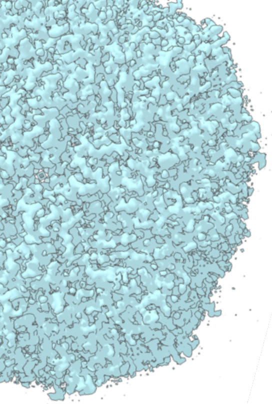
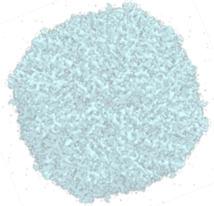
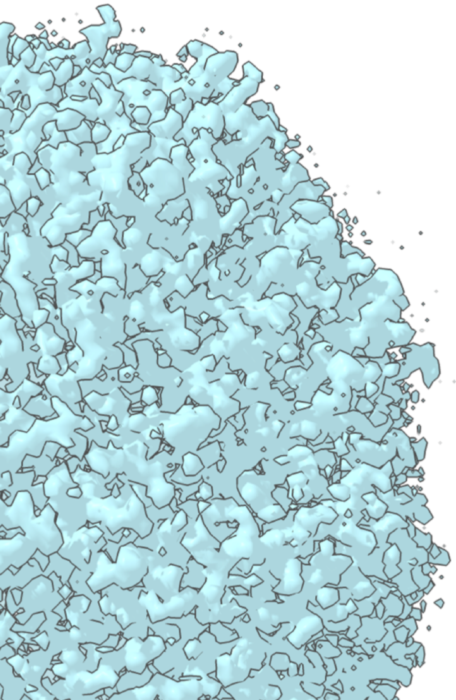




High-speed single-particle data collection protocols using a 200 keV cryoTEM



Joshua Strauss Ph.D.

Research Assistant Professor

Department of Biochemistry and Biophysics

Director of the CryoEM Core Facility

University of North Carolina at Chapel Hill

https://www.med.unc.edu/cryo-em/

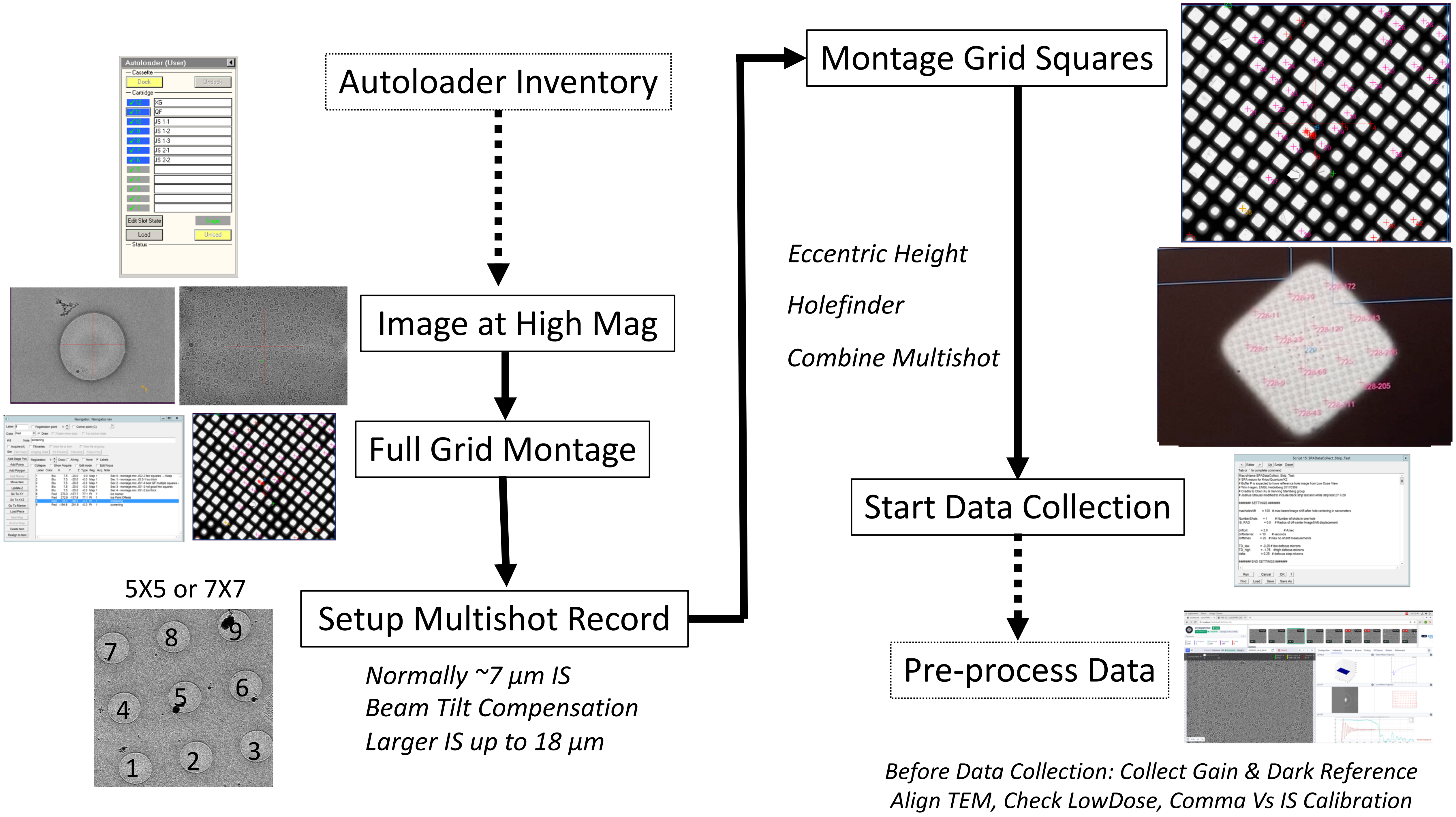
Why is Data Collection Speed Important

- Maximize use of cryoTEM beam time
- Data collection is informative for screening
- Limit bottleneck and wait time for access to a cryoTEM
- Some heterogenous samples require lots of data >10,000 movies
- Screening and “smart data collection” pipelines

UNC CH CryoEM Core

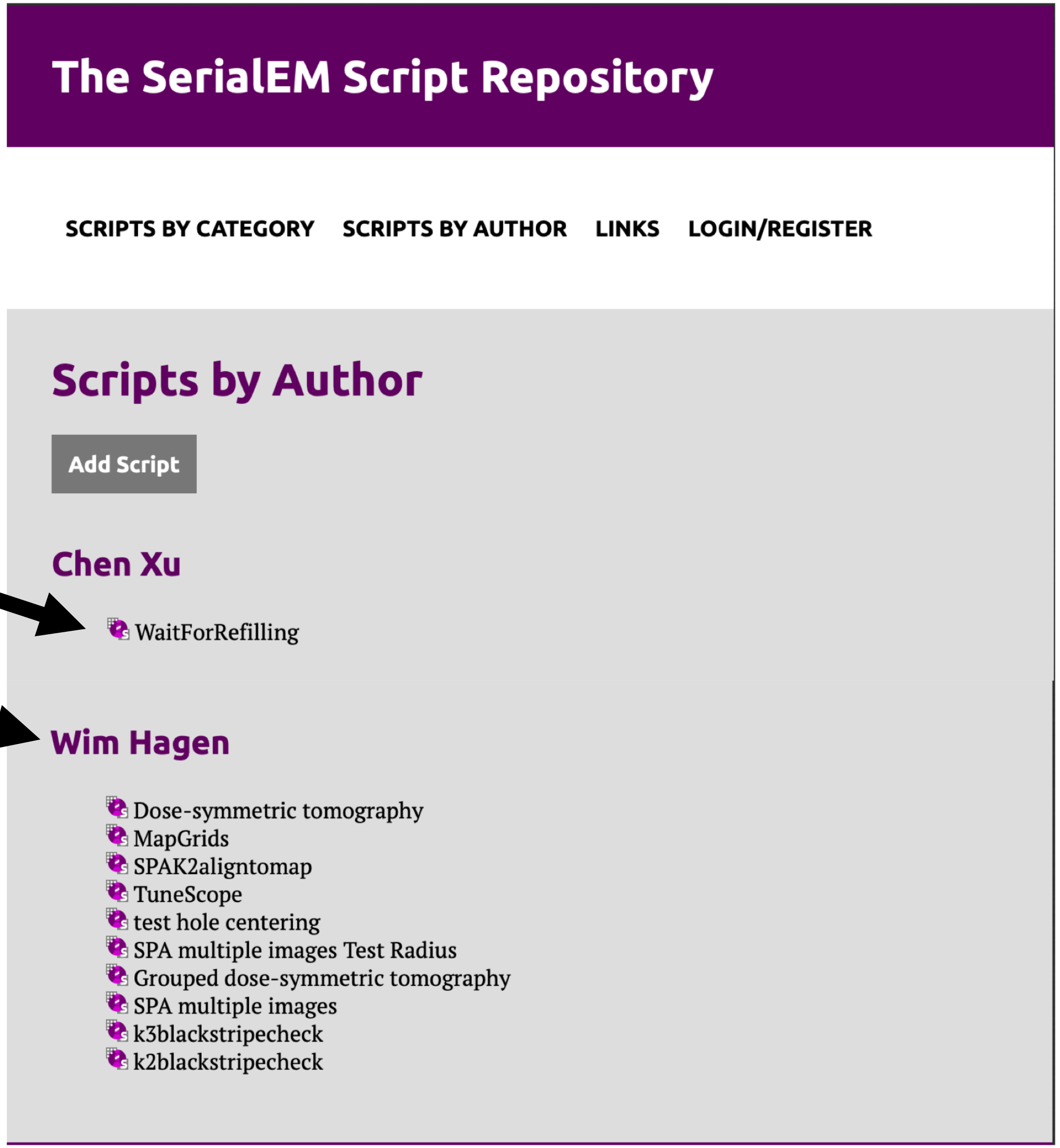
Talos Arctica G3

Mon, 14 Feb	Tue, 15 Feb	Wed, 16 Feb	Thu, 17 Feb	Fri, 18 Feb
09:00 AM - 01:00 PM [redacted] Screening: Self Use / **Price:** $85.77/hr	**09:00 AM - 02:00 PM** [redacted] Screening: Self Use / **Price:** $85.77/hr	**09:00 AM - 11:30 AM** [redacted] Screening: Self Use / **Price:** $85.77/hr **11:30 AM - 02:00 PM** [redacted] Assisted Use / **Price:** $74.81/hr	**09:00 AM - 02:00 PM** [redacted] Screening: Self Use / **Price:** $85.77/hr	**09:00 AM - 02:00 PM** [redacted] External_MOU_Screening_Self / **Price:** $55.16/hr
01:00 PM - 07:00 PM [redacted] Assisted Use / **Price:** $74.81/hr	**02:00 PM - 12:00 AM** [redacted] Screening: Self Use / **Price:** $55.16/hr Data Collection O/N	**02:00 PM - 08:30 PM** [redacted] Assisted Use / **Price:** $74.81/hr CryoCycle	**02:00 PM - 12:00 AM** [redacted] Screening: Self Use / **Price:** $85.77/hr Data Collection O/N	**02:00 PM - 12:00 AM** [redacted] Screening: Self Use / **Price:** $55.16/hr Data Collection O/W

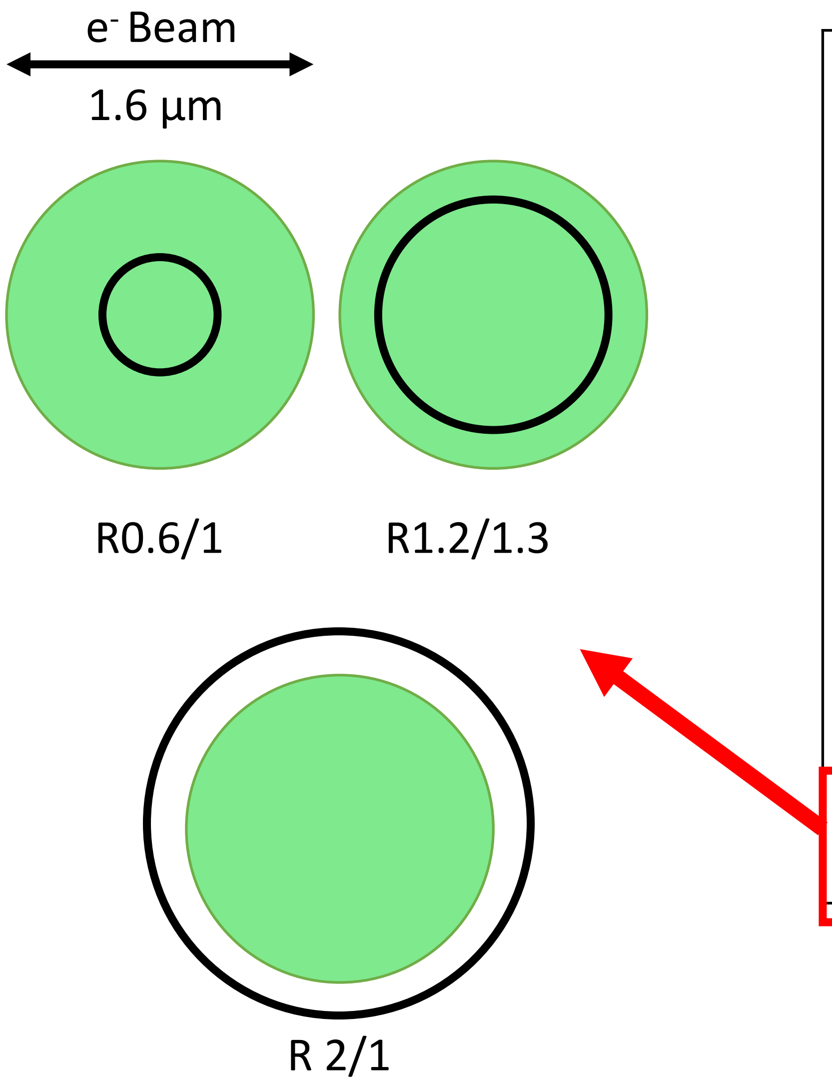

Autoloader (User)
Cassette
Dock
Undock
Cartridge
XG
QF
JS 1-1
JS 1-2
JS 1-3
JS 2-1
JS 2-2
Edit Slot State
Load
Unload
Status
Autoloader Inventory
Image at High Mag
Full Grid Montage
5X5 or 7X7
7
8
9
4
5
6
1
2
3
Setup Multishot Record
Normally ~7 µm IS
Beam Tilt Compensation
Larger IS up to 18 µm
Montage Grid Squares
Eccentric Height
Holefinder
Combine Multishot
Start Data Collection
Pre-process Data
Before Data Collection: Collect Gain & Dark Reference
Align TEM, Check LowDose, Comma Vs IS Calibration

CryoEM SPA Data Collection Script

```
MacroName UpdatedDataCollection_V3
# SPA macro for Krios/Quantum/K2
# Buffer P is expected to have reference hole image from Low Dose View
# Wim Hagen, EMBL Heidelberg 20170309
# Credits to Chen Xu & Henning Stahlberg group
# Joshua Strauss modified to include black strip test
# Joshua Strauss modified 20200824 hole center and defocus check
# Joshua Strauss modified 201024 will save LastView for troubleshooting
# Jared Peck modified to add SaveLog
# JF mod touch on/off toggle in settings and colval on
# JVP 36K has defocus offset of +0.5
# JDS Black Strip View False + adjust threshold 980
# JVP modified time reporting and trimmed out some delays, needs testing
# JDS removed WH IS for Multishot
################################################# SETTINGS #################################################

maxholeshift        = 100   # max beam/image shift after hole centering in nanometers

driftcrit            = 2.0      # A/sec
driftinterval        = 10         # seconds of delay between drift measurements
drifttimes          = 7 # max no of drift measurements

TD_low              =  -0.5 # low defocus microns
TD_high             =  -1.5   #high defocus microns
delta                    = 0.5   # defocus step microns 0.1

touch                   = 1   #set to 0 for off
bmsft                   = 0.22

############################################ Start of collection ###########################################
```



SPA Data Collection Talos Arctica Gatan K3



50 µm Condenser Aperture

Spot Size	% C2 Parallel Illumination	Beam Diameter (µm)	Flux (e^-/pixel/sec)	Dose Rate (e^-/Å²/sec)
1	46.461	1.73	29.94	36.16
2	44.829	1.599	25.54	30.84
3	43.2	1.67	15.58	18.81
4	41.891	1.632	10.47	12.64
5	40.826	1.695	6.16	7.44
6	39.288	1.567	3.84	4.64
7	38.468	1.64	1.82	2.2
8	37.952	1.62	0.6	0.72
Average Beam Diameter (µm)		**1.64**		

Measurements made at nominal magnification of 54,900 X at the detector level, corresponding to pixel size of 0.88 Å.
The objective and selected area aperture were removed.
Gun lens was set to 4.

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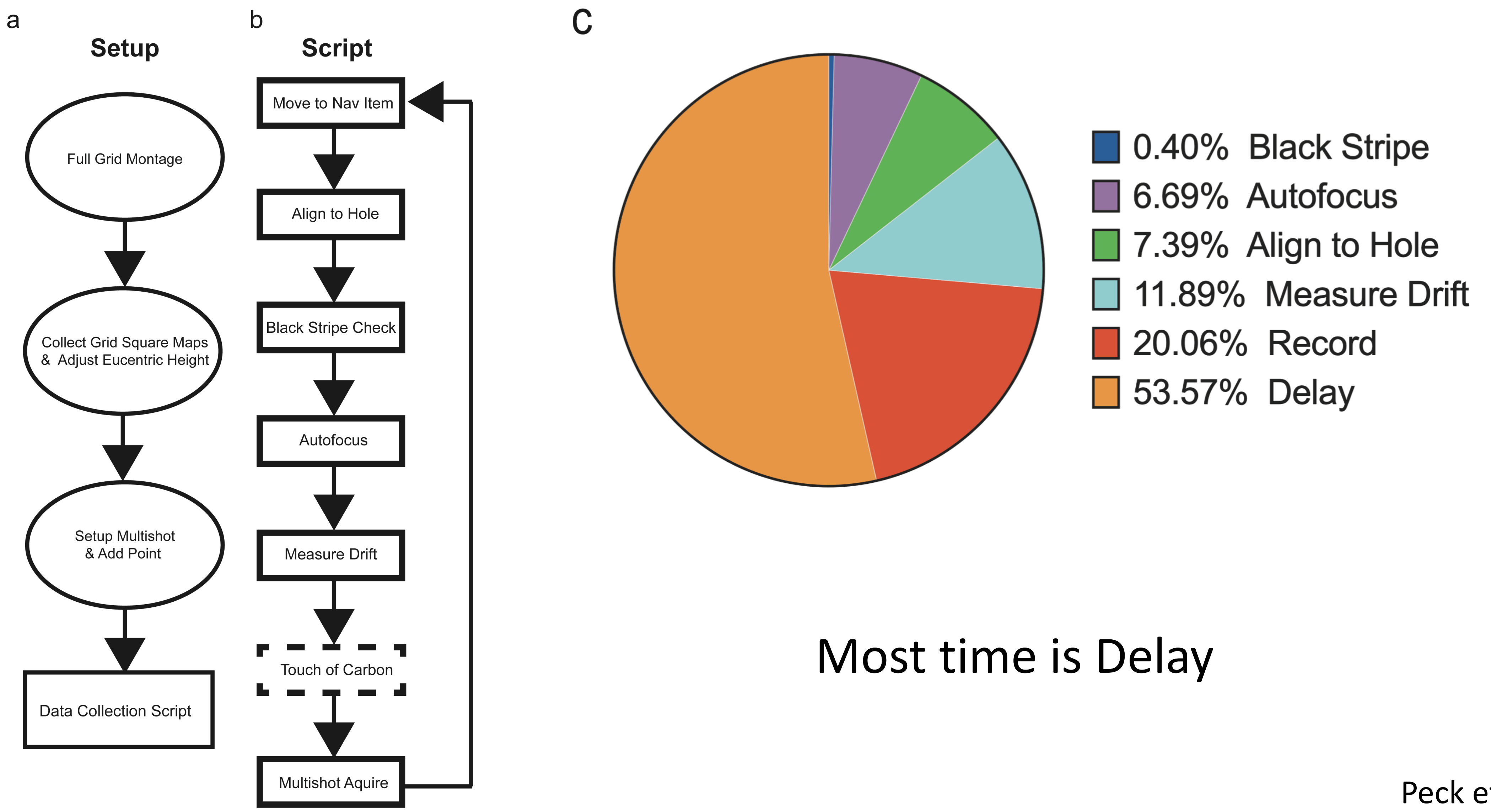
SPA Data Collection Setup and SerialEM Scrip



Most time is Delay

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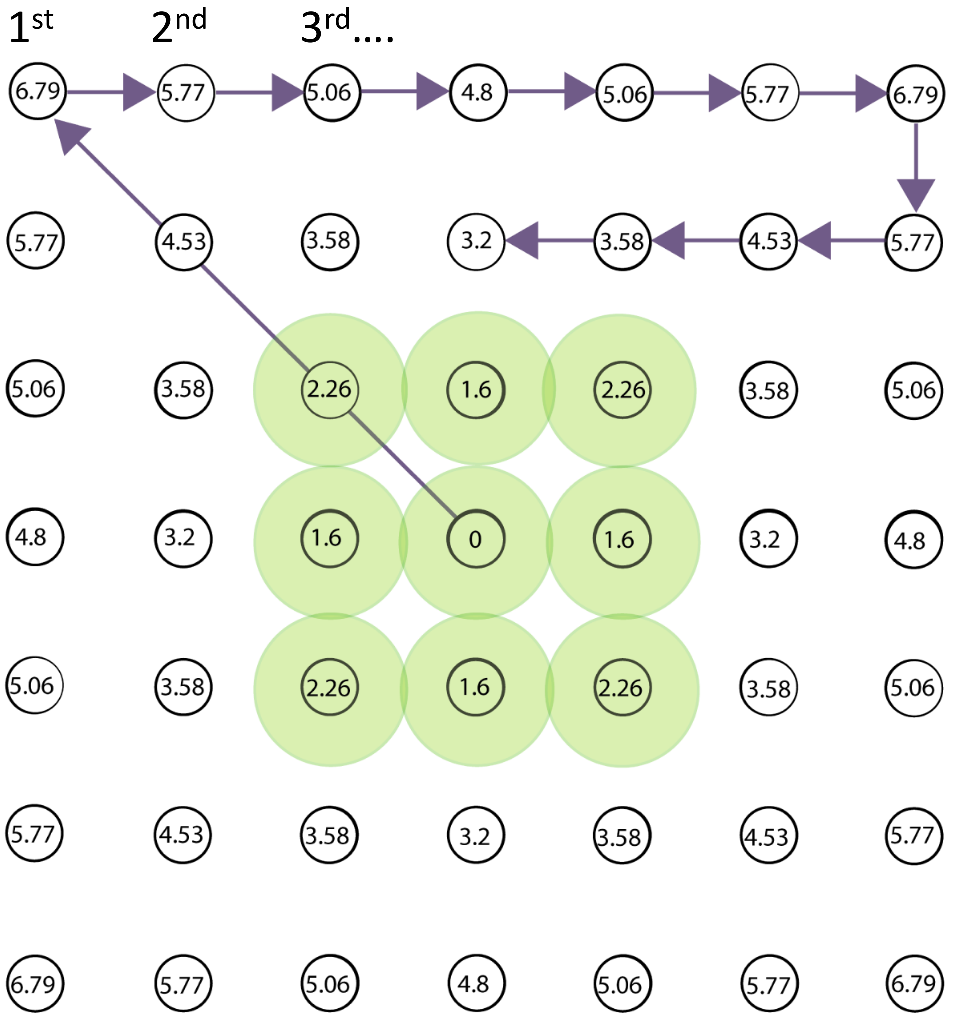
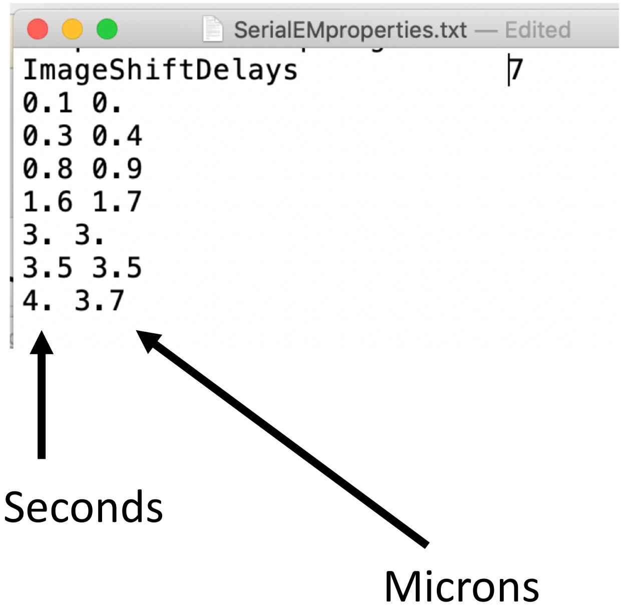
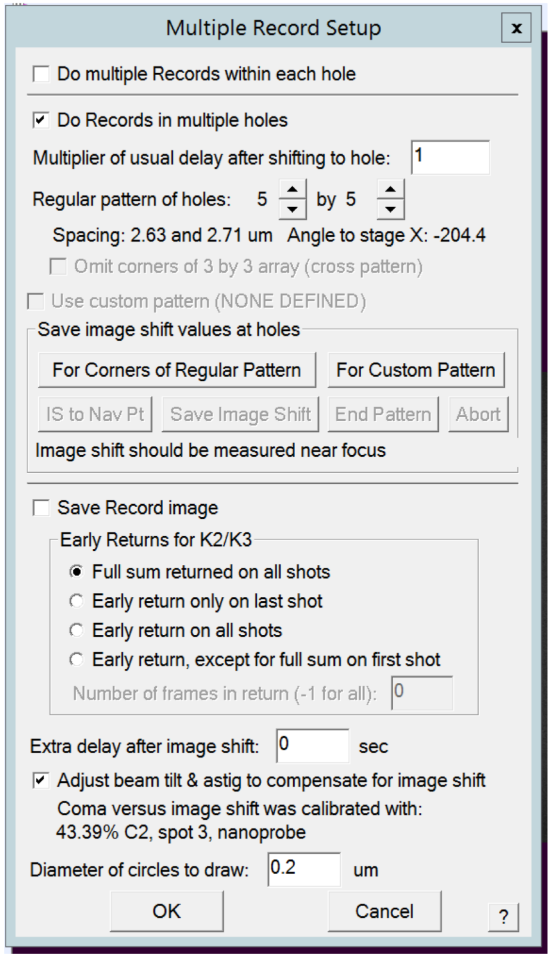
Multishot setup for Talos Arctica on R0.6/1 Quantifoil TEM Grid



IS Delay is proportional to the IS distance





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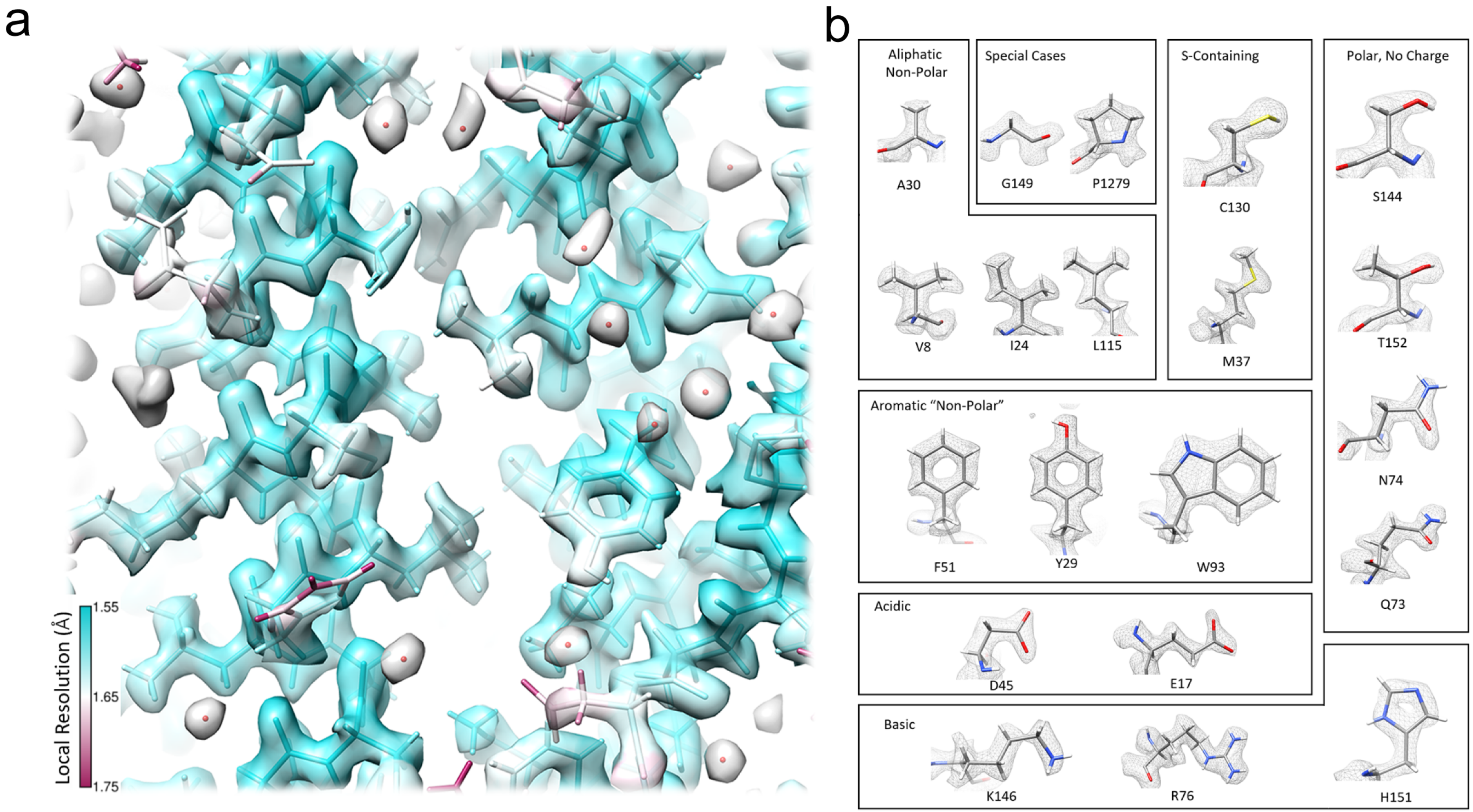
EM Map Apoferritin Collected Overnight



0.5 second IS delay conservative to speed up data collection

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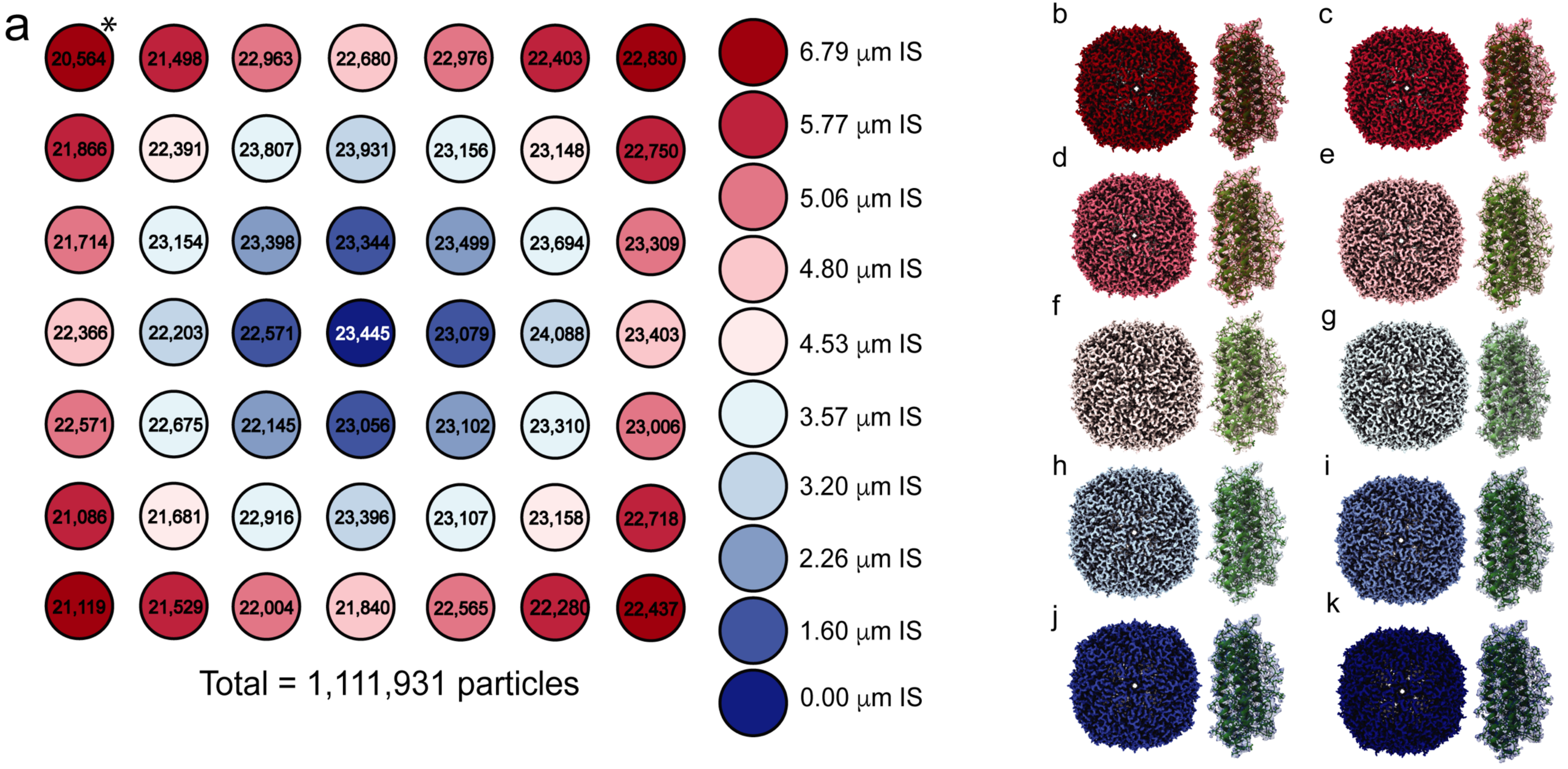
Does IS Long Distance Reduce Data Quality?

Particle Contribution to Final EM Map by Multshot Position



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Subgroup analysis of overnight data collection

Image Shift (µm)	Number of Holes	IS Delay (sec)	Number of Particles in Each Group	Number of Particles in EM Map	Resolution (Å)	Beta Factor
6.79	3	2.6	66386	20564	2.06	41
6.79	1	7.8	20564	20564	2.06	42.5
6.79	4	7.8, 2.6	86950	23445	2.06	43.4
5.77	8	2.6	176130	23445	2.02	41.5
5.06	8	2.6	181108	23445	2.04	42.6
4.8	4	2.6	90289	23445	2.04	43.1
4.53	4	2.6	90378	23445	2.03	41.7
3.58	8	2.6	185819	23445	2.02	42
3.2	4	2.6	93618	23445	2.03	42.5
2.26	4	2.6	92144	23445	2.03	42.2
1.6	4	2.6	92050	23445	2.06	42.2
0	1	2.6	23445	23445	2.08	43

IS Delay

IS Distance

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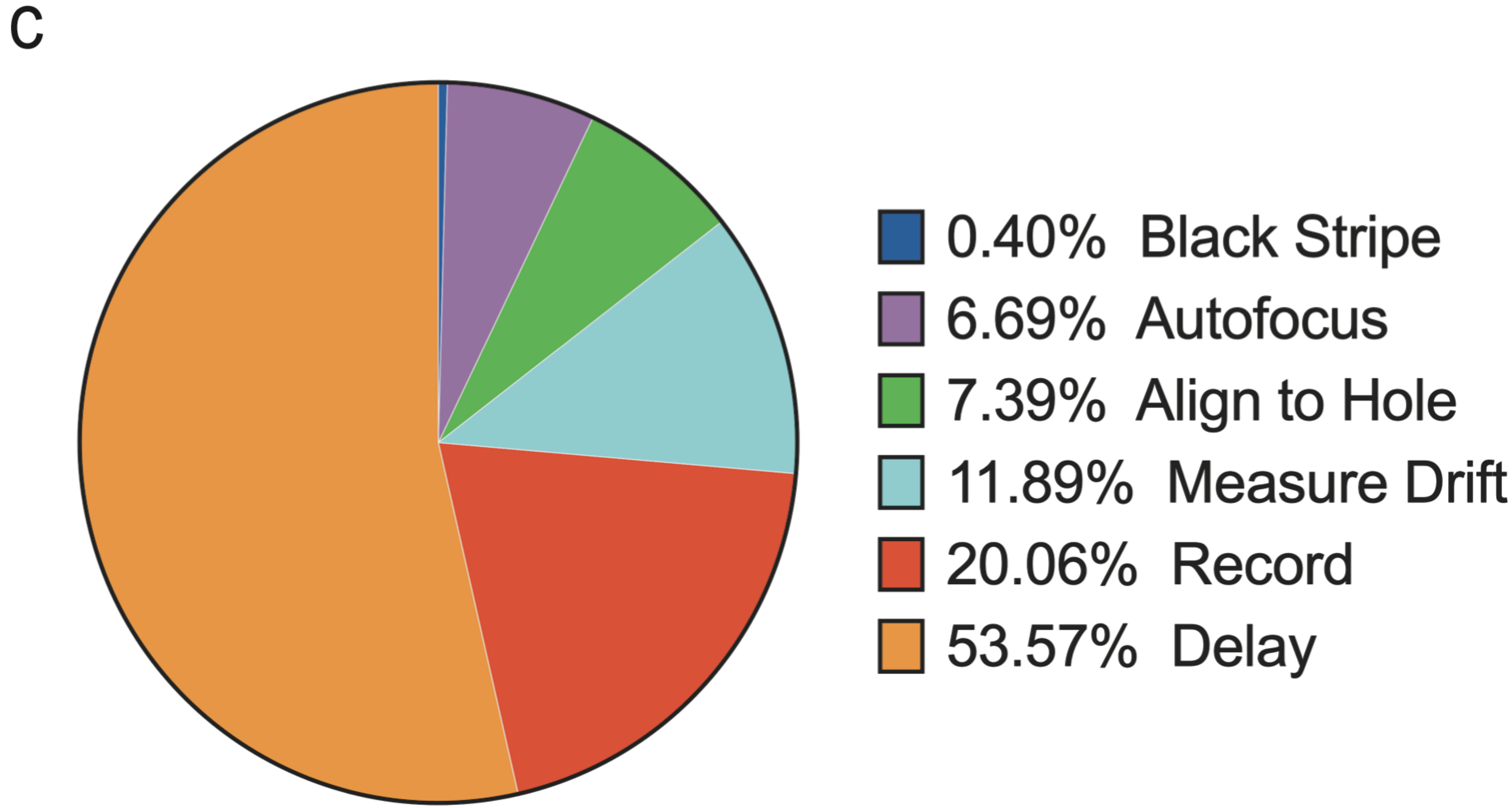
Is Less IS Delay Detrimental ?



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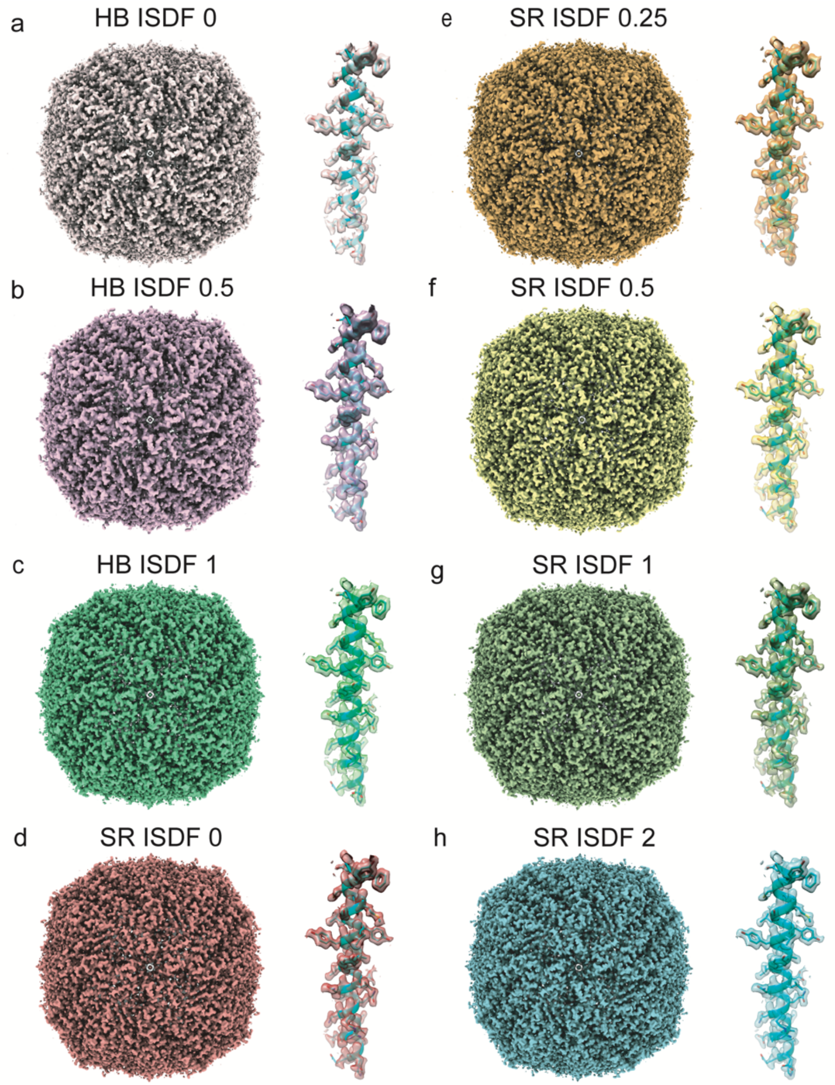
Data collection speeds using different image shift delay factors

Table 1
Data collection and EM map statistics.

Data collection	Super-resolution	Hardware-binned
Microscope	Talos Arctica G3	Talos Arctica G3
Voltage (keV)	200	200
Nominal magnification	54900	54900
Detector	Gatan K3	Gatan K3
Pixel size (Å)	0.44	0.88
Multishot	7 × 7	7 × 7
Image shift delay factor	0, 0.25, 0.5, 1.0, 2.0	0, 0.5, 1.0
Cumulative exposure ($e^- Å^{-2}$)	45.367	45.367
Exposure rate (e^- pixel^{-1} s^{-1})	12.9	12.9
Exposure per frame ($e^- Å^{-2}$)	0.756	0.756
Defocus range (μm)	0.46–1.59	0.46–1.49
Movies collected	490	490
Data collection rate (movies per hour)	346, 348, 342, 296, 240	524, 488, 426
EM Map		
Final particles (No.)	313040	313040
Symmetry imposed	O	O
Resolution at FSC 0.143 Å (unmasked/masked)	2.1/1.85, 2.0/1.83, 2.0/1.81, 2.0/1.81, 2.0/1.80	2.0/1.83, 2.1/1.90, 2.0/1.81
Map-sharpening *B*-factor (Å)	60.2, 56.8, 55.2, 55.7, 55	57.4, 68.9, 55.9
Fraction of physical Nyquist	0.95, 0.96, 0.97, 0.97, 0.98	0.96, 0.93, 0.97



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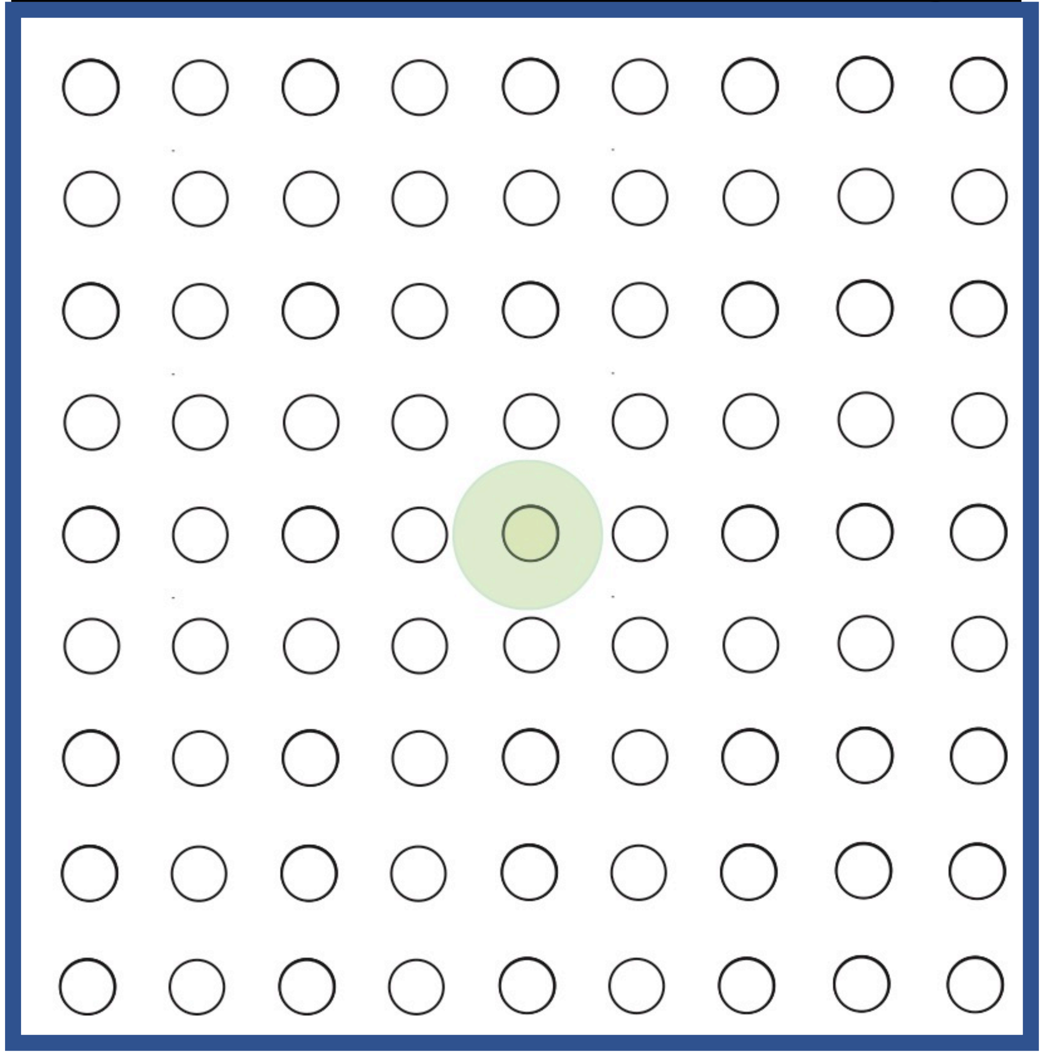
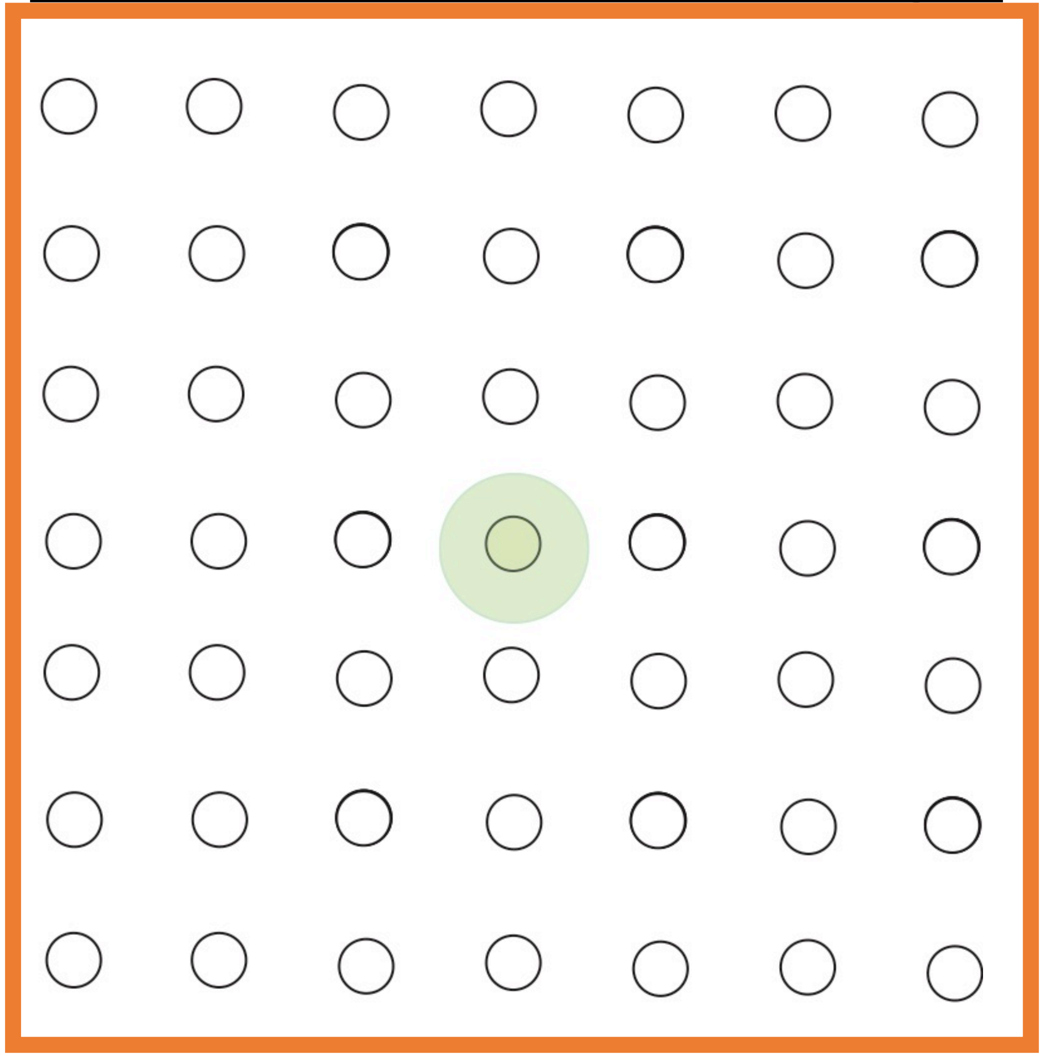
Can We Go Faster?

What if we had a better grid?

R06/06 9X9 Multishot 81 Images

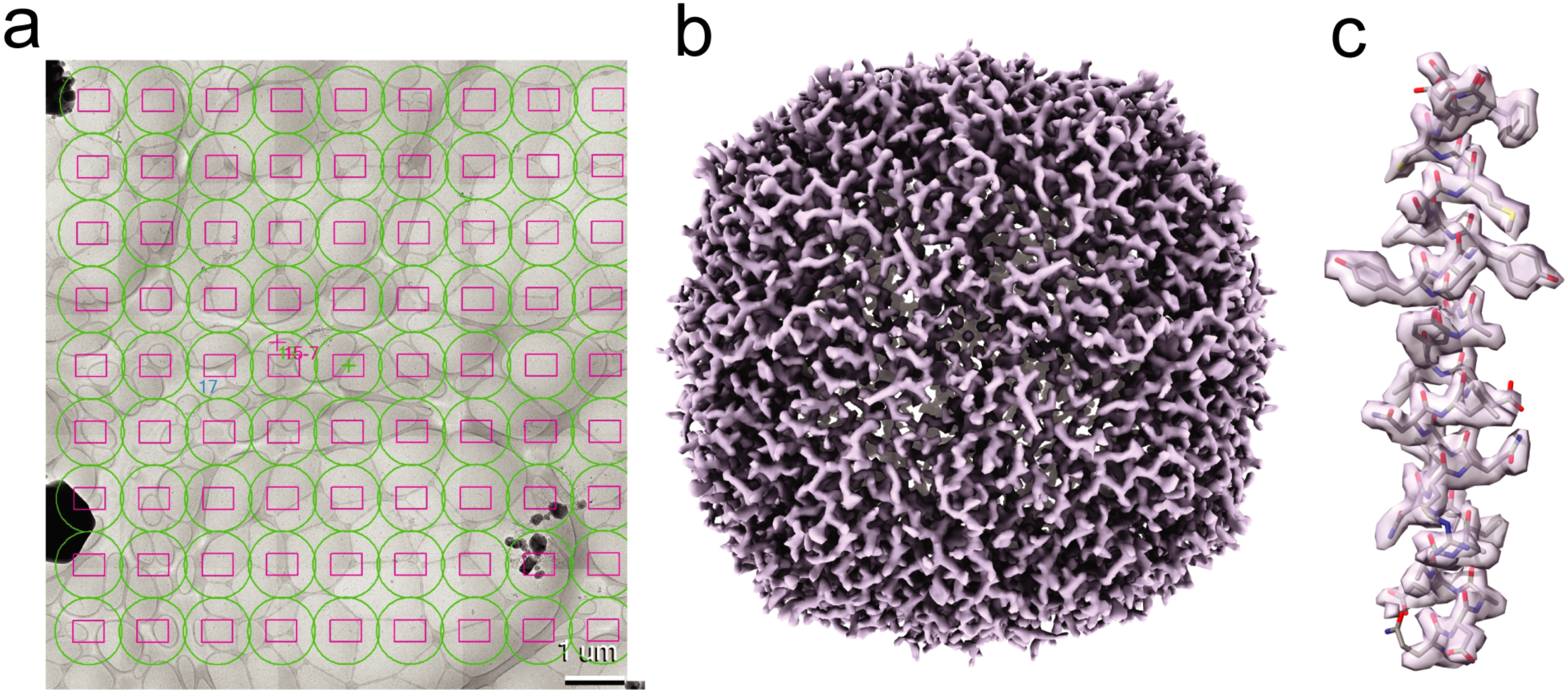


R06/1 7X7 Multishot 49 Images



66 % more holes per unit area

High-speed Data Collection on a Lacey TEM grid: Proof of concept while R06/06 grid is being made



Reduced exposure to 0.9 seconds and collected 20 frames

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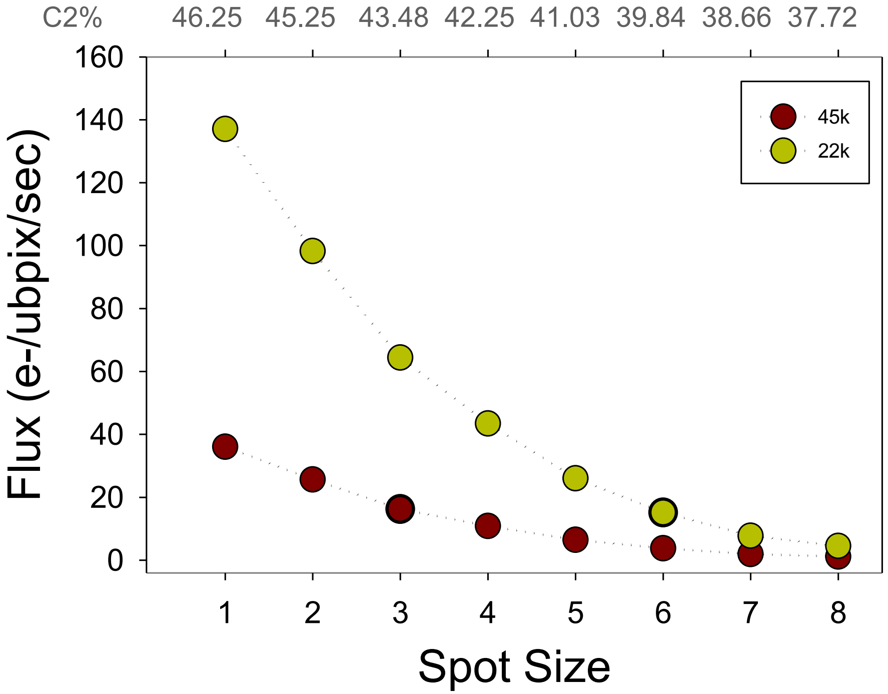
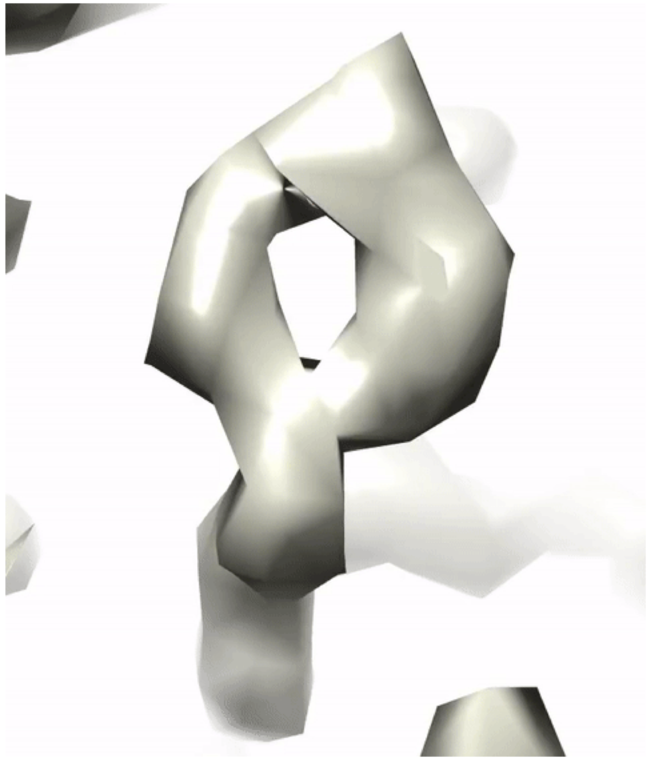
Particles per hour Super Resolution and Hardware Binned at different magnifications and spotsizes



Nominal Magnification	45k HWB	22k SR
Pixel size	0.88 Å/pix	0.89 Å/pix
Cryo Grid	R1.2/1.3 UF	R1.2/1.3 UF
Voltage (keV)	200	200
Multishot	5 x 5	5 x 5
Micrographs Used	400	420
Initial Particles	294,624	909,100
Final Particles	255,443	792,589
GSFSC Symmetry O	1.91 (P92J90)	2.00 (P97J57)
Frac Phys Nyquist	1.09	0.56
Bfactor	58.9	74.2
Defocus (SD)[a]	1.0 (0.3)	0.7 (0.5)
Range	0.1-1.8	0.1-2.0
Record time 25mic/min	3.25+0.04	7.1+0.5
Micrographs/hr	293 (+80%)	163 (-44%)
Particles/hr	214,396 (-39%)	348,942 (+63%)
GSFSC for ~0.74 mm^2 (micrographs/particles)	2.49 (P92J89) 4/2577	2.70 (P93J62) 1/2572
GSFSC for 250,000 particles		



Peck et al 2022 (Unpublished, M&M2022)

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UNC CH Dept BCBP

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