Combining electron counting and beam-induced motion correction to achieve near atomic resolution single particle cryoEM

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K2 camera with the Tecnai Polara

* K2 is fully embedded into our data acquisition procedures, both with UCSFTomo and UCSFImage4.
* Motion correction is implemented both on-the-fly with data acquisition and during image processing.
* Identified optimal dose rate for data collection.

DQE and dose rate

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* Identified optimal dose rate for data collection.
* DQE at 1 e-/(pixel*sec) dose rate is close to an ideal camera.
K2 is suitable for high-resolution low-dose imaging

* thin PtIr film recorded at a magnification of 39kX, ~1Å/pixel;
* ~17e/Å² on specimen and ~17e/pixel on camera;
* D: super resolution image, 23kX, ~1.8Å/pixel, dose rate ~31 e/pixel*sec,
  ~28e/Å² on specimen and ~93 e/pixel on camera;

Chris Booth

K2 image of frozen hydrated protein samples, T20S

* 300kV, 31kX mag, ~10e/pixel/sec; ~1.2Å/pixel, 25e/Å², 3.5sec exposure;

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**Charge induced image blurring can now be corrected**

* instead of recording one image at a time, we fractionate a single exposure into 24 subframes. By re-aligning all subframes, we can correct motion induced image blur.
* We can restore most of images to near perfect quality suitable for atomic resolution single particle cryoEM, ground breaking leap in high-resolution image acquisition.

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**Beam induced motion**

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**We achieved resolution comparable with X-ray crystallography**

* We determined a 3D reconstruction of archaeal 20S proteasome to the resolution of ~3.3 Å, comparable to the resolution of X-ray crystal structure, 3.4 Å.
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Xueming Li

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