Event driven Timepix3 hybrid pixel detector for cryo-EM at 200 kV

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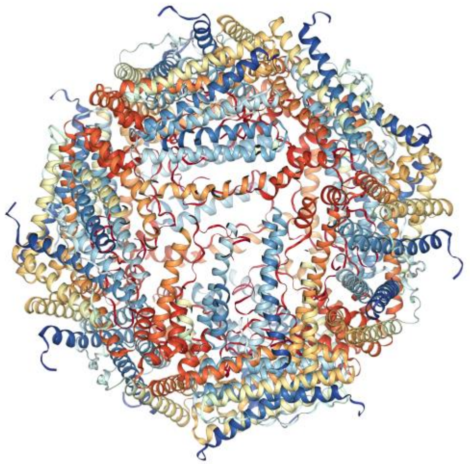
Meeting: Riva del Garda

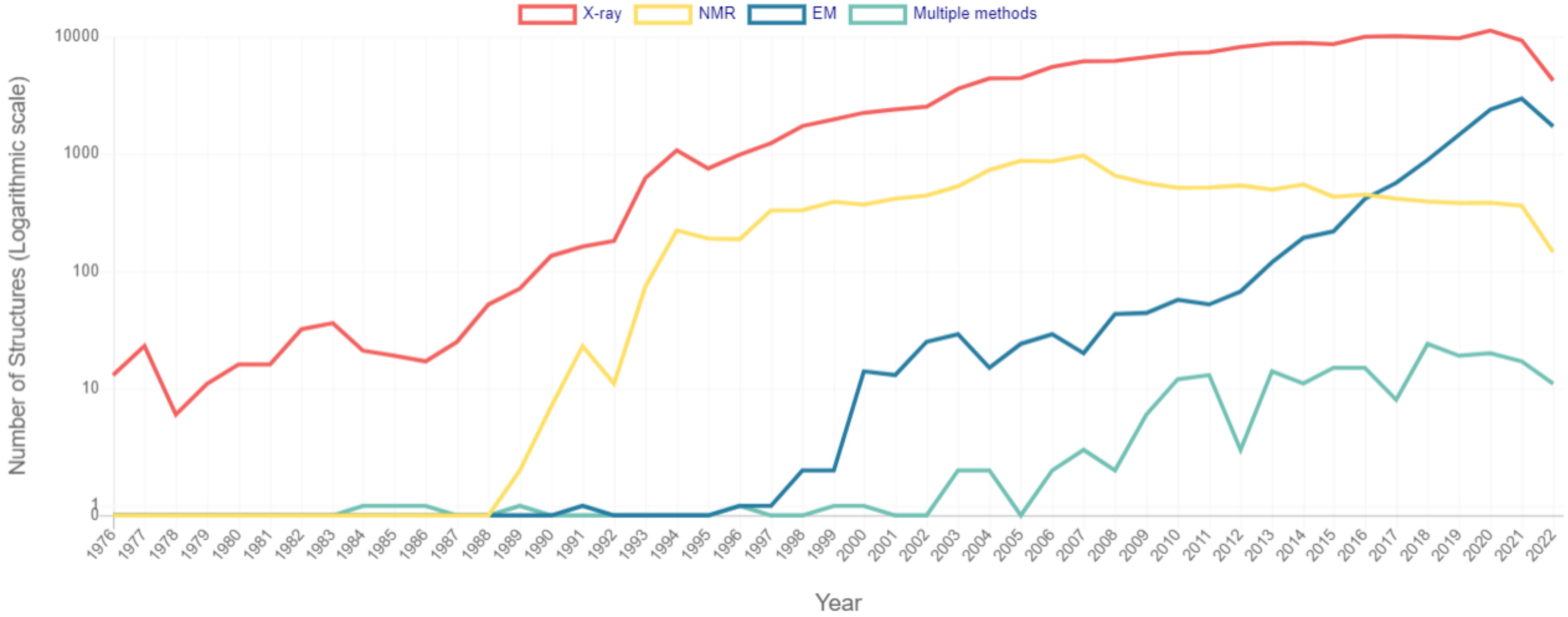
Date 27-06-2022

Maastricht University M4I

Cryogenic Transmission Electron Microscopy for Structural Biology

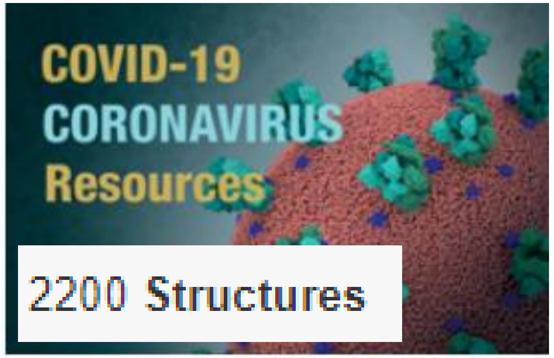


Number of Structures in Protein Data Bank



RCSB PDB
PROTEIN DATA BANK

191565 Biological Macromolecular Structures Enabling Breakthroughs in Research and Education

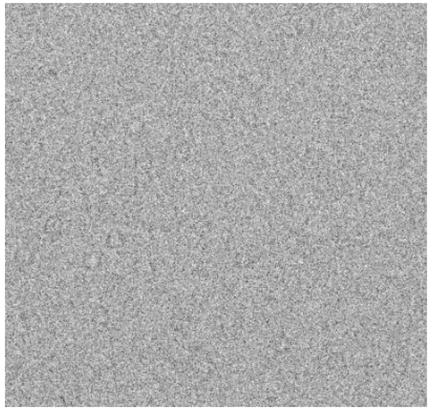


EXPERIMENTAL METHOD

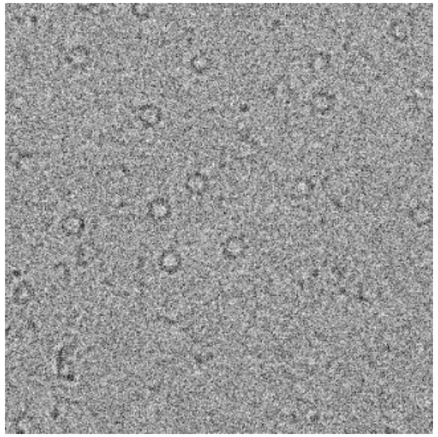
- X-RAY DIFFRACTION (1409)
- ELECTRON MICROSCOPY (780)
- SOLUTION NMR (9)
- NEUTRON DIFFRACTION (7)
- SOLID-STATE NMR (2)

Why detectors?

Weak phase objects



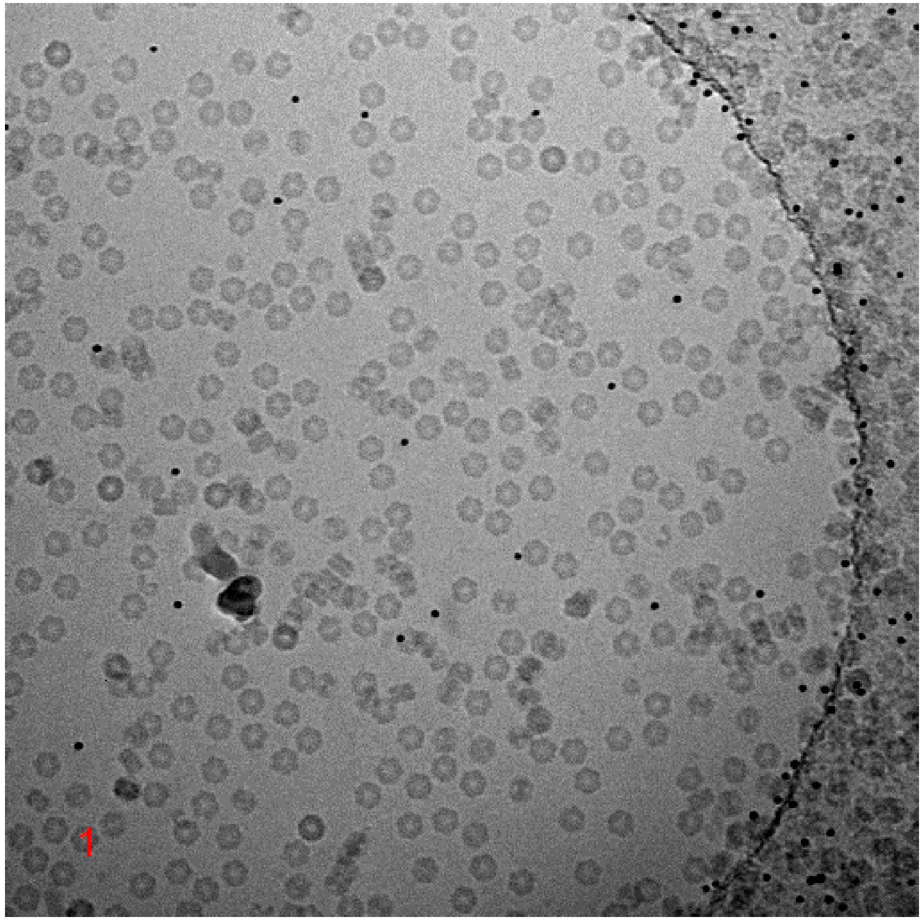
Δf = -0.6 um



Δf = -2.3 um

High defocus

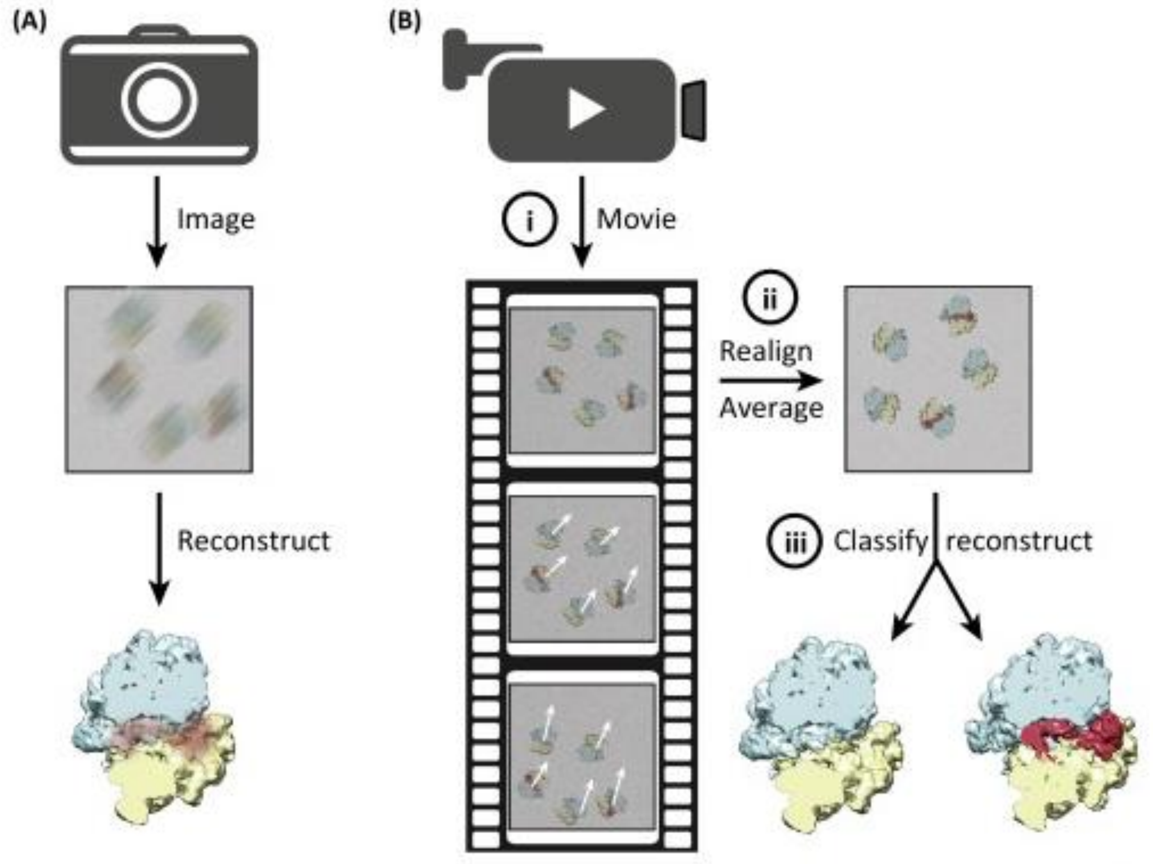
Radiation damage



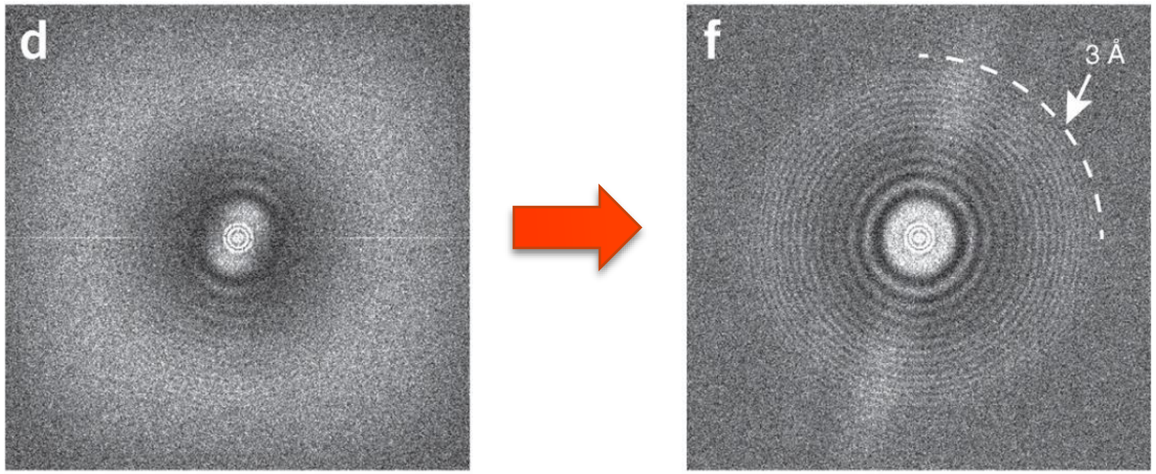
Flux: 50 e · $Å^{-2}$ s^{-1} , 100 images, 1 s integration time each.

Karuppasamy et al., 2011.

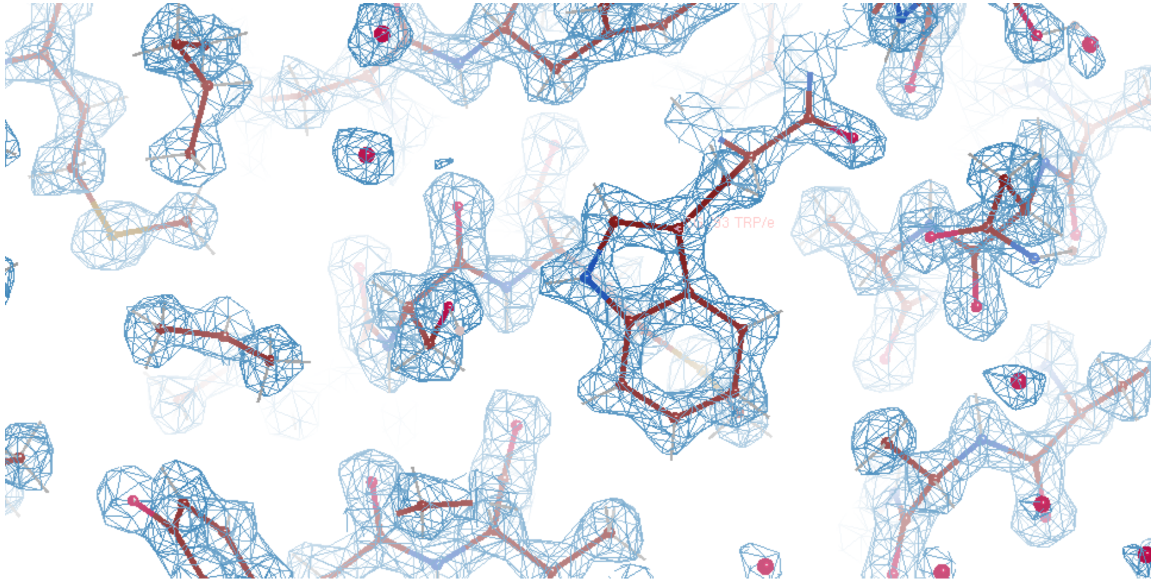
Detector makes the difference



Bai et al., 2015



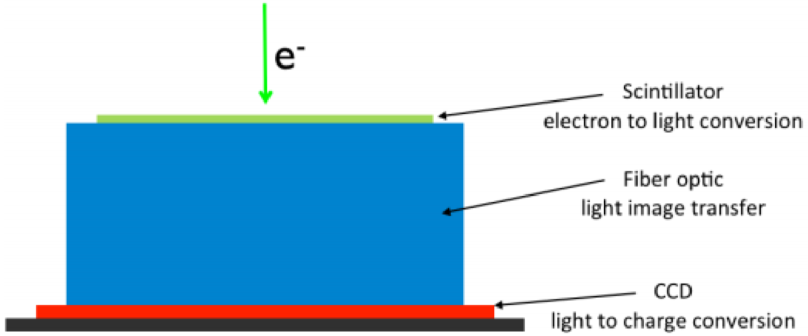
Li et al., 2015



Yip et al., 2020

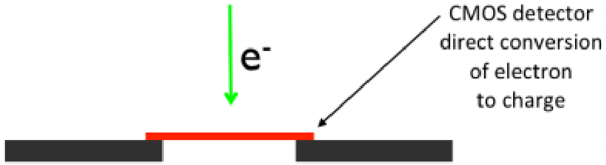
Type of electron detectors

Charge Coupled Device (CCD)



Scintillator adds noise
Scintillator deteriorate PSF
Limited dynamic range
Readout noise

Monolithic Active Pixel Sensor (MAPS)



Frame based
Low throughput ~ 40 s
0.01–0.025 electrons/pixel/frame
Works best at 300 kV
Radiation sensitive
Limited dynamic range

Better detector for cryo-EM?

Contents lists available at ScienceDirect
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journal homepage: www.elsevier.com/locate/ultramic

The energy dependence of contrast and damage in electron cryomicroscopy of biological molecules

Mathew J. Peet, Richard Henderson, Christopher J. Russo*
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radiation damage
JOURNAL OF SYNCHROTRON RADIATION
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Single-particle cryo-EM: alternative schemes to improve dose efficiency

Yue Zhang,[a] Peng-Han Lu,[b] Enzo Rotunno,[c] Filippo Troiani,[c] J. Paul van Schayck,[a] Amir H. Tavabi,[b] Rafal E. Dunin-Borkowski,[b] Vincenzo Grillo,[c] Peter J. Peters[a] and Raimond B. G. Ravelli[a]*

Article | Open Access | Published: 21 February 2020

Serial protein crystallography in an electron microscope

Robert Bücker, Pascal Hogan-Lamarre, Pedram Mehrabi, Eike C. Schulz, Lindsey A. Bultema, Yaroslav Gevorkov, Wolfgang Brehm, Oleksandr Yefanov, Dominik Oberthür, Günther H. Kassier & R. J. Dwayne Miller

Nature Communications 11, Article number: 996 (2020) | Cite this article

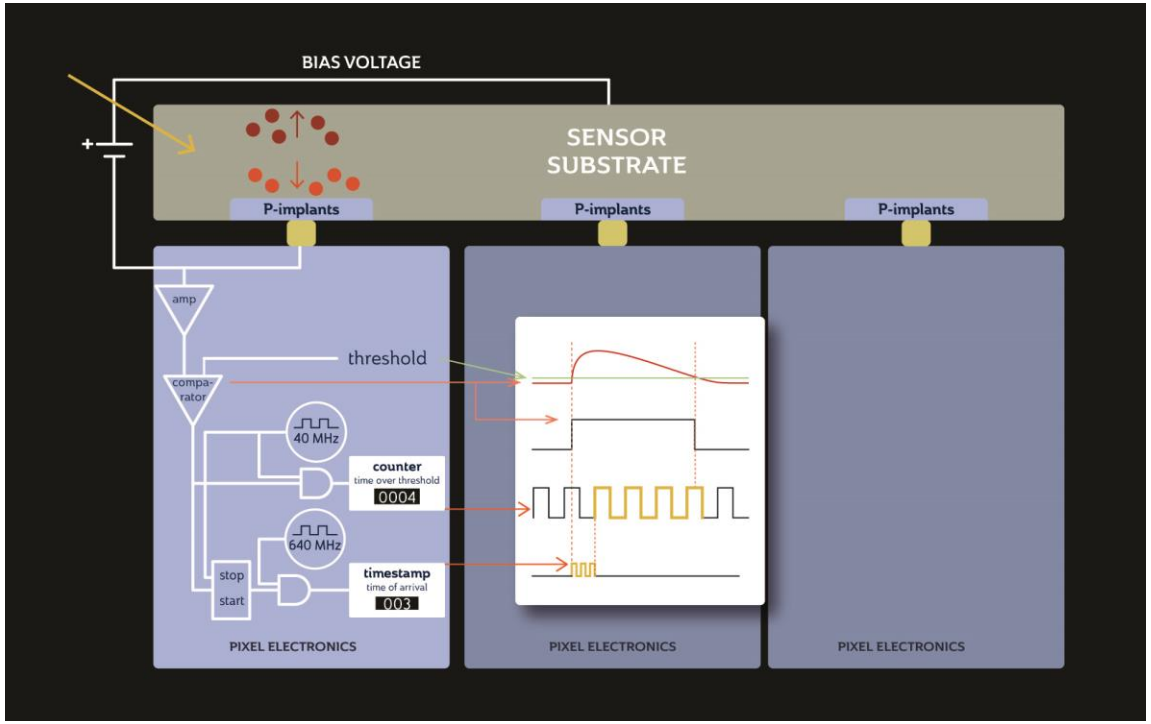
nature communications

Article | Open Access | Published: 02 June 2020

Low-dose phase retrieval of biological specimens using cryo-electron ptychography

Liqi Zhou, Jingdong Song, Judy S. Kim, Xudong Pei, Chen Huang, Mark Boyce, Luiza Mendonça, Daniel Clare, Alistair Siebert, Christopher S. Allen, Emanuela Liberti, David Stuart, Xiaoqing Pan, Peter D. Nellist, Peijun Zhang, Angus I. Kirkland & Peng Wang

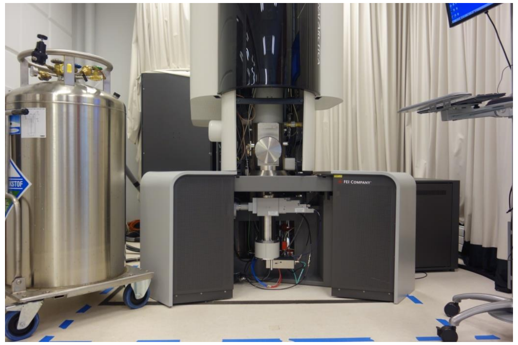
Timepix3: An event driven Hybrid Pixel Detector

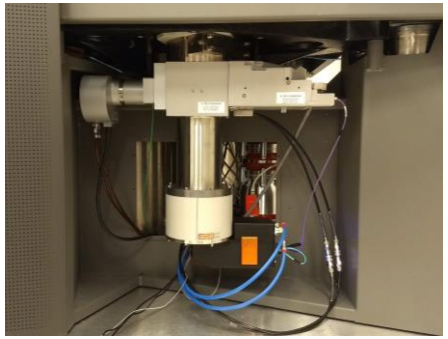


- Event driven
- Radiation hard
- Simultaneous readout of
  - Time over Threshold (energy)
  - Time of Arrival (timing)
- 640 MHz clock
- 120 Mhit/s (quad)
- Noiseless readout

SPIDR readout from Nikhef.

AMSTERDAM SCIENTIFIC INSTRUMENTS





Mounted on FEI Tecnai Arctica (200 kV)

Integration of an event-driven Timepix3 hybrid pixel detector into a cryo-EM workflow

1. Event localisation of electrons
2. Measuring MTF, NPS and DQE
3. Correcting for chip edge
4. Integration of Timepix3 in SerialEM and microscope for automated data collection
5. Single particle analysis

Timepix3 for cryo-EM

1. Event localisation of electrons
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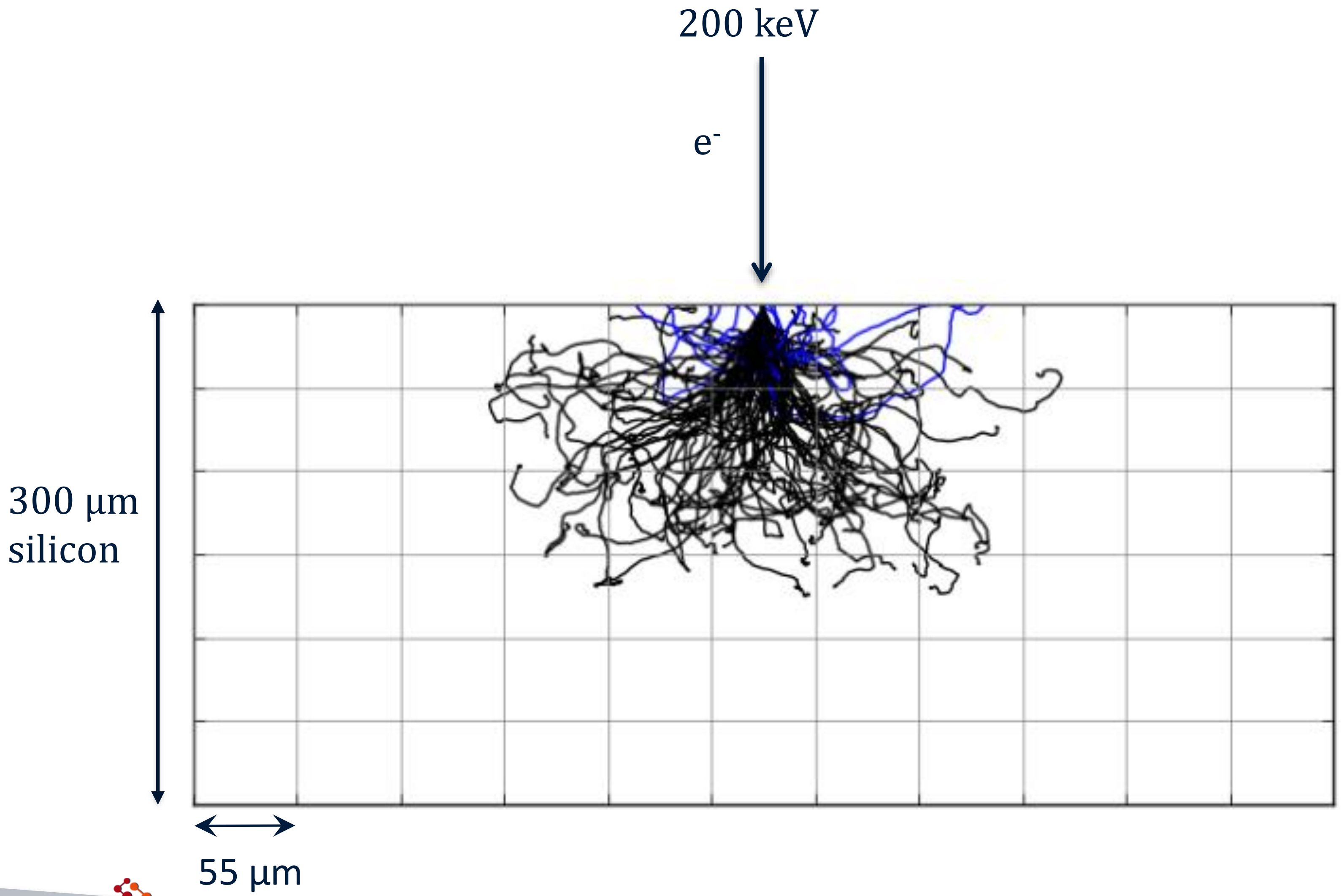
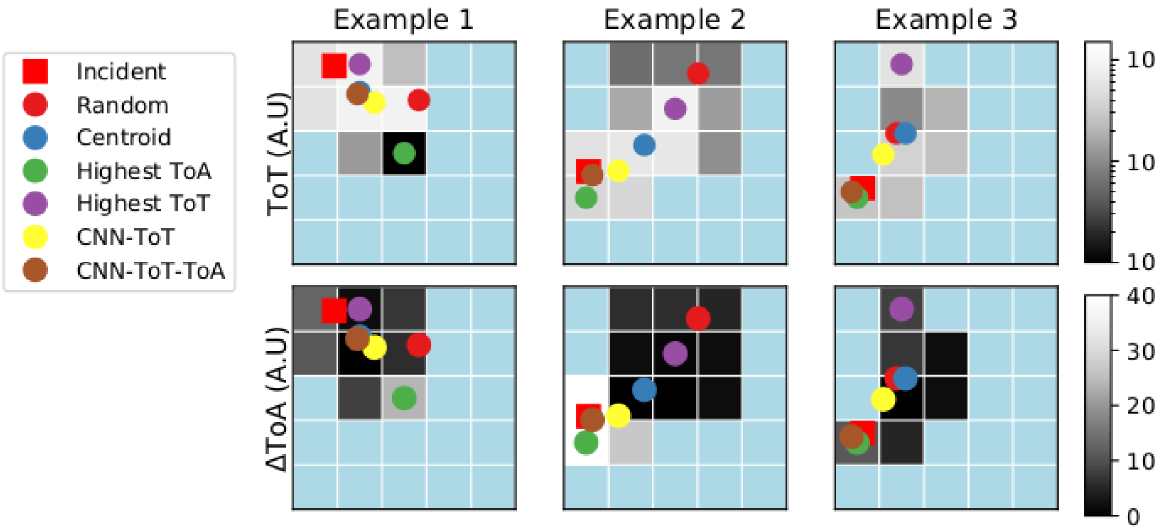
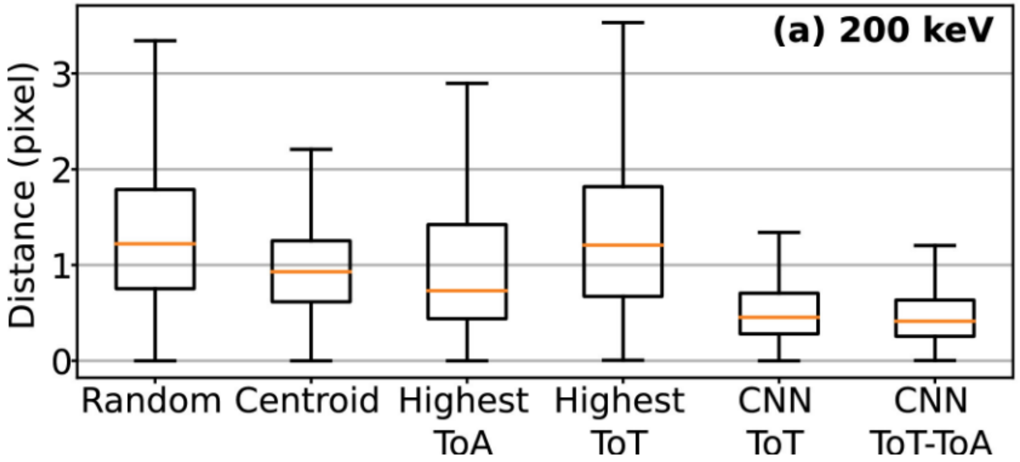
The challenge of detecting electrons in their incident position



Localising the incident position using Timepix3



Simulated Timepix3 output (Geant4Medipix)



Prediction accuracy on simulated data

van Schayck et al., 2020

Timepix3 for cryo-EM

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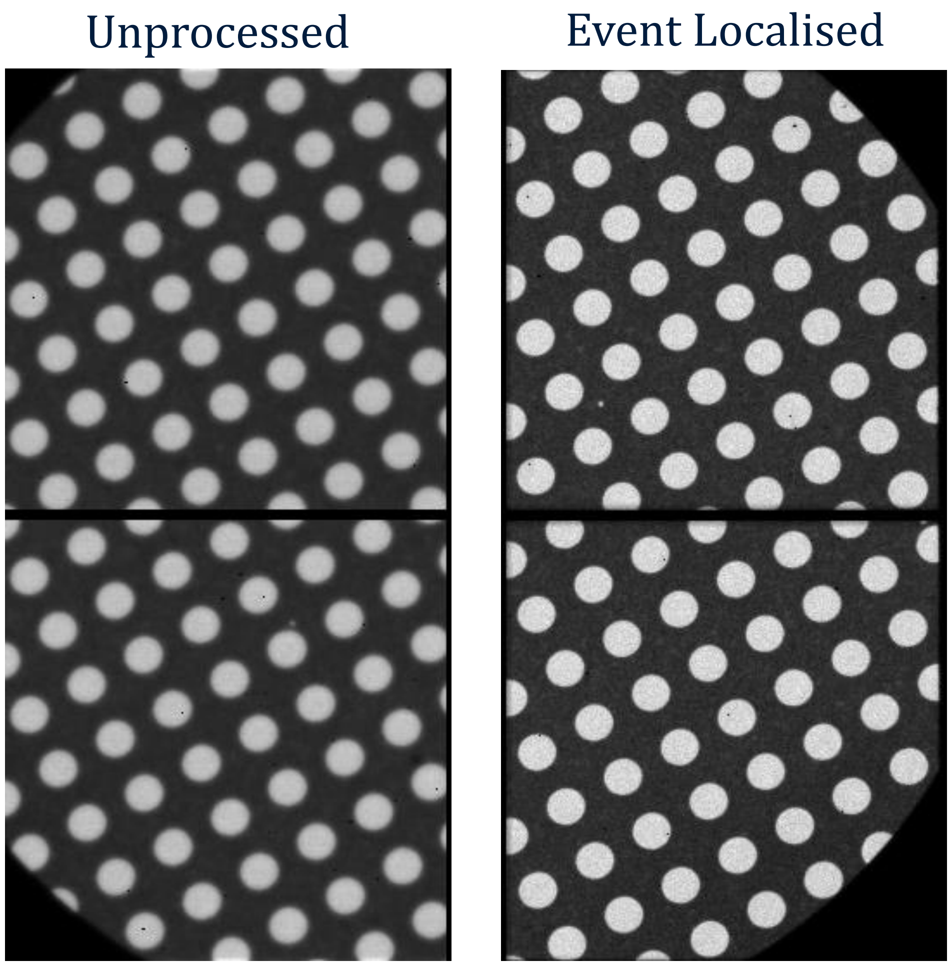
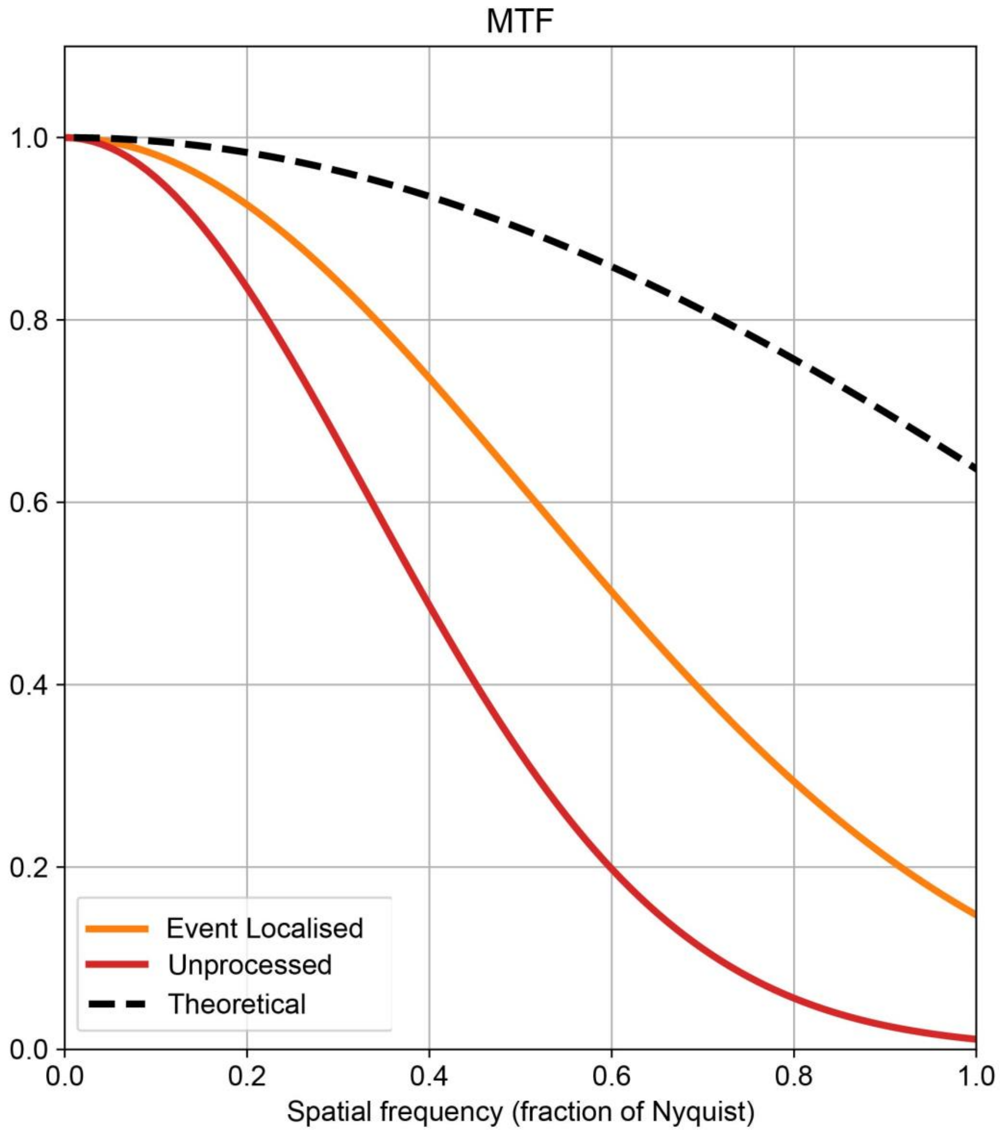
Validation: Modulation Transfer Function (MTF)



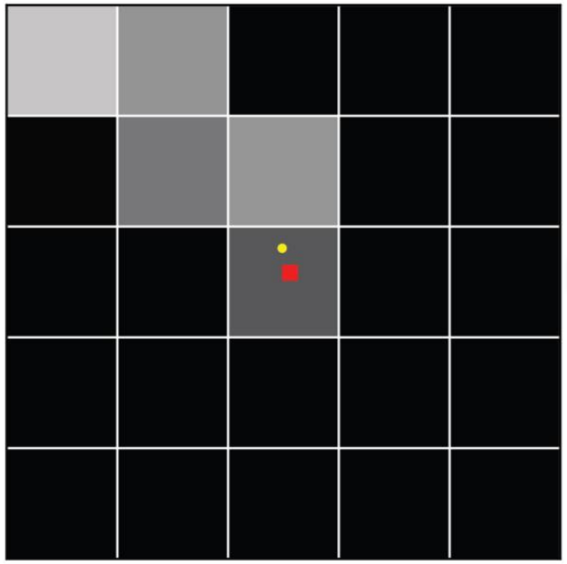
Uncorrected and corrected image of UltrAU grid foil (225x)



Deterministic Blur

$$\uparrow DQE = \frac{SNR_{in}^2}{SNR_{out}^2} = DQE(0)\frac{MTF^2(\omega)\uparrow}{NNPS(\omega)\downarrow}$$

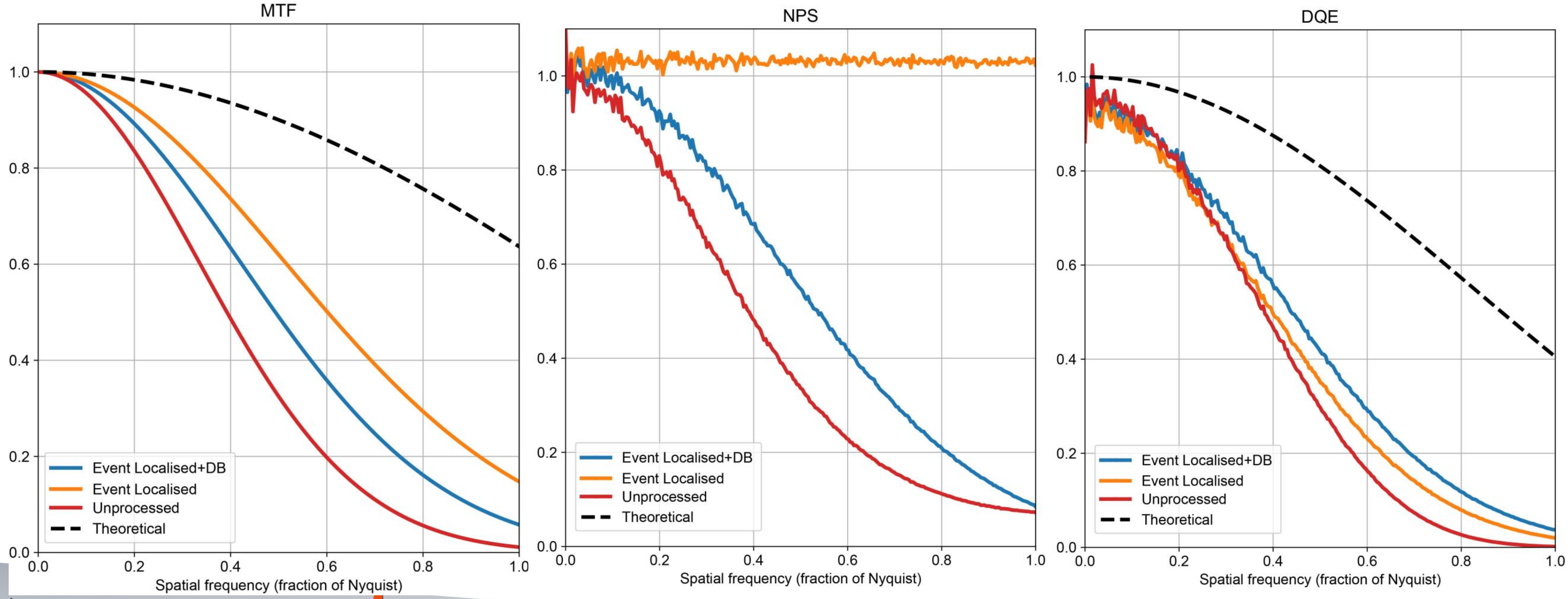
DQE = Detective Quantum Efficiency
SNR = Signal to Noise Ratio
NNPS = Normalised Noise Power Spectrum
MTF = Modulation Transfer Function



Unprocessed Timepix3 output
Simulated incident position (yellow circle)
Predicted incident position (red square)

Validation: Detective Quantum Efficiency (DQE)

$$DQE = \frac{SNR_{in}^2}{SNR_{out}^2} = DQE(0)\frac{MTF^2(\omega)}{NNPS(\omega)}$$



DQE(0) from Paton et al., 2021.

Timepix3 for cryo-EM

1. Event localisation of electrons
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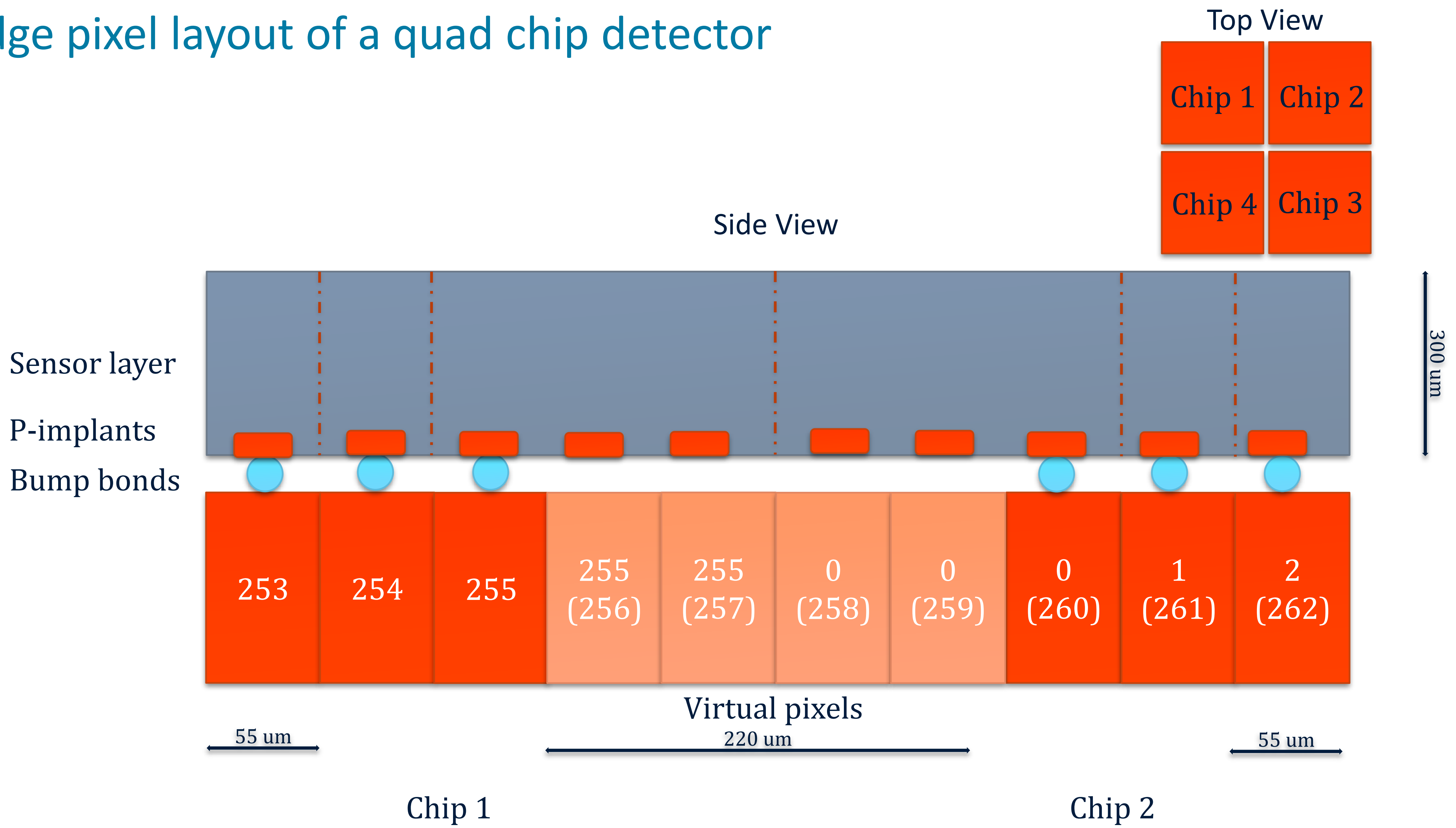
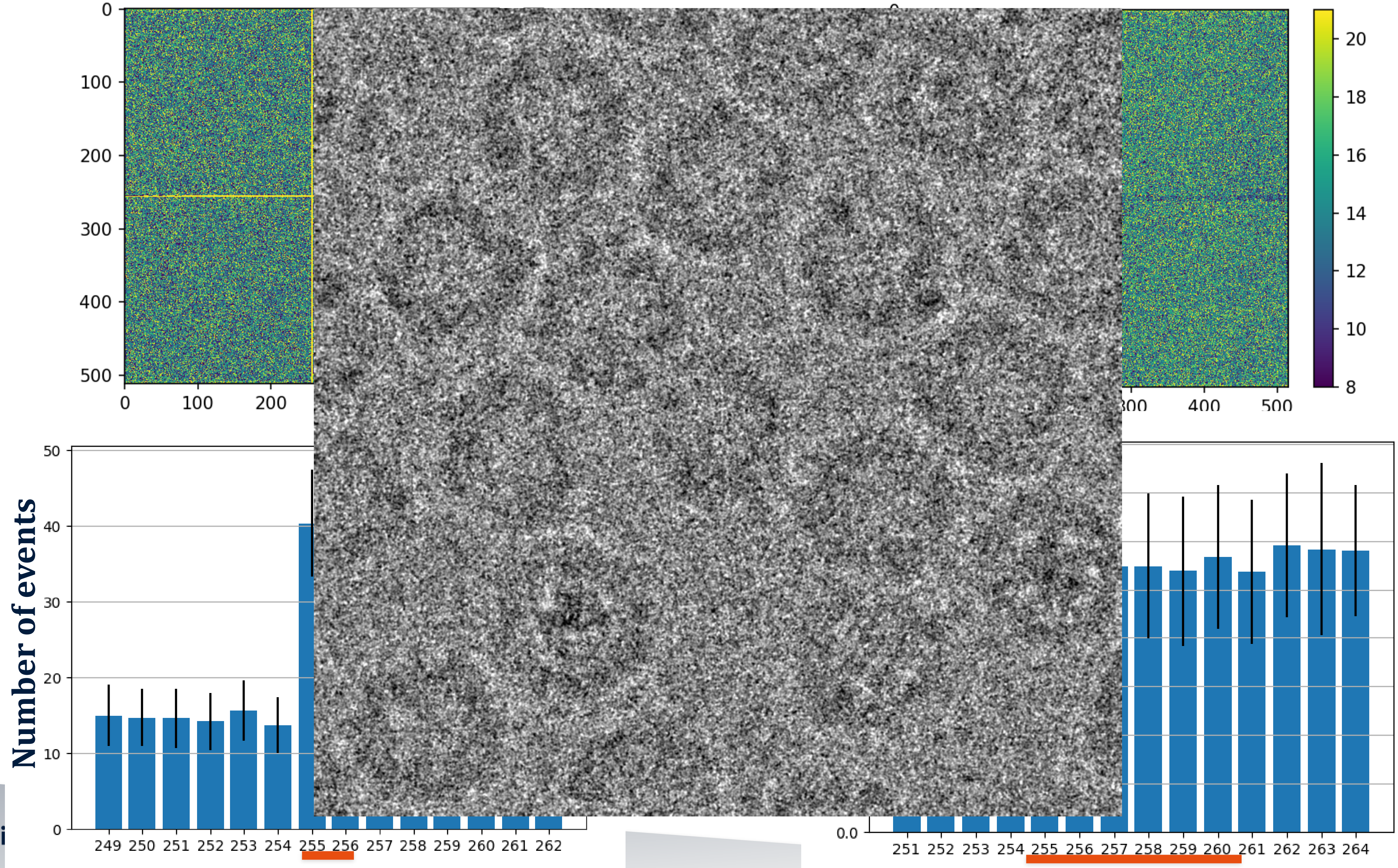
Edge pixel layout of a quad chip detector



Correction for chip edge



Timepix3 for cryo-EM

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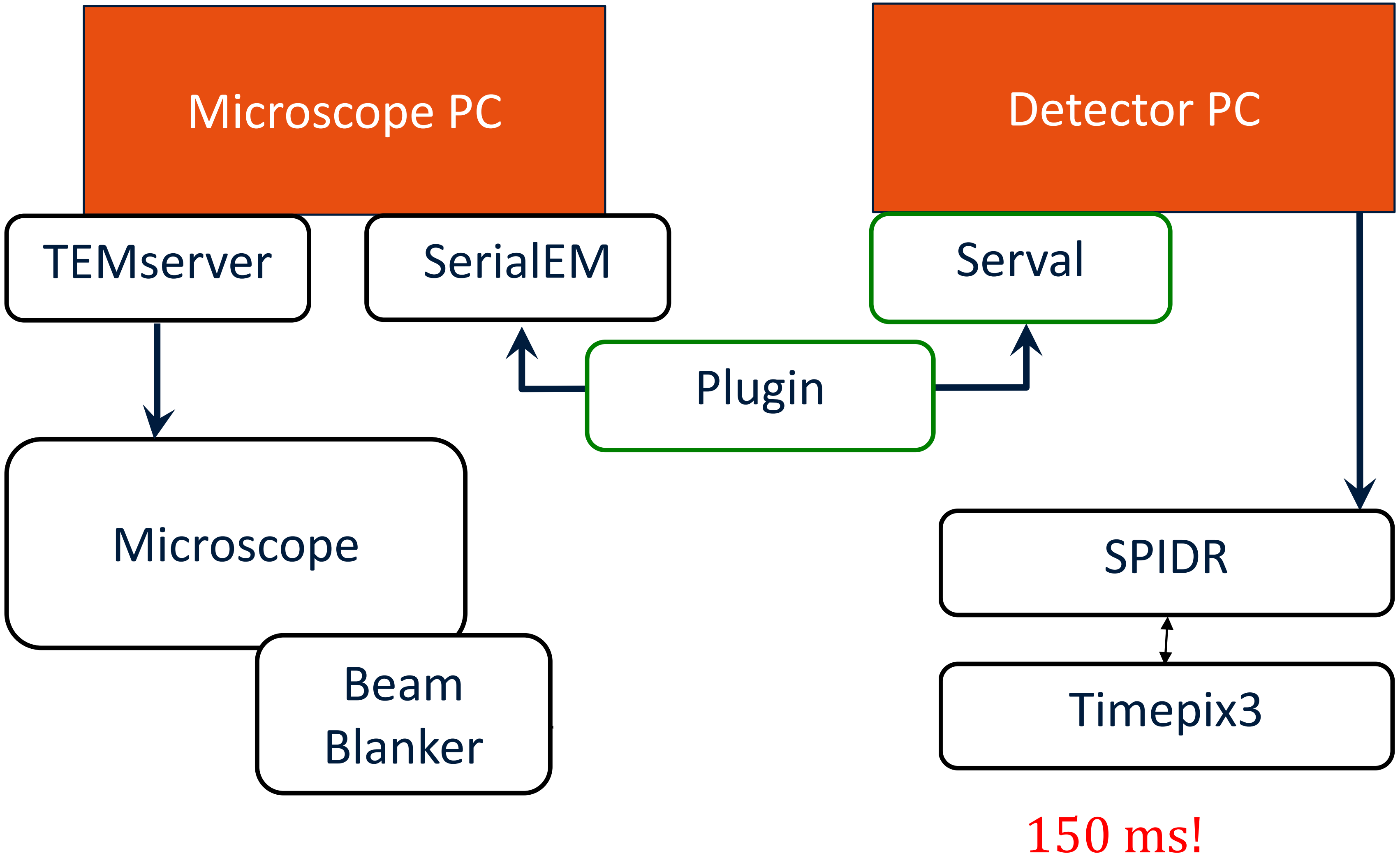
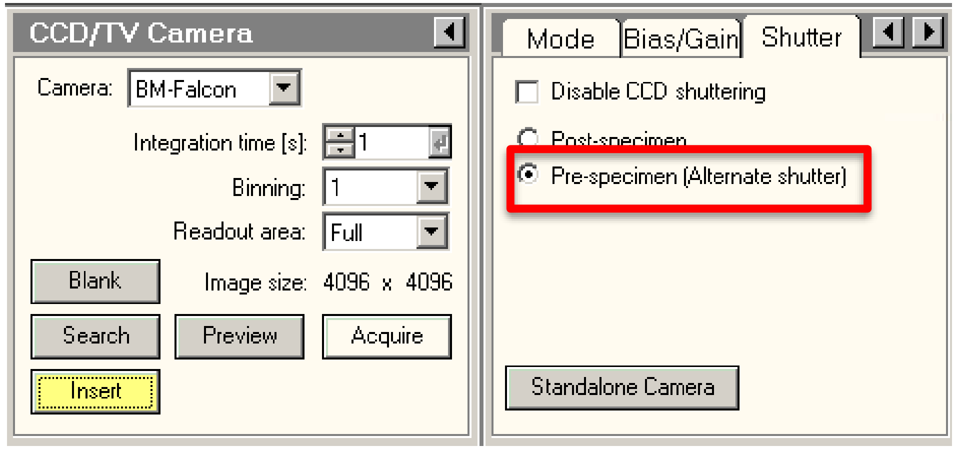
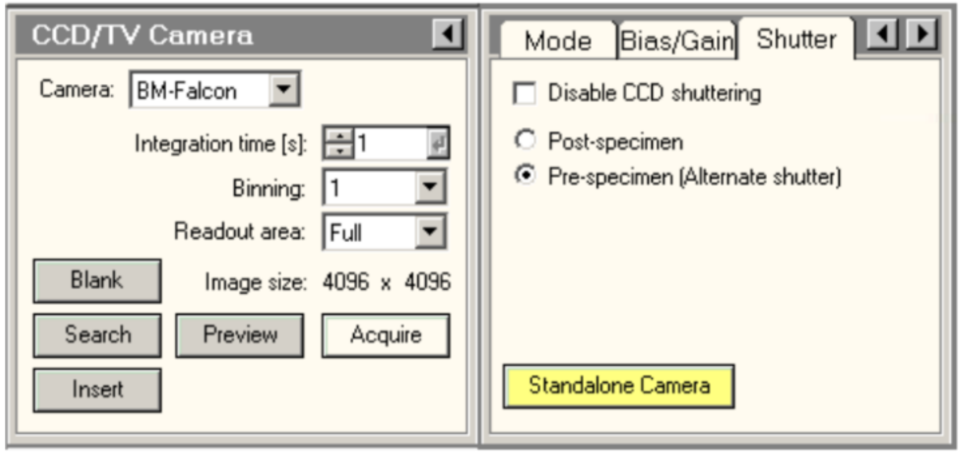
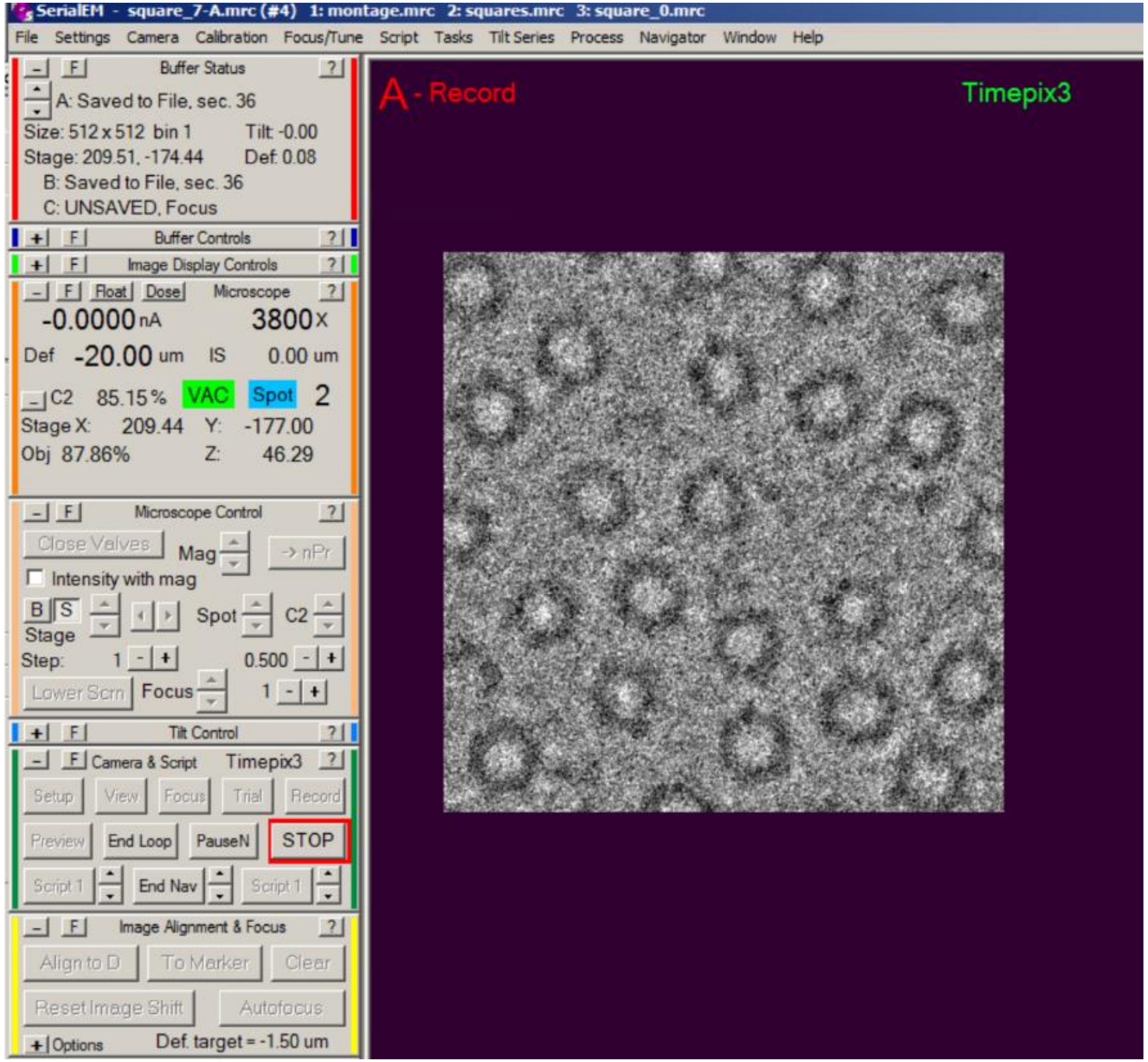
Integrate Timepix3 and the Microscope



Integrate Timepix3 to the Microscope







< 0.3 ms!

1.5s exposure /50 frames
1 frame is 30 ms
300 us is only 1% of the first frame!

Timepix3 for cryo-EM

1. Event localisation of electrons
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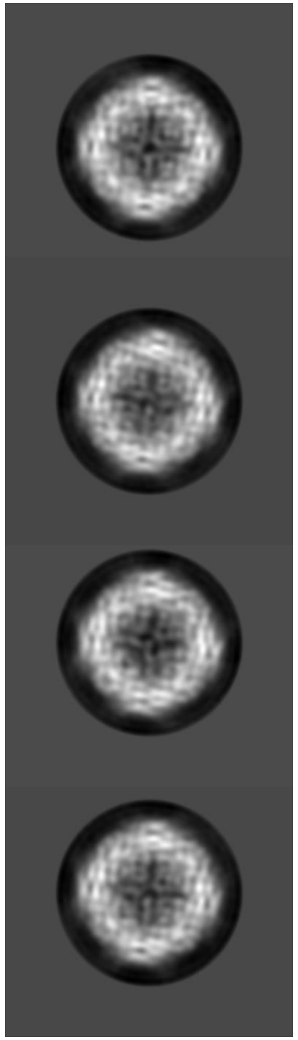
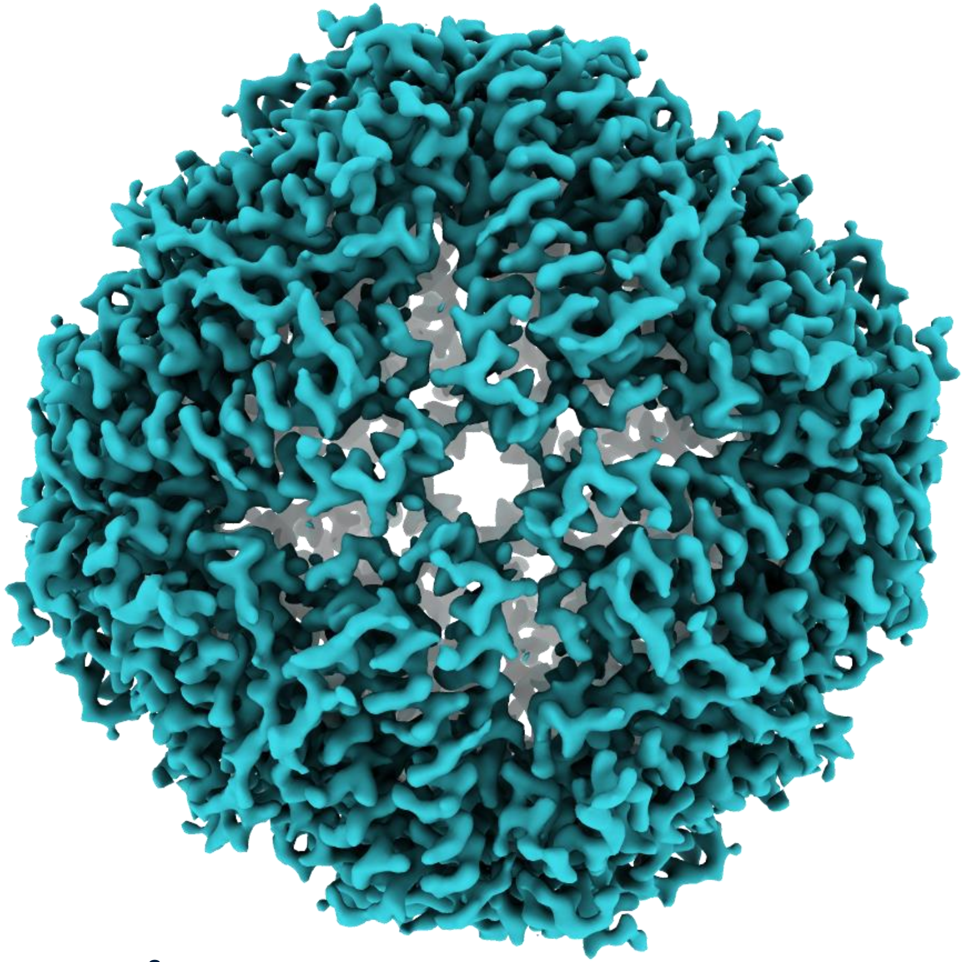
5. Single particle analysis

Single particle analysis with TPX3





3.05 Å map reconstruction of BfrB

Pixel size: 1.2 Å (215 kx)

Superres pixsize: 0.6 Å with Deterministic Blur

B-factor: -100

Exposure: 1.5s

Flux: 35 $e/A^2/s$

Fluence: 50 e/A^2

Event localised: CNN-ToT 2x super resolution

Microscope: FEI Tecnai Arctica (200 kV)

Protein reconstruction map resolution

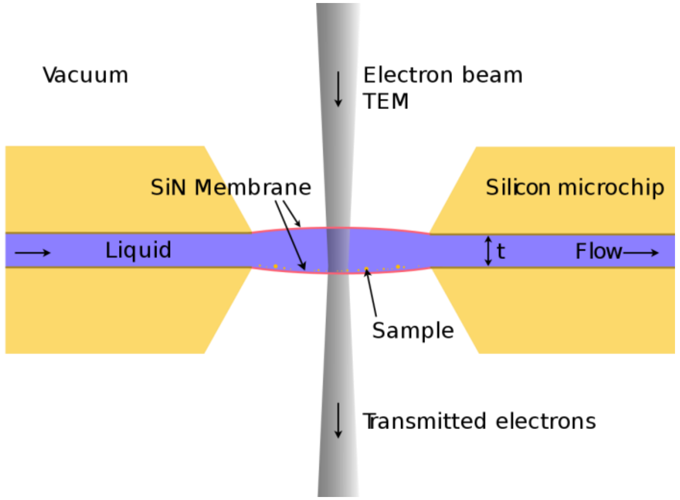
	Unprocessed
Nr. particles	10414
Resolution (Å)	3.85
B factor ($Å^2$)	-201

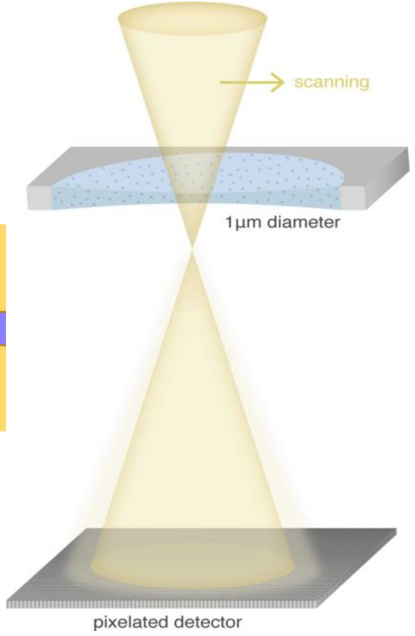
Summary

- Integrate Timepix3 to cryo-EM workflow
- Accurate event localisation of electrons using ToT (and ToA) data using a convolutional neural network
- MTF and DQE improvement from event localisation
- Timepix3 is a versatile detector for diverse cryo-EM workflows

Outlook

- Improving event localisation
- Analyse first few moments of exposure
- Liquid cell electron microscopy
- Cryo Ptychography





Acknowledgments

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Jord Prangsma

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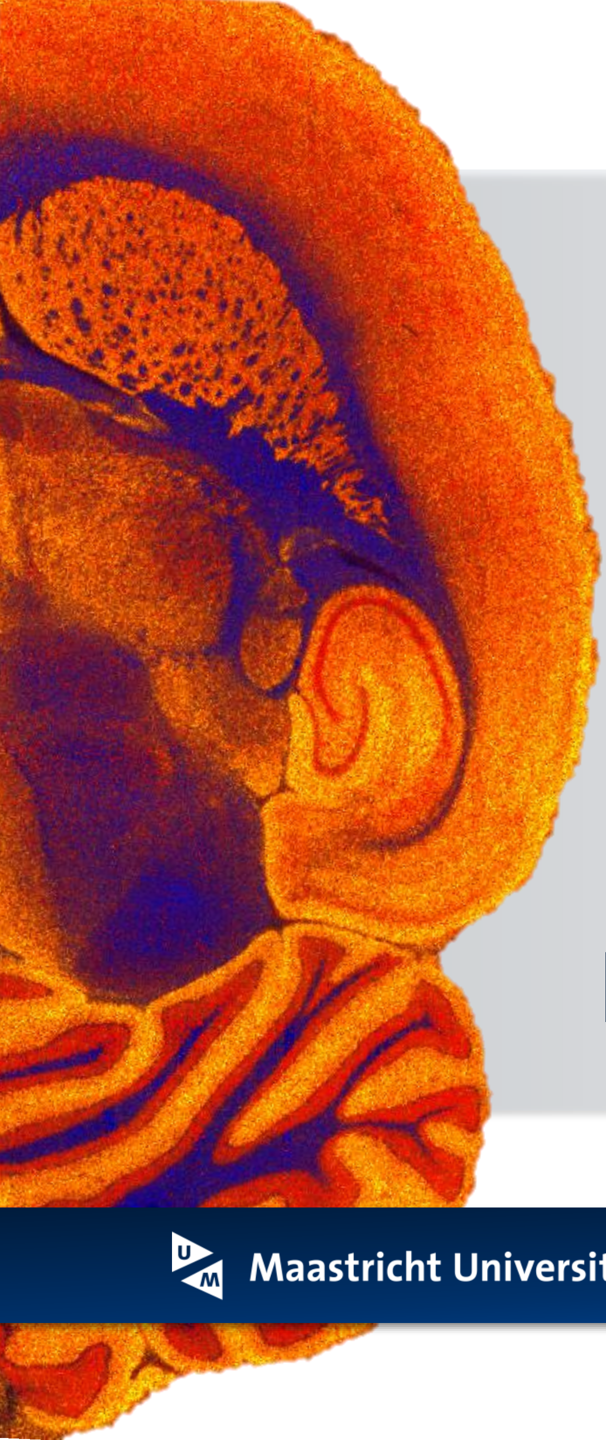
Alexander Clausen

IDEE Instrument Development Engineering & Evaluation

Laurent Schijns

NL Health~Holland SHARED CHALLENGES, SMART SOLUTIONS
Q-SORT
provincie limburg
NWO Netherlands Organisation for Scientific Research



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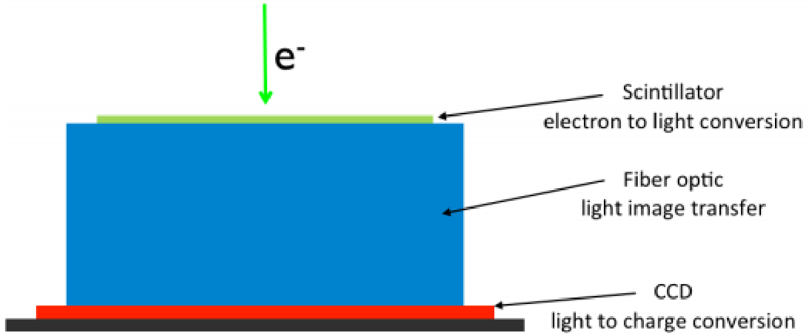
MAASTRICHT FOR IMAGING

Maastricht University | @Maastricht4Imaging | yue.zhang@maastrichtuniversity.nl

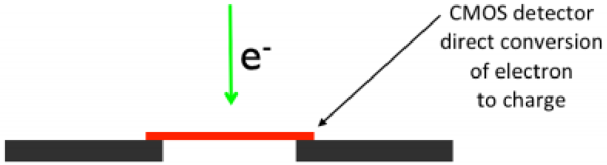
Type of electron detectors

Charge Coupled Device (CCD)



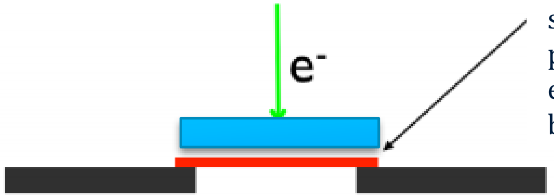
Scintillator adds noise
Scintillator deteriorate PSF
Limited dynamic range
Readout noise

Monolithic Active Pixel Sensor (MAPS)



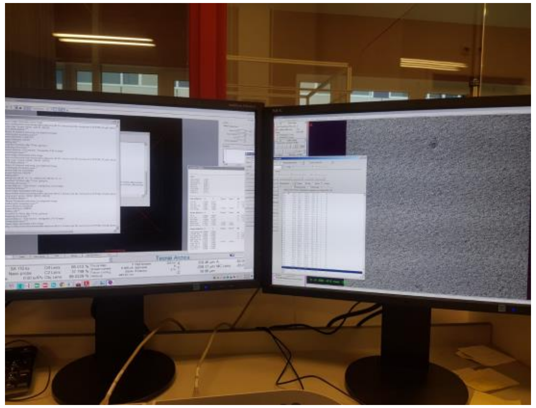
Frame based
Low throughput ~ 40 s
0.01–0.025 electrons/pixel/frame
Works best at 300 kV
Radiation sensitive
Limited dynamic range

Timepix3 Hybrid Pixel Detector

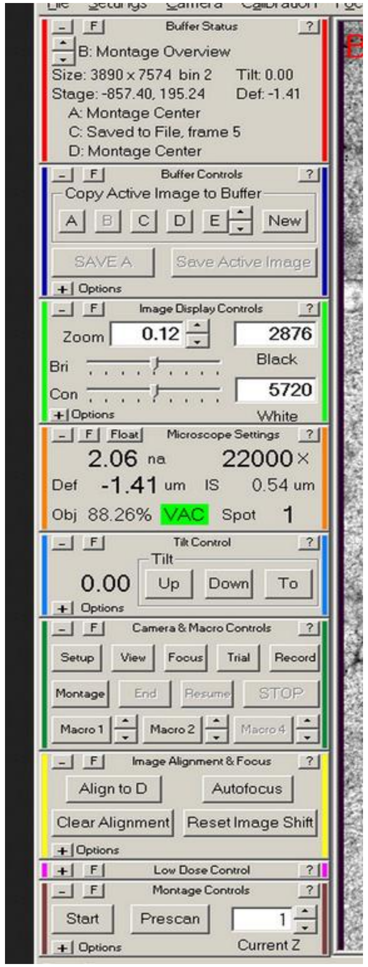


True counting detector
Throughput: < 1 second
Can work at all energies

Triggering the beam blanker 1



Microscope PC



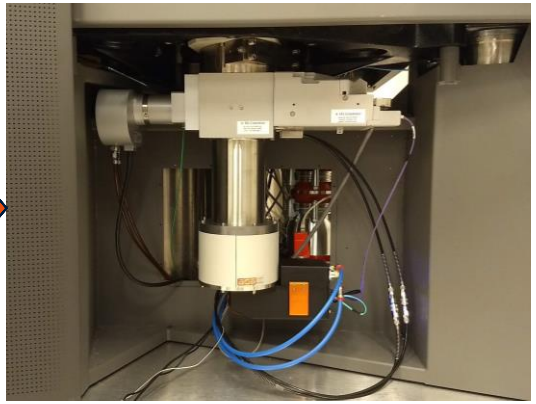
SerialEM

Serval

Triggering the beam blanker 2

Timepix3 PC

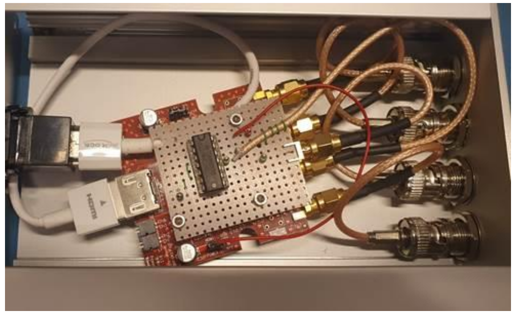
10 gbit fiber cable



Timepix3 + SPIDR

Open Shutter (HDMI cable)

Triggering the beam blanker 3



Signal inversion

ASI TriggerBox

BNC

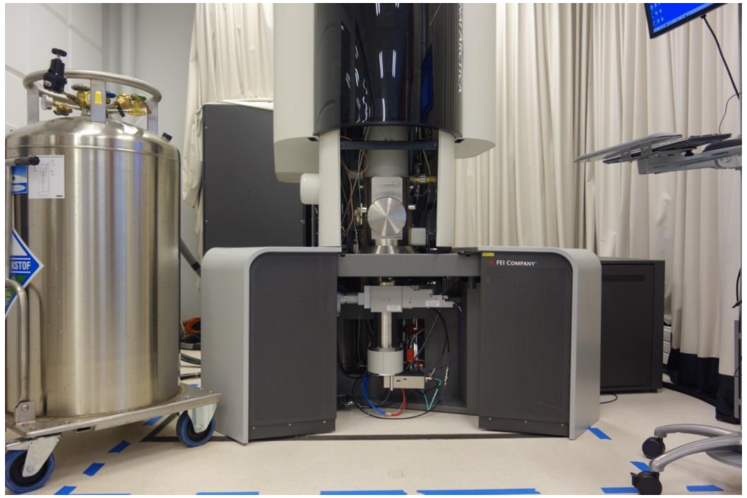
Microscope Blanker control

BNC

Laurent Schijns (IDEE)

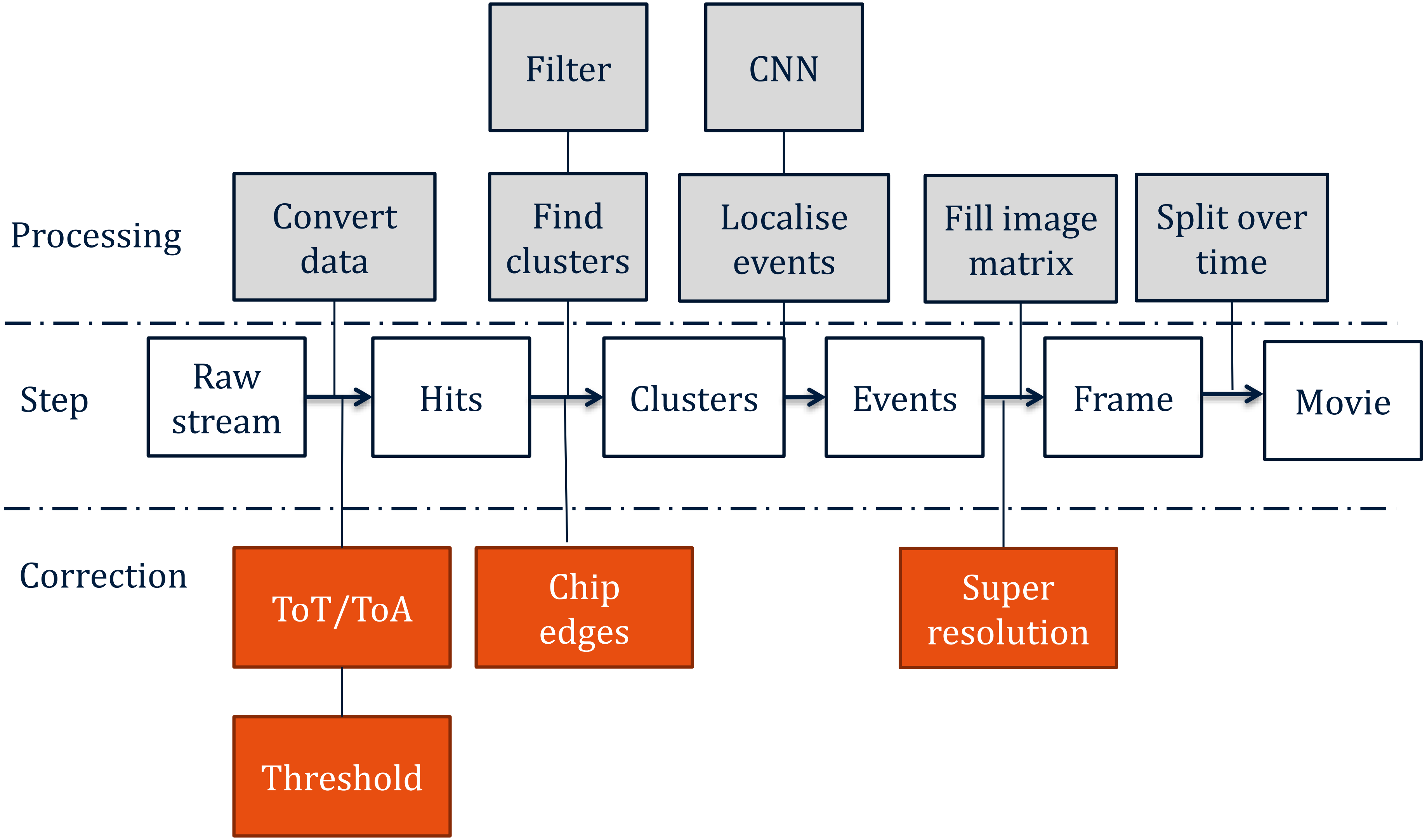
Triggering the beam blanker 4

Microscope electronics box

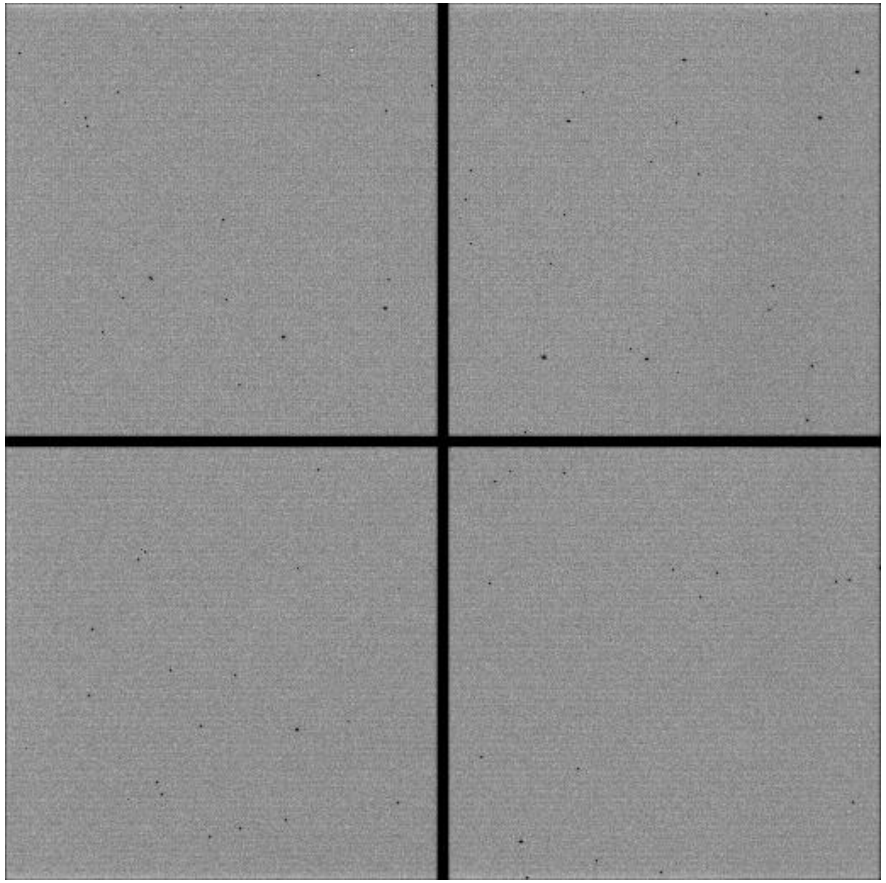


Beam deflector in microscope

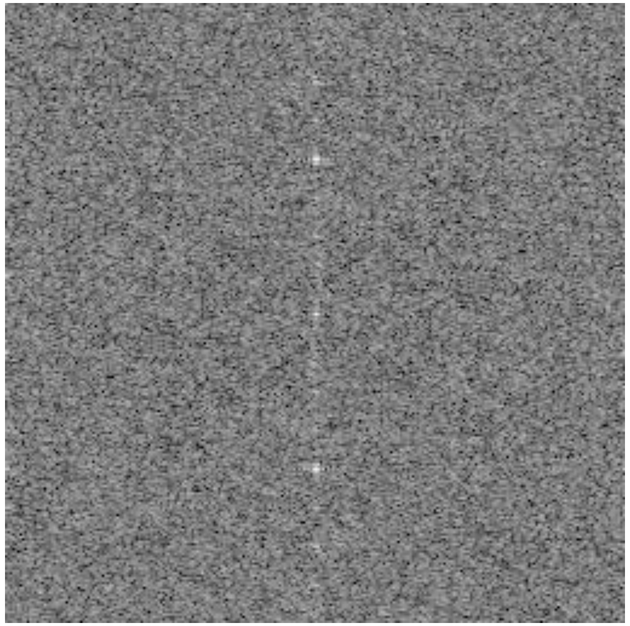
Data Processing



Systematic row pattern seen after CNN-ToT-ToA event localisation

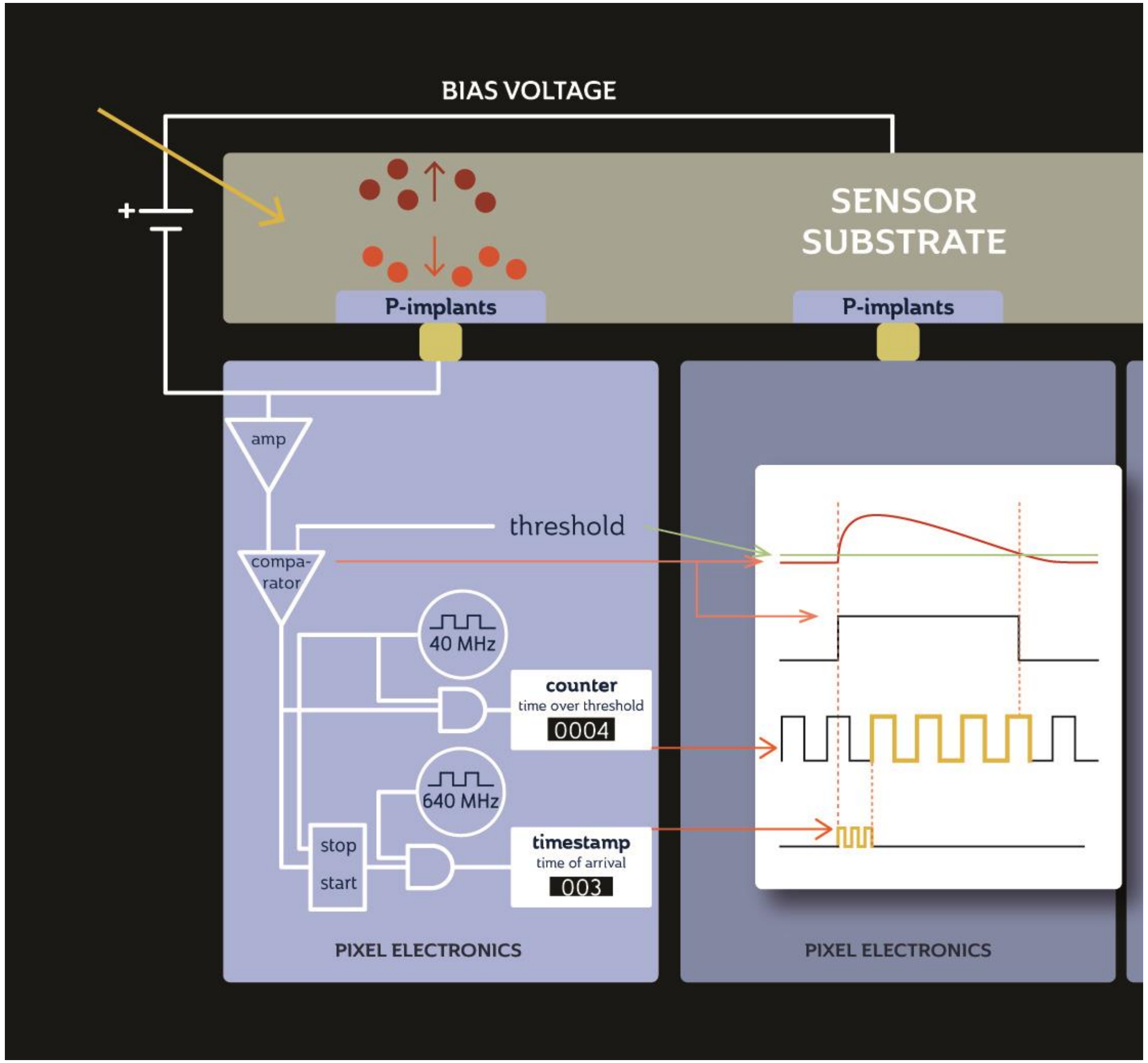


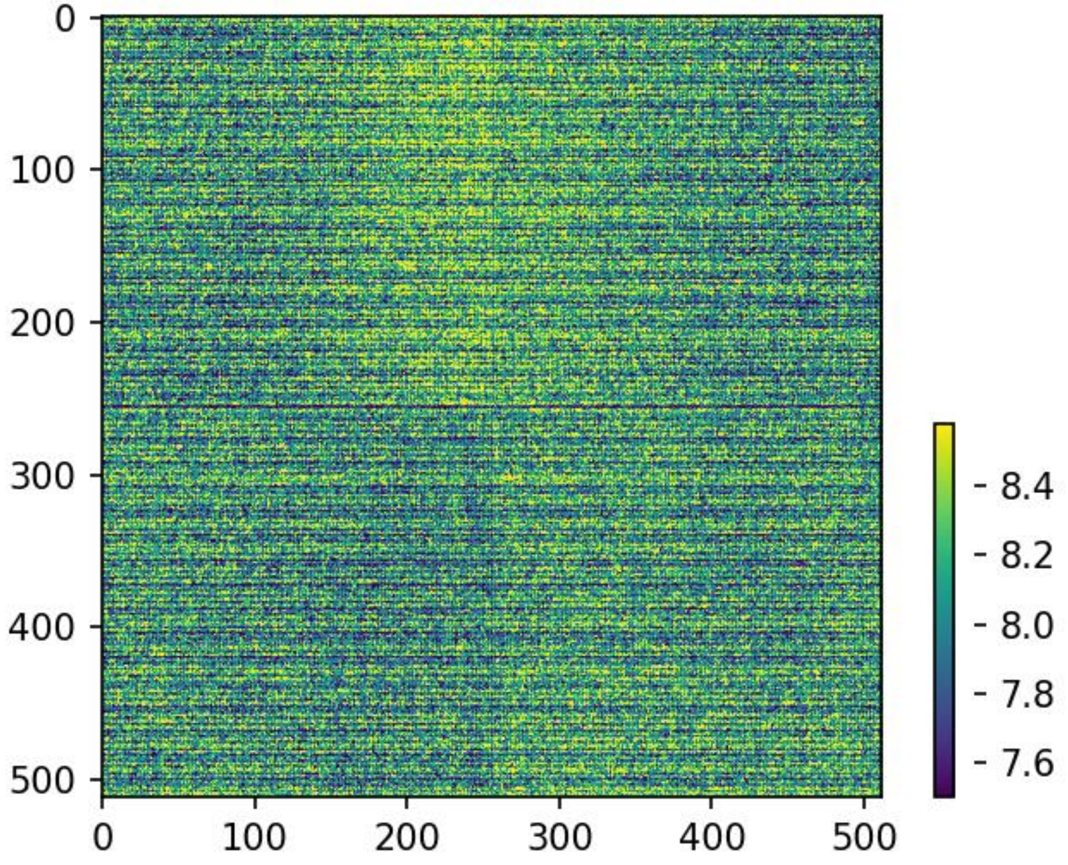
200 kV flat beam, with CNN-ToT-ToA event localisation



Power spectrum (FFT)

Systematic row pattern seen after CNN-ToT-ToA event localisation

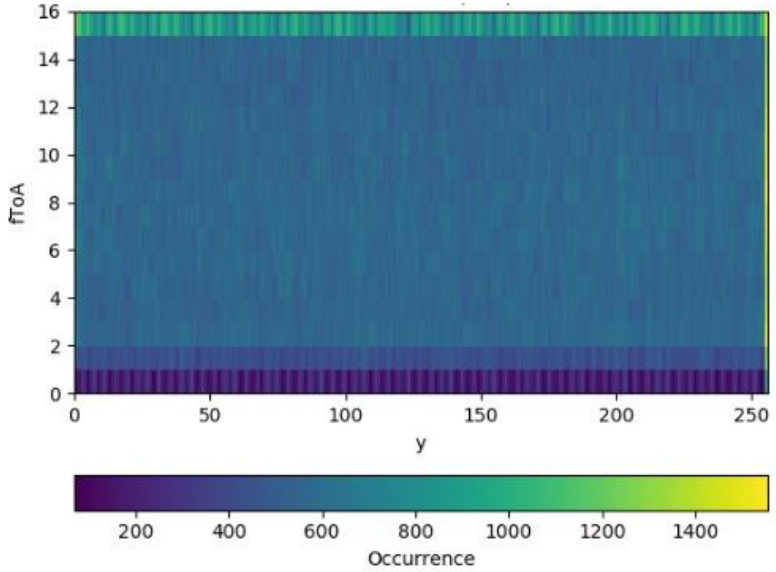




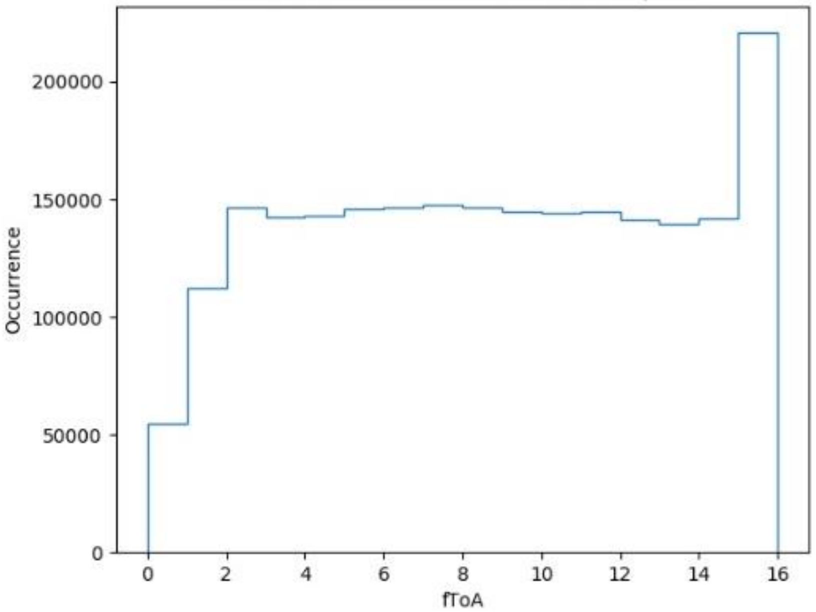
Average fine-ToA value

Pattern seen in fine-ToA timer of Timepix3

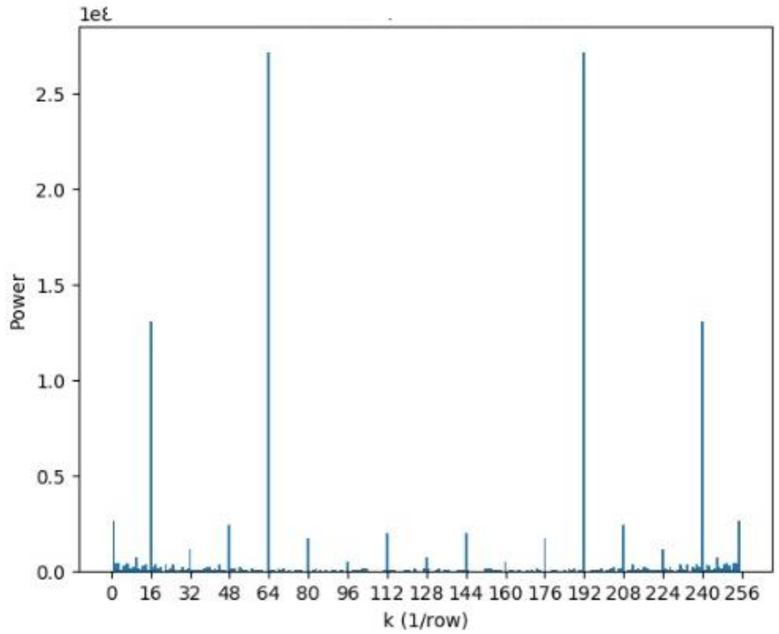
fToA distribution for each row



Overall fToA distribution

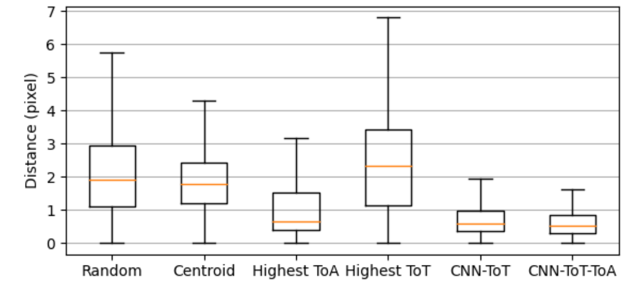


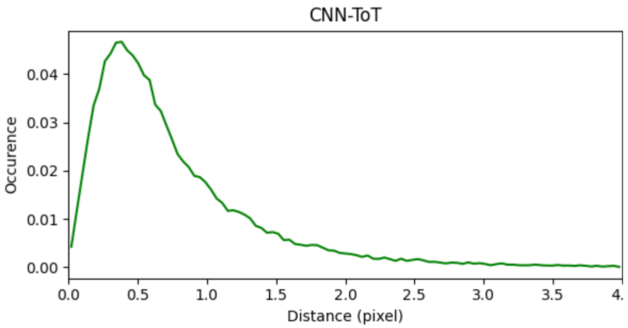
FFT of bin15 across the rows

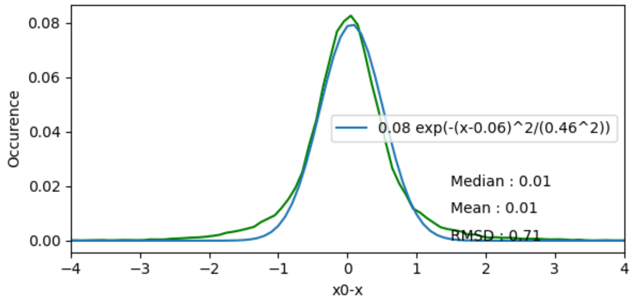


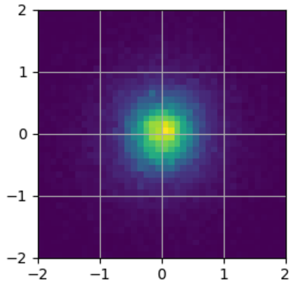
Due to phasing setting of the fine-ToA signal?

Gaussian event place backs



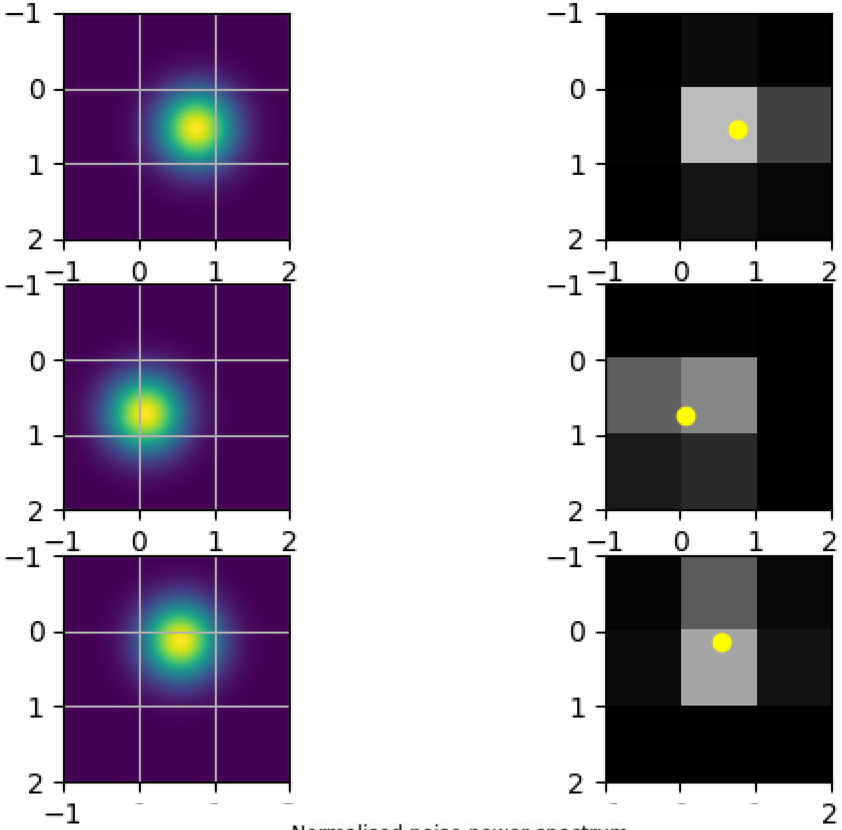


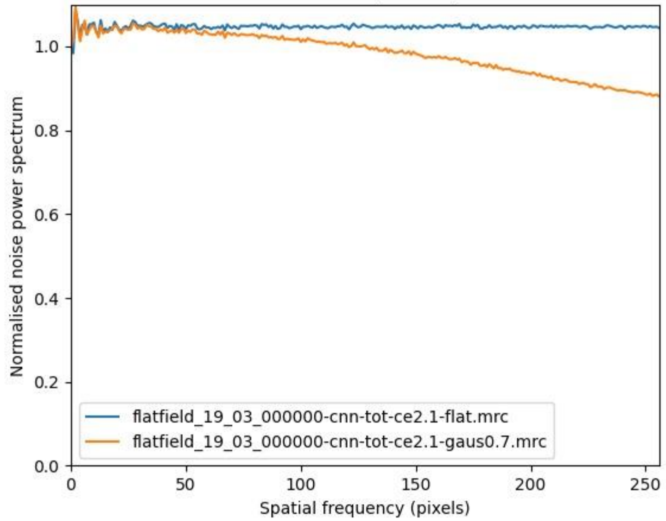




Simulated PSF

Binned simulated Gaussians





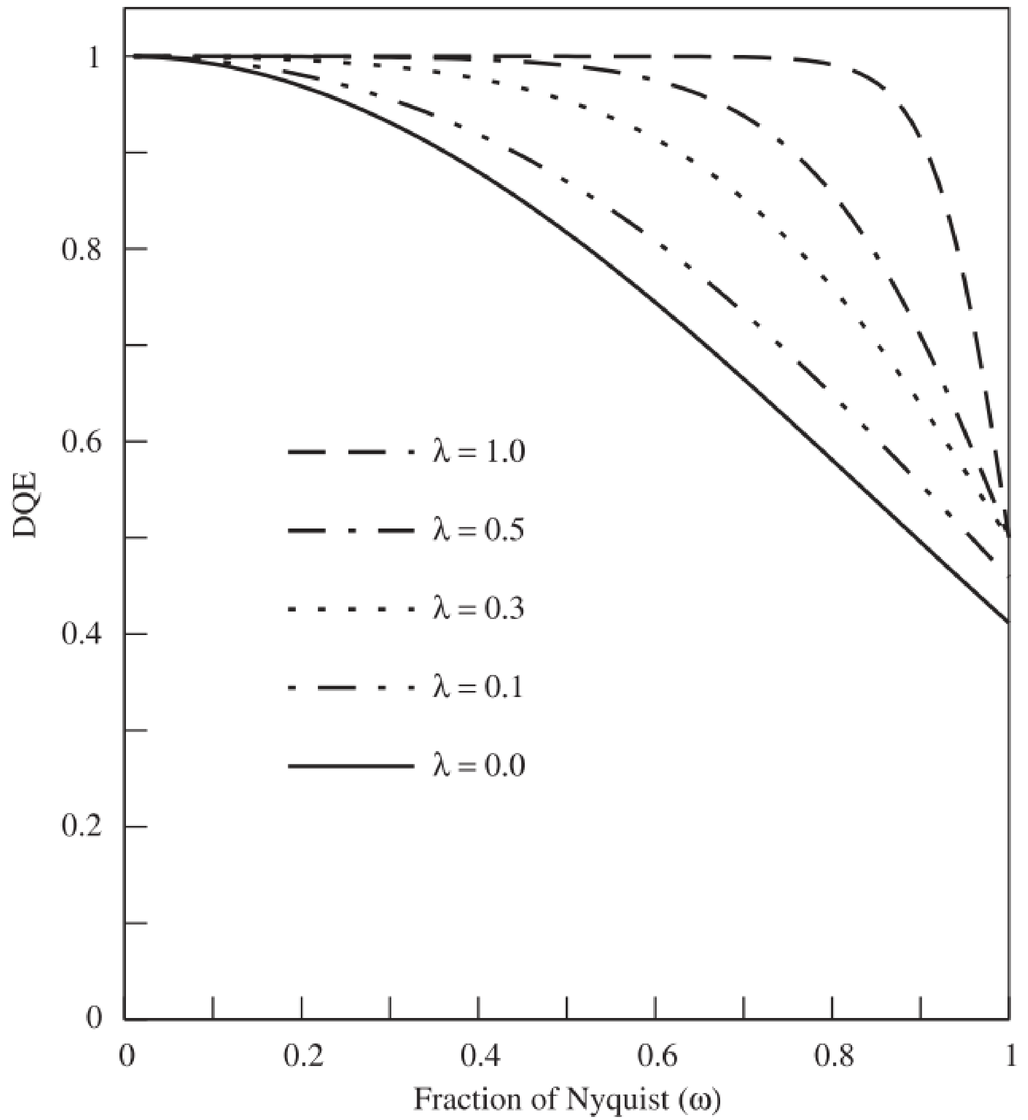
Deterministic blur

dimensional generalisation of Eq. (2). For an incident beam obeying Poisson statistics, with on average n electrons per pixel and giving an average output signal of d_n (so that that the average signal per primary electron is d_n/n)

$$\mathrm{DQE}(\omega) = \frac{d_n^2 \mathrm{MTF}^2(\omega)}{n\mathrm{NPS}_n(\omega)} \tag{3}$$

gain linear filter. In the absence of aliasing, deterministic blur does

not affect the DQE [12] as the signal and noise terms are damped equally. The damping will however be greater for the aliased noise terms at higher frequency and so the relative reduction in their contribution leads to an increased DQE at higher spatial frequencies. This is illustrated by the calculation shown in Fig. 1 where the expected DQE is plotted as a function of spatial frequency for various amounts of deterministic blur in an otherwise perfect pixel detector. As the blurring reduces the signal, having low readout noise is essential. Although it can produce a useful improvement in DQE at 0.75 Nyquist, the DQE at Nyquist is always limited to at most 50%. It is important to stress that this result only applies if the reduction in MTF is due to deterministic blur. The effect will also be less when the input signal has an intrinsic width and hence a natural damping of higher spatial frequency components such as when the input signal is better described by a Gaussian with non-zero width rather than a delta function.



CNN-ToT vs Hits and Timepix 3 VS FalconIII EC

	Super resolution CNN-ToT	Hits
Nr. particles	7741	10414
Resolution (Å)	3.05	3.85
B factor ($Å^2$)	-100	-201

	Timepix 3	FalconIII EC
Nr. particles	7741	124577
Resolution (Å)	3.05	2.54
B factor ($Å^2$)	-100	-93
Nr. Micrographs	2977	979