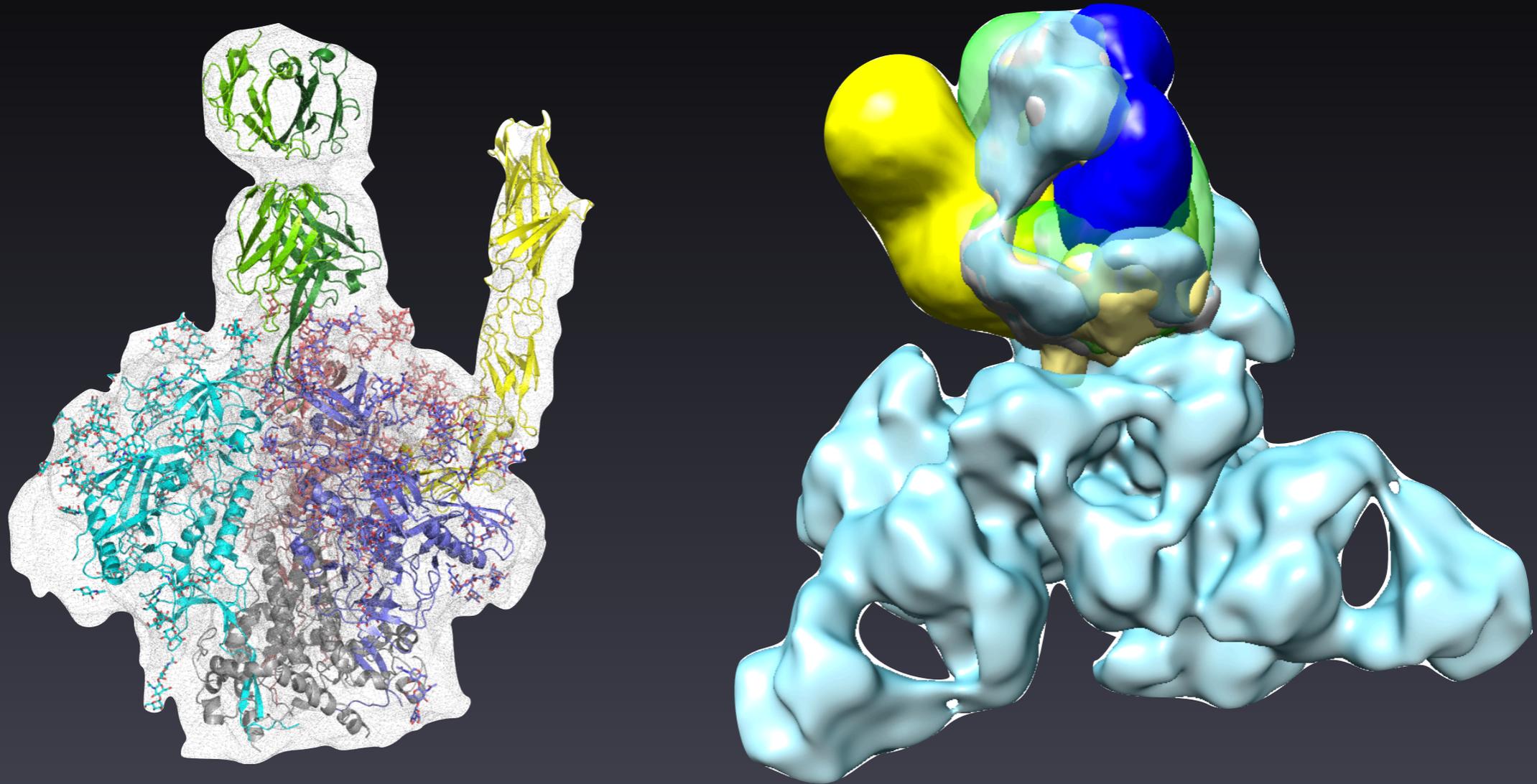


Ed Eng



Venkat Dandey

# Defining HIV Entry Pathway for Vaccine Design



Priyamvada Acharya  
Embedded Post Doc.

Qingbo Liu, Priyamvada Acharya, Michael A. Dolan, Peng Zhang, Christina Guzzo, Jacky Lu, Alice Kwon, Deepali Gururani, Huiyi Miao, Tatsiana Bylund, Gwo-Yu Chuang, Aliaksandr Druz, Tongqing Zhou, William Rice, Christoph Wigge, Bridget Carragher, Clinton S. Potter, Peter D. Kwong, Paolo Lusso. Vaccine Elicitation of Fusion Peptide-Directed Antibodies that Neutralize HIV-1. (2017) NSMB



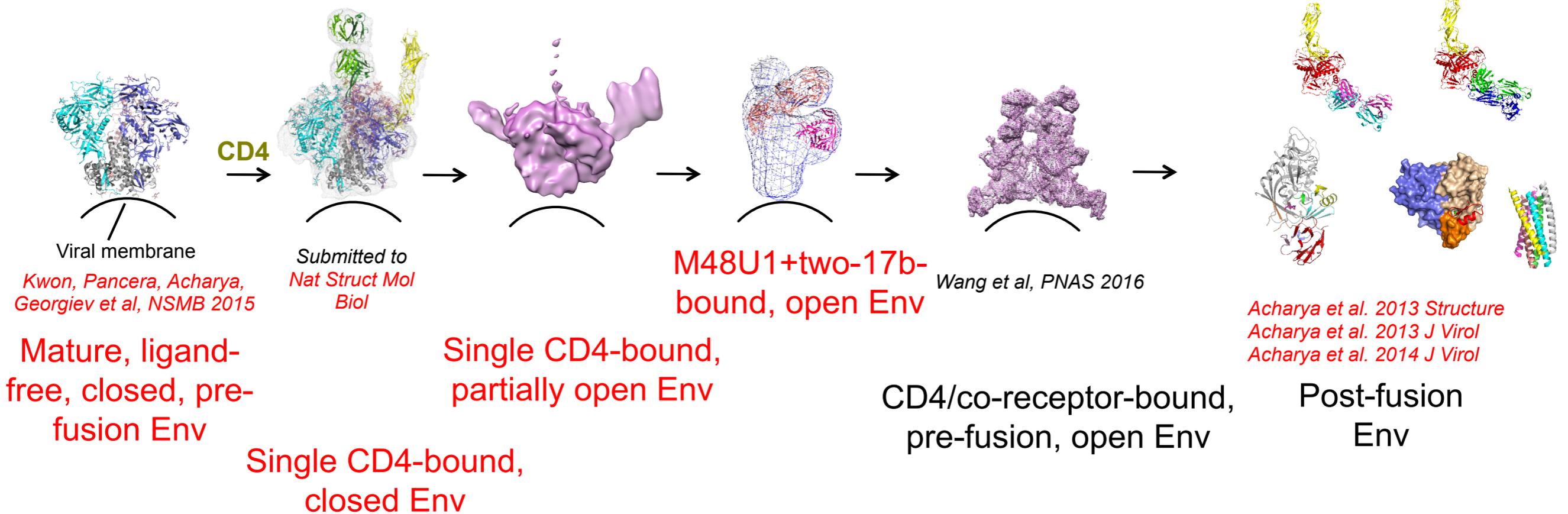
Peter Kwong  
VRC, NIH

# Defining HIV Entry Pathway for Vaccine Design

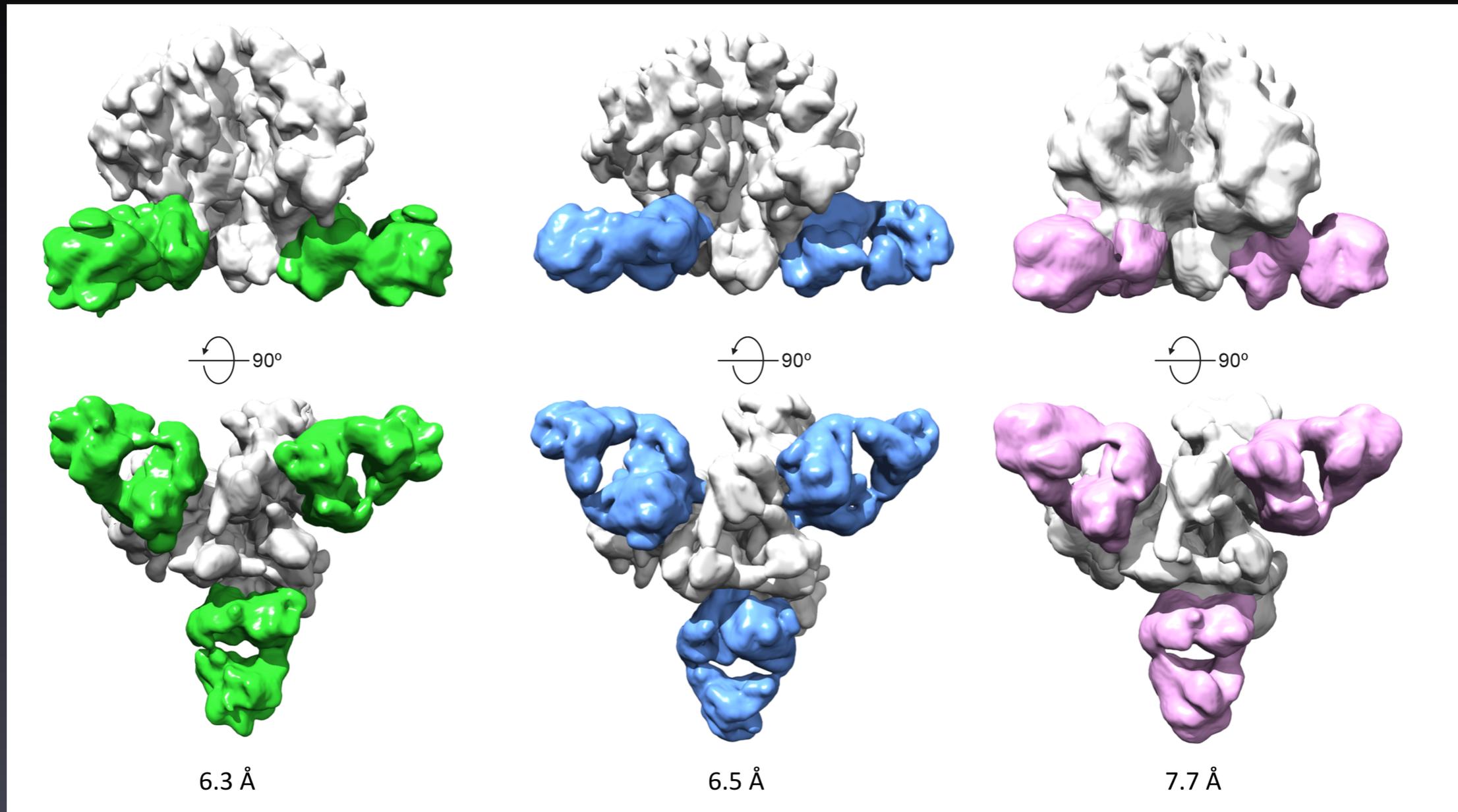
## CD4 binding

## Co-receptor binding

## Fusion and entry



# Defining HIV Entry Pathway for Vaccine Design



Spotiton grid: 1; # images collected: 150; tilt angle: 40 degrees; time: ~5 hours;  
frame alignment (motionscorr2), gctf, particles picked (~40K): during data acquisition  
per particle estimation (gctf): ~3 hours; Relion 2d classes: ~4 hours  
CryoSparc ab initio: 30 minutes; CryoSparc refinement: 30 minutes



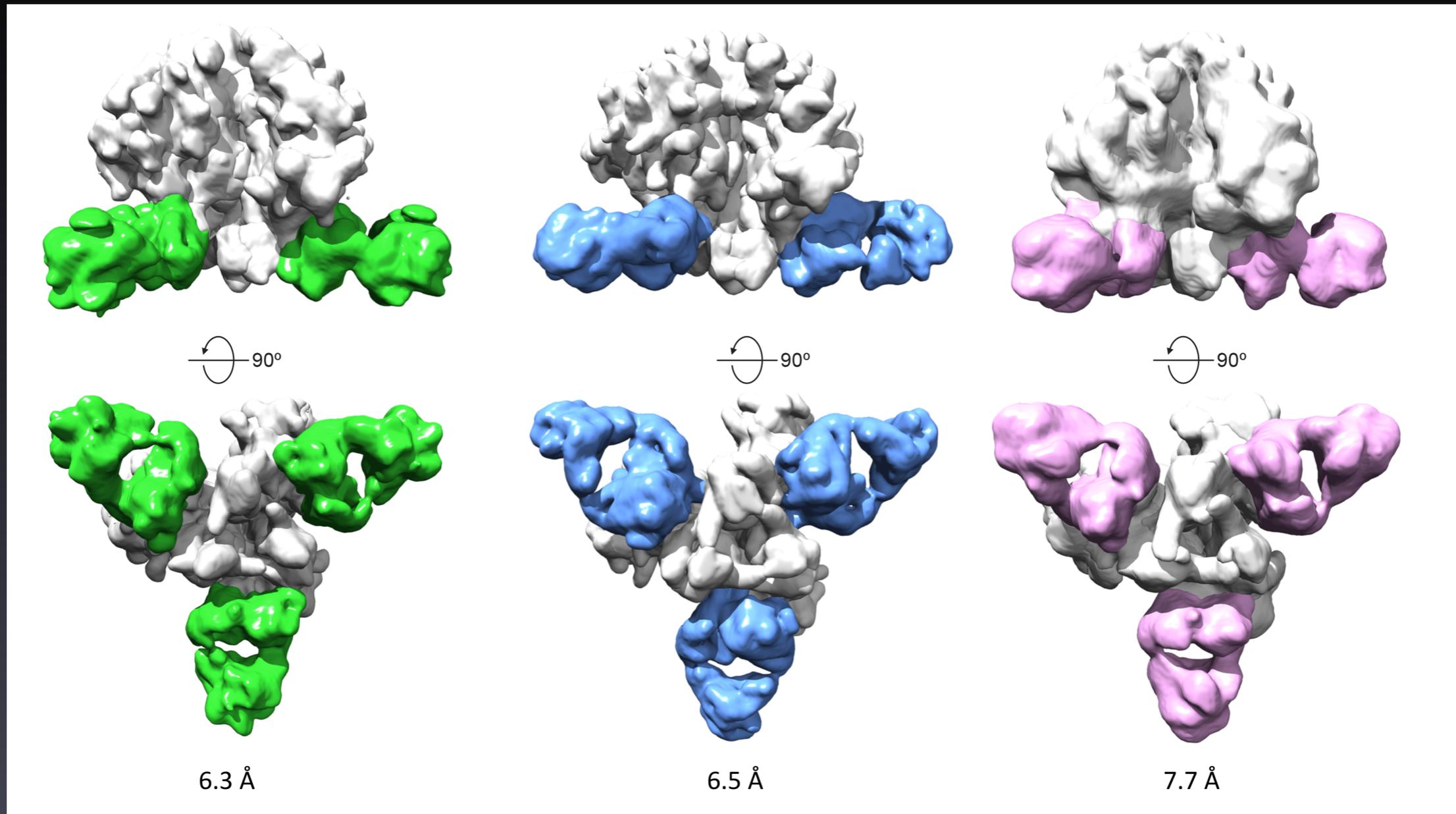
Priyamvada Acharya  
Embedded Post Doc.

**Cryo-EM maps of HIV-1 Env complexed with FP-directed antibodies  
(3 maps within 24 hours)**



Peter Kwong  
VRC, NIH

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**Cryo-EM maps of HIV-1 Env complexed with FP-directed antibodies  
(3 maps within 24 hours)**



Yong Zi Tan



Priyamvada Acharya  
Embedded Post Doc.

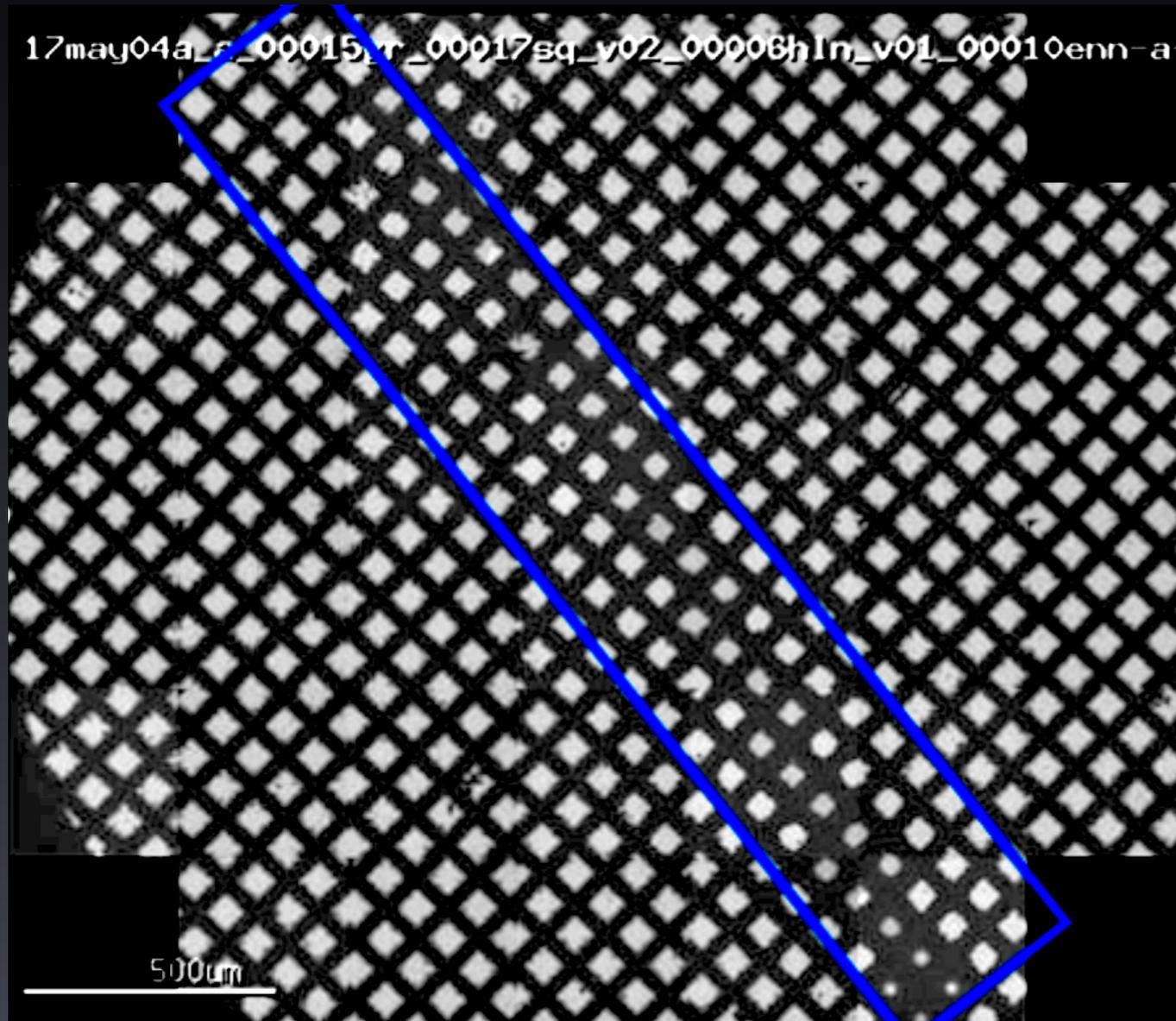


Dmitry Lyumkis



Peter Kwong  
VRC, NIH

# Defining HIV Entry Pathway for Vaccine Design



20 minutes between grid atlas acquired and first high magnification image acquired from a queue of 50 images

25 of 28 vitrified “squares” suitable for data collection



Priyamvada Acharya  
Embedded Post Doc.

Cryo-EM maps of HIV-1 Env complexed with FP-directed antibodies  
(3 maps within 24 hours)



Peter Kwong  
VRC, NIH

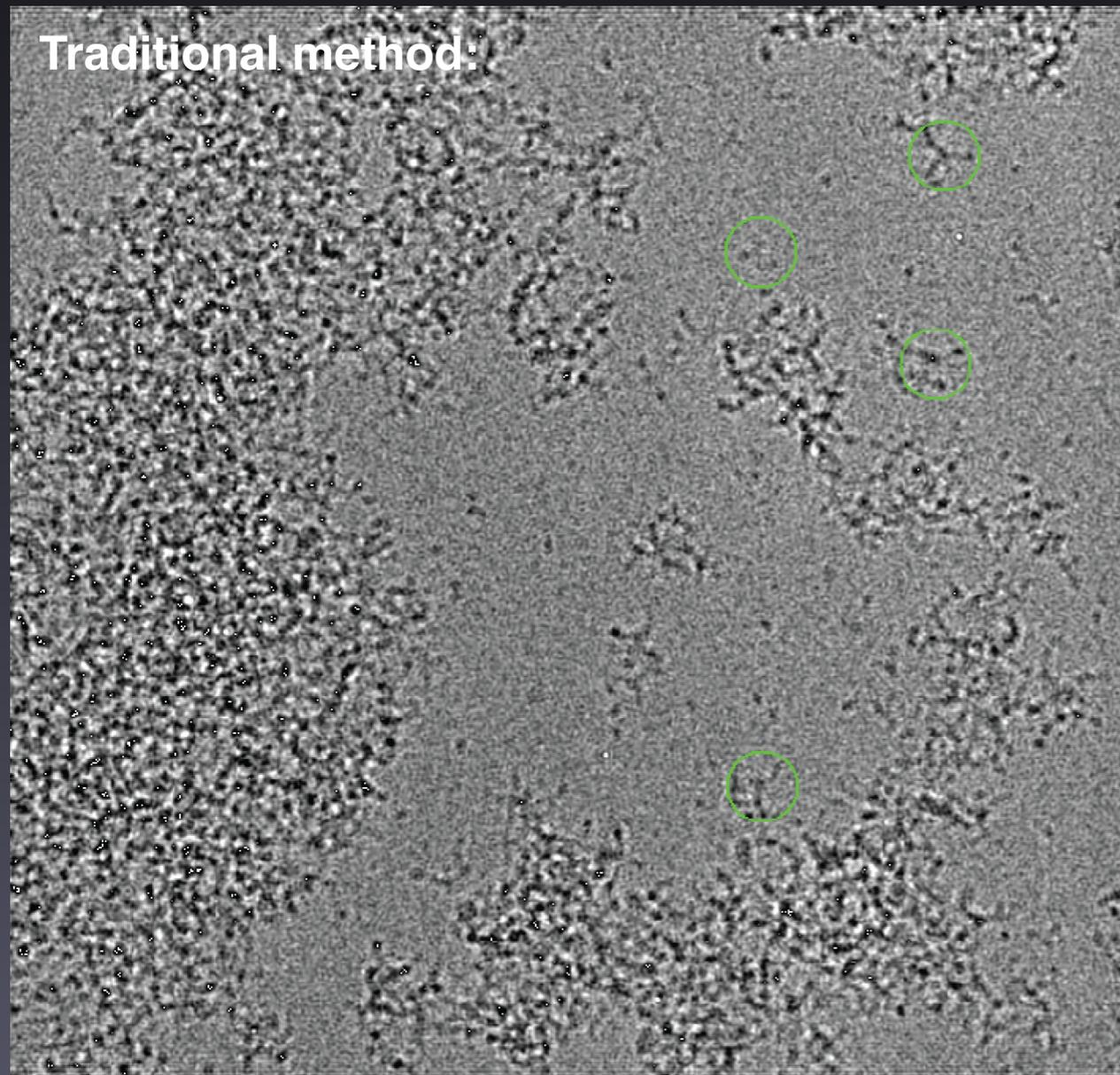
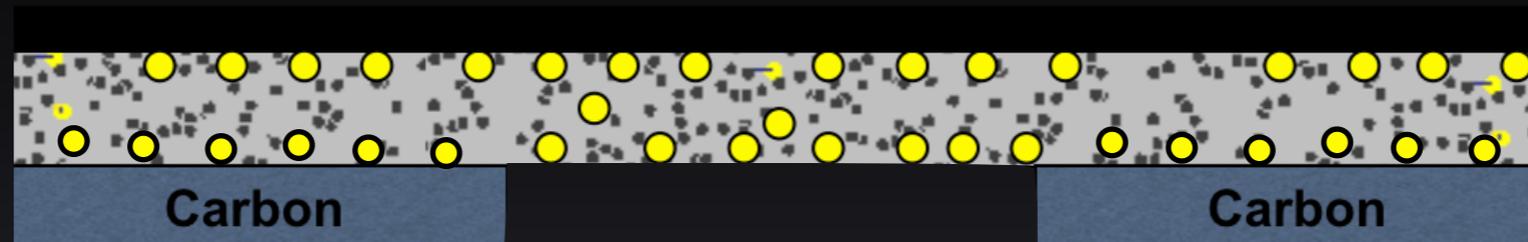
# Spotiton: Other potential advantages

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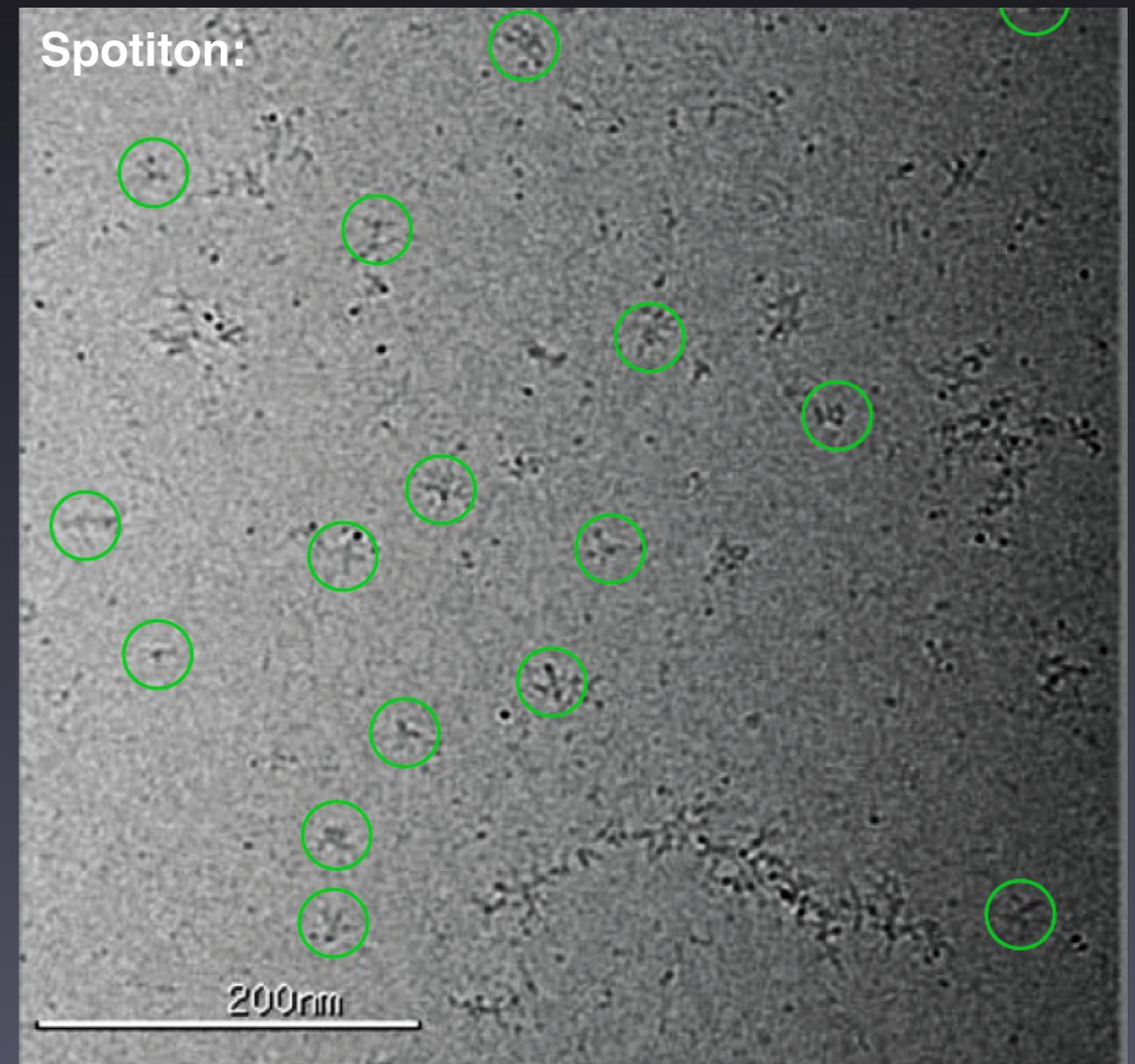
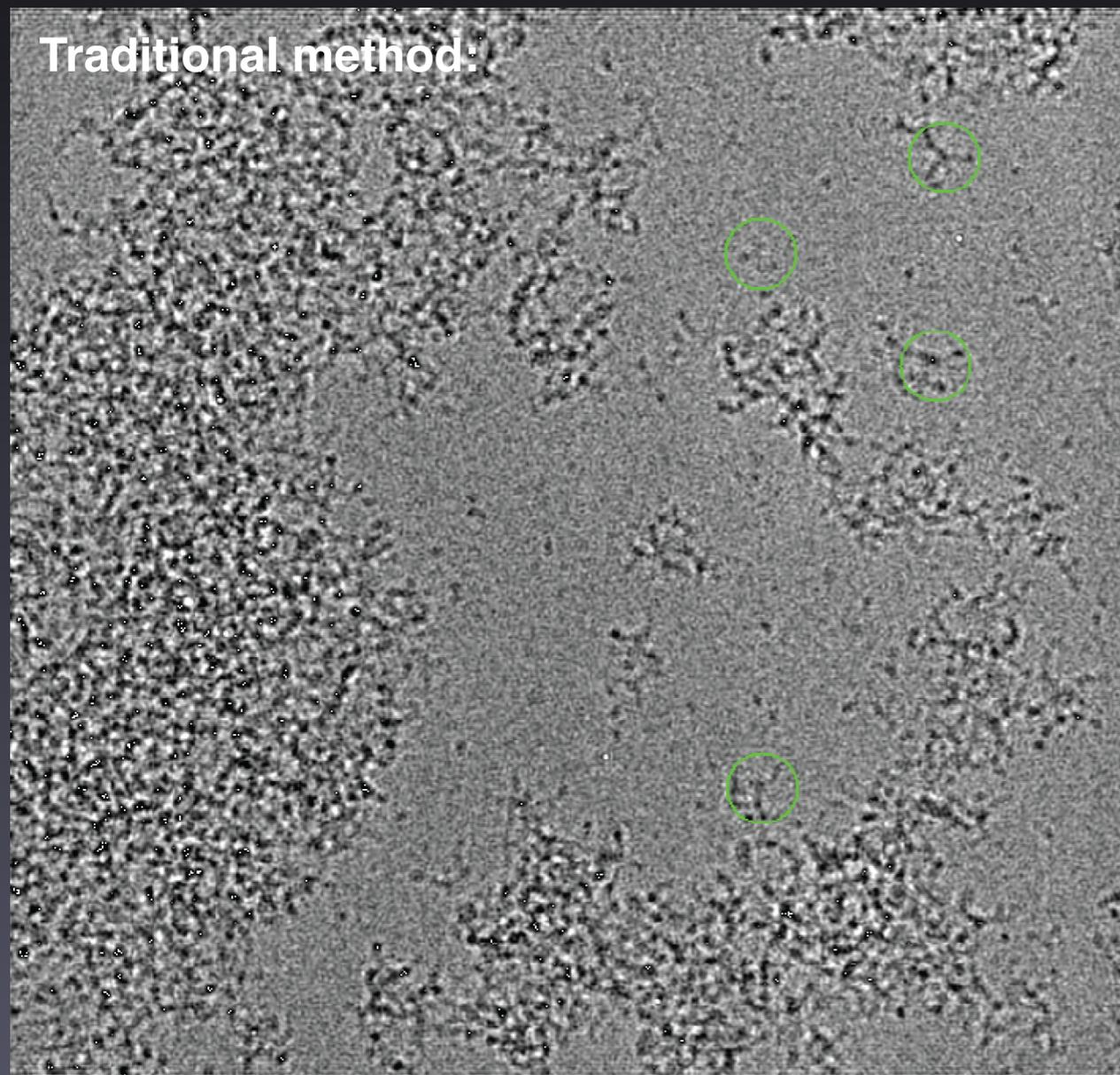
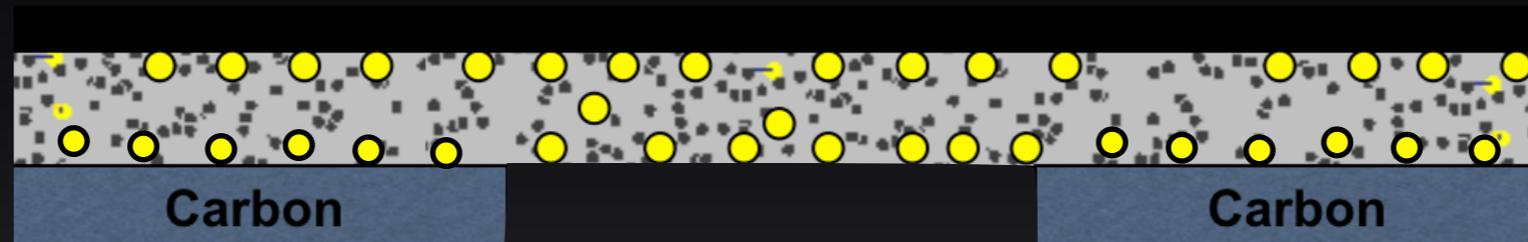


# Spotiton: Other potential advantages



Fusion protein (~300kDa); Luke Chao and Steve Harrison

# Spotiton: Other potential advantages

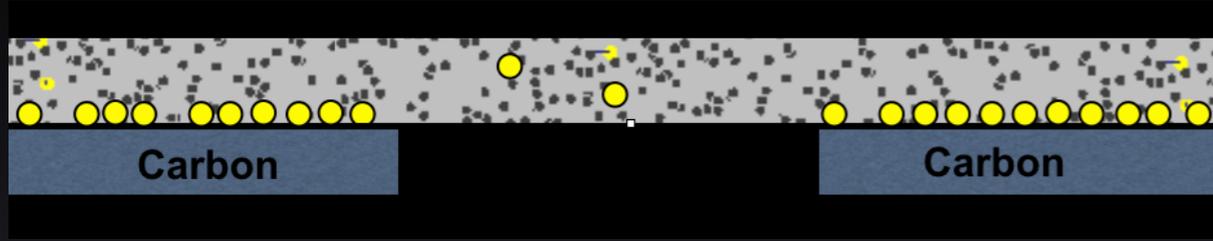


Fusion protein (~300kDa); Luke Chao and Steve Harrison

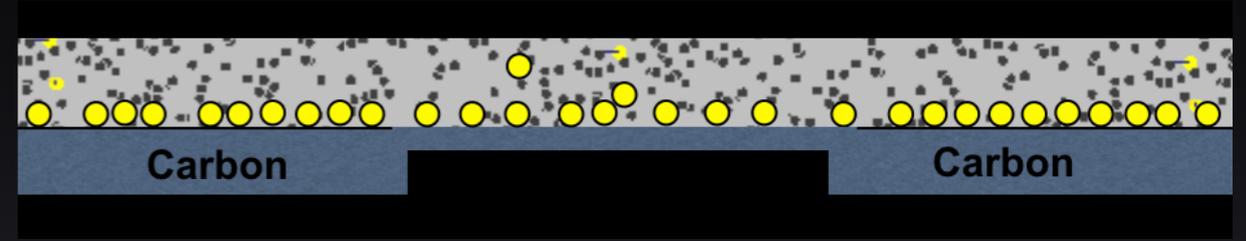
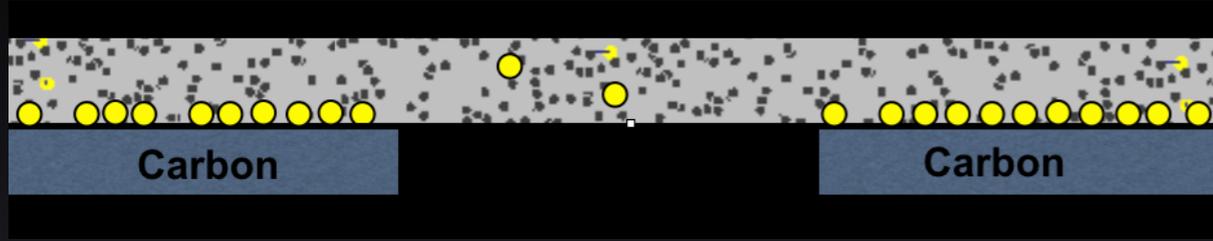
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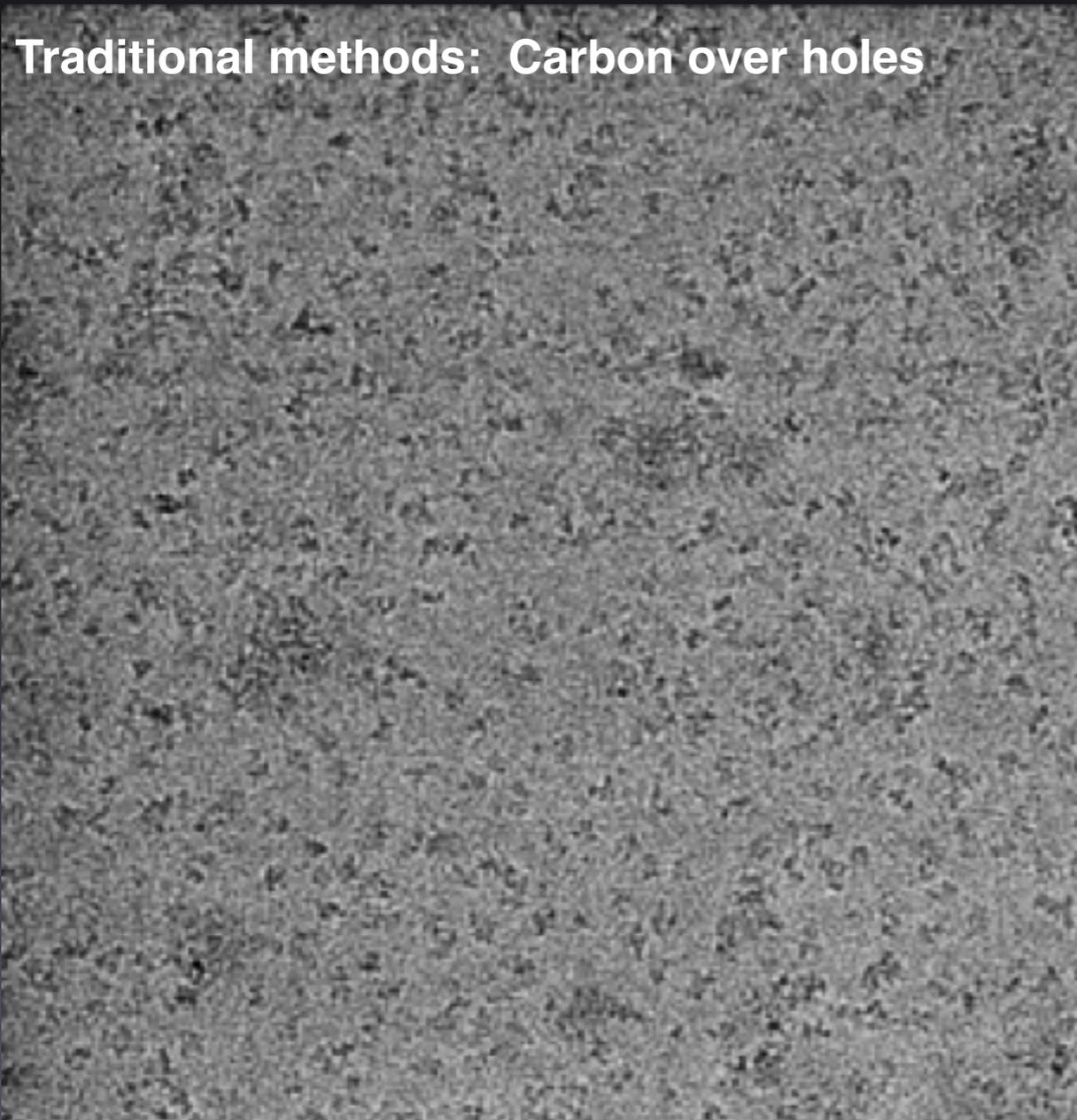
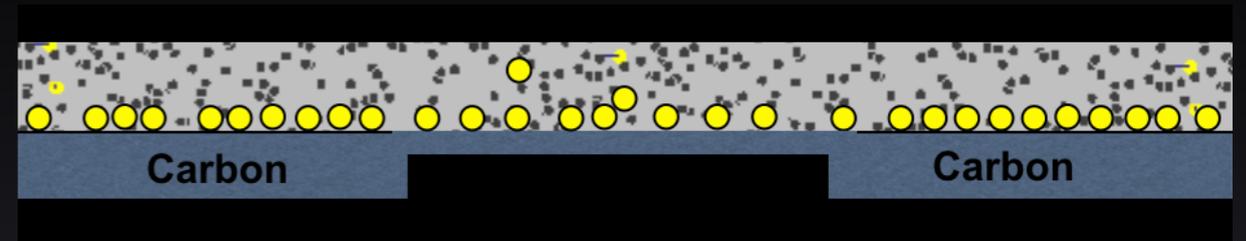
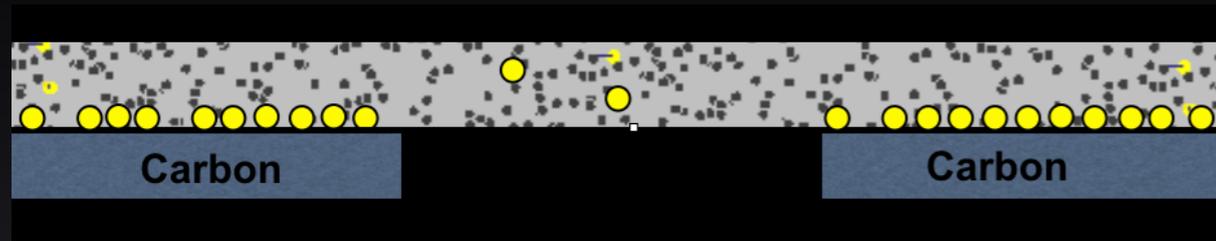
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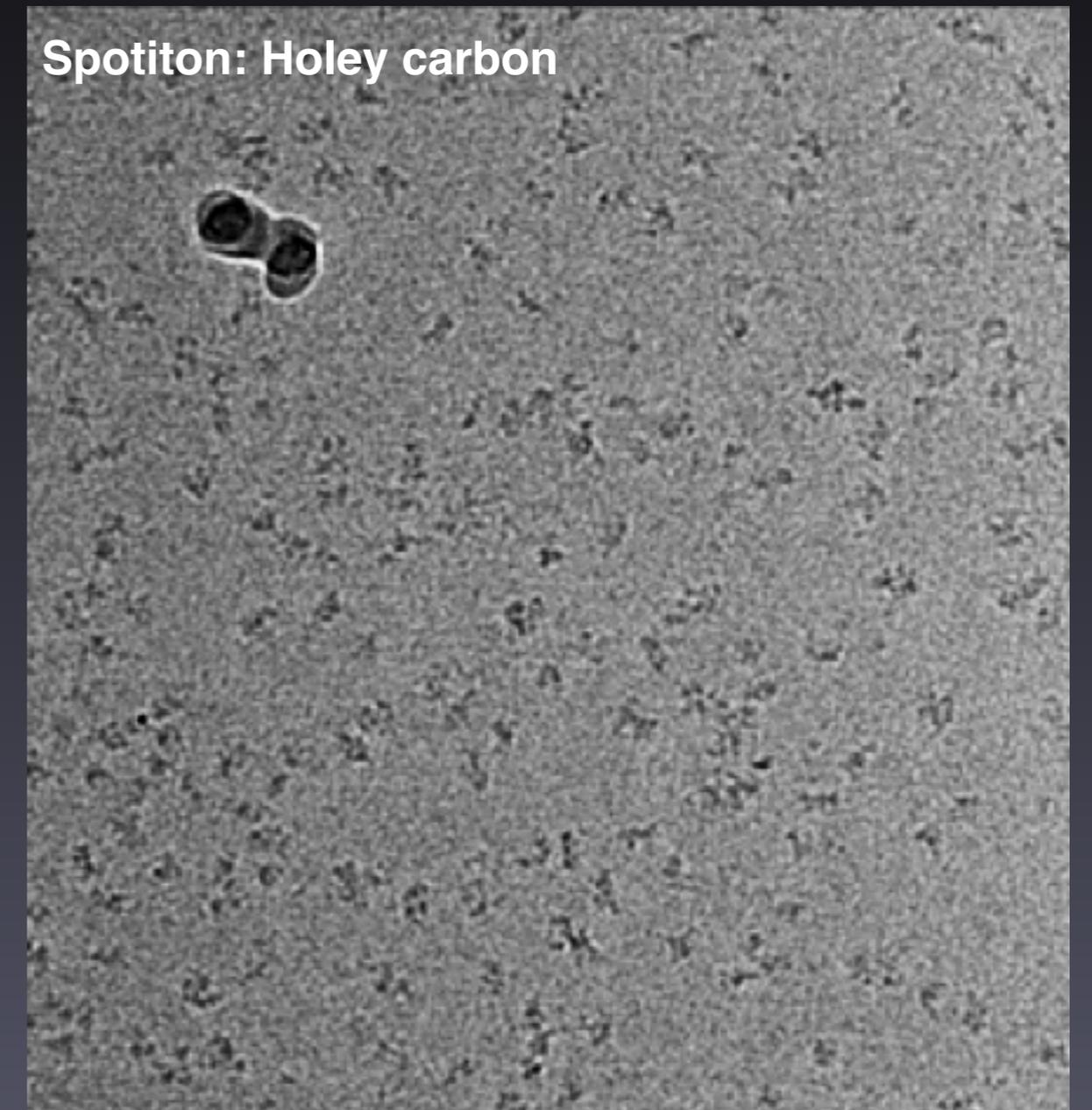
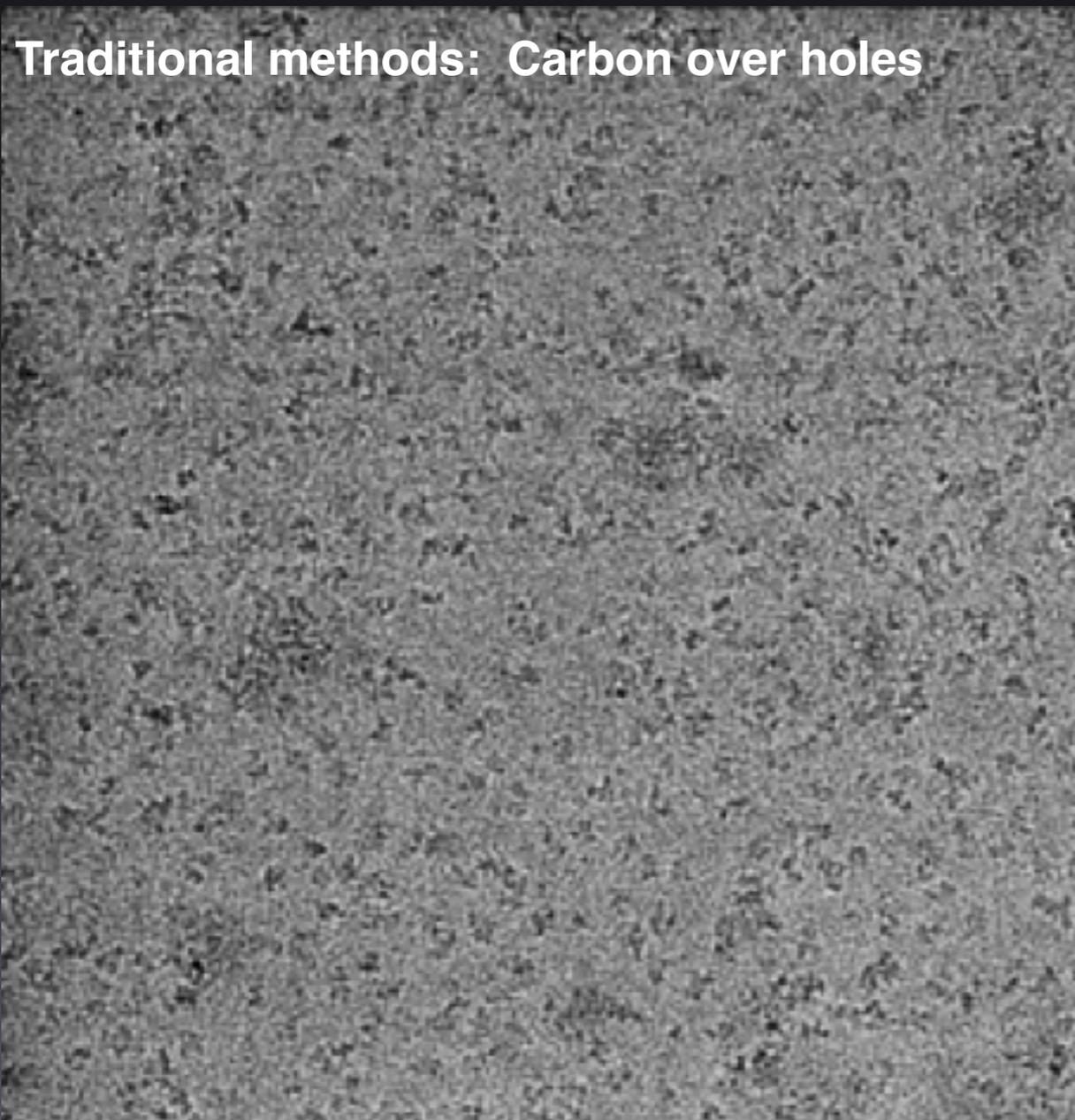
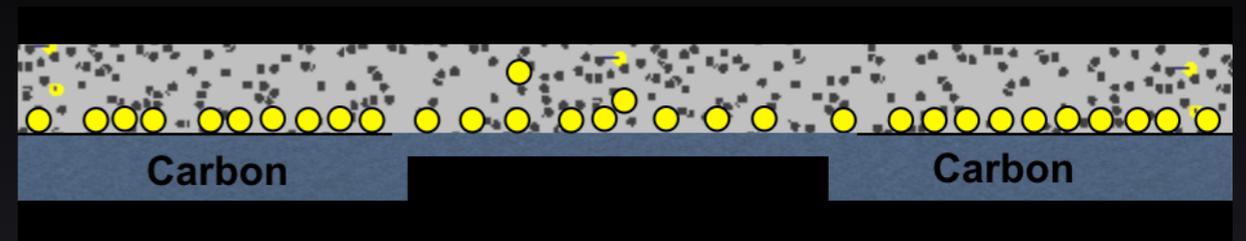
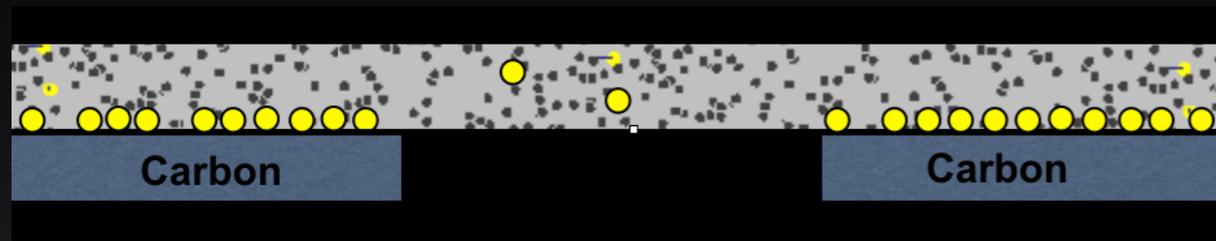


# Spotiton: Other potential advantages



Human protein complex (~300kDa); Vignesh Kasinath and Eva Nogales, UCB

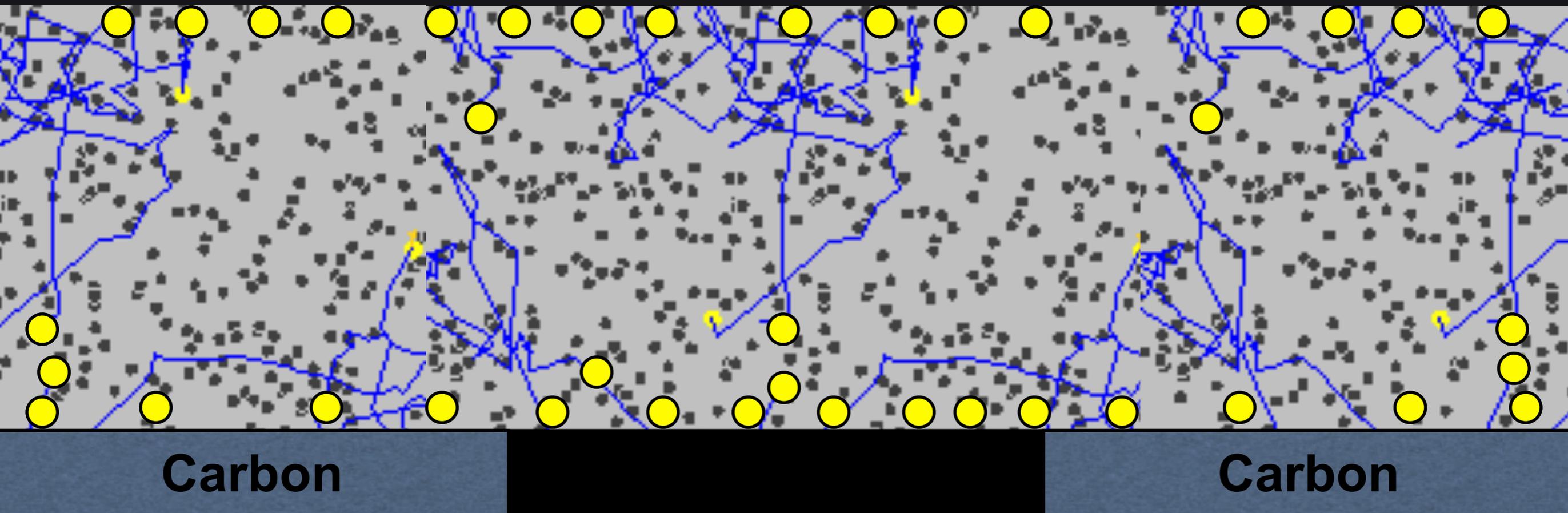
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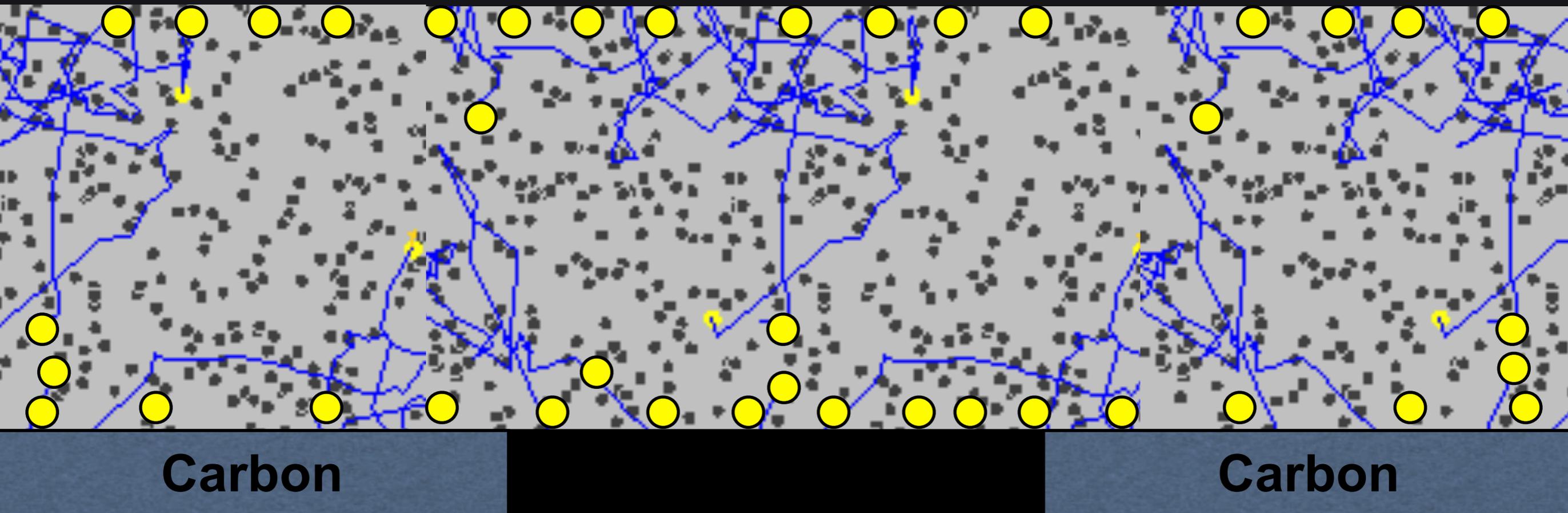
# But still many other challenges to address:

A hypothetical scenario during cryoEM grid preparation



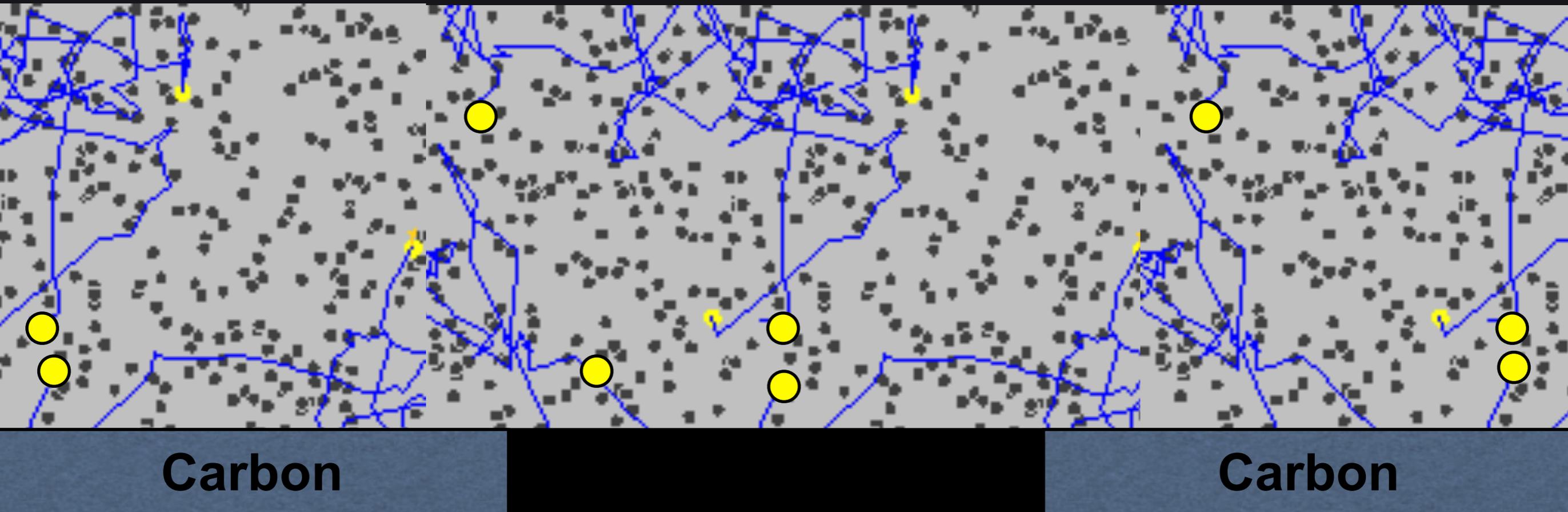
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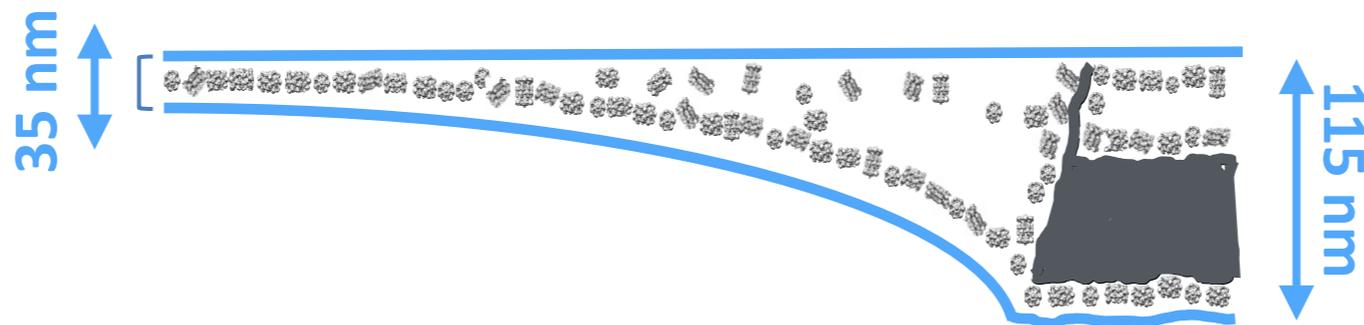
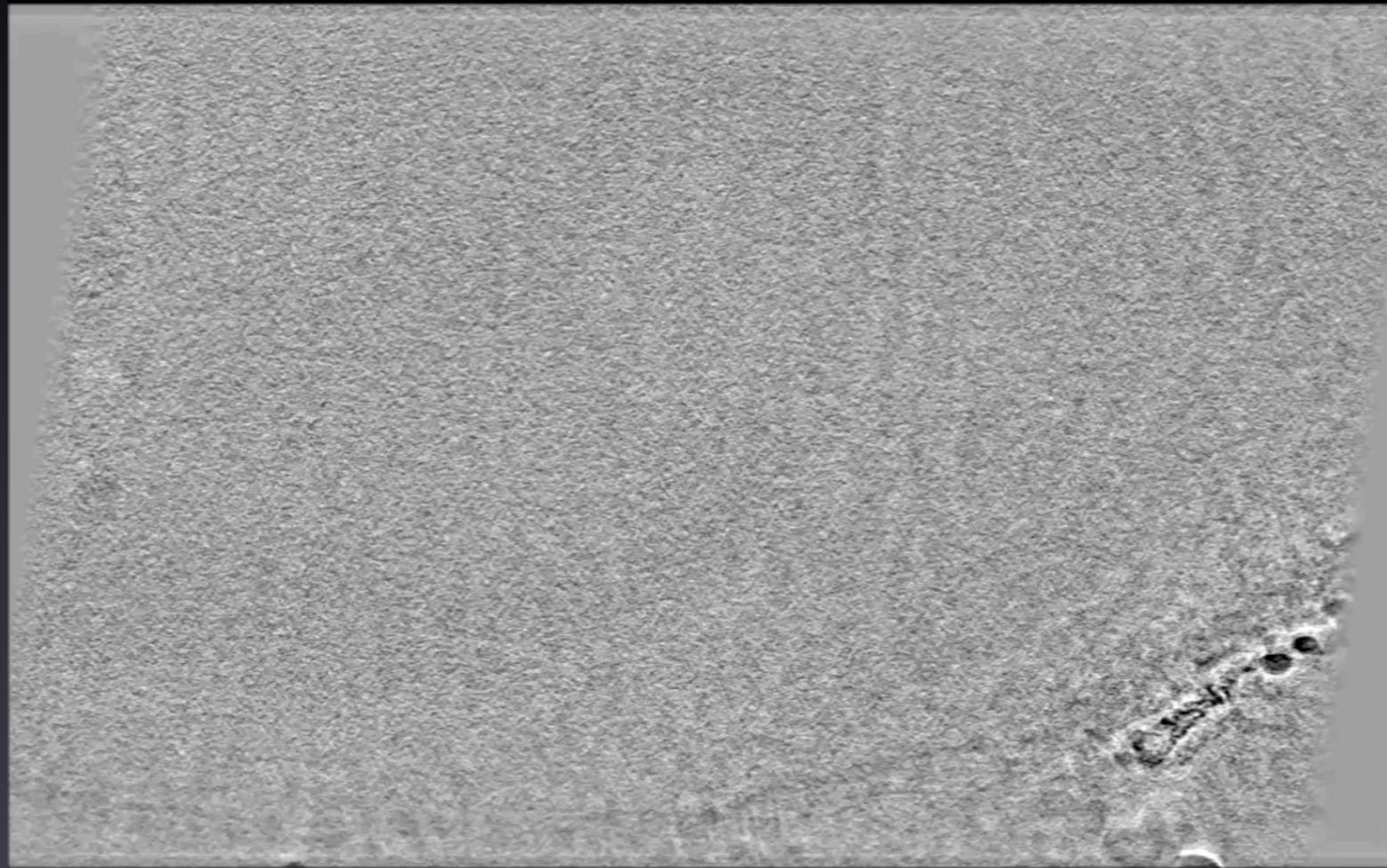
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## Routine tomography of single particle samples:



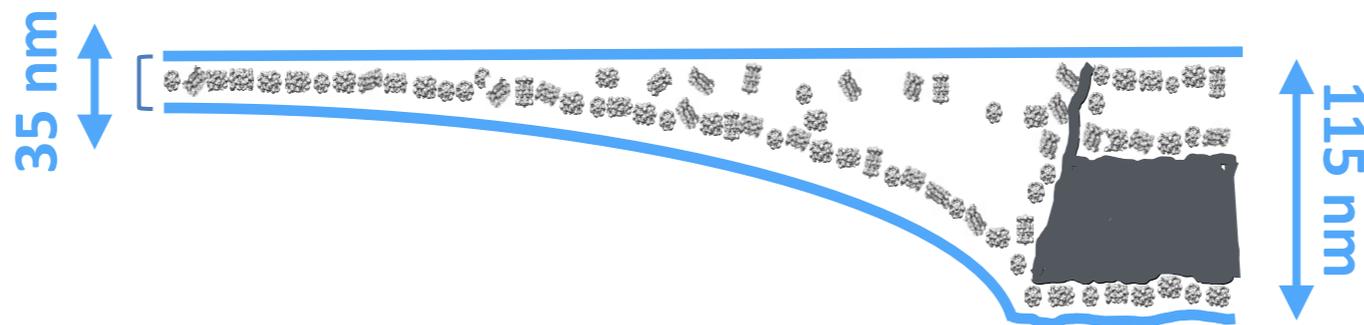
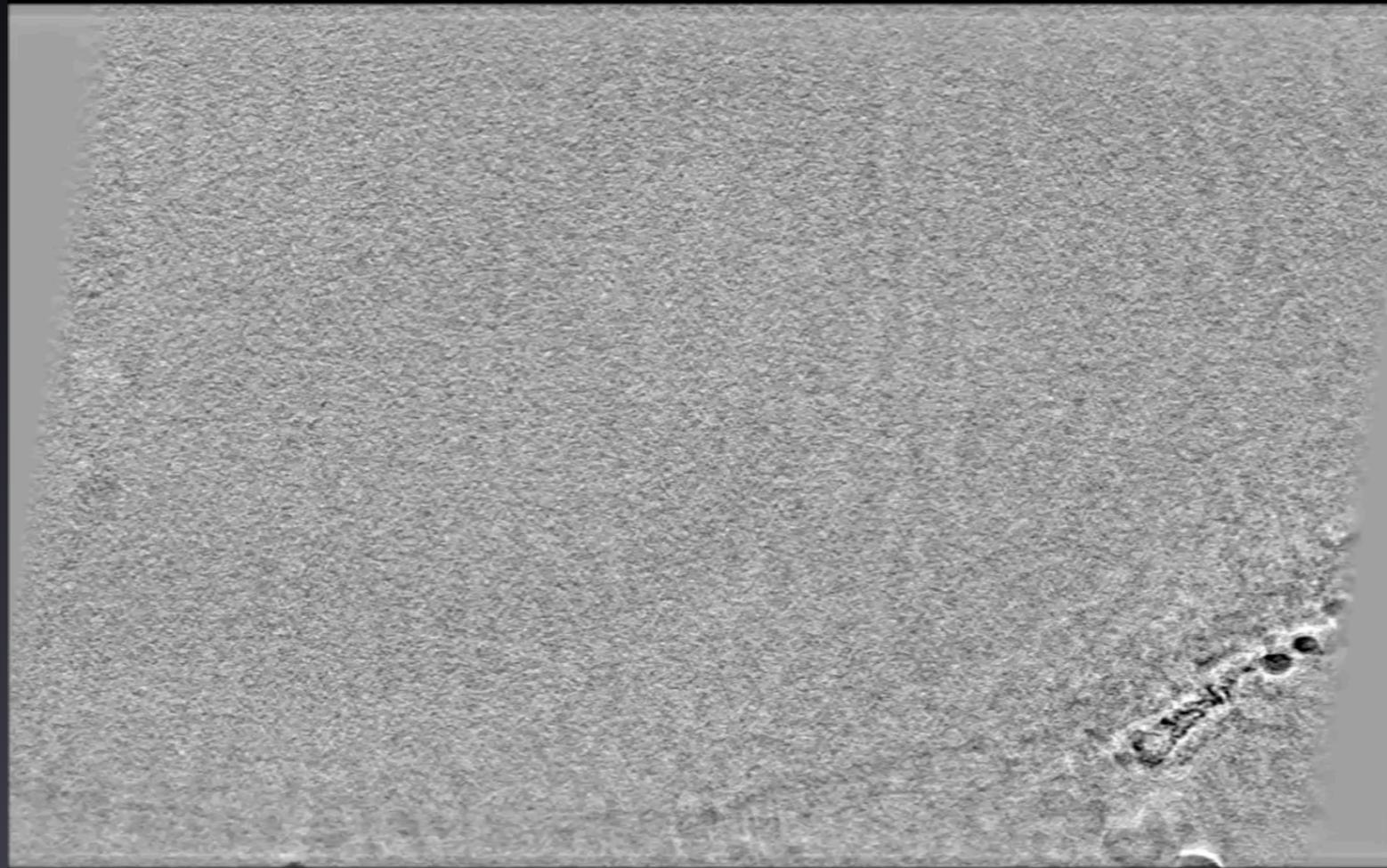
Noble AJ, Stagg SM. Automated batch fiducial-less tilt-series alignment in Appion using Protomo. *J Struct Biol.* 2015;192(2):270-8. PMID: 25711111.



Alex Noble

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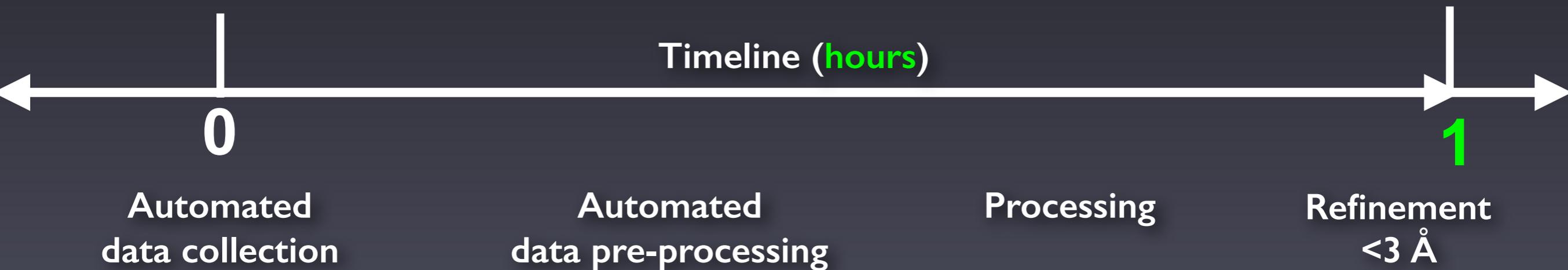
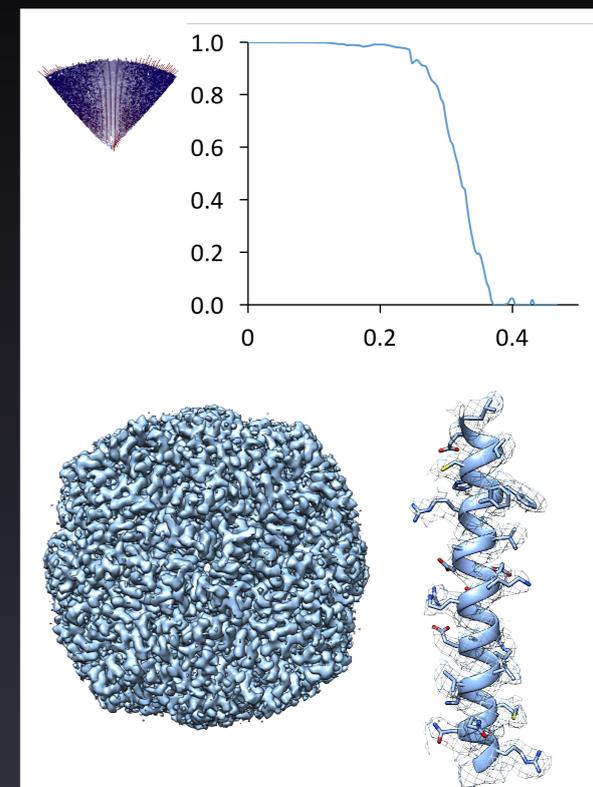
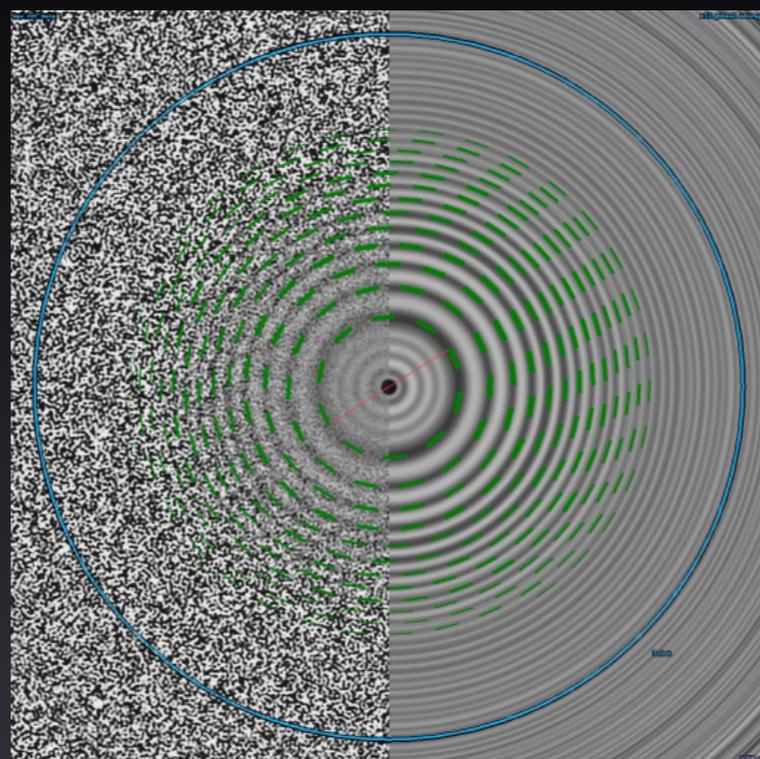
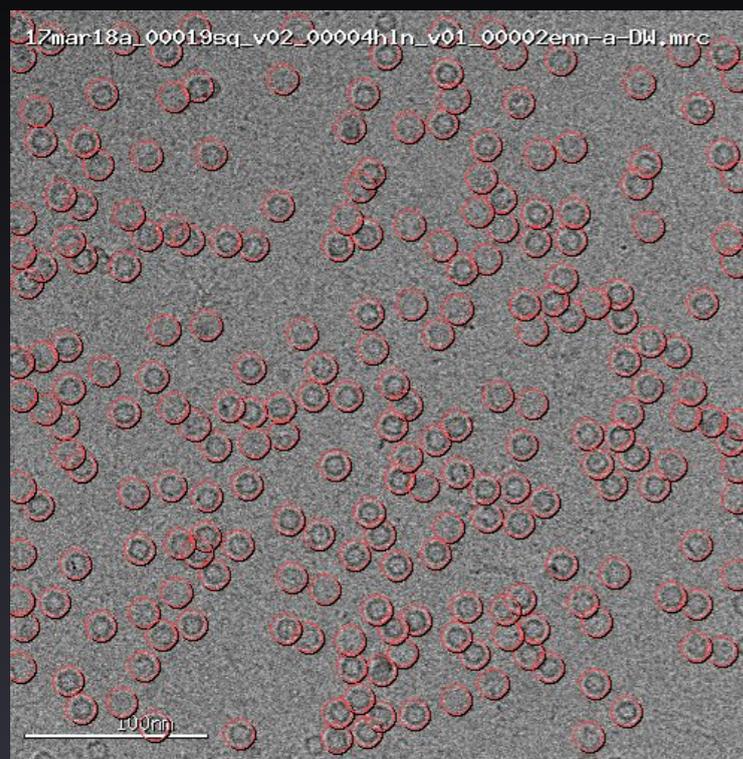


Noble AJ, Stagg SM. Automated batch fiducial-less tilt-series alignment in Appion using Protomo. *J Struct Biol.* 2015;192(2):270-8. PMID: 4633401.

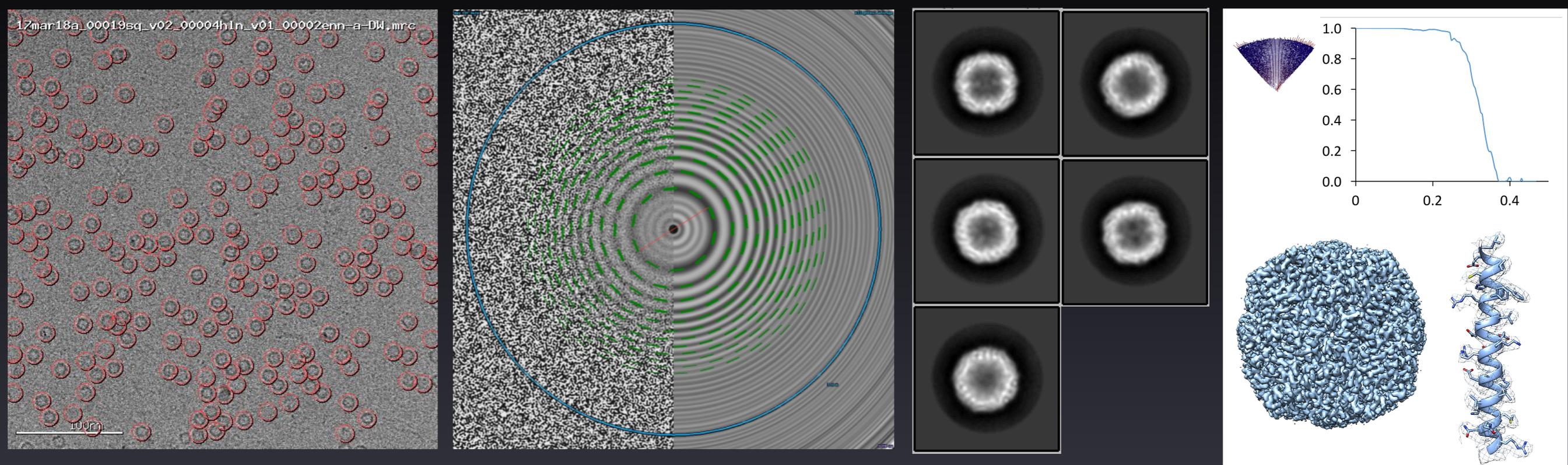


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# CryoEM: Challenges and Opportunities



# CryoEM: Challenges and Opportunities



Timeline (hours)

0

1

Automated data collection

Automated data pre-processing

Processing

Refinement <math><3 \text{ \AA}</math>

Many more users  
Dynamic and heterogeneous particle populations (~millions of particles)  
Drug target screening?



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EM Manager



Ed Eng  
Staff Scientist



Ashleigh Raczkowski  
Senior Technician



Kelsey Jordan  
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Technician



Crystal Premo  
Administratrix



Yong Zi Tan  
Grad. Student



Micah Rapp  
Grad Student



Venkat Dandey  
Post Doc.



Alex Noble  
Post Doc.



Priyamvada Acharya  
Embedded Post Doc.



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Embedded Post Doc.



Giovanna Scapin  
Embedded Scientist



Anchi Cheng  
Res. Staff Scientist



Sargis Dallakyan  
Res. Programmer



Zhening Zhang  
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