CAMERAS FOR MICROSCOPY

Matt Preston

What do we want a sensor to do?

- Take a image – full marks have a medal….

- Quickly / As fast as we can
- From many different light levels
- With good dynamic range
- From signals of differing emission wavelengths
- With enough resolution to see detail
- With limited noise

Content

- How does a camera work
- Sensitivity
- Resolution
- Noise
- Camera settings
  - Gain
  - Binning

•EMCCD's

CCD Fundamentals

The Charge-Coupled Device (CCD)

- Invented in 1970 at Bell Labs
- A silicon chip that converts an image to an electrical signal
- Image is focused directly onto the silicon chip
- Widely used in TV cameras and consumer camcorders
- Special high-performance CCDs made by:

  Eastman Kodak (Rochester, NY)

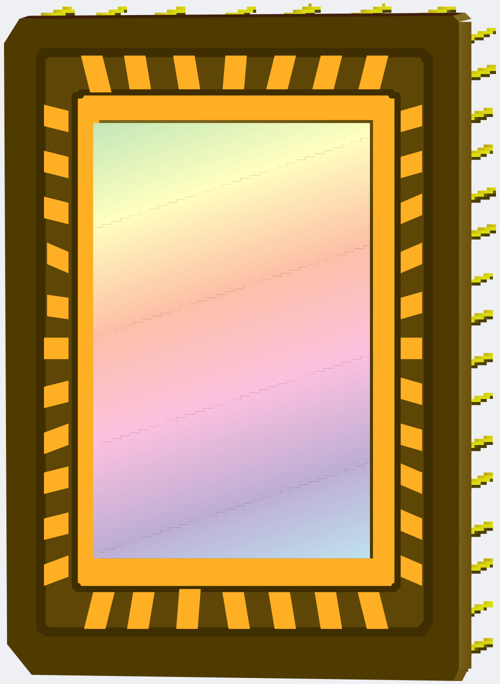
  Thomson CSF (France)

  Marconi (formerly EEV — England)

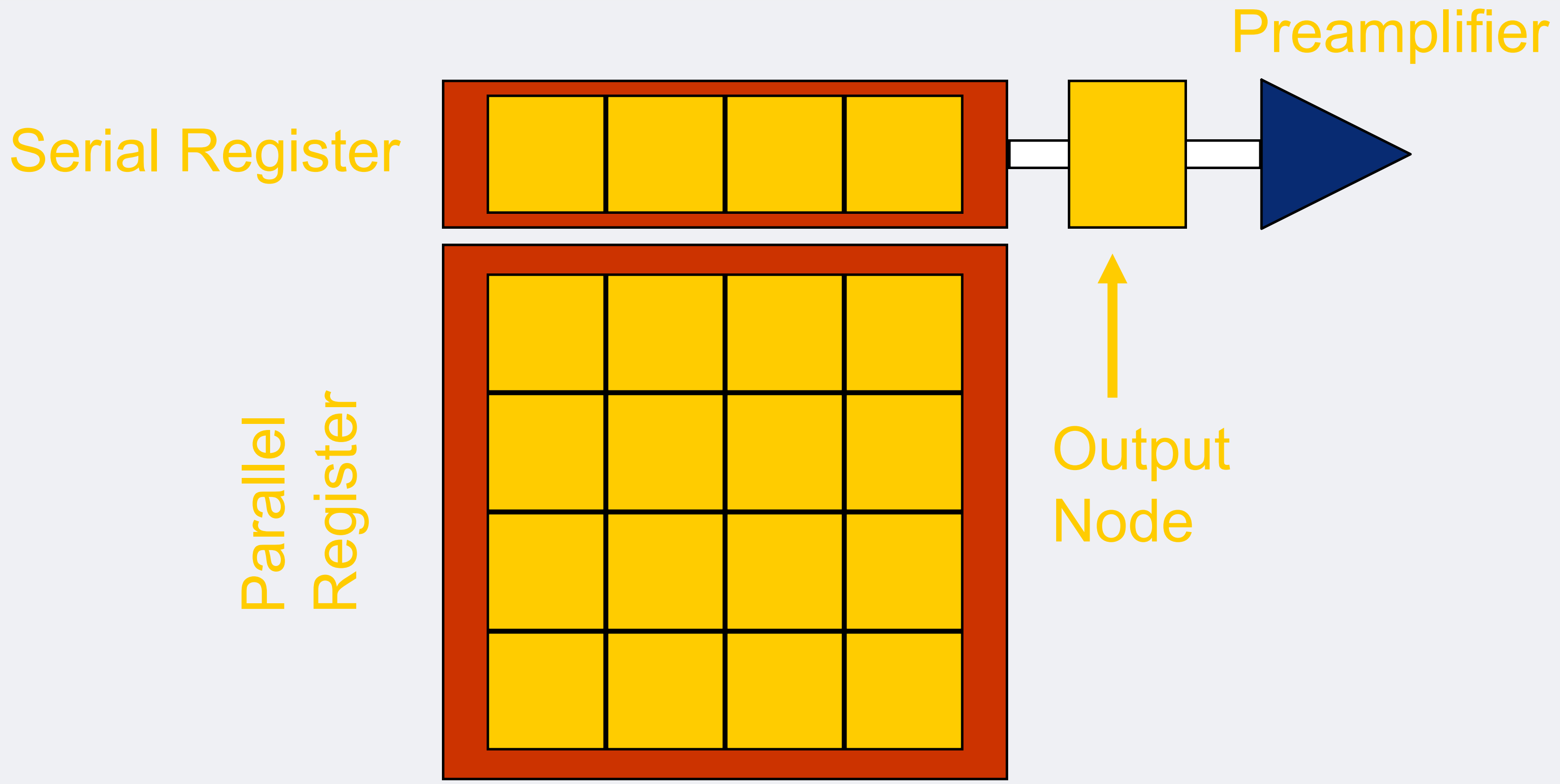
  SITe (Beaverton, OR)

  Sony

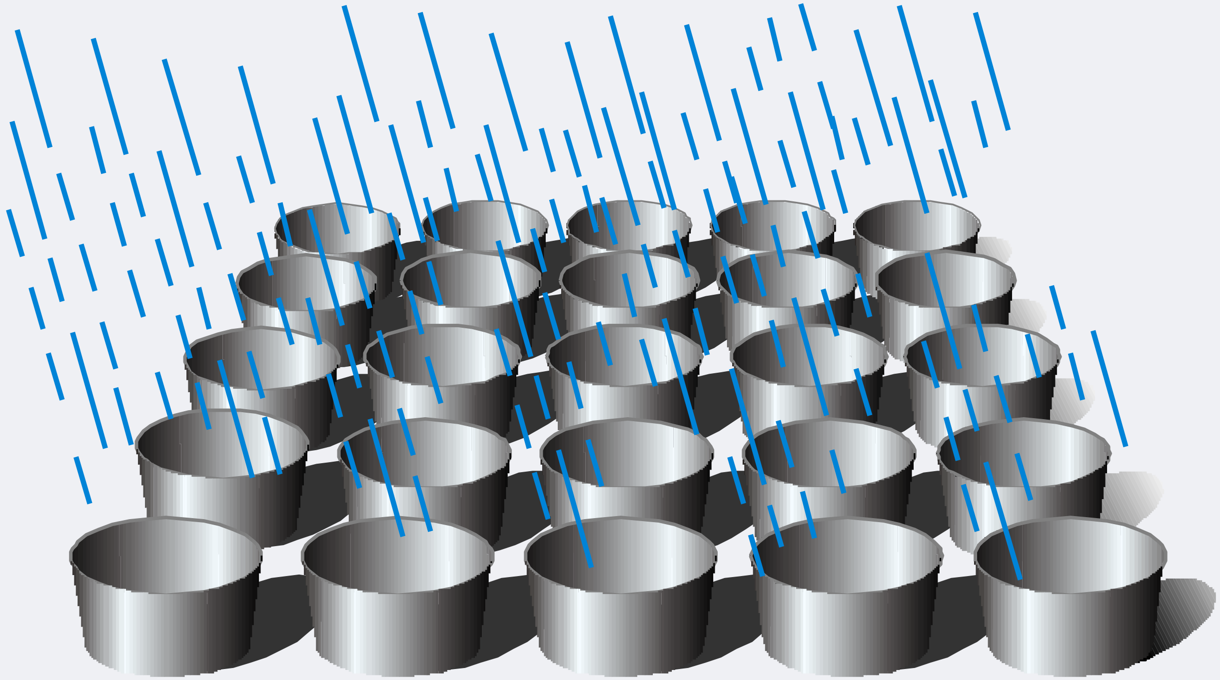
  Others



CCD Fundamentals

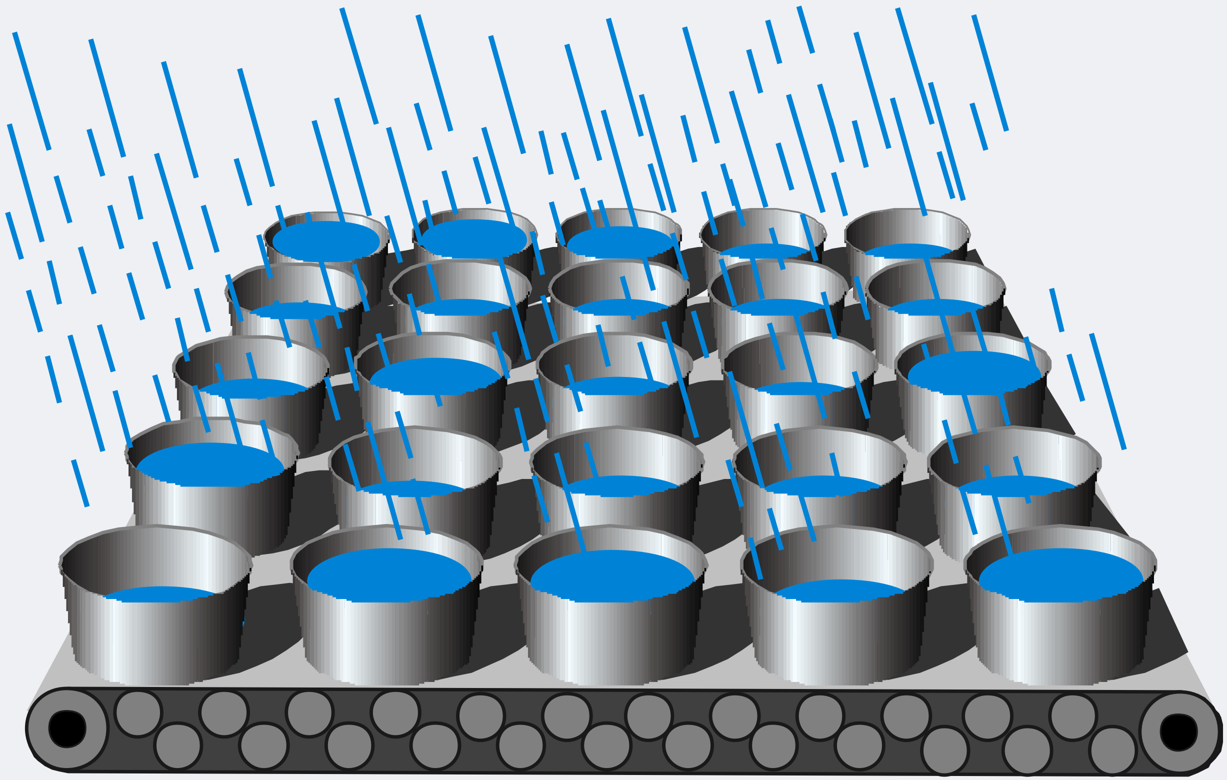


CCD Fundamentals



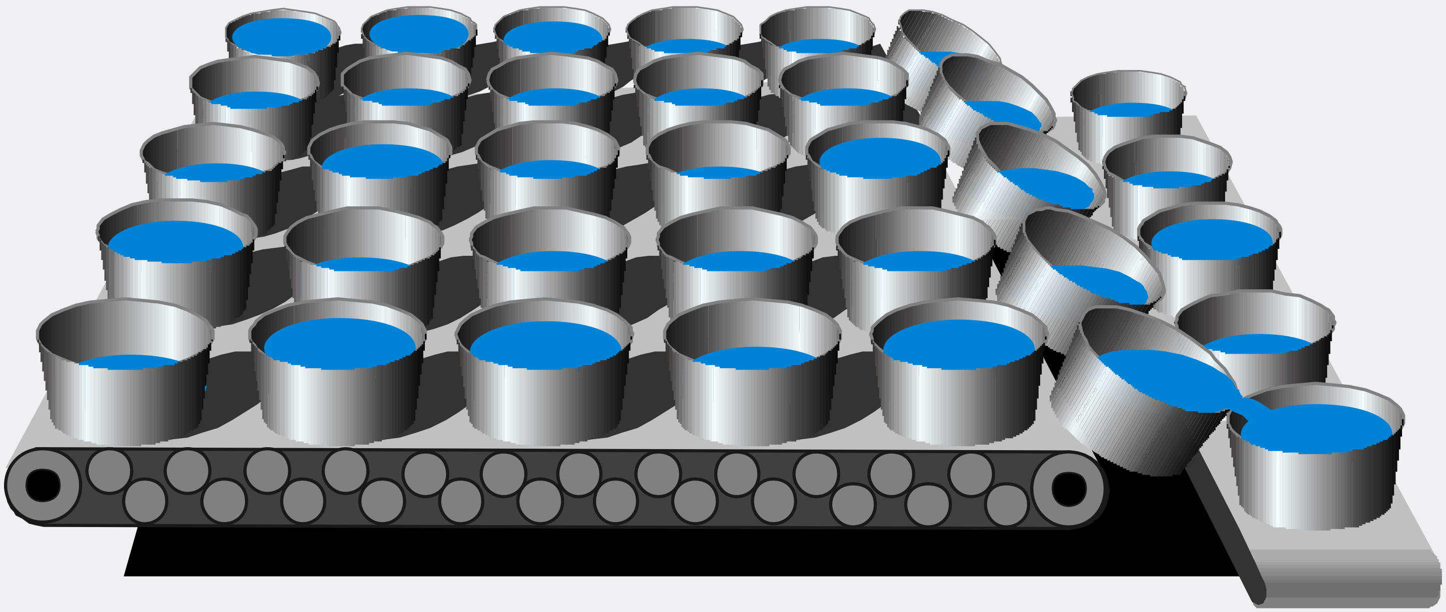
Array of Discrete Photodetectors

CCD Fundamentals



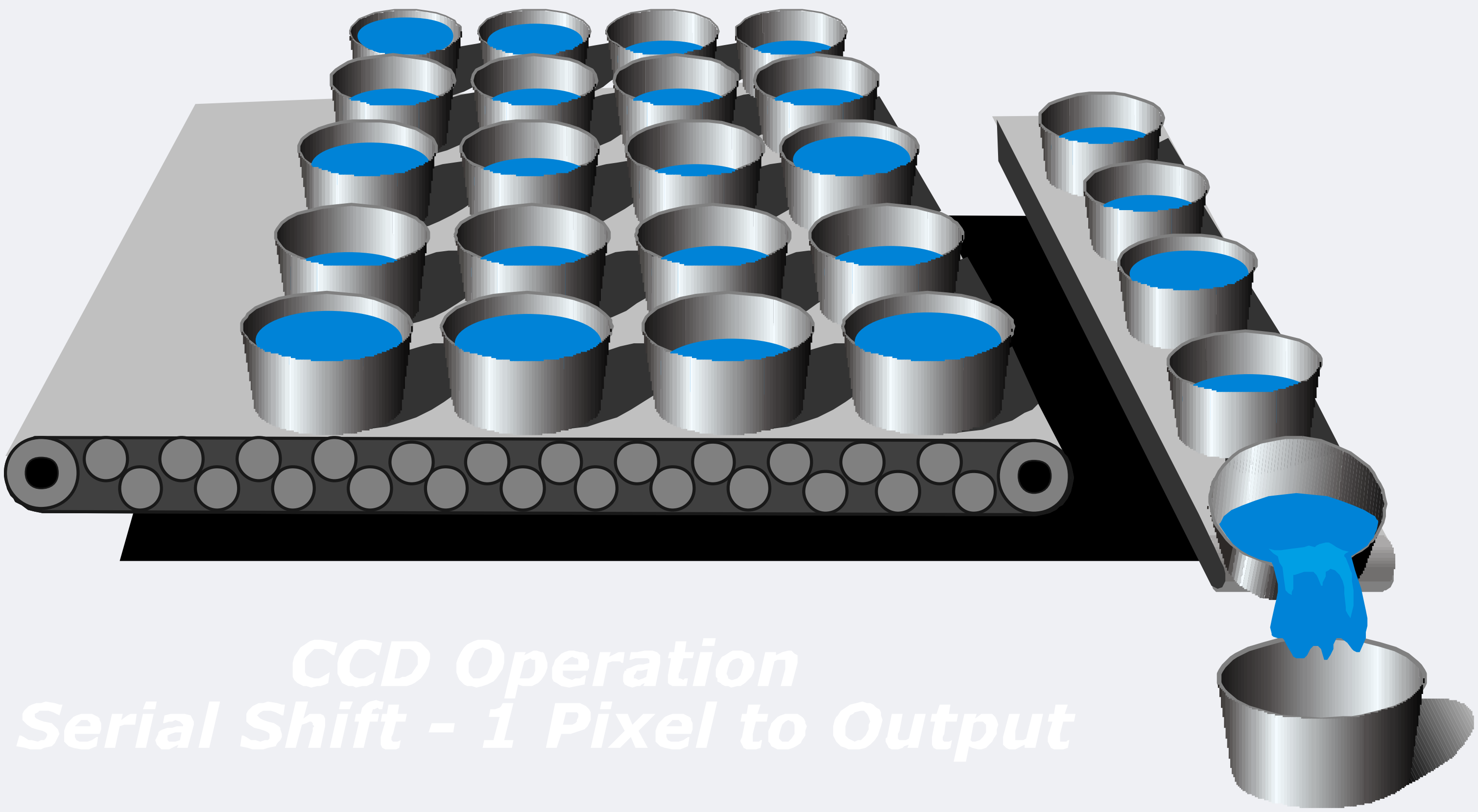
CCD Operation
Integration of Photo-Induced Charge

CCD Fundamentals



CCD Operation
Parallel Shift - 1 Row

CCD Fundamentals



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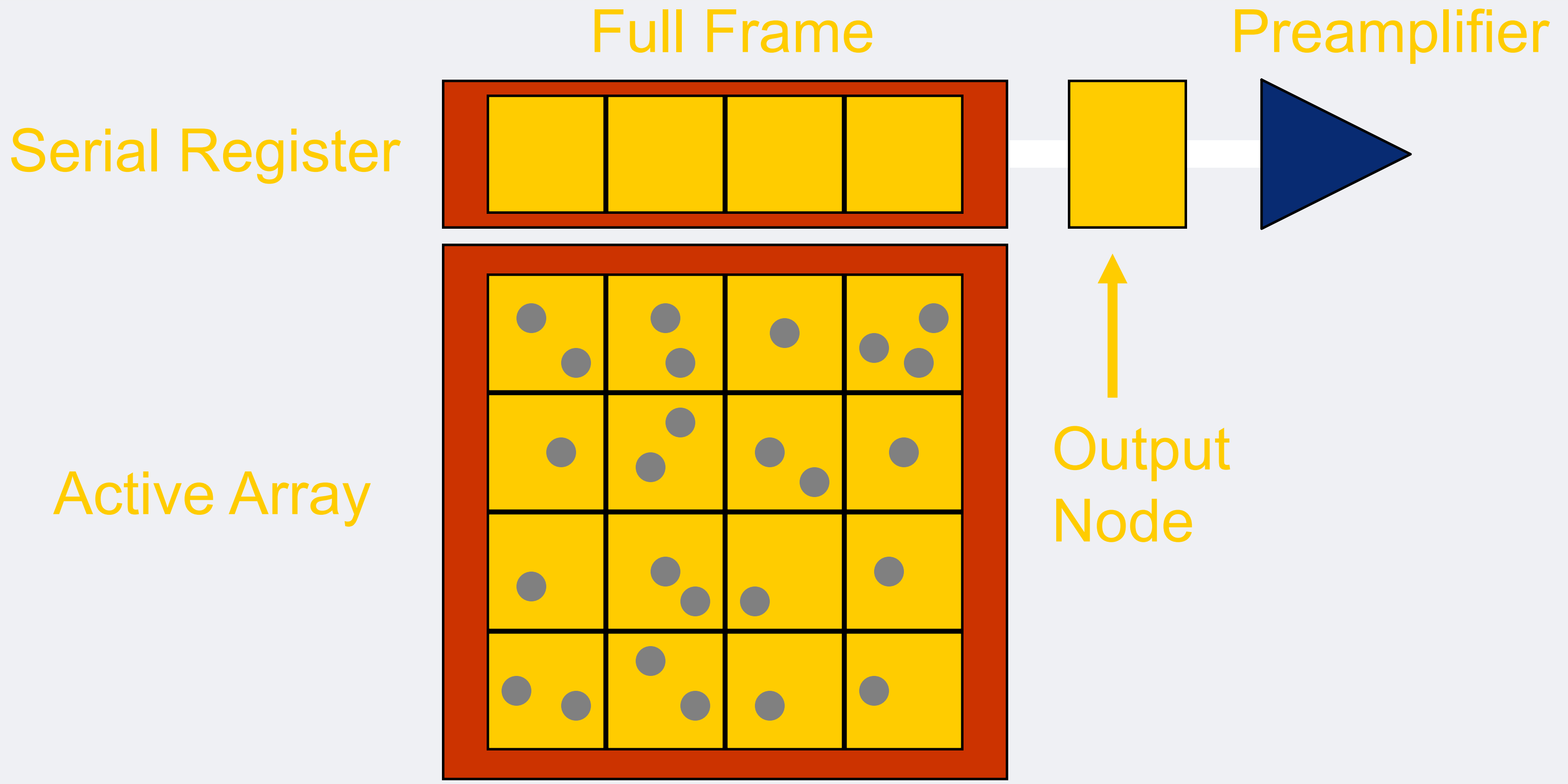
CCD Fundamentals



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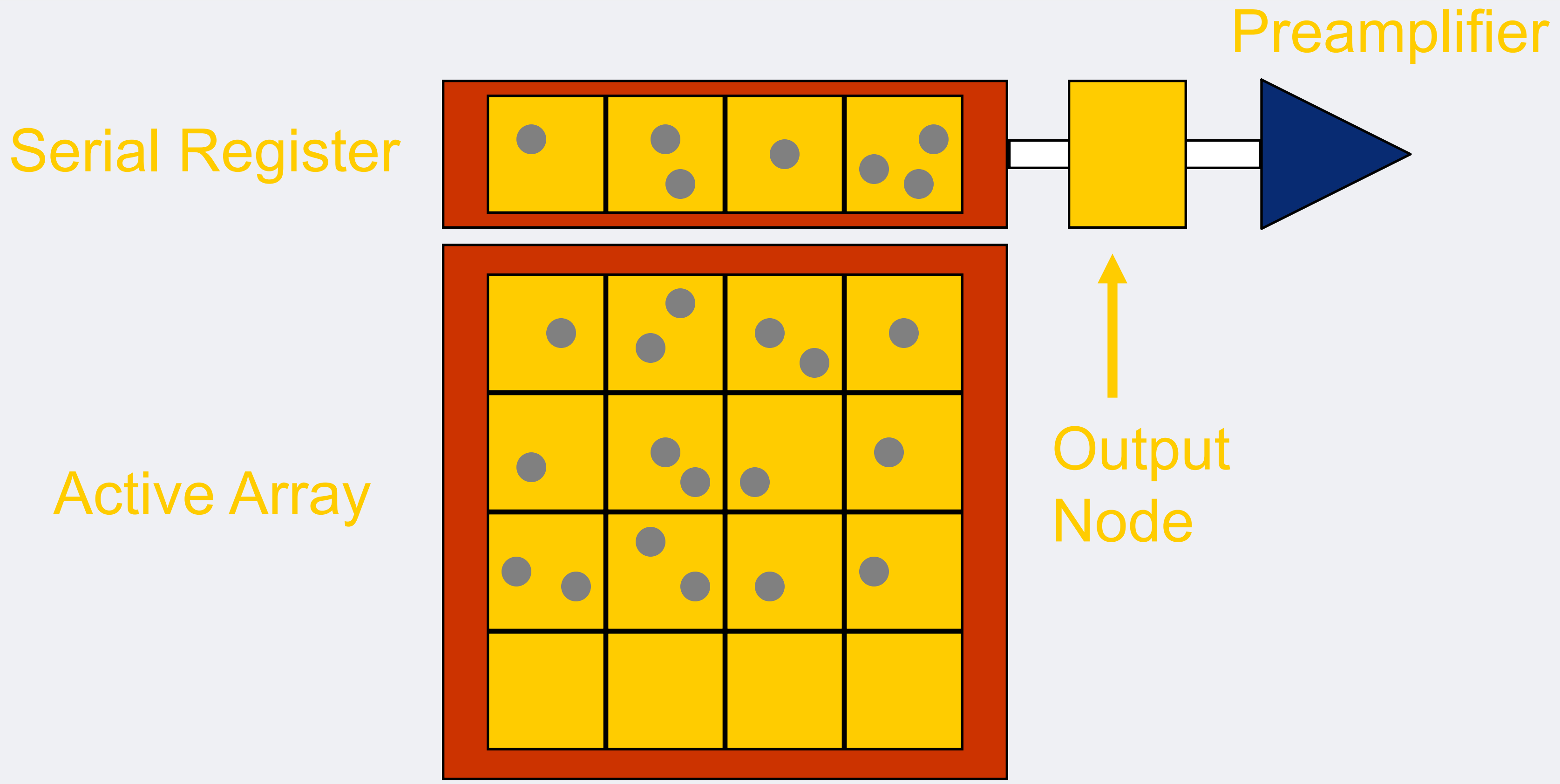

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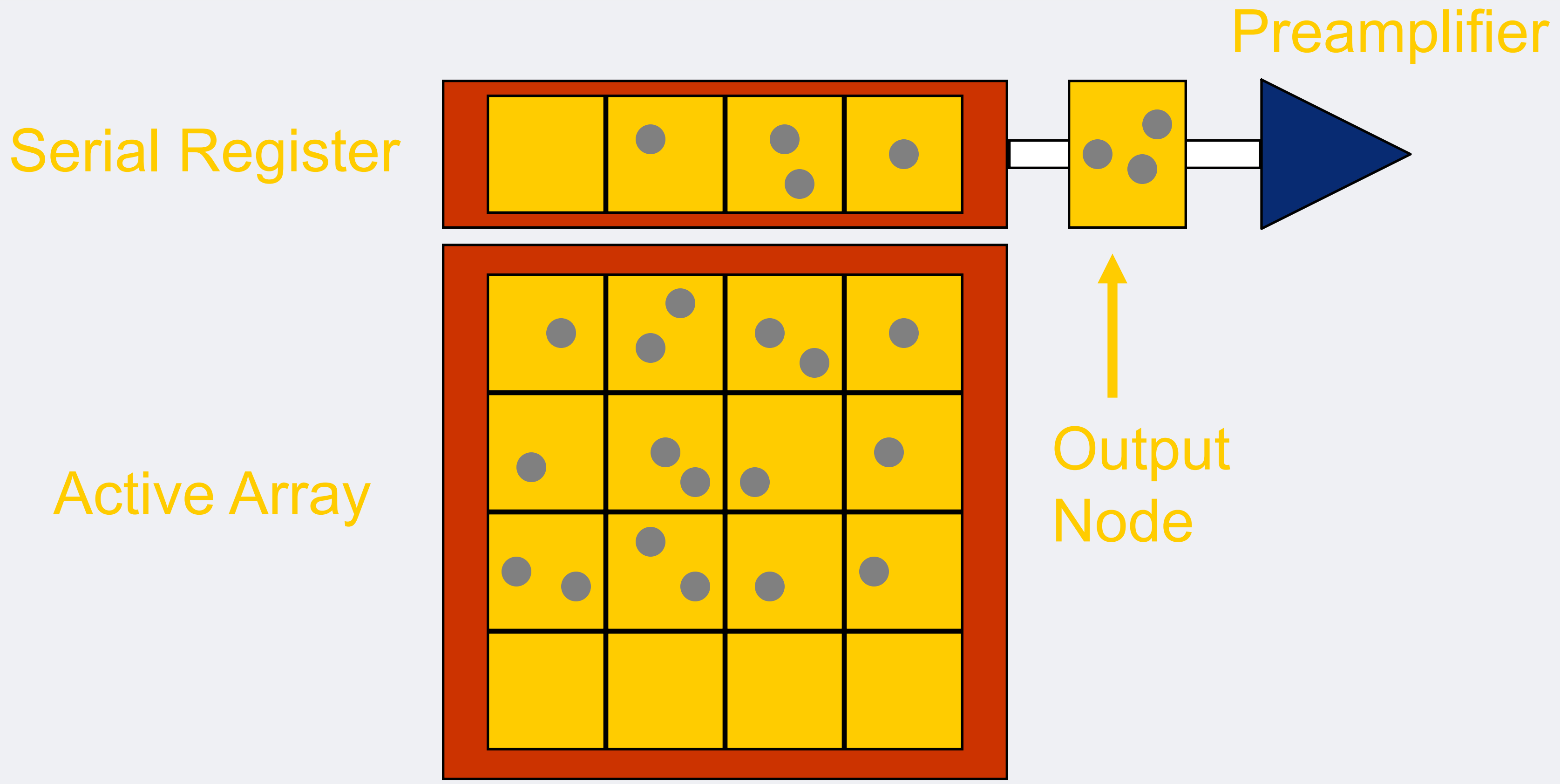
CCD Fundamentals



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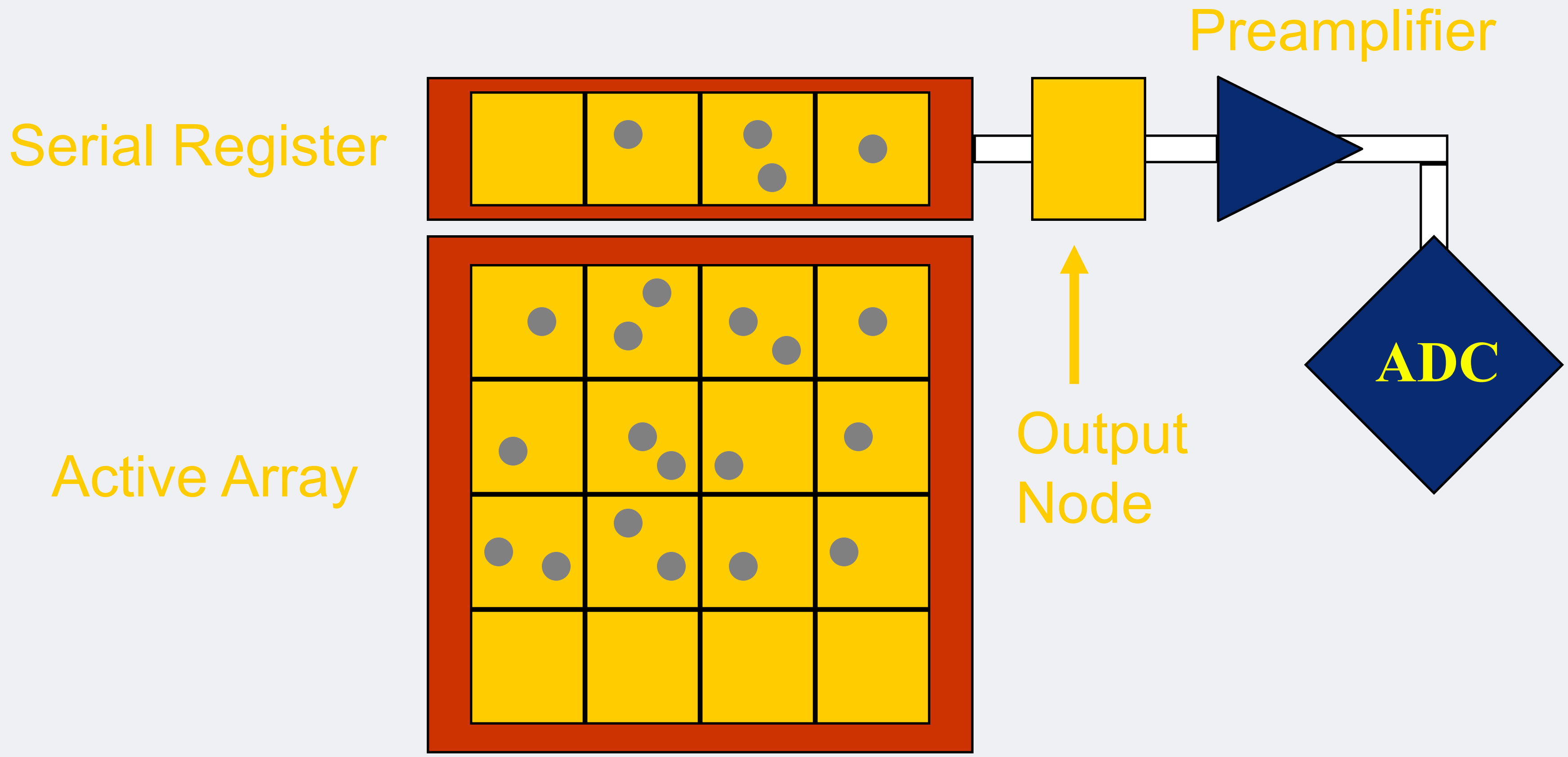

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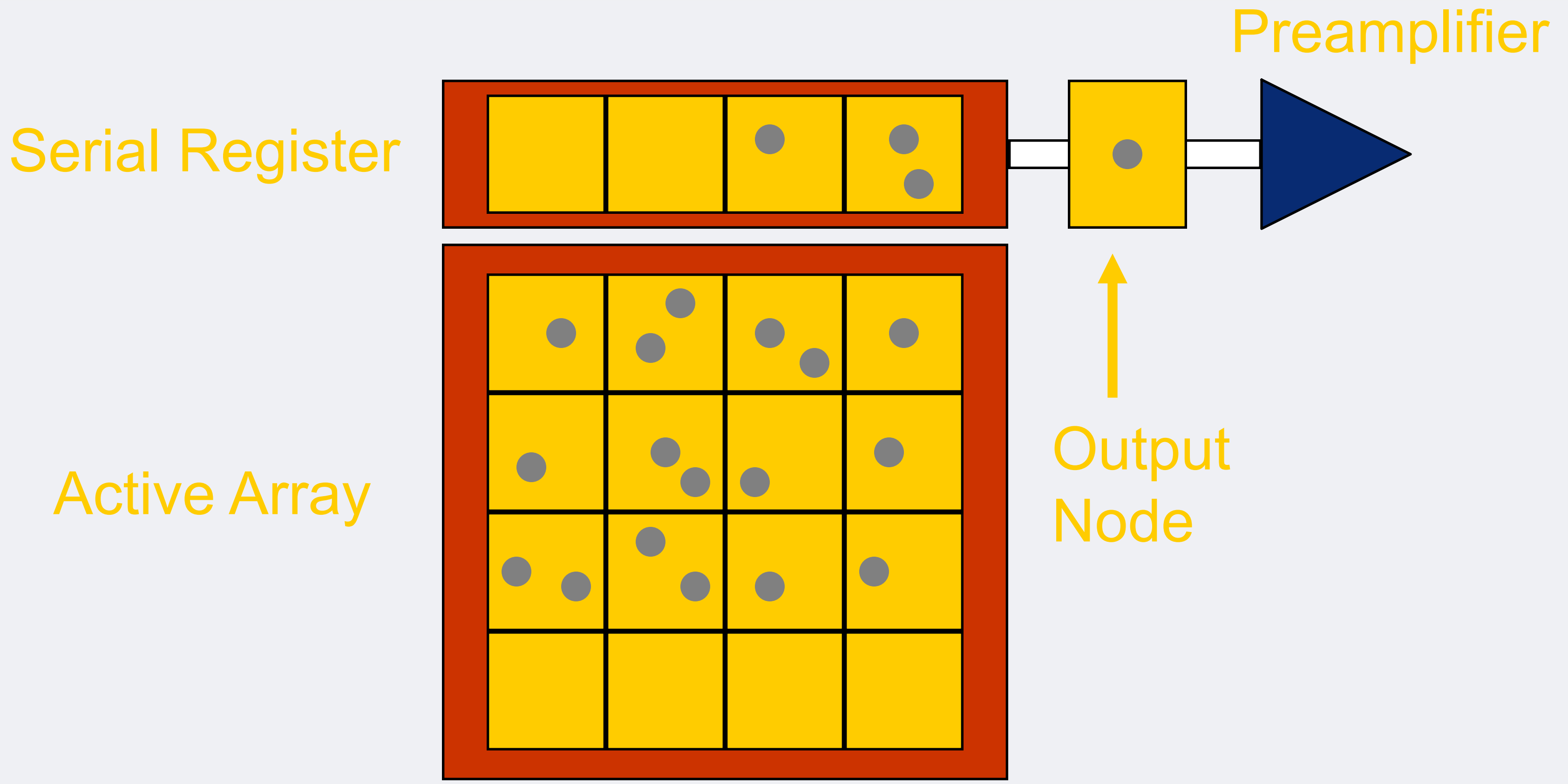

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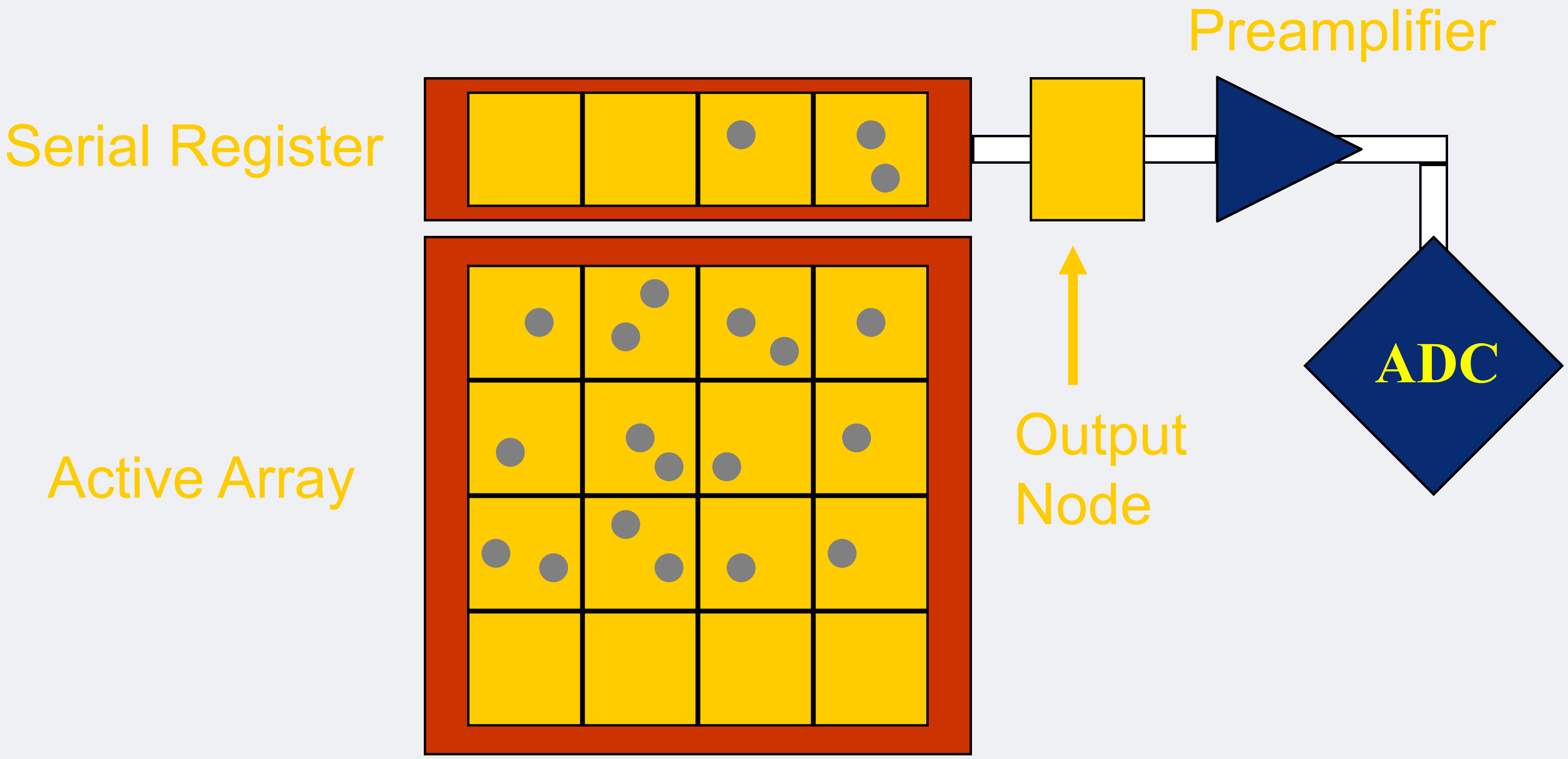

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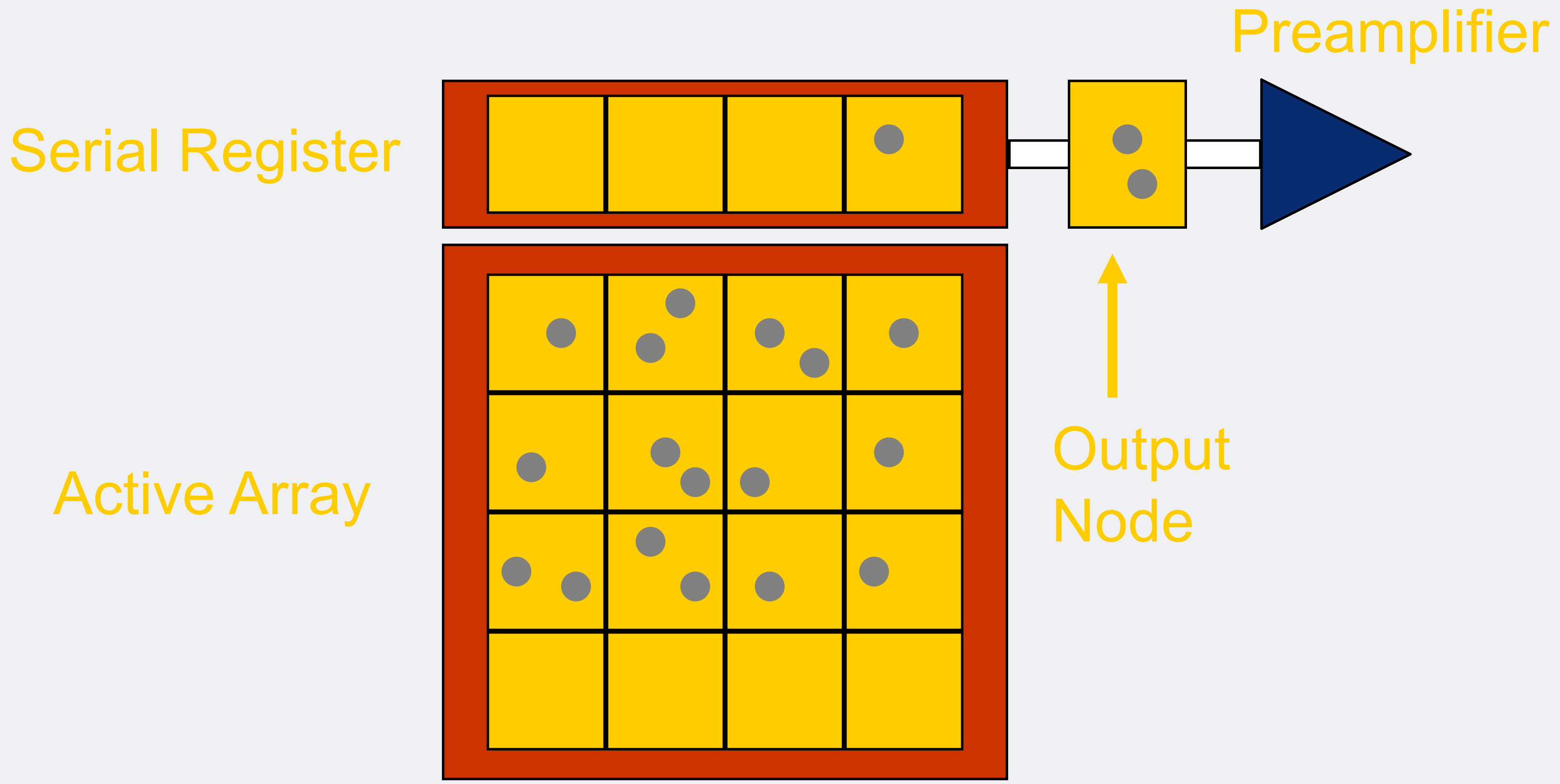

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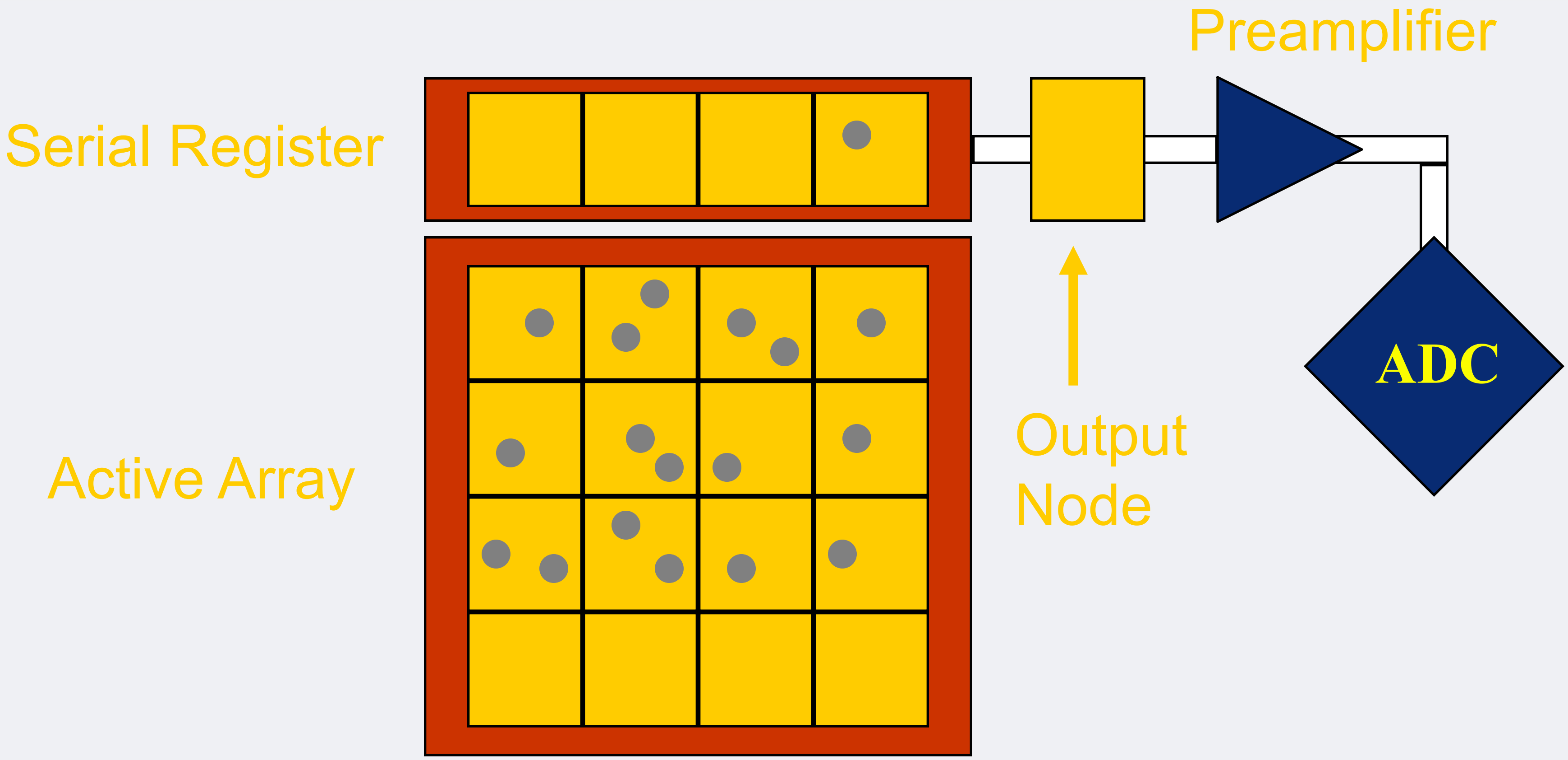

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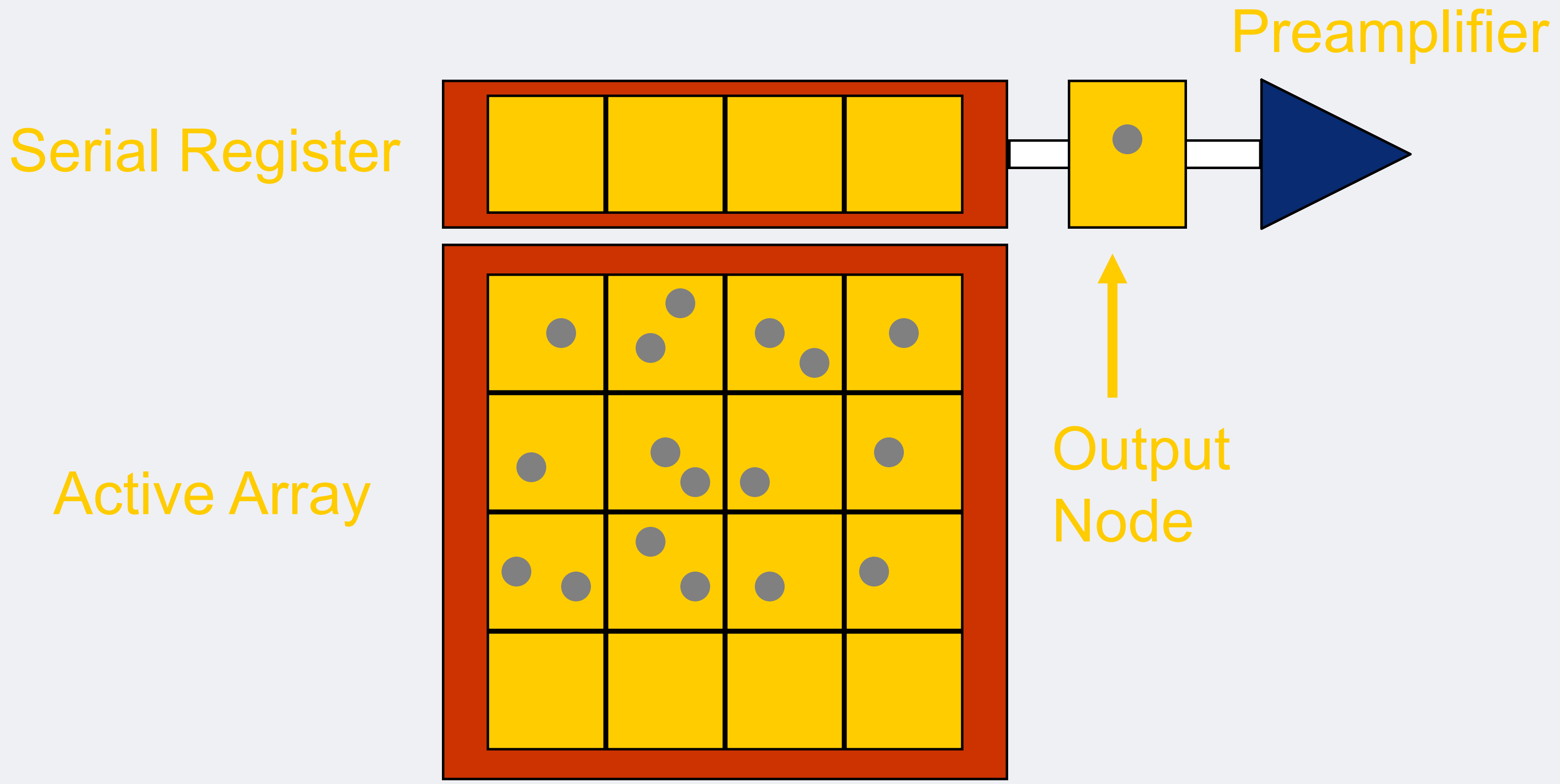

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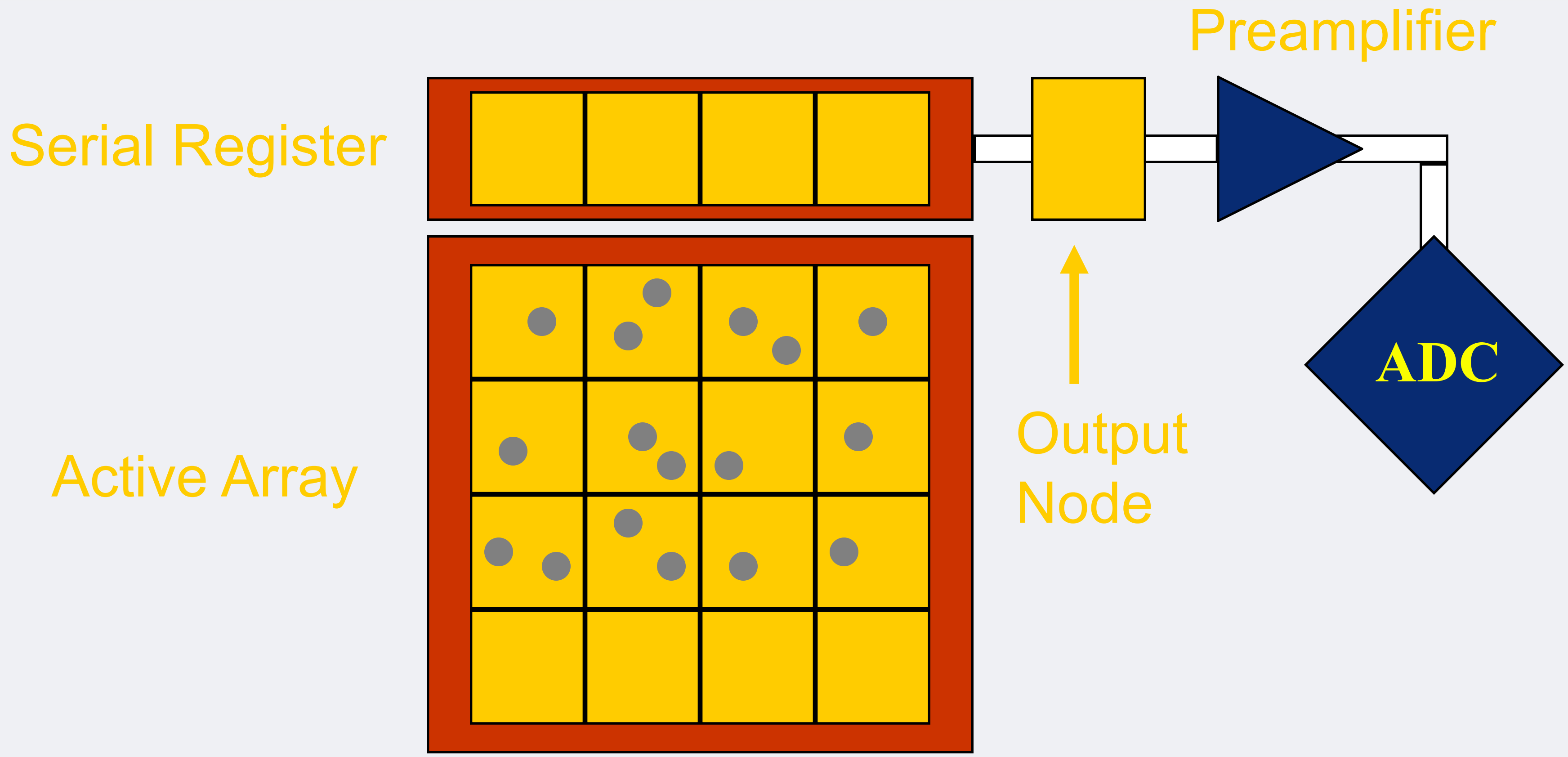
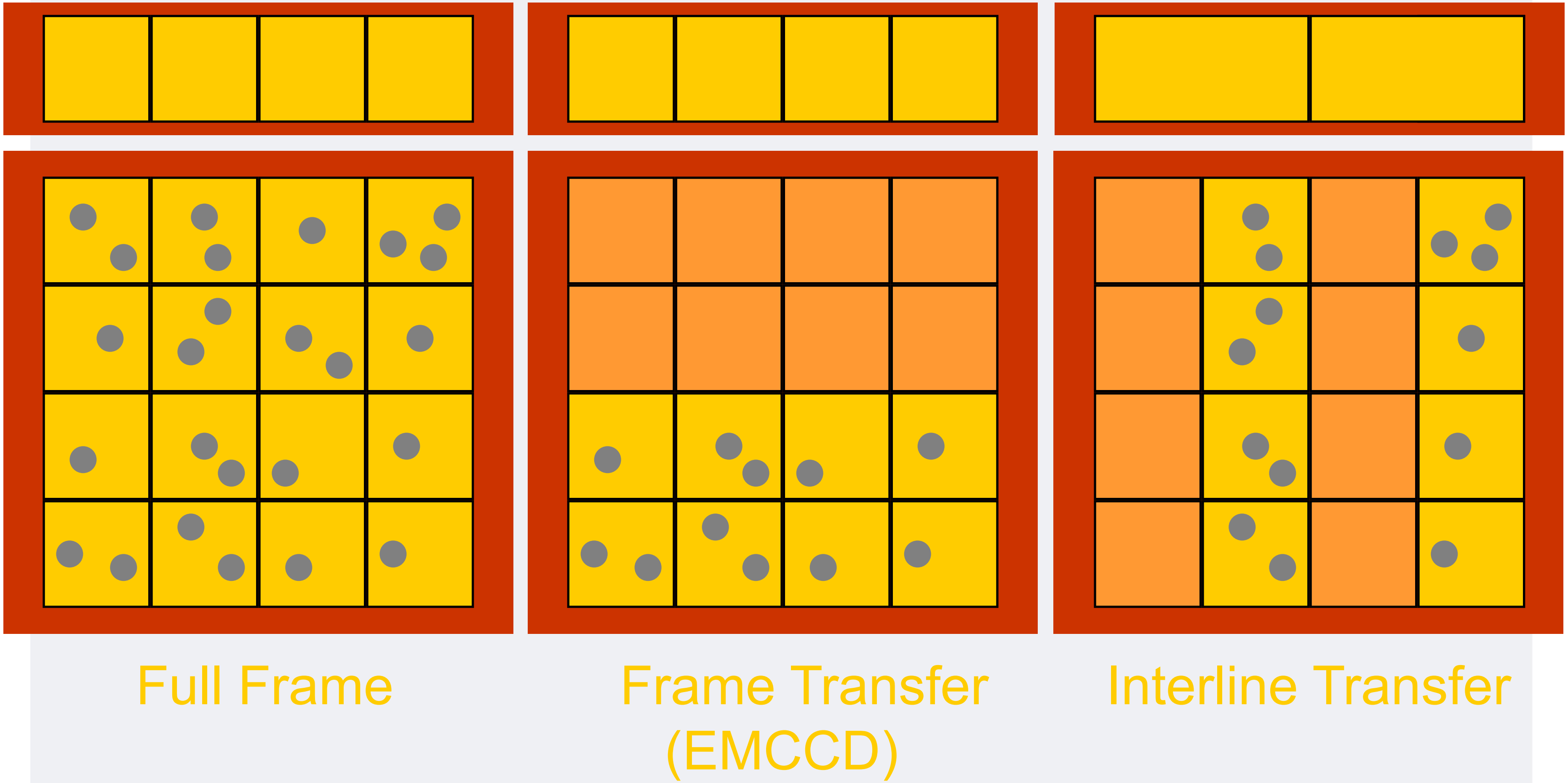


CCD Fundamentals



Sensitivity

- Sensitivity is a horrible word which is often confused with Quantum Efficiency, Pixel Size, Signal and Signal to Noise.

We do know some key facts:

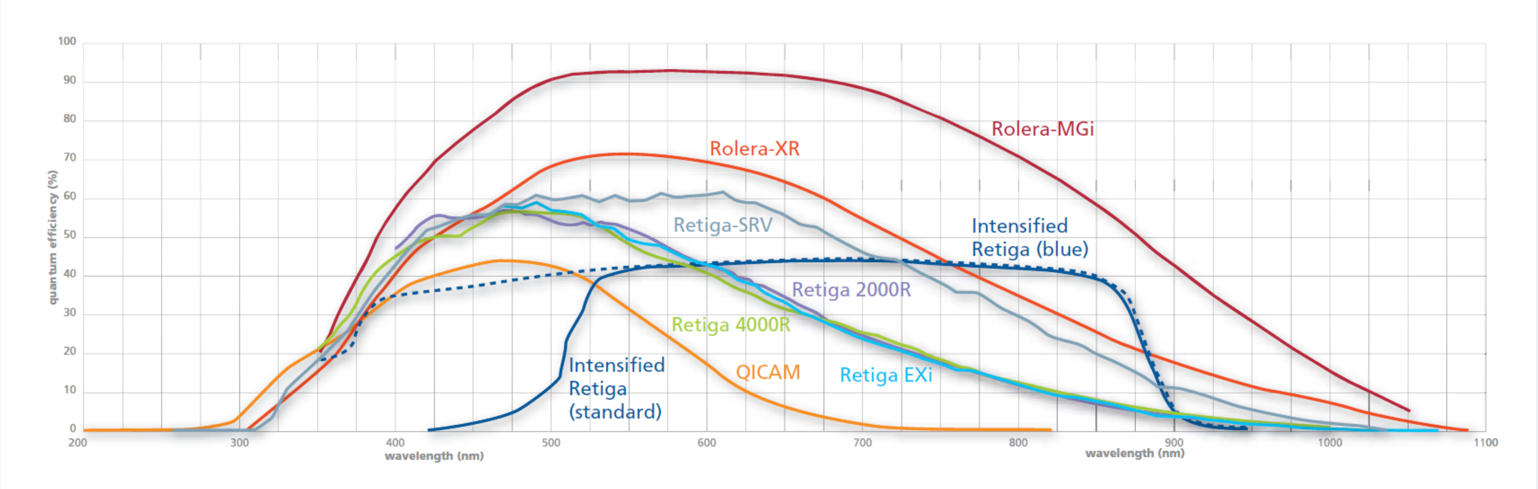
- Photons convert to electrons in sensors and they can then be measured – this conversion rate is defined as Quantum Efficiency

- Sensors convert photons of some wavelengths better than others

- The number of photons that interact with our pixel will depend on the physical size of the pixel

- We can have a sensitive sensor but if our signal to noise is low we get a noisy image with data we cannot decipher

QE Curves



- Spectral response curves are often shown on camera specification sheets.
- Some manufacturers claim higher responses than are achievable , but note these often vary from sensor to sensor
- Some manufacturers will also quote a relative response from 0 to 1
- The battle for good QE is fought in the flatness, max peak and responses to red dyes such as Cy5 (670nm)
- A QICAM is not suitable at this part of the spectrum as QE is only 5% at 670nm

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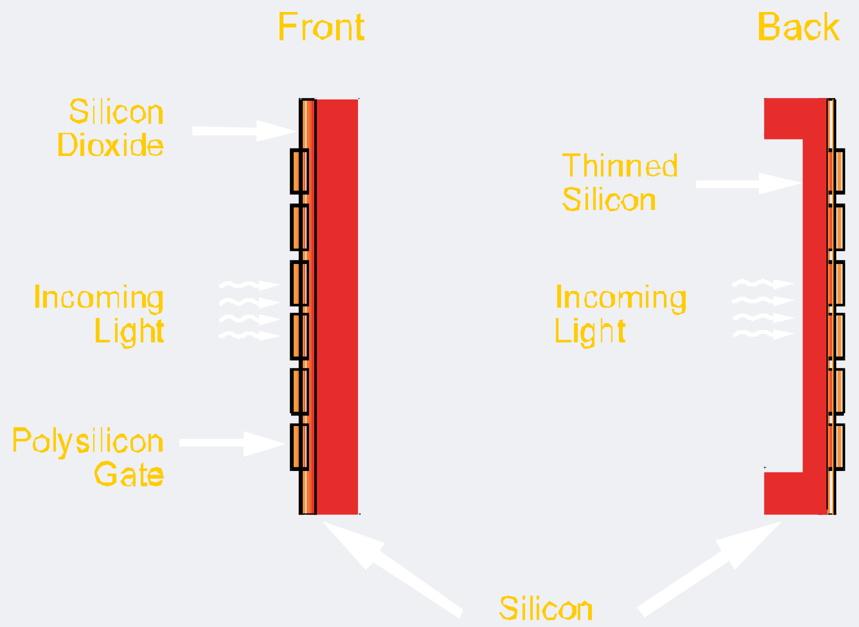
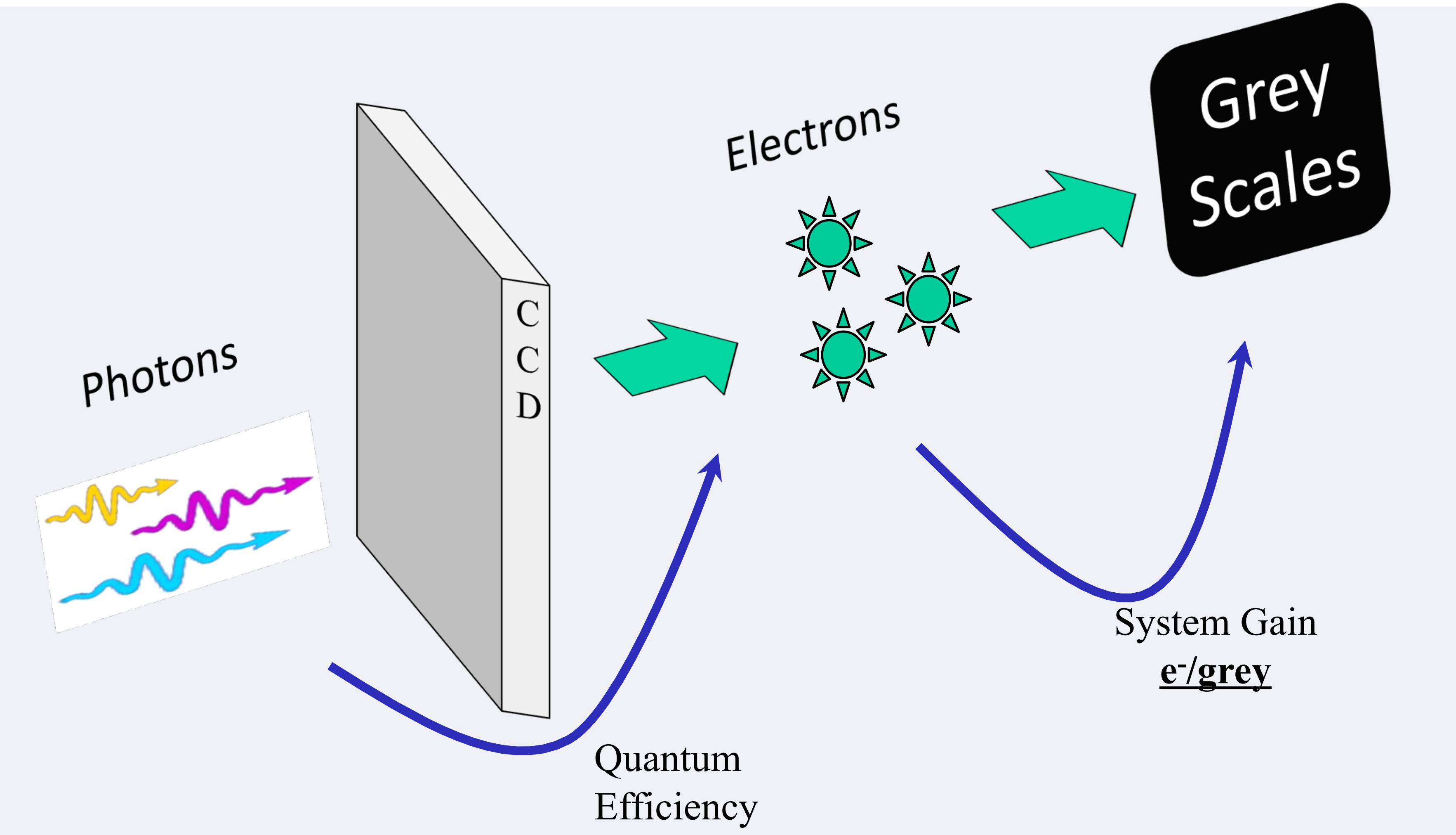
Front and Back illumination

- Some cameras are back thinned and back illuminated to be as efficient as possible with incoming light
- Typical front illuminated QE 40-60% at Lambda Max
- Typical Back illuminated QE 90% at Lambda Max



What is actually happening at each Pixel?



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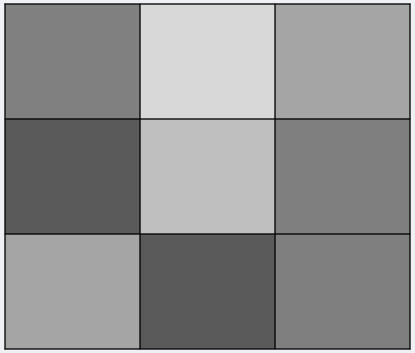
What is Noise ?

- Noise is uncertainty
- Noise is Plus or Minus
- Noise is driven by Statistics
- Noise can be calculated

•Noise is not background

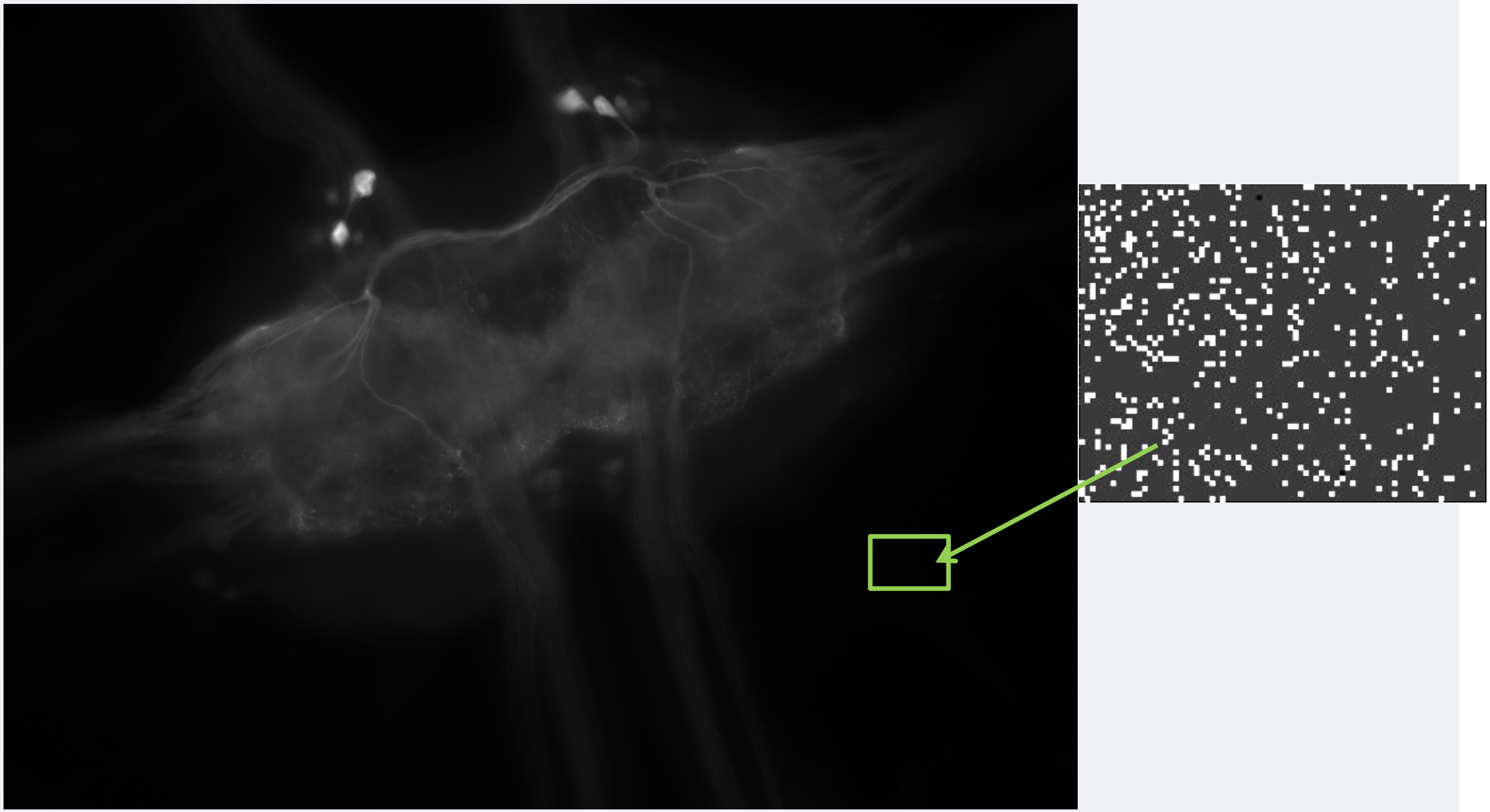
Standard Deviation is an easy way for us to measure noise.



8	12	6
6	10	8
10	6	8

Living with Noise

Noise exists on every camera and in every measurement



Dependent on the image scale used you may or may not see it.

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Why do we see noise ?

- We normally see noise when the signal we have is low in comparison to our required exposure

Reasons for trying to get a short exposure:

- Need to monitor at high speed
- Need to minimise sample damage
- Need to focus at live rate

- If you measure a signal of 100 electrons in one pixel and 102 in another, are they different values?

- Noise distorts measurements and increases the uncertainty in measurements.

Noise Sources

CCD systems suffer from 3 types of noise:

1. ***Dark Current*** – noise from heat and cosmic noise - exposure dependent

2. ***Read Noise*** – noise of reading the signal - fixed

3. ***Photon Shot*** – square route of signal - signal dependent

Other Noises

1. Excess Noise Factor – EMCCD
2. Clock Induced Charge – All but mainly observed in EMCCD
3. Random Telegraph Noise - CMOS

Signal to Noise

- Standard CCD SNR Equation:

- $SNR = [S*QE] \div \sqrt{[S*QE^2 + D + \sigma_R^2]}$

- S = Signal in Photons (converted to electrons by * QE)
- QE = Quantum Efficiency of light at that emission
- D = Dark Current Noise = Dark Current * Exposure Squared
- σ_R = Read Noise

- All values must be compared in electrons

Resolution: The Rules

1. Resolution is ultimately dependent on the N.A. of the objective or lens used

2. Microscope resolution in your camera is dependent solely on the size of the pixel

3. Number of Pixels can affect resolution in non-microscope applications

4. Dynamic range plays a significant role in resolution

5. How big a field of view you see is determined by size of the chip

6. DPI is only an output resolution. This number represents the resolution of a printed image

Optical Resolution

$$d(\mu m) = \frac{1.22 * \text{Wavelength } (\mu m)}{NA_{obj} + NA_{Cond}^{*}}$$

* Fluorescence use ($2*NA_{obj}$)

Fluorescent App: FITC Emission

Example 1: Plan Apo 60x oil (NA 1.4)

$$d(\mu m) = \frac{1.22 * .510}{1.4 + 1.4} = 0.22\ \mu m$$

Example 2: Plan Fluor 10x dry (NA 0.3)

$$d(\mu m) = \frac{1.22 * .510}{0.3 + 0.3} = 1.037\ \mu m$$

Magnification Factor

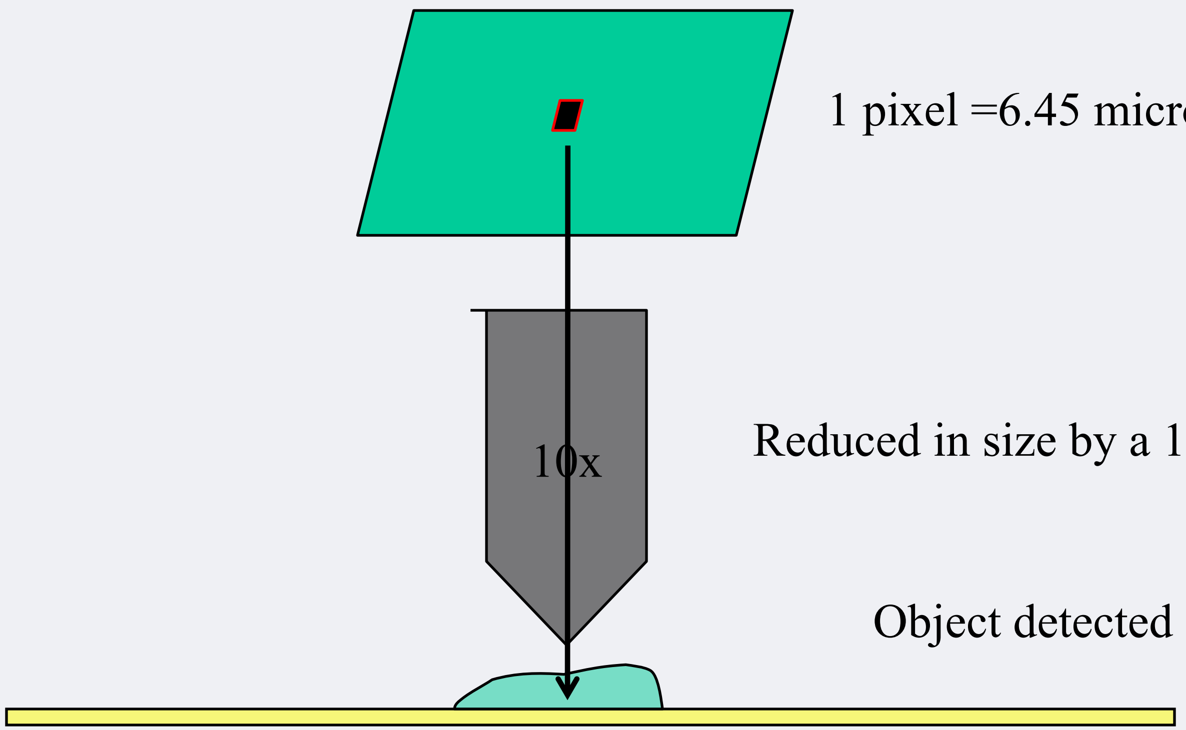
- How big is our pixel in the Object space?

Calibrated pixel size = Pixel size / Total Magnification

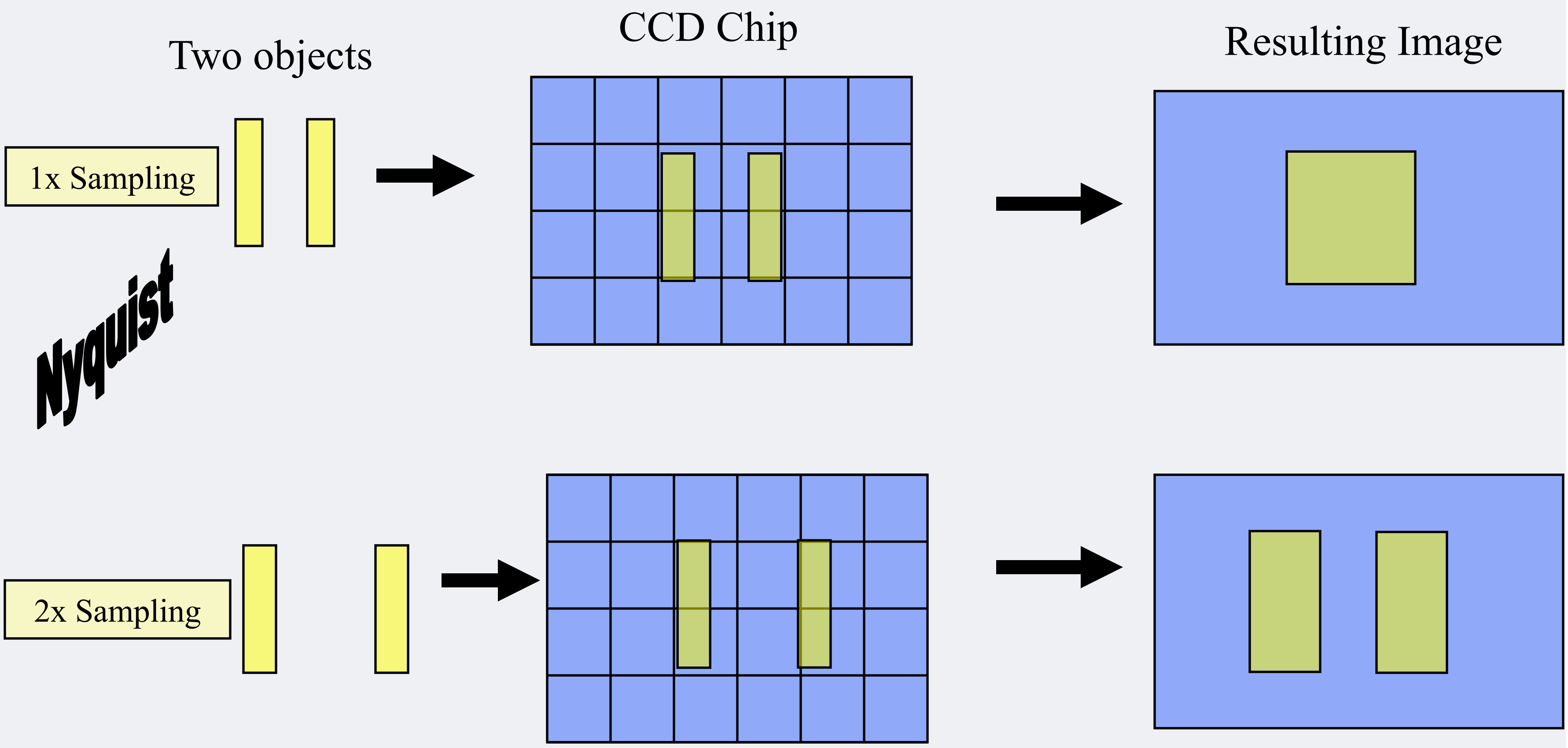
Easy Maths: 60x objective, Sony 285 senor

Calibrated pixel size = 6.45 µm / 60x = **0.1075 µm/ pixel**

What is the area covered by an objective?



Sampling Requirements



Does our Camera match the Resolving power of our 60x 1.4 NA objective?

Rayleigh Criterion: Plan Apo 60x oil (NA 1.4) = 0.22 µm

Nyquist: requires a sampling interval equal to twice the highest specimen spatial frequency

Pixel size in Object Space = 6.45 µm / 60x = **0.1075 µm/ pixel**

Correct Sampling = 0.1075 x 2 = 0.215 **µm**

Gain

- Gain is a way of amplifying signal relative to the image scale allowing users to lower the exposure time to achieve the same grey scale values

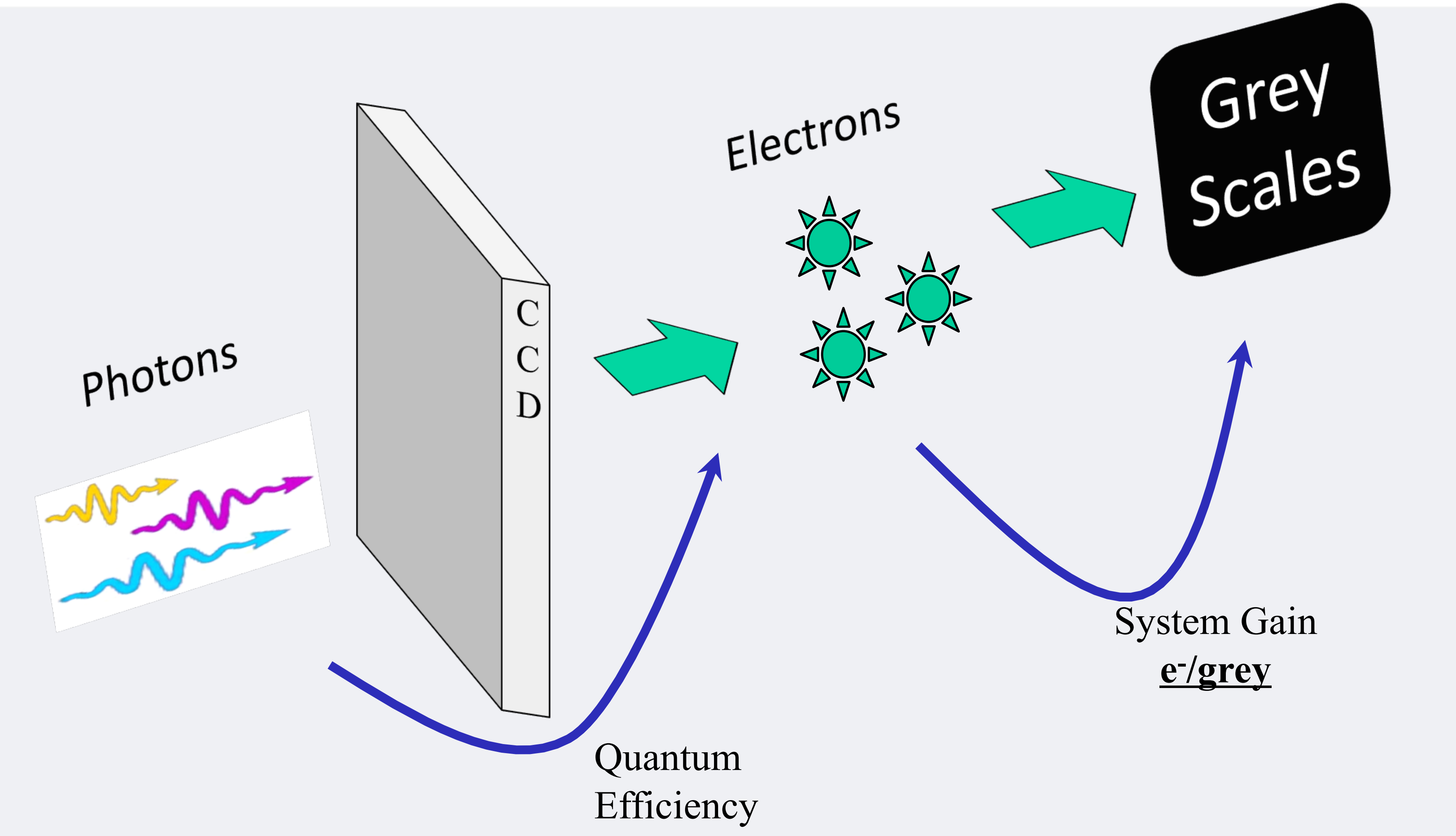
- Gain really can be thought of as electrons per ADU

- ADU = grey scale value

- Gain is thought to increase noise - this is not necessarily true as noise does not really change, but the grey scales which represent it do increase

Increasing gain effectively lowers dynamic range

What is actually happening at each Pixel?



- System Gain = Single Pixel Full Well (e^-) / Bit Depth (ADU)

Single Pixel Full Well = 16,000 e^-

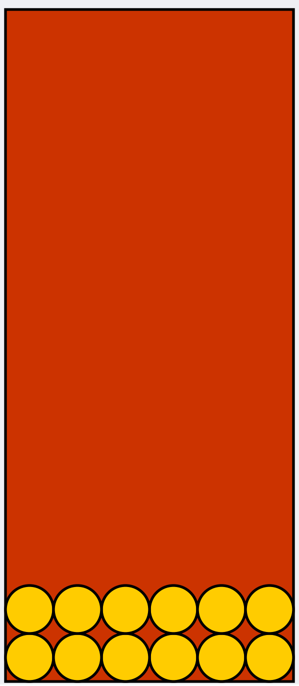
System Gain = 16,000e^- / 4,095ADU

A/D Converter Bit Depth = 4,095

1x Gain = 4e^- /ADU
using 12 bit A/D

What would it be for
a 14 bit camera?

Full Well = 16,000 e^-



4 electrons = 1 ADU

A/D Converter Bit Depth = 4,095

1x Gain = $4e^-$ /ADU

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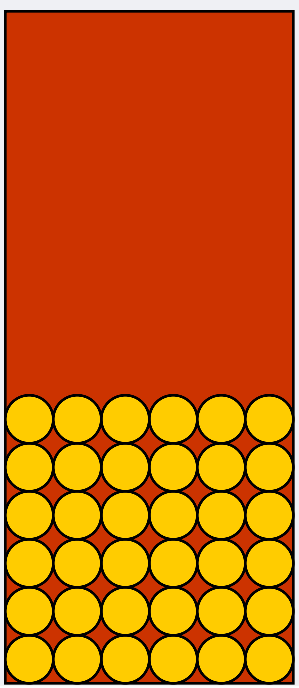
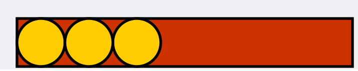
Full Well = 16,000 e^-



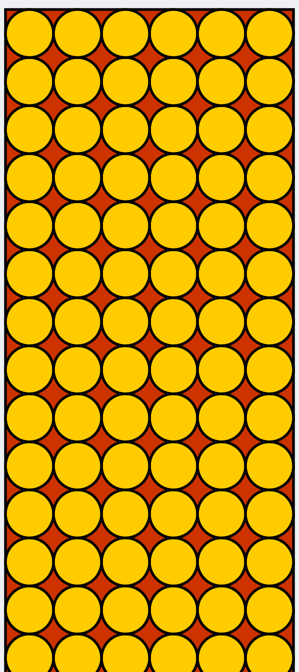
This $4e^-$:1ADU ratio continues until both the CCD Full Well and the A/D converter are filled completely and at the same time.

A/D Converter Bit Depth = 4,095

1x Gain = $4e^-$ /ADU



Full Well = 16,000 e^-

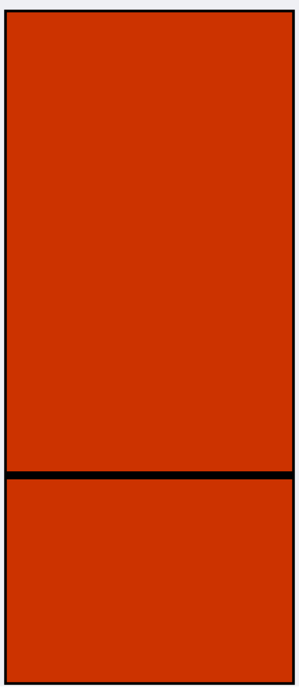


When the CCD and A/D are full, the system has reached Full Well and the A/D limit.

A/D Converter Bit Depth = 4,095

1x Gain = $4e^-$ /ADU

4x User Gain



4x gain effectively lowers the full well of the CCD by 1/4. In this example, the CCD's effective full well is now 4,000 electrons.

4x single pixel Full Well = 1x single pixel Full Well /4

$= 16{,}000^{-} /4$

$= 4{,}000e^{-}$

A/D Converter Bit Depth = 4,095

4x Gain = $1e^{-}$/ADU

4x User Gain

Full Well = 4,000e^-

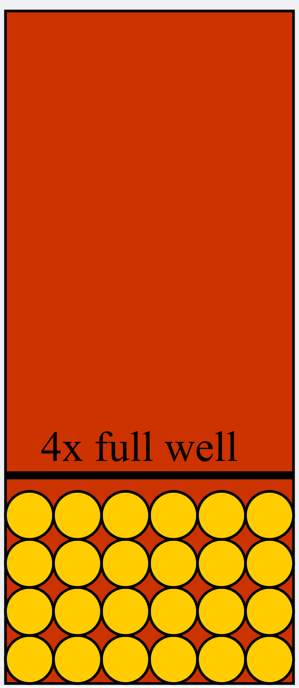
Now, at 4x gain, 1 electron will produce 1 ADU.

A/D Converter Bit Depth = 4,095

4x Gain = 1e^-/ADU

4x User Gain

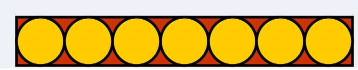
Full Well = 4,000e$^-$



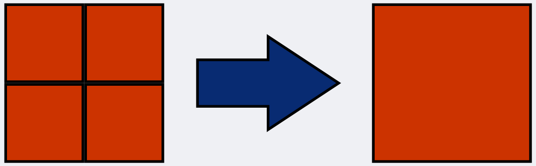
This 1e$^-$:1ADU ratio continues until the A/D converter has reached its limit.

A/D Converter Bit Depth = 4,095

4x Gain = 1e$^-$/ADU



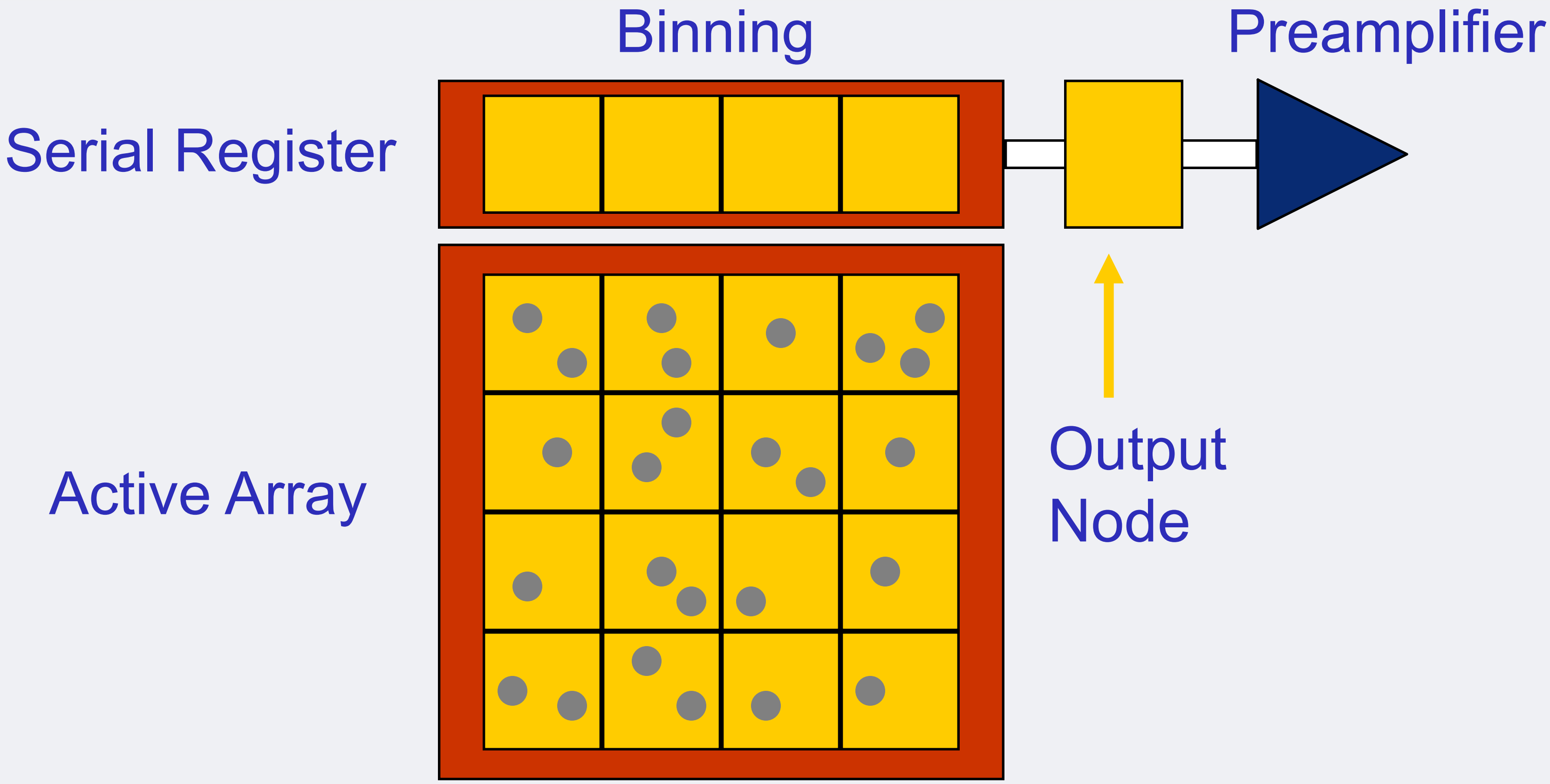
Binning



- Higher Dynamic Range
- Higher Signal-to-Noise Ratio
- Faster Readout
- Dynamically Change Pixel Size/Aspect Ratio

Above all gained at the expense of Spatial Resolution!!

CCD Fundamentals



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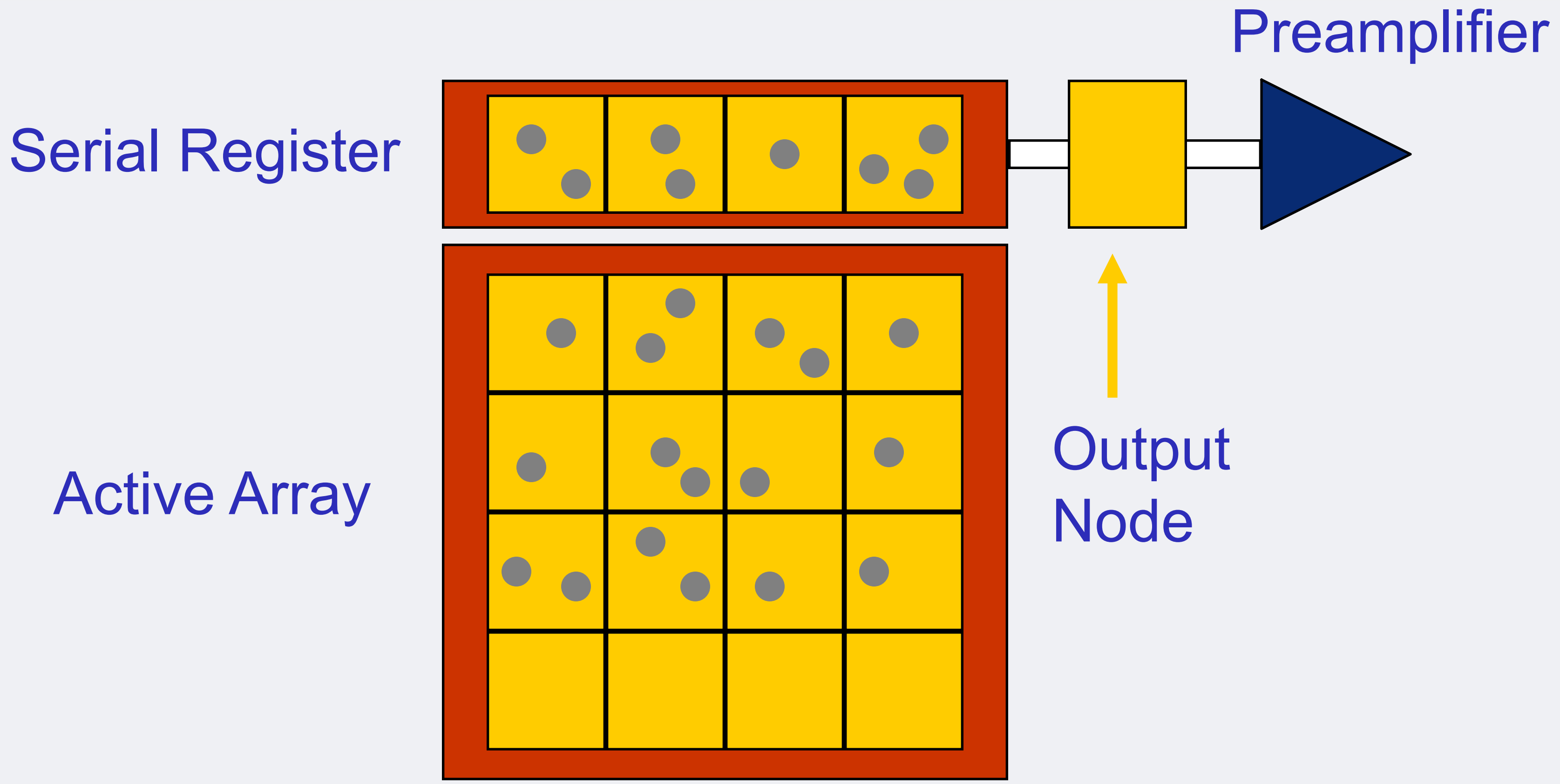

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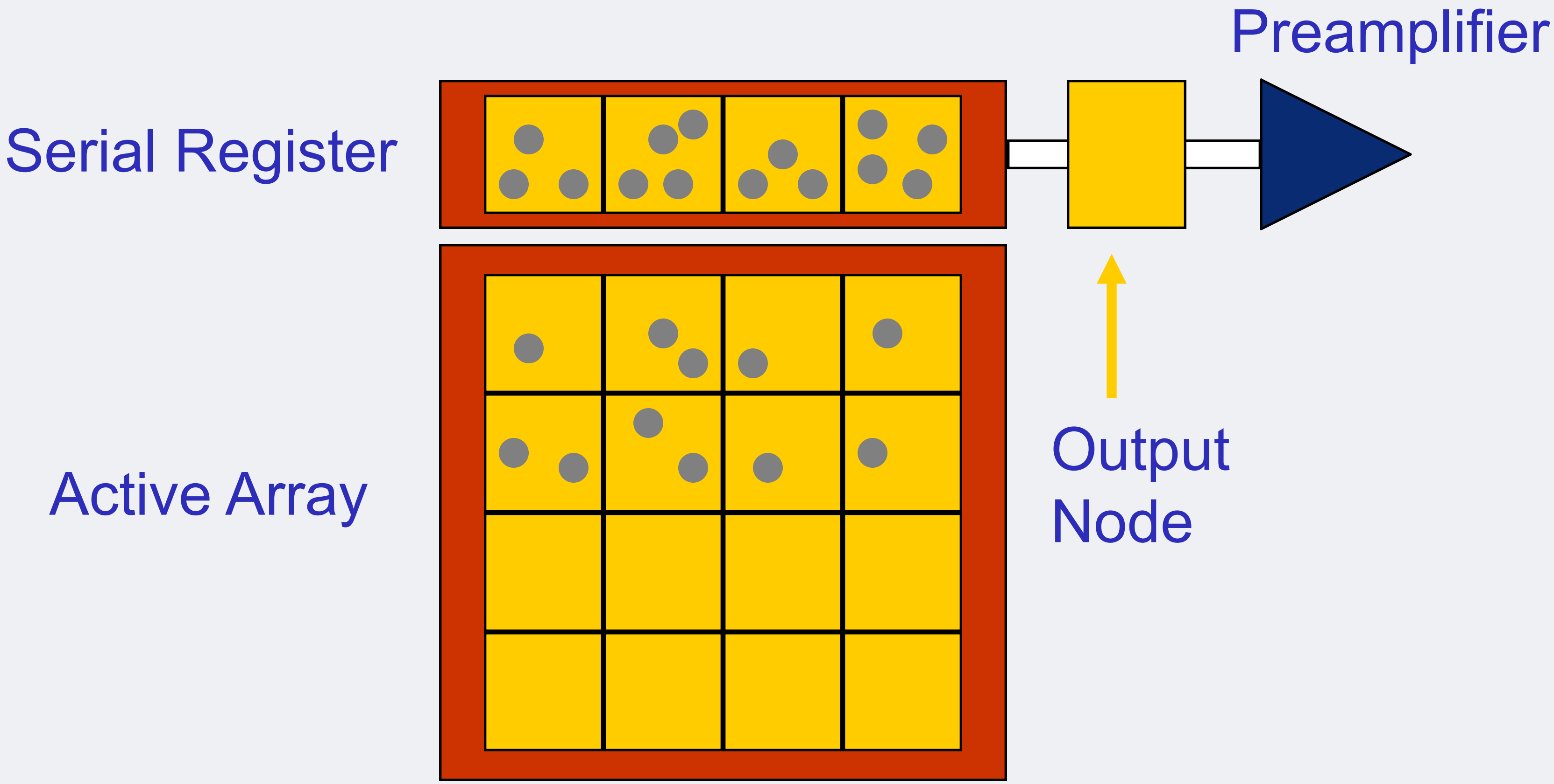

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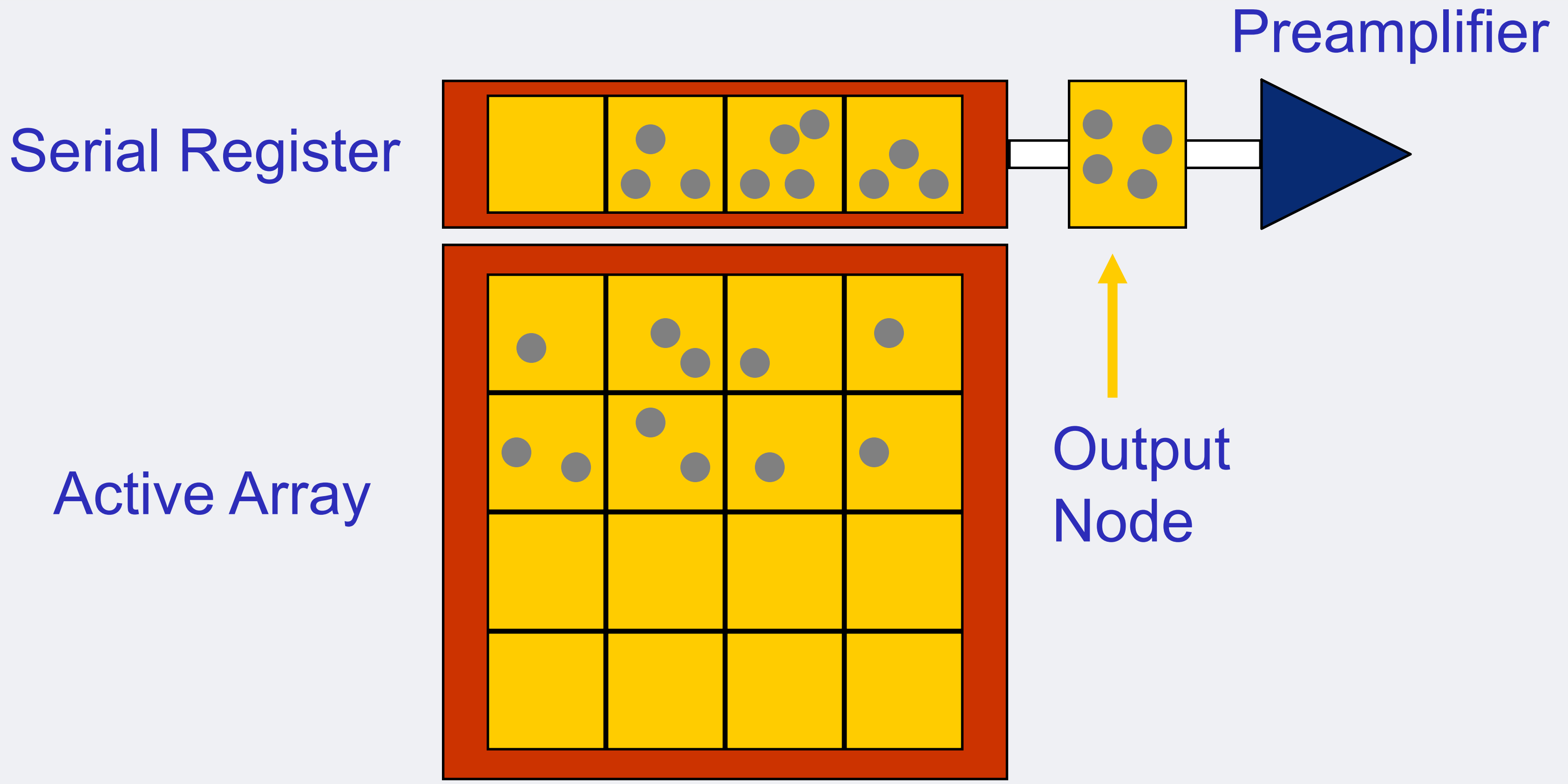

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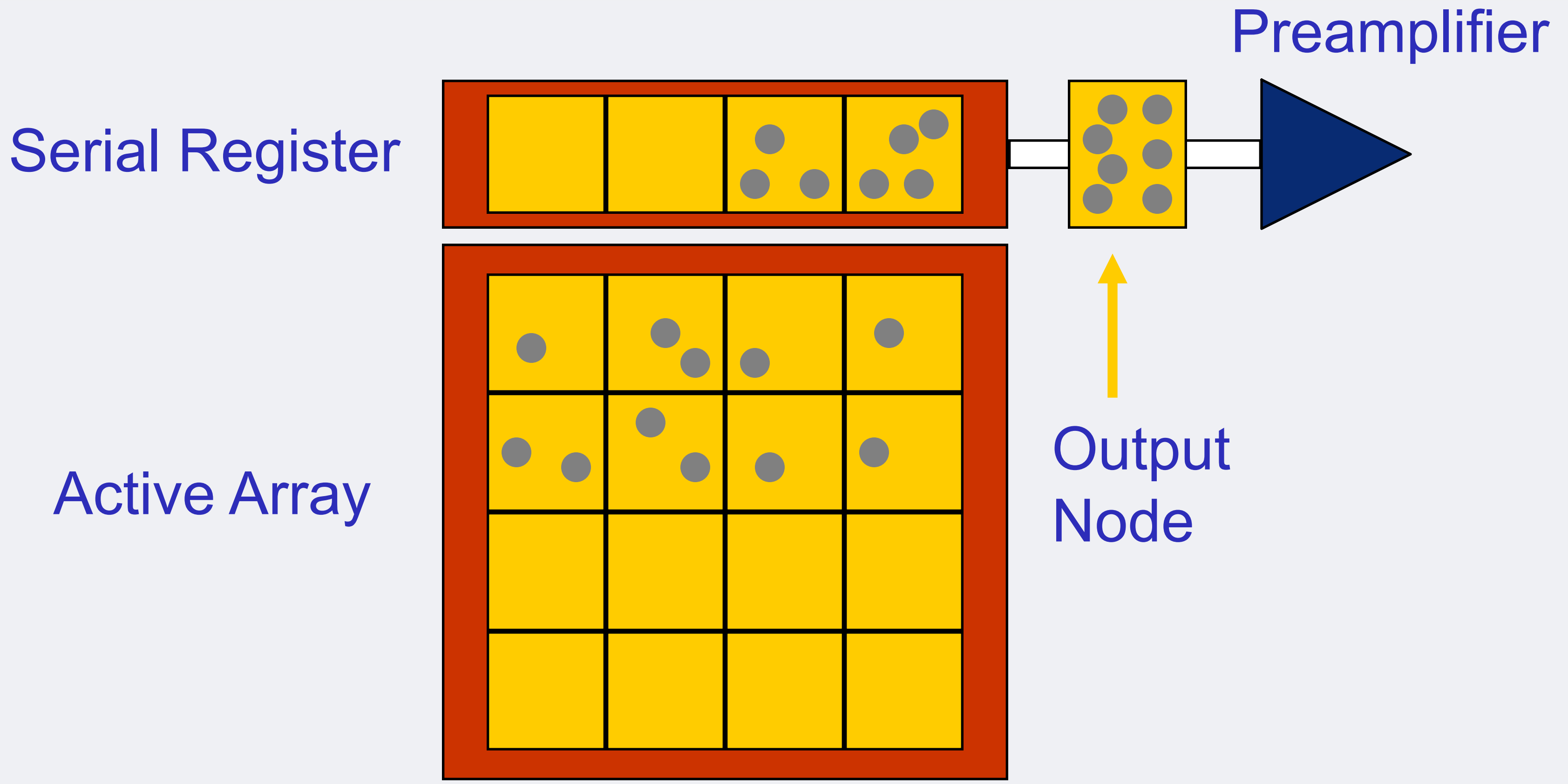

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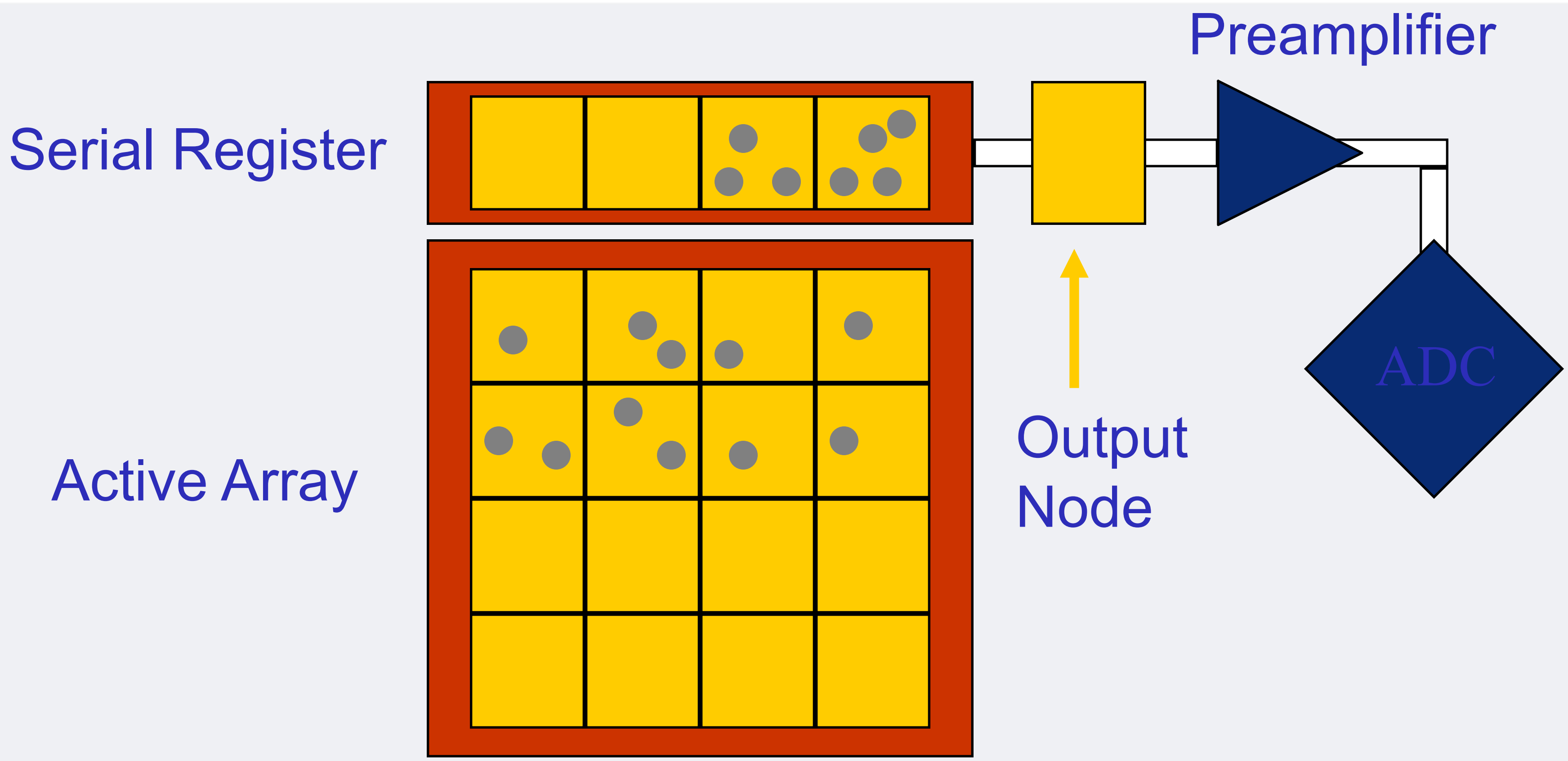

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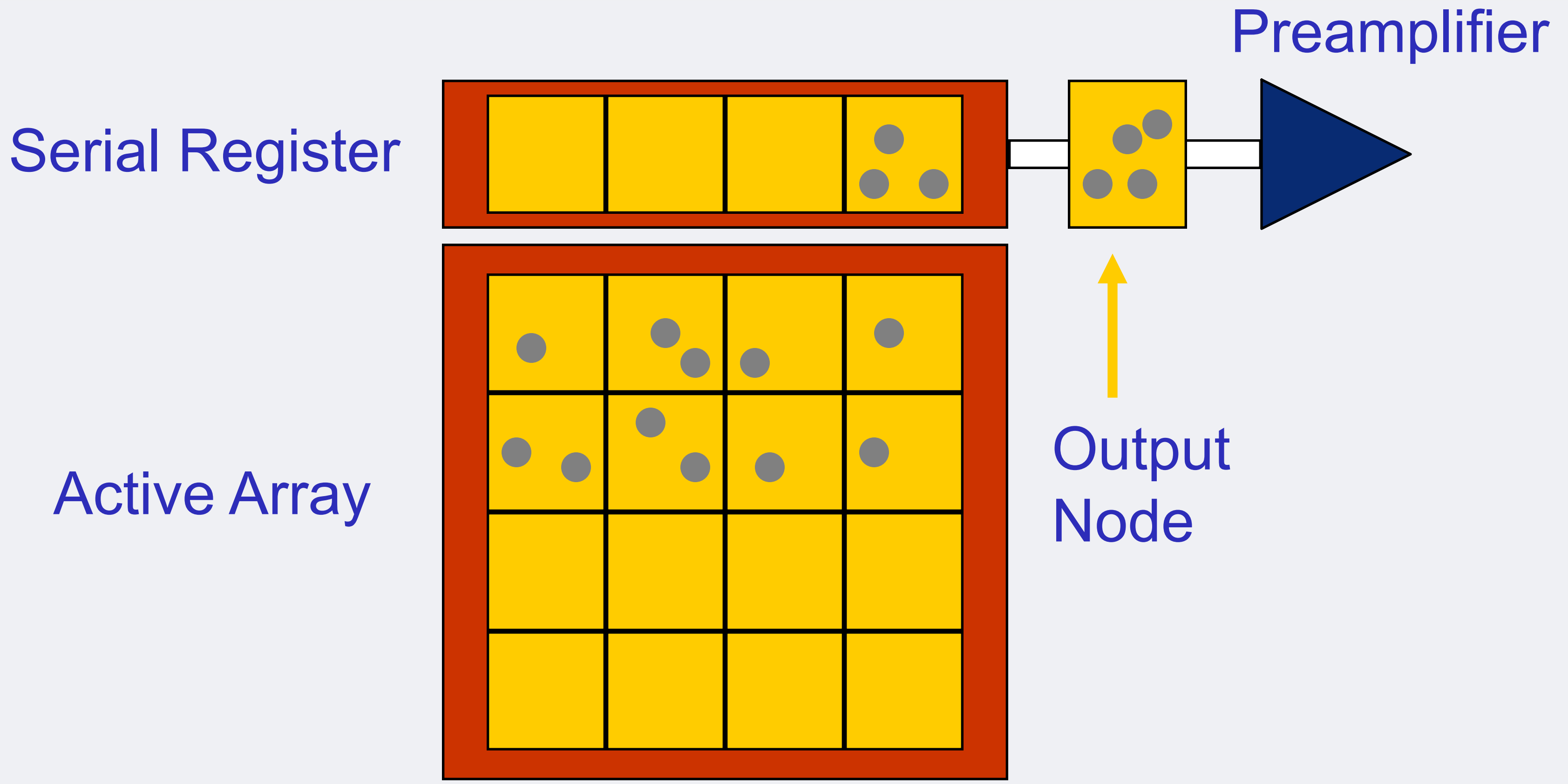

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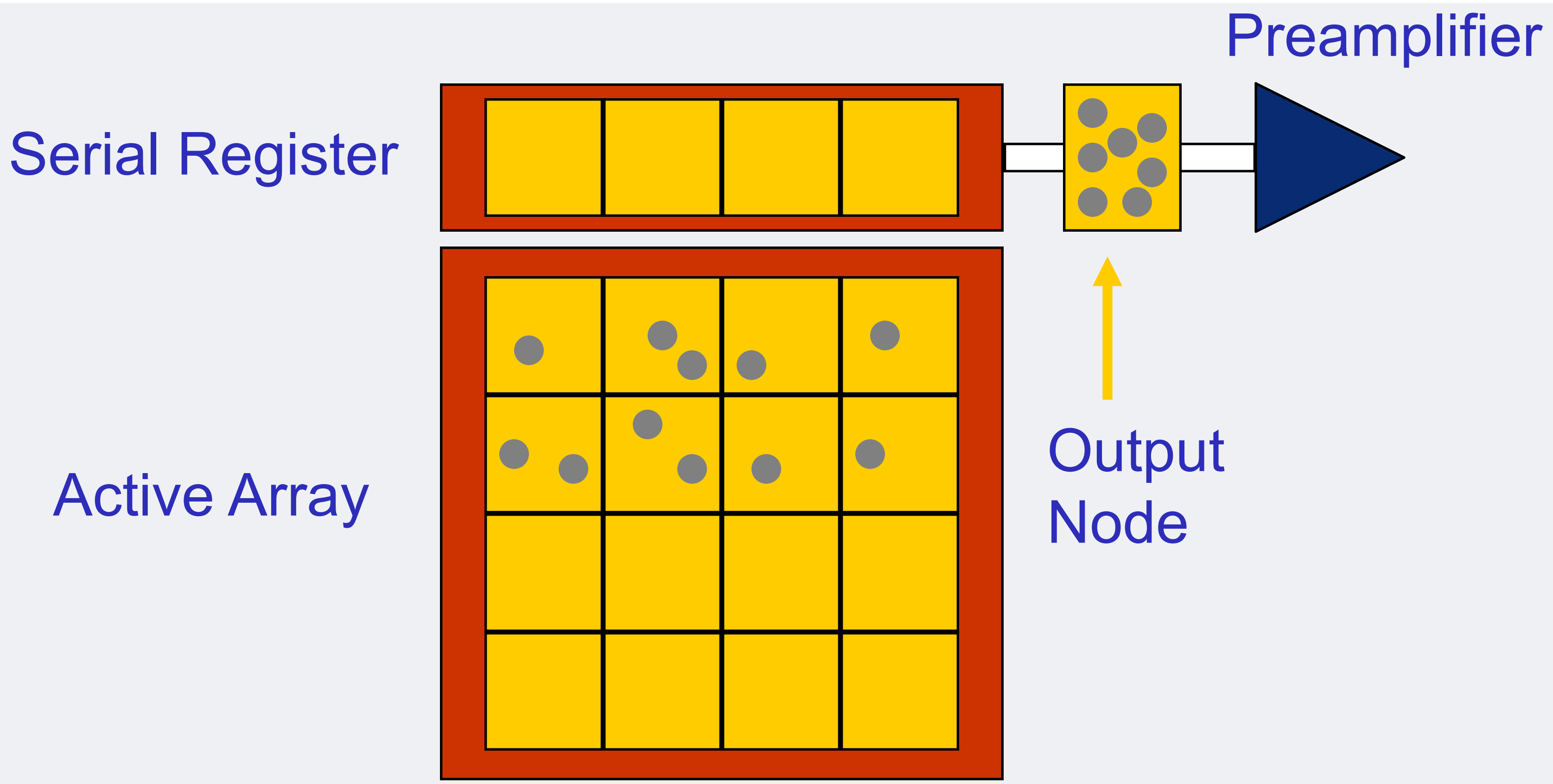

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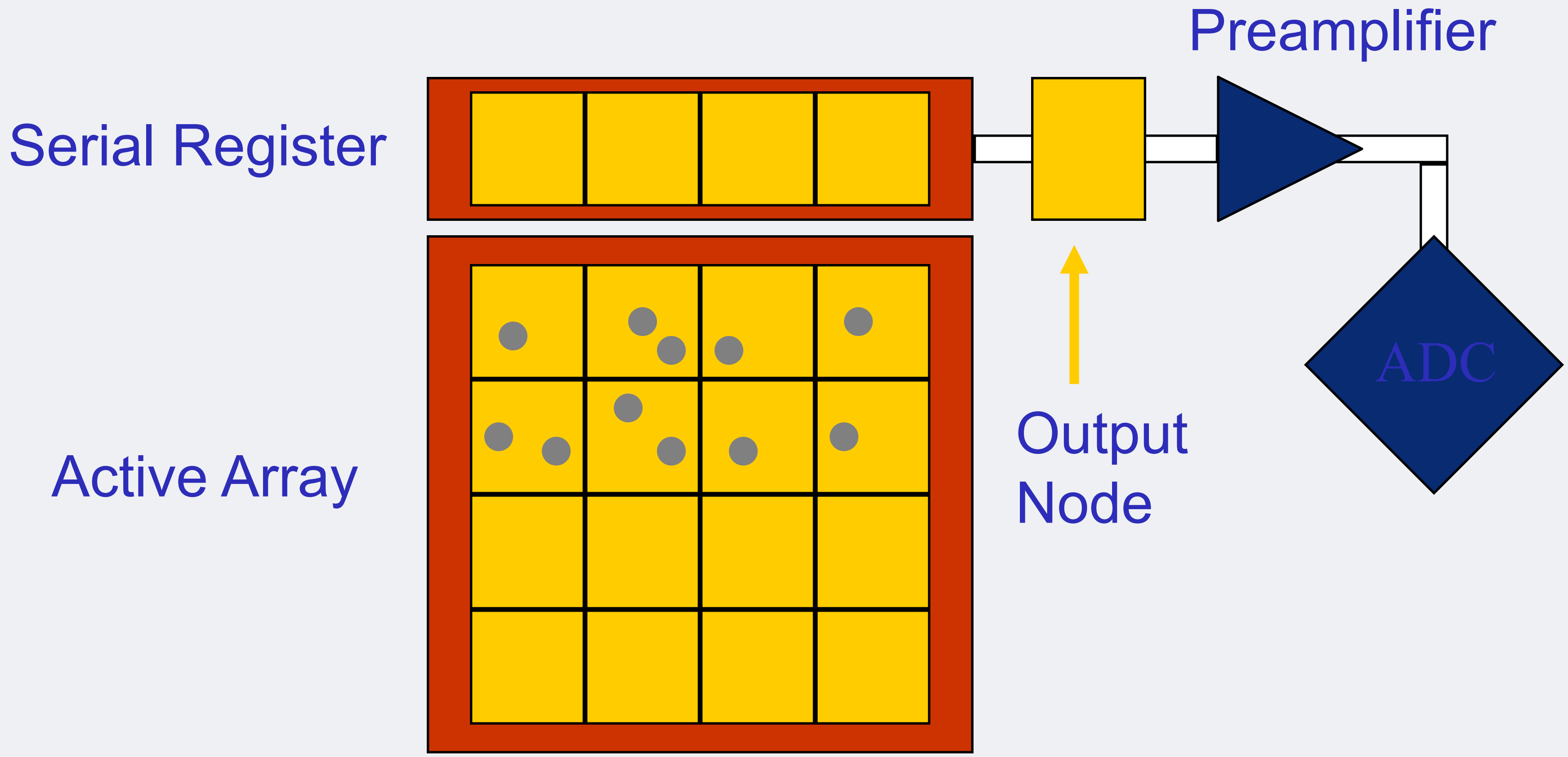


EMCCD

EM CCD – Electron Multiplied CCD sensors have been in place for over 15 years ago and are used for scientific, military and surveillance applications

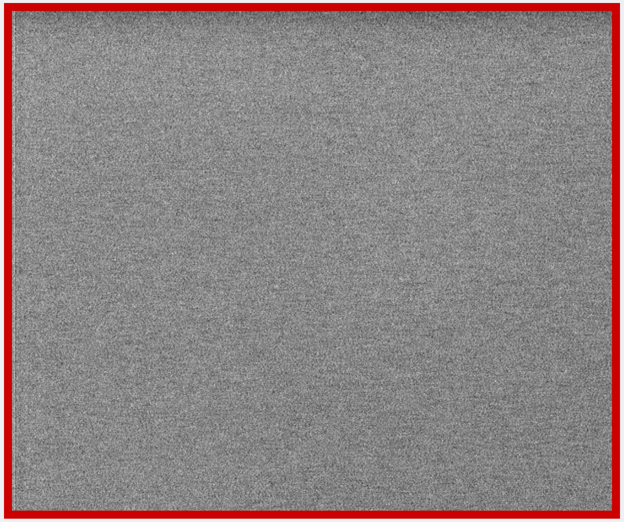
Photometrics introduced the first scientific grade camera (Cascade 650) in 2000 to enable customers with low light to achieve higher speed dynamic imaging

Based on CCD technology, the advancement comes from the addition of an Electron Multiplication register enabling higher signals to be achieved relative to the fixed camera noise - Read Noise

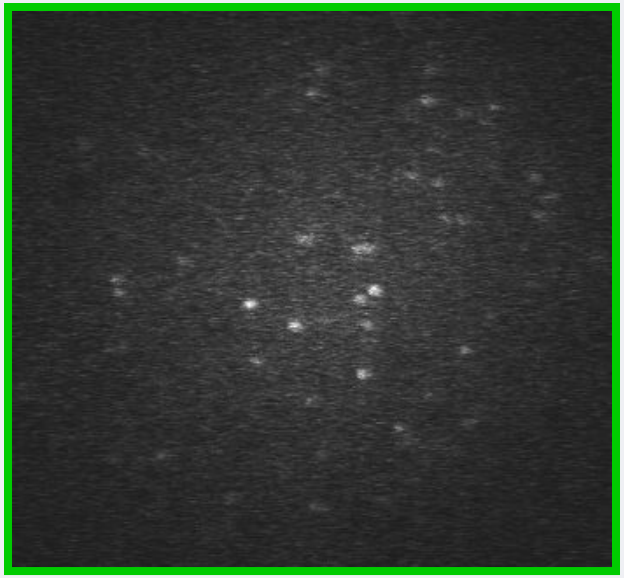
The Read Noise Limitation

The low-light level applications are read noise limited

i.e. the signals below the read noise cannot be seen



Read noise limited



By minimizing the read noise

Example: single molecule fluorescence

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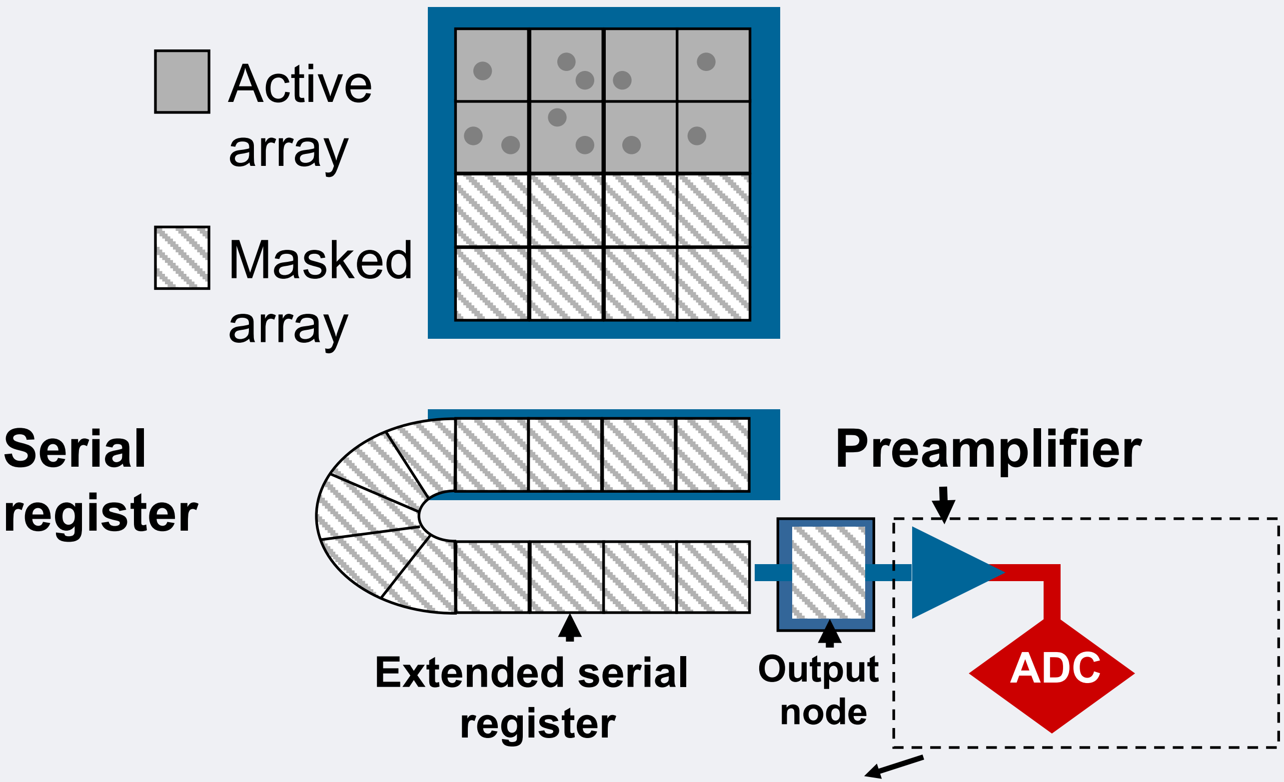
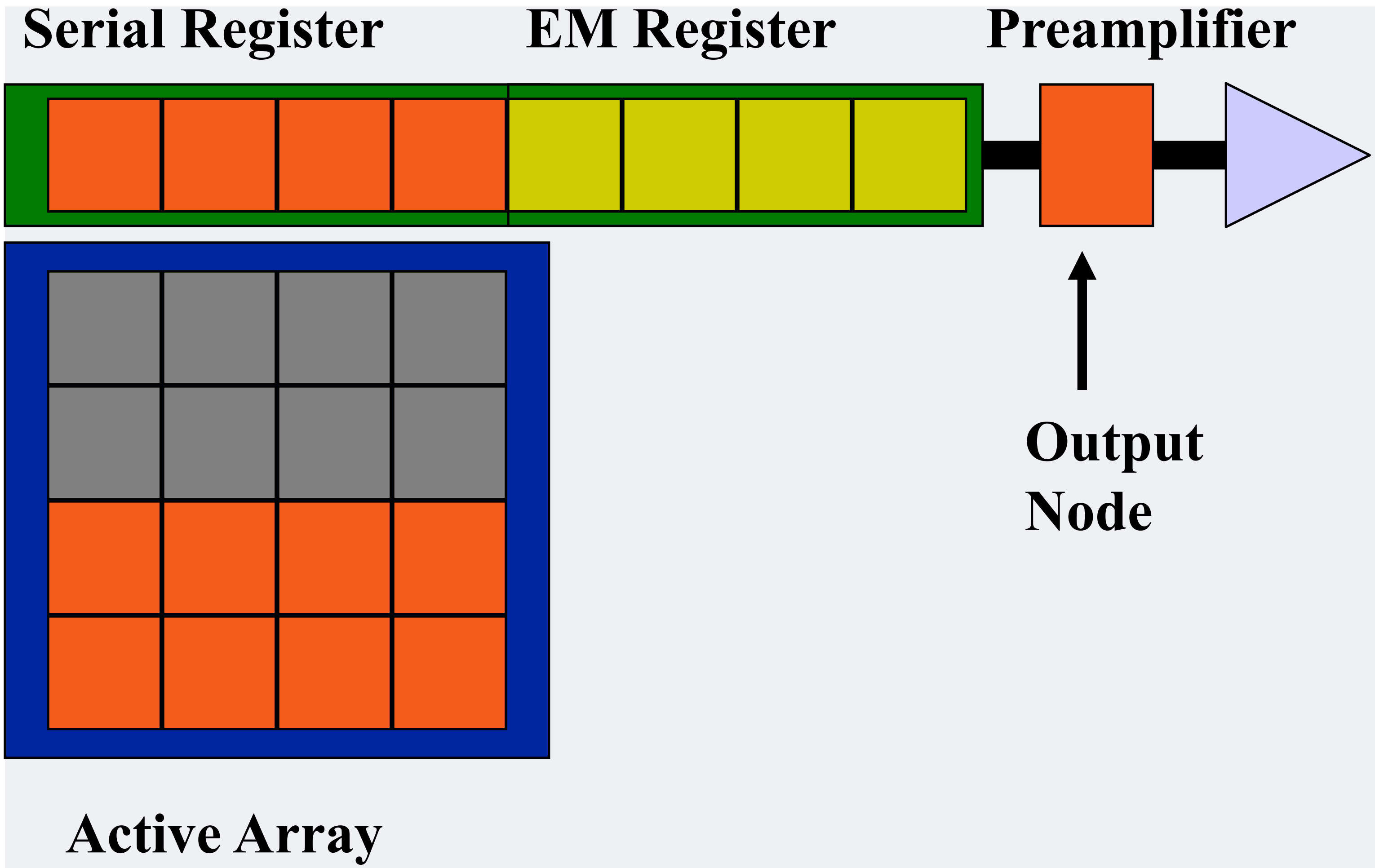
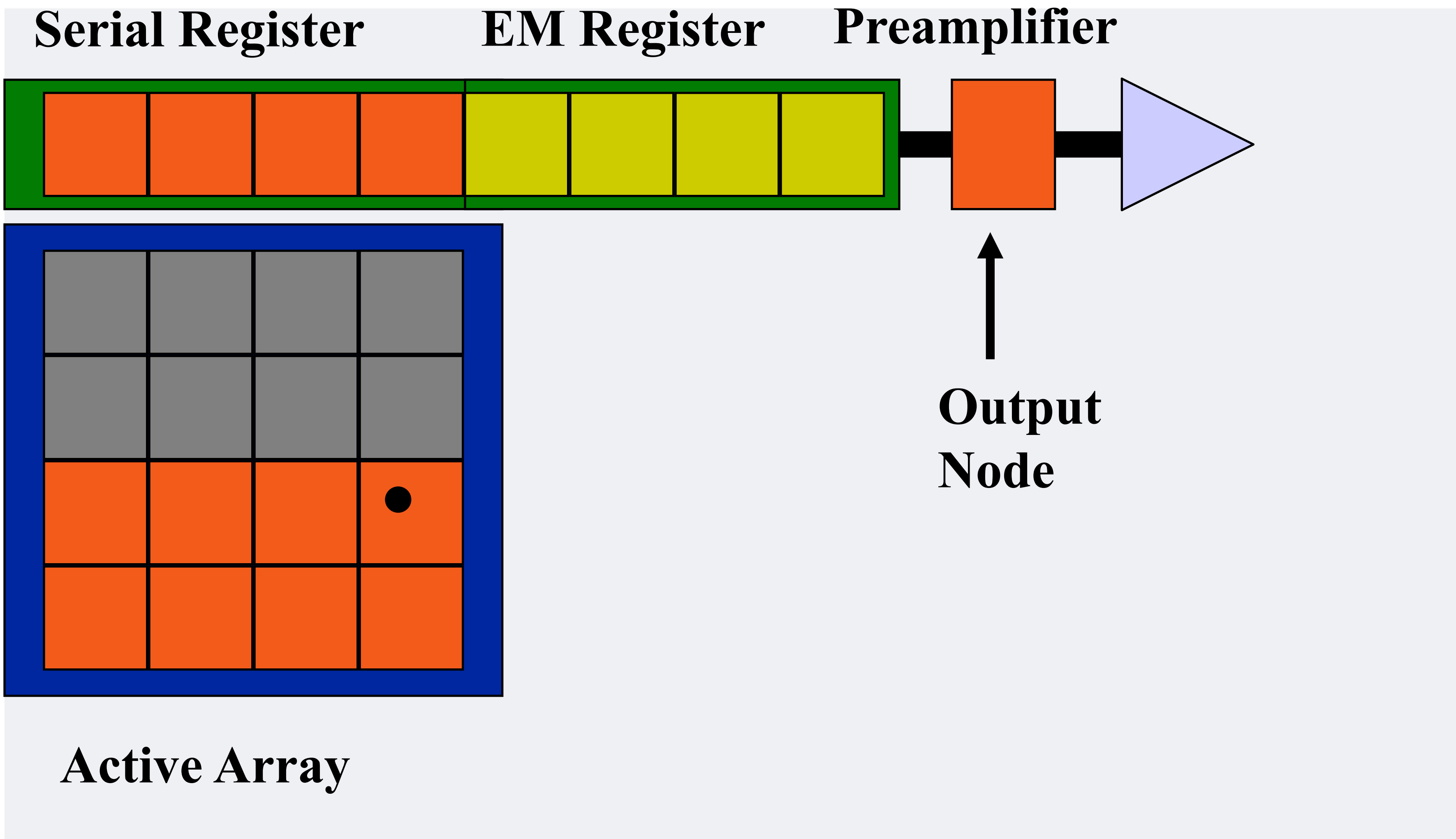
Theory of Operation

On-chip multiplication gain CCD



Pre-amp/electronics noise is effectively overcome by multiplying the signal


Serial Register
EM Register
Preamplifier
Output Node
Active Array



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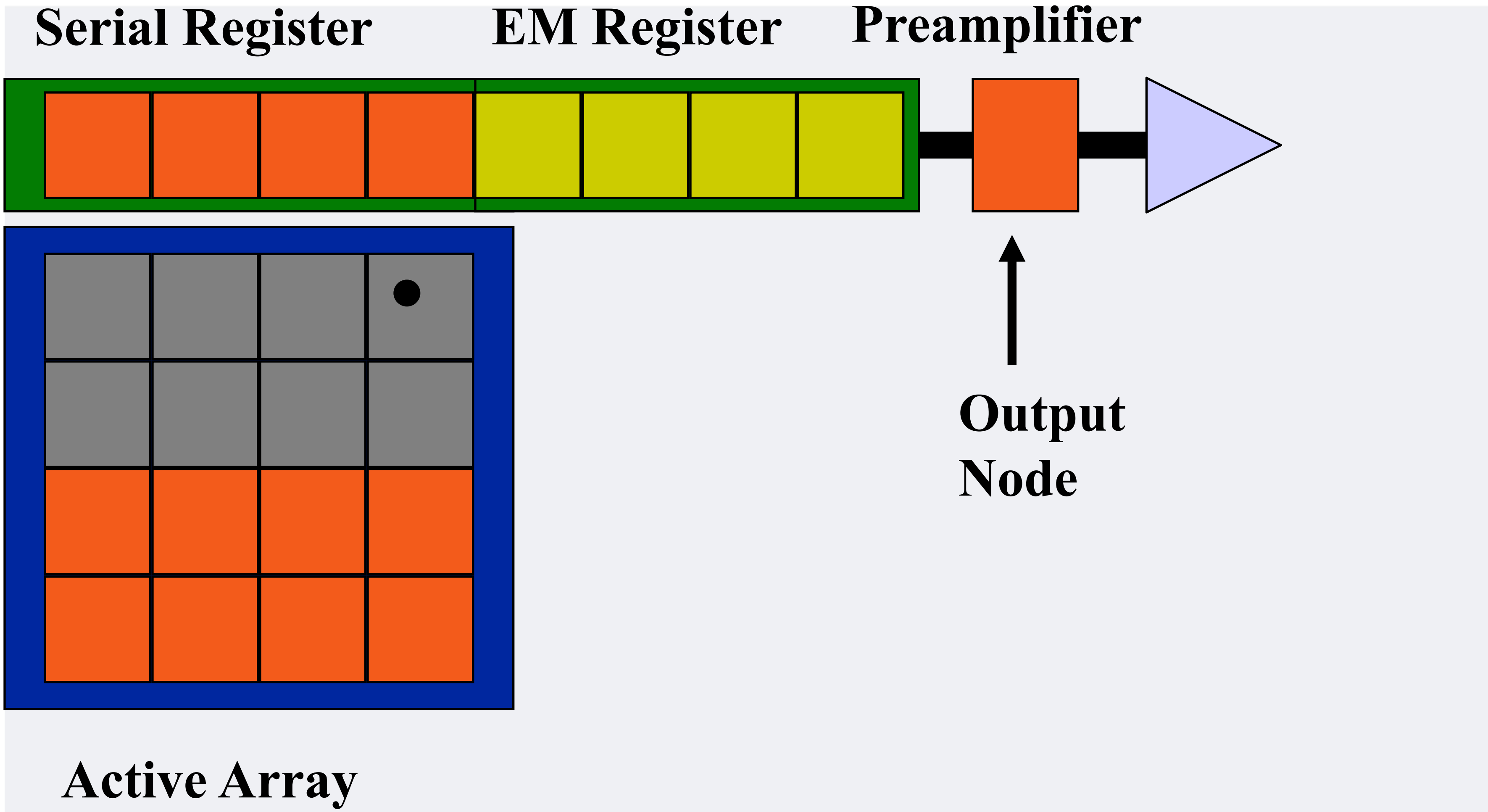
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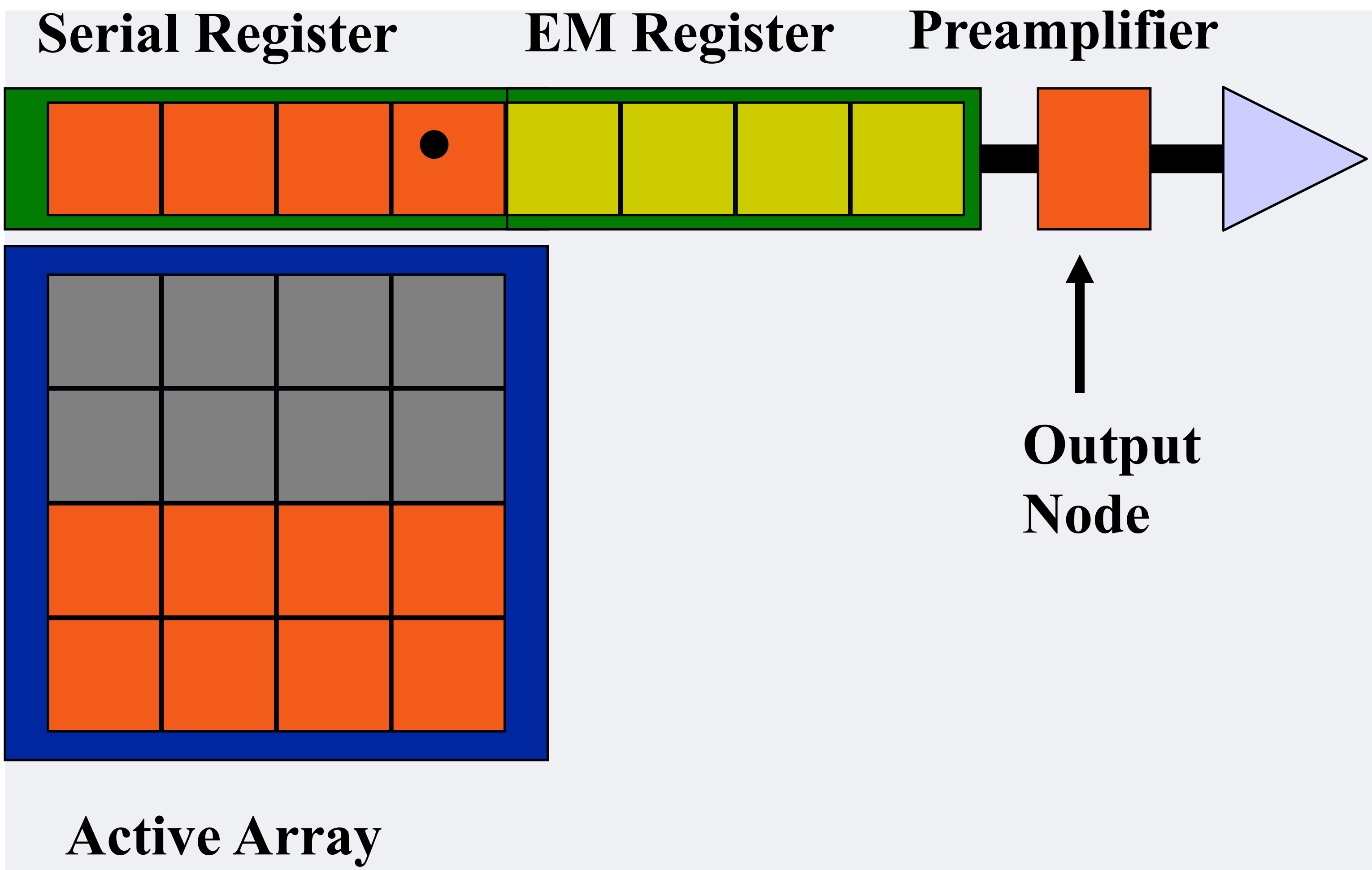
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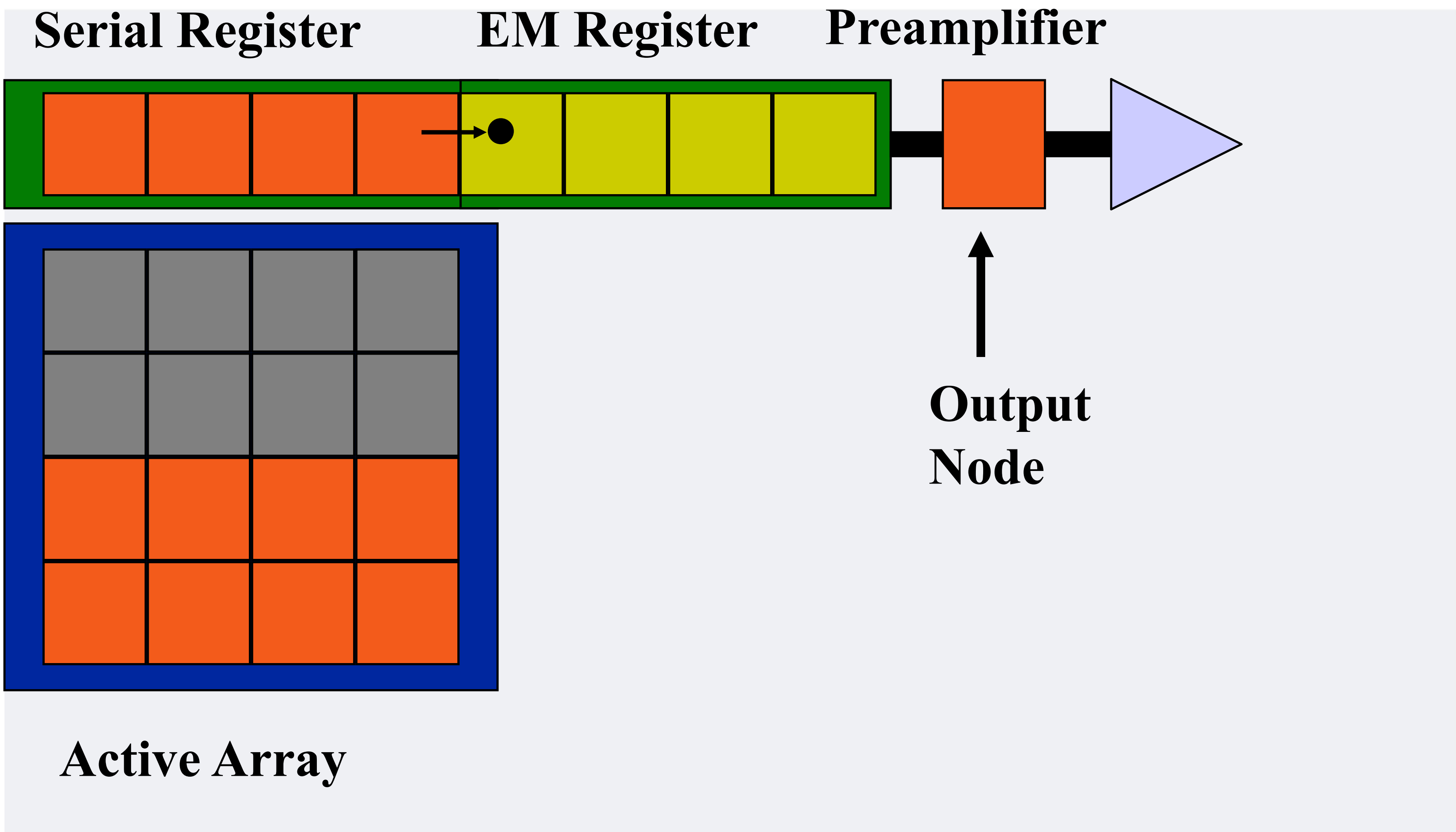
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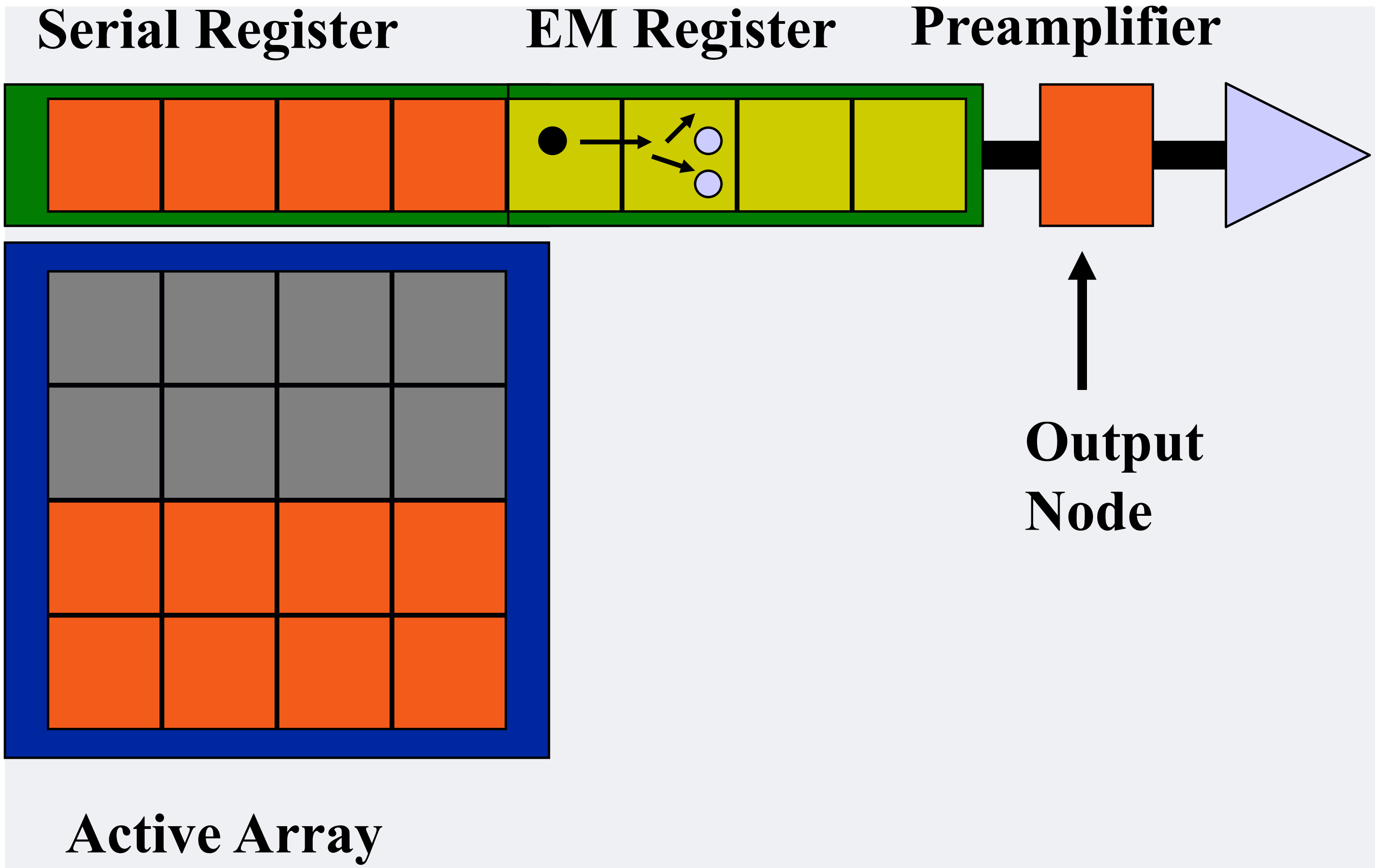
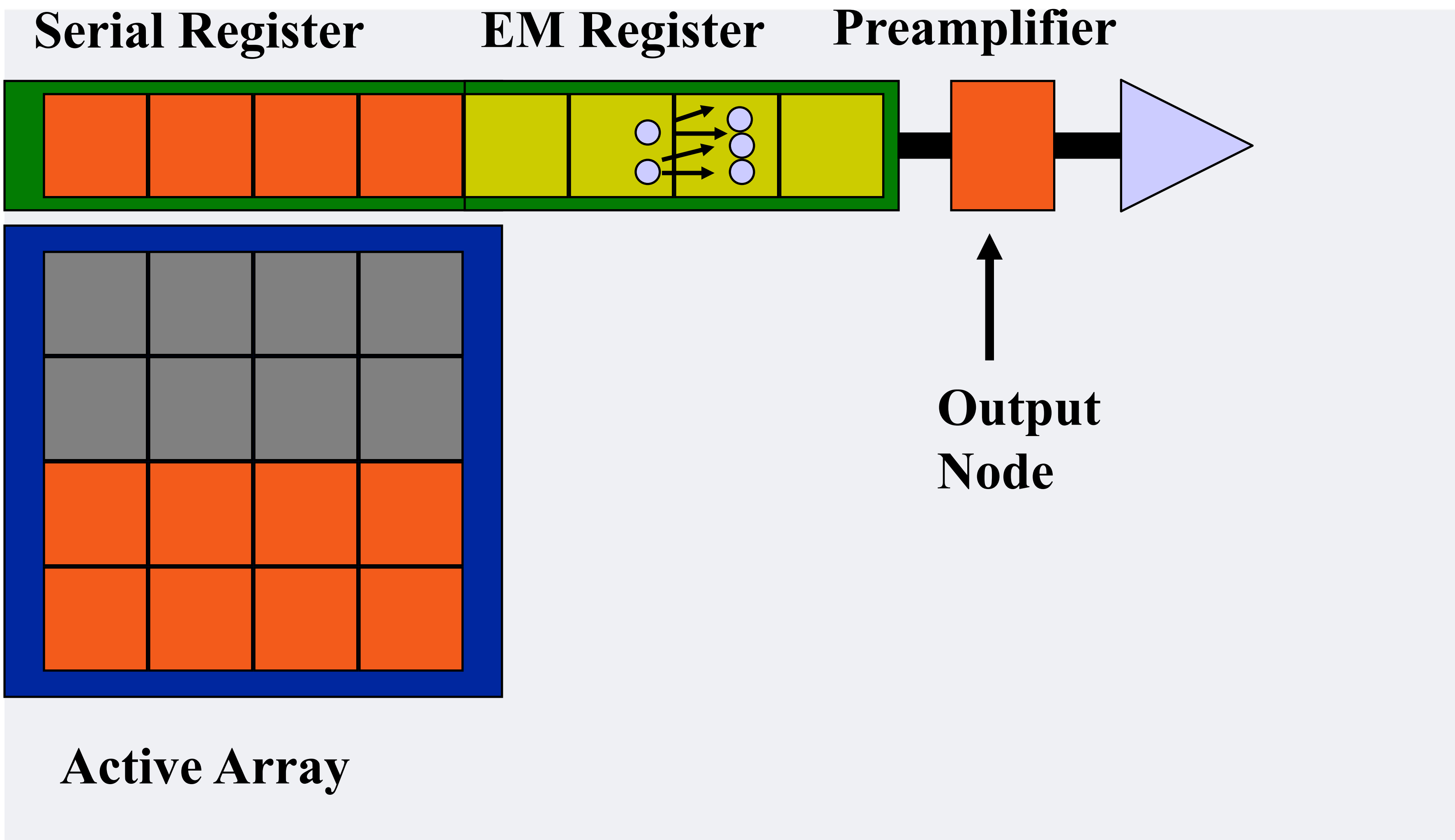

Serial Register
EM Register
Preamplifier
Output
Node
Active Array



Serial Register
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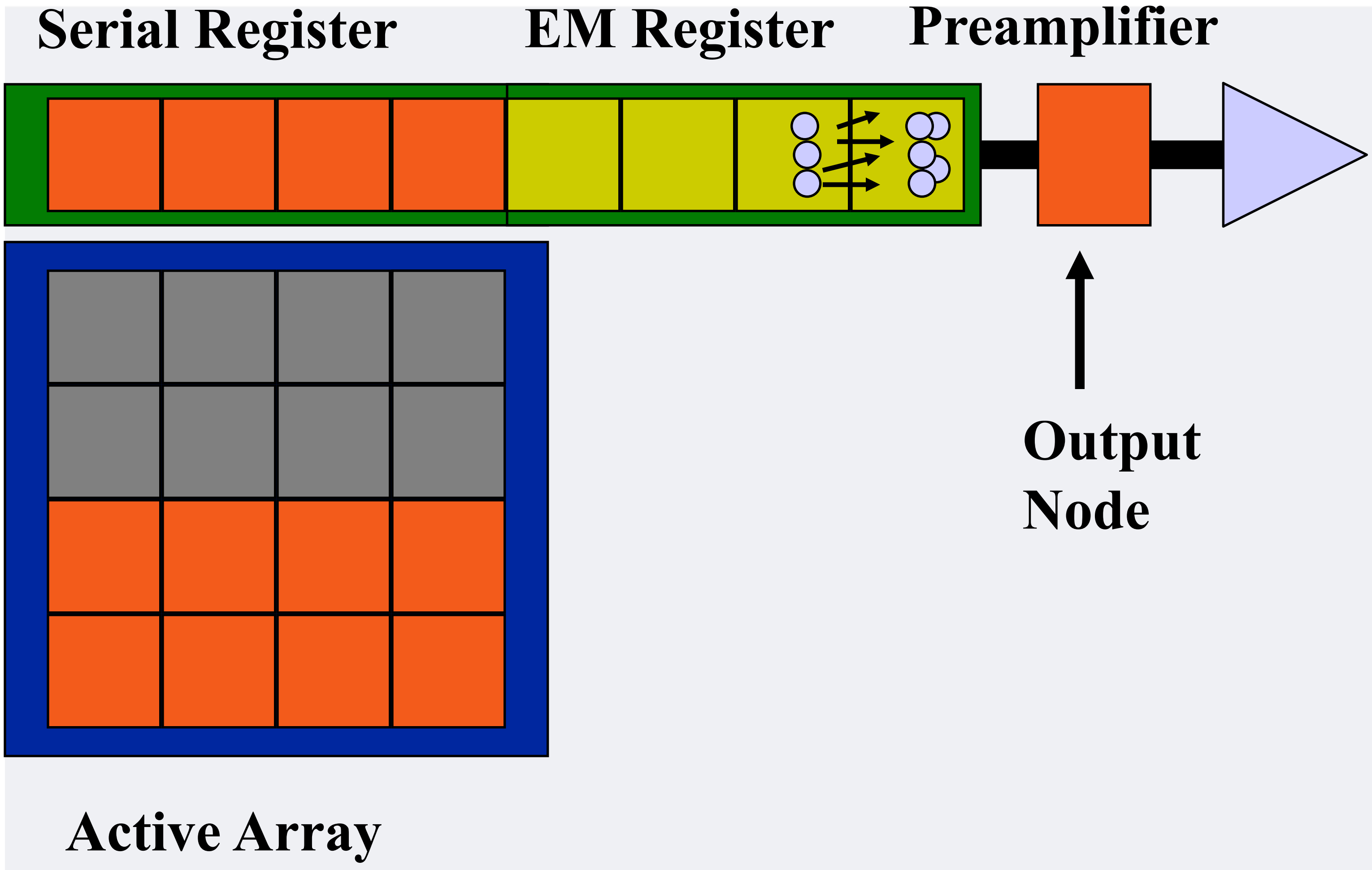

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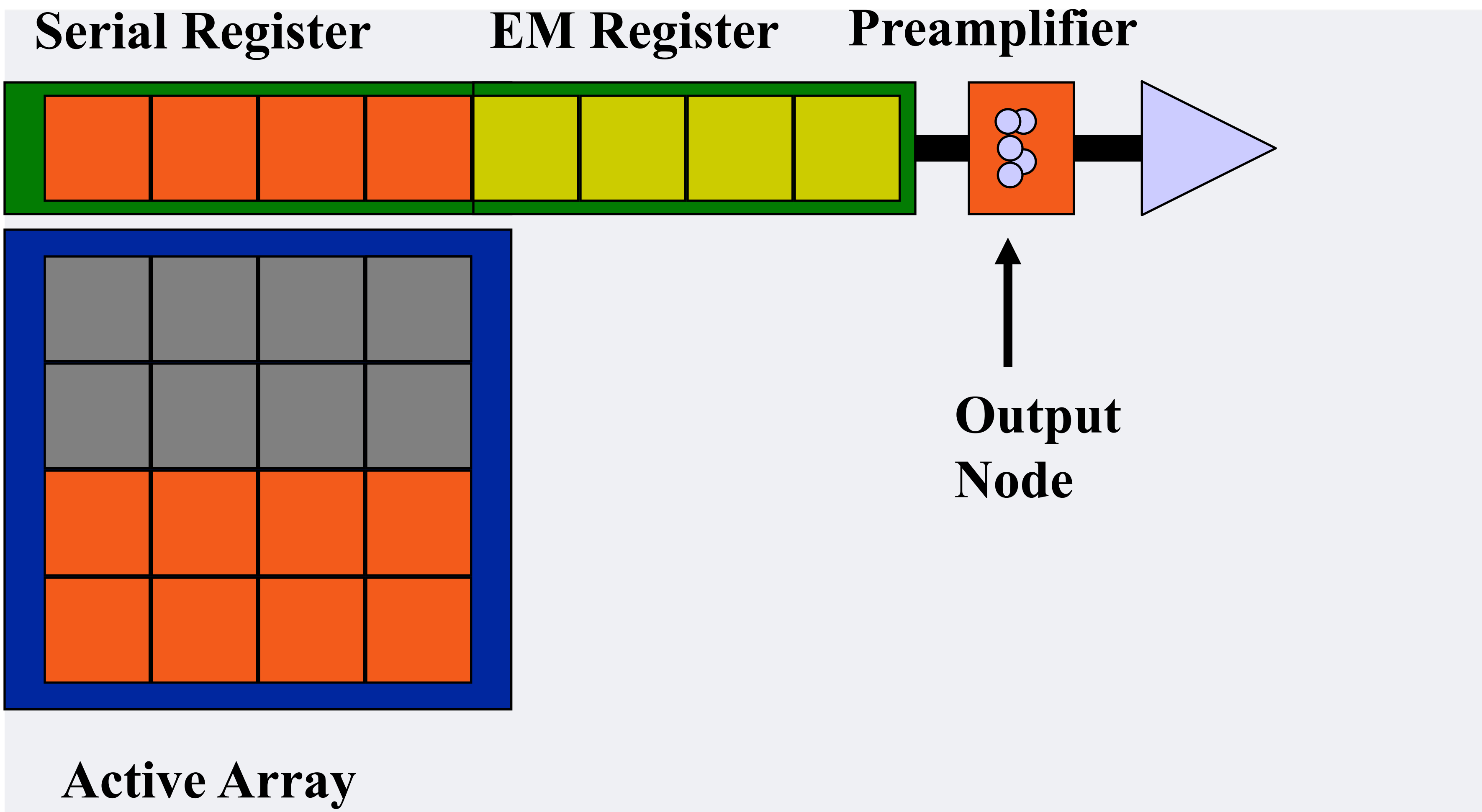

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What is the Excess Noise factor?

- The process of impact ionization is NOT FREE!!
- There is an inherent unpredictability factor of the EM process.
- Some electrons may multiply more than others when going through the extended register.
- This leads to an uncertainty in your measurement, *hence* the excess noise factor.
- Let’s look at EMCCD camera noise.

Excess Noise – Low Signal

- Consider a signal a typical interline signal of 20 electrons

- CCD sensor
- Photon Noise +/- 4.5 e
- Signal to Noise (assume 6.5e of read) 2.5 :1

- EMCCD sensor
- Photon Noise +/- 6.3
- Signal to Noise (assume 100x EMGain) 6.2 :1

* Note this is slightly unfair as the CCD and EMCCD have different pixel sizes and Quantum Efficiencies

Excess Noise – High Signal

- Consider a signal - a typical interline signal of 10,000 electrons

- CCD sensor
- Photon Noise +/-100
- Signal to Noise (assume 6.5e of read) 100:1

- EMCCD sensor
- Photon Noise +/-140
- Signal to Noise (assume 100x EMGain) 77:1

* Note this is slightly unfair as the CCD and EMCCD have different pixel sizes and Quantum Efficiencies

SNR: The new equation

On-Chip Multiplication Gain CCD SNR:

$$SNR = [S*QE] \div \sqrt{[S*QE*F^2 + D*F^2 + (\sigma_R/G)^2]}$$

Note: F is the excess noise factor.

Conclusions

- Need to think about what matters for your experiment –
  - is it just photo-documentation
  - does resolution matter
  - how fast do you need to image
  - at what wavelengths
- Different cameras and settings on each camera can be optimised depending on what you need
- EMCCD cameras are a great option if you need high speed at very low light levels.

QUESTIONS?