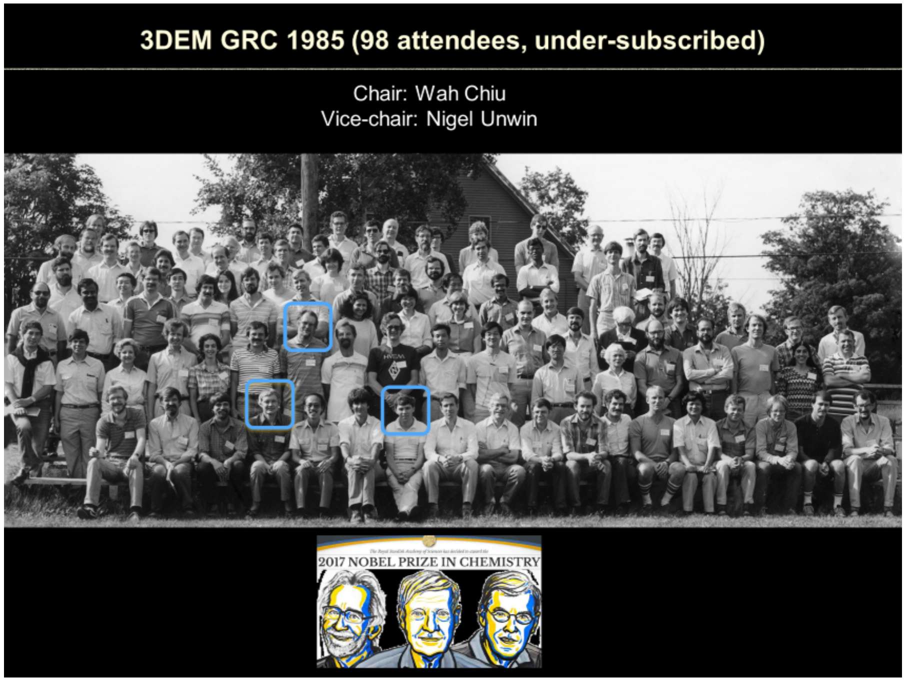
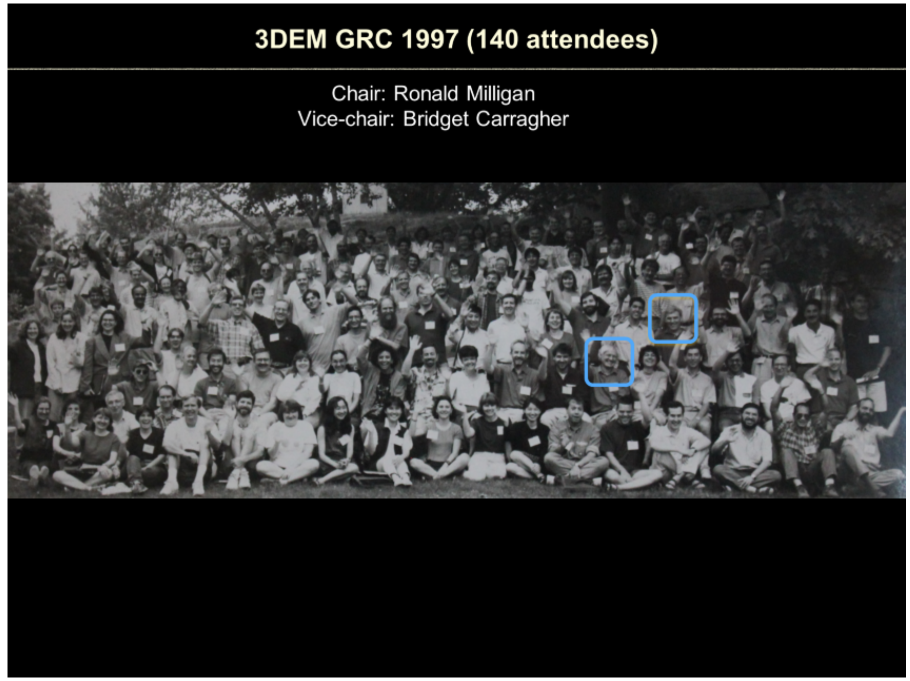
# Grids optimization for Spotiton/chameleon

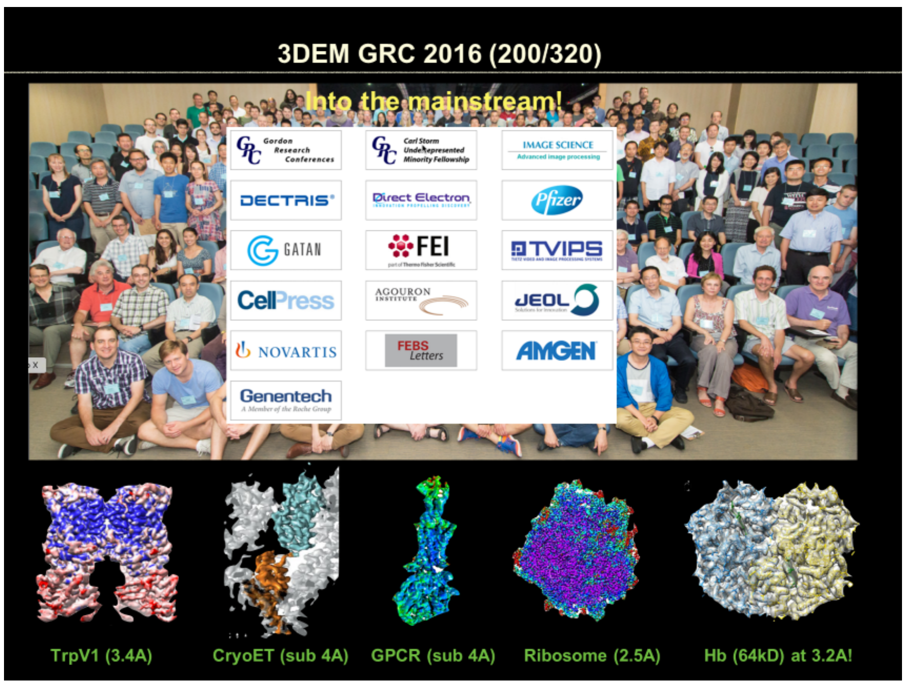
Hui Wei

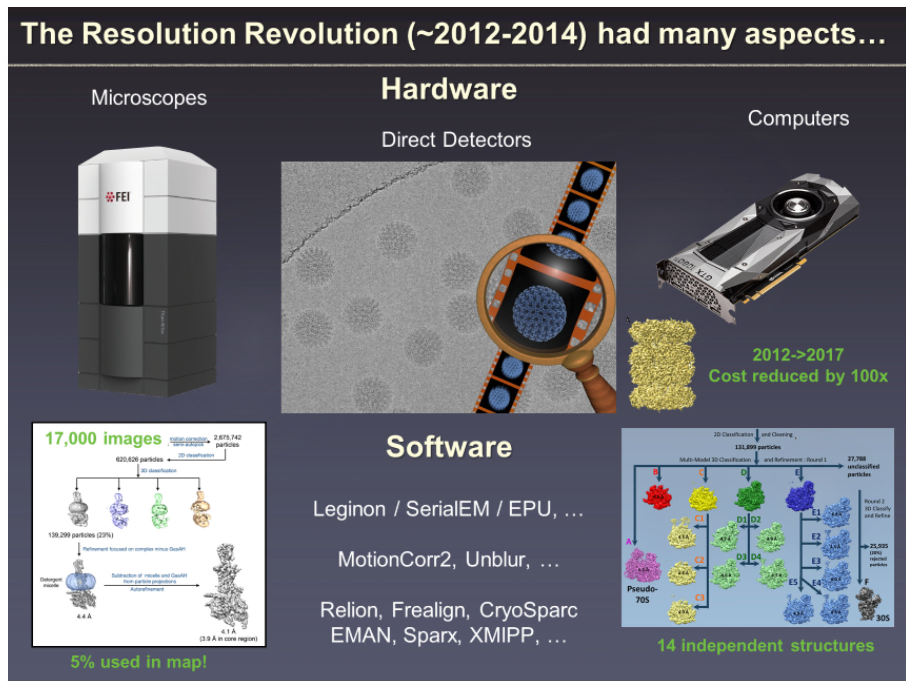
6/4/2020

# Rapid development of cryo-EM in the past 20 years

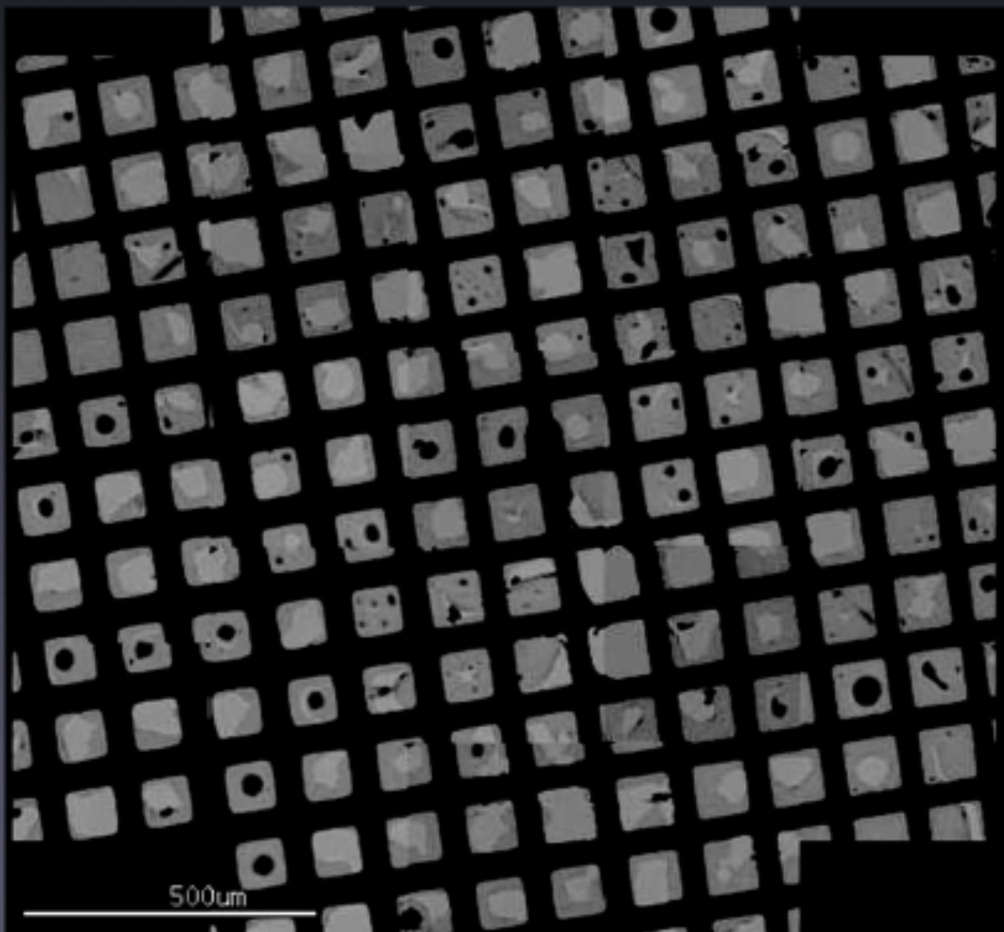


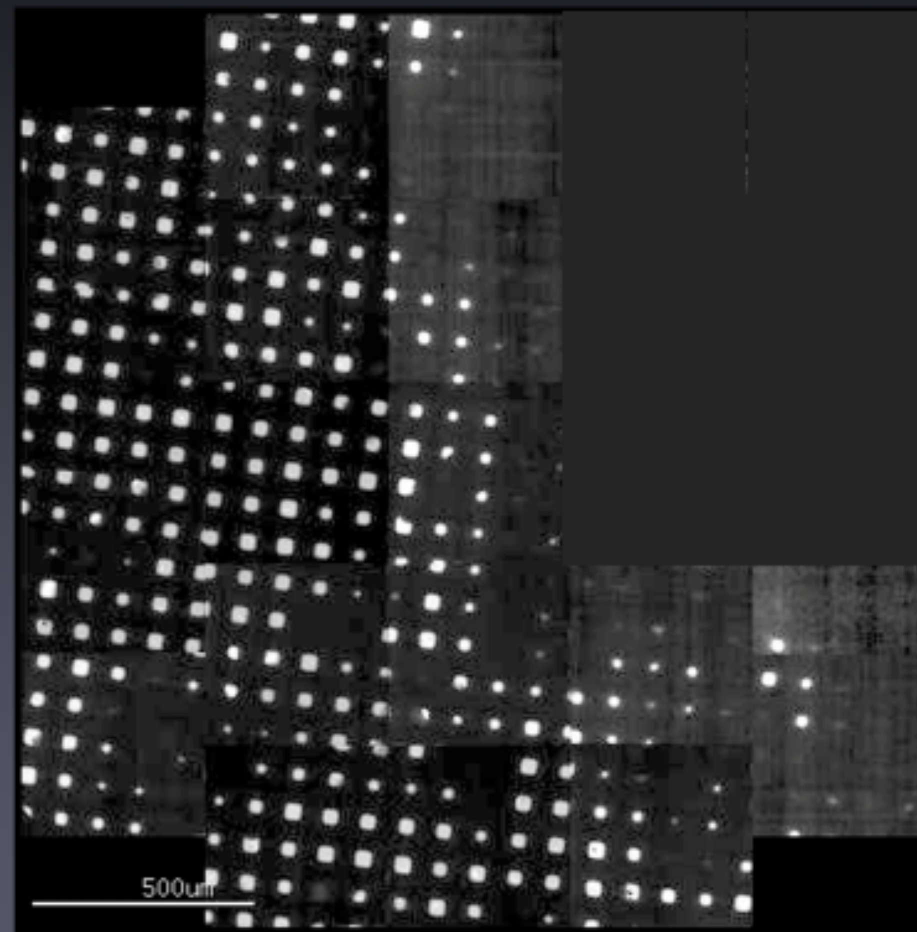



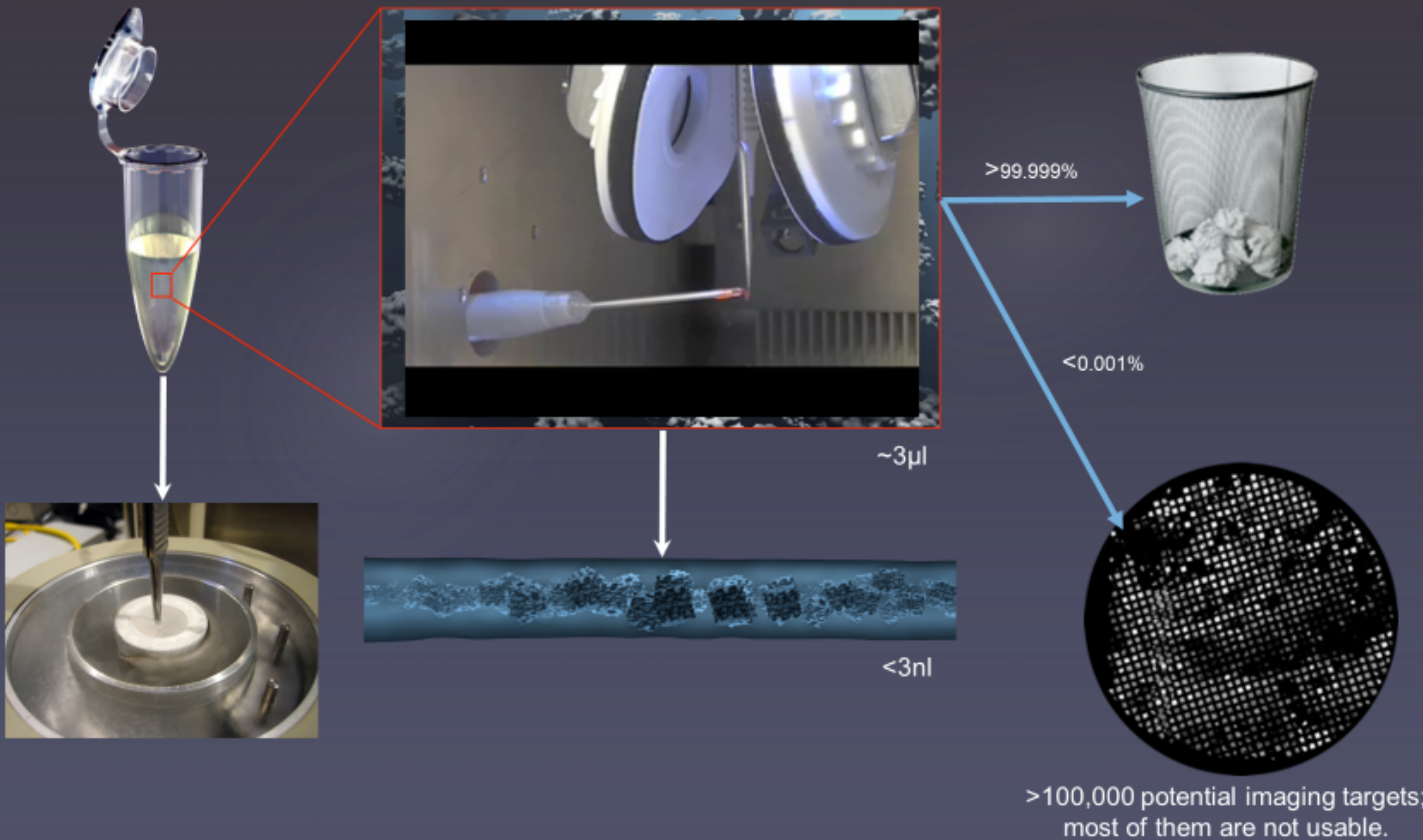



# Grid preparation can still be a challenge…



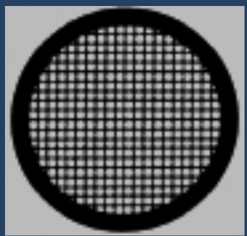



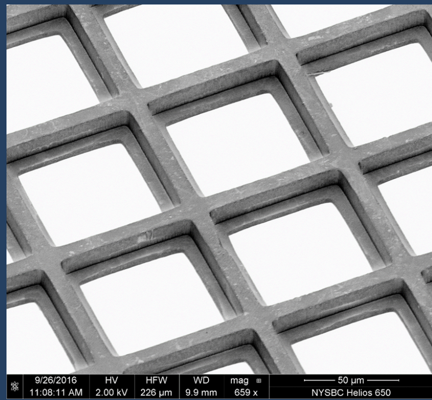
# The Spotiton Project:
# Improving Current CryoTEM Grid Preparation Methods

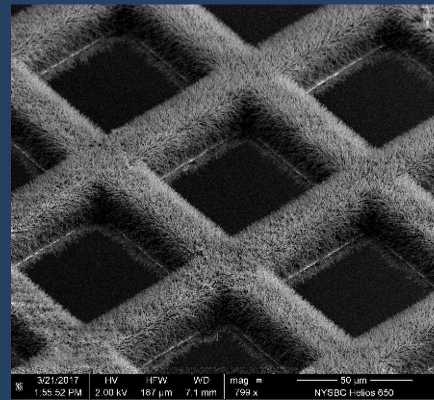


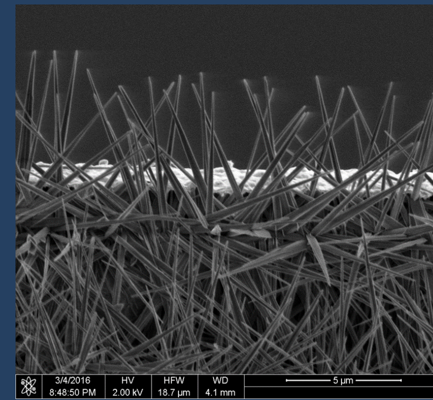

Graphics courtesy Gabe Lander

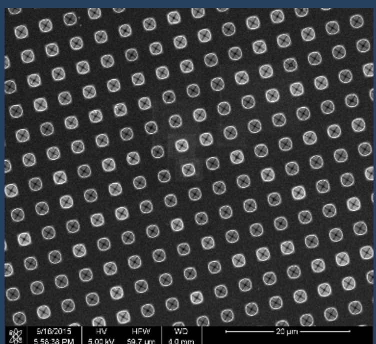
# How is the nanowire gird made and what kind of variations we have

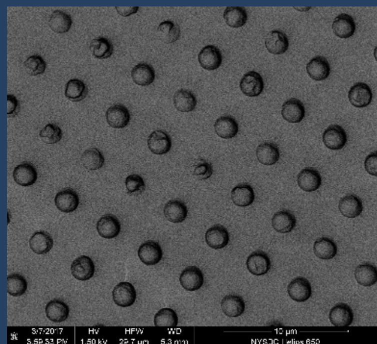


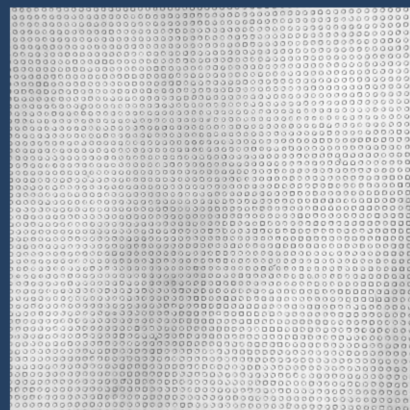


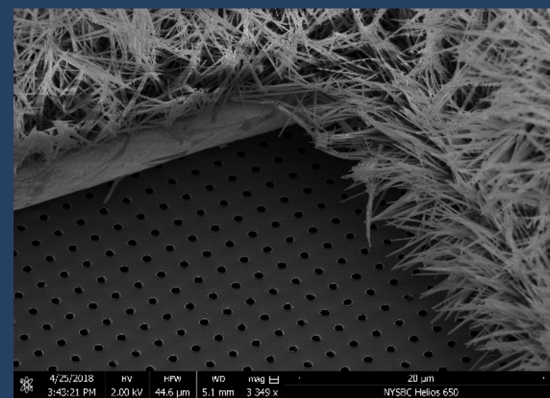






## Prepare glass slide

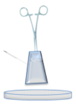
- Soak slides in acetone for at least 4 hours in the glass "slides container box" in the fume hood.
- Pull out the inner glass rack from the staining dish and let slides dry.
- Soak slides tween20 0.03% solution for 2 hours in glass Coplin containers.
- Rinse slides by transferring to Coplin jar full of water.
- Pull out of the water and observe that the water flow off leaving only few drops.
- Let the slides dry

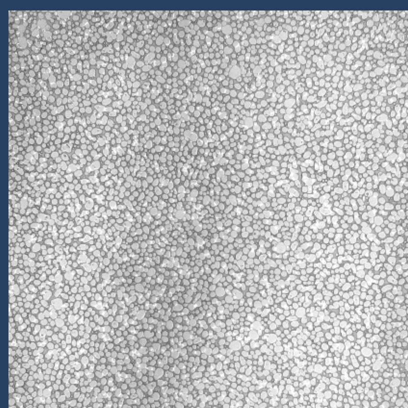
slides container box

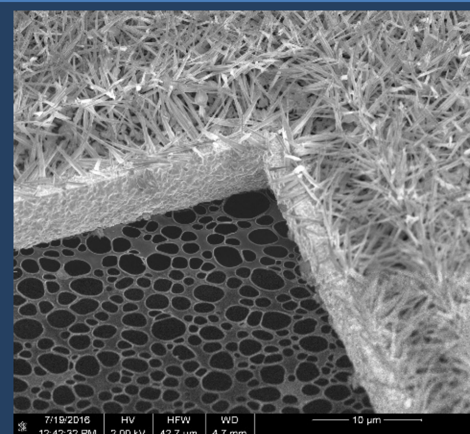
Coplin containers

## Holey film preparation

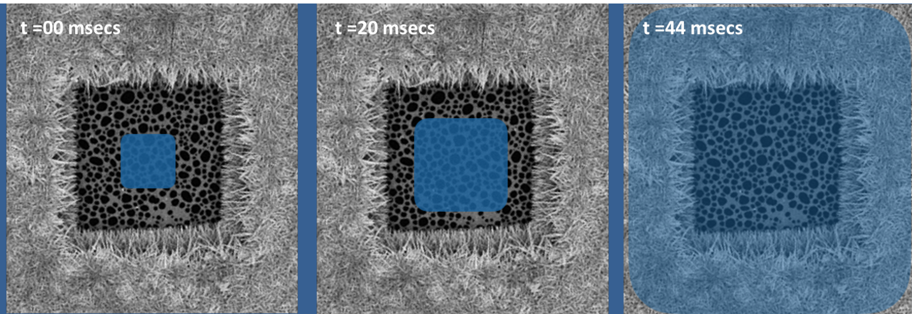
- Prepare the humidity chamber by spray water on the wall (with paper towler attached). The humidity should be at least about 60% at room temp.
- Quickly transfer the slide from outside the chamber into humidity chamber and immediately squirt a nice continuous smooth layer of plastic solution ≈1.5ml of 0.25% plastic in one wide stream by squirting across the top age of each side in one motion.
- Do this move a petri dish lined with tissue to collect run-off and dab the end of the slide to blot the accumulate solution at the bottom edge.
- Let the slide dry. You should see it turn from cloudy to transparent as the water droplet evaporate
- Keep the slide in the chamber until it completely dry
- *****The size of hole and hole/carbon ration is depend on difference dew point between room and humidity chamber*****



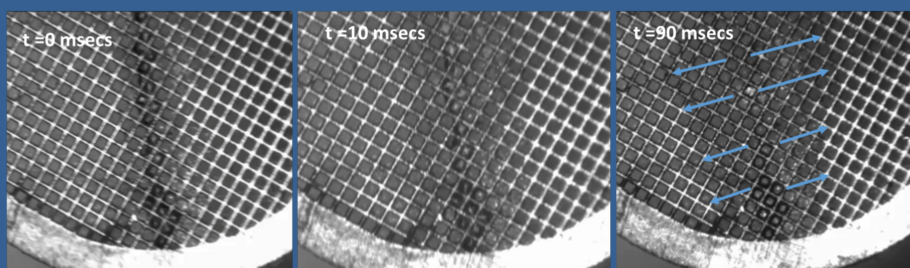




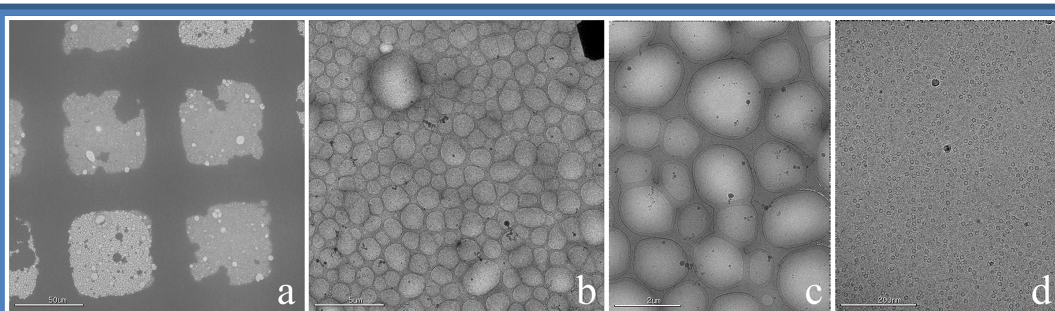
# How is the nanowire help to wick out the excess liquid



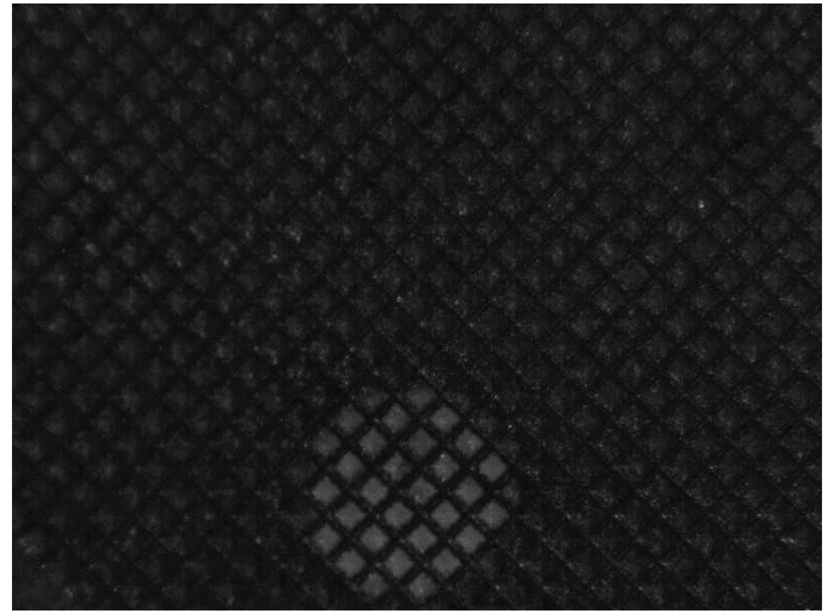

Schematic of a our understanding of the behavior of self-wicking grids. Sequential time steps show a drop contacting the substrate (left), spreading out (middle) and then rapidly wicking to a thin film when it comes into contact with the nanowires.



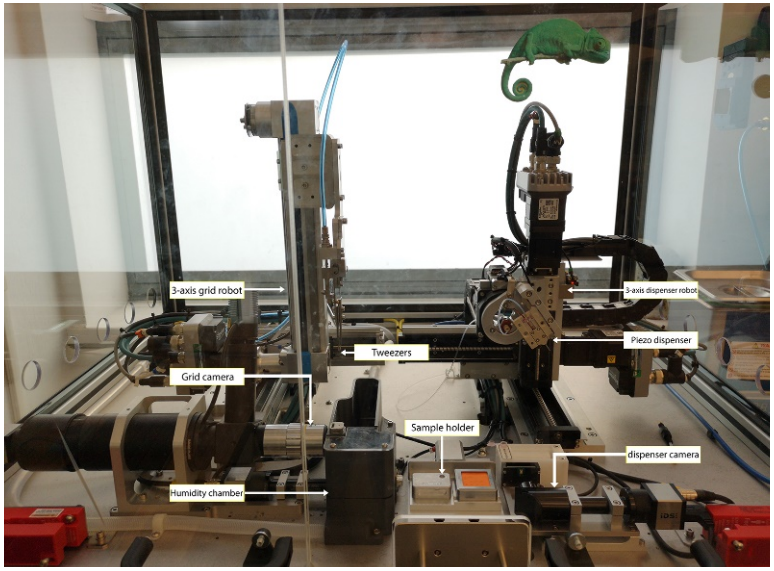

Observed behavior of stream of droplets (~30 pLx80 drops) applied to a grid moving rapidly past the piezo head. Sequential time steps show that the droplets spread rapidly as soon as they come into contact with the nanowires.



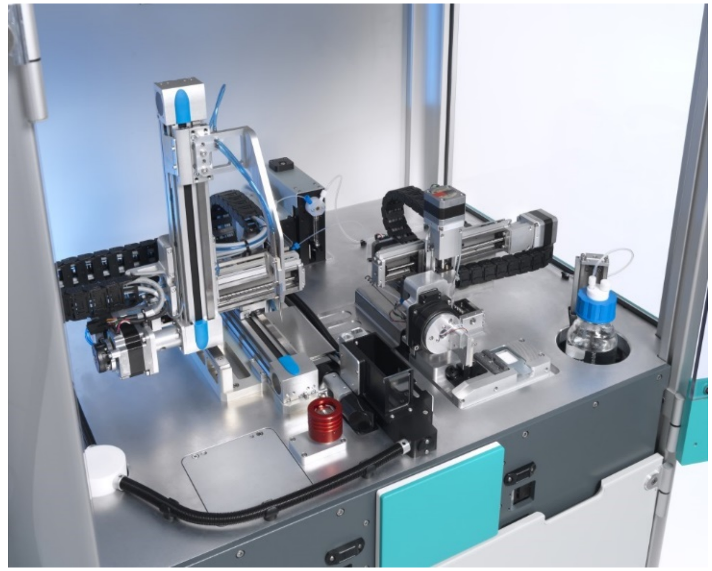

Sequentially increasing magnification images of a vitrified sample prepared using a carbon lacey supporting film on a nanowire grid. a, b, c, and d are the sequential images indicate that the ice is of consistent good quality and particles are well distributed.



# From Spotiton to commercial product




Experimental Prototype at NRAMM

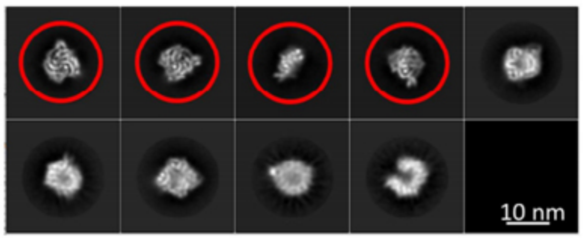


Chameleon (deck view)

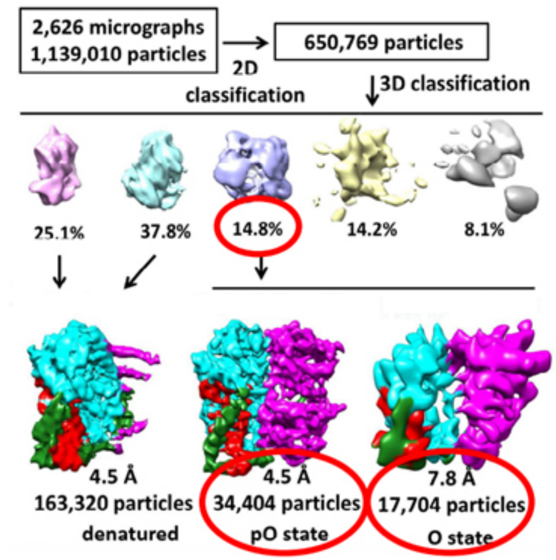

Chameleon (whole system)

# A couple of success examples using chameleon #1

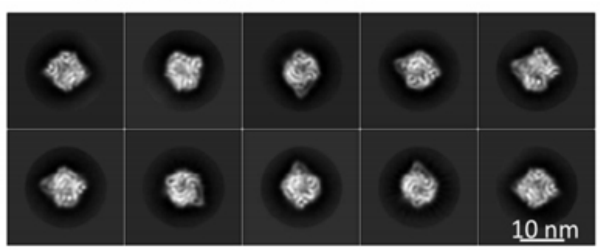
- 2-domain protein – hydrophobic & charged
- On Vitrobot: only 15% of particles have both domains




Wei-Jen Tang at the University of Chicago,
Hui Wei, Bridget Carragher, Clint Potter at NYSBC




- 2-domain protein – hydrophobic & charged
- On Vitrobot: only 15% of particles have both domains
- **On chameleon: 73% of particles have both domains!**

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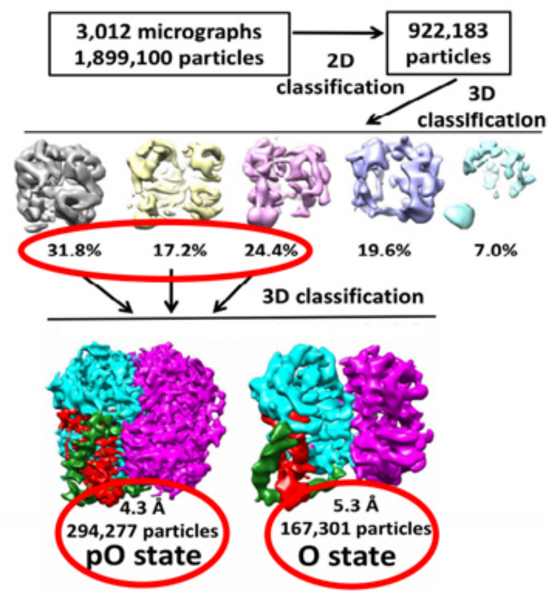
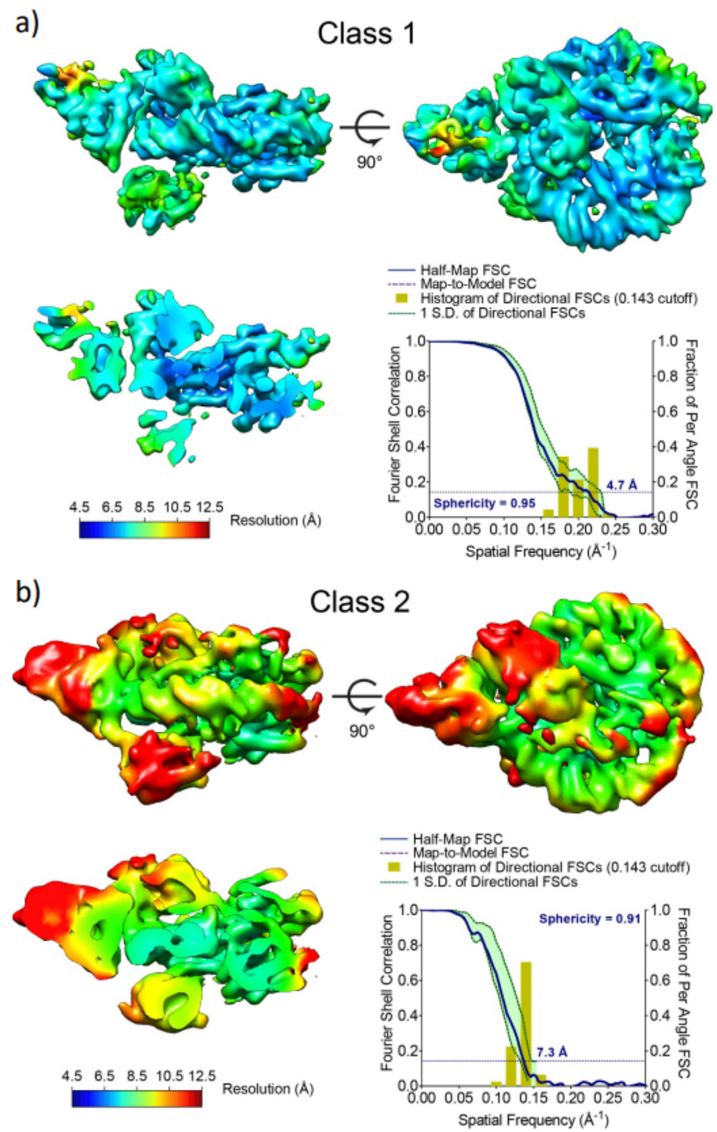


# A couple of success examples using chameleon #2

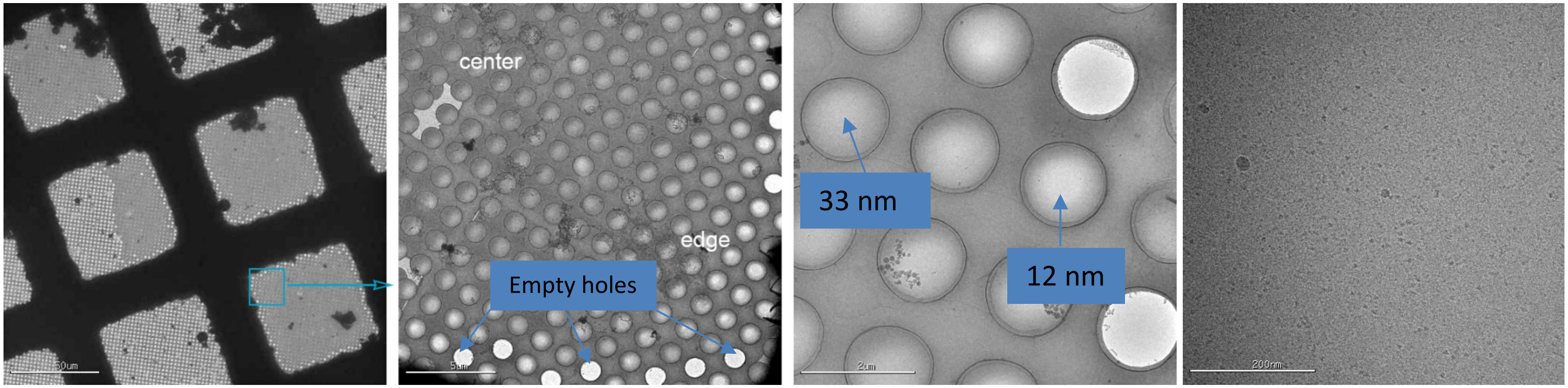
We are able to solve the structure of The histone chaperone 'Facilitates Chromatin Transcription'

(FACT with subunit of SPT16 and SSRP1) with the help of cryo grids made from chameleon which hold the structure together during vitrification.

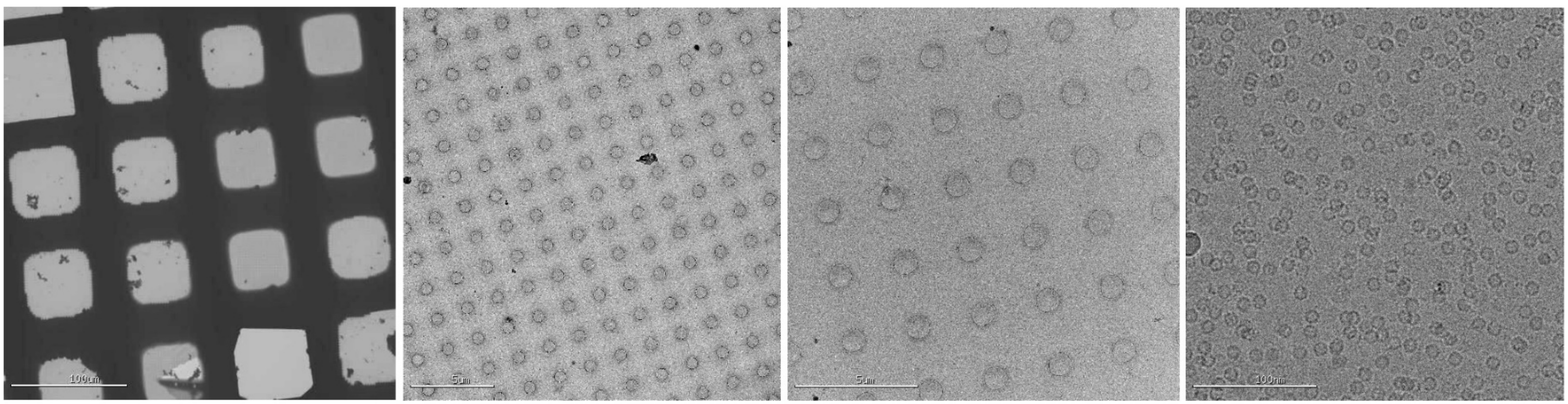
EM data validation (local resolution map, FSC curve, etc.)



# Typical grid we are getting by fast plunging

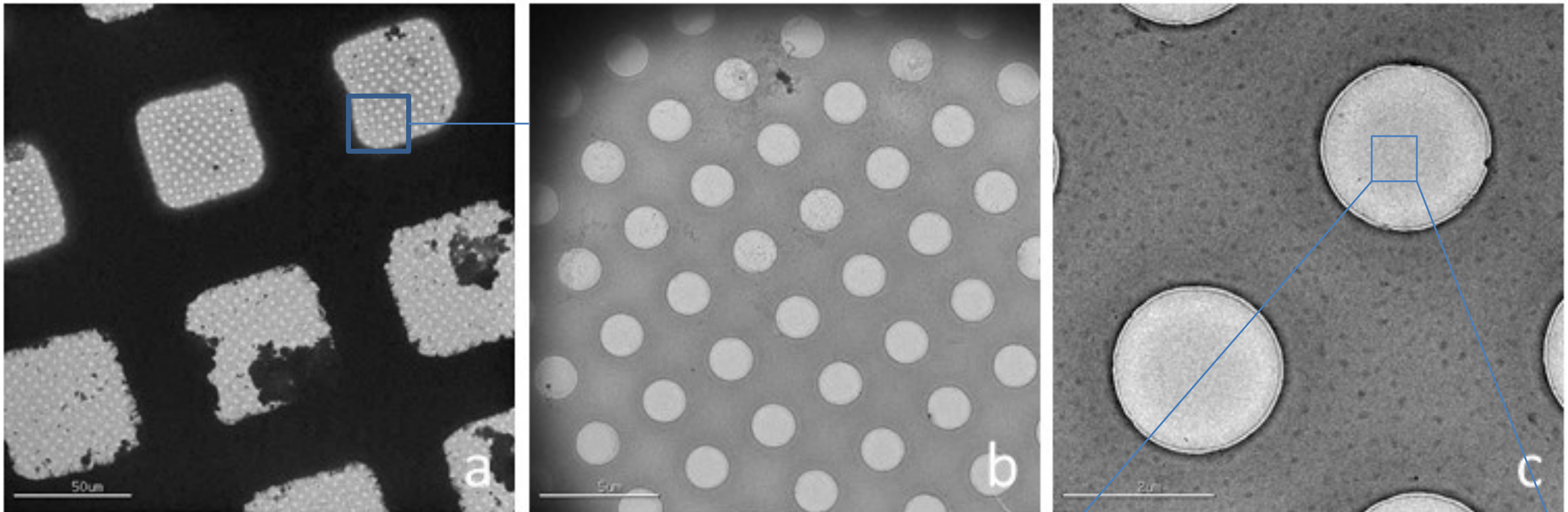


Fast plunged grid at 133 ms

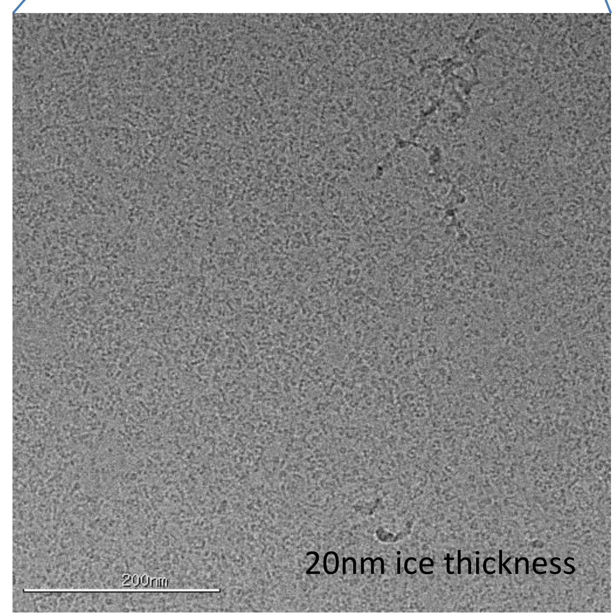

Slow plunged grid at ~400 ms

Fast plunge speed requires fast wicking but sometime it leads to uneven ice distribution (top panel) across grid square. In this case the ice near the edge of the square is either empty or much thinner (12 nm range) than the ice in the middle of the square (can be as thick as 80 nm). Slow plunged grid (lower panel) shows even ice distribution.

# Maintain wicking can help make the ice more even across the whole square




Modifying grid preparation conditions (lower the plasma clean time) in order to maintain wicking when the grid is frozen can produce much better ice across the whole grid square. The square is also intact without any empty holes near the edge.



# Time-resolved cryoEM with Spotiton


enclosed system housing:

nitrogen gas

operation terminal

fluid reservoir

sled for sample holder

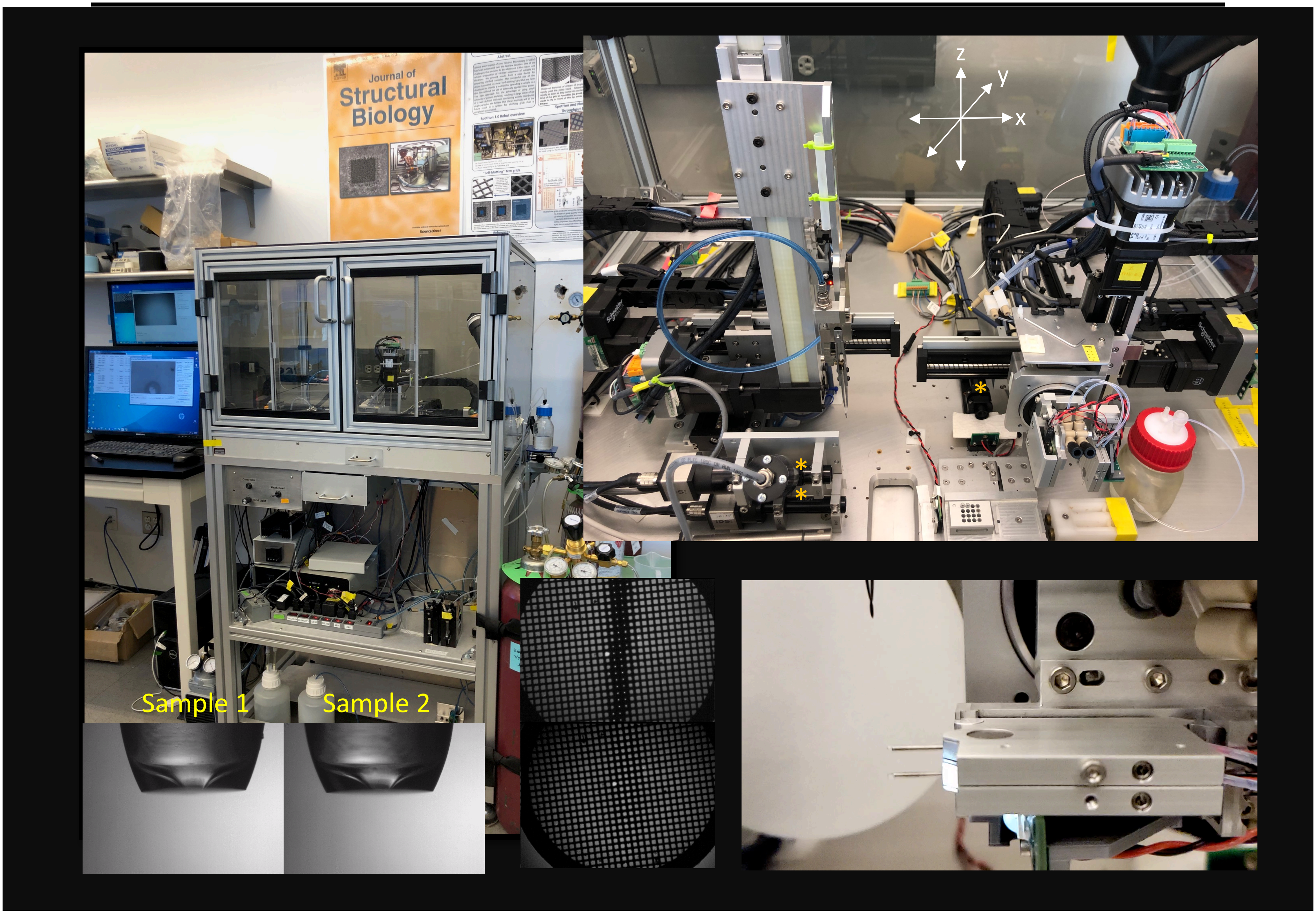
sled for cryogen bowl

humidity monitor

dispensing head
controllers

syringe pumps

ethane


Journal of
Structural
Biology
z
y
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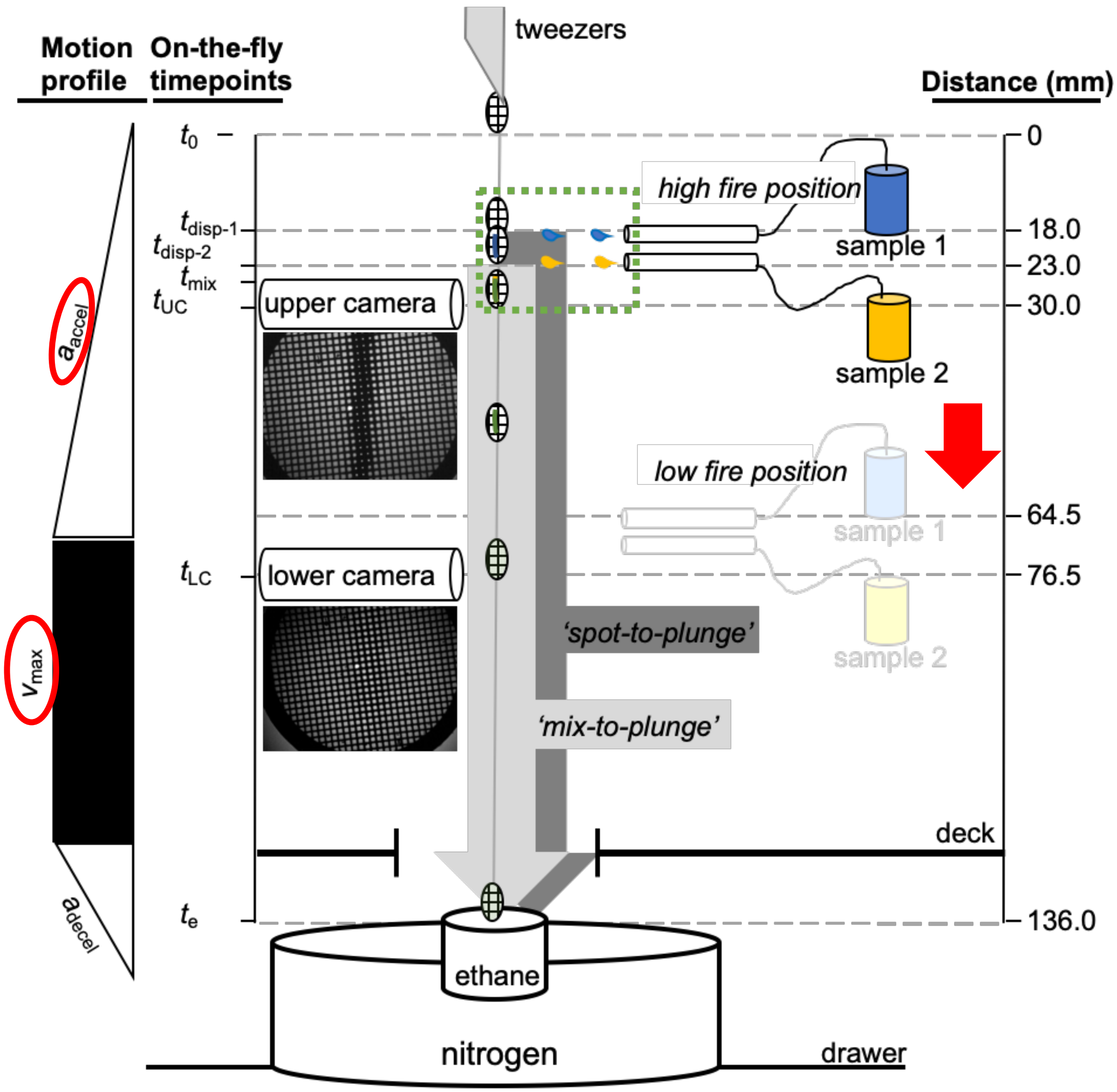
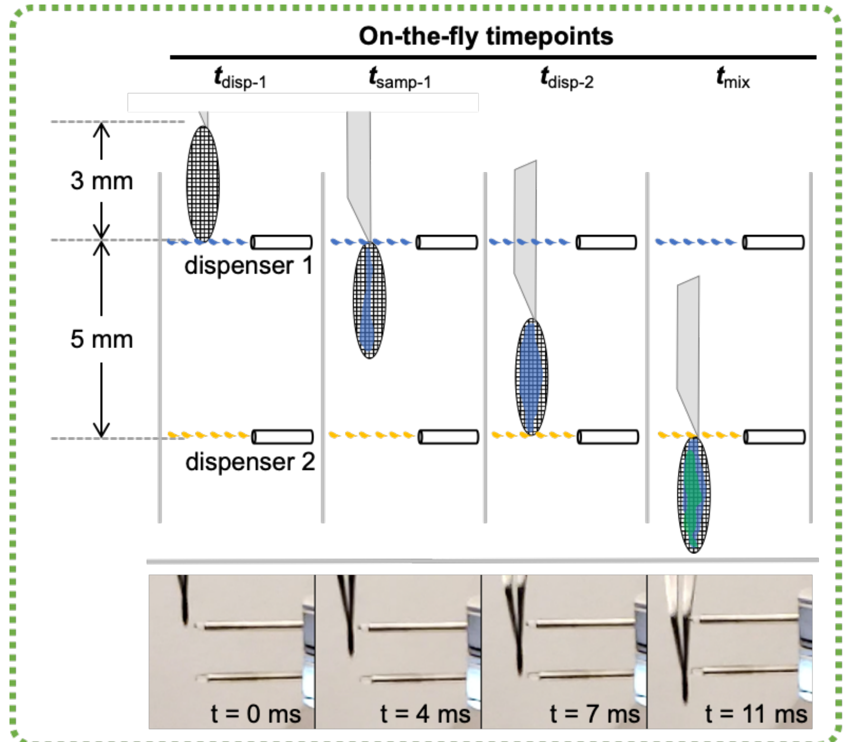
Sample 1
Sample 2



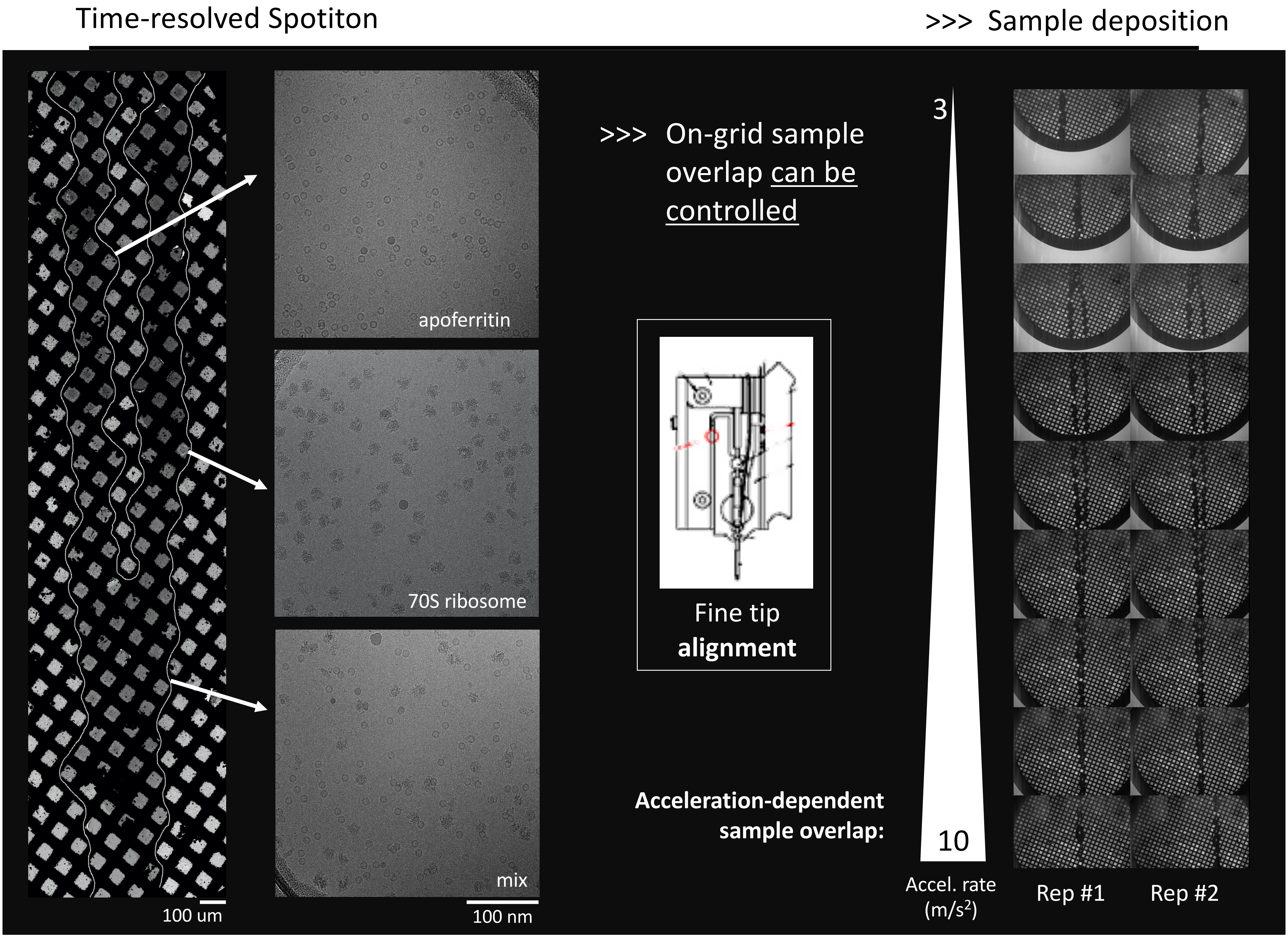




Adjustable parameters:

- Grid acceleration
- Grid max velocity
- Dispenser distance above cryogen

Achievable mixing times:

- Typical: 150 ms
- Minimum: 89 ms
- Maximum: > 500 ms? [limited by wicking on grid]



Time-resolved Spotiton
>>> Sample deposition
apoferritin
70S ribosome
mix
100 um
100 nm
>>> On-grid sample overlap can be controlled
Fine tip alignment
Acceleration-dependent sample overlap:
3
10
Accel. rate (m/s2)
Rep #1
Rep #2