New Approaches to Specimen Preparation for Molecular TEM

Clint Potter

National Resource for Automated Molecular Microscopy

The Scripps Research Institute
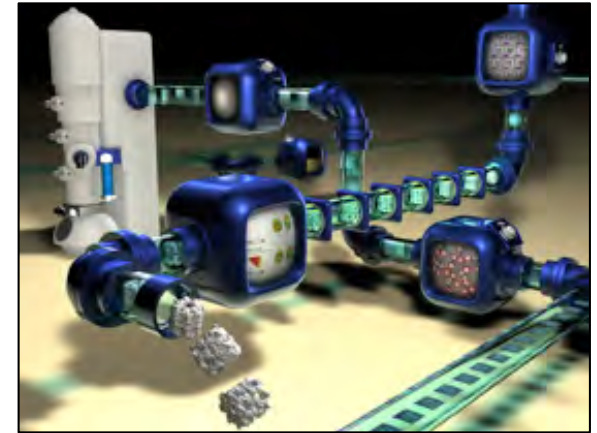
NRAMM Workshop
10 November 2014

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
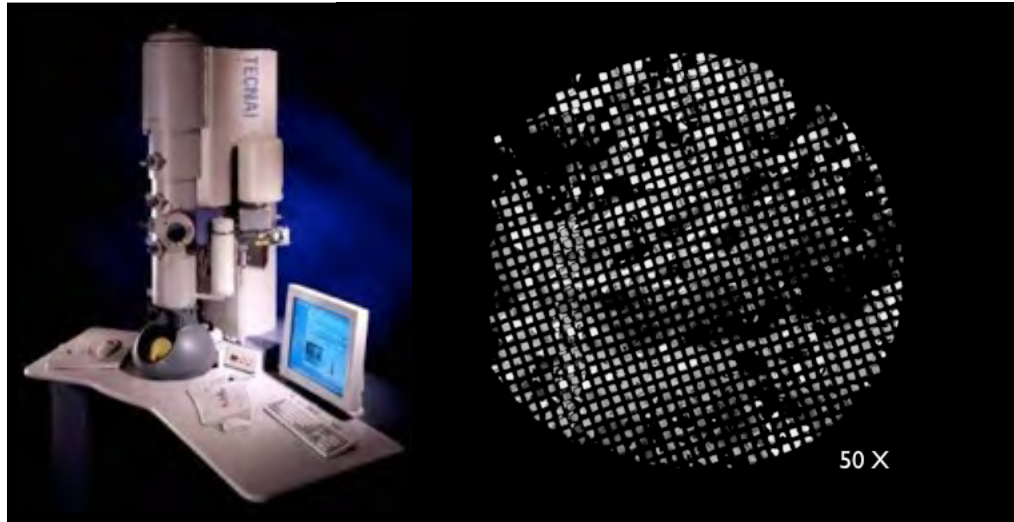(may need to analyze 1,000,000's molecules)

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

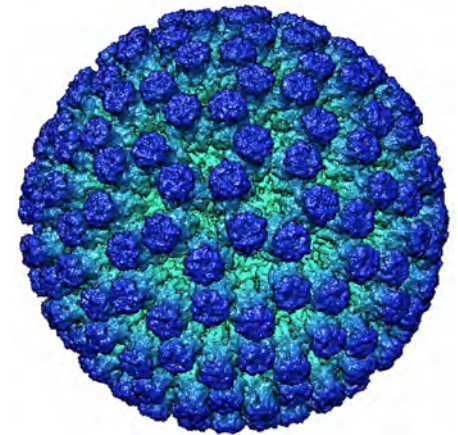**Investigation of the structure, function and dynamics of molecular machines**

EM Automation:
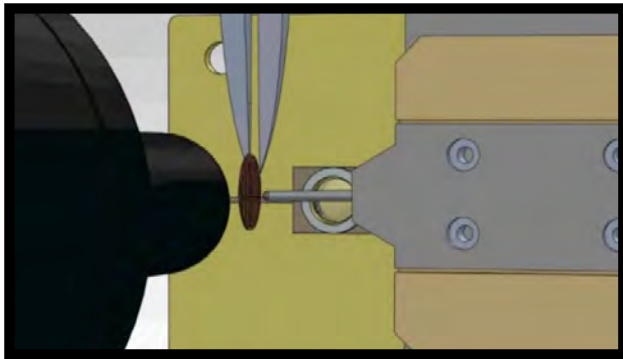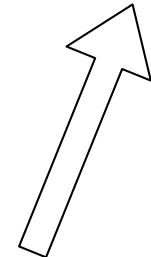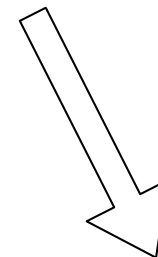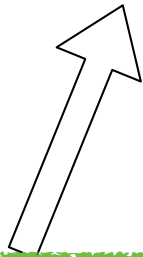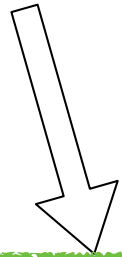# Investigating structure and dynamics of molecular machines
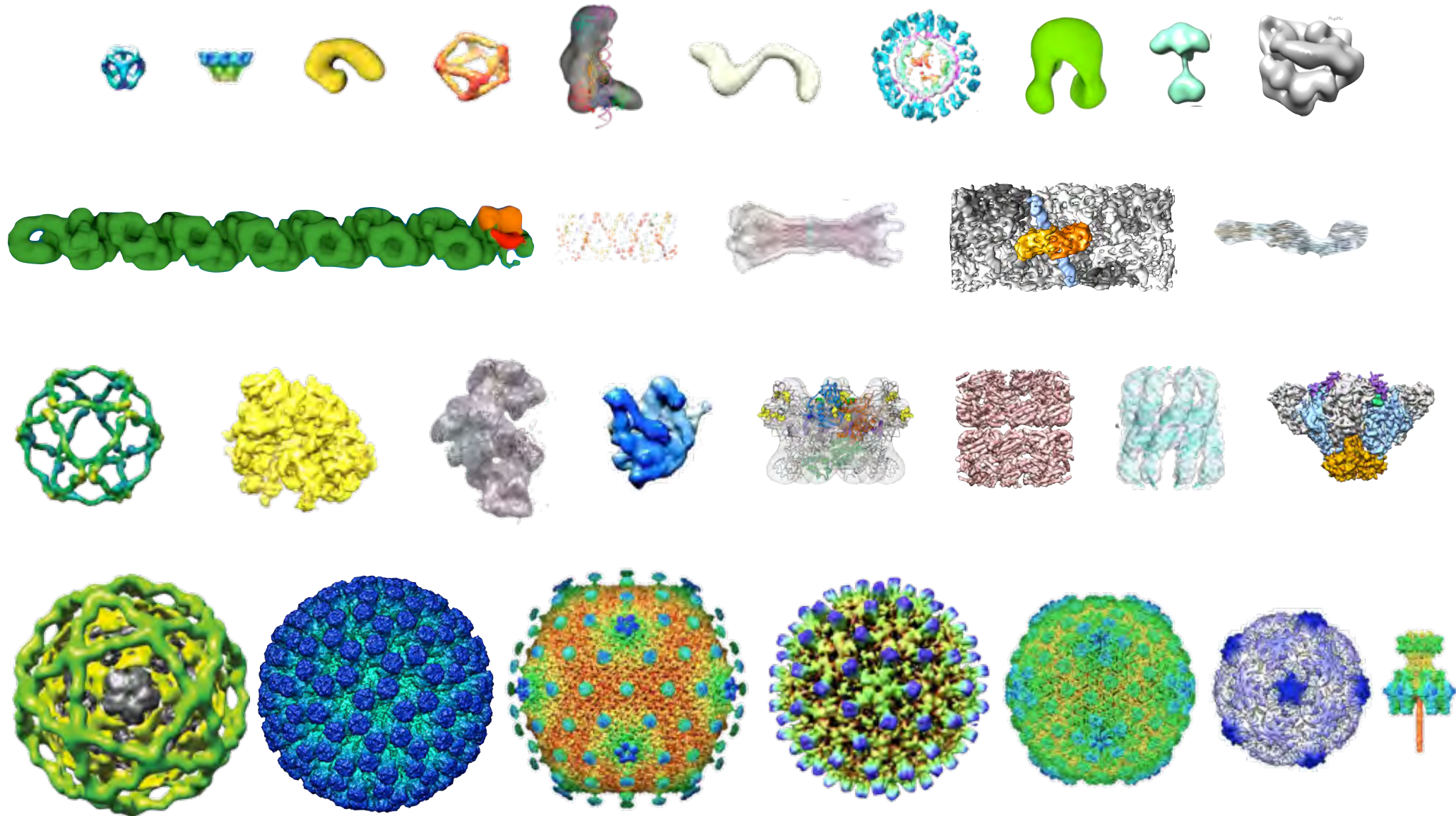
Core Technologies: A Streamlined and Automated TEM Pipeline
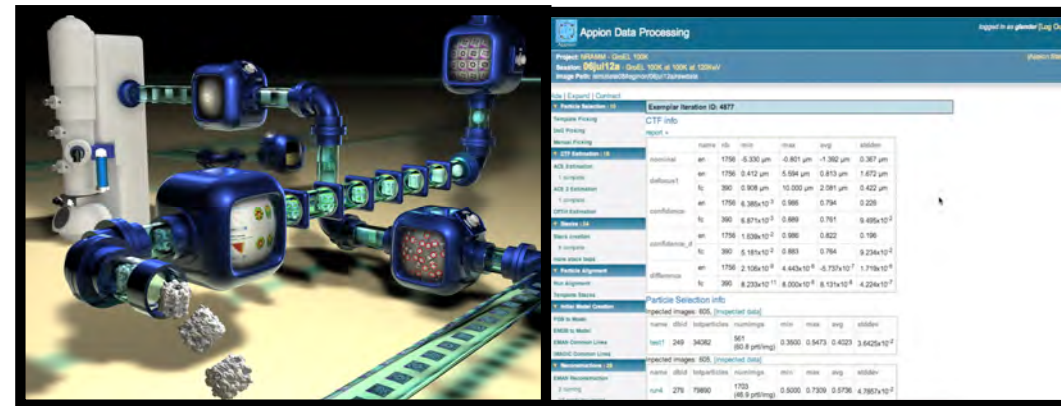
Sample

EM Density

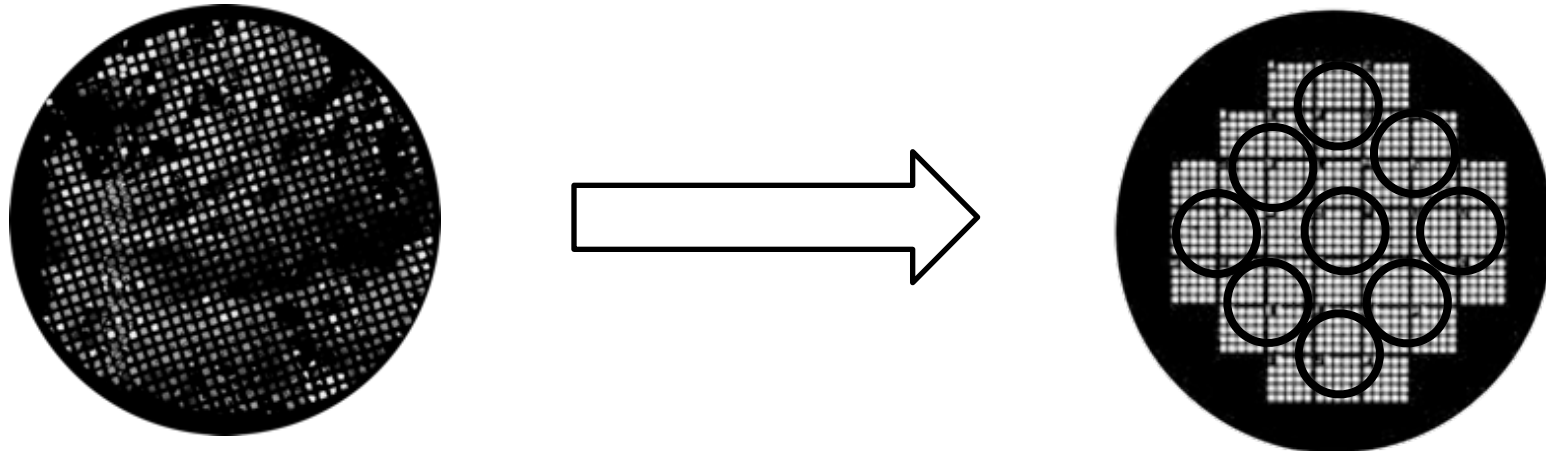Automated Data Collection
(Leginon)

Specimen preparation
(Spotiton)

Streamlined Processing
(Appion)

Current CryoTEM Specimen Preparation

3µl

3nl

99.9%

0.1%

>100,000 potential targets for imaging; most of them are not usable.
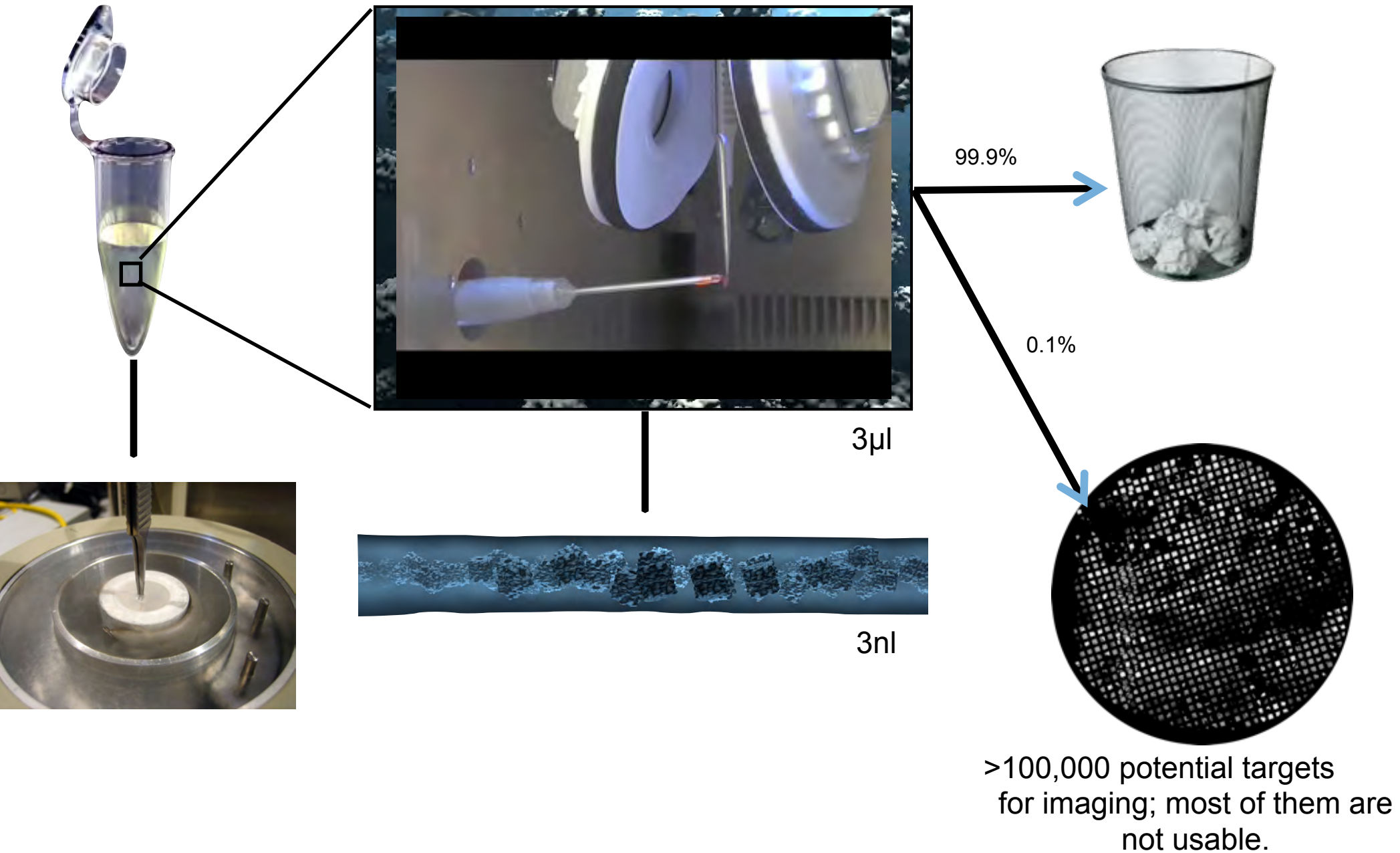
A New Approach to Specimen Preparation:

Current method:

1 sample per grid ⟶ ↑ 10x ⟶ 9 samples per grid

3µl ⟶ ↓ 1000x ⟶ 30 pL

10% usable area ⟶ ↑ 10x ⟶ 100% usable area

Proposed picoliter sample dispensing:

Inkjet dispensing

C   Voltage waveform on dispense head

+ -   + -   + -

Programmable number of droplets

New substrates

Droplet impact

Droplet spreading

Contact with barrier "pinned"

Center thinning
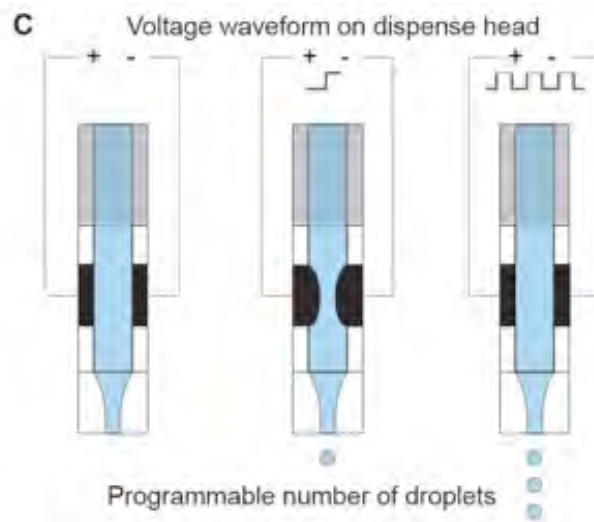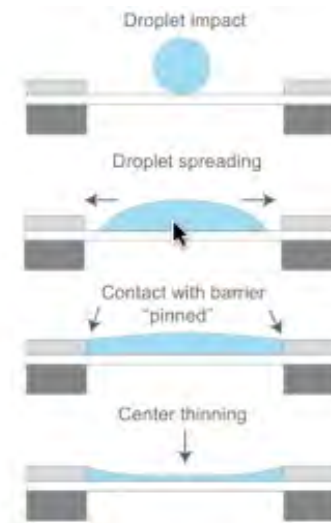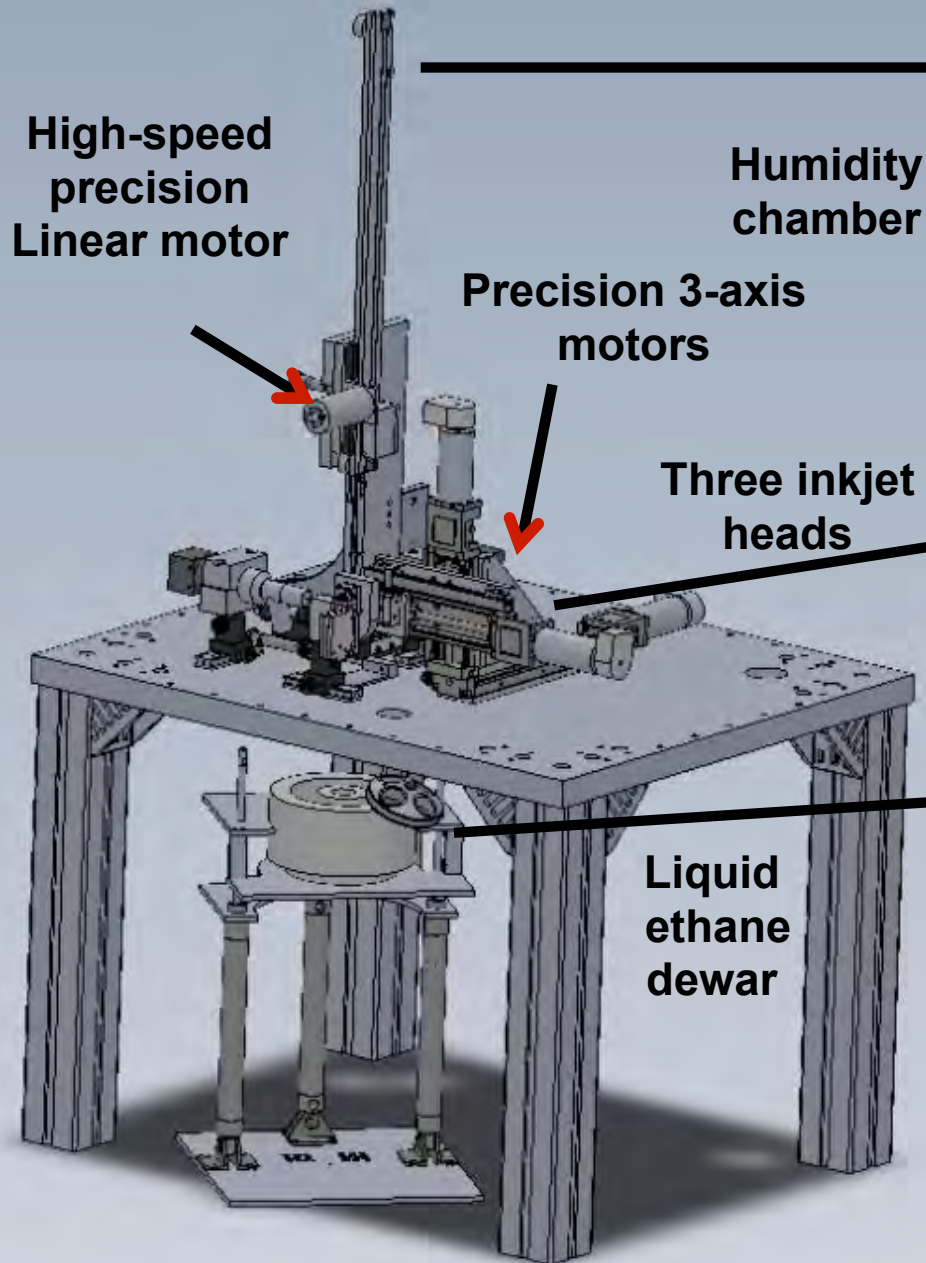
Spotiton v0.75

(Engineering Arts custom made, automated, three inkjet heads 24 um nozzle, 32 pL drops)

High-speed precision Linear motor

Precision 3-axis motors

Three inkjet heads
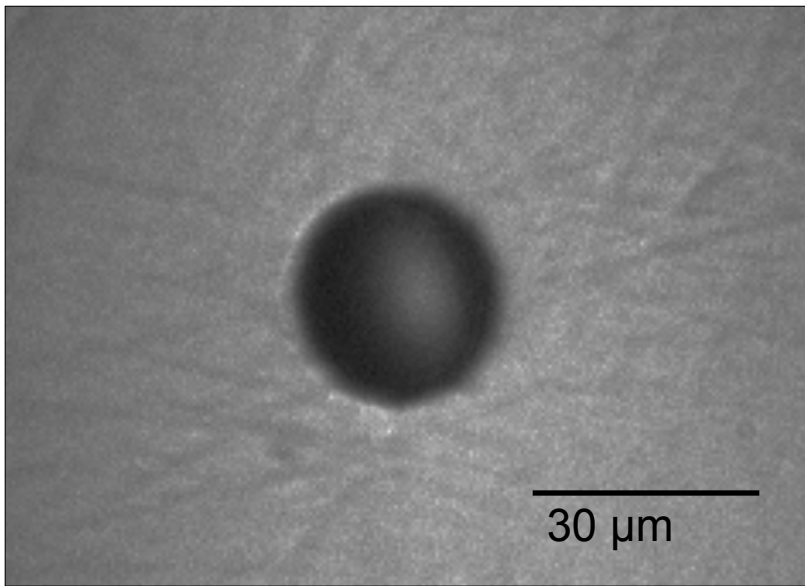
Liquid ethane dewar

Humidity chamber
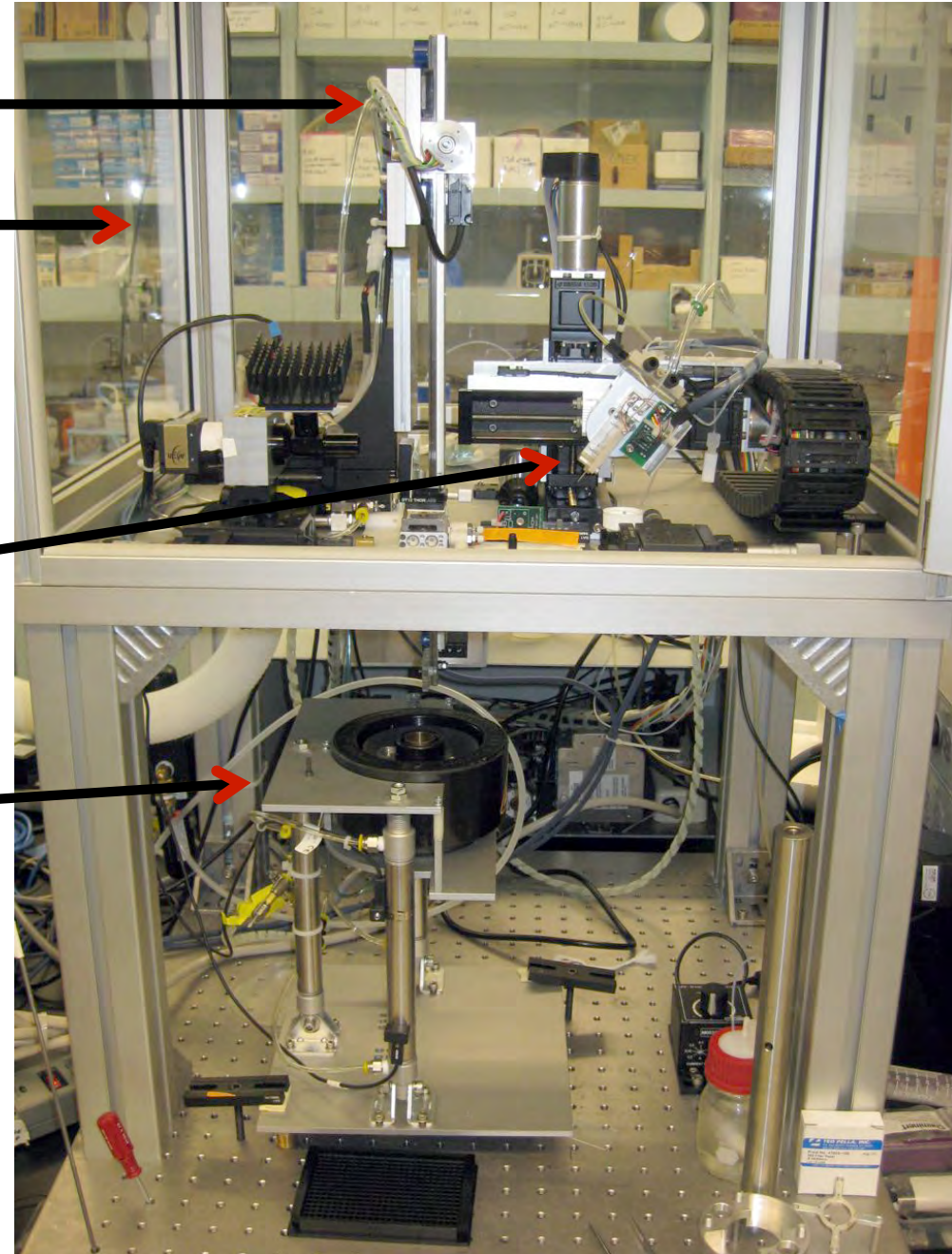
Sample volume can be precisely controlled
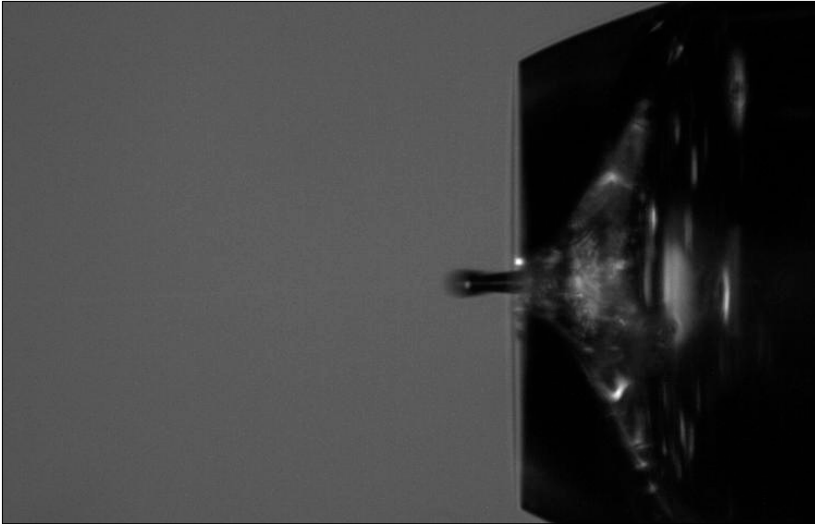
1 droplet (32 pl)
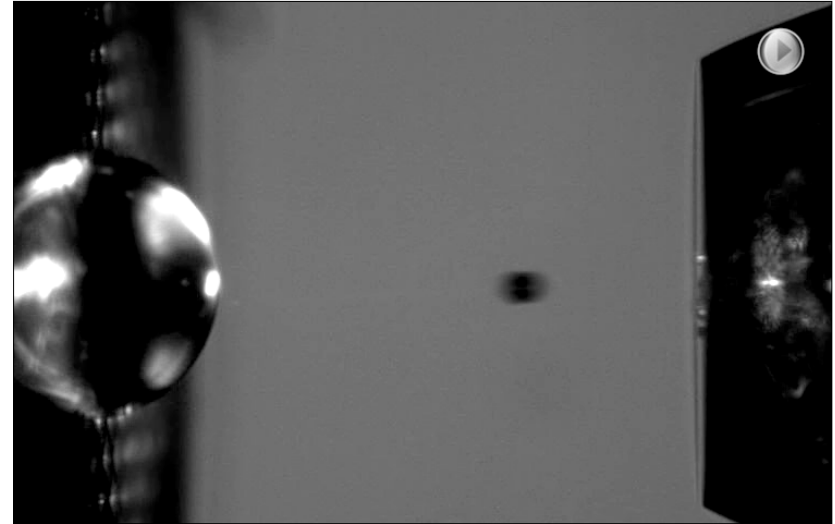
1000 droplets (32 nl)

Dispense tip front view

30 µm
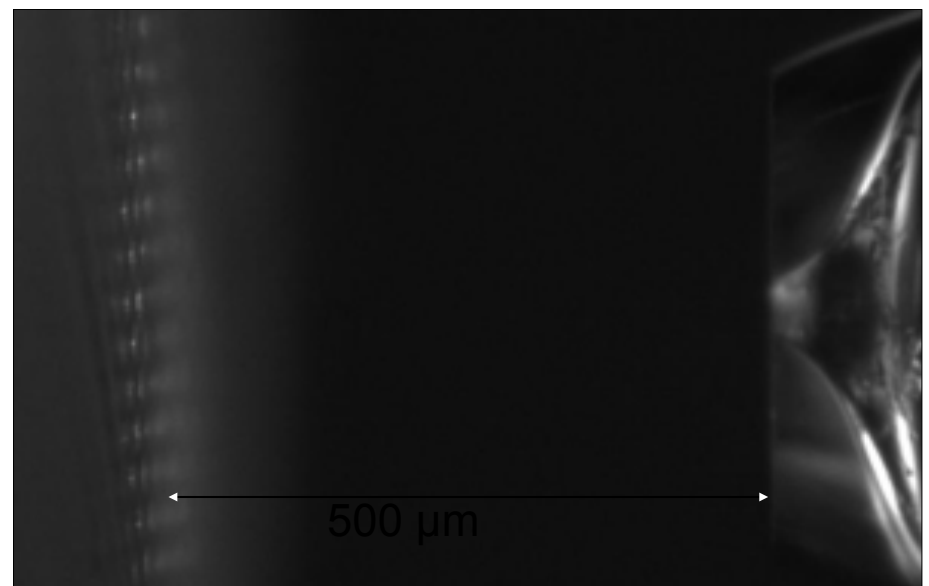
Grid-tip positioning
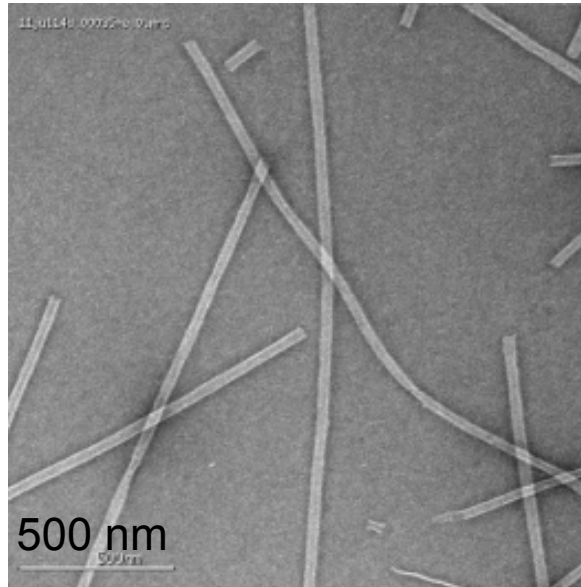
500 µm

Spotiton v0.75 in action.
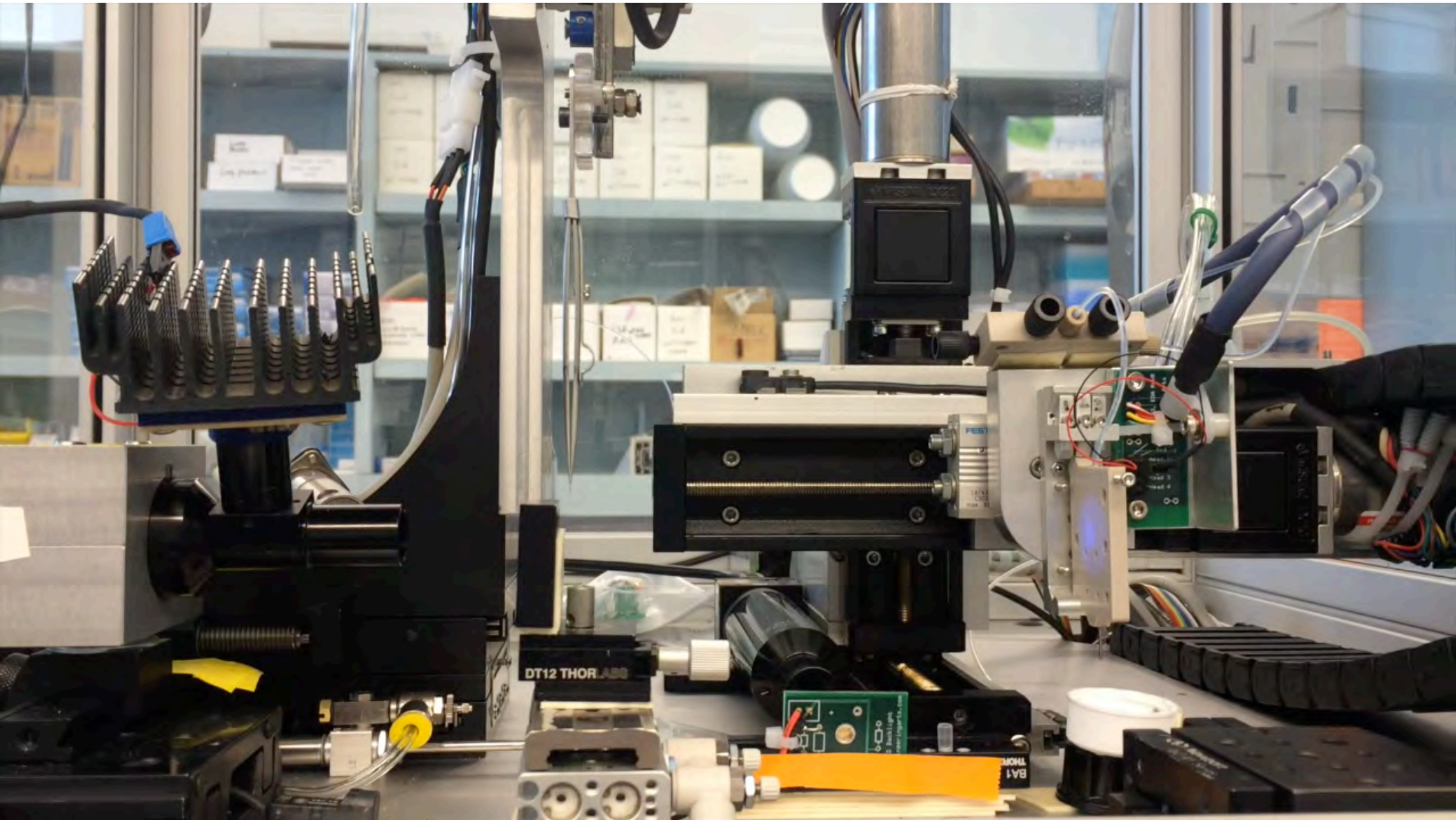
Stability of particles dispensed using inkjet

TMV
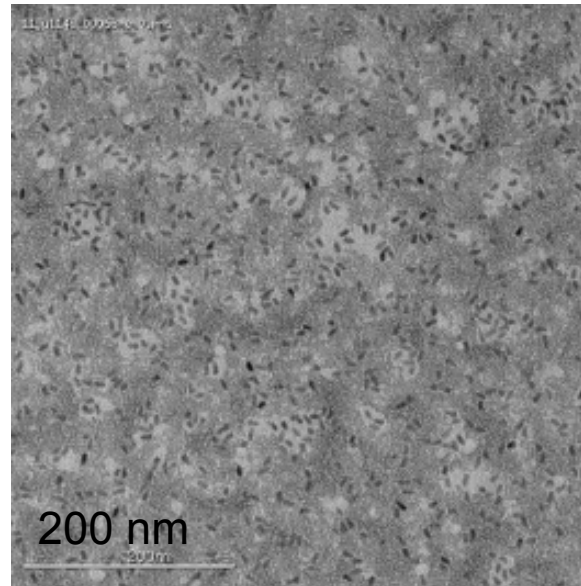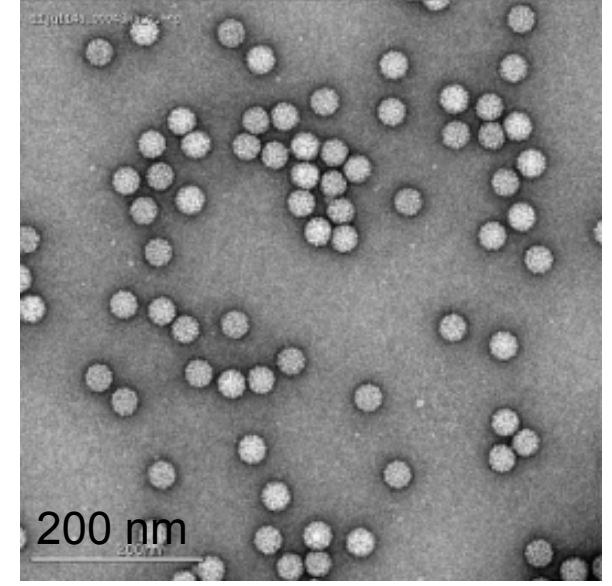100 nm
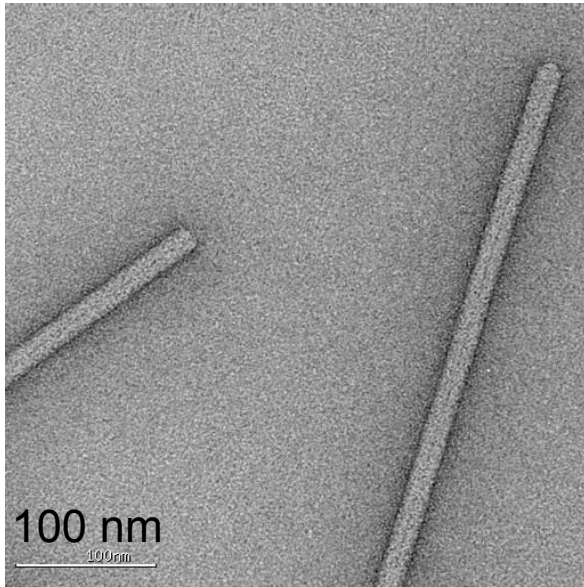
Microtubules
500 nm

GroEL
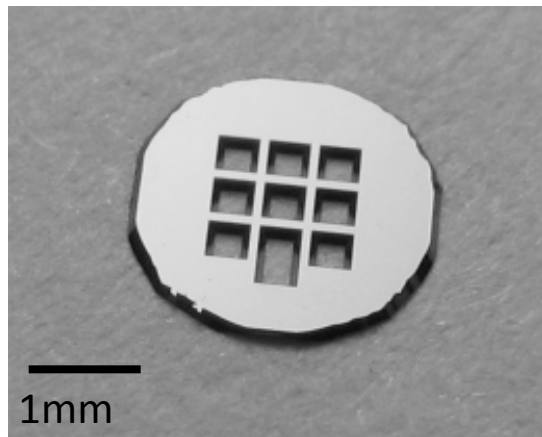200 nm
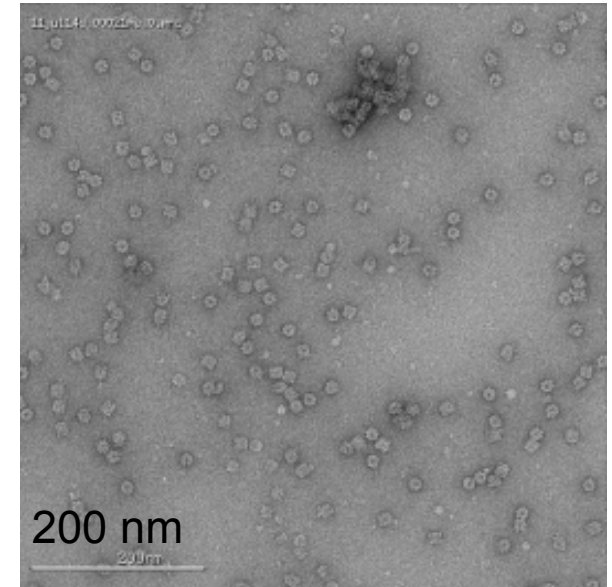
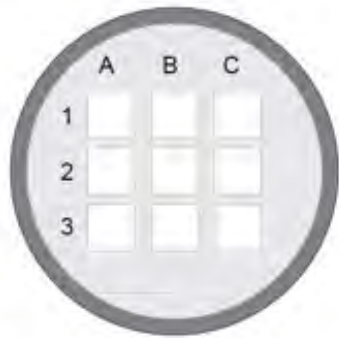Lipid nanotubes
2 µm
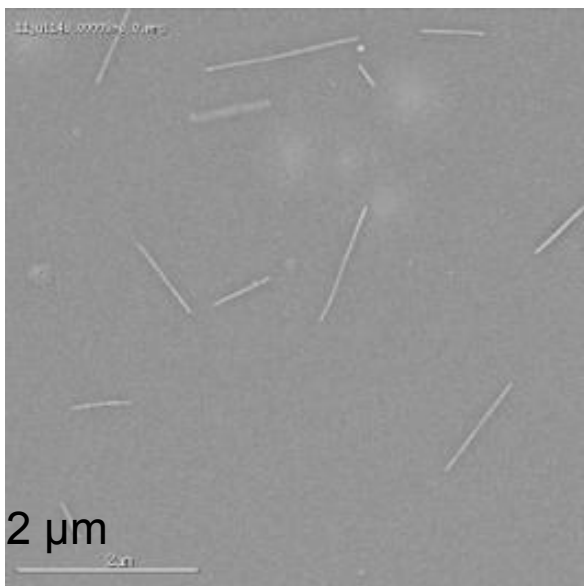
Antibody-labeled QDots
200 nm

CNV
200 nm

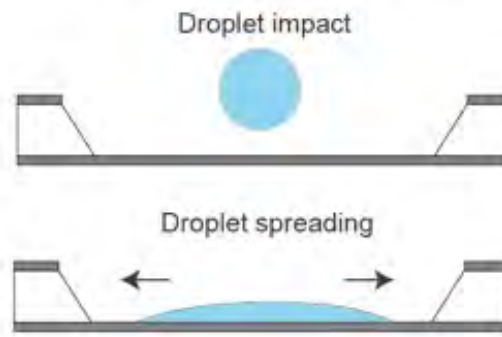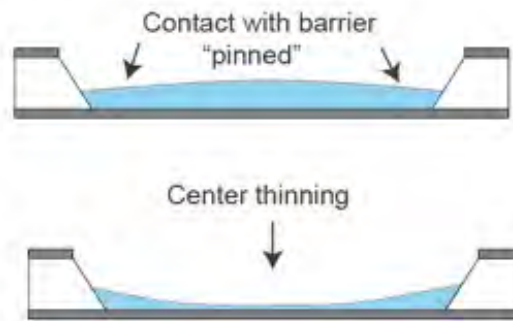# Applying specimens to microfabricated grid substrates

EM grid with nine windows

A B C

1

2

3

1mm

Edge thinning

Droplet impact

Droplet spreading

Center thinning
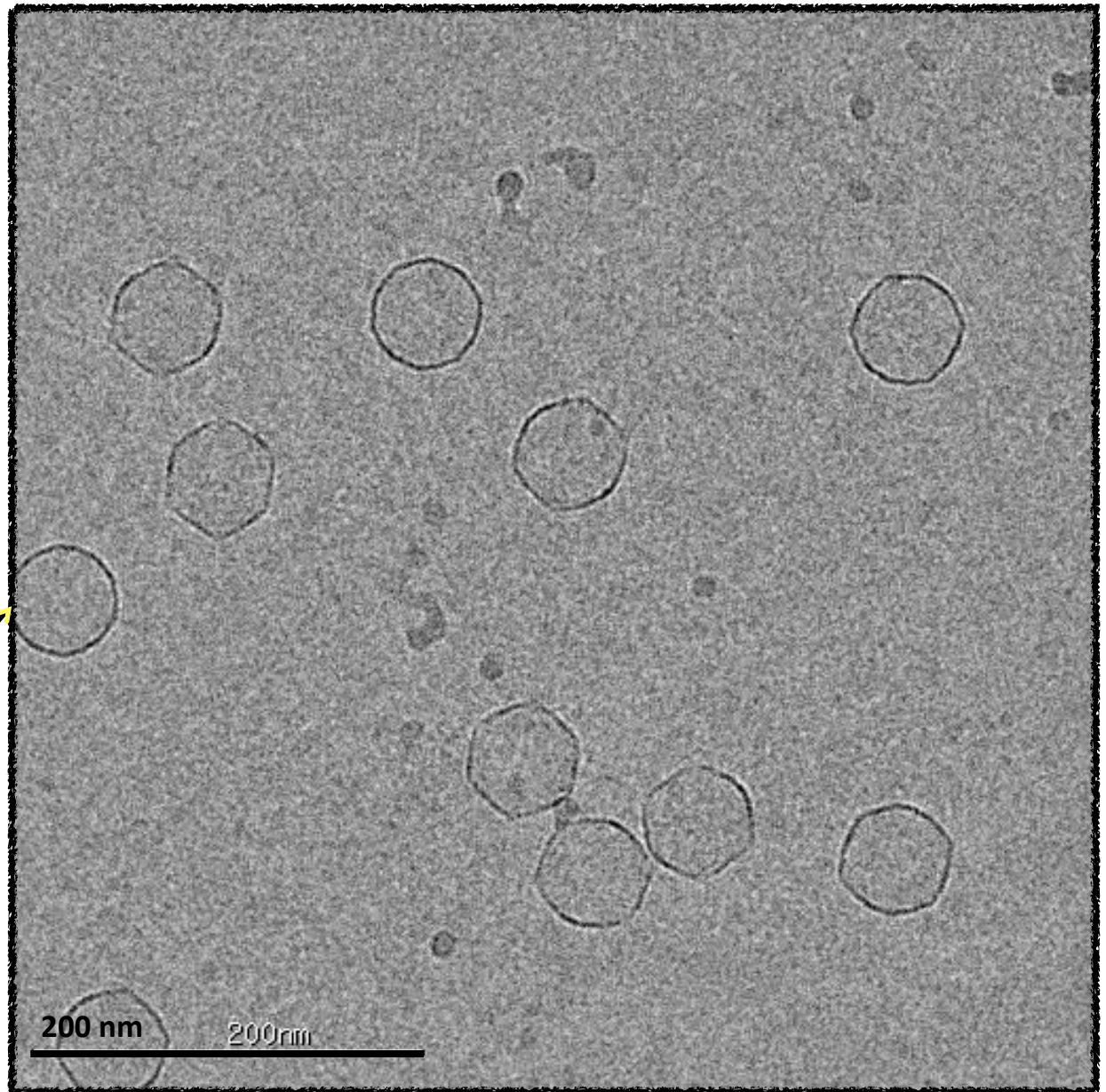
Contact with barrier "pinned"
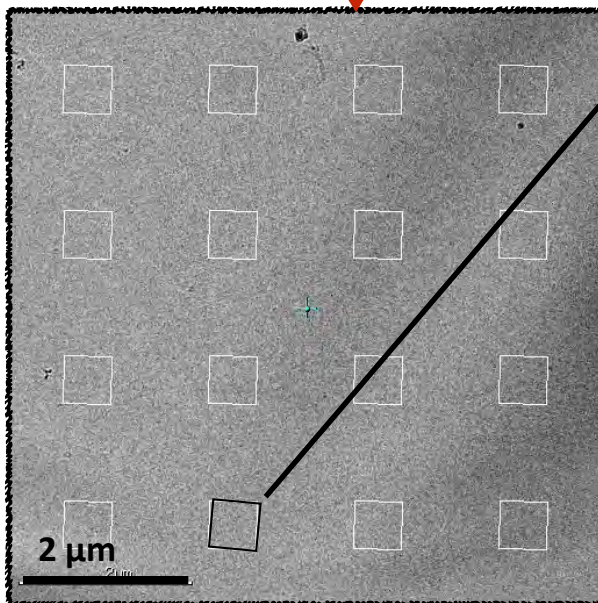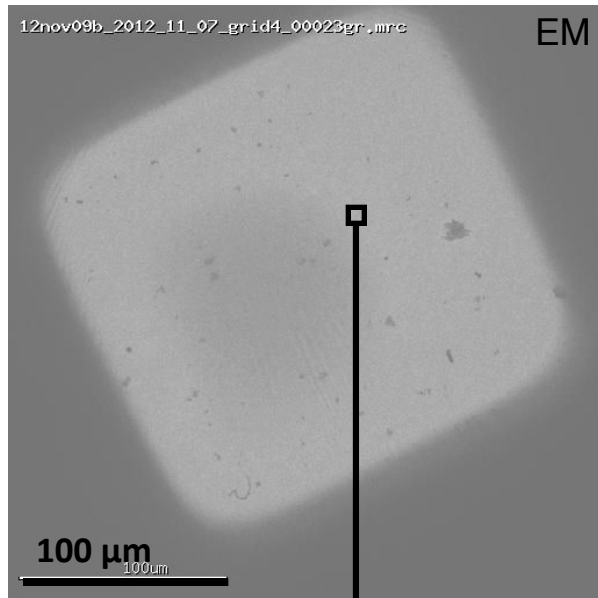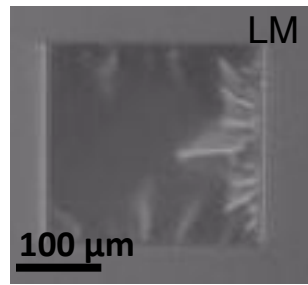
Center thinning

Hydrophobic
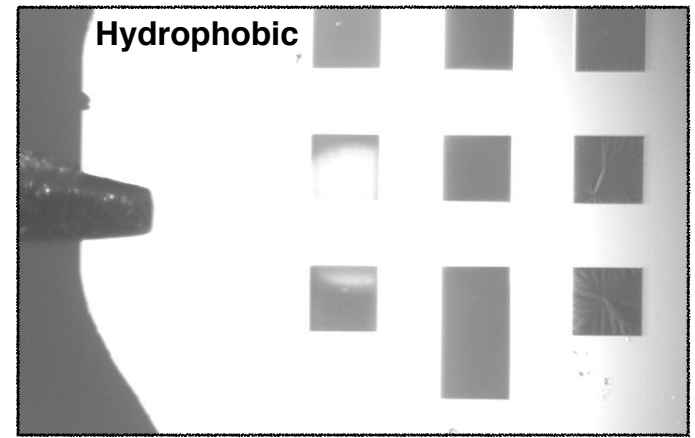
Hydrophilic
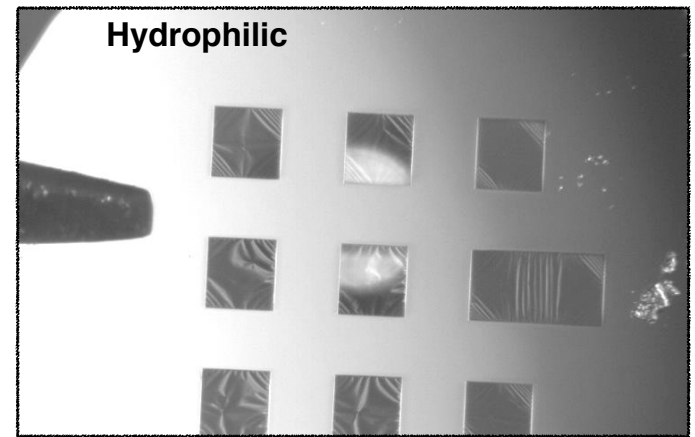
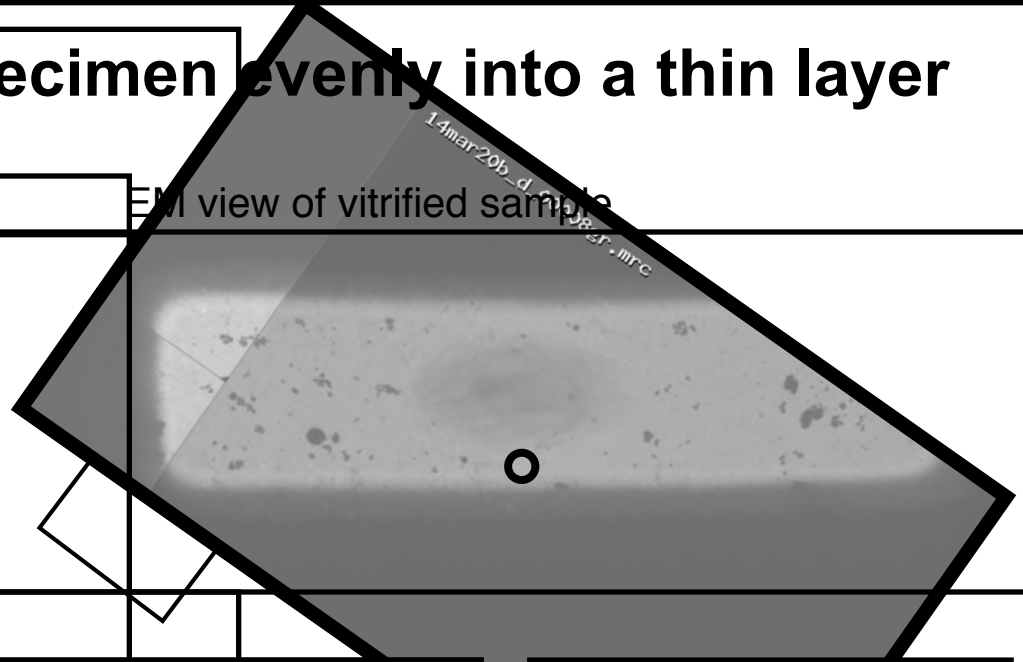# Vitrification across a 250 micron Si₃N₄ window

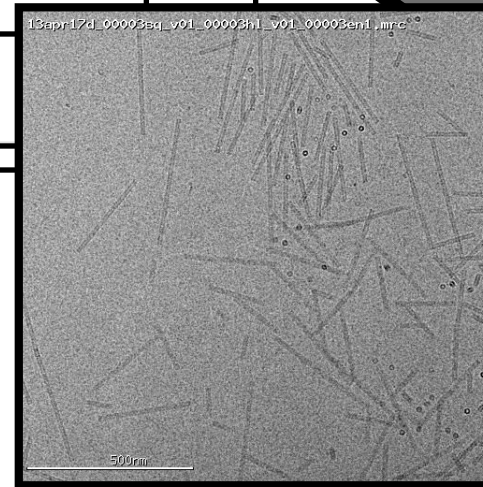# Challenge is to spread the specimen evenly into a thin layer

LM view of drop dispense

EM view of vitrified sample

20nm thick SiO_2
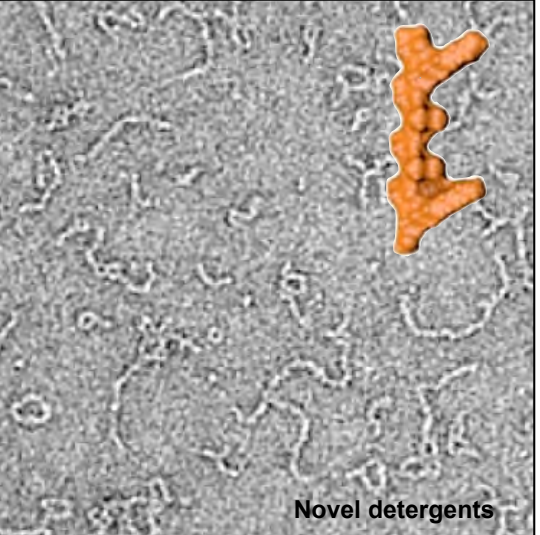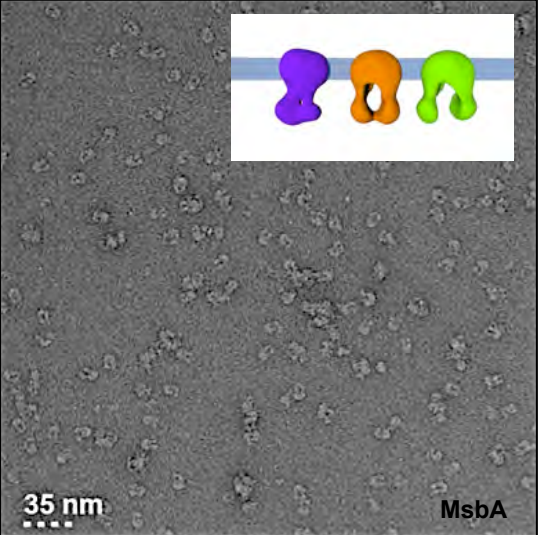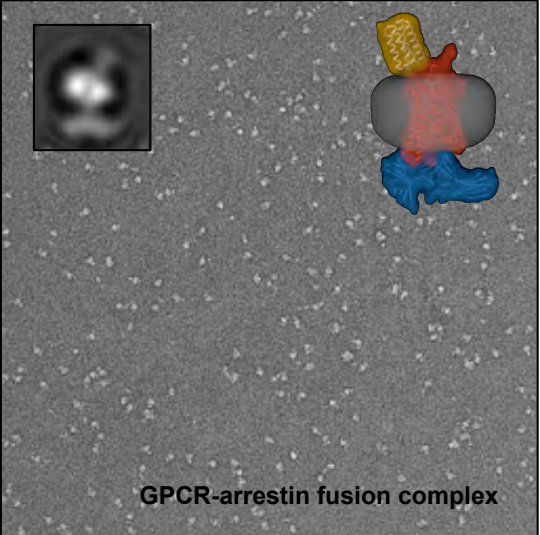100 x 350 um window
Plasma cleaned
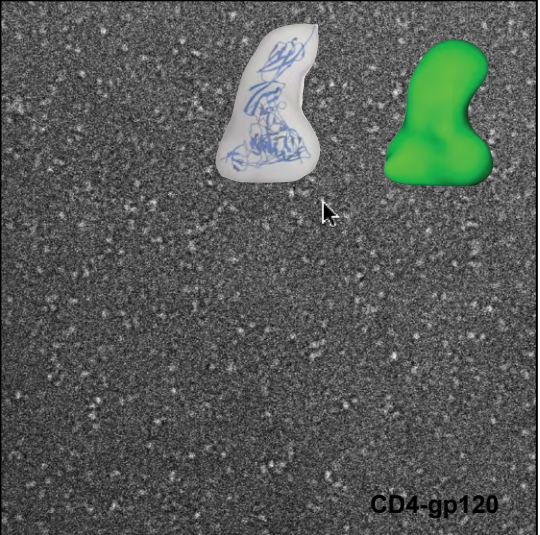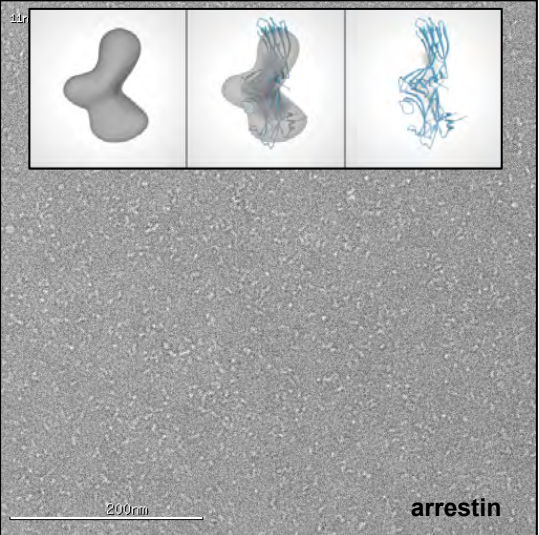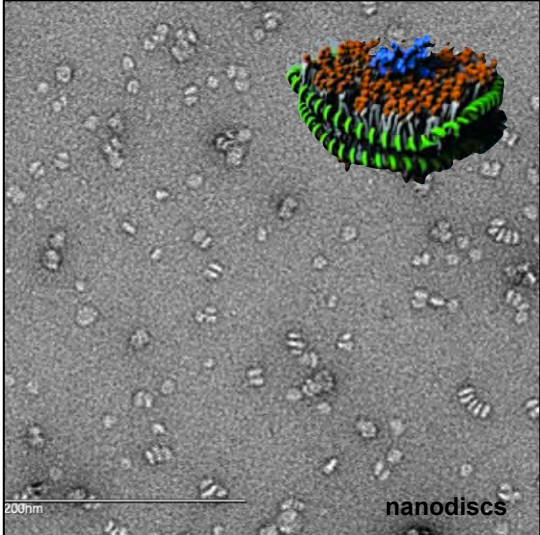72pL (2 drops)
0.5mg/mL TMV

Current status

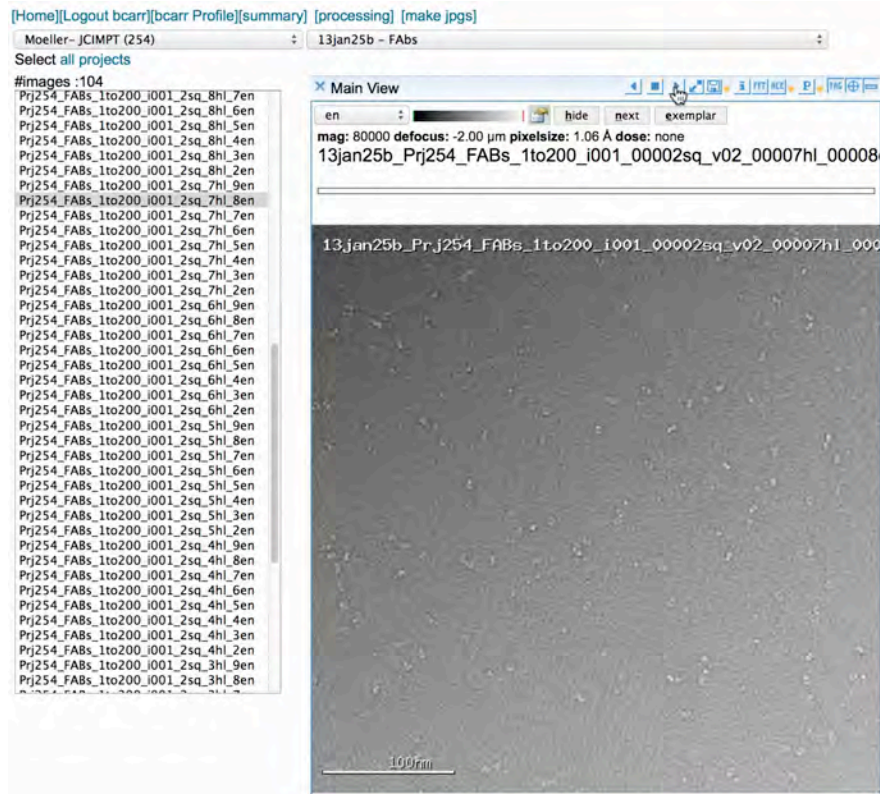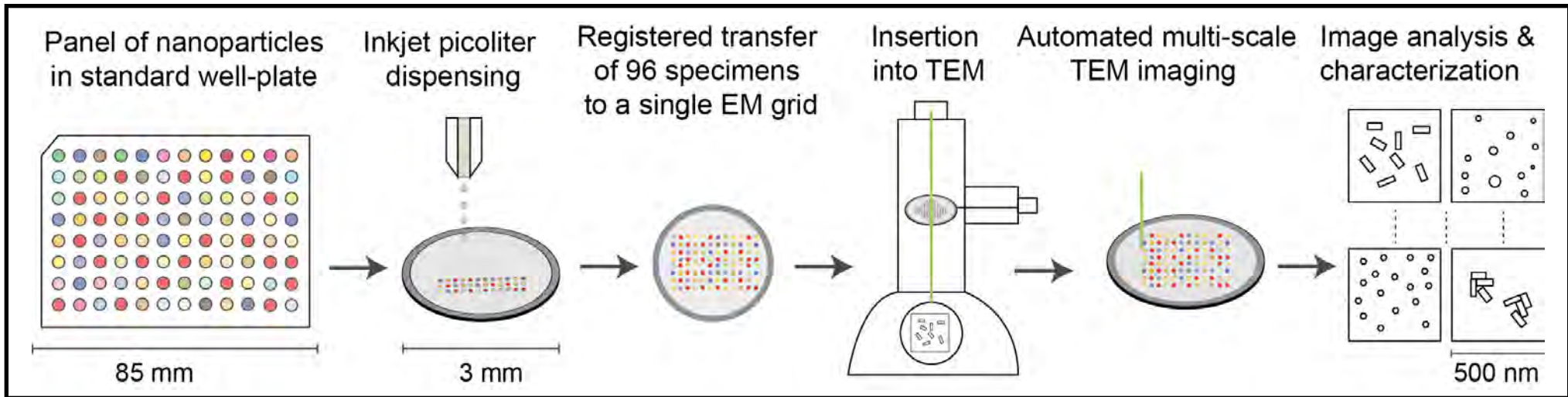Spotiton inkjet dispensing system fairly robust. Now refining for v1.0.

Capable of producing thin ice with well preserved specimens

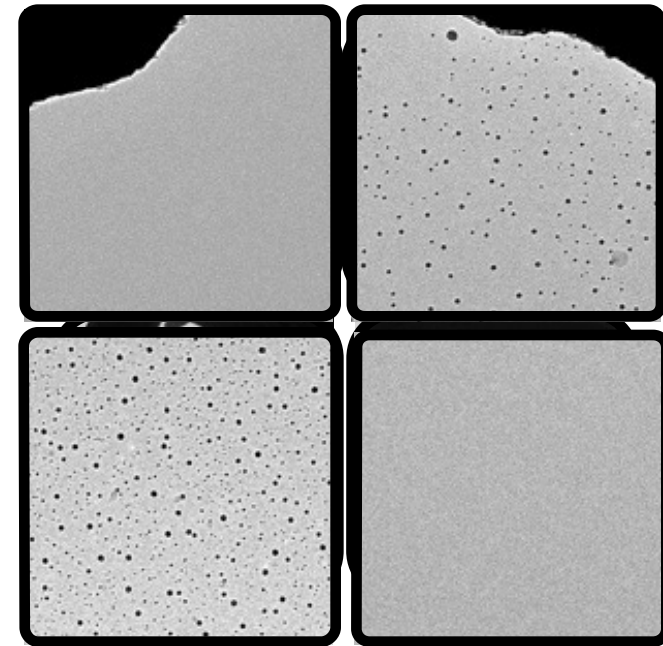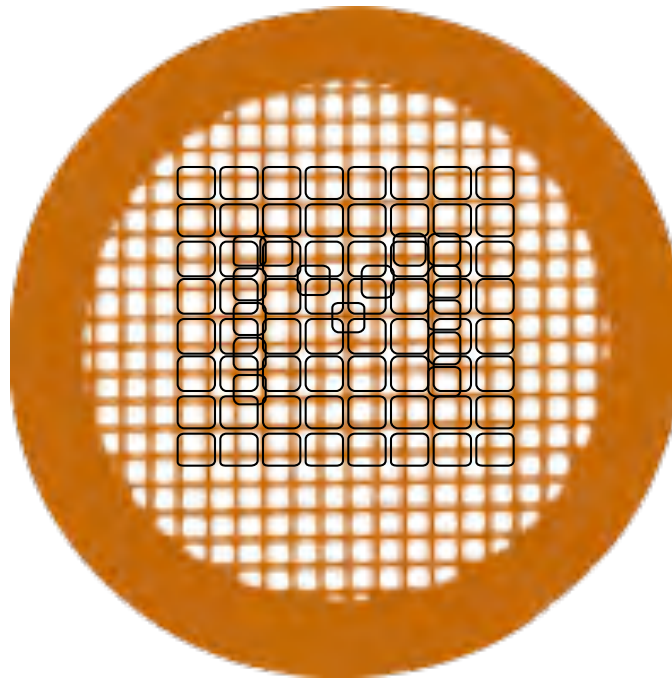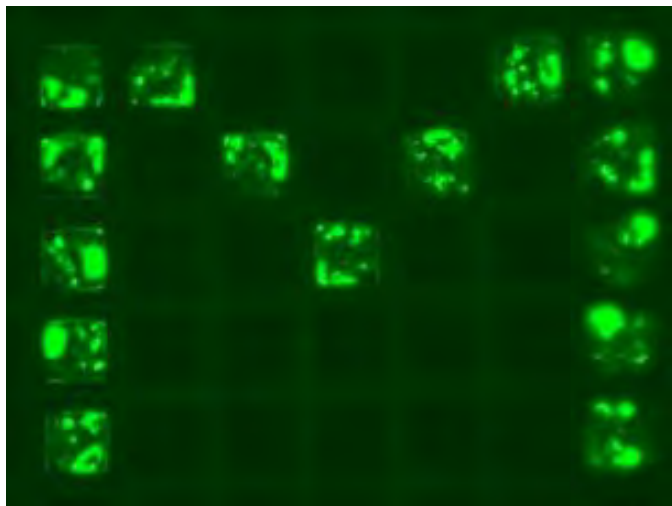Now focusing on even drop spreading and wetability of substrates

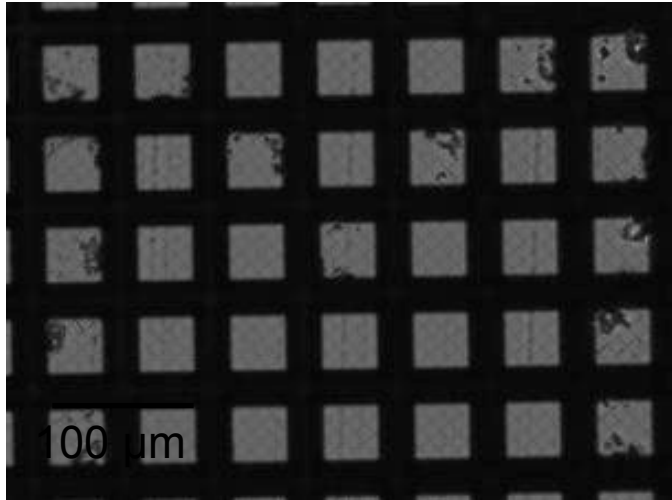# A different application for specimen preparation automation:
Screening and rapid characterization

Typhon (The Concept)

Panel of nanoparticles in standard well-plate → Inkjet picoliter dispensing → Registered transfer of 96 specimens to a single EM grid → Insertion into TEM → Automated multi-scale TEM imaging → Image analysis & characterization
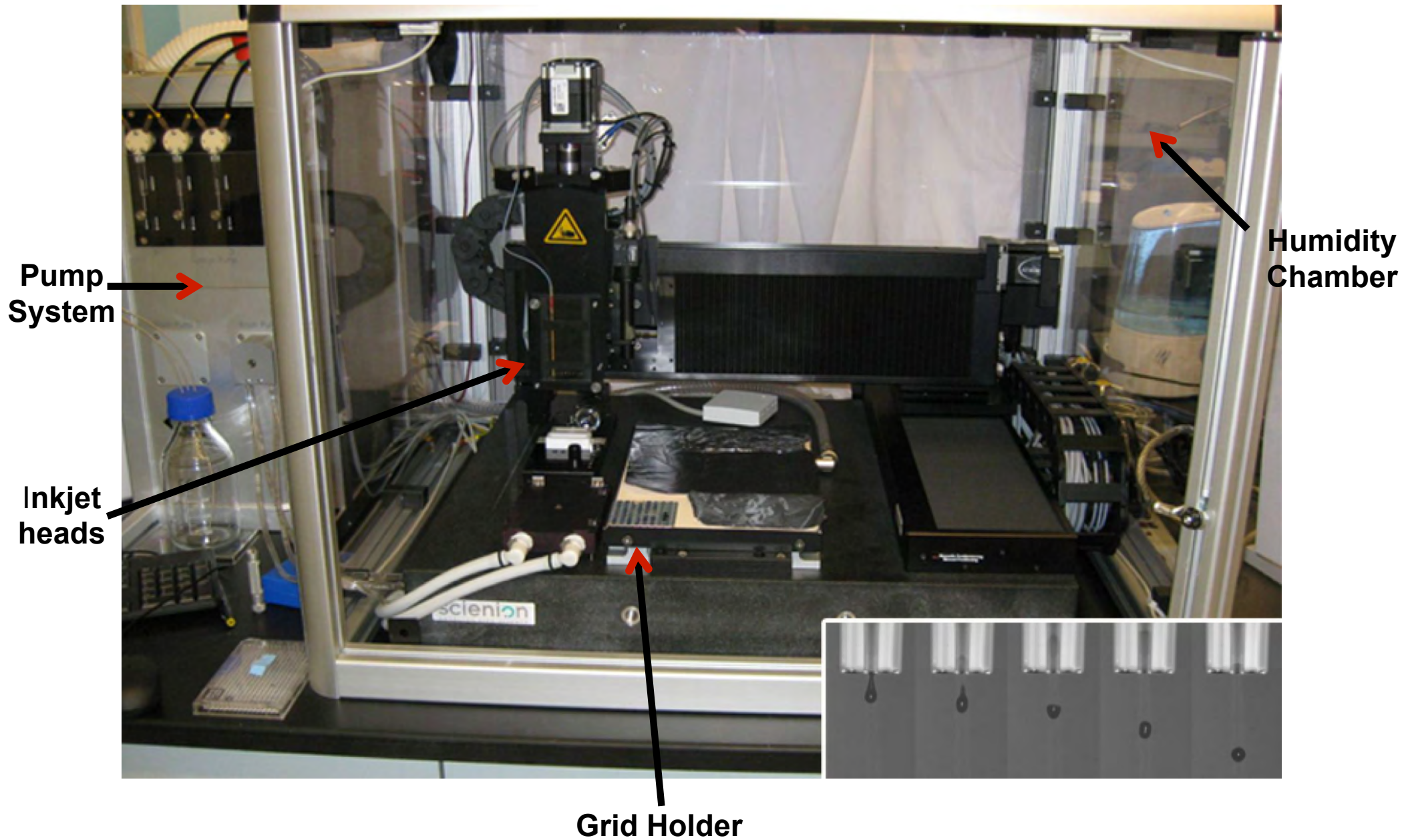
85 mm — 3 mm — 500 nm

Sample can be precisely targeted and the grid is a very large space
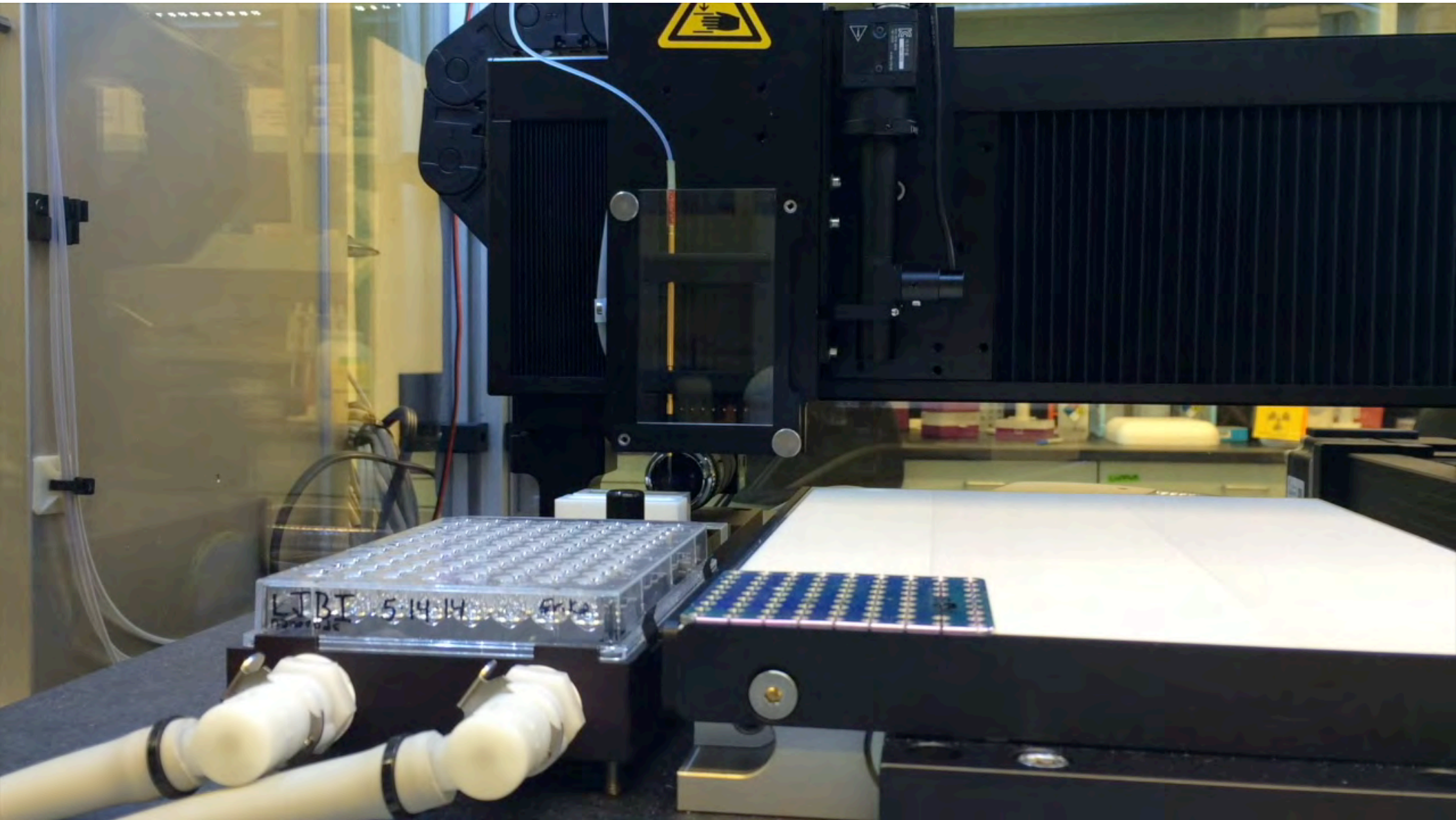

100 μm

☐ ~1,000 grid squares available

Typhon v0.5

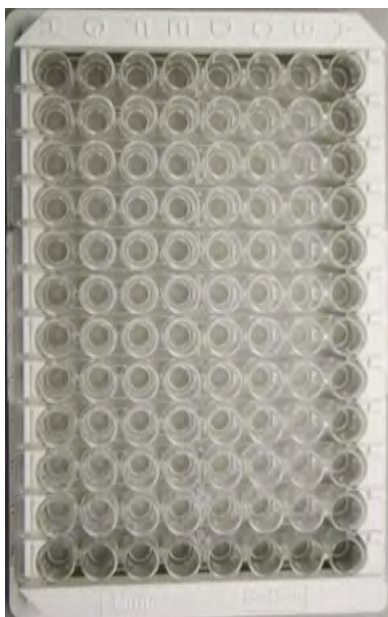## Scienion sciFLEXARRAYER S3, 8 inkjet heads, ~100 pl drops

Typphon v0.5 in action
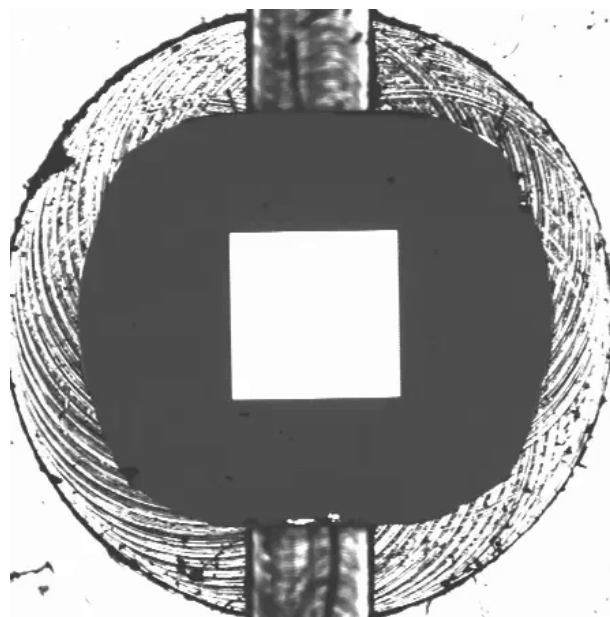
Typhon v0.5
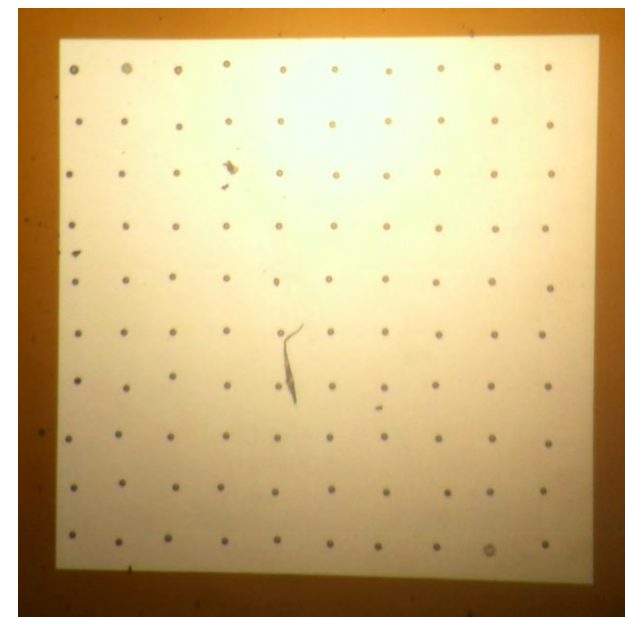
Microtitre Plate Direct Transfer to EM Grid
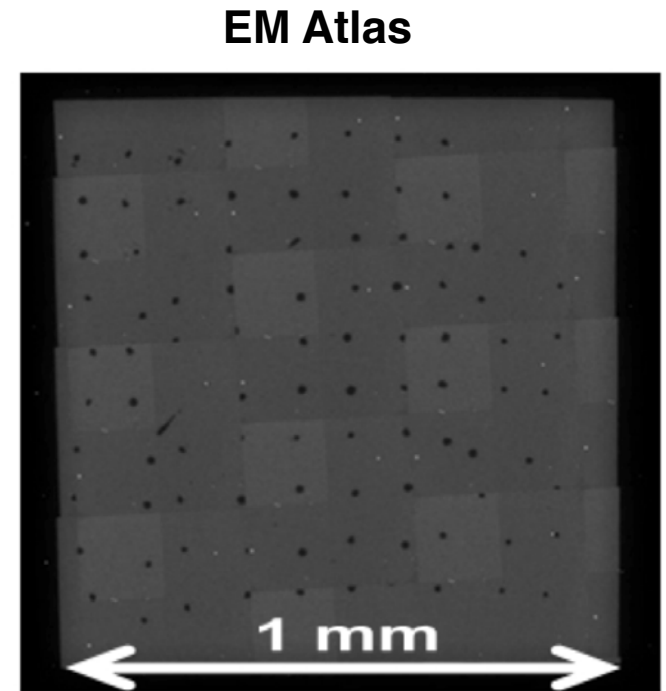

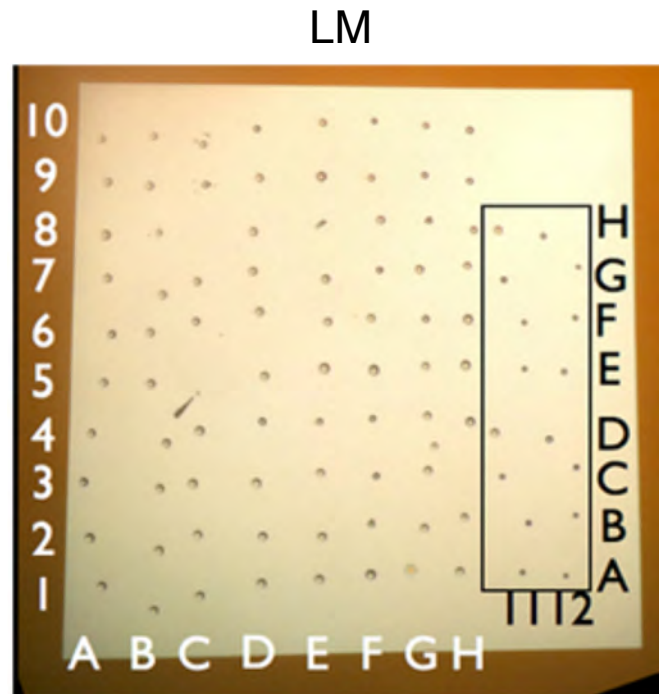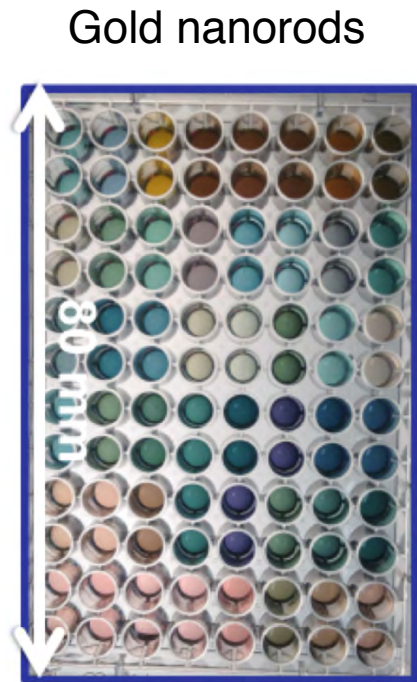Microtiter plate

80 mm

Video

3 mm

LM

1 mm

Typhon v0.5

Application: Screening of nano particles (John Nolan group, Scintillon Inst.)

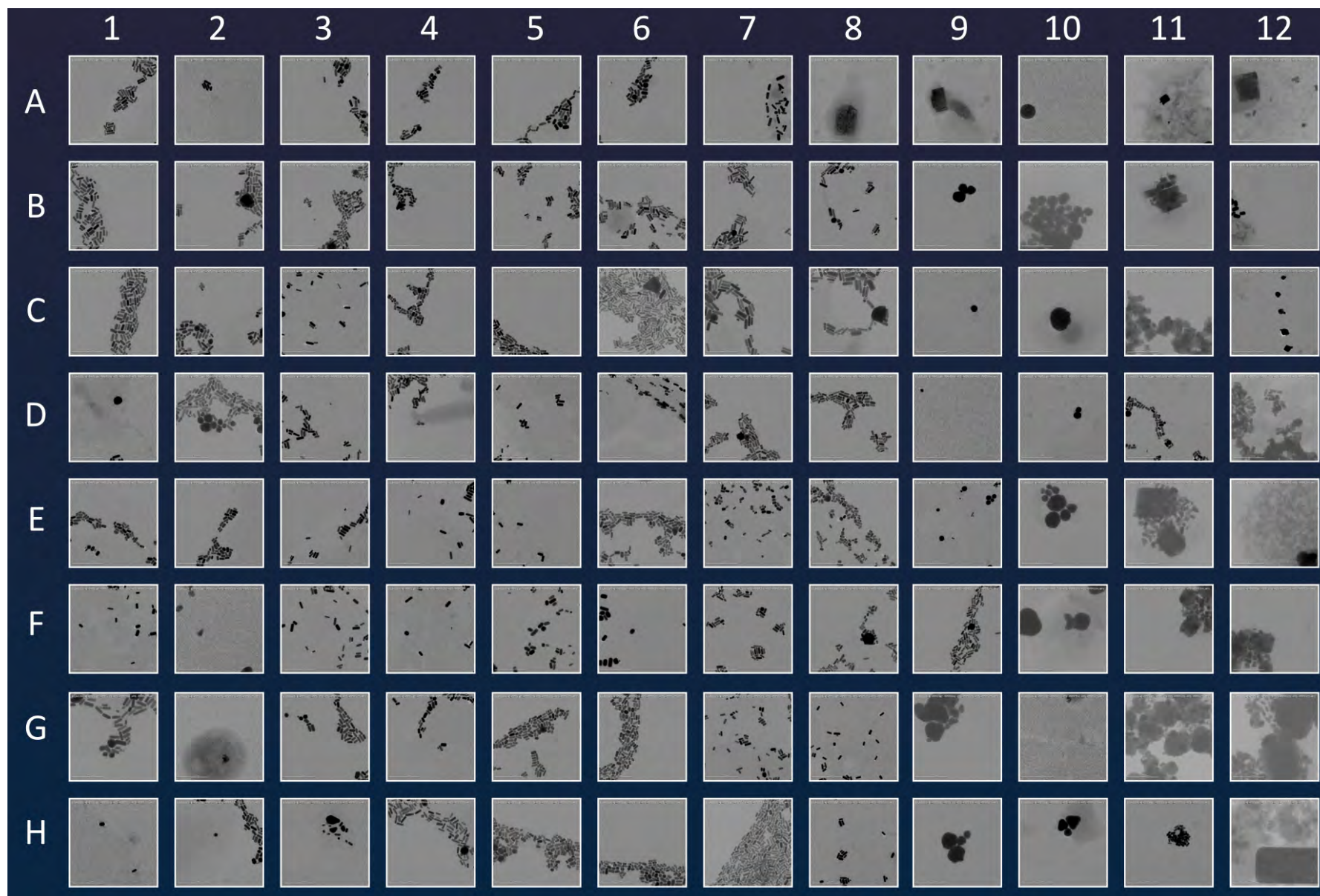Gold nanorods

LM

1 mm

EM Atlas
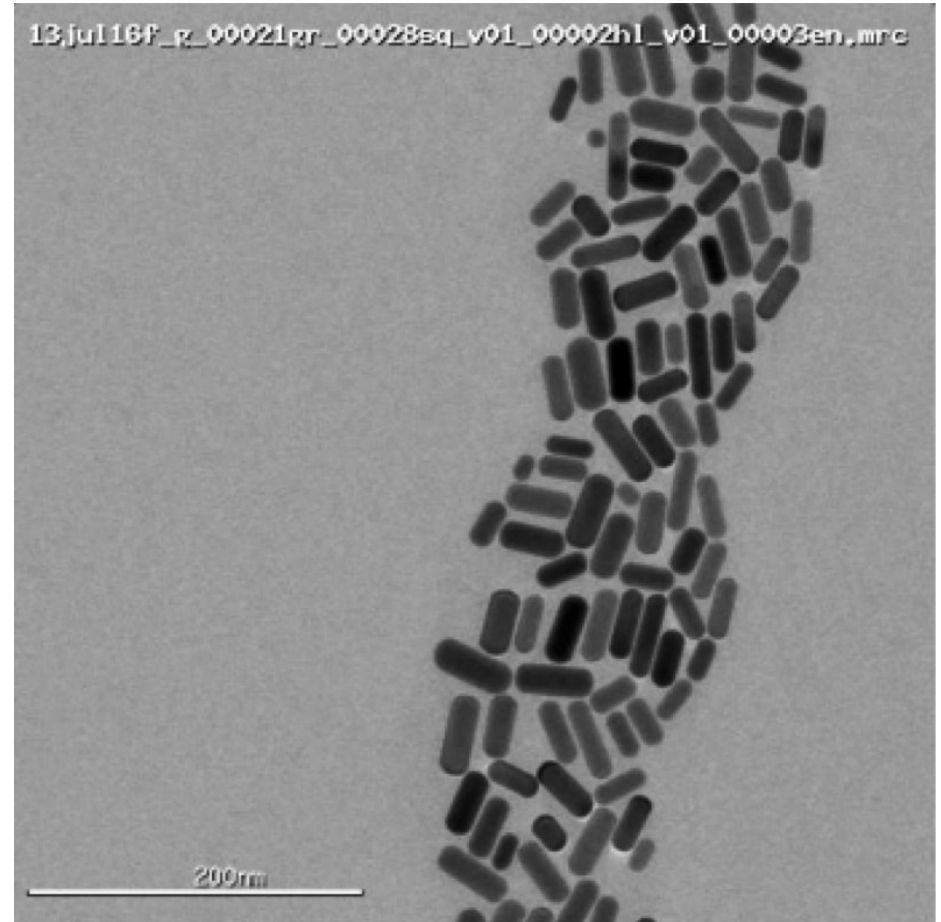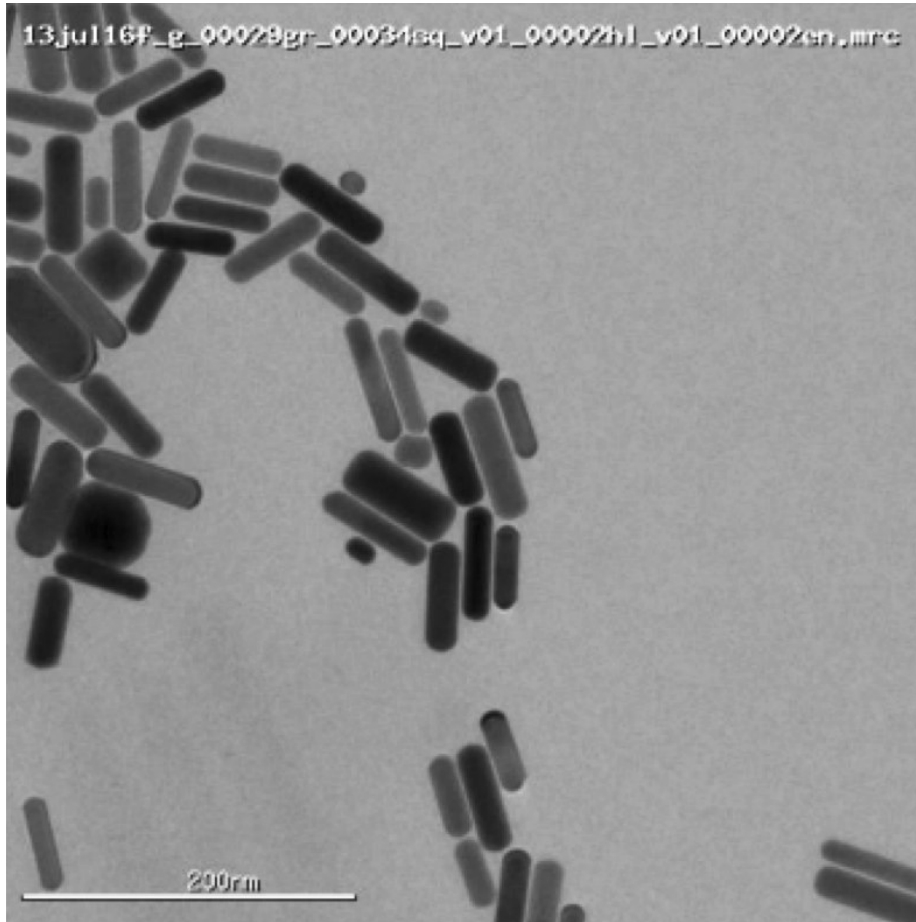
1 mm

Semi-automated multiscale imaging
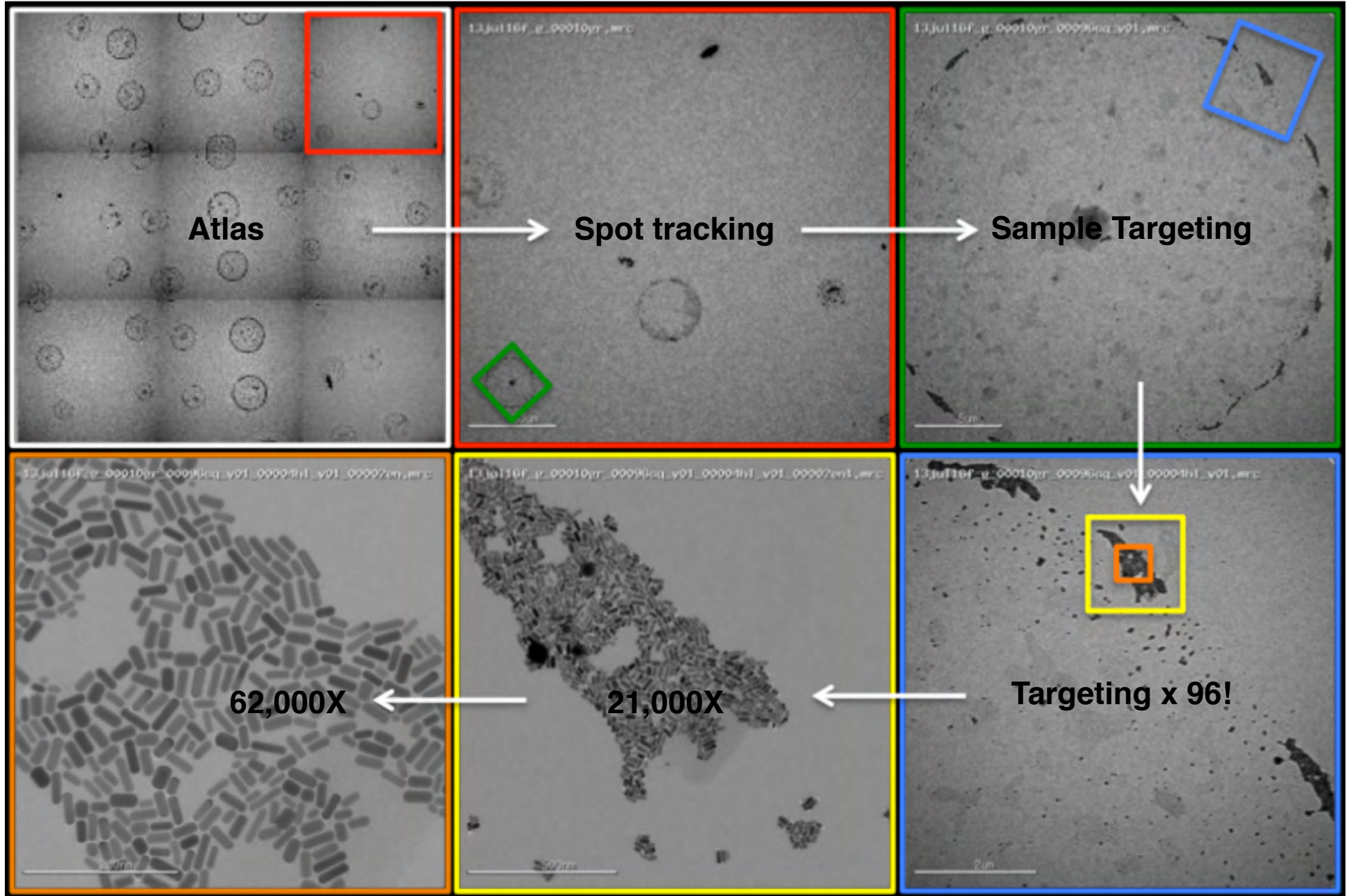
Typhon v0.5

High magnification thumbnail images of 96 individual samples

Examples of high magnification image of two samples

Suitable resolution and detail for downstream analysis in CellProfiler
**Distribution of sizes and shapes can be accurately determined**
10-20 images of each sample have enough particles for statistical characterization
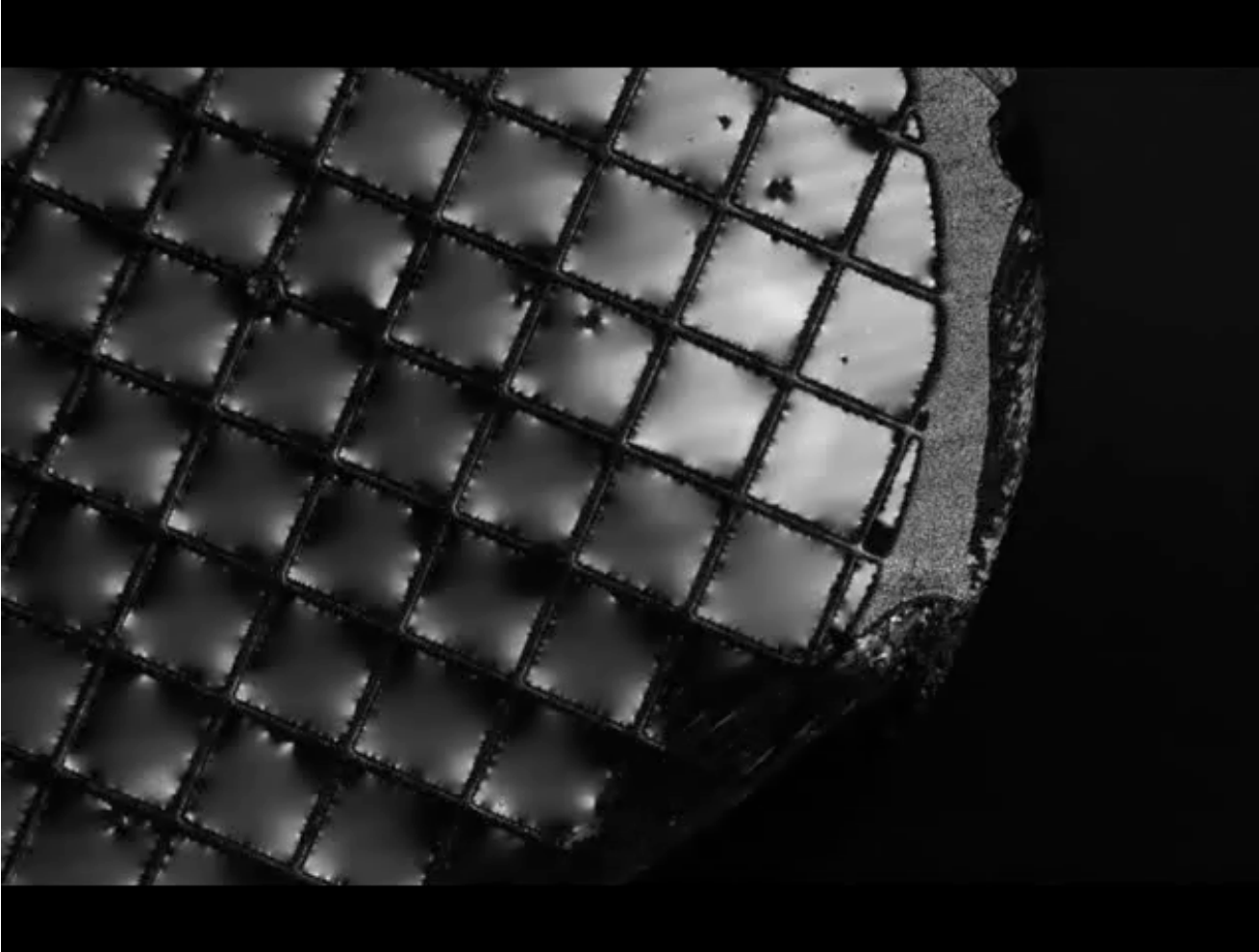
Typhon v0.5 Status

Flexible system based on commercially available liquid handler

Capable of placing 96 samples on a single grid and acquiring high magnification images in 24 hours

Focus is now on optimizing process and adapting for negative staining of proteins
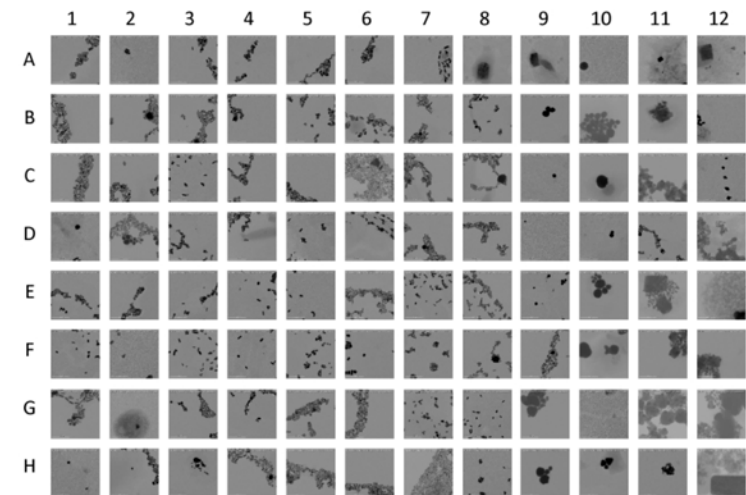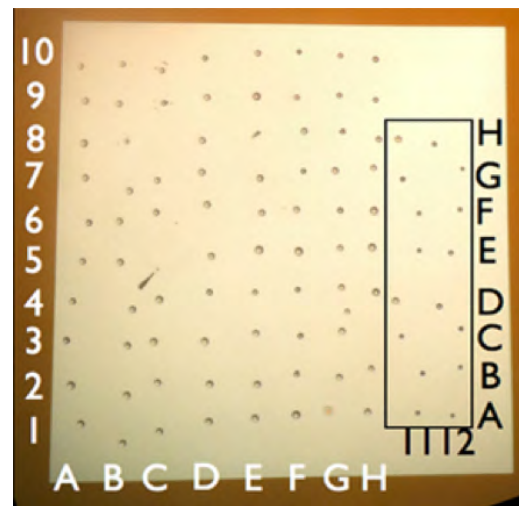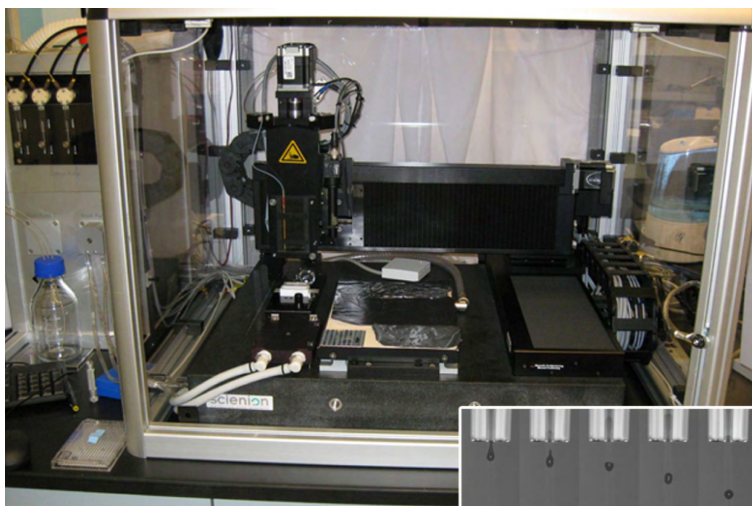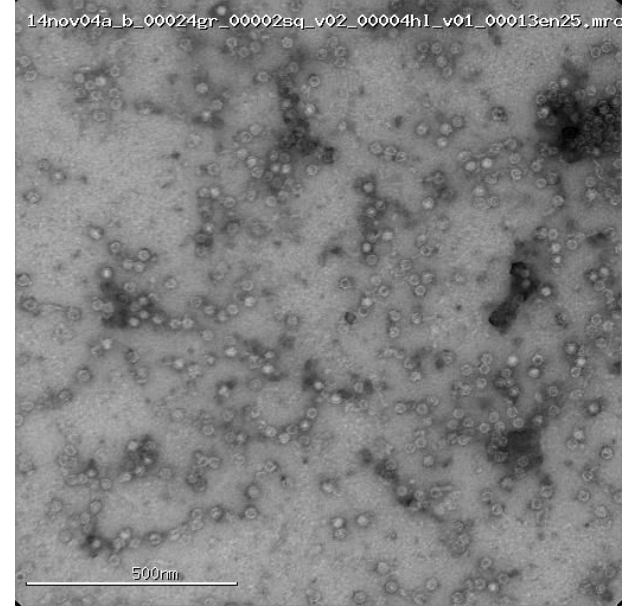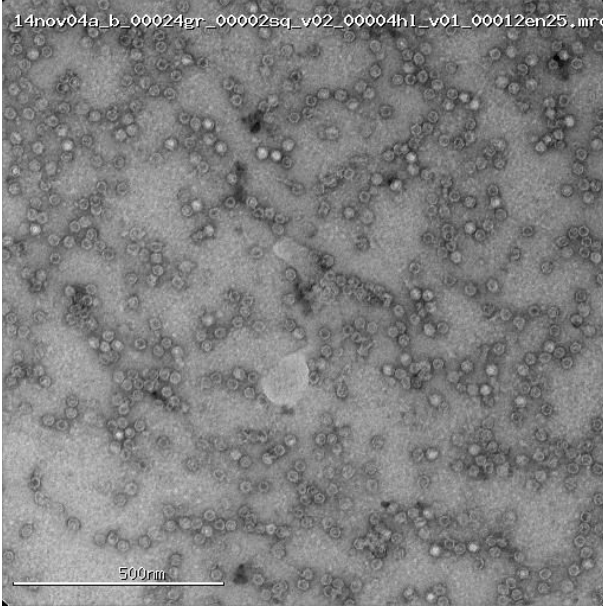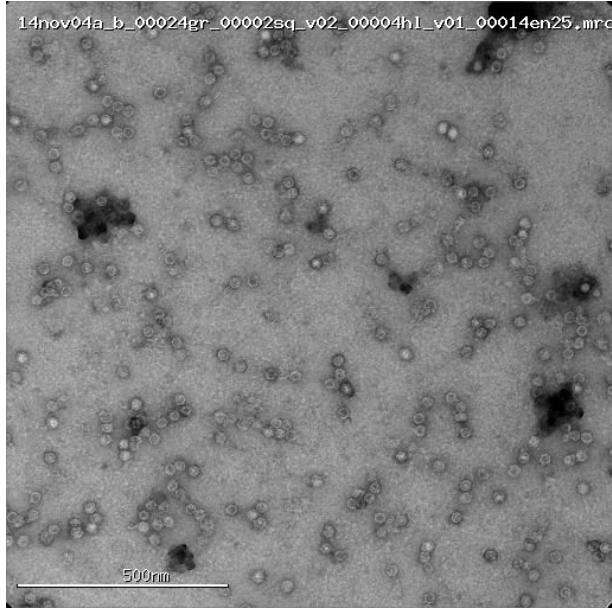
Negative Staining of Proteins

100 mesh Cu grid

Carbon on Formvar

Protocol: 1.8nL sample followed by 1.8nL 2% UF
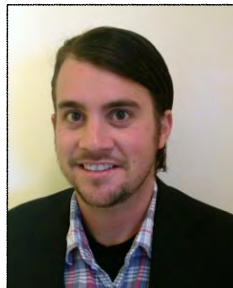
CPMV 0.65mg/mL

The Automated Molecular Microscopy Group

Anchi Cheng

Sean Mulligan

Ivan Razinkov

Jeff Speir

John Crum

Sargis Dallakian

Melody Campbell

David Veesler

Emily Greene

Lorraine Lathrop

Clint Potter

Bridget Carragher

THE SCRIPPS RESEARCH INSTITUTE

National Resource for Automated Molecular Microscopy
http://nramm.scripps.edu