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Particles Per Hour as a Metric for Single-particle Cryo-EM Data Collection Speed When Comparing Super-resolution and Hardware-binned Data

Jared V. Peck[1], Joshua D. Strauss[1], and Jonathan F. Fay[1]*

[1.] Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
* Corresponding author: fayj@unc.edu

Optimization of single-particle cryo-electron microscopy (cryo-EM) data collection routines is critical for ensuring efficient use for cryo-TEM beam time. Recently, we showed that it is possible to collect high-resolution cryo-EM data on a 200 keV Talos Arctica G3 cryo-TEM equipped with a Gatan K3 direct-electron detector at speeds of up to 720 movies per hour [1]. EM maps of mouse apoferritin were collected with SerialEM [2] using beam-image shift (BIS) data collection. Speed was dependent on multiple factors including the number micrographs collected within each multishot (TEM grid hole size and spacing), the image-shift delay factor, and detector binning. We found data collection using super-resolution mode (SR) to proceed slower compared to hardware-binned mode (HWB). The resulting apoferritin maps reconstructed from data sets collected at a nominal magnification of 54,900x corresponding to a physical pixel size of 0.88 Å in either SR or HWB mode were comparable, with the estimated resolution ranging from 1.8-1.9Å.

Following up on our previous work, we explore another useful metric of data collection speed: particles per hour. By reducing the magnification from our previous data collections by half to 28,400x (physical pixel size of 1.78 Å), we can increase the field of view and effectively quadruple the number of particles in each micrograph. Collecting at this magnification allows for potentially faster data collection as measured by particles per hour, with the added benefit that collecting in SR mode makes is possible to surpass the physical Nyquist limit and maintain a comparable relative pixel size [3].

Here we sought to compare data collection speeds, measured in particles per hour at 54,900x magnification in HWB mode (physical pixel size 0.88 Å) and 28,400x magnification in SR mode (SR pixel size of 0.89 Å). The wider field of view at 28,400x magnification necessitated the use of UltrAufoil R1.2/1.3 TEM grid, which resulted in slower data collection rates than can be achieved on an R0.6/1 UltrAufoil grid using ~7 µm BIS. Moreover, lowering the magnification required us to change the spot size from 3 to 6 in order to maintain a comparable flux (~ 16 e^-/unbinned pixel/second) between datasets (Fig 1). The reduction in beam intensity in turn required a longer exposure time (2.7 to 5.4 seconds) to maintain the same exposure dose (~54 e^-/A^2).

We collected 2 datasets, collecting 400 movies in ~1.5 hours in HWB mode and 420 movies in ~3 hours in SR mode. Data collection rate as measured in micrographs per hour for SR mode proceeded 44% slower than in HWB mode, but there was a 63% increase in particles per hour due to the larger field of view (Table 1). EM maps were similar in appearance, as best illustrated by comparing aromatic amino acids Y29 and F41 (Fig 2). Although the relative pixel size was essentially the same, we found that the higher magnification HWB data produced slightly higher resolution structures (for maps with either C1 or O symmetry applied) (Table 1) using substantially fewer particles.

Lowering the magnification by half and utilizing SR mode enabled the collection of substantially more particles per hour compared to the higher magnification, HWB counterpart. However, data collection in

HWB mode required substantially fewer particles to obtain reconstructions of slightly higher resolution, making HWB data collection effectively faster than the lower magnification, SR counterpart for obtaining EM map of similar resolution in less time.

Table 1

Nominal Magnification	54,900x	28,400x
Pixel size (Å)	0.88	0.89
Cryo Grid	R1.2/1.3	R1.2/1.3
Voltage (keV)	200	200
Multishot Array	5 x 5	5 x 5
Micrographs Used	400	420
Initial Particles	296,708	909,100
Final Particles	276,645	792,589
GSFSC (Å) Symmetry O	1.88	2.00
Symmetry C1	2.25	2.50
Fraction Physical Nyquist	0.94	1.79
Bfactor	58.4	74.2
Defocus in µm (SD)	1.0 (0.3)	0.7 (0.5)
Range	0.1-1.8	0.1-2.0
Record time (min/ 5X5 shot)	3.25±0.04	7.1±0.5
Micrographs/hr	293	163
Particles/hr	215,913	348,94
GSFSC (Å) for ~0.74 µm^2	2.49	2.70
micrographs used	4	1
total particles	2577	2572

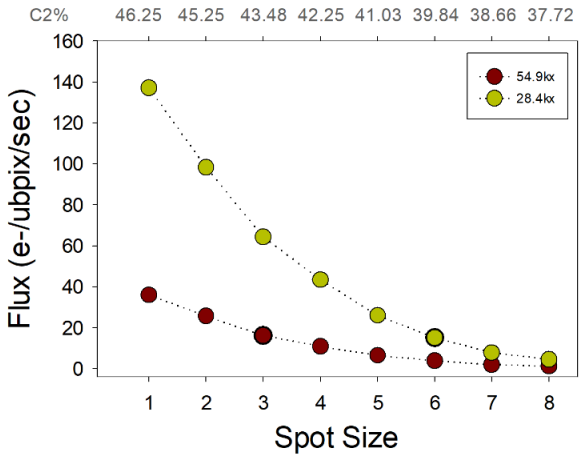

Figure 1. Flux as a function of spot size at 28.4kx and 54.9kx nominal magnifications at parallel illumination. In this comparison study we used a spot size of 6 or 3 for the respective magnifications commensurate with the appropriate C2 lens strength (top gray) to provide parallel illumination.

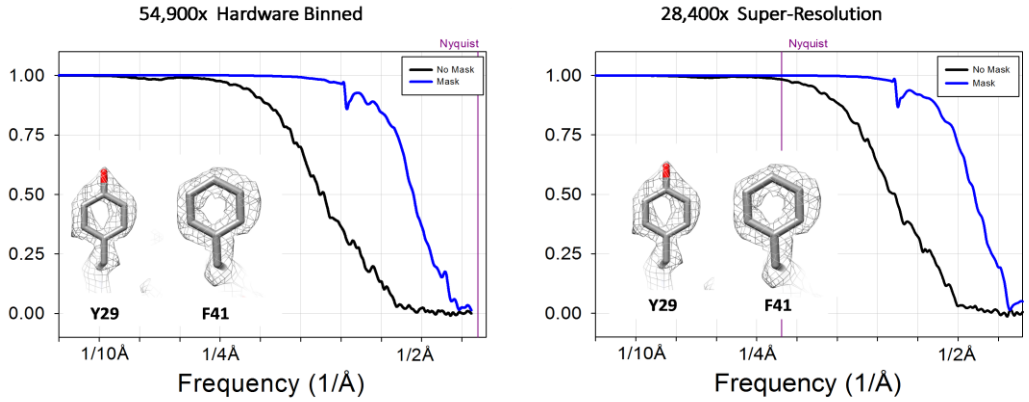

Figure 2. Gold-standard Fourier shell correlation (FSC) curves calculated from two independently refined half-maps indicate the overall resolution to approach Nyquist for 54,900x HWB data and surpass the physical Nyquist limit at 28,400x SR mode. Inset are coulombic potentials for amino acids Y29 and F41 for respective three-dimensional reconstructions of mouse heavy chain apoferritin. Reference line in purple is the physical Nyquist limit of 1.78Å and 3.56 Å for the respective magnifications. Blue FSC traces were calculated from masked half-maps and black FSC traces are unmasked half-maps.

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