Automating MicroED/3DED data acquisition in DigitalMicrograph with Latitude D and Alpine Vista

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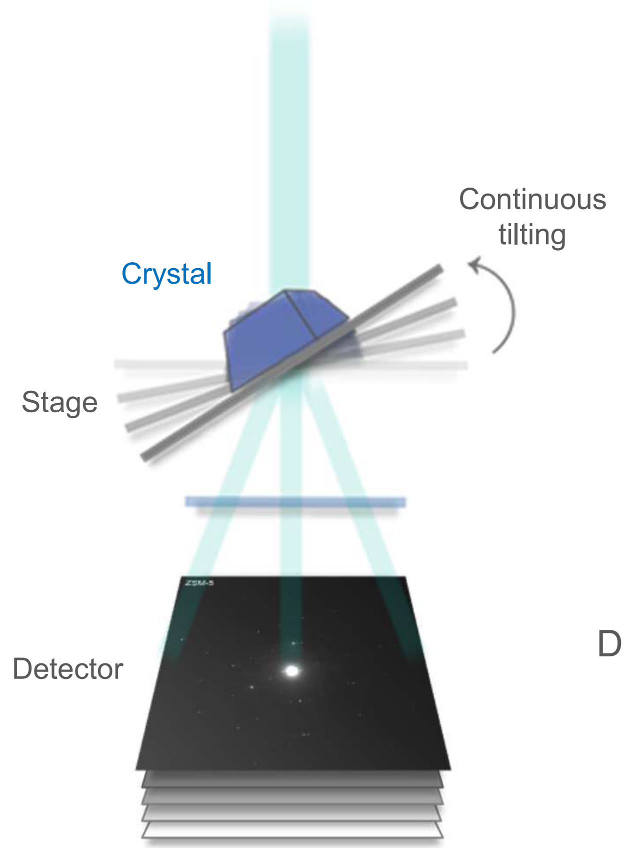
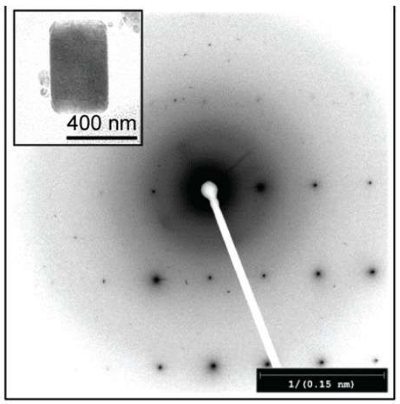
MicroED Concept

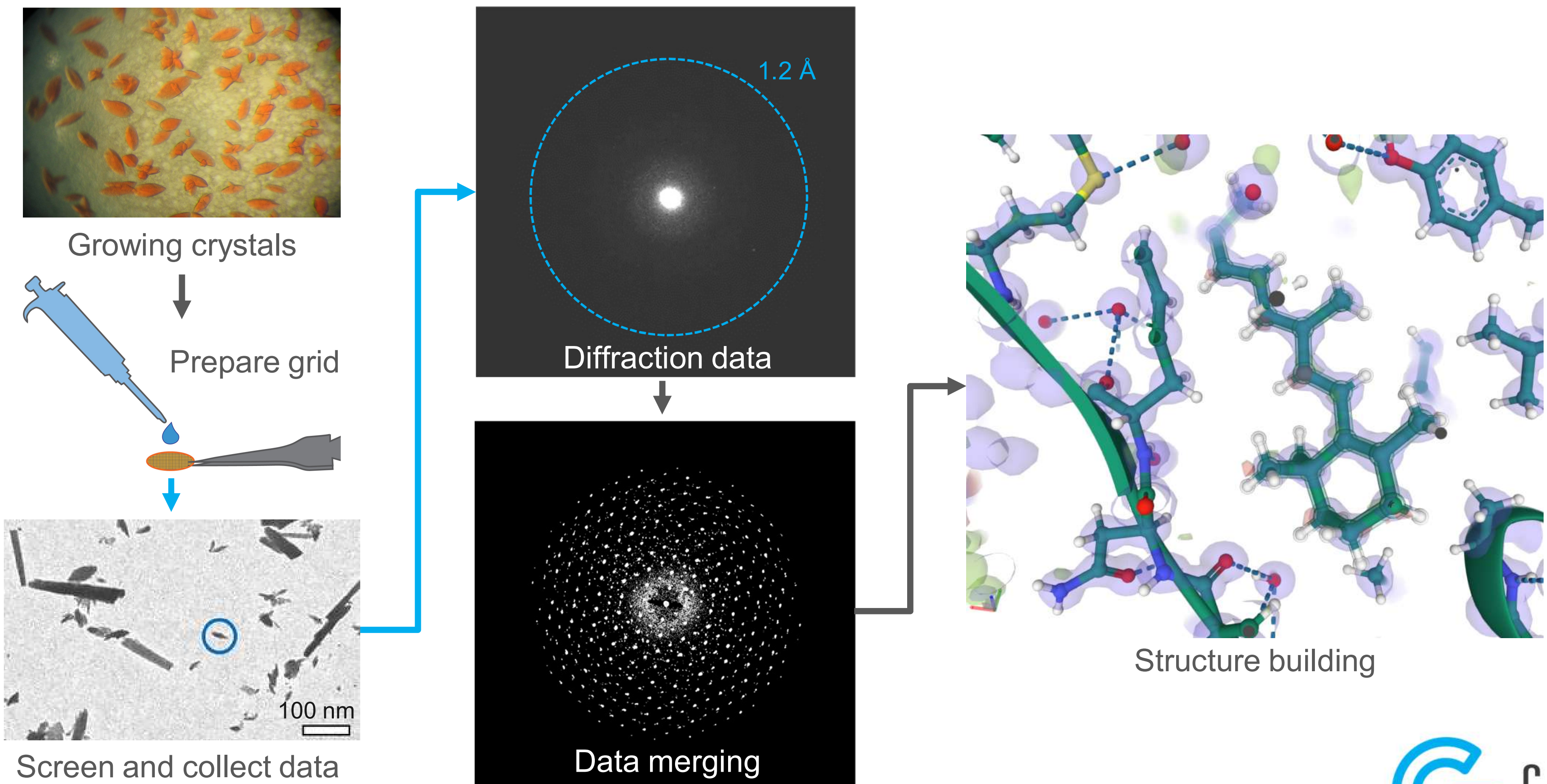
- Collect a diffraction dataset while continuously tilting the sample





Diffraction pattern from a lysozyme crystal (*inset*)

Extended microED workflow



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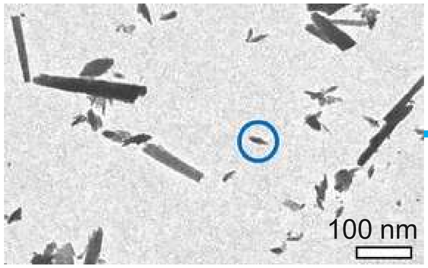
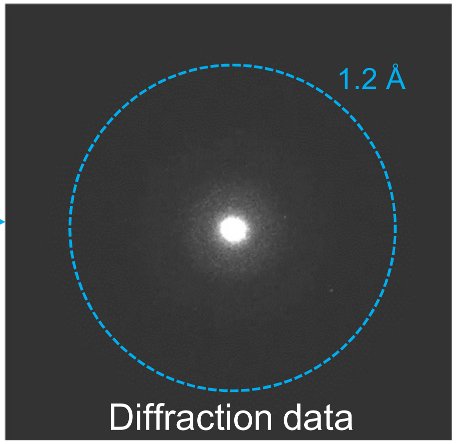
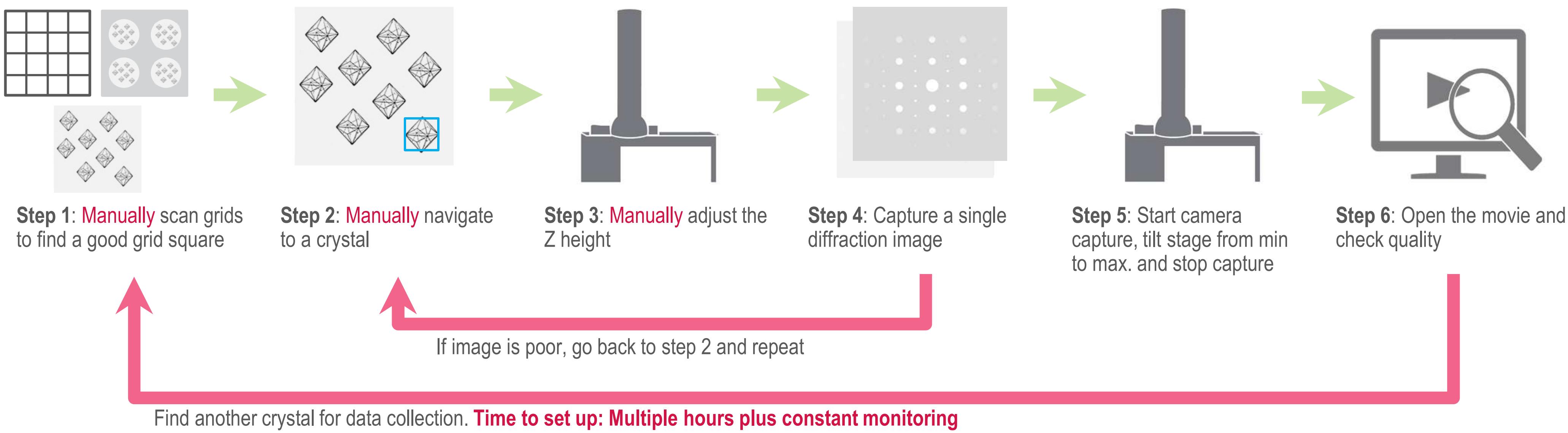
Gatan domain in the microED workflow



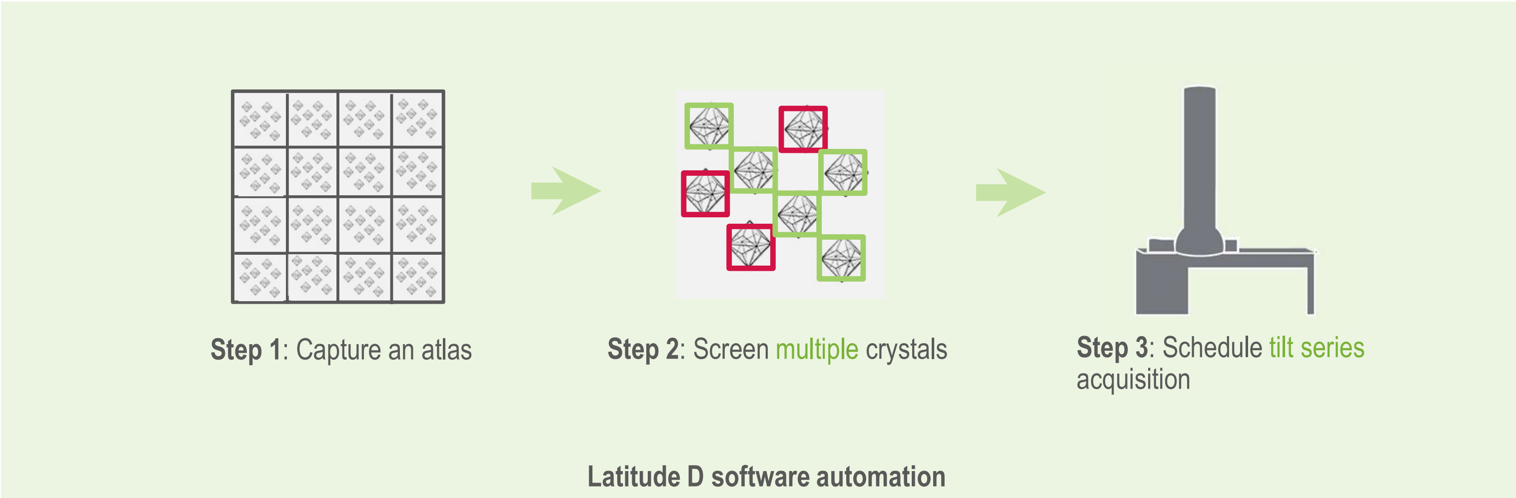
Screen and collect data





Traditional workflow – Manual data collection

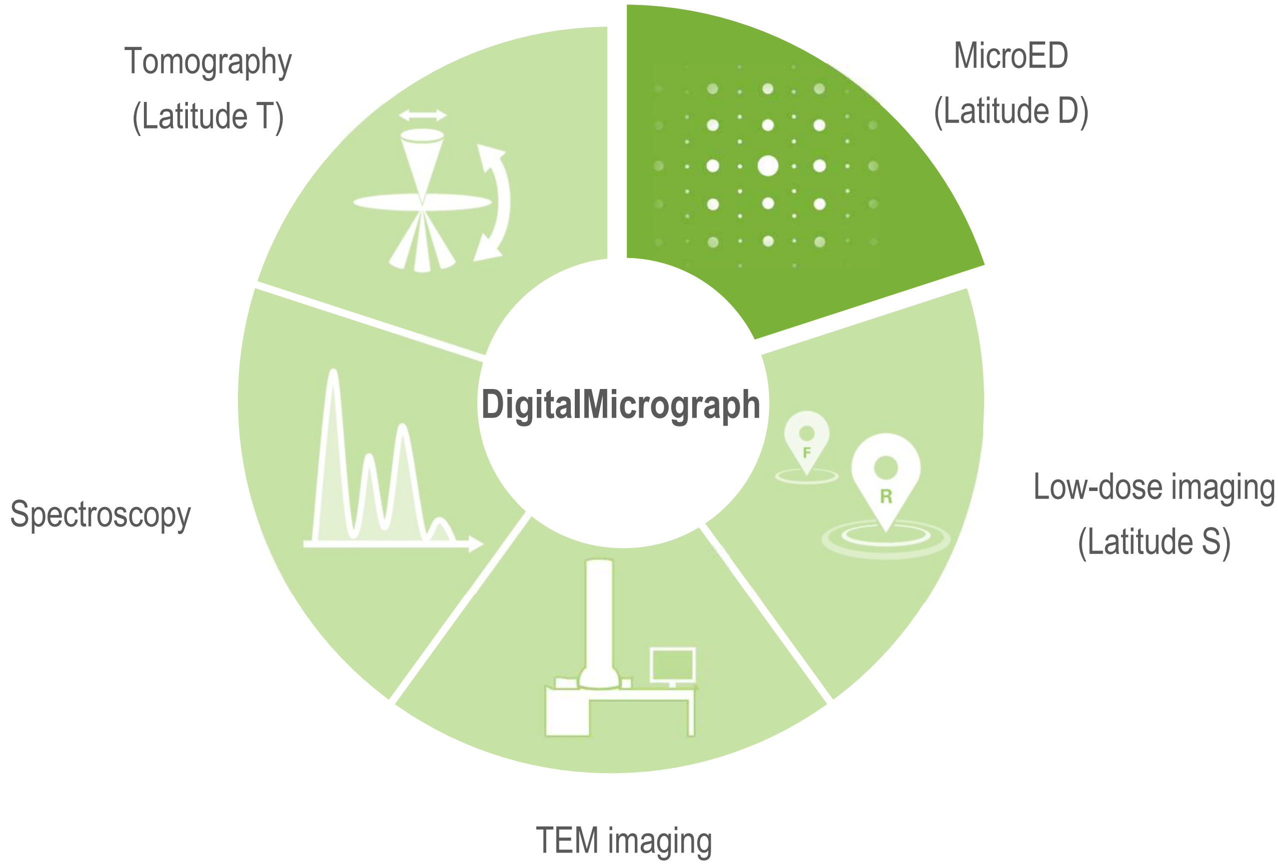
- Requires repetitive manual navigation, Z-height adjustment, and crystal screening
- Needs continuous manual input of microscope and camera parameters during data collection
- Manual intervention across multiple software interfaces reduces efficiency and slows time to results



One setup for hundreds of crystals. Time to set up 5 – 10 min, then walk away for remaining run

Latitude D workflow – Automates data collection

- Simply select crystals and screen them with Latitude D
- Latitude D automatically navigates, sets microscope parameters, and acquires the data for you
- Schedule new tasks and monitor the progress **while collecting data**



Broadens the capabilities of your existing microscope

- Adds MicroED to your existing TEM using the Latitude D module
- Utilizes a single DigitalMicrograph interface to setup and acquire data
- Compatible with pre- and post-GIF cameras

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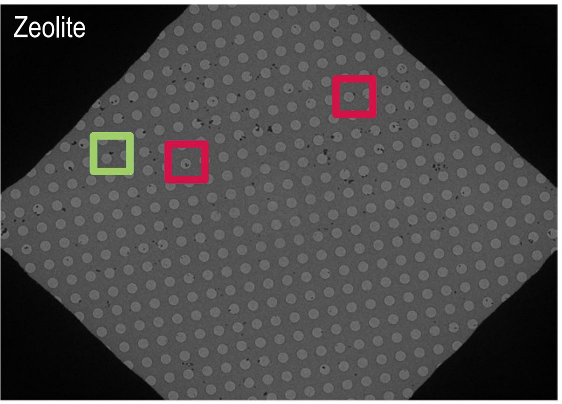
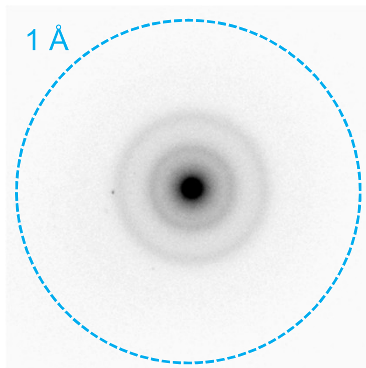
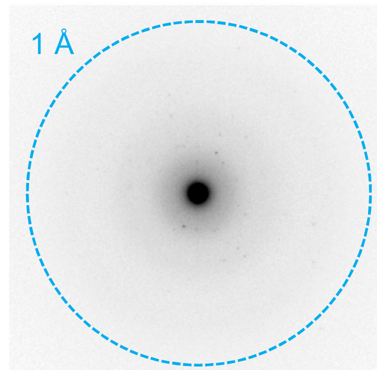


Pick crystals

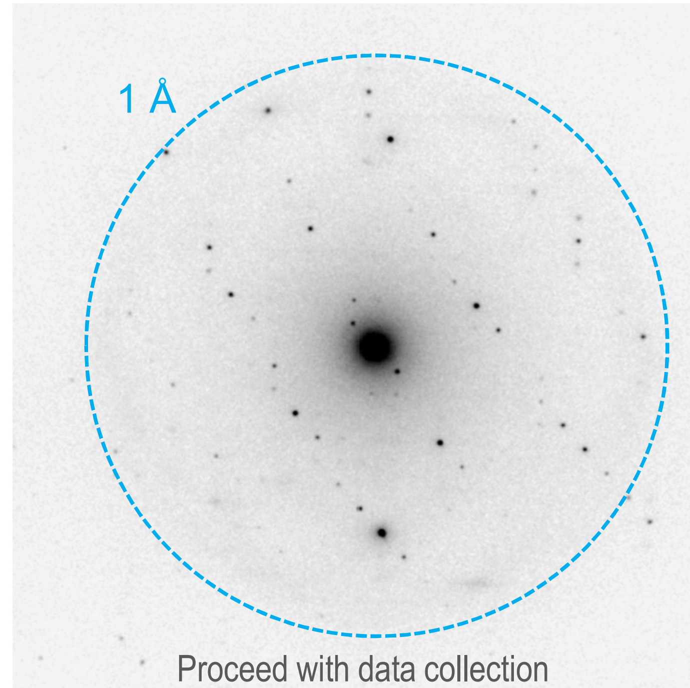
No usable spots: **Not good**



Few spots: **Not good**

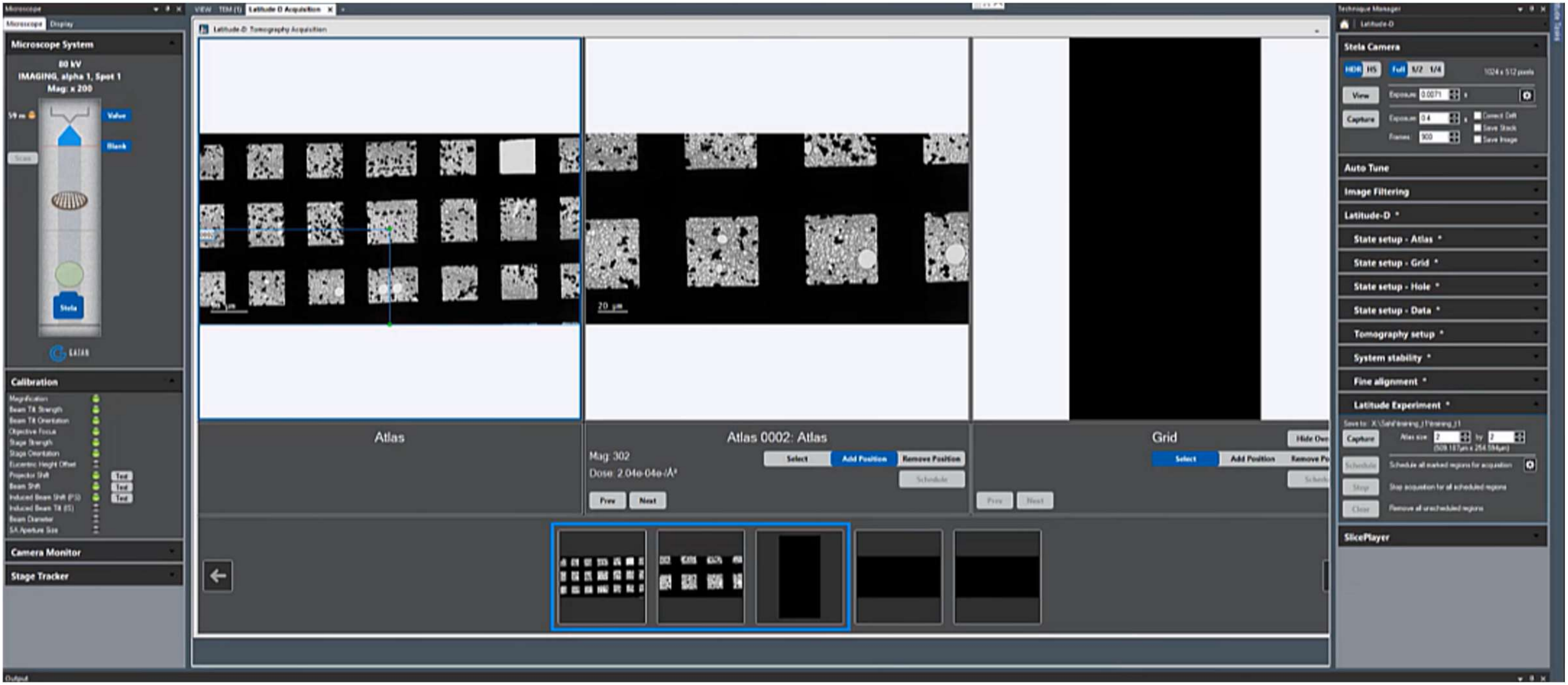


High-resolution spots: **Good**



Captures diffraction data from your best crystals

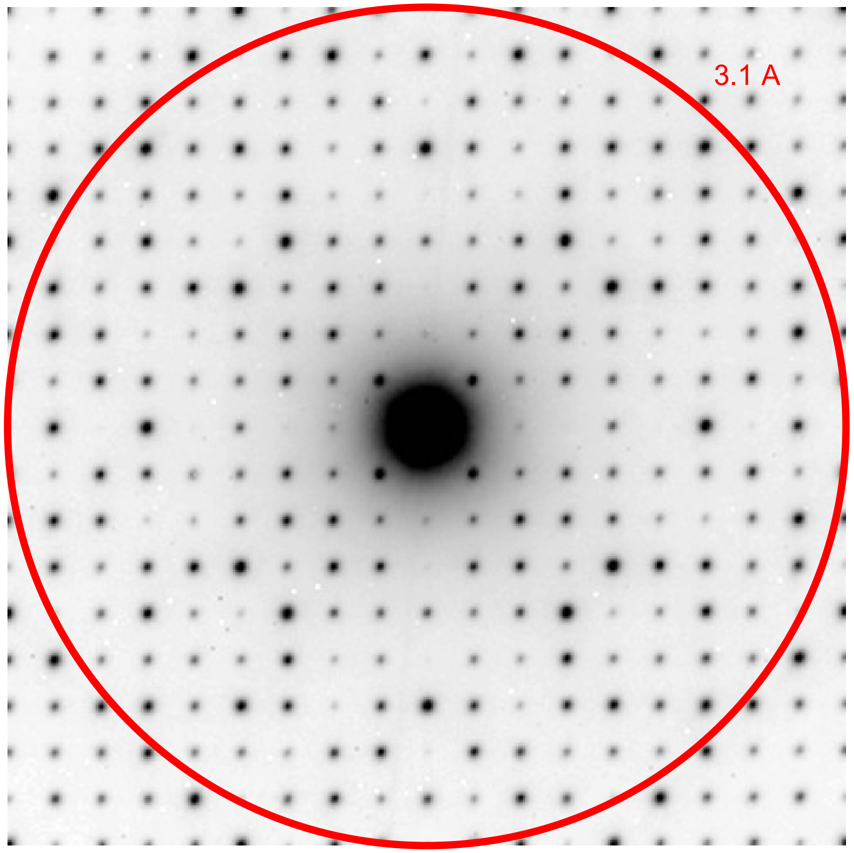
- Enables you to quickly identify the finest crystals for high-resolution data collection
- Only collects/stores data from good crystals
- Ability to collect data on hundreds of crystals with simple point-and-click control



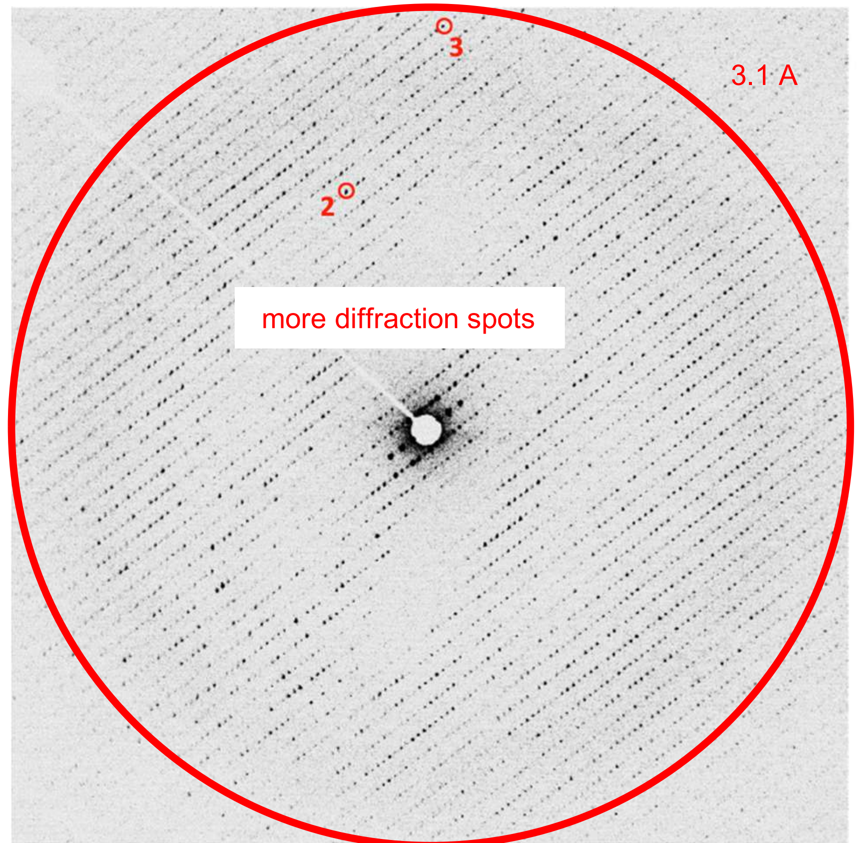
In the Latitude Experiment panel, specify the Atlas size and click Capture to create an atlas

What does high-quality microED data look like?

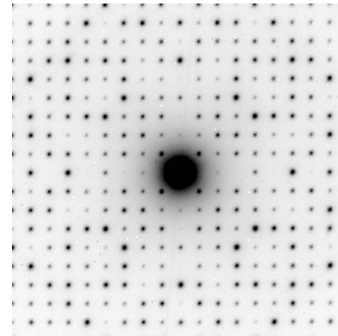
Small molecule ($K_2O{\cdot}7Nb_2O_5$)

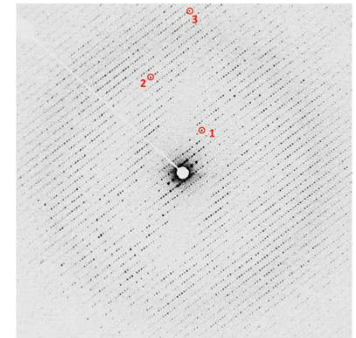


Protein (Catalase)



What do we need to collect high-quality microED data with a detector?





- **Capture weak diffraction spots under low dose conditions**

  Needs a detector with high sensitivity and signal to noise

- **Capture diffraction images at high speed to support continuous tomography**

  Needs a detector with high framerate capabilities

- **Capture intense and weak diffraction spots without saturating pixels**

  Needs a detector with high dynamic range

- **Capture small spots over a large area of diffraction space**

  Needs a detector with a wide FOV and high number of pixels

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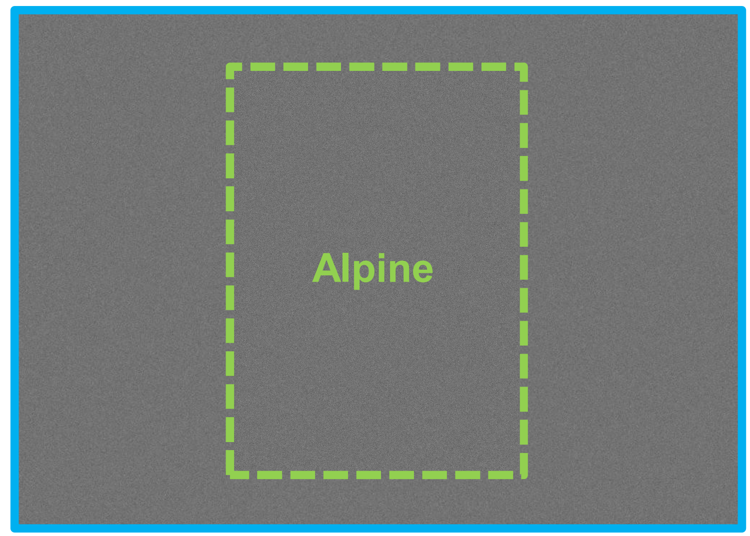
At NYSBC: Alpine direct detection cameras

Two models available to match the field of view with your application needs:

Alpine
2,304 x 3,240 pixels
Medium imaging throughput

Alpine Vista
5,760 x 4,092 pixels
Highest imaging throughput

Alpine Vista



- **Captures superior results on your existing 80 – 200 keV TEM**

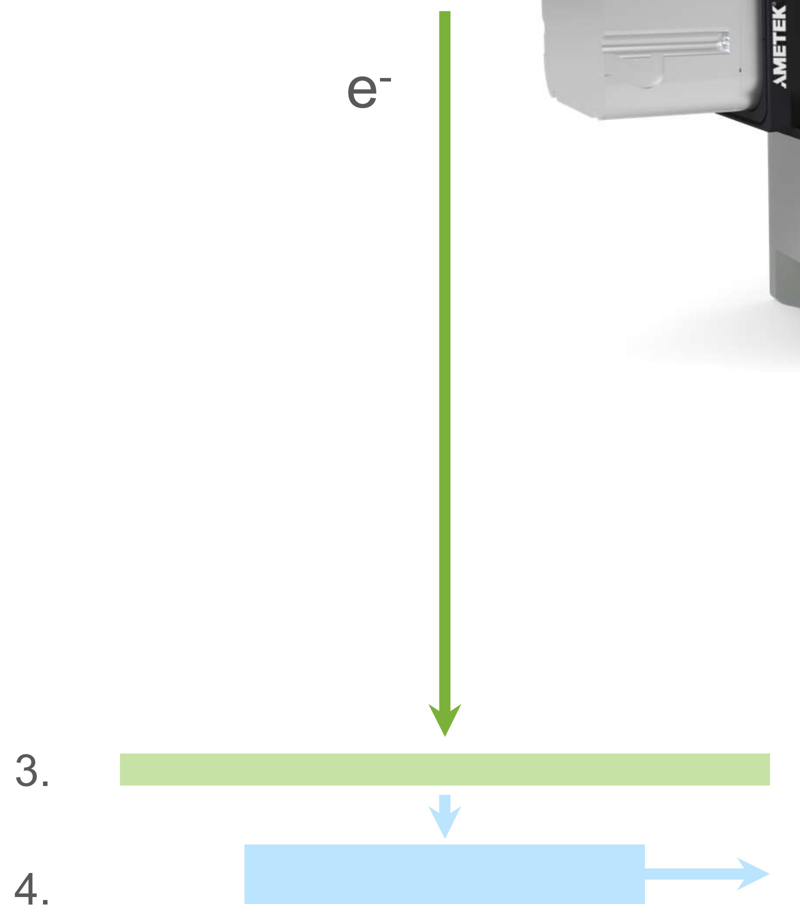
Direct detection camera

Direct detection refers to using a detector which is directly exposed to the e- beam to create a signal

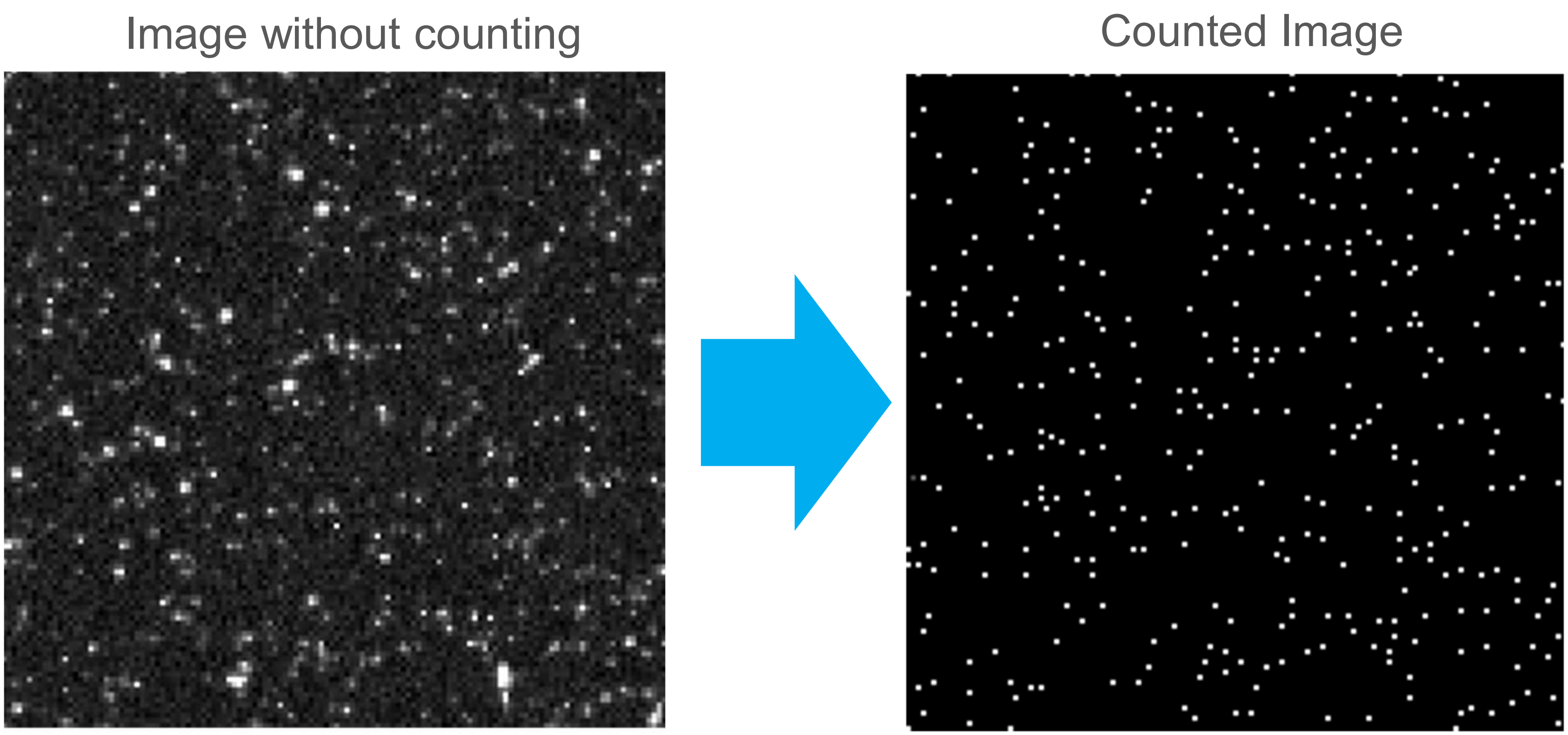
~~1. Convert electrons to light~~

~~2. Transfer light~~

3. Detect **electrons** and convert to signal
4. Electronically transfer signal and read-out to form image

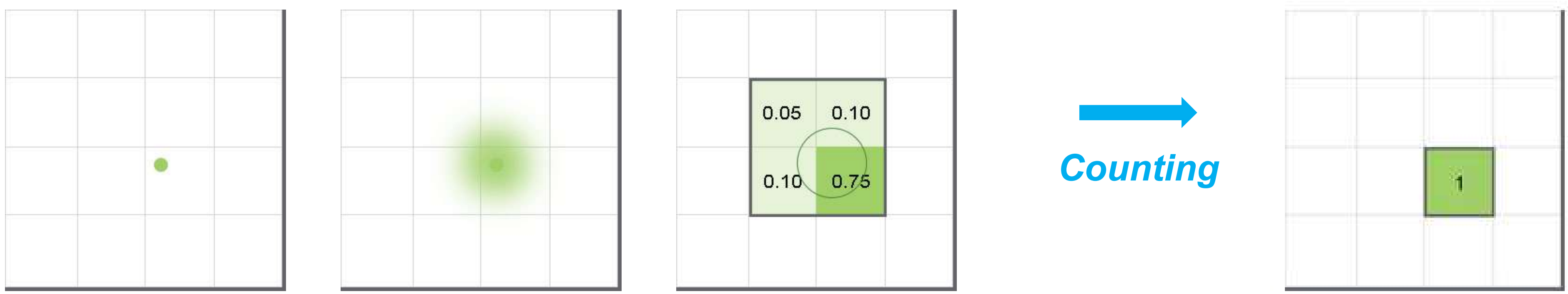


Electron counting in a direct detection camera



Electron counting in a direct detection camera

- As each electron strikes the sensor it creates a cloud of charge that spans a few pixels
- The charge is collected in each pixel
  Note: If the charge in each pixel is added now, it is termed "linear accumulation" and does NOT benefit from counting
- As the inline counting algorithm is applied to each frame, a complex "centroiding" algorithm reduces the multi-pixel charge to a single pixel



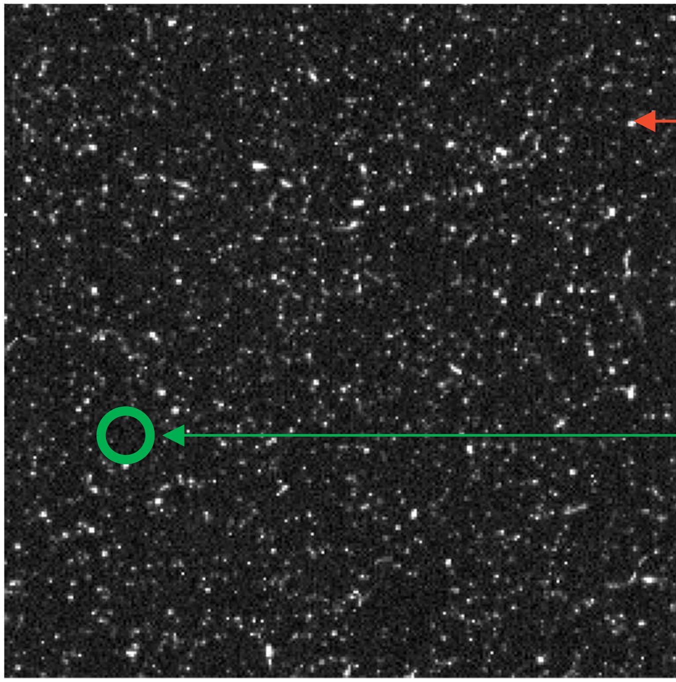
Electron enters Detector.

Electron signal is scattered.

Charge collects in each pixel.

Events reduced to highest charge pixels.

Electron counting makes all the difference

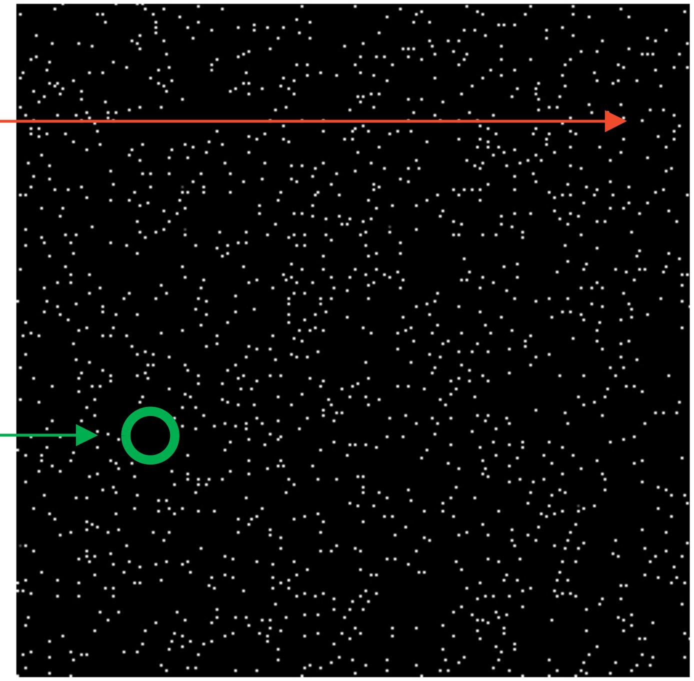


Single high speed frame using integrated charge read-out

Counting removes noise:

1) **Variable energy (signal) variation** *(events reduced to 0's and 1's)*

2) **Read noise eliminated** *(background is black)*



Same frame after counting

Both effects greatly restore DQE

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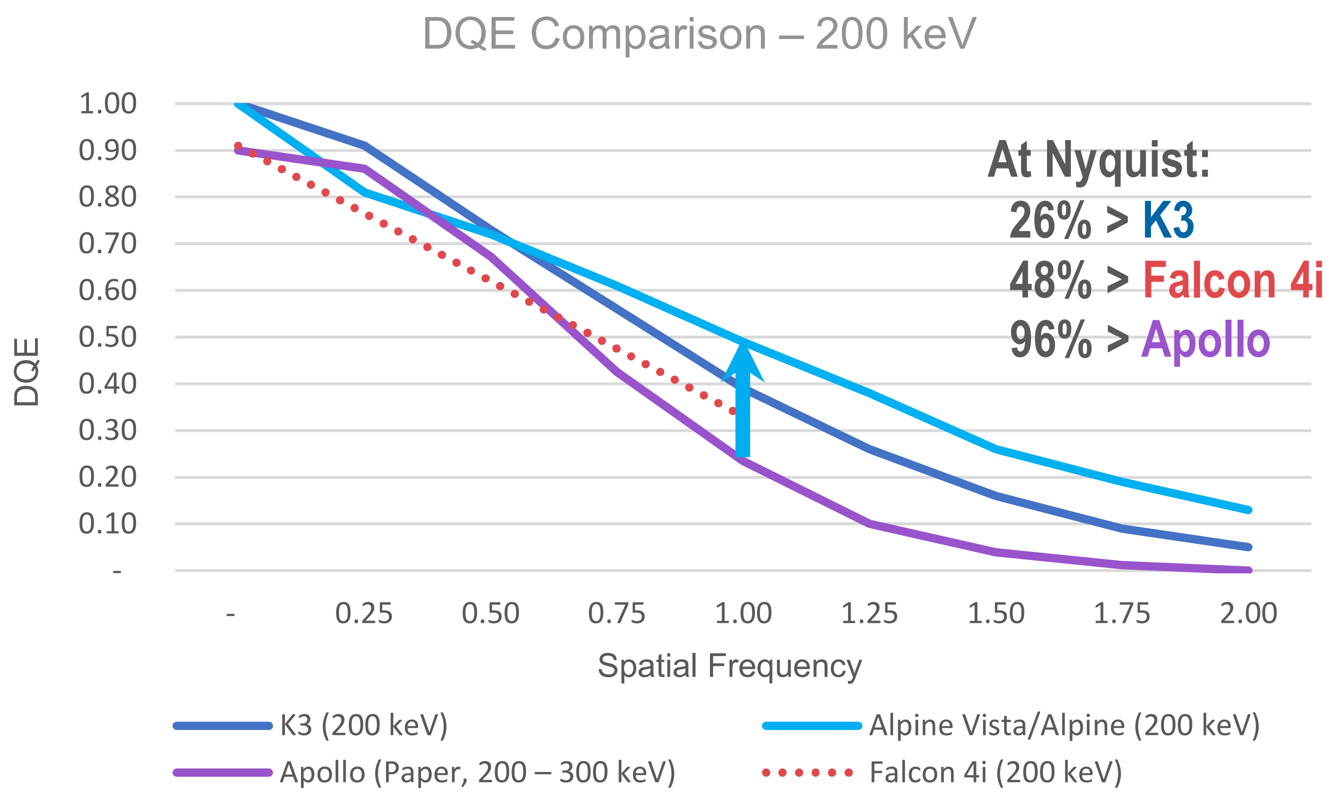

Delivers remarkable DQE results at 200 keV

- Proprietary sensor rejects read noise and variability associated with electron scattering
- Dramatic DQE improvement across all spatial frequencies

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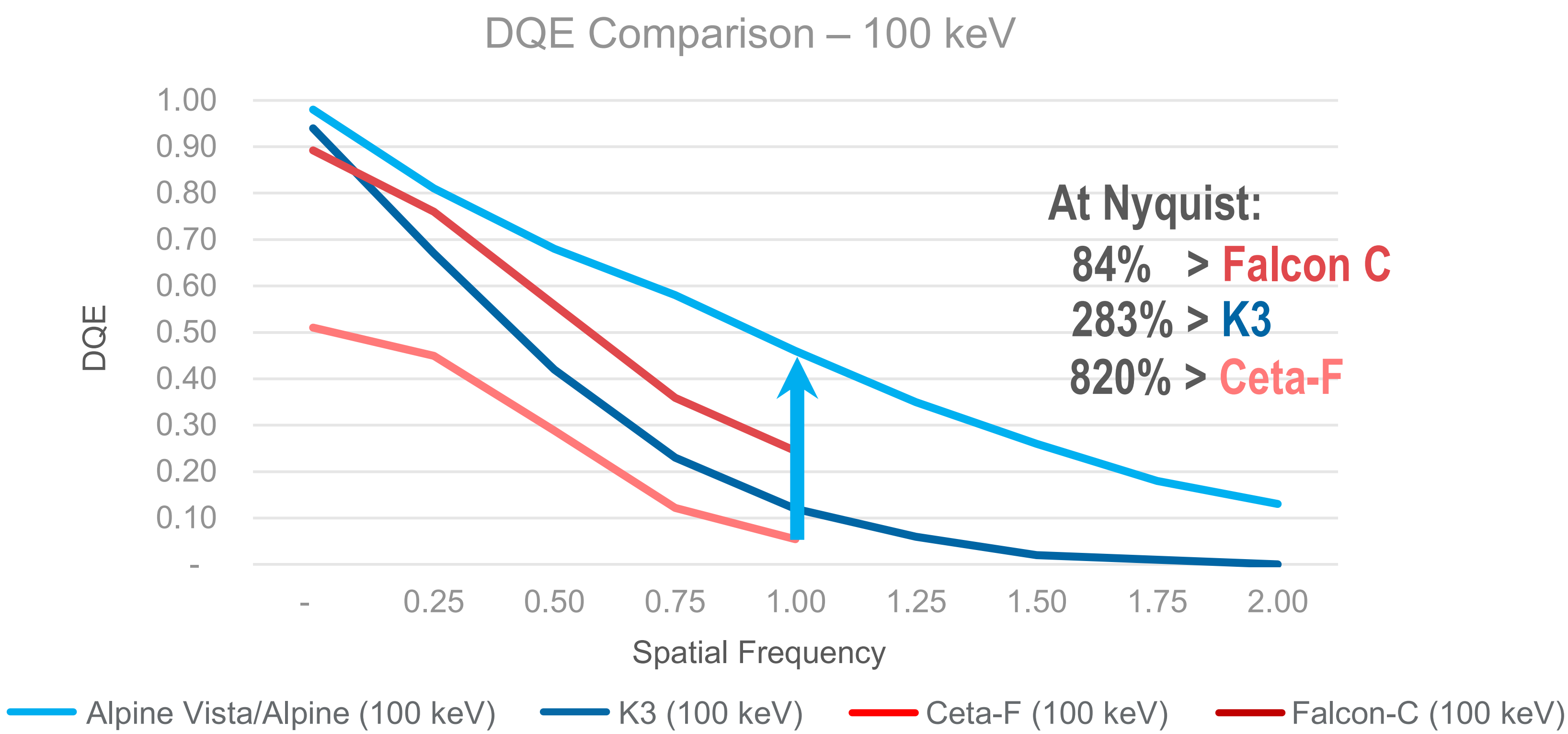

Delivers remarkable DQE results at 100 keV

- Proprietary sensor rejects read noise and variability associated with electron scattering
- Dramatic DQE improvement across all spatial frequencies
- Alpine Vista and K3 deliver super-resolution readout

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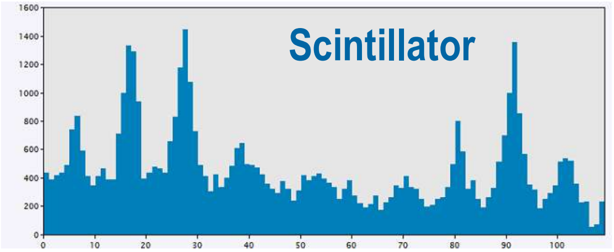
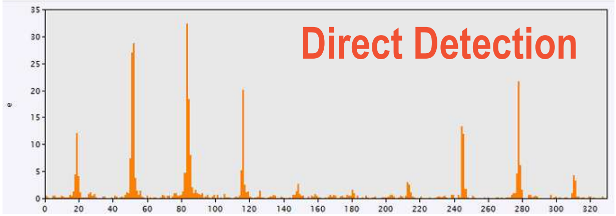
Capture higher quality diffraction

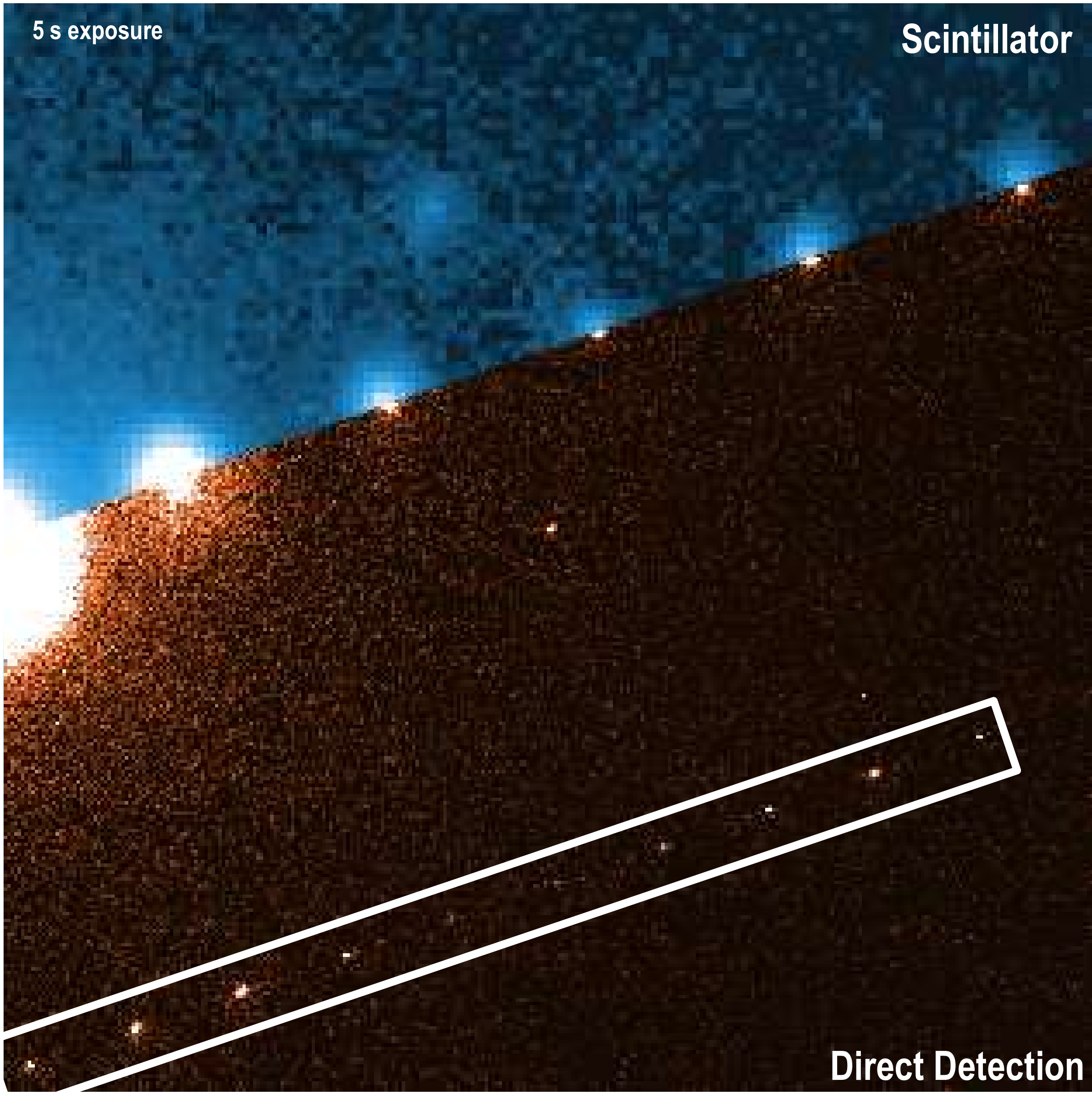
Scintillator cameras can detect many faint diffraction spots…

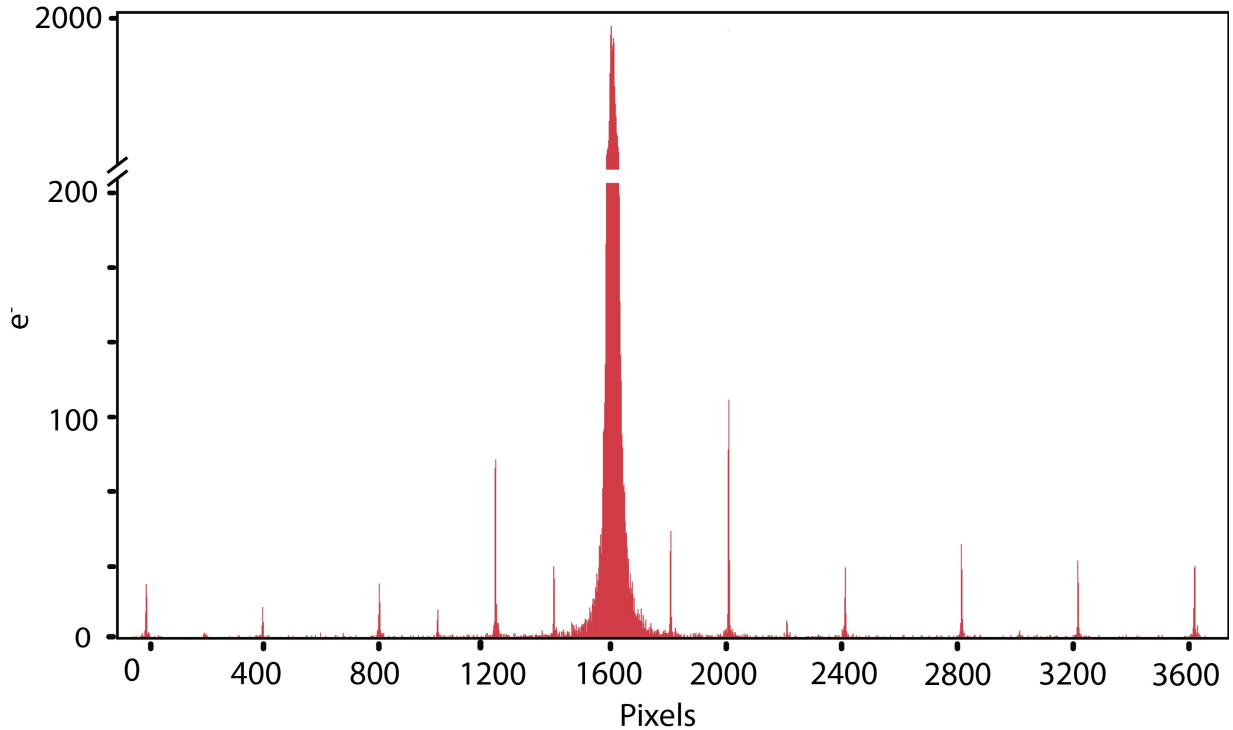


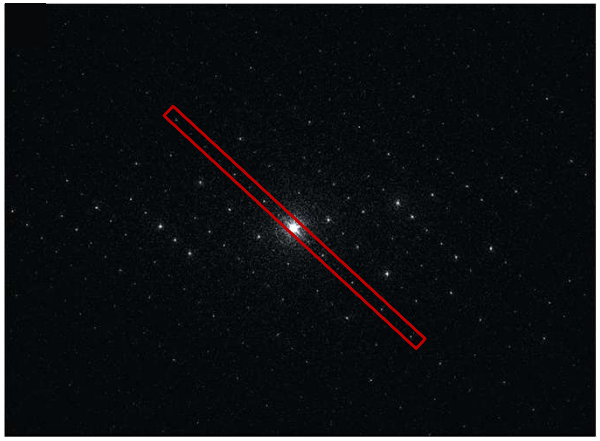
Direct detection provides sharper detail and less background noise



Allows you to more accurately characterize crystals and defect structures







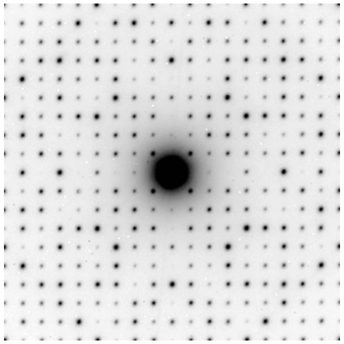
Data from K3 camera. Specimen: Zeolite, diffraction image size: 5760 x 4092, exposure: 1 s

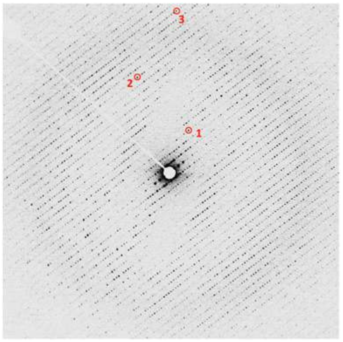
Diffraction Uncompromised – direct detection cameras

- Electron counting maximizes the SNR to detect weakest diffraction reflections
- Frames are summed to get high dynamic range
- High dynamic range allows detection of weak and intense peaks at the same time

Sample courtesy of Dr. Dos Reis, Northwestern University.

What do we need to collect high-quality microED data with a detector?





- Capture weak diffraction spots under low dose conditions
- Capture diffraction images at high speed to support continuous tomography
- Capture intense and weak diffraction spots without saturating pixels
- Capture small spots over a large area of diffraction space

Higher resolution, more complete structures

Higher throughput

Explore a wider range of beam sensitive samples

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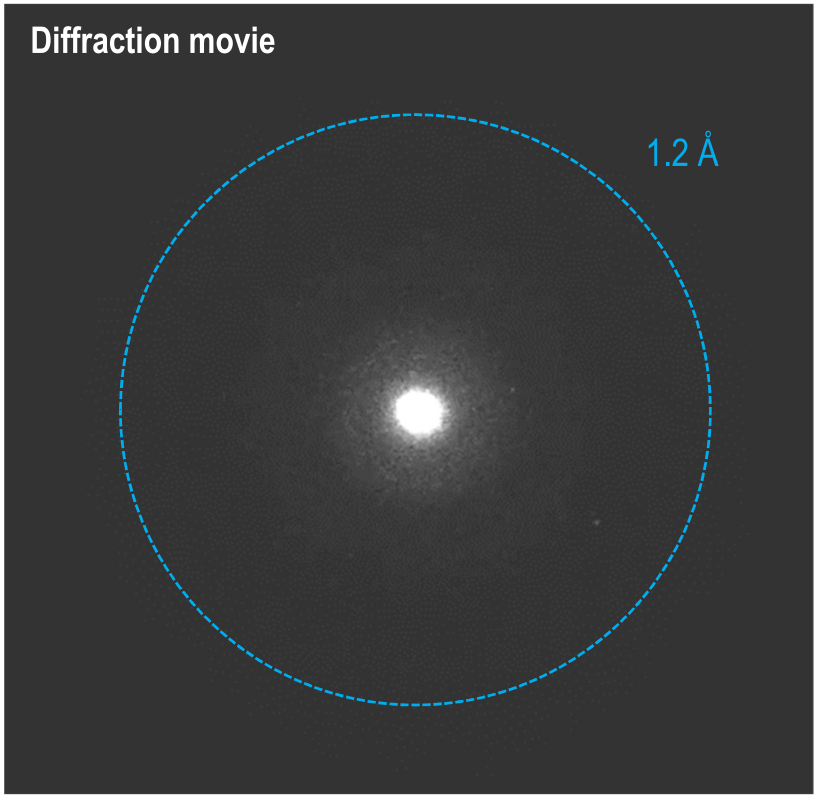

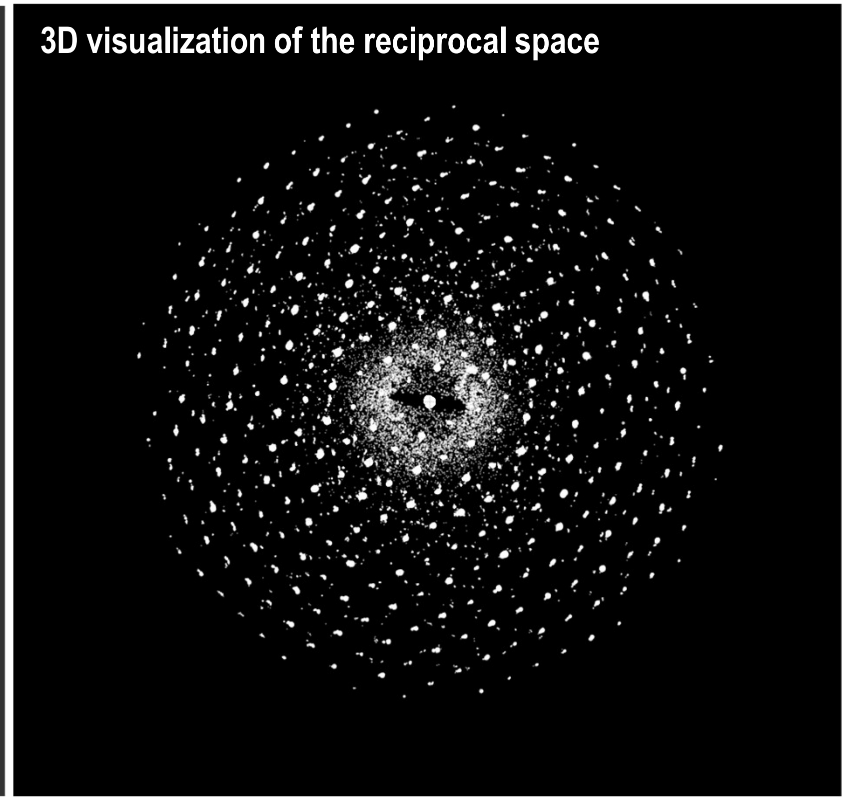

MicroED of acetaminophen – A small molecule

- High-SNR, low noise, and fast frame rate allow Stela to solve high-resolution structures of small molecules
- Electron counting maximizes the SNR to detect the weakest diffraction reflections

3D reconstruction courtesy of Dr. dos Reis, Northwestern University.

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Summary

- Latitude D leverages Gatan hardware & DigitalMicrograph to enable high-throughput data acquisition for microED
- Gatan's direct detection electron counting cameras enable the high speed, high sensitivity, and high dynamic range acquisition of diffraction images required for microED
- Even more camera types and configurations exist (e.g. hybrid-pixel detectors) – what is the ideal balance between speed, SNR, and pixelation for your experiment?

Thank you!

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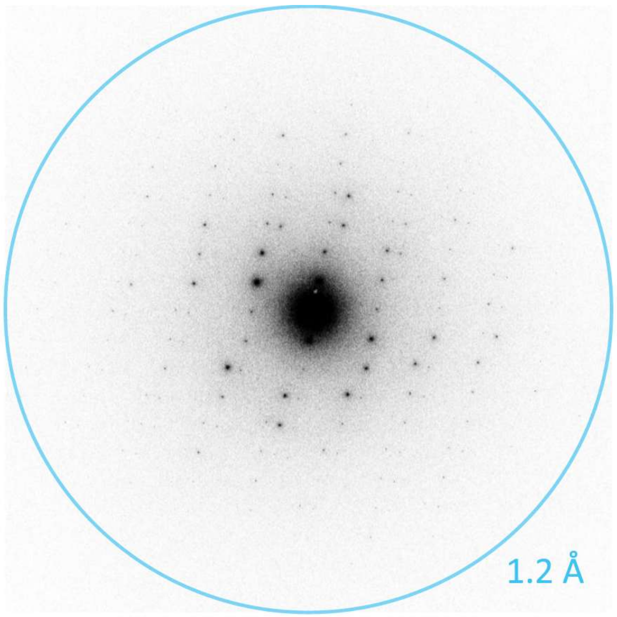
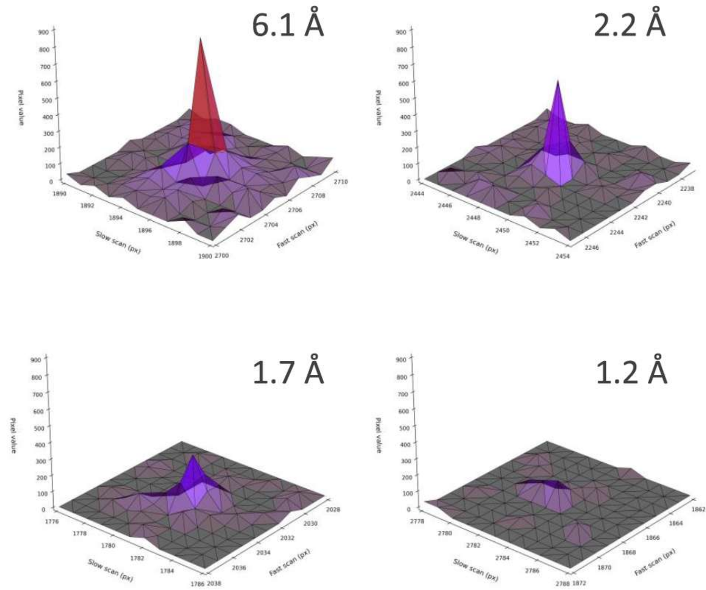
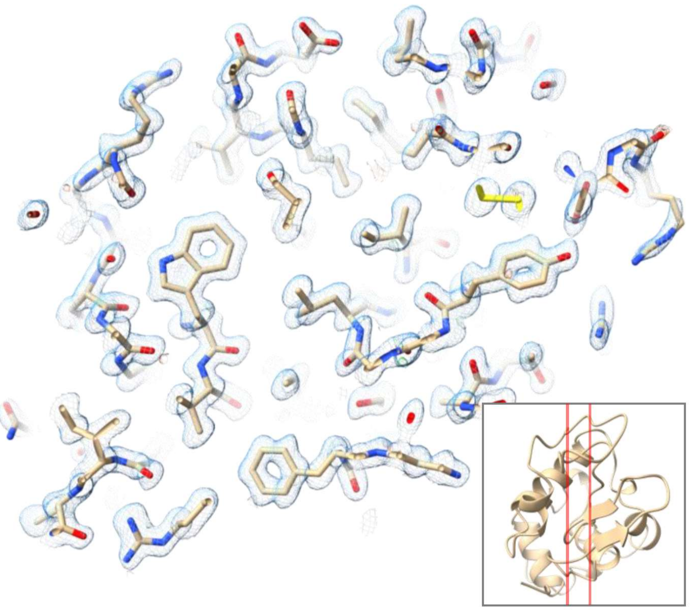


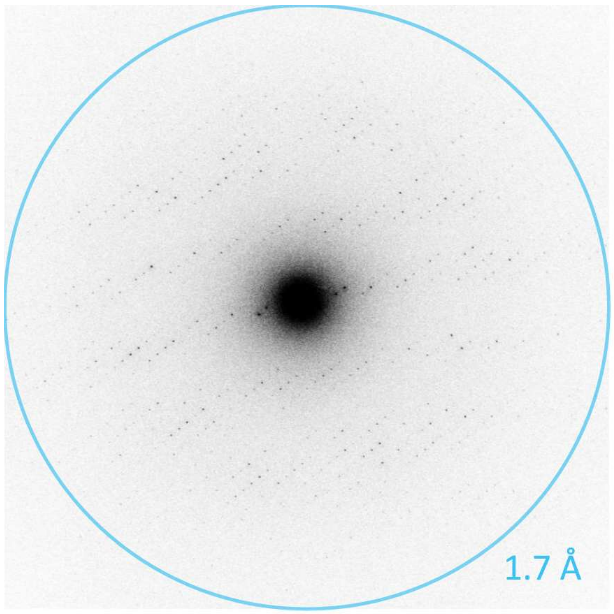


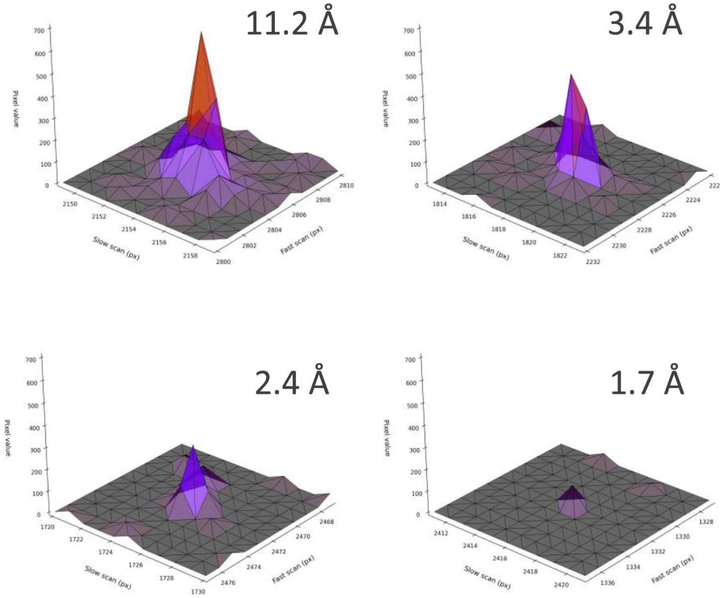


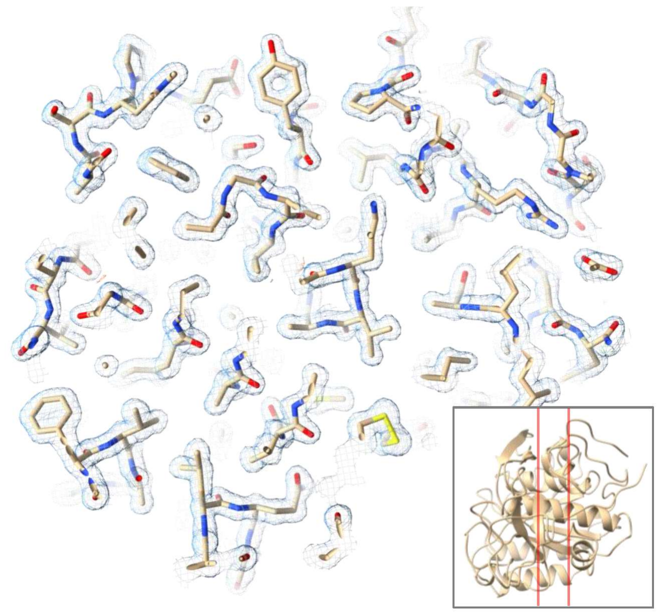
High-resolution microED results: Lysozyme

- No beam stop
- 1.2 Å structure of lysozyme with K3 camera (great resolution for proteins)

Max T.B. Clabbers, **Michael W. Martynowycz**, Johan Hattne, Brent L. Nannenga, Tamir Gonen
Electron-counting MicroED data with the K2 and K3 direct electron detectors, Journal of Structural Biology, Volume 214, Issue 4, 2022, 107886





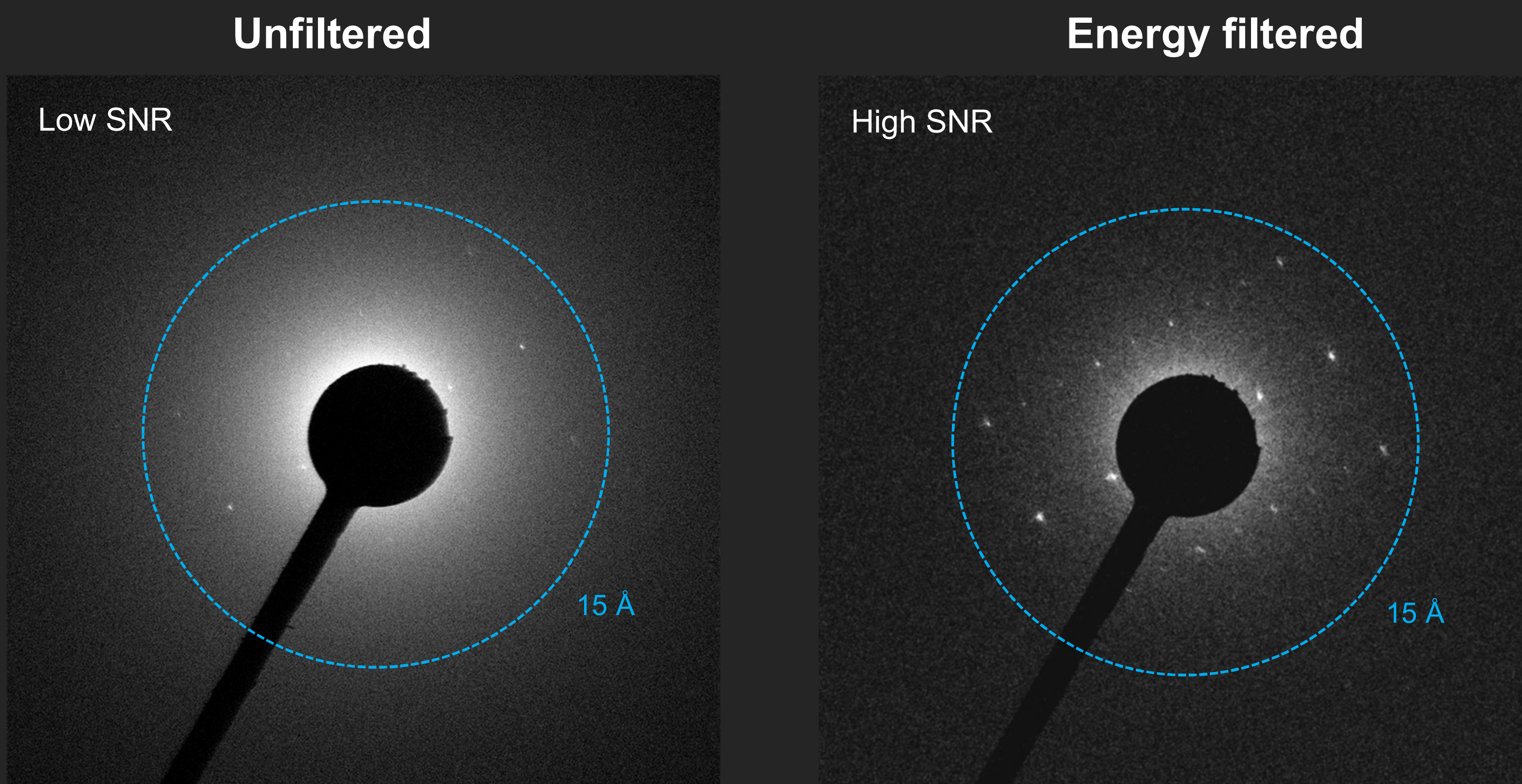


High-resolution microED results: Proteinase K

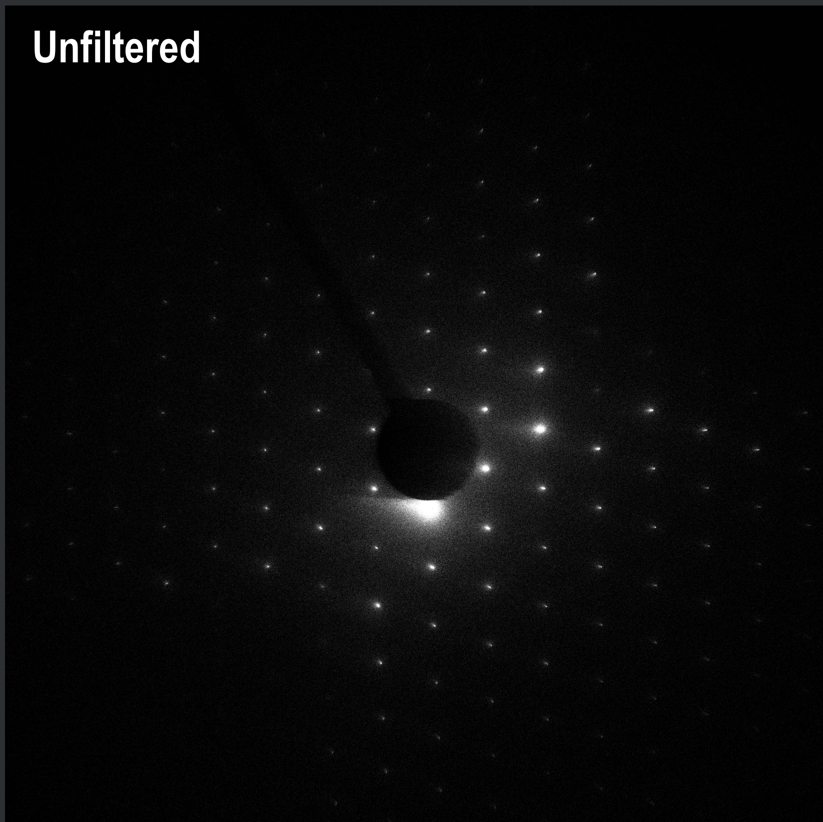
- No beam stop
- 1.7 Å structure of proteinsase K with K3 camera

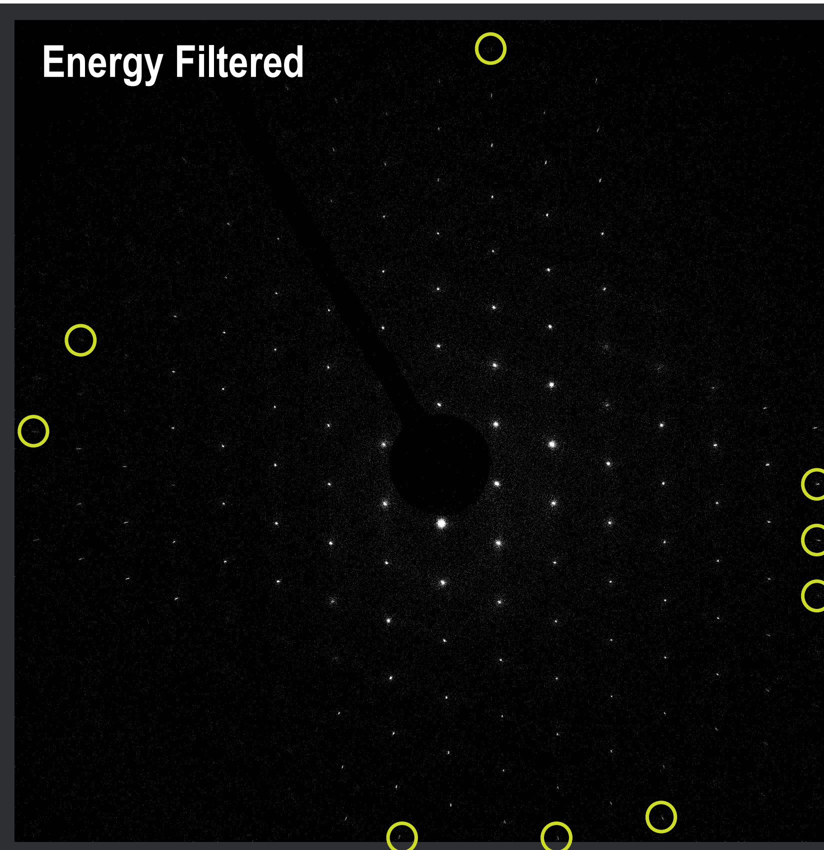
Max T.B. Clabbers, **Michael W. Martynowycz**, Johan Hattne, Brent L. Nannenga, Tamir Gonen
Electron-counting MicroED data with the K2 and K3 direct electron detectors, Journal of Structural Biology, Volume 214, Issue 4, 2022, 107886

Energy filtering improves signal to noise for diffraction acquisition



Catalase protein diffraction example





High-Res Energy-Filtered Tilt Series Even on Thick Specimens

- Collects information out to Nyquist