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Cryo-EM Movie-Mode Imaging with Direct Detection Cameras

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For more information, go to:

www.directelectron.com

or contact: Benjamin Bammes, Ph.D. Director of Applications & Marketing bbammes@directelectron.com +1 858.384.0291 x105

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Microscopy Science Theater 3000

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or contact: **Benjamin Bamme** Director of Applications bbammes@directele. n.cor

Ph.D. Marketi

Outline

Introduction to Direct Detectors **Performance Comparison Movie-Mode Processing** Motion Correction Damage Compensation **Example Result**

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Introduction to Direct Detectors

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We Need to Better Detectors

"Although photographic film has long been the standard to beat (especially for 300 keV electrons), it leaves much room for improvement in terms of detective quantum efficiency under low-exposure conditions. New types of area detectors that are currently being developed for EM not only improve on the readout speed of CCD cameras but also promise to improve the point-spread function (i.e., resolution) relative to the pixel size of the detector."

Glaeser & Hall, Biophys J 100 (2011), 2331-2337.

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Principle of Direct Detection



CCD Camera

Direct Detection Camera

TRADITIONAL TEM DIGITAL IMAGING.

Performance is limited by the scintillator and fiber optic coupling.

NEXT GENERATION TEM IMAGING. High-performance direct detection of primary electrons.

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TEM CMOS APS Development



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DDD Development History

Over a decade of continuing development, in partnership with academia and the NIH.

Generation	1	2	3	4	5	6	7	8	9	10
Year	2002	2002	2003	2005	2006	2008	2010	2011	2012	2014
Pixel Size (μm)	20	5, 10, 20, 30	5	5	5	6	6	6	6.4	6.5
Array Size (pixels)	128 × 128	Various	550 × 512	1024 × 1024	560 × 460	4096 × 3072	4096 × 3072	4096 × 3072	5120 × 3840	8192 × 8192
Pixel Design	3T	3T	3T	3T	3T	> 3T	> 3T	> 3T	> 3T	> 3T
Feature/ Milestone	4 quad	4 sections	Single pixel design; MTF/DQE	Larger format; cryo-tomo	ADC per column; electron counting	Large format	Faster; more radiation hard	Thinned; improved at 200 kV	Larger; reduced noise; improved MTF	Ultra-large format; improved SNR, dynamic range, and radiation hardness
Developer/ Funding	University of California, San Diego NIH RR018841				Direct Electron, LP NIH RR024964 & NIH GM103417					

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Overview of DE-Series Cameras

- Direct detection of primary electrons from 80 keV to 1.25 MeV
- Continuous streaming with user-adjustable frame rate and full access to raw data
- \circ 6 6.5 µm pixel pitch, with >3T pixel design (CDS)

N PROPELLI	DE-12	DE-20	DE-16	DE-64
Generation	8	9	10	10
Pixel Size (µm)	6.0	6.4	6.5	6.5
Array Size (pixels)	4096 × 3072	5120× 3840	4096 × 4096	8192 × 8192
Maximum Frame Rate (full frame)	40	3 2	60	40



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Unique, Industry-Leading Features

	Direct Electron	Alternative 1	Alternative 2	
Pixel Size (µm)	6.0 – 6.5	14	5	
Array Size (pixels)	Various sizes up to 8192 × 8192	4096 × 4096 (17% smaller than DE-20; 300% smaller than DE-64)	3838 × 3710 (38% smaller than DE-20; 370% smaller than DE-64)	
Pixel Design	>3T (CDS to reduce noise)	ЗТ (High noise)	ЗТ (High noise)	
Frame Shutter	Global or rolling	Rolling (Introduces gradients across images)	Rolling (Introduces gradients across images)	
Backthinned Yes		Yes	Yes	
Radiation Hardness	Excellent	Excellent	Excellent	
Optimal Exposure Rate	Consistent performance at any exposure rate	Worse performance at low exposure rates	Only very low exposure; < 3 e ⁻ /pixel/s is optimal; e.g., at 10 e ⁻ /pixel/s performance drops ~30% ¹	
Dynamic Range > 400 e ⁻ /pixel/s		At ~60 e⁻/pixel/s² At ~60 e⁻/pixel/s qua efficiency decreases		

¹ Ruskin et al., J Struct Biol 184 (2013).

² McMullan et al., Ultramicrosc 147 (2014).

³ Li et al., J Struct Biol 184 (2013).

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Unique, Industry-Leading Features

	Direct Electron	Alternative 1	Alternative 2	
Frame Rate	Flexible; Up to 30 fps full frame; 1000+ fps for subarrays	18 fps; Typically users may only save 7 frames	40 fps transfer rate to computer	
Survey Sensor	Integrated near-axis 2048 × 2048 survey sensor	Separately purchased and maintained	Separately purchased and maintained	
Exposure Measurement	Integrated Faraday plate above the DDD sensor	None	None	
Sensor Protection Shutter	Yes, patented	None	None en e	
Retractable	Yes, mechanical	Yes	Yes, pneumatic	
Mounting Position	Film chamber (JEOL) or standard bottom mount	Bottom mount	Bottom mount	
Magnification Factor	Film chamber: 1.0× Bottom mount: ~1.4×	arsa ars≃1.4× ar	~1.4×	
Data Acquisition Software	Multiple options; Open API for integration	Proprietary	Proprietary	
Movie Processing Software	Flexible and open-source; patented algorithms	Minimal	Proprietary	

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Direct Electron Imaging Manager (DE-IM)

DE-IM Data Collection Experiment	In Califrations Aperlinen Neardoure (About	_ @ x
Imaging	Live Preview	Capiture
Camera 🔺	Capture: Genome	
Camera DE20 DE20 Survey	• • • +	ал х он х л
Binning		
Esposure Time (s) 2.		
Mode		
Standard Advanced		
Cooling		
Weber Temperature ("CJ 19/40		
Setup 🔺		
Position Extended Retracted		
Positian Status Litended		
Auto Retract On On		
Camera Delay (s) 70		
Autosave 🔺		
Filename Suffix BMV		
Final Image On Off		
Raw Frames		
Last Exposure		
Total Number of Friends 32		
Does Rate (electrons/pixel/s) 20.90 Does Rate (electrons/A/V) 24.50		1/0.330 mm 🗶
Total Desr (electrons/A') 49.13		
	TEM Magnification 50,000 Internity - 13.4	7 / Prositium: 858, 18 / Stat: 1834 / 1024

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56,000× nominal magnification (0.71 Å/pixel).



Collected on a FEI Titan with a DE-12 Camera.

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43,000× nominal magnification (0.91 Å/pixel).



Collected on a FEI Titan with a DE-12 Camera.

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27,000× nominal magnification (1.78 Å/pixel).



Collected on a FEI Titan with a DE-12 Camera.

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21,000× nominal magnification (1.82 Å/pixel).



Collected on a FEI Titan with a DE-12 Camera.

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Consistent High-Resolution Performance

Camera Resolution (Fraction of Nyquist)



Specimen Resolution (Absolute Spatial Frequency) 40kx 1.5 1.2 0.9 Peak 1 0.6 ∿ Peak 2 Peak 3 0.3 50kx 1.5 1.2 0.9 0.6 0.3 60kx 1.5 1.2 0.9 0.6 0.3 80kx 1.5 1.2 0.9 0.6 0.3 100kx 1.5 1.2 0.9 0.6 0.3 0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 Spatial Frequency (1/Å)

High-resolution (e.g., 3.5 Å) SNR is maintained at at nearly the same level at 40kx mag. (80% Nyquist) as it is at 100kx mag (30% Nyquist). This indicates a consistent high-resolution performance, meaning that you can image at lower magnification (for a larger fieldof-view) *without* sacrificing high-

Lower magnification means a larger field-of-view!

resolution SNR.

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Performance Comparison

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SNR from Each Incident Electron



Courtesy of Greg McMullan (MRC) and Dan Clare (Birkbeck).

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DQE Comparison (200 kV)



Curves from Ruskin et al., J Struct Biol 184 (2013).

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DQE Improvement from DE-12 to DE-20



CCD and DE-12 curves from Ruskin et al., J Struct Biol 184 (2013). DE-20 curve calculated independently. Results for 200 keV electrons.

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Published Virus Reconstructions (200 kV)

RED indicates differences between the two experiments, either worse results or experimental parameters that should have generated better results.

	Direct Electron DE-12 Campbell, et al. (2012) <i>Structure</i> 20, 1823-8.	Gatan K2 Summit Veesler, et al. (2013) <i>J. Struct. Biol.</i> 184, 193-202.
Sensor Size	4096 × 3072 with 6 μm pixels, (Note: DE-12 gen1 was NOT backthinned)	7424 × 7680 (super-resolution), backthinned binned by 2× yielding 3712 × 3840 with 5 μm pixels
Magnification	1.42 Å/pixel (42,135× magnification)	1.21 Å/pixel (41,322× magnification)
Exposure Time	0.64 s (saved 16 frames @ 25 fps)	4.00 s (saved 16 frames @ 4 fps)
Dose	$32 e^{-}/Å^{2}$ (rate = 99 e ⁻ /pixel/s)	
Total Images	561 acquisitions (movies) 1915 particles originally extracted 807 particles used for the final reconstruction	754 acquisitions (movies) 4490 particles originally extracted 4446 particles used for the final reconstruction
Drift Corr.	Per particle drift correction	Drift correction of 2k × 2k regions
Refinement	CTFFIND3 & Frealign	CTFFIND3 & Frealign
Symmetry	Icosahedral (with 13-fold NCS averaging on the coat)	Icosahedral
Resolution	Virus = 6.2 Å (46% Nyquist) / Coat = 4.4 Å (65% Nyquist)	Virus = 6.1 Å (40% Nyquist) / Coat = 4.4 Å (55% Nyquist)
Specimen	Rotavirus DLP (70 MDa)	Sulfolobus turreted icosahedral virus (STIV) (75 MDa)
		A LALAN AND A REAL

Example of Visible Side Chains





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Direct Comparison Between Direct Detectors



- Comparison between Direct Electron DE-20 and Gatan K2-Summit.
 - Identical cryo-EM experiment on two different cameras.
- Same microscope (FEI Polara 300 kV).
- Same specimen preparation (TMV).
- Similar imaging conditions and number of particles.
- Same image processing.

Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

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Direct Comparison Between Direct Detectors

DE-20:

- FEI Polara (300 kV)
- Spot size 6, C2 52.7%
- 39,000x TEM
- Pre-GIF (no energy filter)
- 1.221 Å/pixel
- 25 e⁻/Å² total exposure used for reconstruction
- 15 fps, at 2.1 $e^{-}/Å^{2}/frame$
- Exposure time for reconstruction 0.8 s
- DE_process_frames.py with whole frame alignment (quanta 1) and no damage compensation
- 11,000 particle segments boxed and used

K2-Summit:

- FEI Polara (300 kV)
- Spot size 8, C2 51.8%
- 160,000x EFTEM
- Post-GIF with 30 eV energy filter slit
- 0.695 Å/virtual pixel (super-resolution mode),
- 1.390 Å/physical pixel
- 25 e⁻/Å² total exposure used for reconstruction
- 5 fps, at 0.59 e⁻/Å²/frame
- Exposure time for reconstruction 8.4 s

Required 10x longer exposure time for an equivalent image

- Tried both IMOD for sub-frame alignment (similar to DE_process_frames.py) and MOTIONCORR from Yifan
 - 11,000 particle segments boxed and used

Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

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Class Averages

After 6 rounds of alignment

DE-20



K2-Summit



Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

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3D Reconstructions (Unfiltered)

DE-20



K2-Summit



Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

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3D Reconstructions (Sharpened)

DE-20

K2-Summit



Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

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Final Resolution (FSC)



Red: DE-20 0.5 FSC = 3.3 Å (0.143 = 3 Å)

Blue: K2-Summit 0.5 FSC = 3.4 Å (0.143= 3 Å) (processed also at 0.695 Å/pix yielding 3.36 Å at 0.5)

Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

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Movie-Mode Processing

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Collect Movies Instead of Static Images

Excellent sensitivity and SNR so that each raw frame contains usable information

Direct detection delivers high resolution

High frame rate with no dead time between frames

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Saving Raw Frames

Comput	er 🕨 FastData	(D:) • RawFran	mes 🕨 DE20 🕨 2	0130828_00126_bea	mstop
Organize 🔻 Include i	n library 🔻	Share with 🔻	Slide show	Burn New fo	lder
Name	Date		Туре	Size	Tags
🗾 FinalImage.tif	8/28/2013 5:2	20 PM	TIF File	38,431 KB	
info.txt	8/28/2013 5:2	0 PM	Text Document	3 KB	
RawImage_0.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_1.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_2.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_3.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_4.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_5.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_6.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_7.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_8.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_9.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_10.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_11.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_12.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_13.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_14.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_15.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
📄 RawImage_16.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_17.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_18.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
RawImage_19.tif	8/28/2013 5:1	9 PM	TIF File	38,431 KB	
52 items					

<u>DE-20</u> 37.5 MB/frame 1.1 GB for 30 frames

<u>DE-64</u> 128 MB/frame 3.75 GB for 30 frames

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The Impact of "Movie-Mode" for Imaging

Motion correction

- Correct the motion of the entire frame (best for tomography).
- Correct the motion of individual particles (best for singleparticle imaging since it corrects for both stage drift and beam-induced specimen motion).
- Dramatically increases data quality.
- Choose your exposure after collecting data
- Damage compensation
 - Use a high dose while maintaining high-resolution features.
 - Dramatically increases contrast.

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Motion Correction

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We Need Stability, Not Motion

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"Finally, we turn to the point that the signal in cryo-EM images shows a much steeper falloff at high resolution than is observed in the electron diffraction pattern of the same specimen... In any event, beam-induced movement that occurs while the image is recorded is responsible for the steep falloff of signal."

Glaeser & Hall, *Biophys J* **100** (2011), 2331-2337.

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Motion Correction Development

 Applications of direct detection device in transmission electron microscopy.

Jin, et al. J Struct Biol. 2008 Mar;161(3):352-8.

- Beam-induced motion of vitrified specimen on holey carbon film. Brilot, et al. J Struct Biol. 2012 Mar;177(3):630-7.
- Movies of ice-embedded particles enhance resolution in electron cryo-microscopy. Campbell, et al. Structure. 2012 Nov 7;20(11):1823-8.
- Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM. Li, et al. Nat Methods. 2013 Jun;10(6):584-90.
- Ribosome structures to near-atomic resolution from thirty thousand cryo-EM particles. Bai, et al. Elife. 2013 Feb 19;2:e00461.

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CPV at 3.5 Å Resolution



Courtesy of Hong Zhou (UCLA), unpublished.

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DE-12 Image (~2 micron defocus)



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Aligning Frames

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Rolling Averages



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Filtering Alignment Images



Bin 6× | Bandpass Filter | Normalize | Taper Edge

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Align with IMOD (tiltxcorr)

-RotationAngle 0 -FirstTiltAngle 0 -TiltIncrement 0 -FilterRadius2 0.30 -FilterSigma1 0.01 -FilterSigma2 0.02 -ShiftLimitsXandY %i,%i -Iterate %i -CumulativeCorrelation -ReverseOrder



Frames to Align

Reference

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Align with IMOD (tiltxcorr)

-RotationAngle 0 -FirstTiltAngle 0 -TiltIncrement 0 -FilterRadius2 0.30 -FilterSigma1 0.01 -FilterSigma2 0.02 -ShiftLimitsXandY %i,%i -Iterate %i -CumulativeCorrelation -ReverseOrder



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Align with IMOD (*tiltxcorr*)

-RotationAngle 0 -FirstTiltAngle 0 -TiltIncrement 0 -FilterRadius2 0.30 -FilterSigma1 0.01 -FilterSigma2 0.02 -ShiftLimitsXandY %i,%i -Iterate %i -CumulativeCorrelation -ReverseOrder



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Align with IMOD (*tiltxcorr*)

-RotationAngle 0 -FirstTiltAngle 0 -TiltIncrement 0 -FilterRadius2 0.30 -FilterSigma1 0.01 -FilterSigma2 0.02 -ShiftLimitsXandY %i,%i -Iterate %i -CumulativeCorrelation -ReverseOrder



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Applying Translations

Apply translations (subpixel) with EMAN2 Transform Class



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Applying Translations

Apply translations (subpixel) with EMAN2 Transform Class



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Specimen Motion



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Typical Motion (2.5 Å Traveled)



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Typical Motion (2.5 Å Traveled)



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Typical Motion (2.5 Å Traveled)



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28% average SNR improvement

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Small Motion (0.25 Å Traveled)



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Small Motion (0.25 Å Traveled)



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3% average SNR improvement

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Large Motion (9.8 Å Traveled)



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Large Motion (9.8 Å Traveled)



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173% average SNR improvement

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SNR Improvement Comparison

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Damage Compensation

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We Need to Overcome Radiation Damage

"The primary limiting factor is radiation damage and, more specifically, the poor signal/noise ratio (SNR) in images recorded with optimal electron exposures... The optimal electron exposure that can be used for cryo-EM depends somewhat on the resolution. For example, it is counterproductive to use exposures (with 300 keV electrons) higher than 2000 electrons/nm² to image features at high resolution, whereas exposures five times larger than that can be used to image features at very low resolution."

Glaeser & Hall, *Biophys J* **100** (2011), 2331-2337.

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Damage Compensation Development

The resolution dependence of optimal exposures in liquid nitrogen temperature electron cryomicroscopy of catalase crystals.

Baker, et al. J Struct Biol. 2010 Mar;169(3):431-7.

Visualizing and correcting dynamic specimen processes in TEM using a Direct Detection Device.

Bammes, et al. Microsc Microanal. 2013 Aug;19(S2):1320-1.

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Cumulative Exposures on Catalase

3 e⁻/Ų



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Cumulative Exposures on Catalase

9 e⁻/Ų



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Cumulative Exposures on Catalase



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Optimal Exposure vs. Resolution



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10 e-/Å2

20 e-/Å2 30 e-/Å2



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10 e-/Å2

20 e-/Å2 30 e-/Å2



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Damage Compensation on Catalase



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Damage Compensation on Catalase



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Contrast Enhancement for GroEL

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25 e⁻/Å²

100 e⁻/Ų

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Example: BMV



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Application: Single-Particle Cryo-EM

Brome mosaic virus (BMV) collected at 50k× magnification (1.07 Å/pixel) with a 2 s exposure and 50 $e^{-}/Å^{2}$, at ~0.7 μ m defocus



Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

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Per-Particle Motion Correction of BMV

Vector plot of the motion of each BMV particle in one image. Vectors are magnified by 50× to improve visualization.



Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

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Thon Rings for a Stack of BMV Particles



Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

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SNR Plot Has Signal Up to 2.7 Å!



Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

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De Novo 3D Reconstruction



Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

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focus on innovation

NNOVATION PROPELLING DISCOVERY

count on exceptional service

generate results

REPATION 1

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