



Digital EMCCD Camera Technology - Overview

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Agenda

- ✓ What is a sensitive camera and why are they important?
- ✓ Competing Technologies.
- ✓ Impact Ionization and EMCCD Gain.
- ✓ Applications in Ultra Low-light Microscopy.
- ✓ Future?

Acknowledgements

- Dr M. Hollywood - QUB.
- Prof S. Chu - Stanford.



When do we need sensitivity?

- **Low concentrations**
- **Short exposures**
- **High photon loss**
- **Lower excitation power**
- **Greater magnifications**
- **Raman and weak emitters**

What makes a detector sensitive?

Two key parameters:

➤ **Quantum Efficiency**



➤ **Noise**



- ✓ Camera must be designed to ensure these parameters are optimised.

Typical Quantum Efficiency

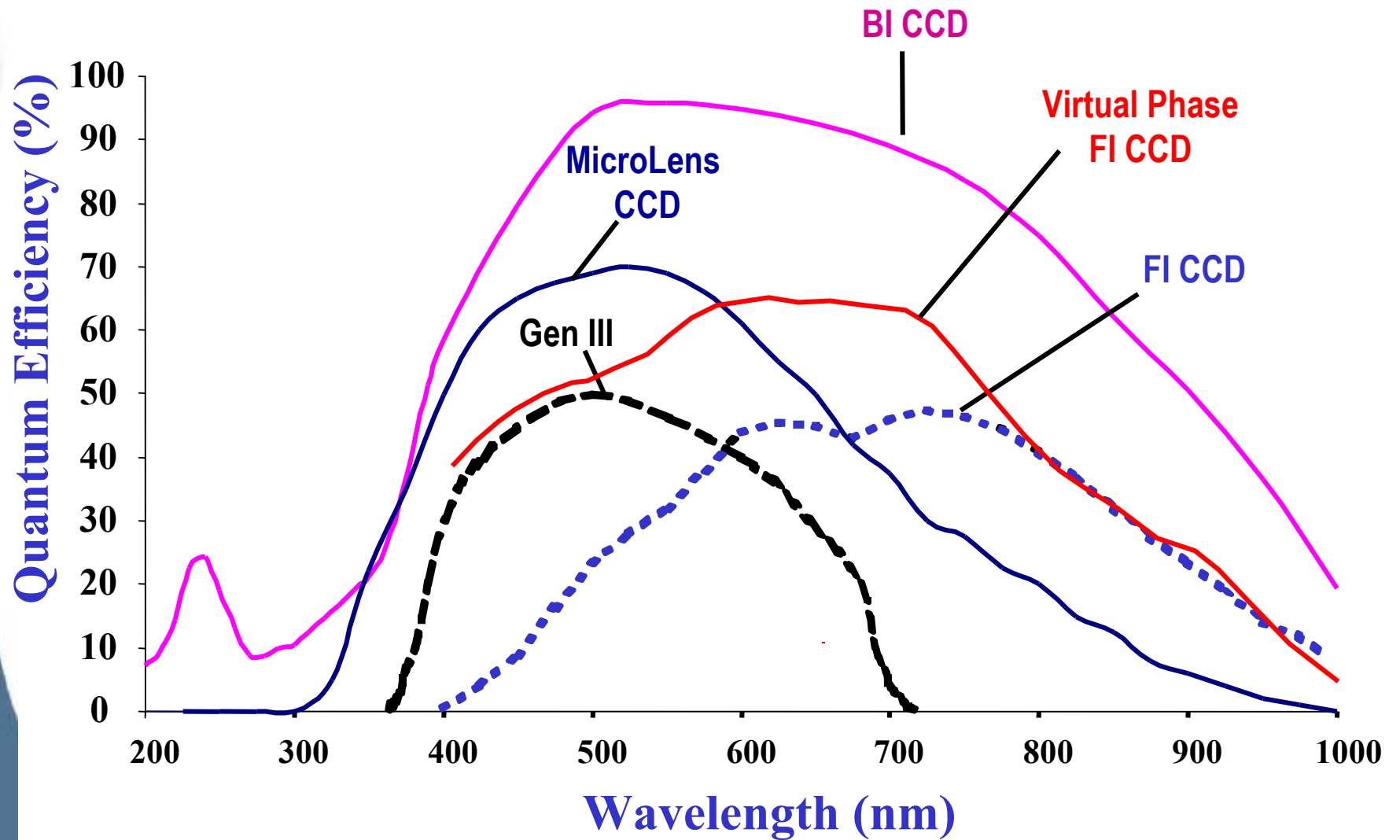
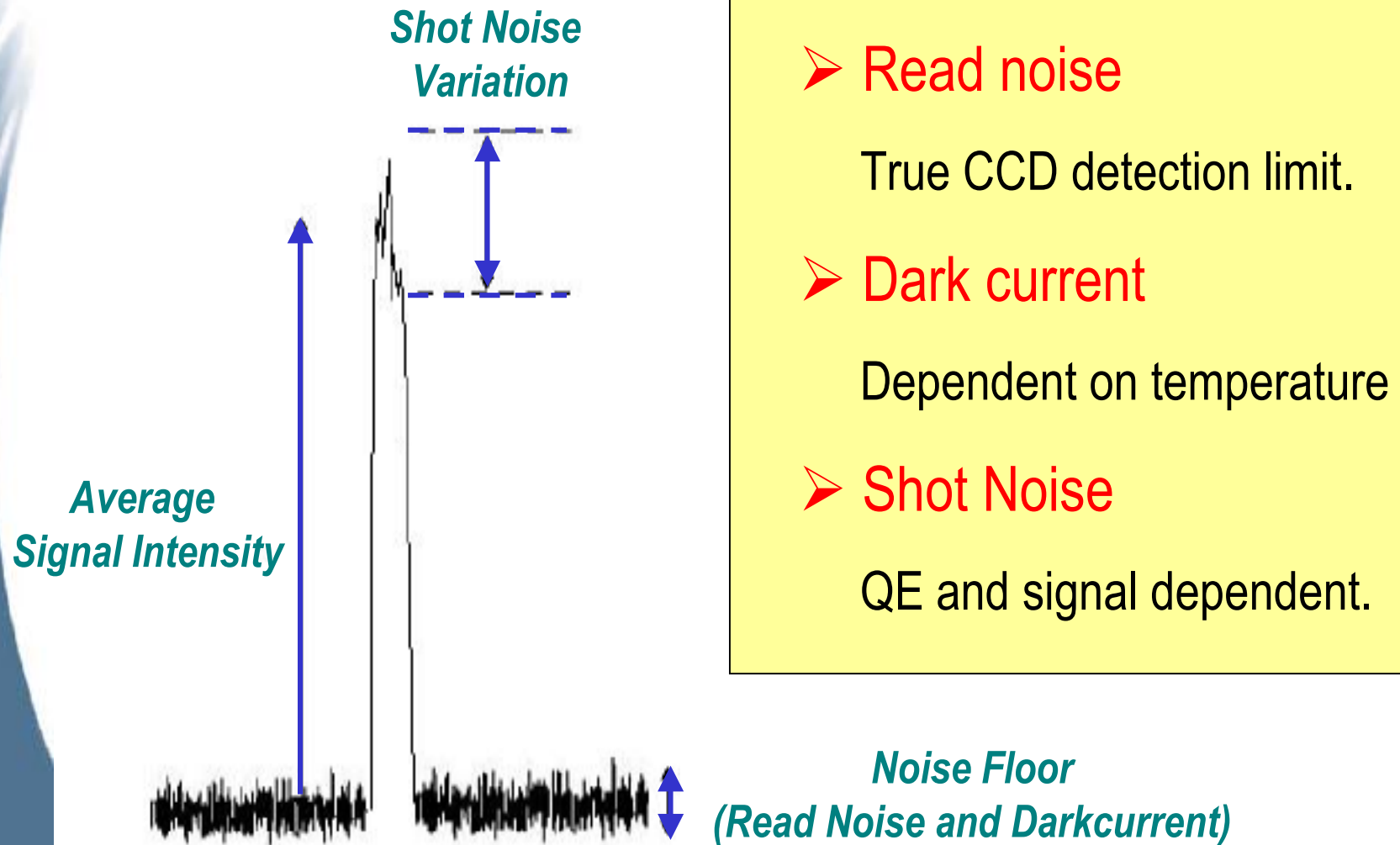


Illustration of Principle Noise Sources



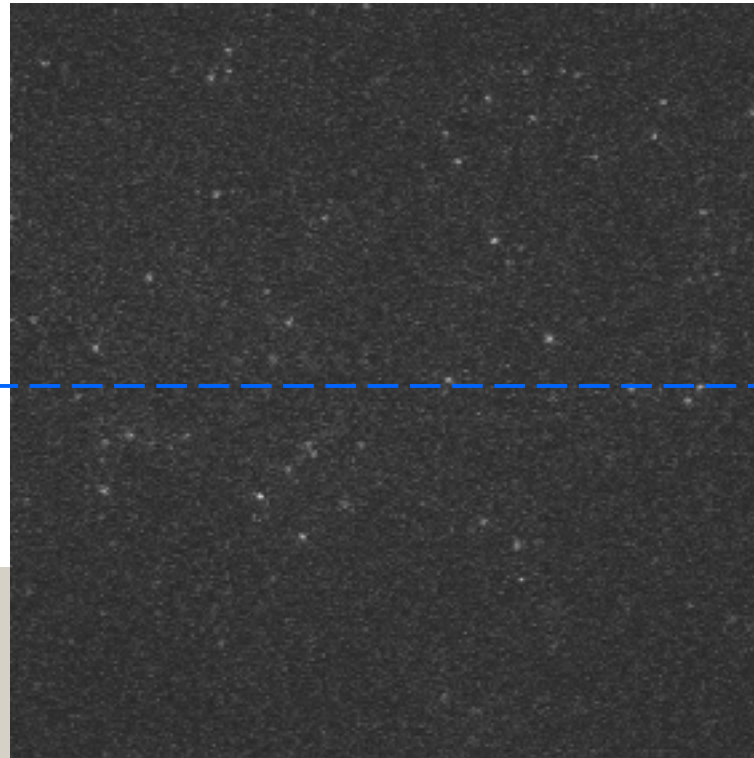
Unwanted background photon noise



Sources?

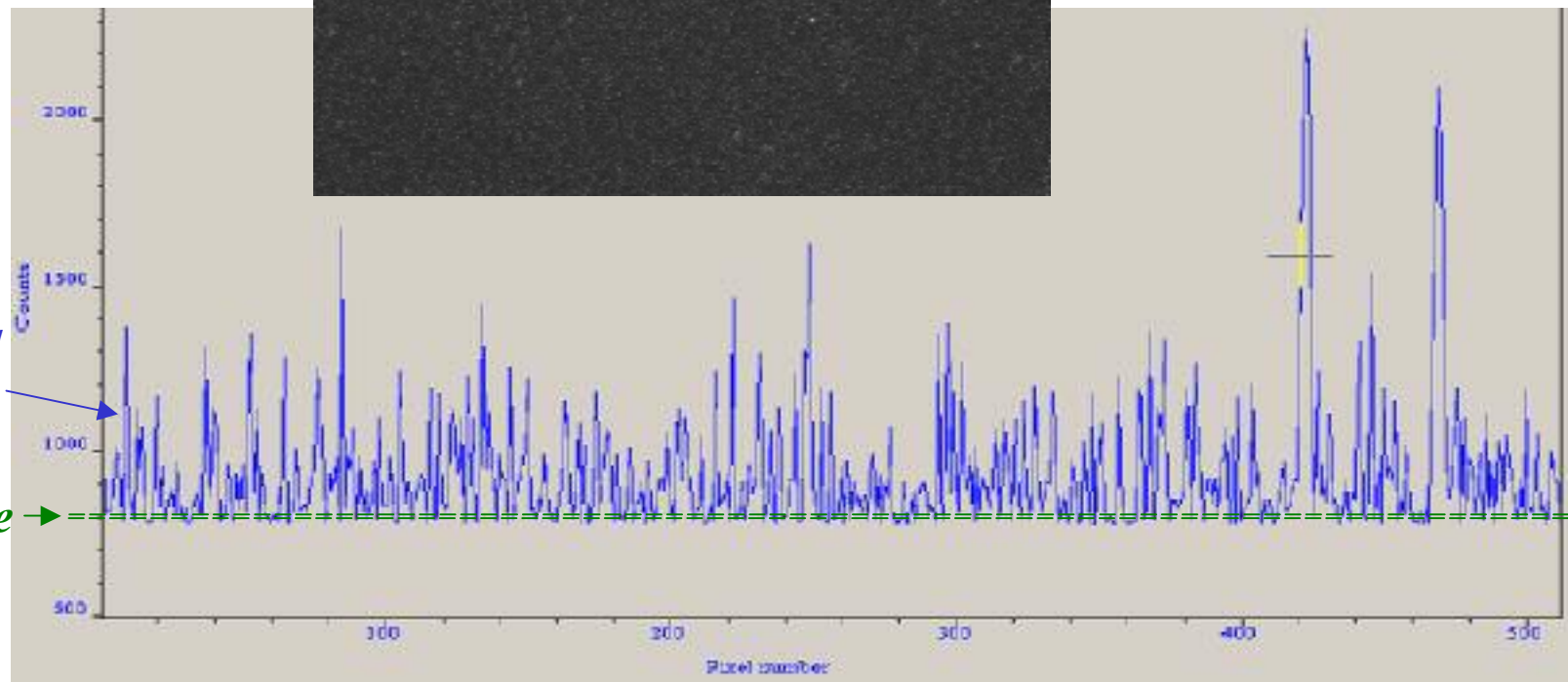
- **Unoptimised optical filters**
- **Stray background light**
- **Non-specific binding of fluoro-phore.**

Need to minimise background photon noise -



*Background
Shot Noise*

Read Noise



Competing Technologies



➤ **CCDs**

- High and broad QE
- Pixel resolution

- Noise floor
(readout restriction)

➤ **ICCDs**

- Single photon sensitive

- Restricted QE
- Cross-talk
- Multiplication Noise
- Artefacts

➤ **EBCCDs**

- Single photon sensitive

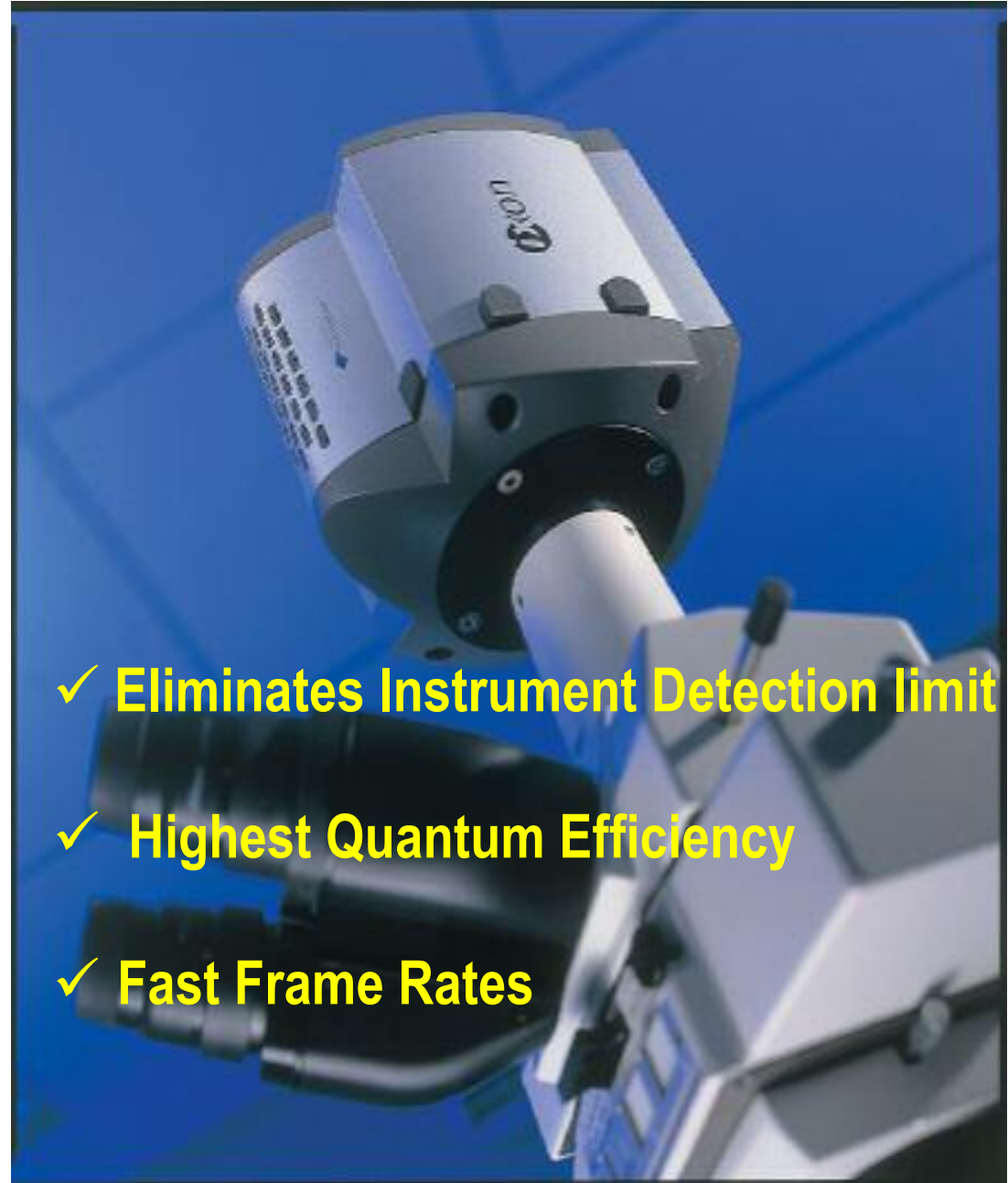
- Restricted QE
- High spurious noise
- Artefacts

The Electron Multiplying Charge Coupled Device

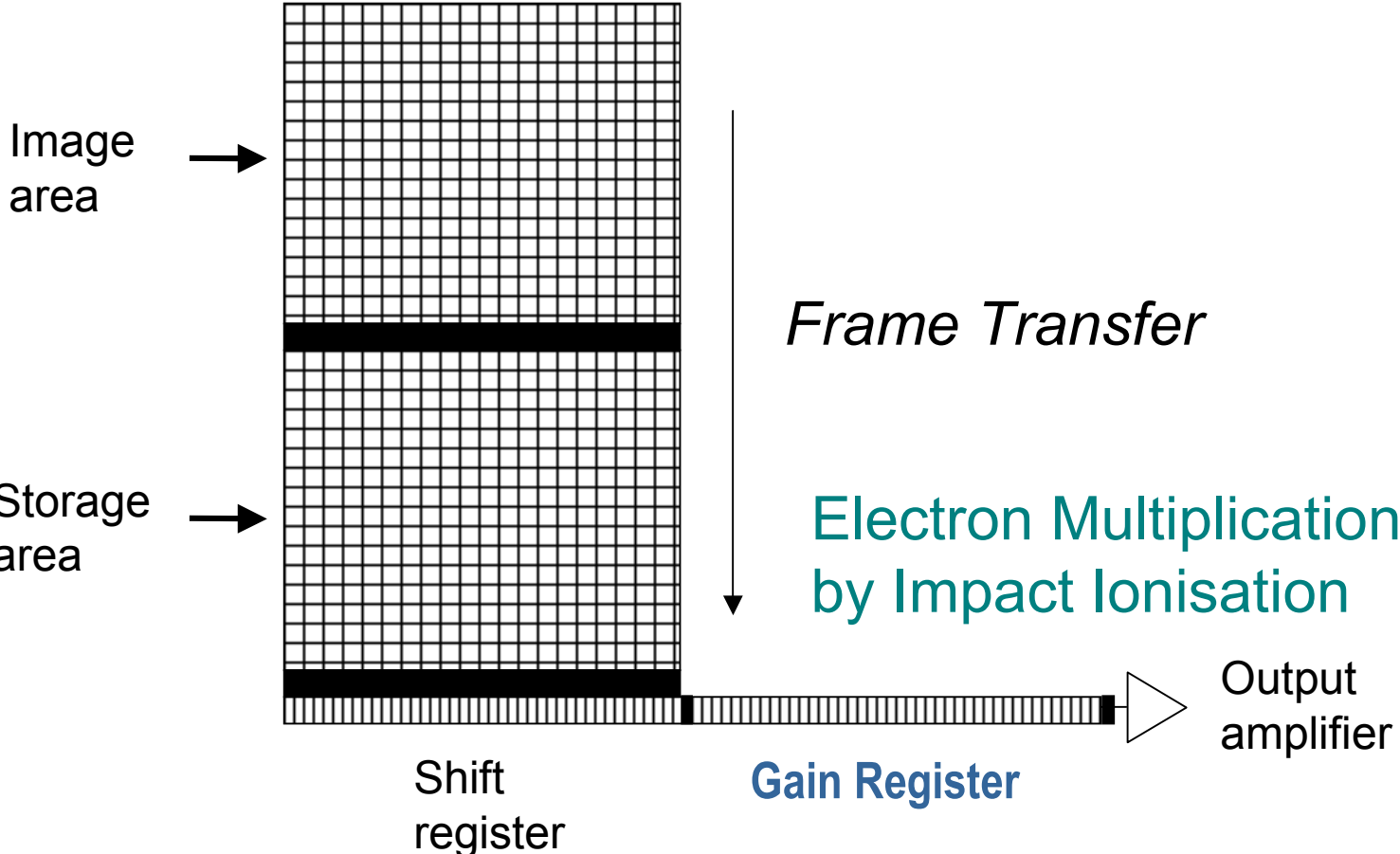
(EMCCD)



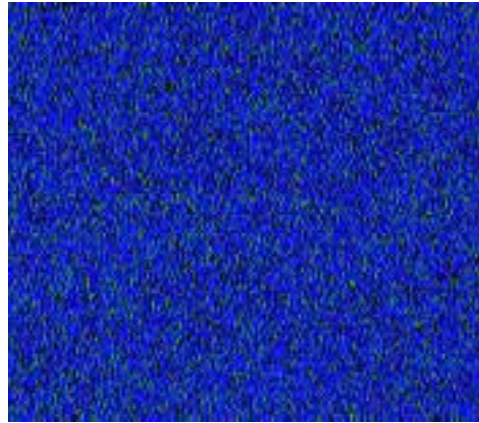
- ✓ Eliminates Instrument Detection limit
- ✓ Highest Quantum Efficiency
- ✓ Fast Frame Rates



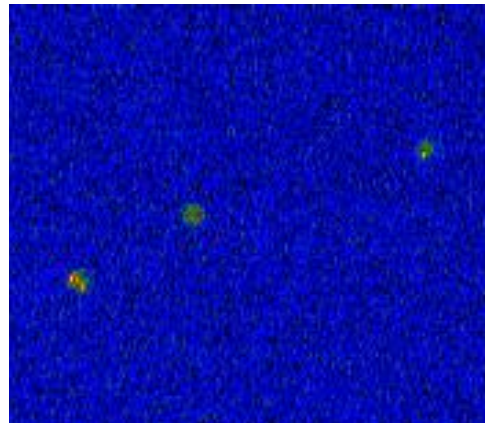
EMCCD Gain



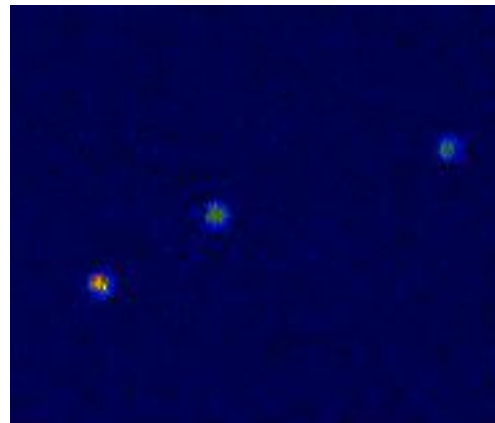
Effect of EMCCD Gain on S:N



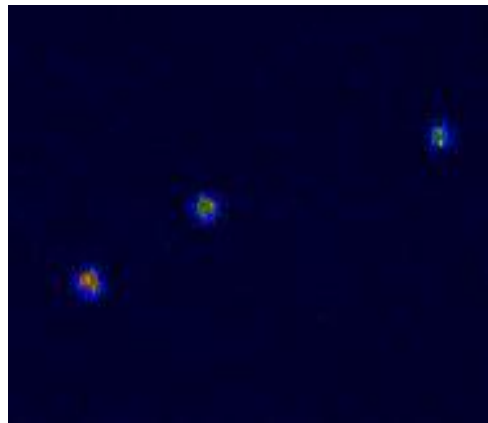
Gain x1



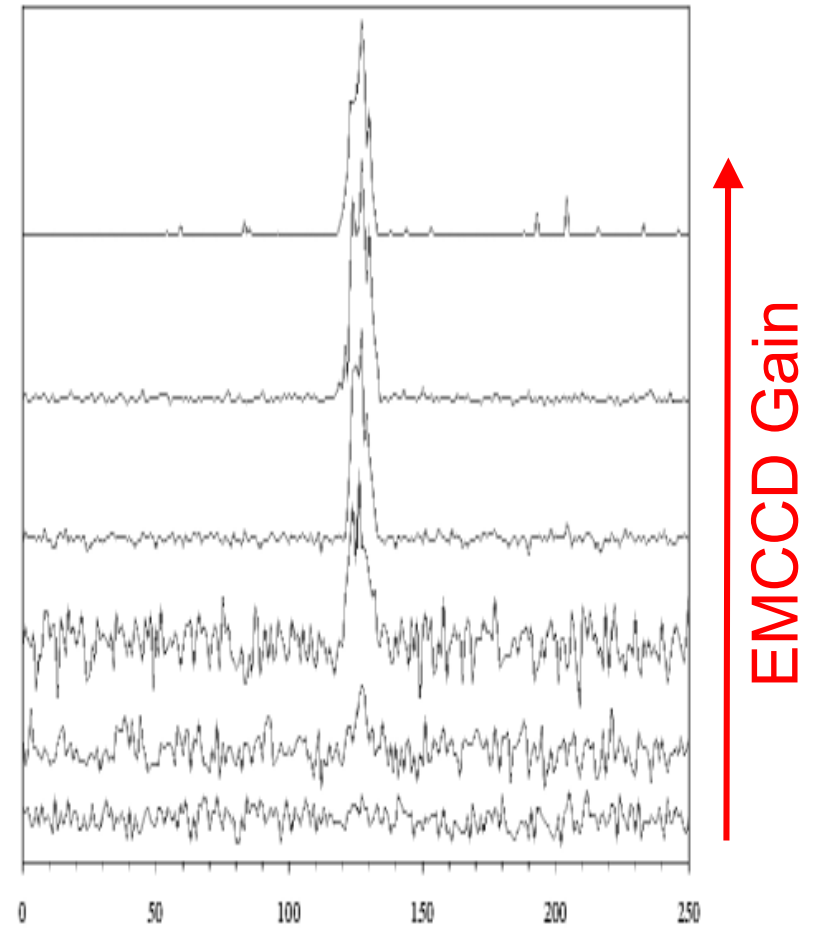
Gain x10



Gain x100



Gain x500



Latest Innovation:

**EMCCD united with
back-illuminated CCD technology:**

✓ **Highest Quantum Efficiency (>90%).**

combined with,

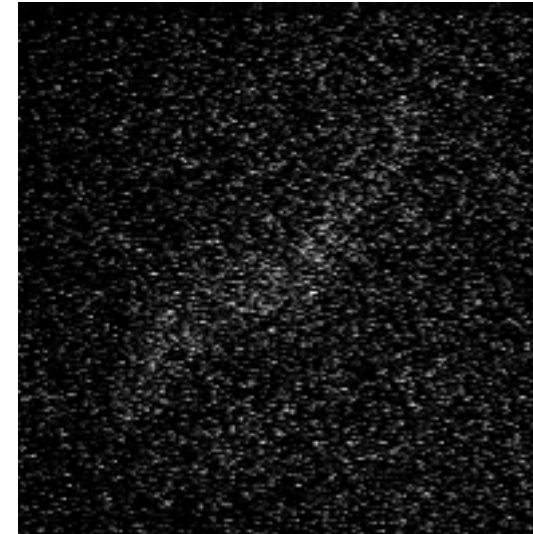
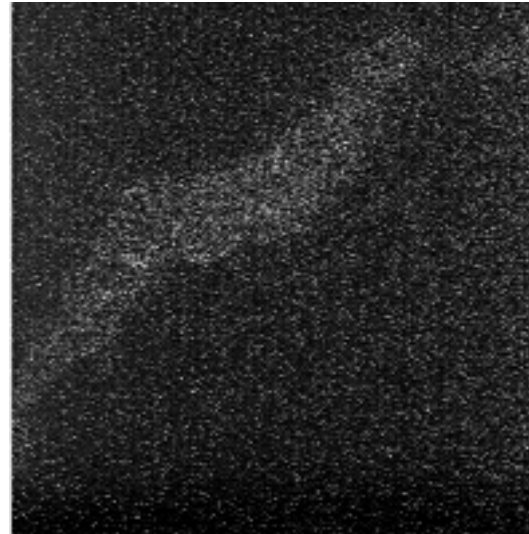
✓ **Single Photon Detection ability.**

Detection Limit for EMCCD V GEN III

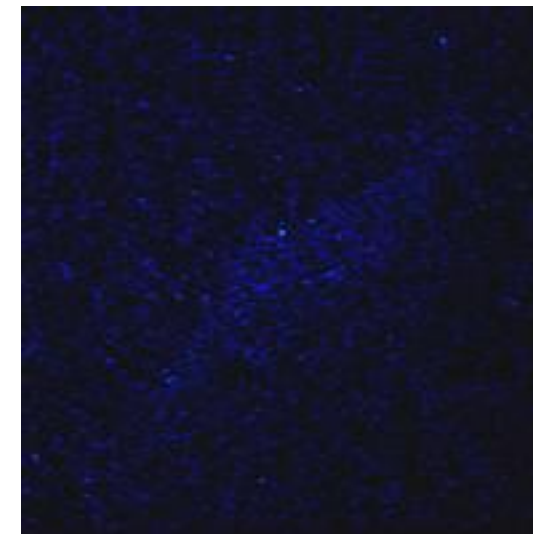
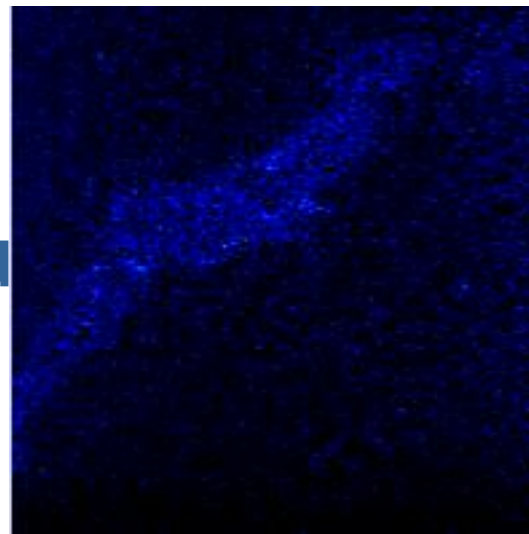
BV-EMCCD (512x512)

Gen III

Raw
Data



Pseudocolour
and Smoothed



Applications for Ixon

e.g.

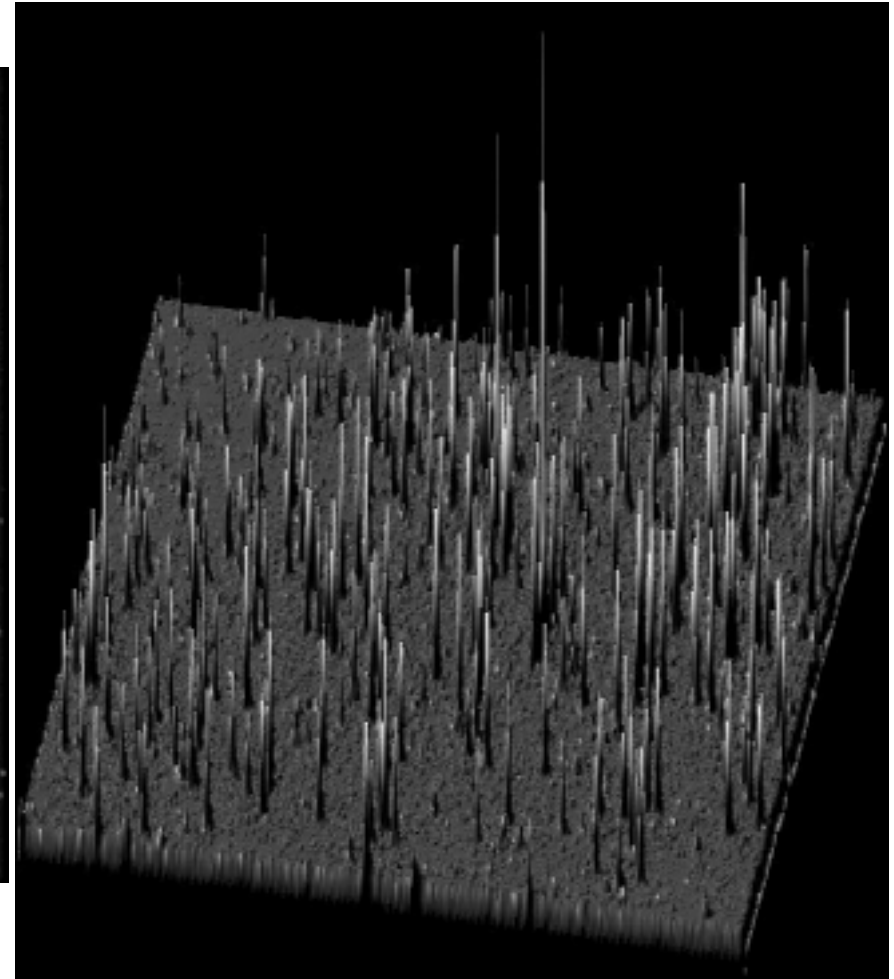
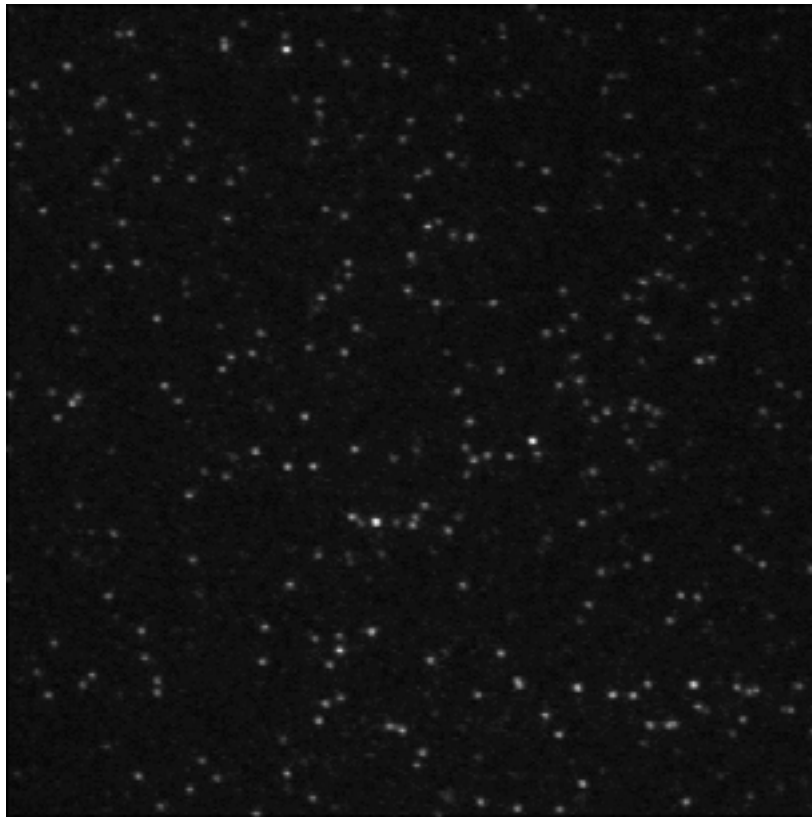
- Ion Signalling Microscopy (Ca²⁺ flux)
- Neural Imaging
- Single Molecule Microscopy
- Cell Motility
- Confocal Live Cell Microscopy
- HTS Kinetic Analysis
- Adaptive Optics
- BEC

- Bioluminescence
- Gene chip reading
- GFP & Chemiluminescence
- Astronomy

*Kinetic
Requirements*

Single Cy3 Fluorophores on Glass Surface - Total Internal Reflectance Fluorescence Microscopy

Recorded with iXon DV887 at 17 fps (full frame)



EMCCD Overview

- ✓ Eliminate read noise floor.
- ✓ Single Photon Detection.
- ✓ Highest QE ($> 90\%$).
- ✓ Minimal Exposure Times.
- ✓ Reduce Excitation Powers.
- ✓ Reduce Fluorophore Concentrations.
- ✓ Increase Magnifications

What is the ideal detector for your application?



- **Sensitivity?**
- **Frame rate?**
- **Number of pixels/Sensor dimensions?**
- **Pixel size?**
- **Dynamic range?**
- **Wavelength Range?**
- **Binning/Sub-array options?**
- **Flexibility of readout options?**
- **Software?**
- **Price/Performance?**