C100 Characterisation of a Novel Wafer-Scale CMOS Detector Optimised for 100keV CryoEM

Herman Larsen – STFC RAL

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Outline

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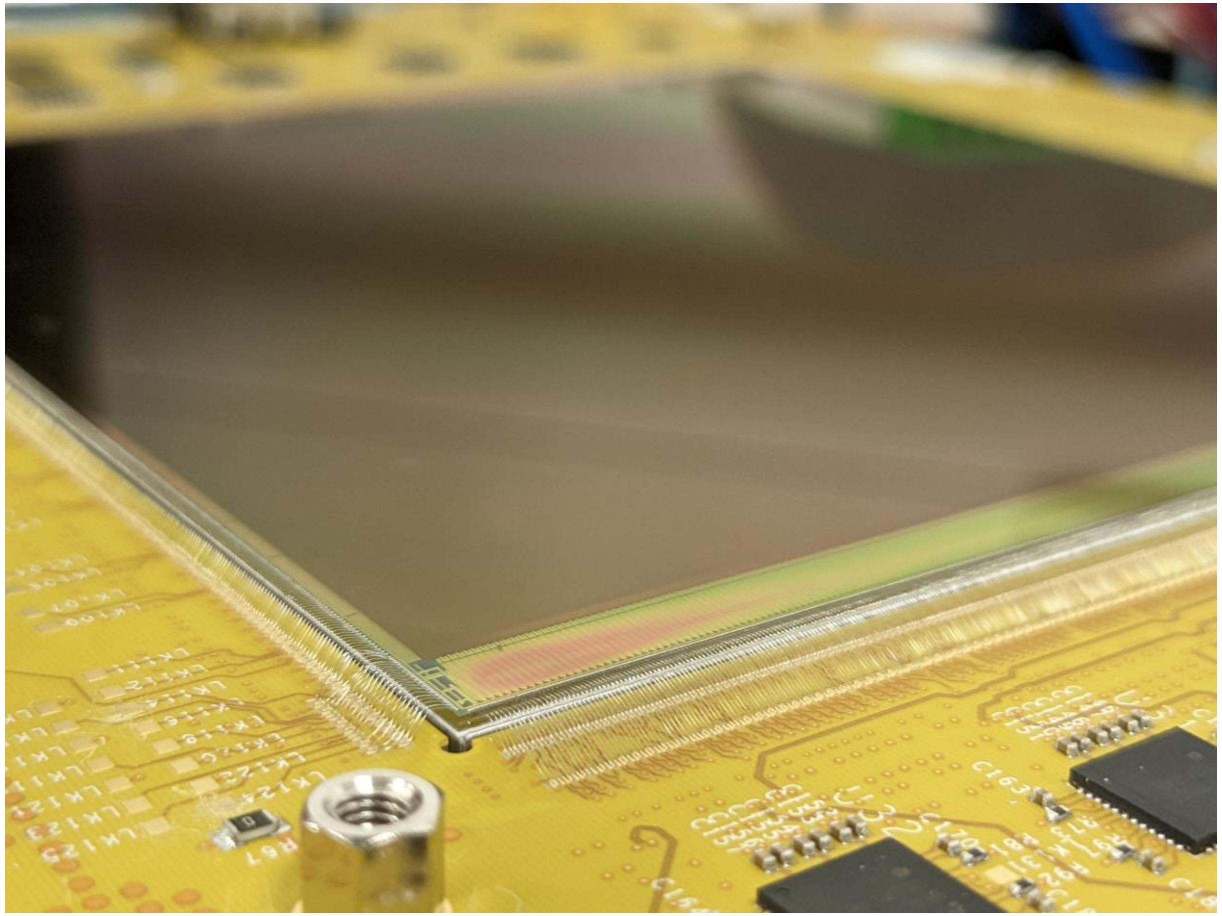
- Conclusions
- Acknowledgements



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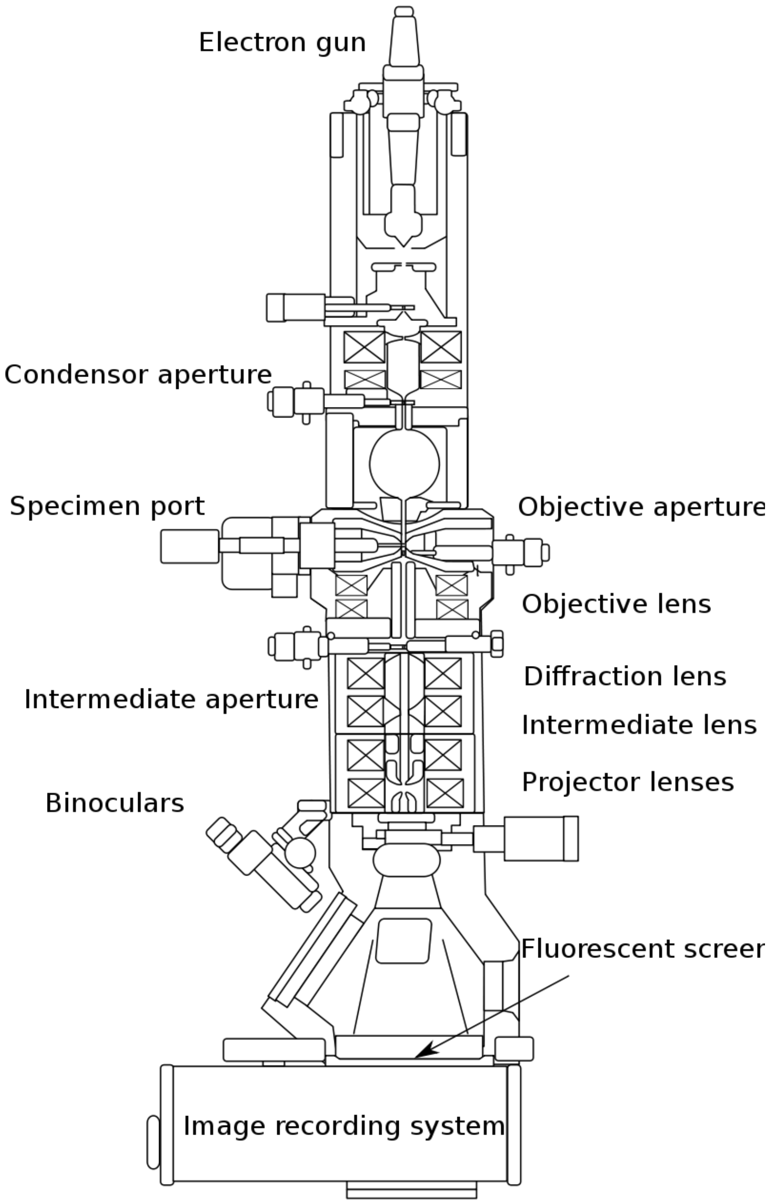
Introduction

Transmission Electron Microscopy (TEM) is a technique that allows to obtain high resolution images of thin samples.
The higher resolution is achieved due to the smaller De Broglie wavelength of the high energy electrons compared to visible light.

Cryo-Electron Microscopy (cryoEM) is a consequent technique used to image biological samples. The sample is prepared by flash-freezing to cryogenic temperatures, which preserves the sample structure and delays sample destruction during imaging.



https://en.wikipedia.org/wiki/Transmission_electron_microscopy

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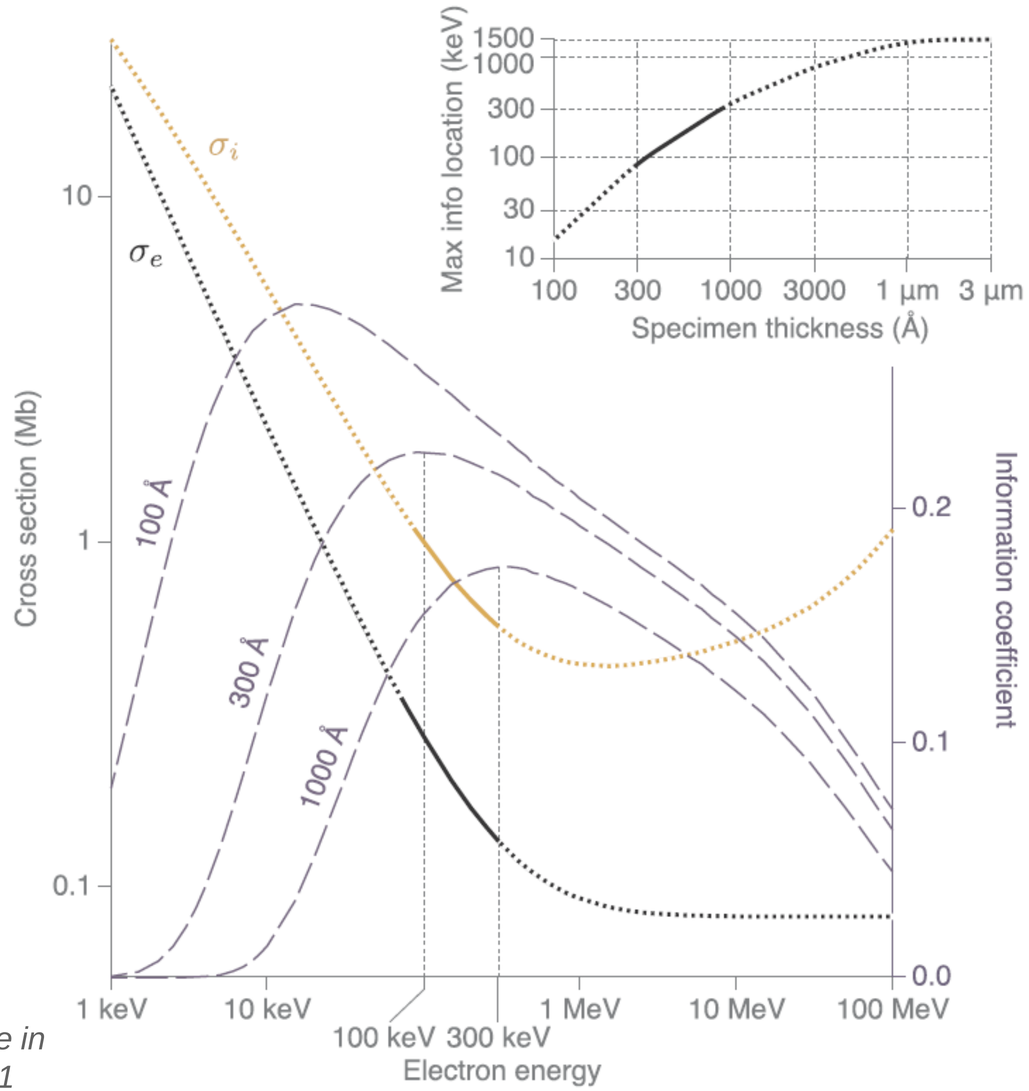
Why 100 keV?

State-of-the-art electron microscopes use an electron energy of 300 keV, but the theoretical expectation is that the ratio of elastic to inelastic cross-sections gets better as the electron energy is lowered from 300 keV to 100 keV.

Recently the elastic σ_e and inelastic σ_i cross-sections, as well as radiation damage to organic and biological specimens as a function of electron energy have been measured.

The results show that moving from 300 keV to 100 keV causes a 25% increase in the ratio σ_e/σ_i, indicating a 25% improvement in the image contrast for a given amount of radiation damage.

From M. Peet, R. Henderson,C. Russo, «The energy dependance of contrast and damage in electron cryomicroscopy of biological molecules» Ultramiscroscopy 203 (2019) 125-131



Scaling of cross-sections and information vs energy. The theoretical relationship between the elastic (σ_e) and inelastic (σ_i) scattering cross-sections for carbon are plotted vs. energy.

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Why 100 keV?

Based on the latest literature, most single-particle cryoEM investigations would benefit from changing the electron energy from 300 to 100 keV.

The present limitation to low dose imaging at 100 keV is the detector.

"Currently available direct detectors are either optimised for higher energies (300 keV and above) or lack the combined features required for cryoEM (DQE, number of pixels and frame rate)."

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

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ARTICLE INFO

Keywords:
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ABSTRACT

We have measured the dependence on electron energy of elastic and inelastic scattering cross-sections from carbon, over the energy range that includes 100 keV to 300 keV. We also compared quantitatively the radiation damage to bacteriorhodopsin and paraffin ($C_{44}H_{90}$) at 100 keV and 300 keV by observing the fading of the diffraction spots from two-dimensional crystals as a function of electron fluence. The elastic cross-section is 2.01 - fold greater at 100 keV than at 300 keV, whereas the radiation damage increased by only 1.57. This implies that the amount of useful information from diffraction patterns or images of most biological structures should be 25% greater using 100 keV rather than 300 keV electrons. Using these measurements, we calculate the energy dependence of the available information per unit damage for a specimen of a particular thickness, which we call the "information coefficient." This allows us to determine the optimal energy for imaging a biological specimen of a given thickness. We find that for most single particle cryoEM specimens, 100 keV provides not only the highest potential for information per unit damage, but would also simplify the instrument while retaining the potential to reach high resolution with a minimum of data. These measurements will help guide the development and use of electron cryomicroscopes for biology.

1. Introduction

Specimen damage is the fundamental limit to all forms of microscopy capable of atomic resolution imaging. Particularly for electron microscopy of biological specimens – comprised of RNA, DNA, proteins and other organic macromolecules in an aqueous environment – cumulative ionisation damage and bond breakage limits the available information that can be obtained by any imaging technique before the specimen is destroyed. Low temperature techniques reduce the damaging effects of ionising radiation and are effective in both electron microscopy and X-ray crystallography [1,2]. Although radiation damage is what limits the usable electron fluence at all specimen temperatures, recent improvements in both software and hardware technology for electron cryomicroscopy (cryoEM), including fast and efficient detectors, new algorithms for image processing, and methods to reduce the movement of the specimen during imaging have all contributed to increases in the signal-to-noise ratio in micrographs collected [3]. It is with this in mind that we revisit the fundamental question of the amount of information available per unit damage caused by irradiation with high energy electrons. We then discuss these measurements in the context of current and future technology for electron cryomicroscopy of biological specimens.

Since the energy of the incident electron is precisely controllable over the range of interest to transmission electron microscopy, 1 keV to 10 MeV, it is of interest to understand how the choice of accelerating voltage affects image quality in cryoEM. While the optics of the microscope may limit the choice of energy for a variety of reasons, the last two decades of development in electron microscope hardware have meant that in principle and in practice, a microscope capable of sub-two Ångstrom resolution can be constructed at energies ranging from 20 keV to 3 MeV [4,5]. The natural question is then: *what is the best energy for cryoEM?* Put in another way, if the non-trivial issues of hardware are set aside and consideration is given only to beam–specimen interaction, what electron energy might provide the best images? To answer these questions, we have determined how the amount of damage to the specimen changes with the energy of the incident electron beam, and in turn how this compares to the scattering contrast available for high resolution imaging.

Previous calculations of the relationship between the elastic and inelastic scattering estimated that the two quantities parallel each other to a reasonable approximation [6–10]. Technology has progressed and now the success of cryoEM methods obliges us to reconsider this relationship with more precision. More complete calculations of the inelastic interactions of electrons with atoms in the specimen indicate

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DEMO1 Test Structure

- Pixel Test Structure
  - 72x128 54 µm pixels
  - Built with previous test camera and test chip circuitry.

Gain	Relative Gain	Gain $(\mu V/e^{-})$	σ (e^{-})	σ (μV)
0	-	-	-	-
1	1	16.72	89.9	1594
2	3.07	50.17	55.5	932
3	5.54	91.09	54.1	914

	Horizontal		Vertical	
Spatial Frequency	0	Nyquist	0	Nyquist
Integrating DQE	0.87	0.40	0.87	0.40
Counting DQE	0.97	0.5	0.97	0.5
MTF		0.66		0.65

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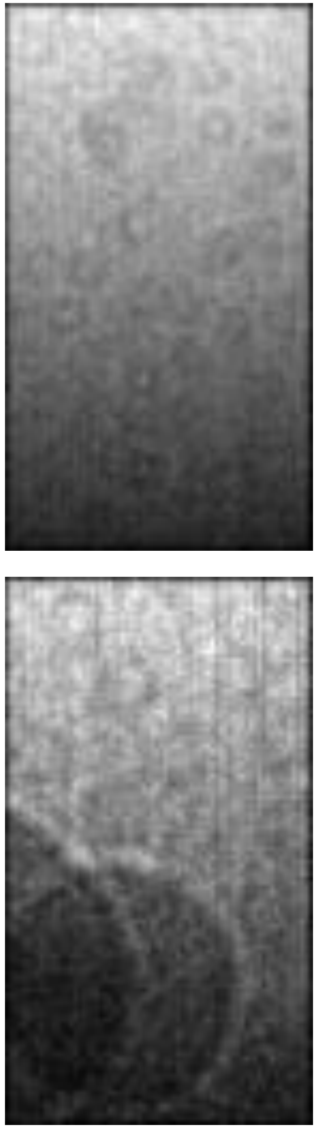
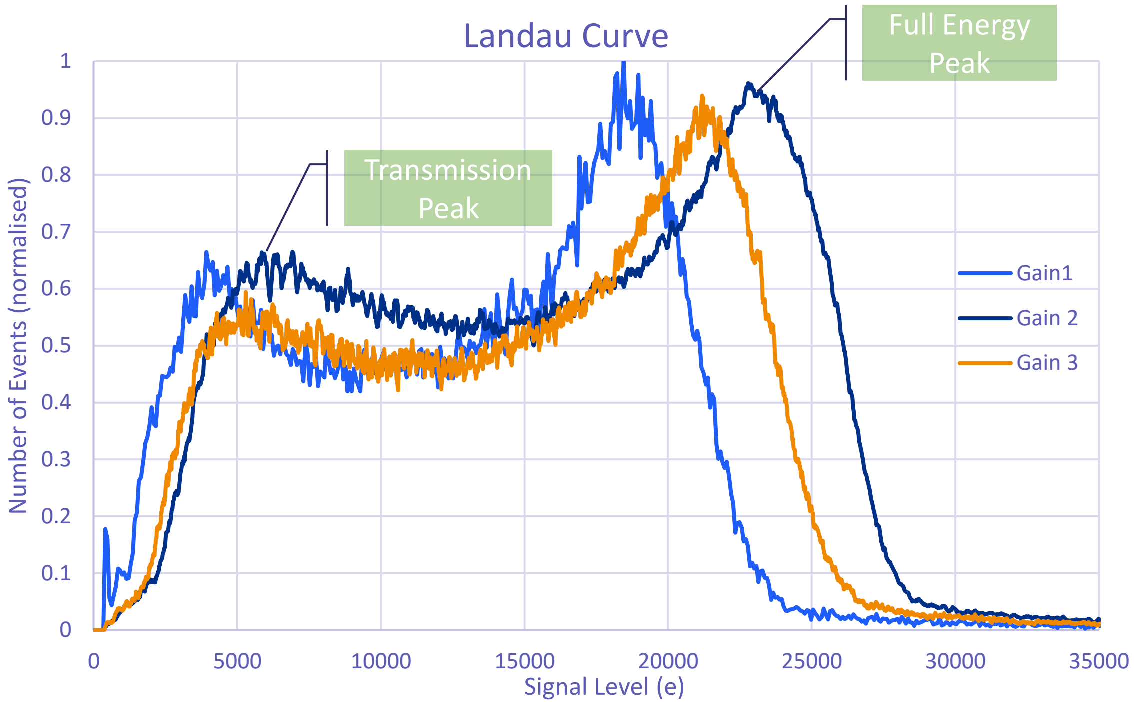
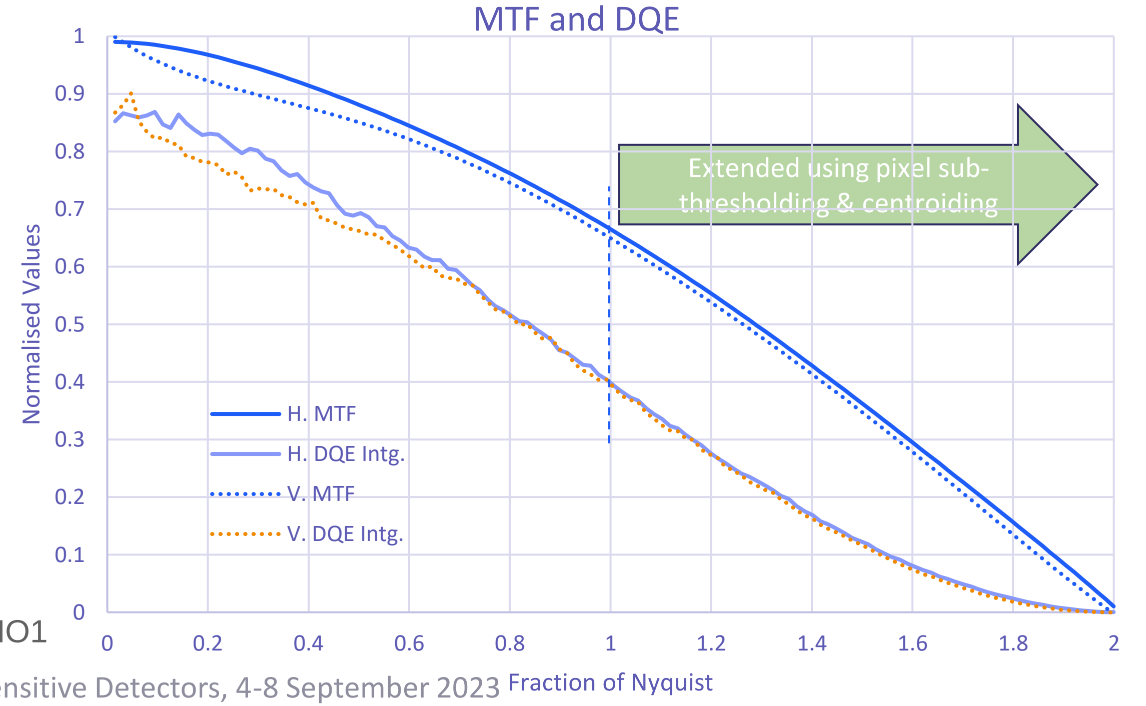


Apoferritin images from DEMO1





C100 Overview

Based on DEMO1 after very promising results.

A collaboration between

- STFC Technology Department
- RFI (Rosalind Franklin Institute)

"Our aim is to increase, by an order of magnitude, the number of biological specimens that can prepared for analysis by structural biology."

- Professor James Naismith,
Director of RFI

The Rosalind Franklin Institute

The C100 camera system will be commercialised by UK company Quantum Detectors.

Quantum DETECTORS

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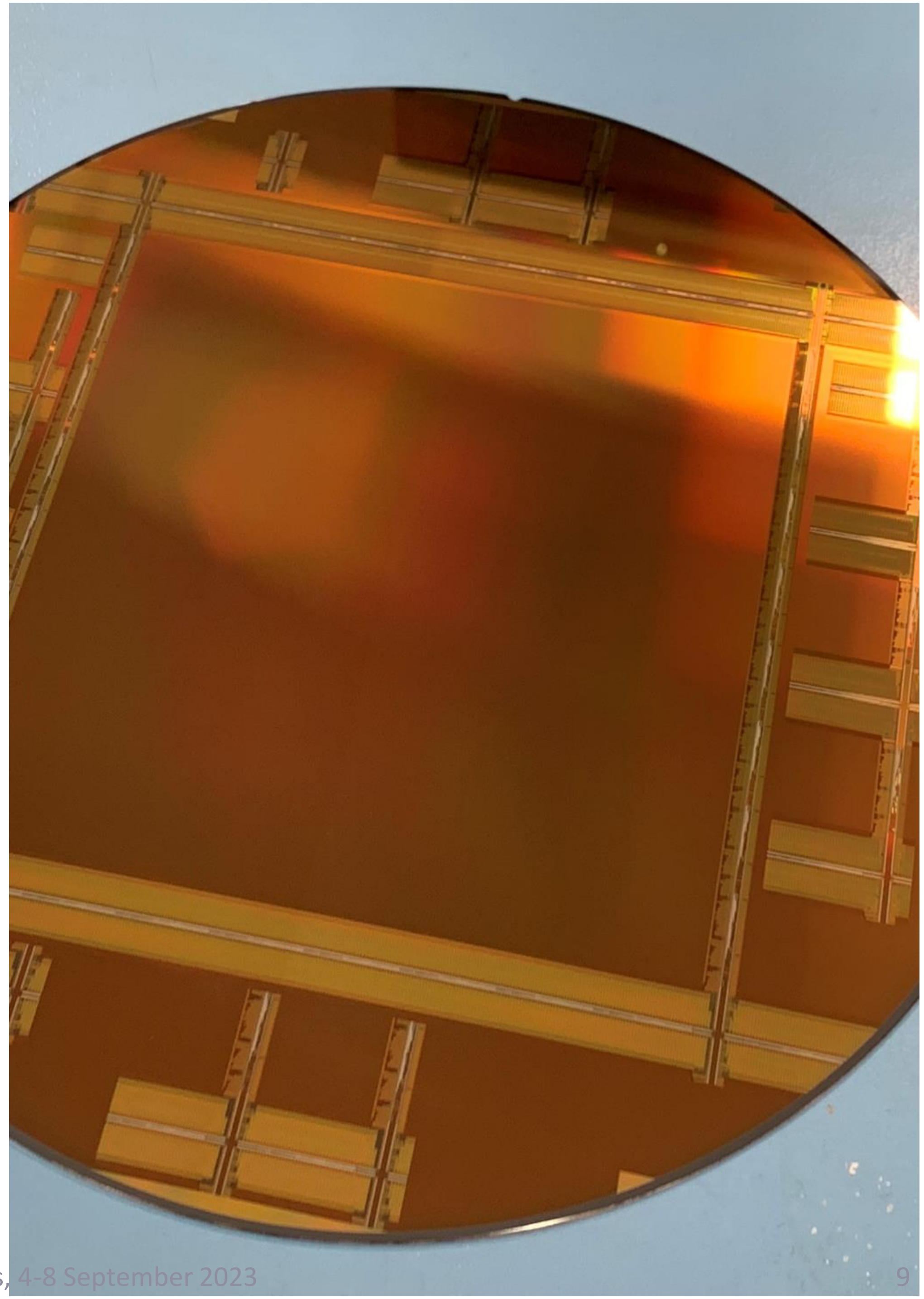

C100 Specifications

- Sensor based on DEMO1:
  - Pixel size minimises effects of scattering whilst ensuring high-resolution imaging.
  - Resolution and frame rate targets the requirements for future cryoEM detectors.
- Stitched CMOS technology allows the manufacturing of large sensors with a non-interrupted sensitive area
- Standard CMOS process enables cost-effective sensors which are constantly evolving.
- High yield design employed to reduce probability and criticality of defects on wafer scale sensor.

Specification	Target	
Sensor format	2048 x 2048	
Pixel pitch	54 x 54 µm	
Frame rate	2000 fps	2500 fps
Bit depth	12 bits	10 bits
Operation mode	Rolling shutter	
Readout mode	Continuous	
Readout type	Analogue CML lines	
Sensor size	200 mm wafer-scale sensor	
Manufacturing process	TowerJazz 180 nm CIS process	
Sensitive area	122.3 cm^2	
Radiation hard	YES	
Back-thinning	NO	
Dark pixels	Only on left and right sides of the pixel array	

From D. Krukauskas, "C100 – CMOS Sensor for 100 keV EM" Rosalind Franklin Institute annual meeting 2019

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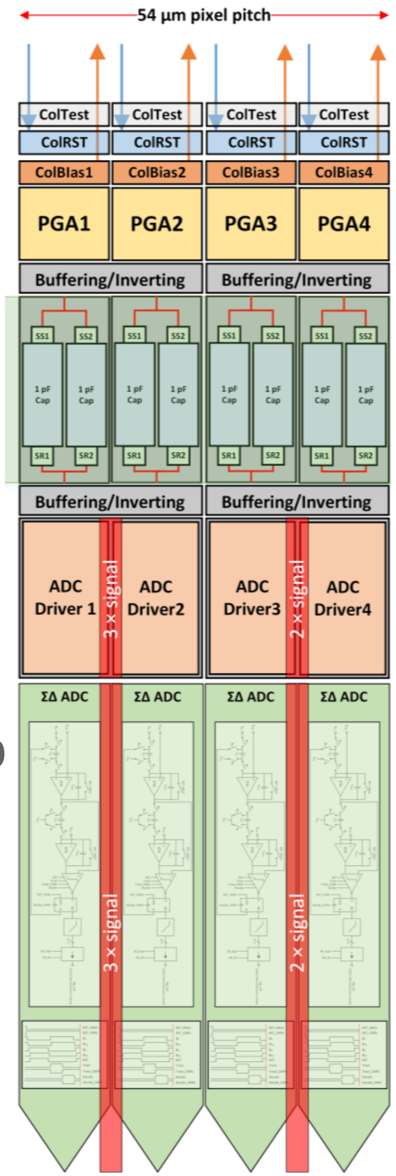
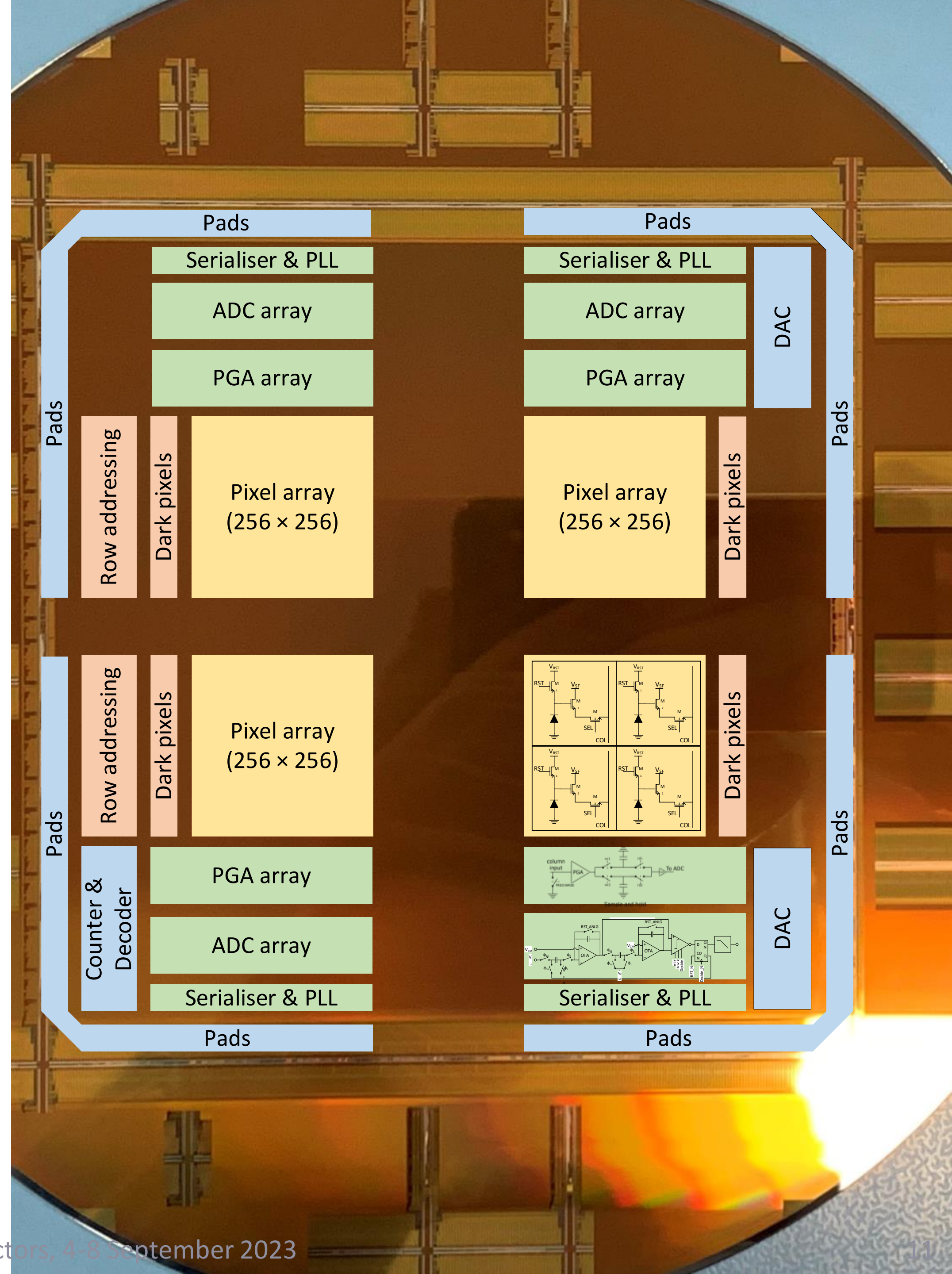
C100 Architecture

- Wafer-scale stitched sensor
  - Allows multiple size options.
- A simple 3T pixel was chosen for radiation hardness and improved yield.
  - Covers ~90% of chip.
- Readout from two sides as line rate is limited by the very long vertical lines.
- 4 ADCs per column pitch, 16k total.
  - ΣΔADC chosen due to robustness to process and mismatch variation.
- High Speed CML outputs compliant with Xilinx Aurora 64b/66b communication protocol.





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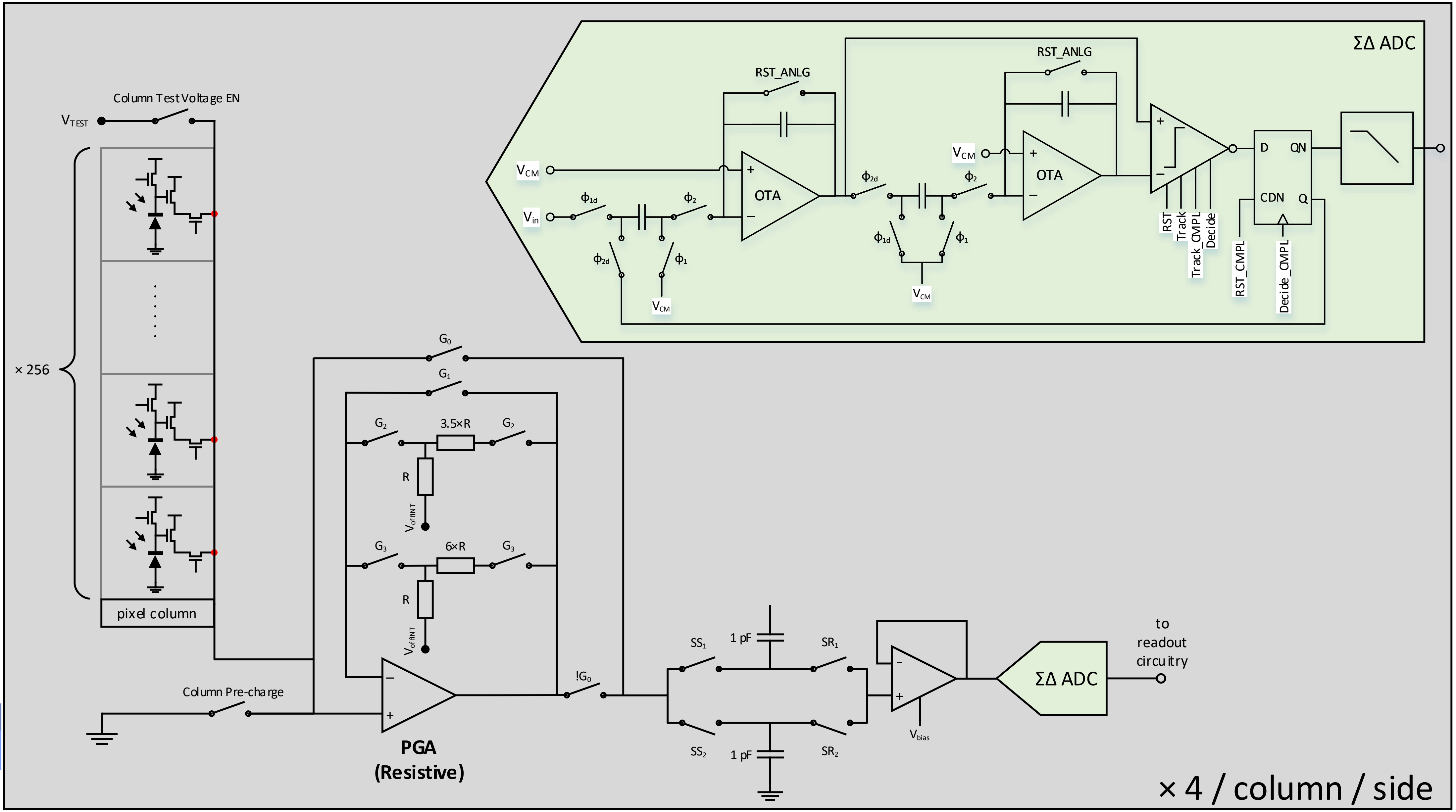
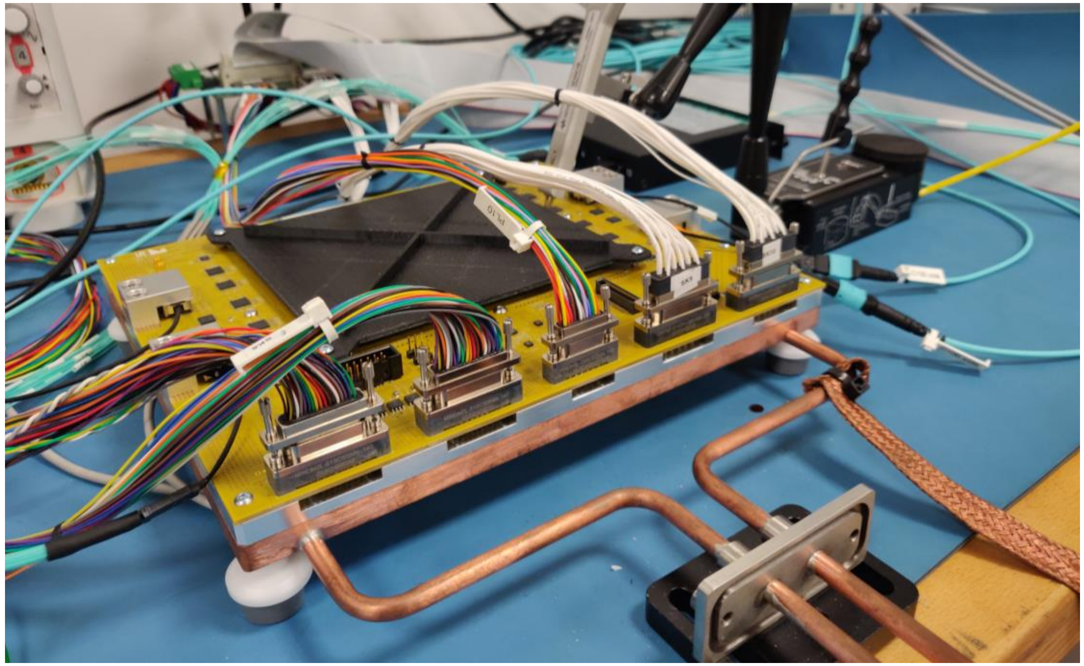
C100 Architecture

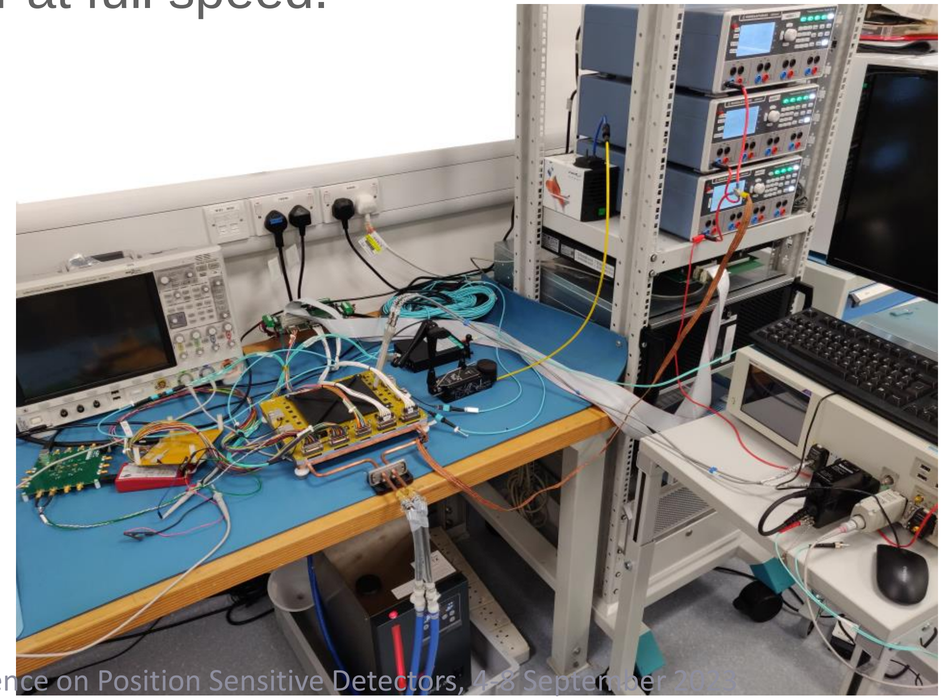


C100 Preliminary Testing

Overview

- Camera housing prototype tested with in microscope.
- All individual circuit blocks have been tested and shown working:
  - PGA, ADC, Serialiser working successfully.
  - Testing revealed an issue with supply coupling causing inability to operate sensor at full speed.
  - An amended version underway!
- Yield has been very promising for wafer scale device.
  - 6 sensors tested so far.
  - No sign of yield issues.





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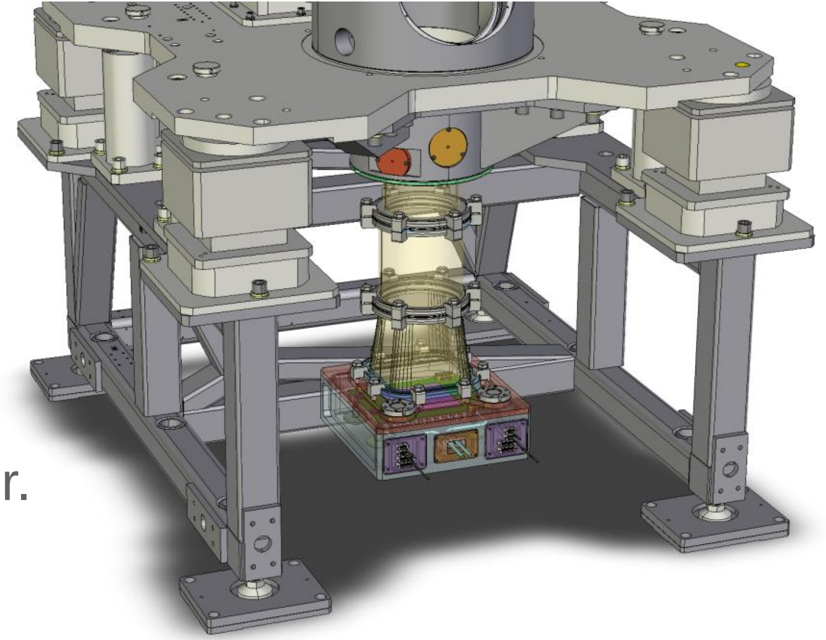
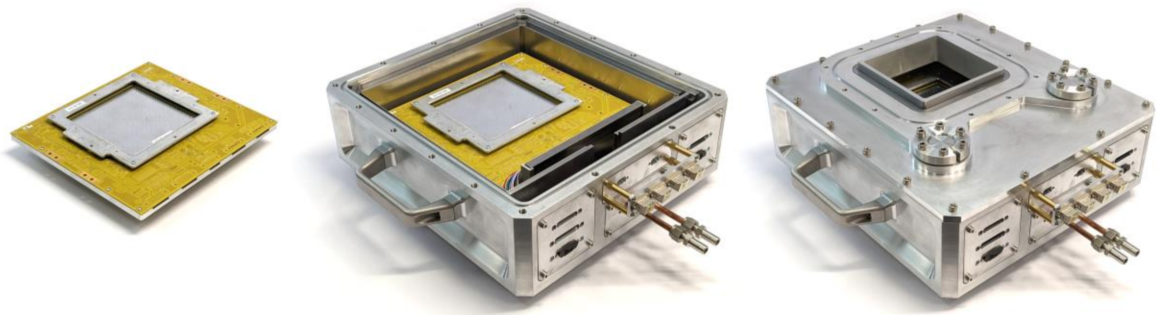
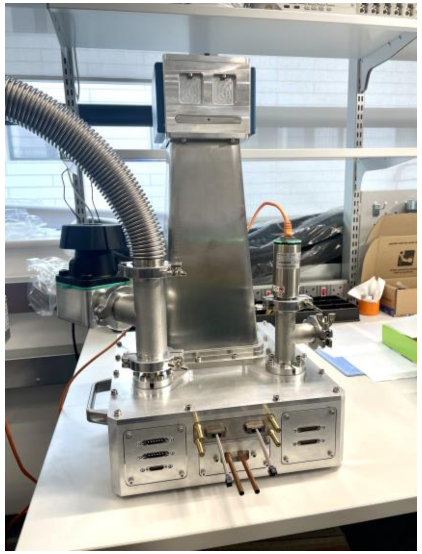
C100 Preliminary Testing

Camera Housing

- C100 must be cooled and operated in a vacuum.
- Co-design of sensor and housing at STFC
  - Vacuum housing design challenging due to the large sensor.
  - Sensor IO limited due to vacuum housing constraints.
- A sensor and housing prototype has been installed and tested (vacuum and electrons) in a JEOL microscope in RFI.







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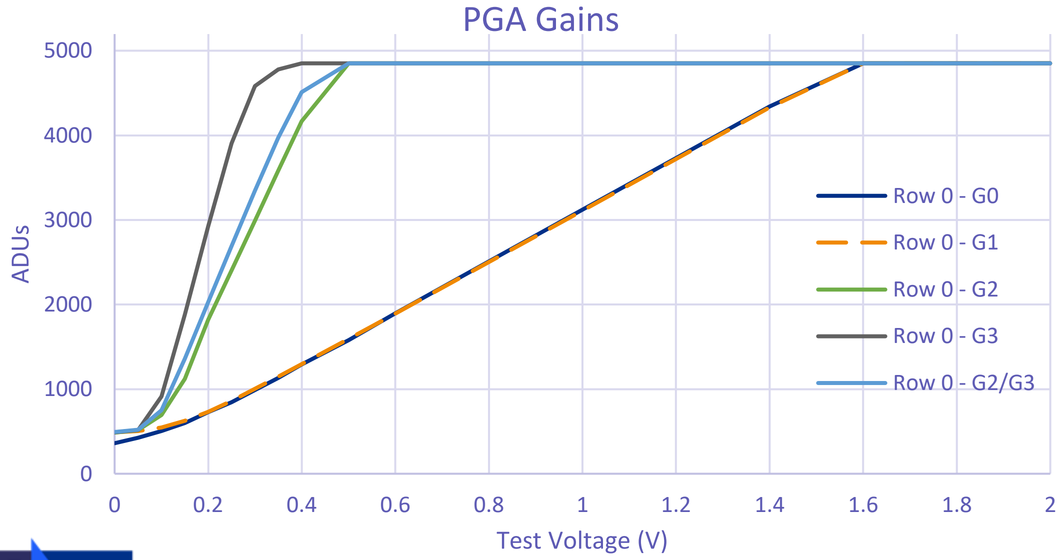
Quantum DETECTORS

C100 Preliminary Testing

Programmable Gain Amplifier

- Test Mode
  - Applying column test voltage on both sides and onto column line.
  - Terminated in opposite side's analogue readout.
- PGA operation with different gains verified.



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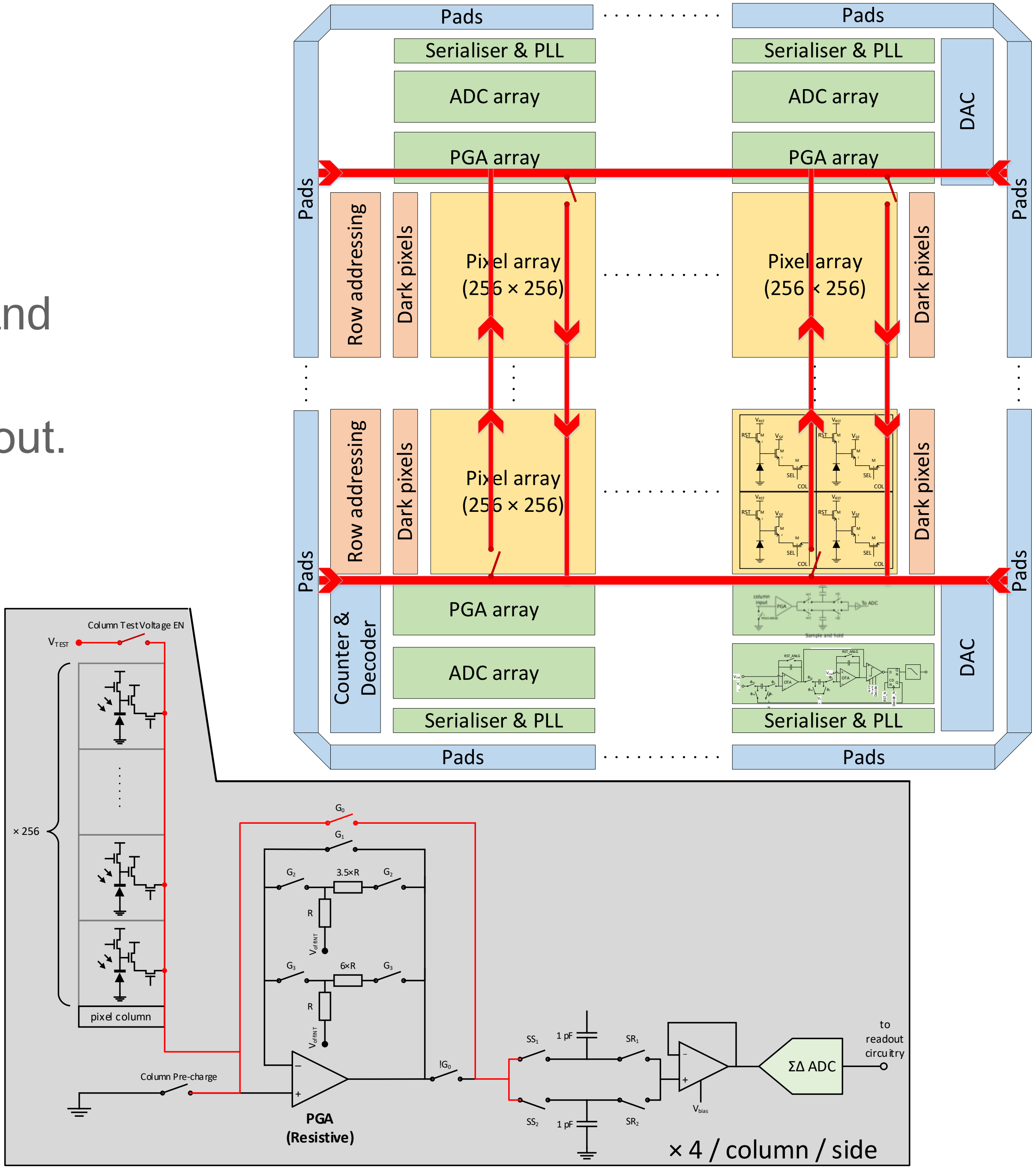


C100 Preliminary Testing

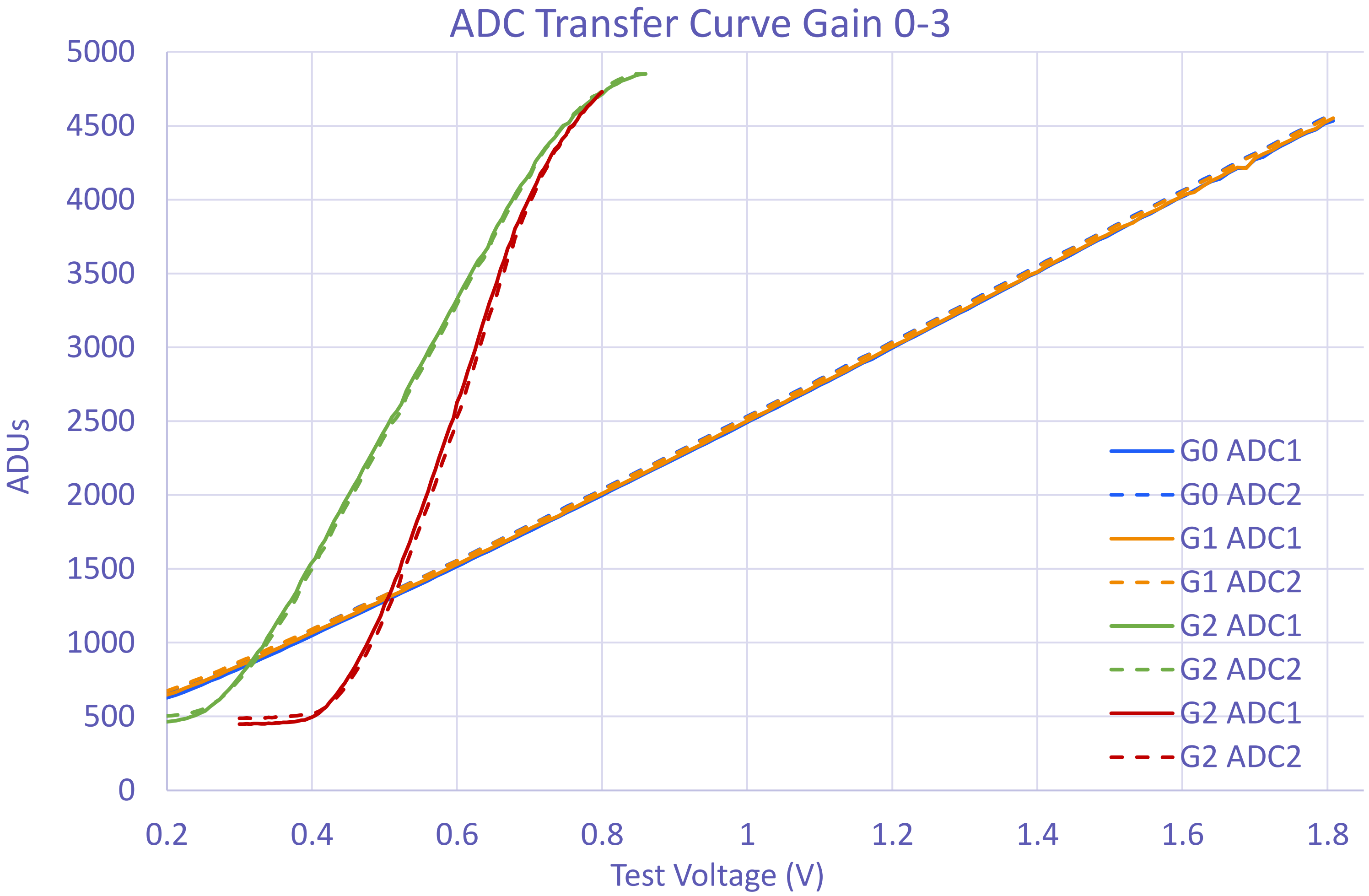
ADC

DEMO1
3.1 & 5.5

Gain	Relative Gain	Gain (ADU/V)
0	-	2469
1	0.997	2460
2	3.586	8853
3	5.800	14317

ΣΔADC σ
x2.7 lower
than DEMO1

Gain	*σ (ADU)*	*σ (μV)*
0	0.899	364
1	1.395	567
2	-	-
3	-	-



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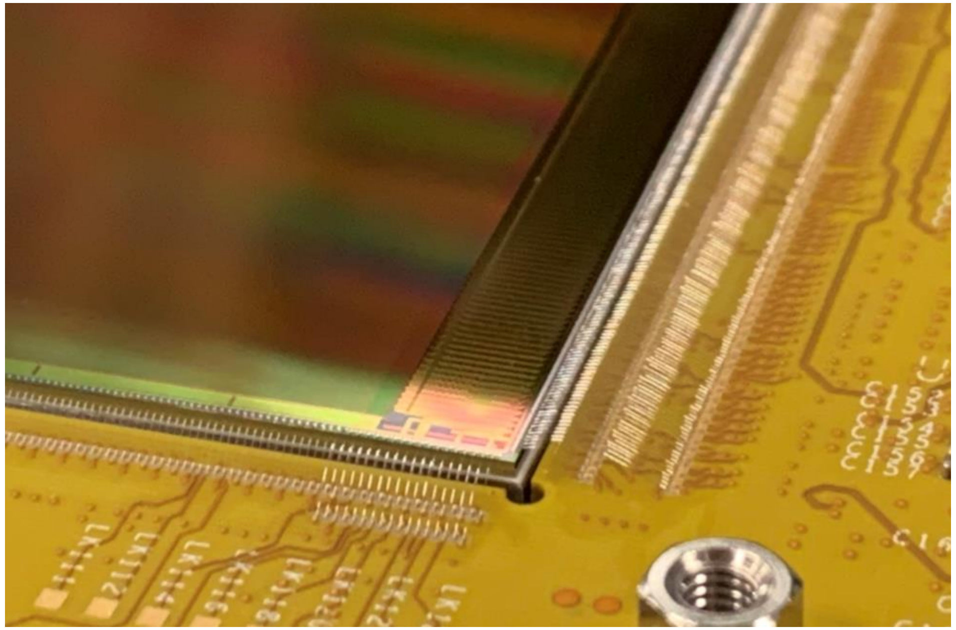
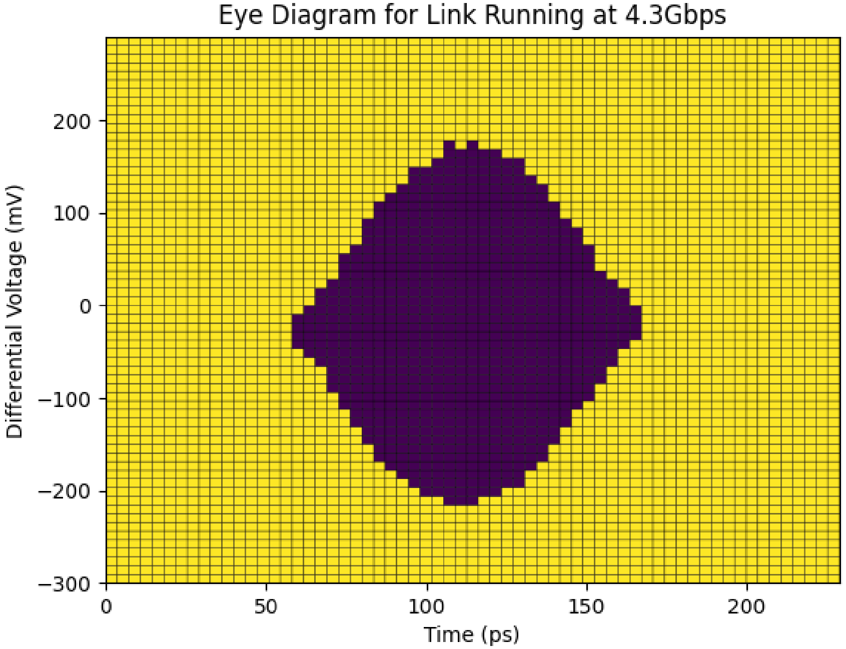
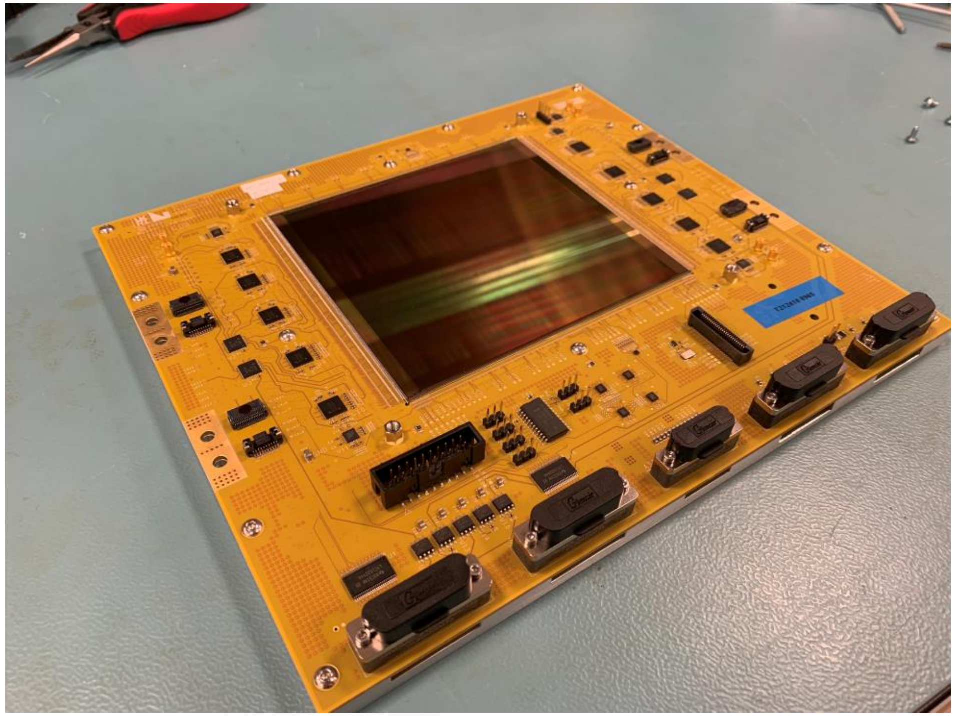
C100 Preliminary Testing

Serialiser

- 34 Aurora Serialisers @ 4.3 Gbps
  - Lane/channel locked for all transceivers
  - Total data rate over 110 Gbps
    - Bit Error Rate (BER) measured to be lower than 7×10^{-15}







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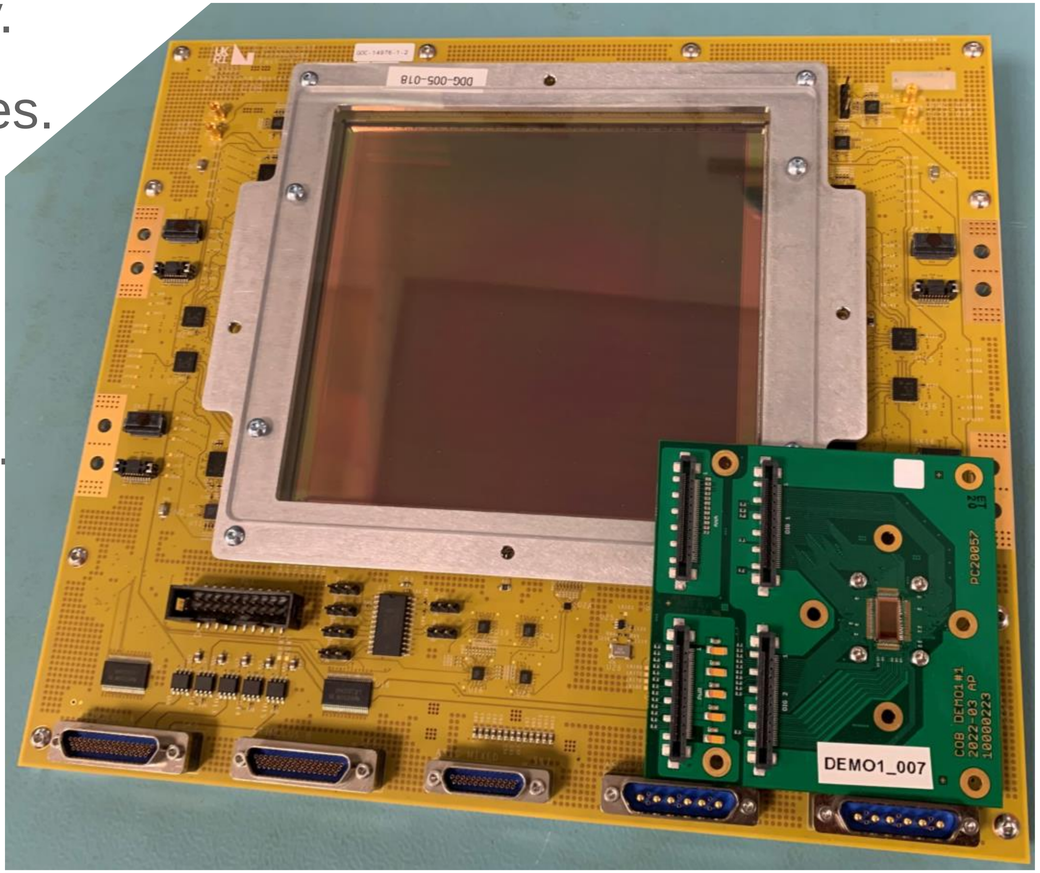
From "I. Sedgwick et al., PRECISE: A 5Gbps Serialiser for Scientific Detectors in a 180nm CMOS Image Sensor Process", proceedings of NSS-MIC 2022, Milano, Italy"

Conclusions

- Theoretical studies suggest 100keV microscopy offers many benefits
  - Increased image contrast for a given amount of radiation damage.
  - Wider employment and accessibility of the technology.
- DEMO1 demonstrated good performance at these energies.
- Based on DEMO1, the C100 full-size device was manufactured and is currently under test.
- All individual components work well:
  - Better noise performance than DEMO1 demonstrated.
  - Second iteration to be tested early 2024.



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Liam O'Ryan
Adriana Klyszejko
Danail Vassiliev
Giulio Crevatin
Ben Bradnick

Matt Callahan
Mohamed El Sharkawy
Josh Wilkes
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Graham Dennis

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Quantum Detectors

UKRI Medical Research Council

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Thank you

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