Advances in Single Particle Analysis Data Acquisition

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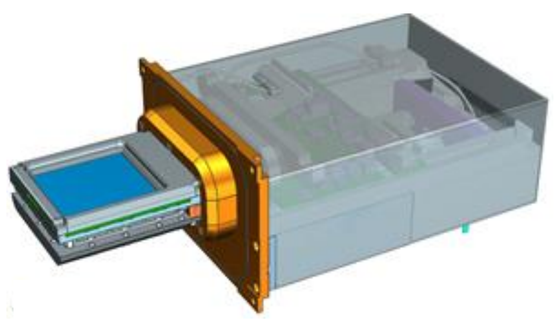
Introduction

Structural biologists have made great progress in revealing the structures of individual proteins and protein complexes via cryoEM at ever higher spatial resolutions (< 2 Å). After the resolution revolution of Single Particle analysis (SPA), the next frontier is the improvement of SPA data acquisition throughput (currently the acquisition of a single data set takes about 48 to 72 hrs).

In this presentation we show the latest advances in throughput within the SPA data acquisition workflow. This includes a faster and better detector, new optical features and optimized acquisition schemes that are implemented within Thermo Scientific EPU data acquisition software and are standard available on Krios G4. In this way optimal productivity is achieved without compromising usability, reliability or data quality.

Falcon 4 direct electron detector



- 250 frames / second allowing up to 6 electrons/pixel/second at full frame (4096x4096 pixels; 14 μm pixel size)
- Efficient in-line image processing and minimized acquisition overhead
- On-the-fly drift correction
- Live viewing & continuous acquisition
- Improved radiation hardness
- Highest Detective Quantum Efficiency (DQE) over full frequency range; Hardware anti-aliasing via low pass filter maximizes DQE on 4k x 4k pixel grid

Imaging performance - DQE

Mode	Dose rate (e-/pixel/s)	Exposure time* (s)	DQE (0)	DQE (1/2 Nq)	DQE (1 Nq)
EC mode	2	11	0.90	0.75	0.35
EC mode	5	4.5	0.80	0.65	0.30
Linear mode	20-100	0.2 – 1.0	0.50	0.40	0.25

* Pixel size: 0.75Å; Total dose: 40 e/Å²

Throughput performance of Falcon4 counting for several use cases

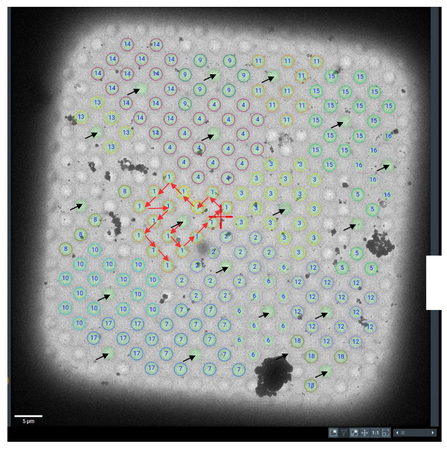
Throughput (images/hr)	Pixel size (Å)	Exposure time (s)	Auto focus every (um)	Stage settling (s)	Beam shift settling (s)	Aberration free image shift	Fringe free imaging	Images per hole	Grid type
98	0.84	7.8	5	10	2	NO	NO	3	R2/2
183	0.84	7.1	10	20	2	YES	NO	4	R2/2
168	0.84	6.4	10	10	0.5	NO	YES	7	R2/2
227	0.84	6.4	10	20	0.5	YES	YES	7	R2/2
243	0.84	6.4	10	20	0.5	YES	YES	3	R1.2/1.3
331	0.53	2.5	10	20	0.5	YES	YES	4	R1.2/1.3

Aberration-free image shift (AFIS) and hole clustering

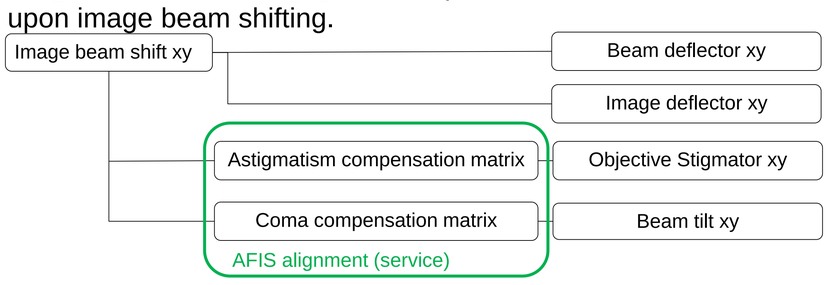
Usually SPA data acquisition uses the stage to move from foil hole to foil hole. With AFIS integrated in EPU, most movements can be done with image beam shift which is faster and in addition, does not create any stage-movement induced sample drift.

Image beam shifts normally create aberrations like coma and two fold astigmatism which can become resolution limiting (phase error > π/4).

With AFIS, the image beam shift is now coupled also to the beam tilt and objective stigmator optical elements such that coma and astigmatism remain negligible upon image beam shifting.



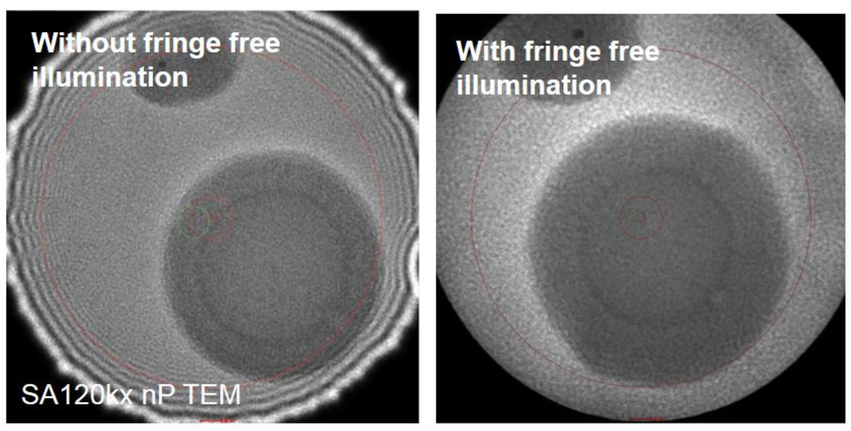
The numbers in the hole indicate the cluster number. The black arrow indicates the hole that is targeted with stage. The surrounding holes within the cluster are targeted with image-beam shift (red arrow). In this example: only 18 stage moves are required to reach 205 holes.



Note1: coma correction method only works for experiments without phase plate.
Note2: Coma correction via beam tilt is not applied in Cs image-corrected systems

An additional throughput feature is that the holes are now organized into clusters and only the primary hole (the one that is positioned with stage) is centered instead of all holes. This removes a considerable non-productive overhead time from the EPU run.

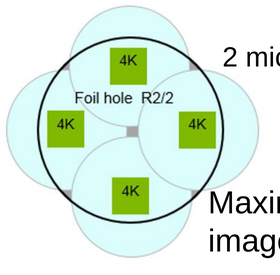
Fringe free imaging



Fresnel fringes from C2 aperture are visible especially at the edges of the beam.

No Fresnel fringes because C2 aperture is imaged on the sample plane (conjugate image planes)

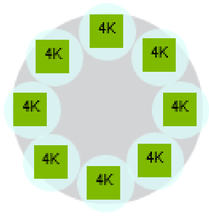
Illumination must be much larger than camera chip as to minimize visibility of the fringes in the image.



2 micron foil hole

Maximum four images / foil hole

Illumination must be only slightly larger than camera chip which means that more images / hole are possible → more images / hole means larger throughput.



Maximum eight images / foil hole

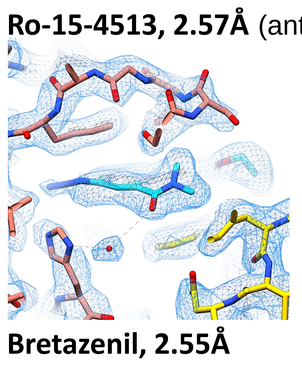
How it is done:

The stage tilt axis is adjusted until fringes are not visible anymore (tens of microns lowered compared to traditional tilt axis position). This is done mechanically so S/TEM tomography remains possible.
The objective lens is then tuned until the TEM image is in focus.
This procedure is performed in TEM nanoprobe at typical SPA data acquisition magnification to allow for best single particle analysis results.

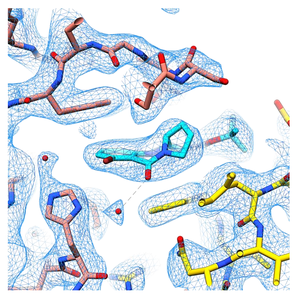
Application data

3D reconstruction of the GABA receptor membrane protein in nano disks bound to various drugs as measured with Single Particle Acquisition.

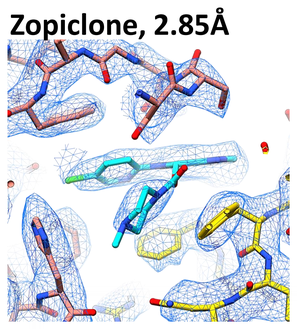
Ro-15-4513, 2.57Å (antidote to ethanol)

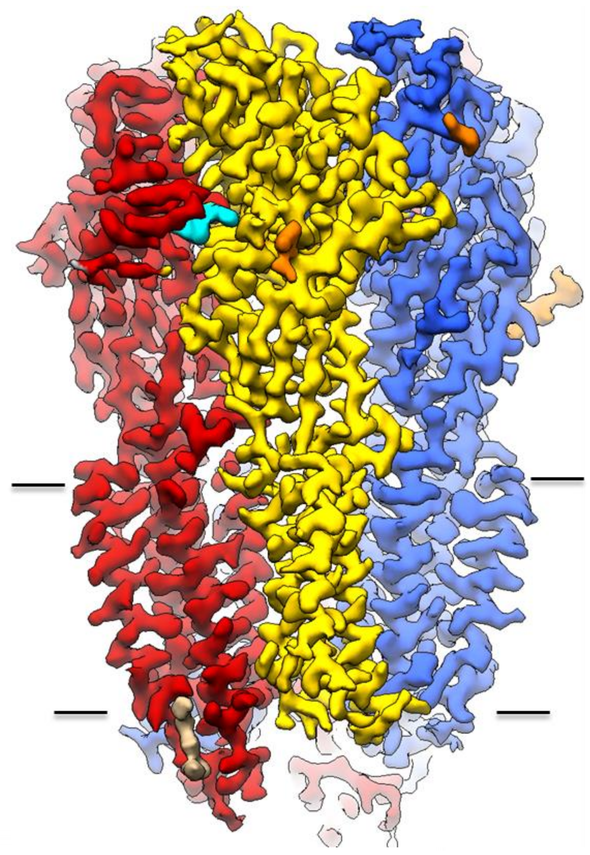


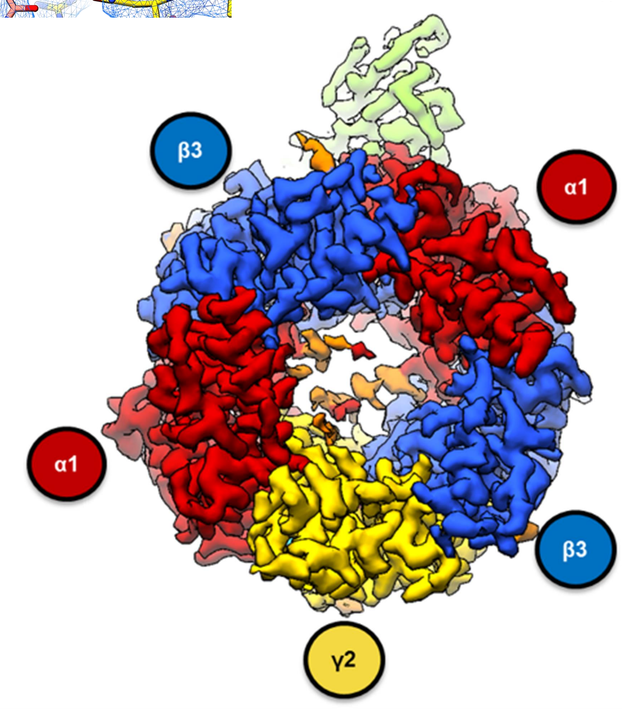
Bretazenil, 2.55Å



Zopiclone, 2.85Å



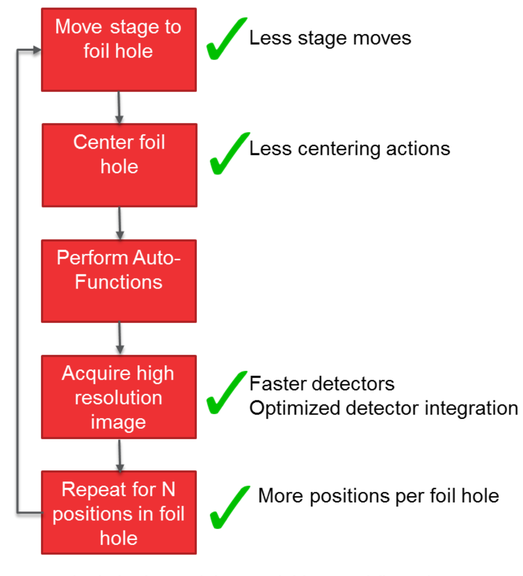




Miller, Masiulis et al, biorXiv, 2019, "*Molecular mechanisms of human GABAA receptor modulation by benzodiazepine site ligands*"

Conclusions

SPA data acquisition was improved on various aspects allowing data acquisition speeds within EPU of over 300 images / hr. Using the new and faster Falcon 4 detector, new optical features and optimized acquisition schemes within EPU, the time to acquire HiRes data sets shall go from several days to several hours.



Typical single particle acquisition workflow via EPU and realized throughput improvements

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