

The
Scripps
Research
Institute

Spotiton: A new approach to EM specimen preparation

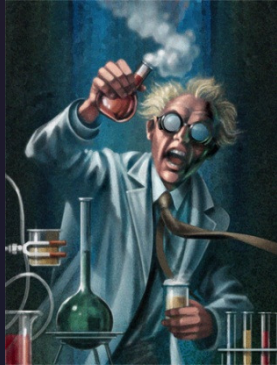
Tilak Jain

Staff Scientist

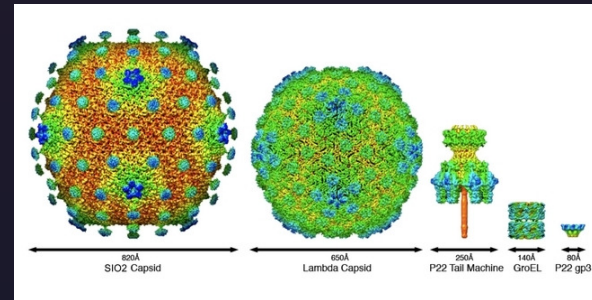
National Resource for Automated Molecular Microscopy
The Scripps Research Institute (La Jolla)

Focus of project

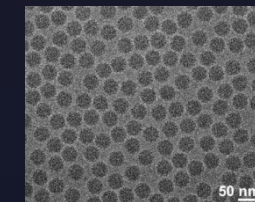
Hard-earned proteins



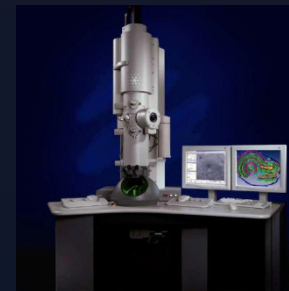
Analysis /
Discovery



3D reconstruction



2D map of particles



Transfer
to EM



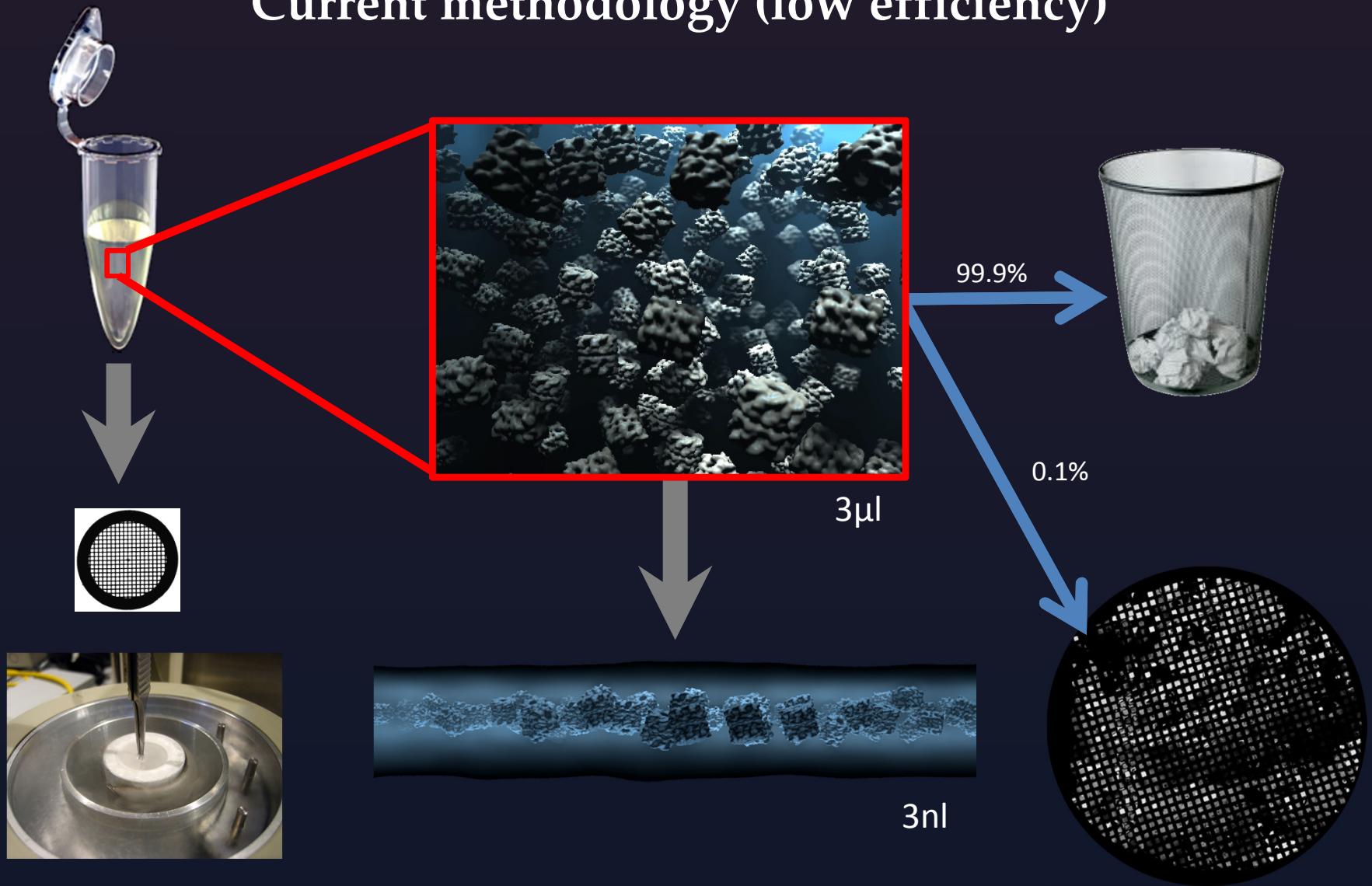
Sample
vitrification
on grids



Samples
transfer

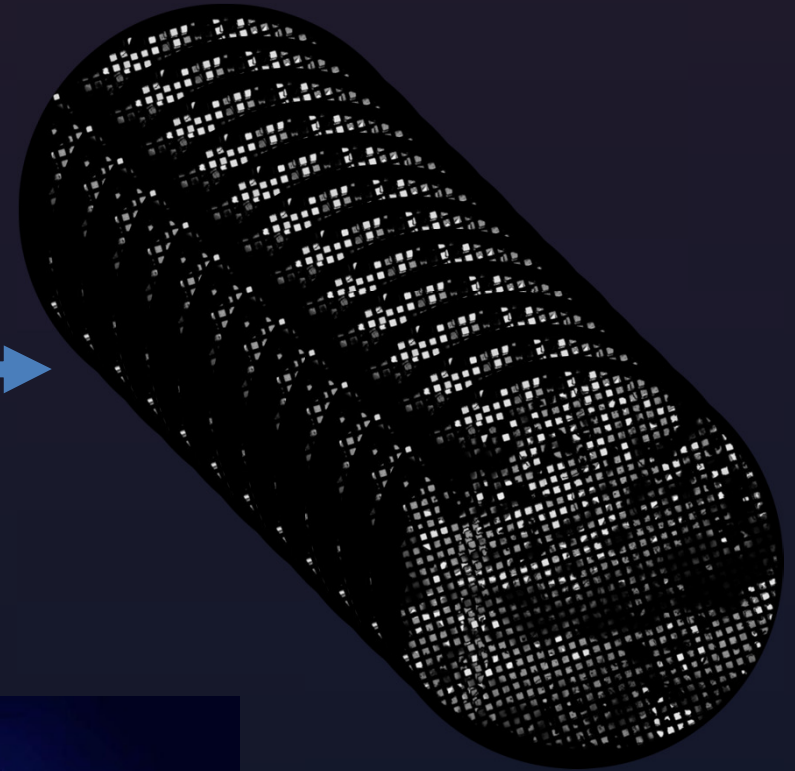


Current methodology (low efficiency)

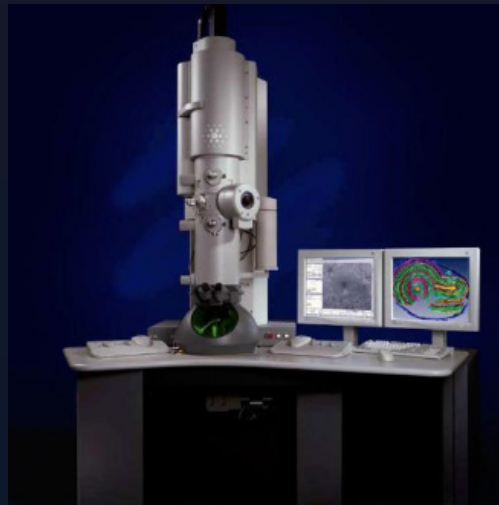


Current methodology (low throughput)

- Buffer conditions
- Concentrations
- Protein states
- Time-points
- Replicates



- Vacuum recovery
- Vacuum crashes
- Contamination
- Manual intensive
- Service requests
- Disillusioned grad students



Next-generation Cryo-EM Specimen Preparation

3 μ l

1 sample

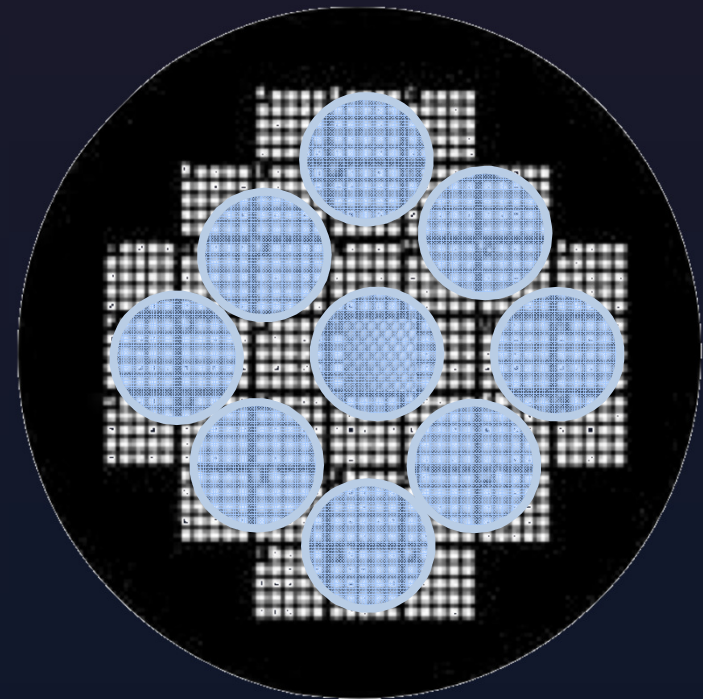
10% usable area



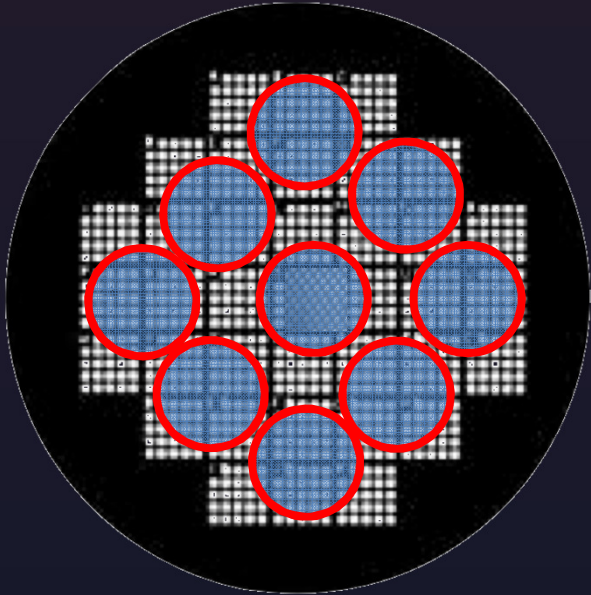
300 p1

9 samples

100% usable area

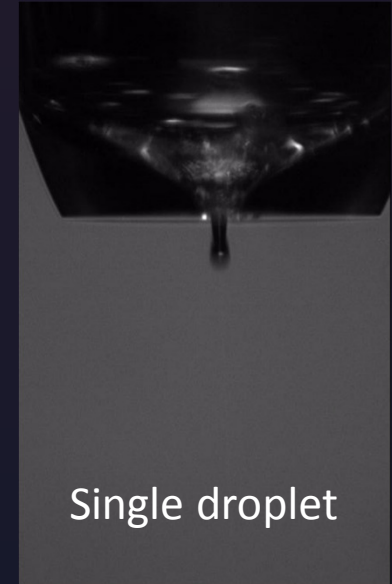
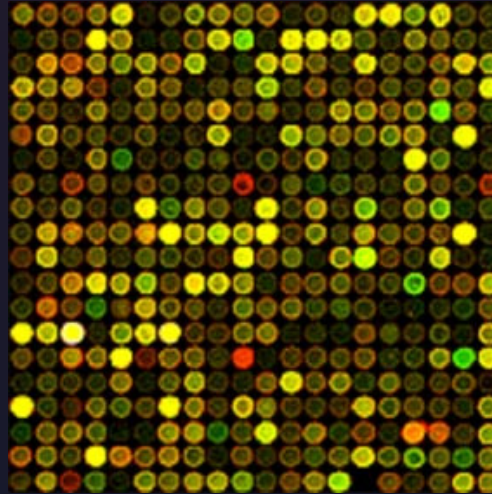


Precision picoliter to nanoliter volume transfer

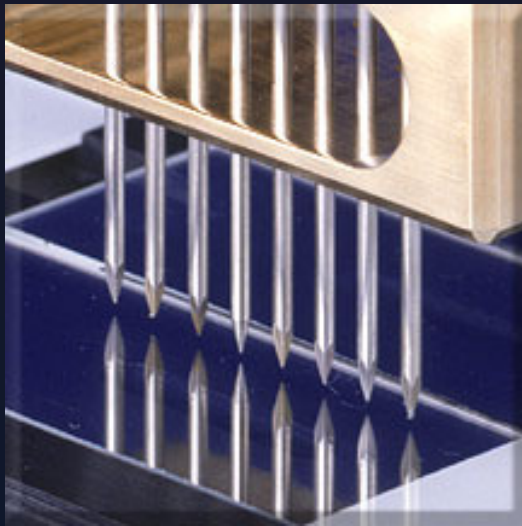


Contact-pin printing*

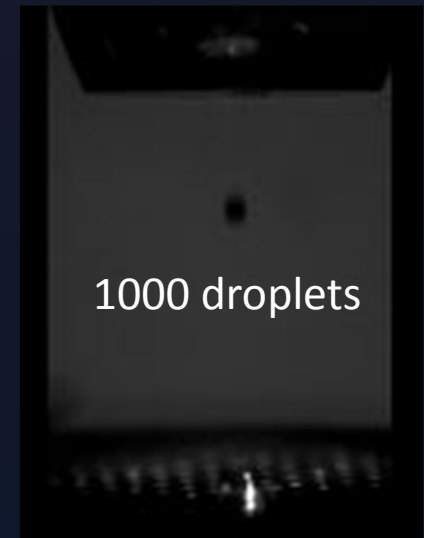
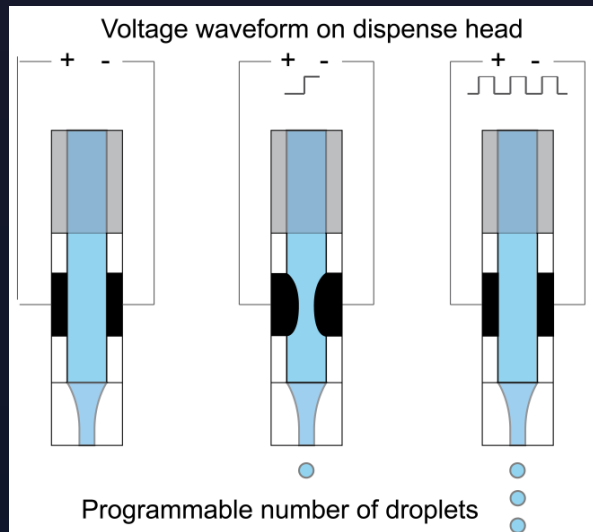
DNA / Protein arrays *



Single droplet

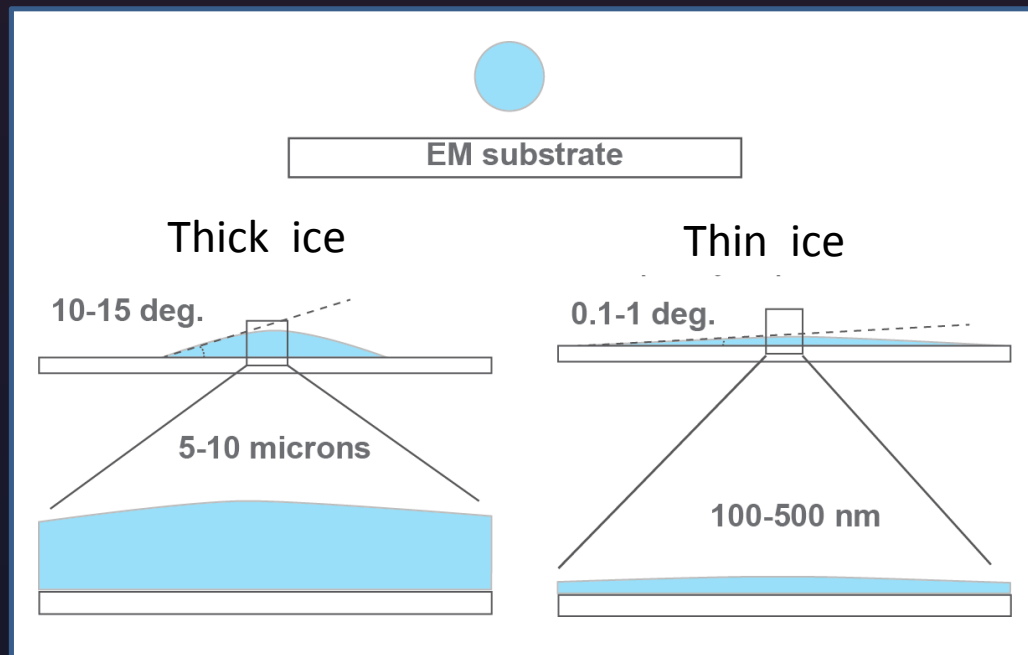
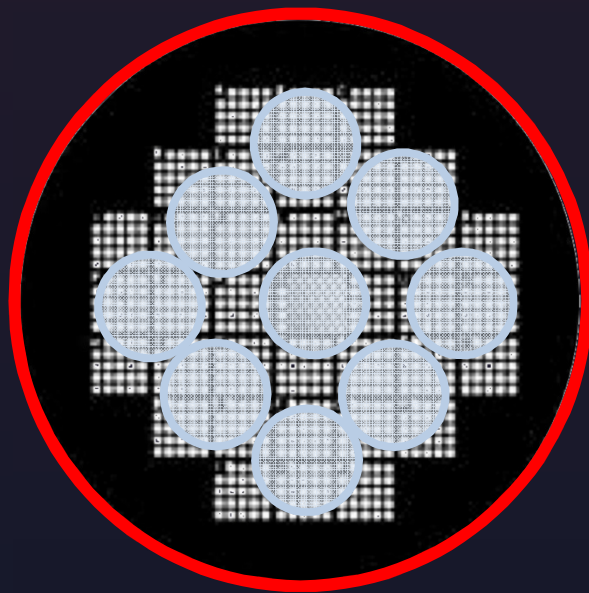


Inkjet dispensing (non-contact)

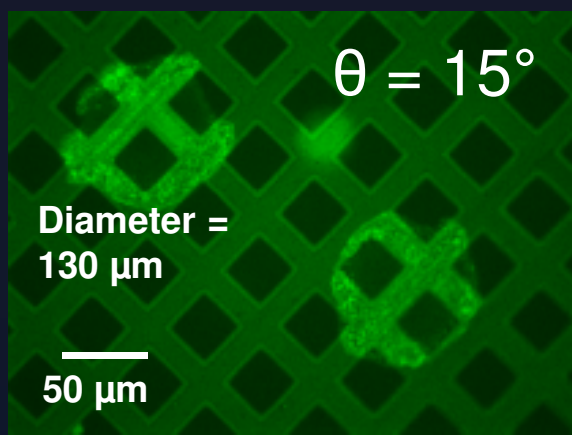


* Images from www

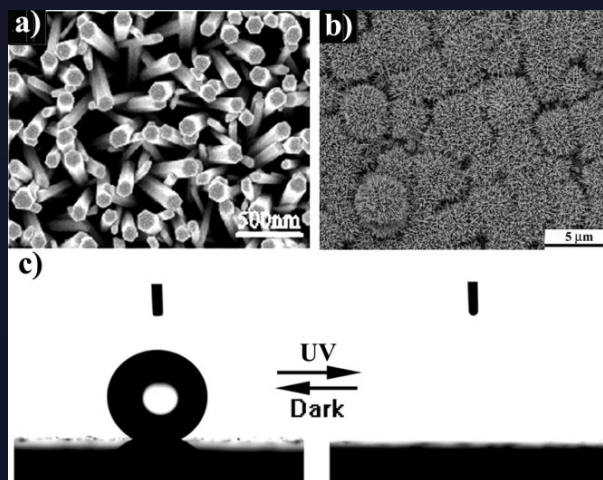
Novel substrates to induce on-grid specimen thinning



Plasma treated carbon



Super-hydrophilic surfaces?



Capillary action?

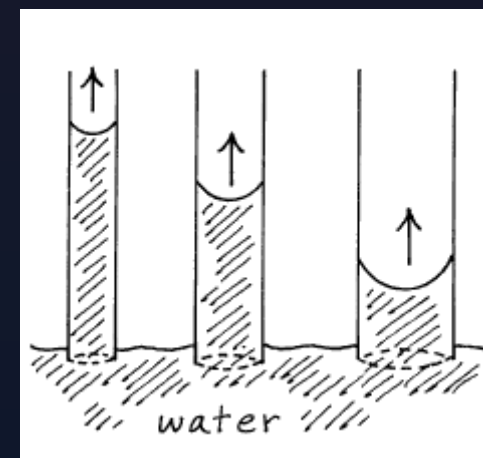
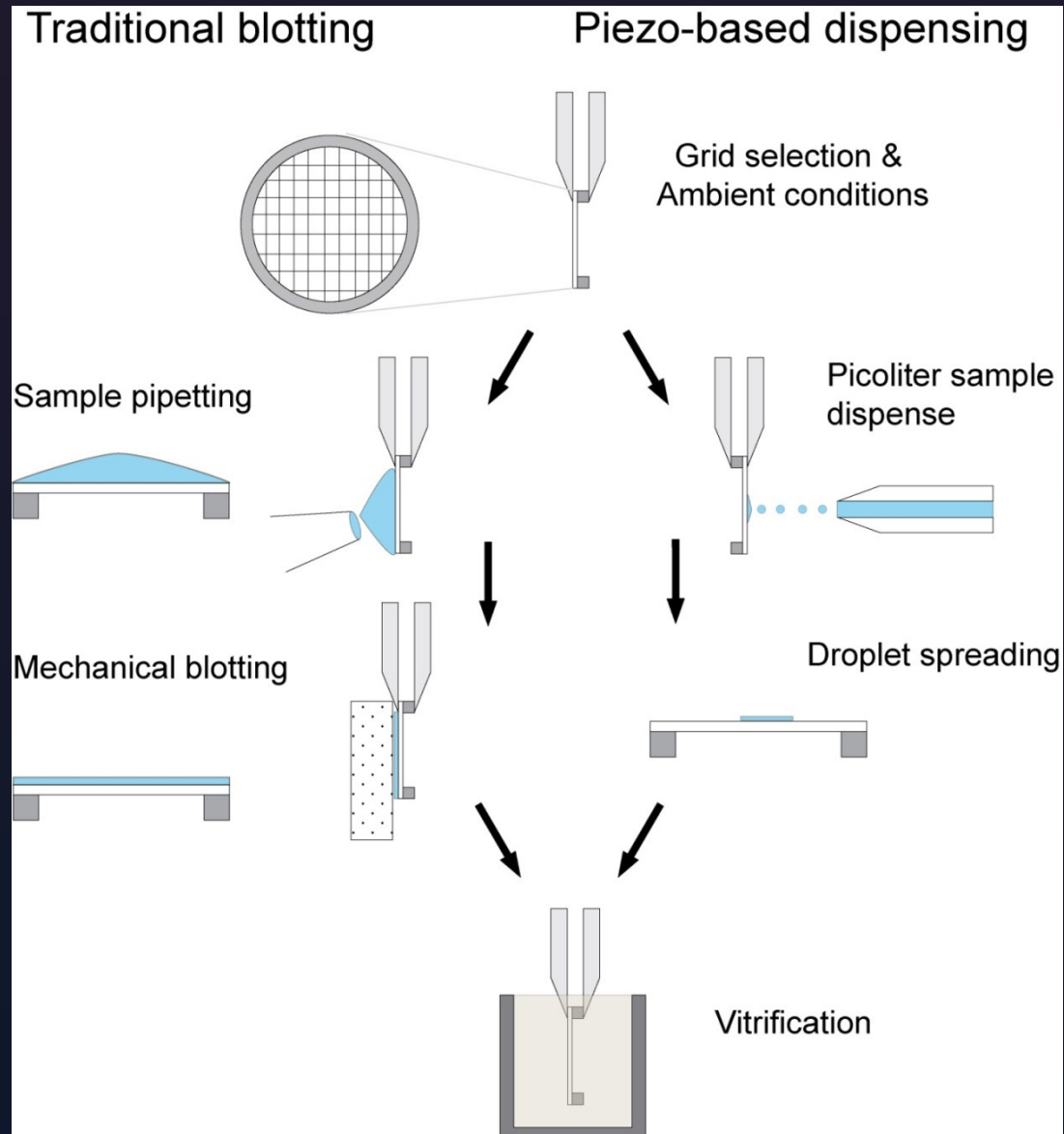
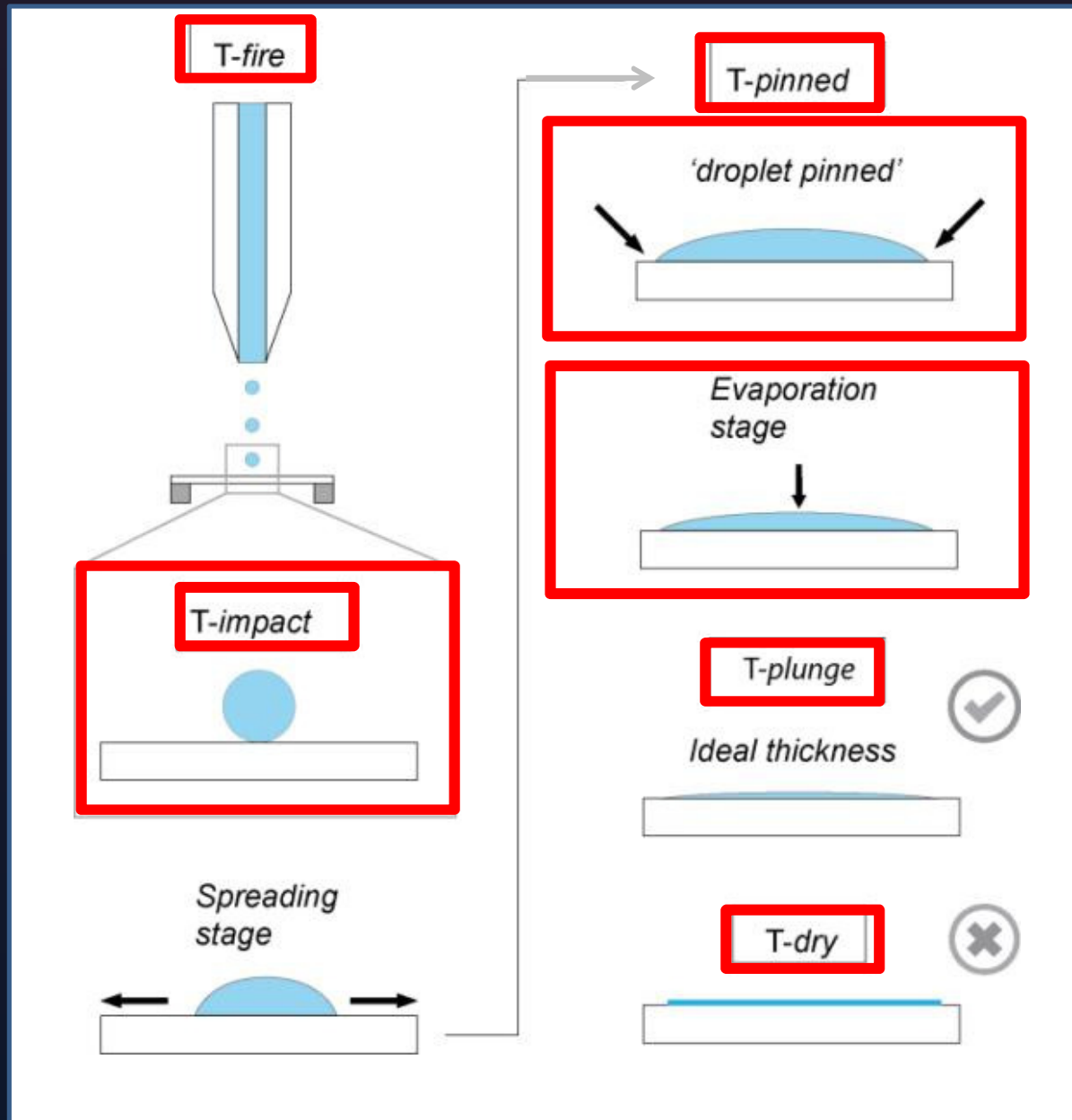


Image from Feng et al, JACS 126 (2004)

Inkjet approach to cryo-EM specimen preparation



Critical elements of approach



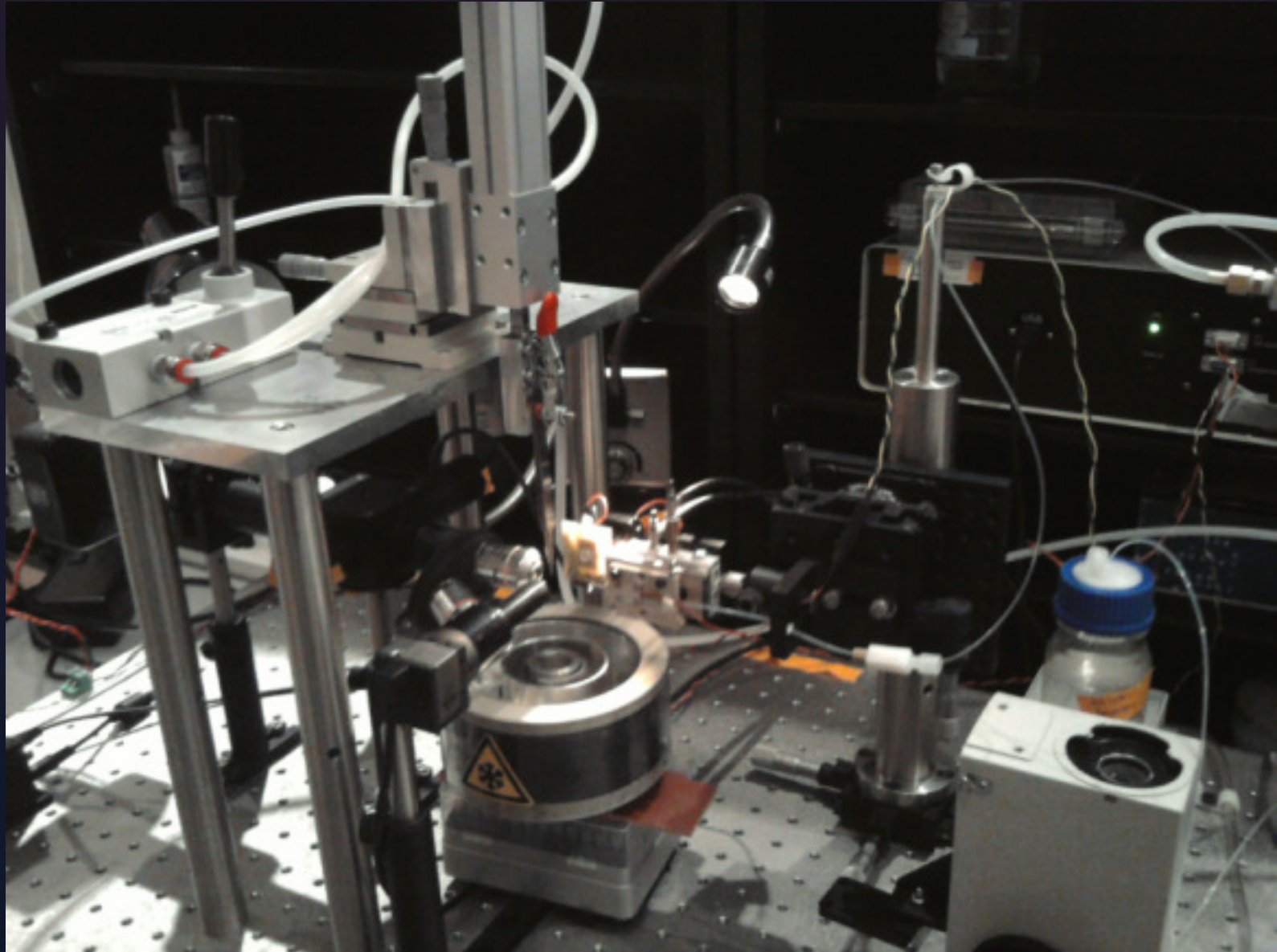
Spatial precision

Surface spreading

Humidity

Timing

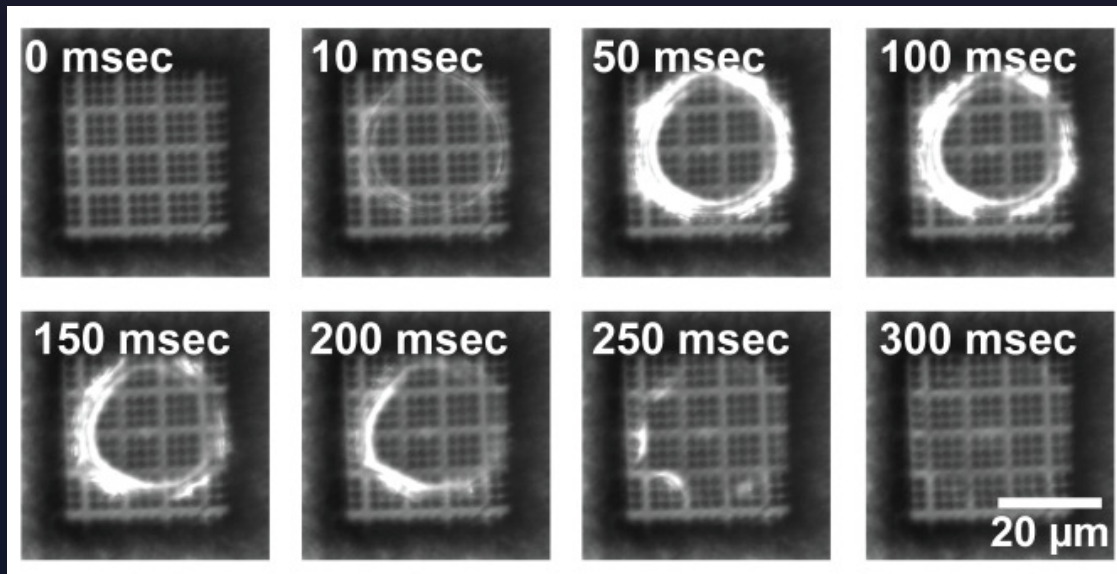
Spotiton system v0.5 (Manual, One inkjet head)



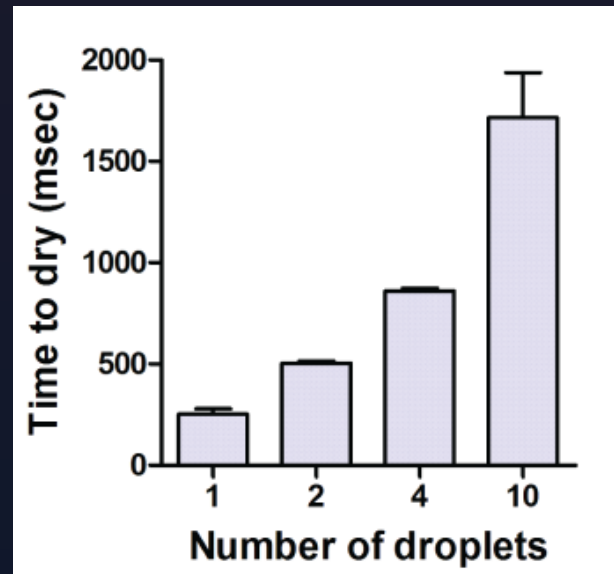
Spatial and temporal precision of specimen dispensing



100 μm



20 μm



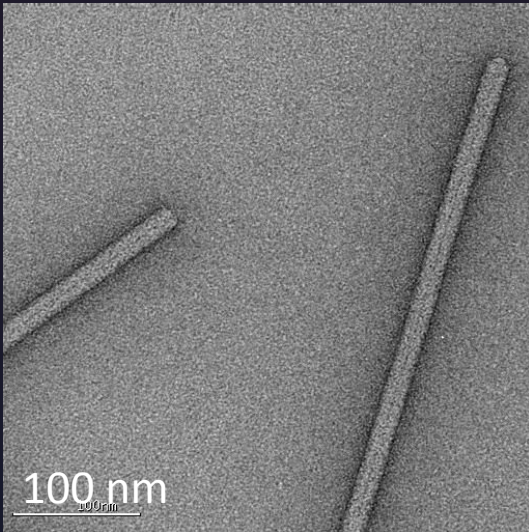
Effect of Relative Humidity (RH%) on evaporation rate

62 pL (2 droplets)
on a glass slides

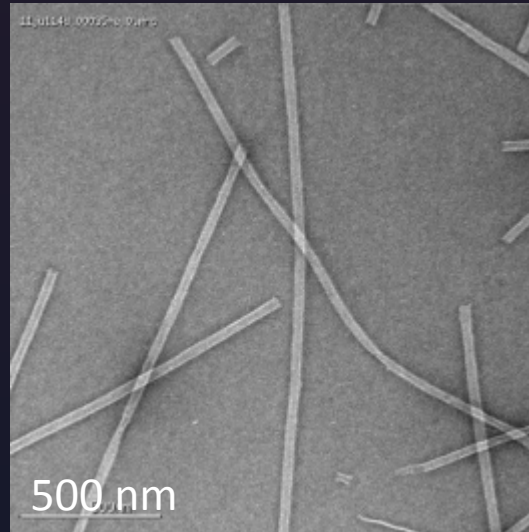
40%	50%	60%	70%	80%	90%	93%
0.8 sec	1.3 sec	2.0 sec	2.2 sec	3.6 sec	23.7 sec	92.0 sec

Stability of particles dispensed using inkjet

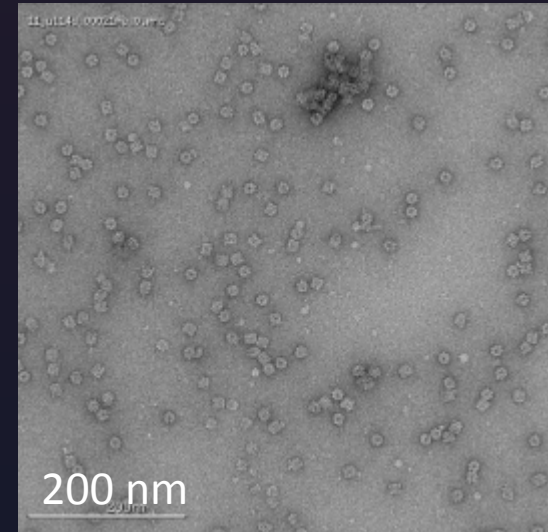
TMV



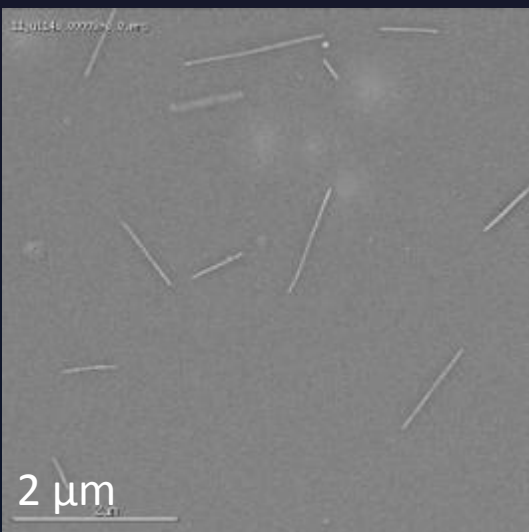
Microtubules



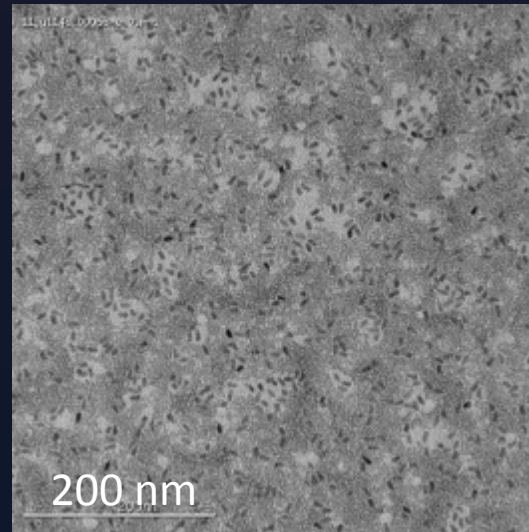
GroEL



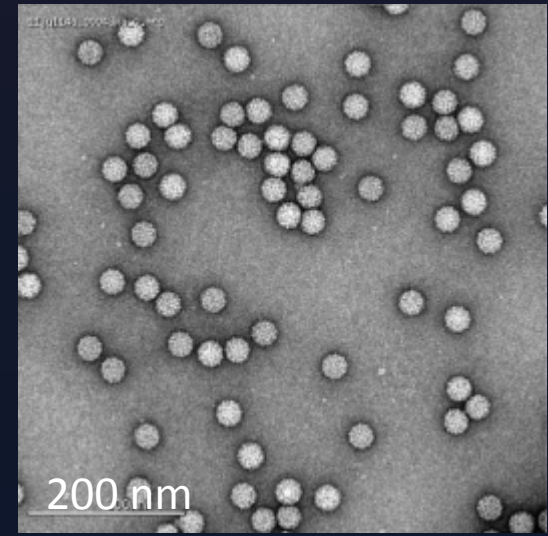
Lipid nanotubes



Antibody-labeled QDots

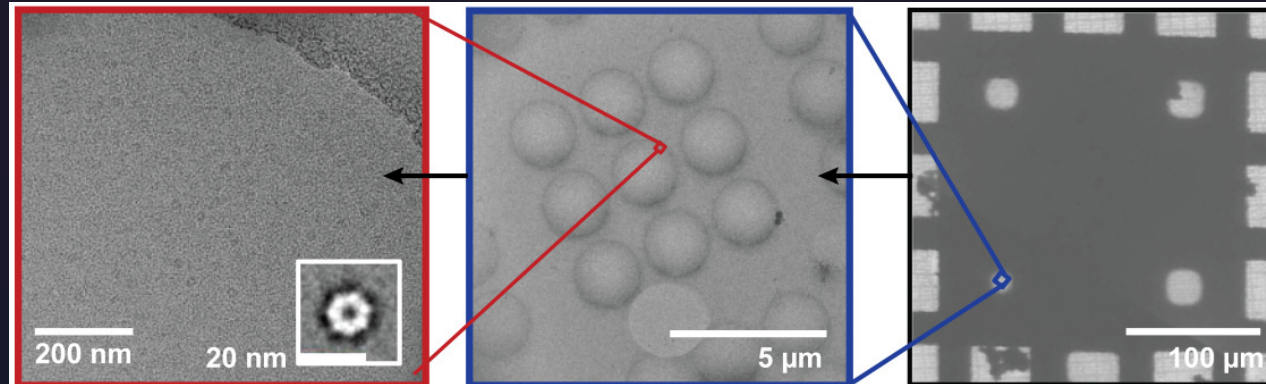


CNV

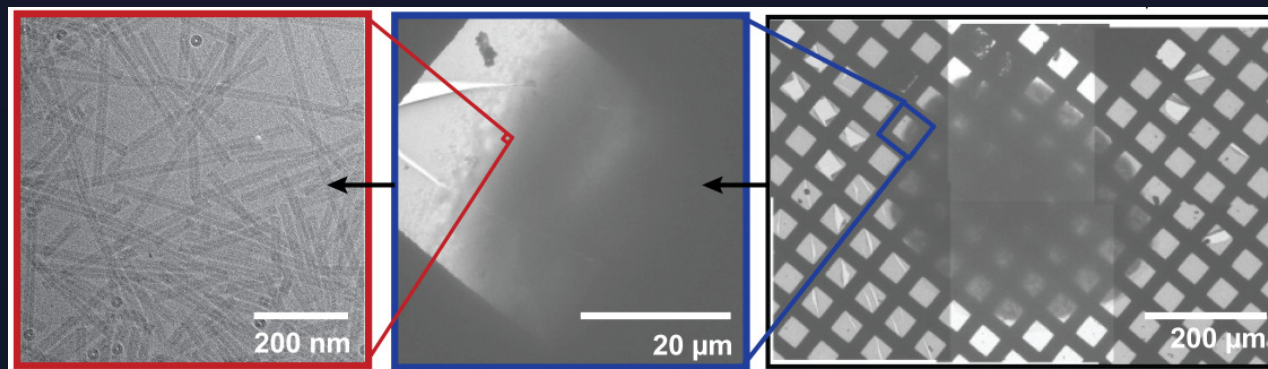


Vitrification of specimens using Spotiton v0.5

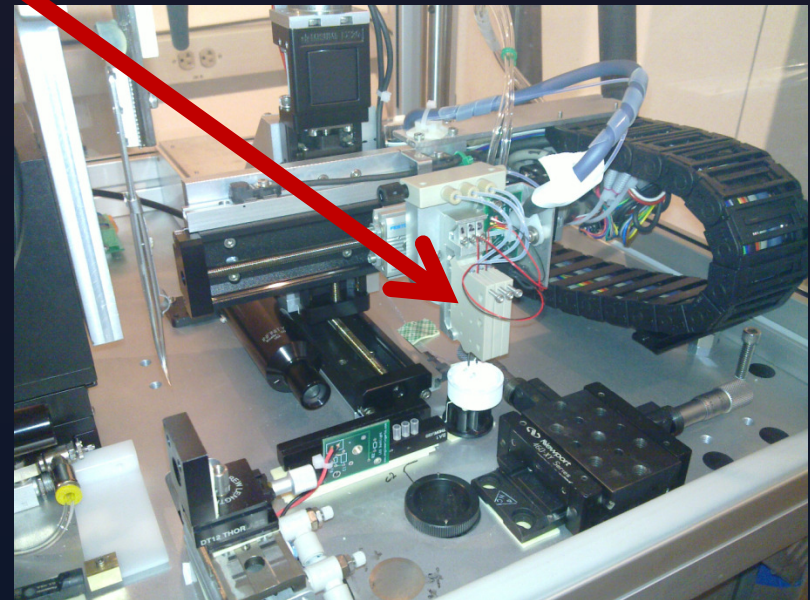
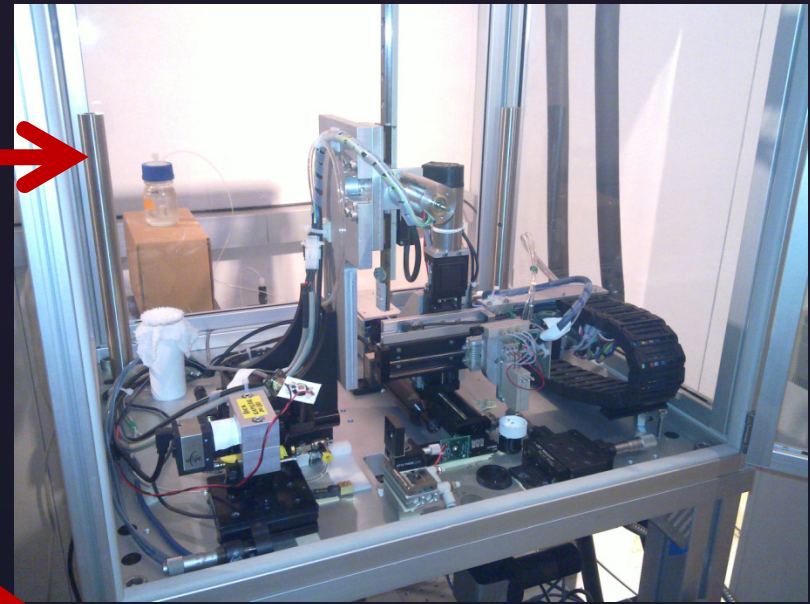
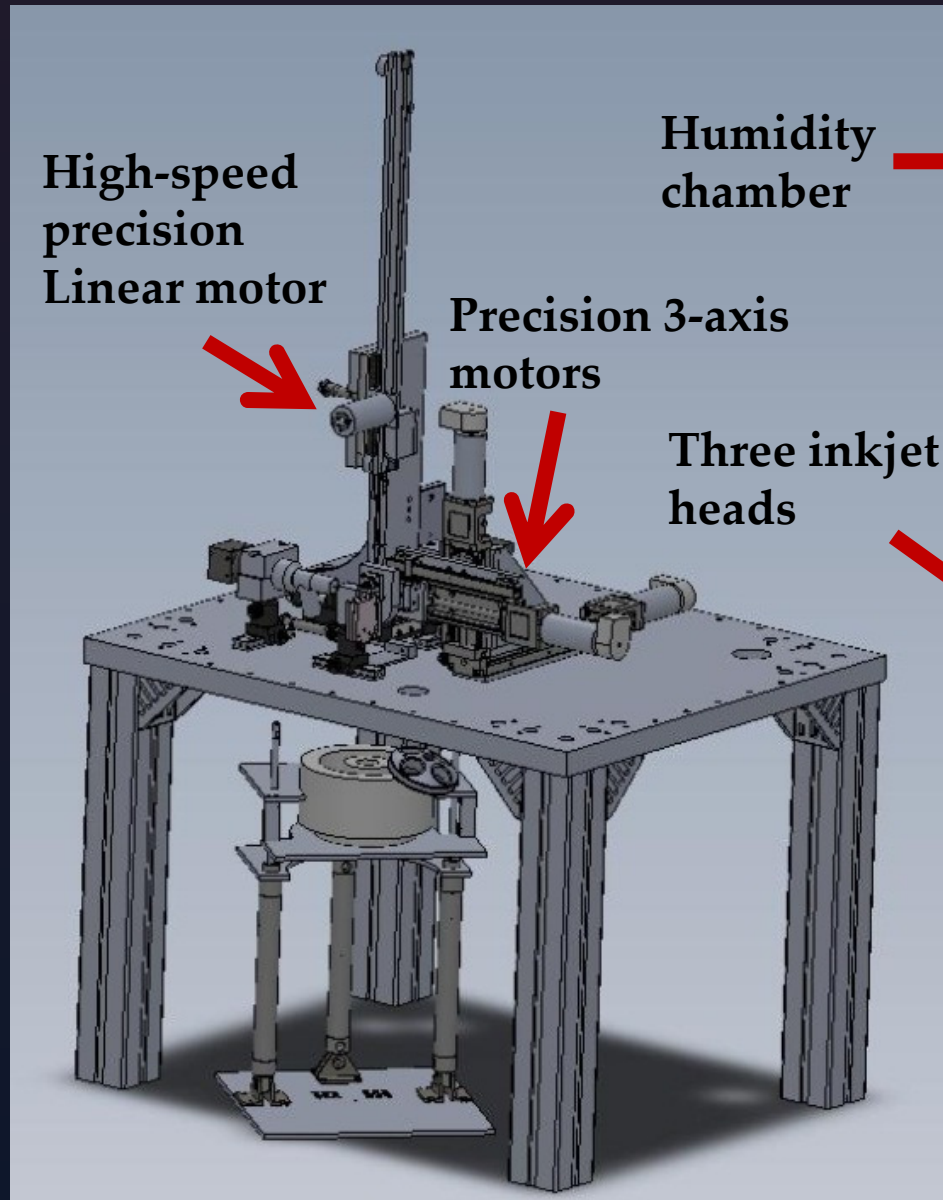
GroEL (1.6 nL dispensed on Holey carbon grids)



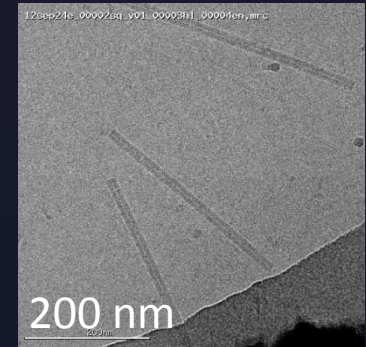
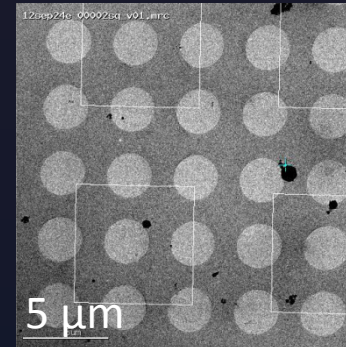
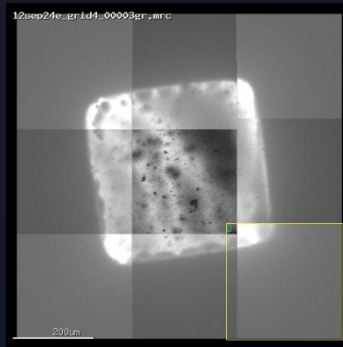
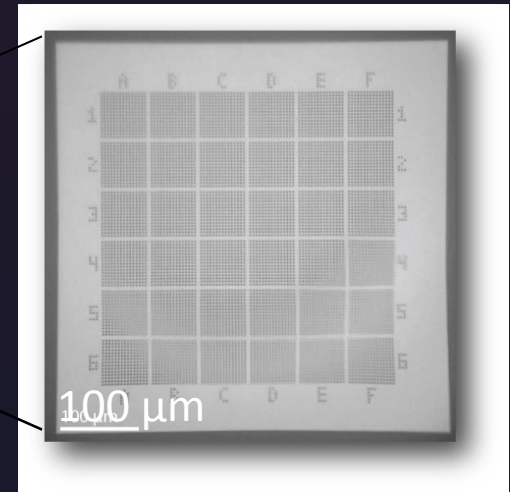
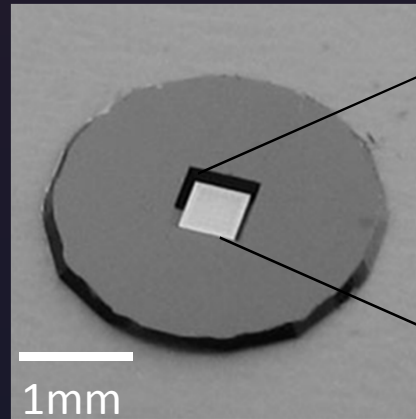
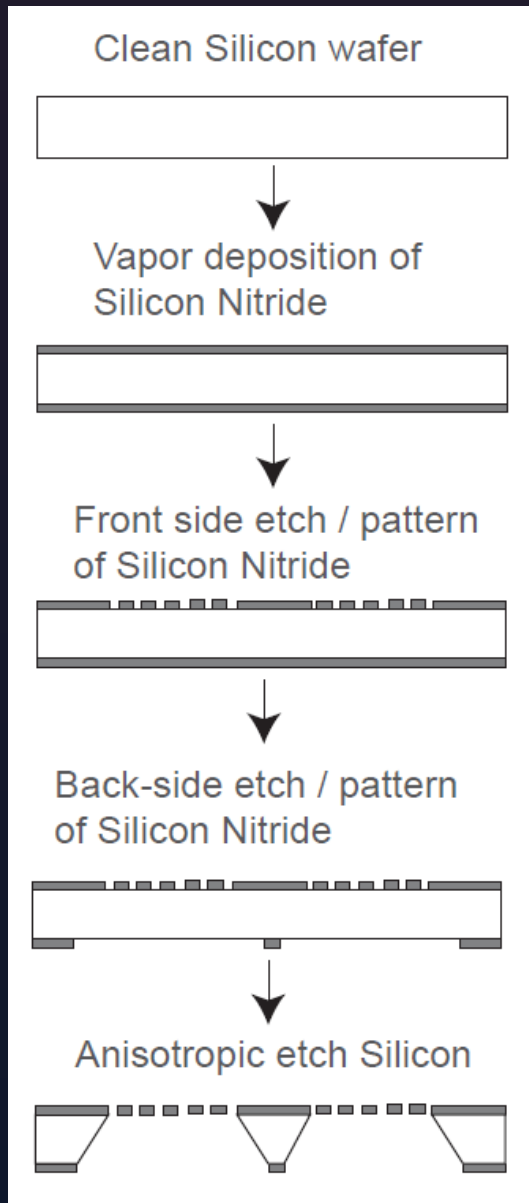
TMV (3.2 nL dispensed on Continuous carbon grids)



Spotiton v0.75 (Automated, Three inkjet heads)



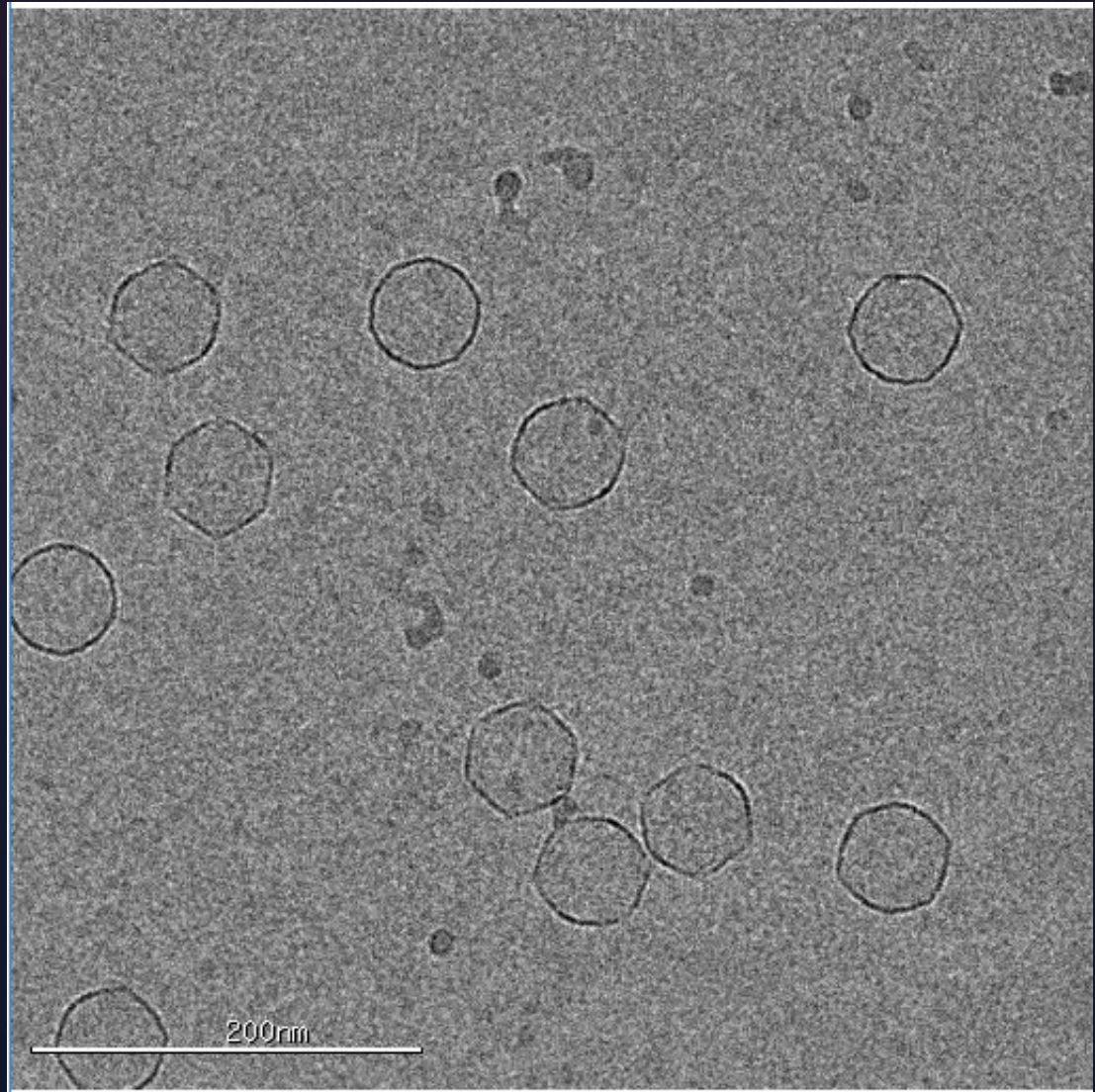
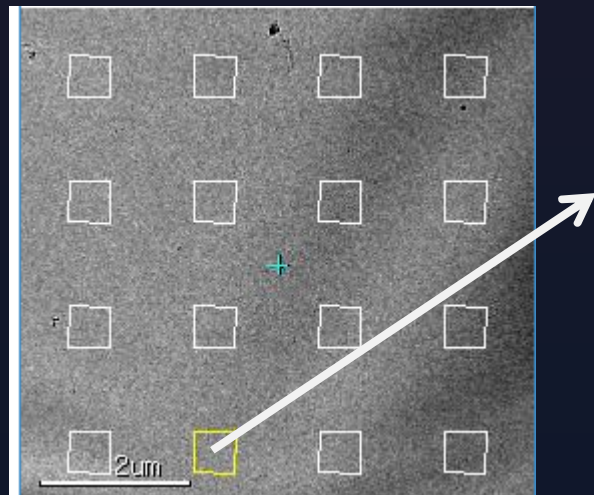
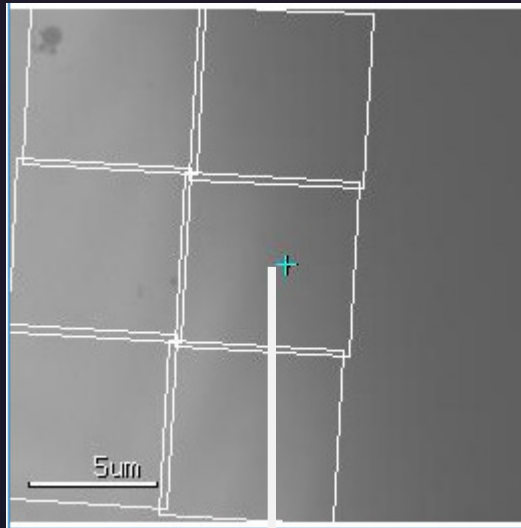
Silicon fabrication technology for cryo-EM



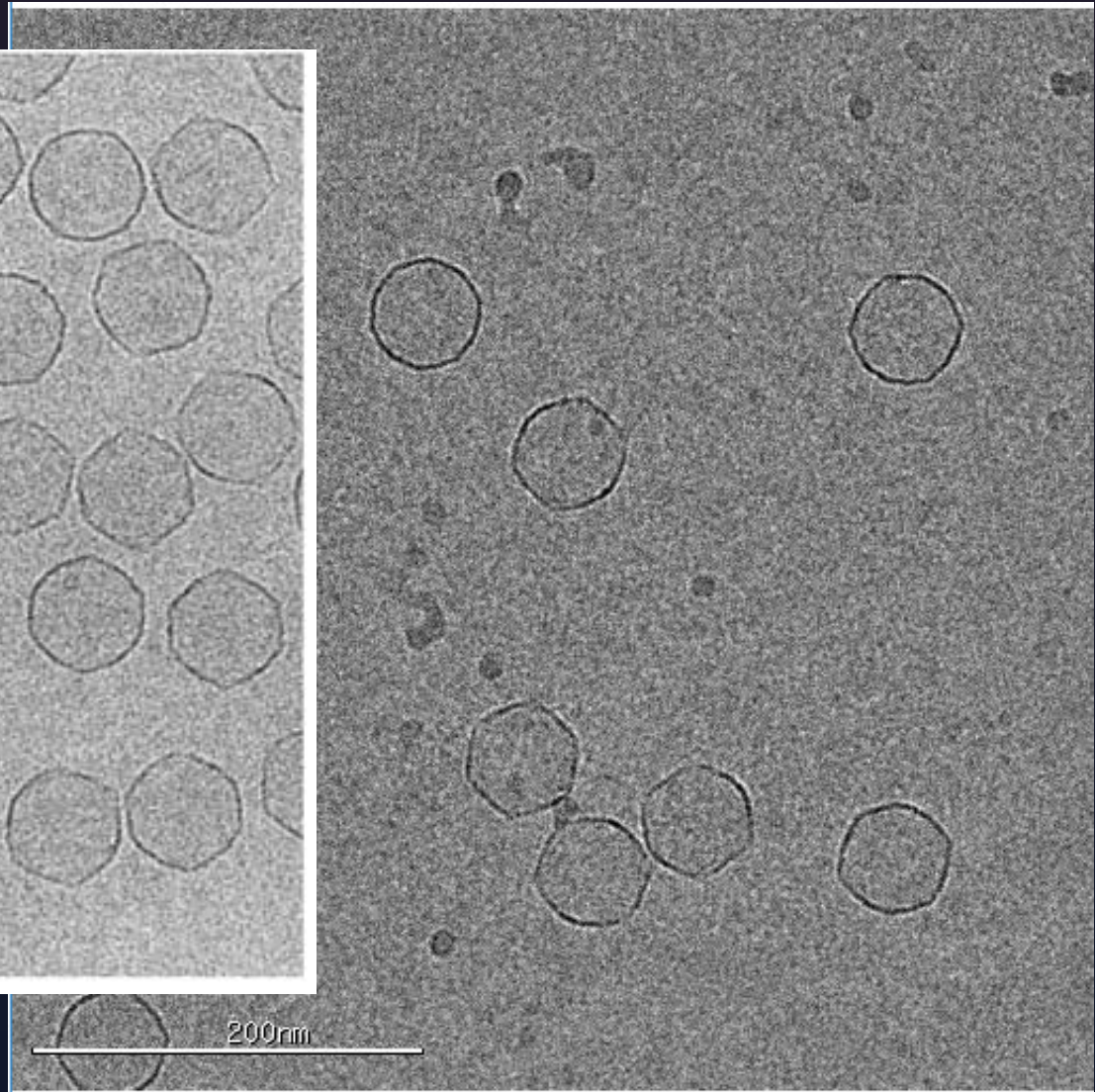
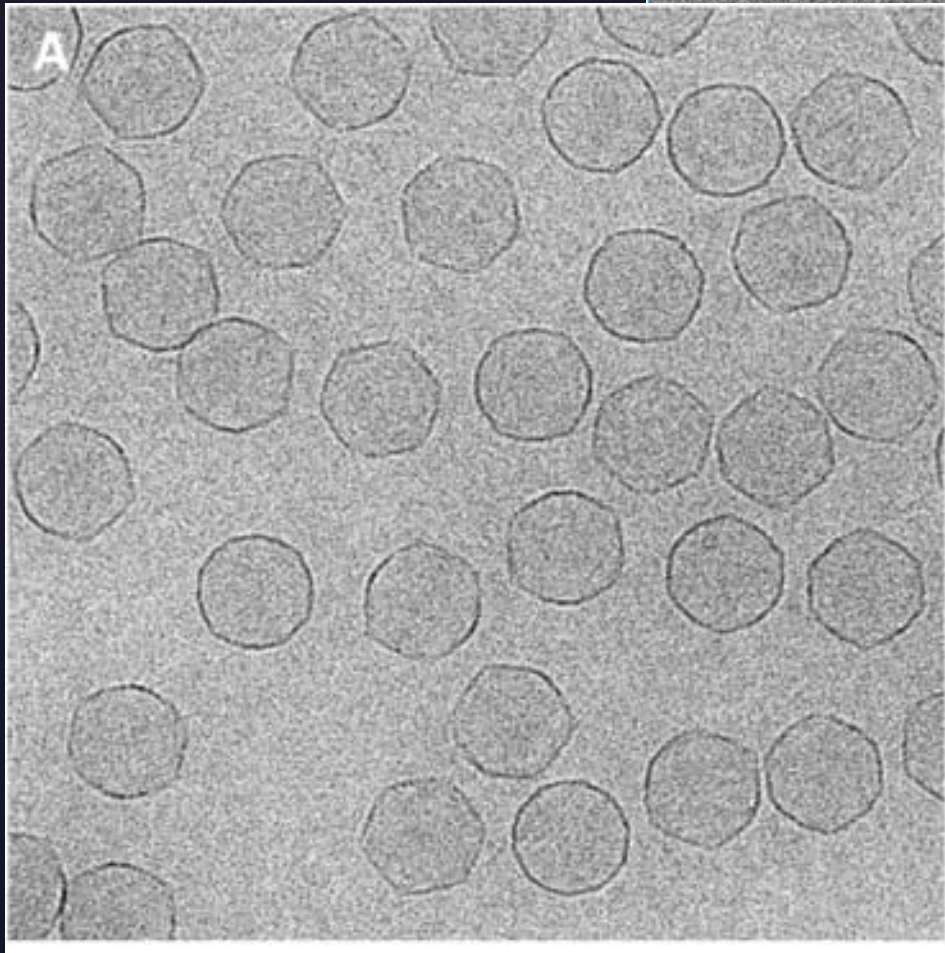
Vitrified specimen within 250 micron window



Vitrified specimen within 250 micron window



Comparison to traditional freeze

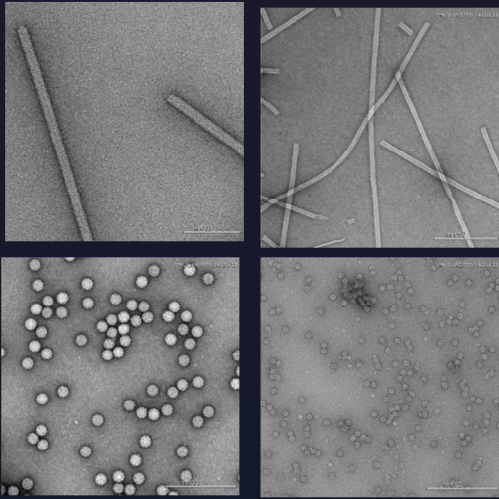


Ross et al. The EMBO Journal
(2005) 24, 1352–1363

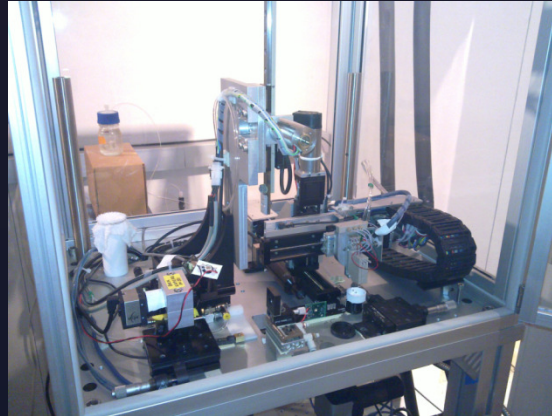
Spotiton frozen

Conclusions

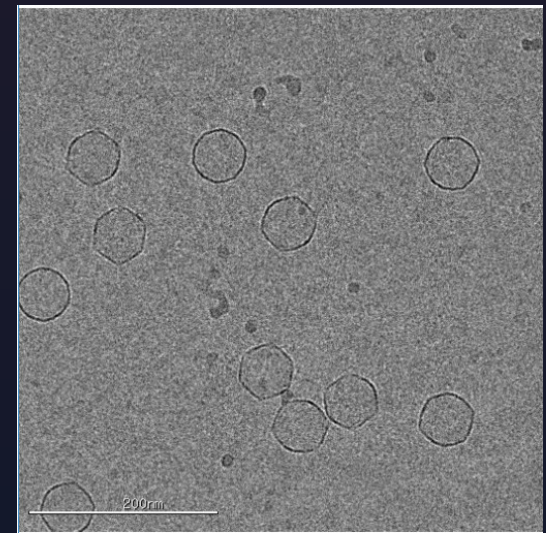
Viability of inkjet technology



Spotiton v0.75



Vitrified specimens



Further developments

Optimize and validation

Novel grid development

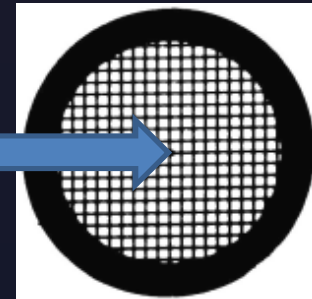
Nine inkjet heads

Food for thought...

96 well-plate



Single grid



Acknowledgments

NRAMM @ TSRI

- Clint Potter
- Bridget Carragher
- AMI members

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- James Roussie

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