CAMERA SENSORS FOR MICROSCOPY

Matt Preston

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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

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

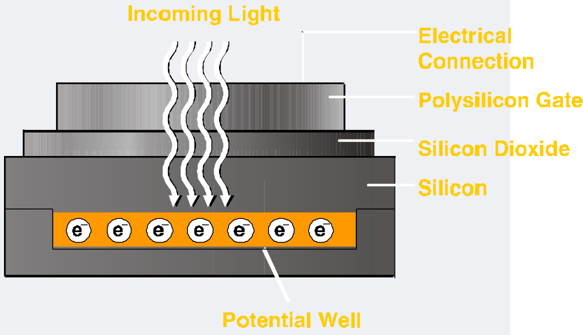
What is Quantum Efficiency?

- Quantum efficiency (QE) is a measure of the effectiveness of an imager to produce electronic charge from incident photons.

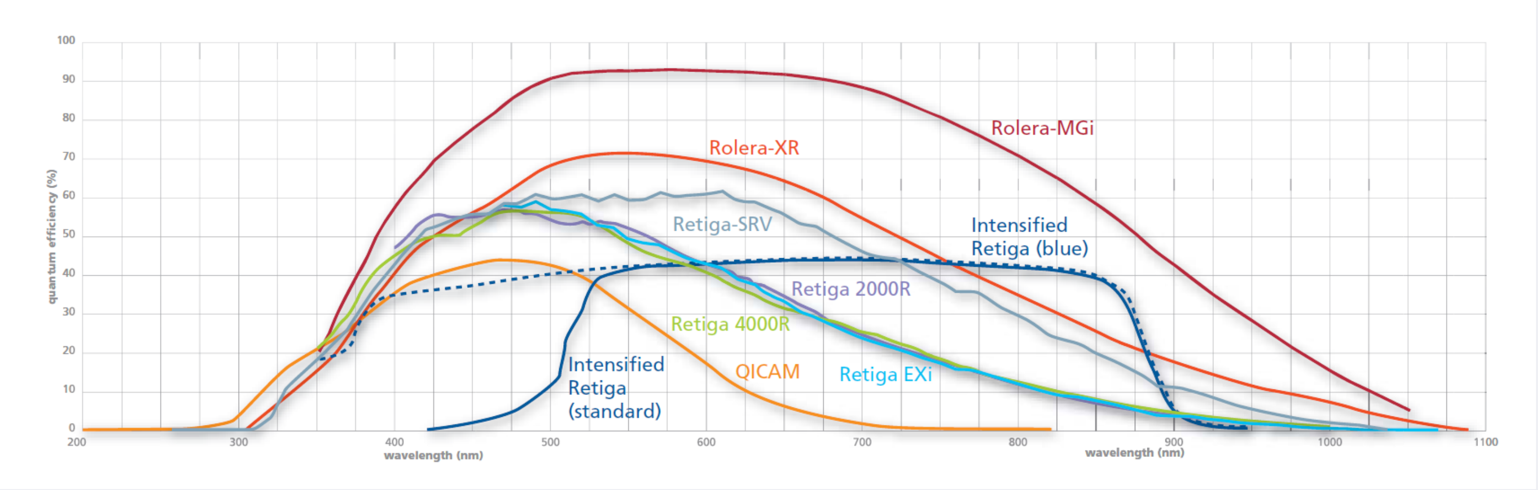
- In the high-purity crystalline form, each atom of silicon is covalently bonded to its neighbour. Energy greater than the band gap energy, about 1.1 eV, is required to break a bond and create an electron/hole pair.

- The wavelength of incoming light and photon absorption depth are directly related; the shorter the wavelength, the shorter the penetration depth into the silicon.

- Light normally enters the CCD through gates of the parallel register (front-illuminated CCD). These gates are made of very thin polysilicon, which is reasonably transparent at long wavelengths, but becomes opaque at wavelengths shorter than 400 nm. Thus, at short wavelengths, gate structure attenuates incoming light.



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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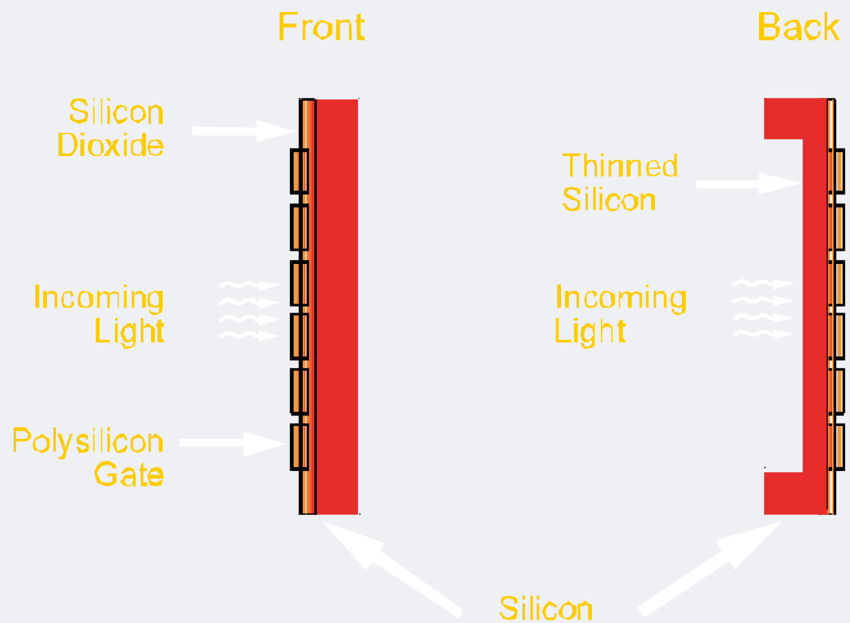
Front and Back illumination

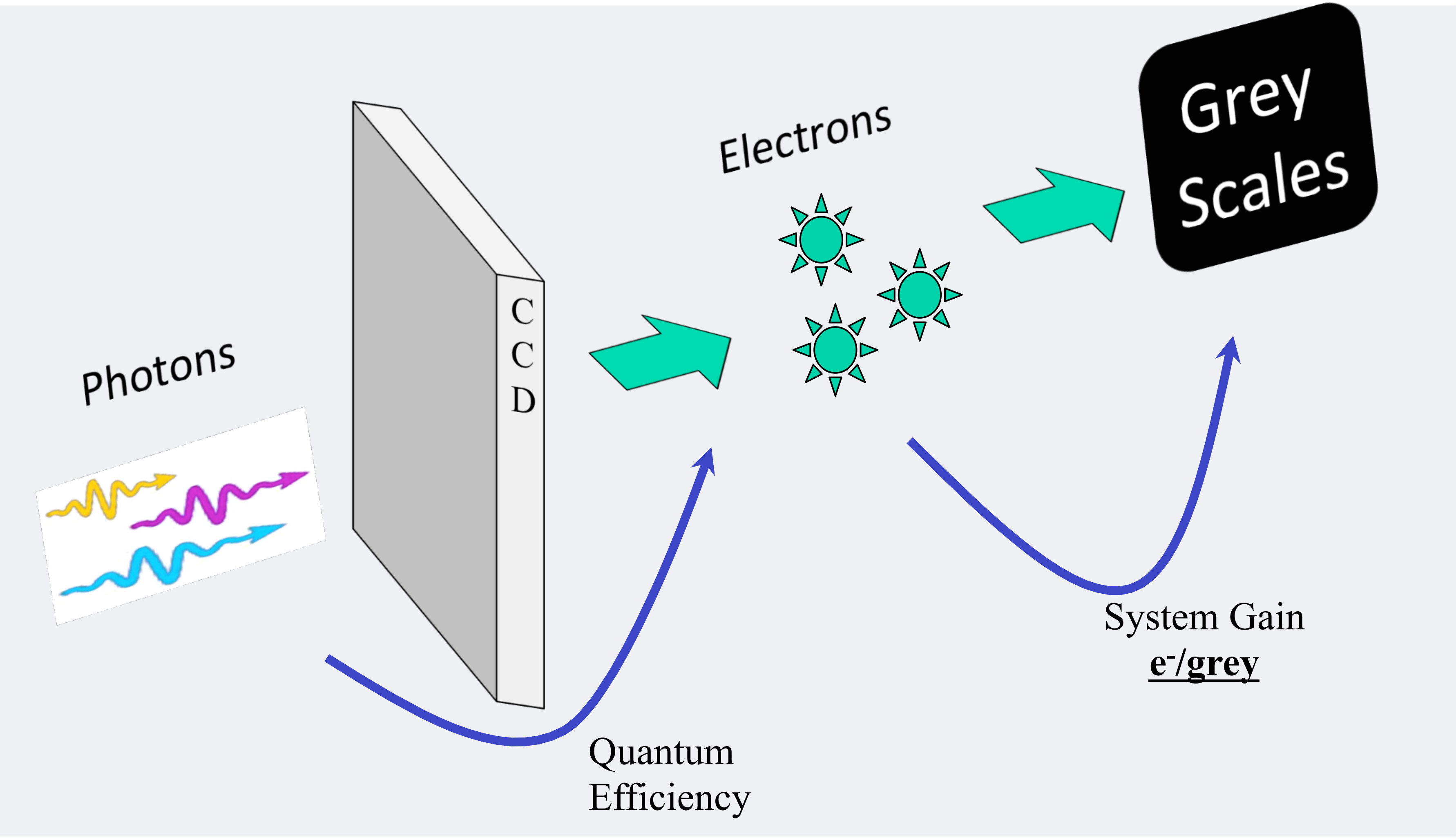
- Some cameras are back thinned and back illuminated to be as efficient as possible with incoming light

- Typical front illuminated QE 40-60% at Lambda Max

- Typical Back illuminated QE 90% at Lambda Max



What is actually happening at each Pixel?



What's happening

1. Photon hits the CCD sensor
2. Photon is then converted to an Electron
3. Electron is then digitised using an Analogue to Digital converter (ADC)
4. Electron value is now converted to a grey scale
5. User measures grey scale (ADU)

Note - The camera is completely in control over grey scale values and changing camera parameters doesn't change the light detected.

Sensors – What are our main types

- CCD – Charged Coupled Device
- EMCCD – Electron Multiplied CCD
- CMOS – Complementary Metal Oxide Semiconductor

CCD Fundamentals

The Charge-Coupled Device

- 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)

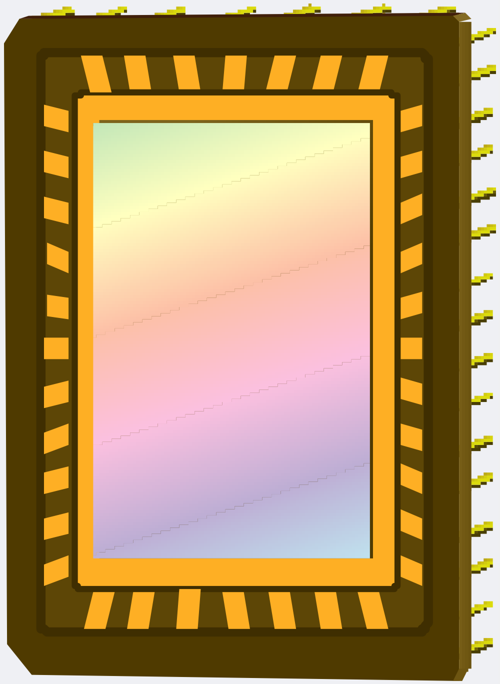
  Thomson CSF (France)

  Marconi (formerly EEV — England)

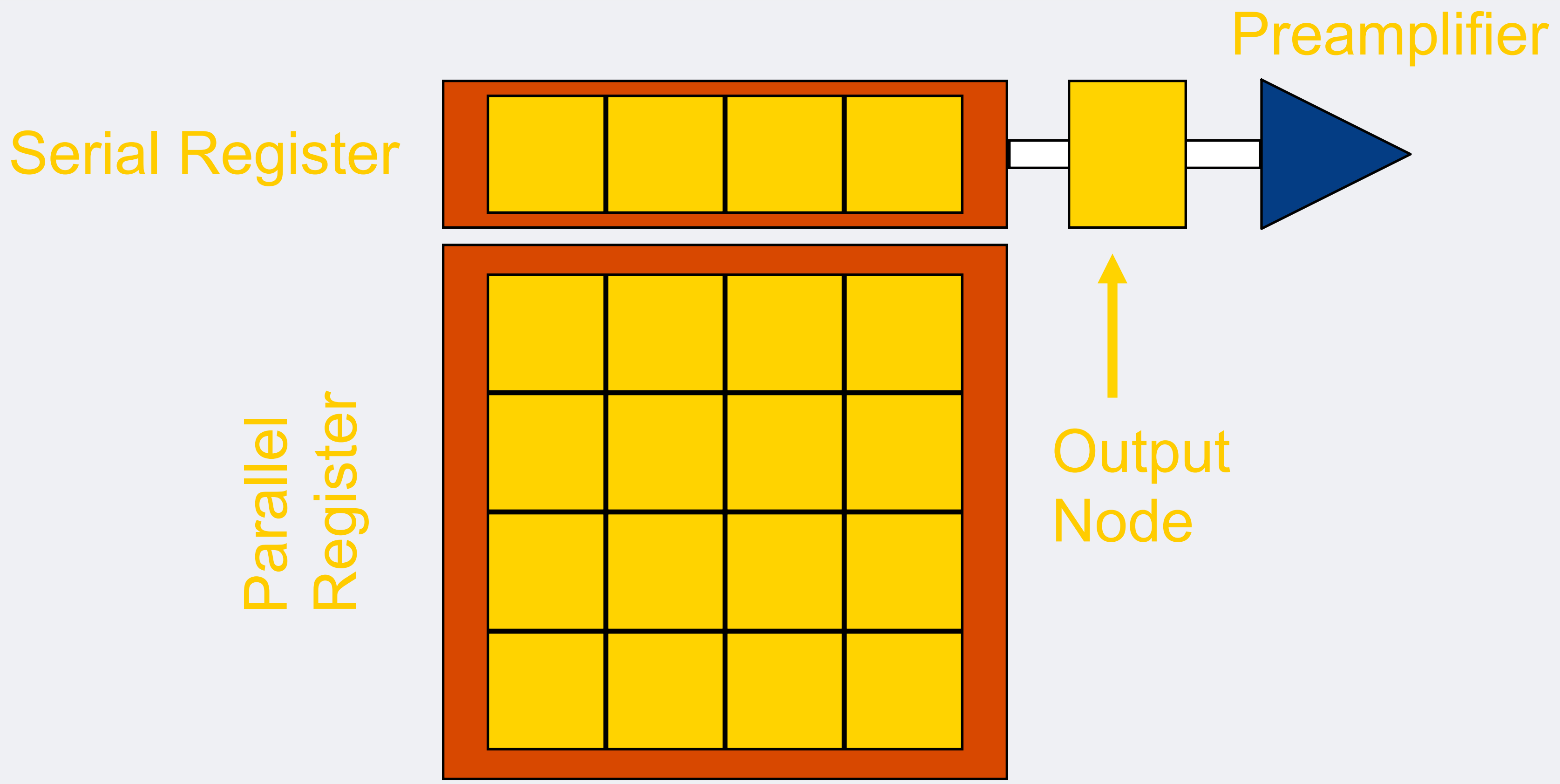
  SITe (Beaverton, OR)

  Sony

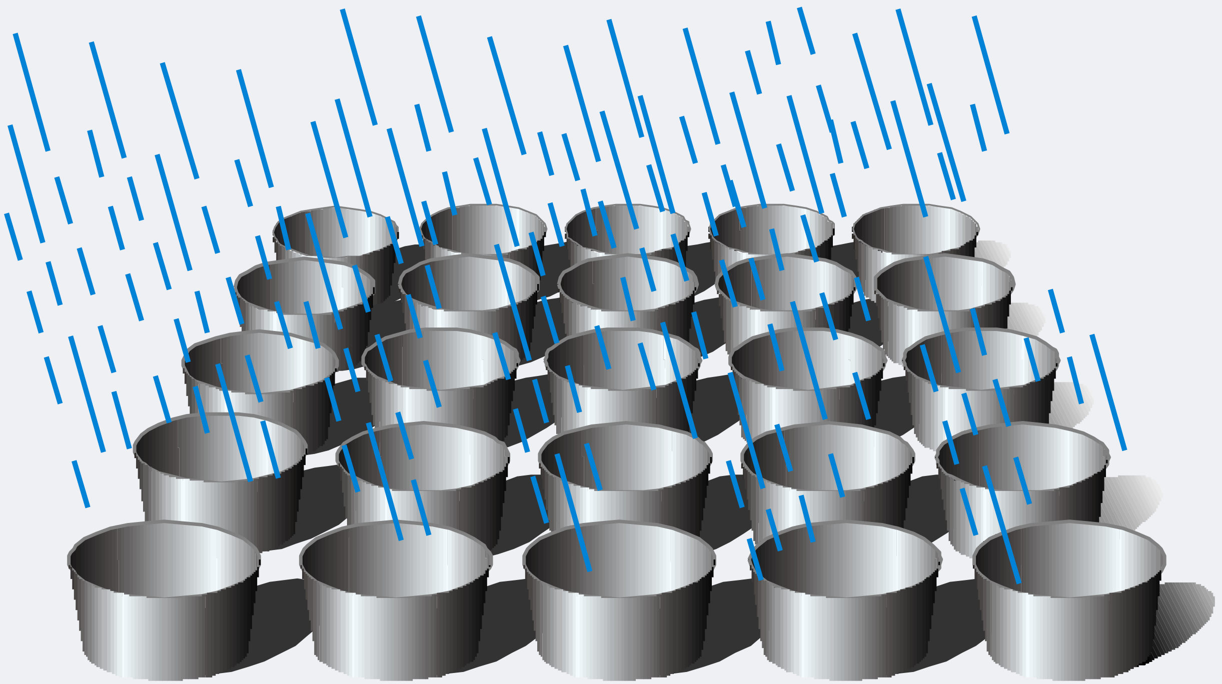
  Others



CCD Fundamentals



CCD Fundamentals



Array of Discrete Photodetectors

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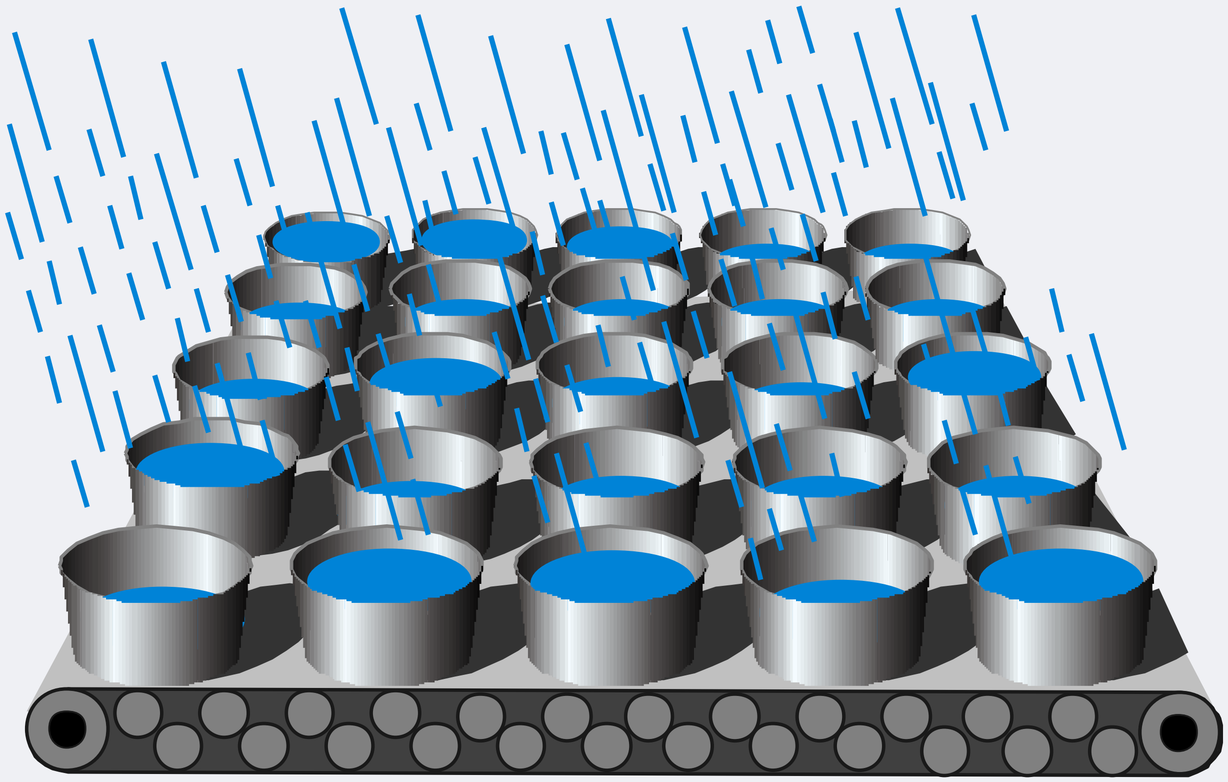
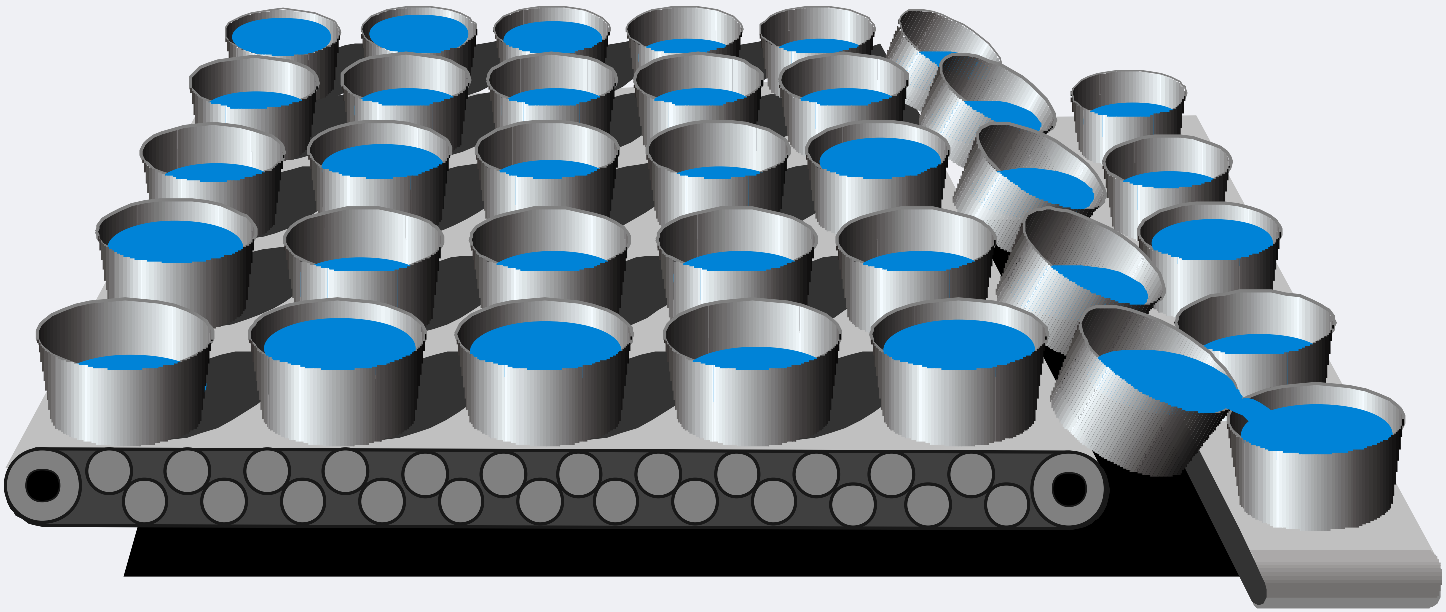
CCD Fundamentals



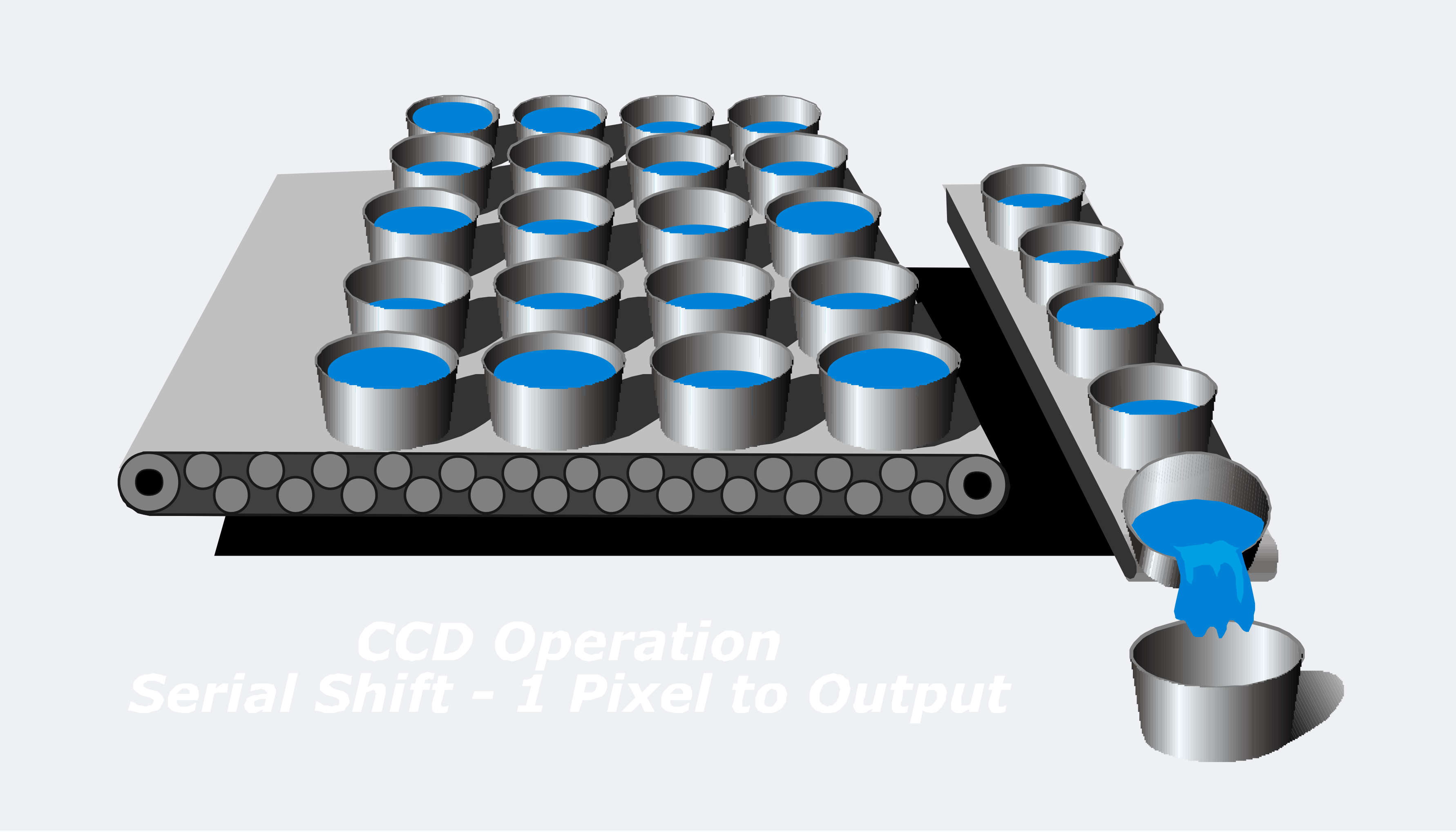
CCD Operation
Integration of Photo-Induced Charge

CCD Fundamentals



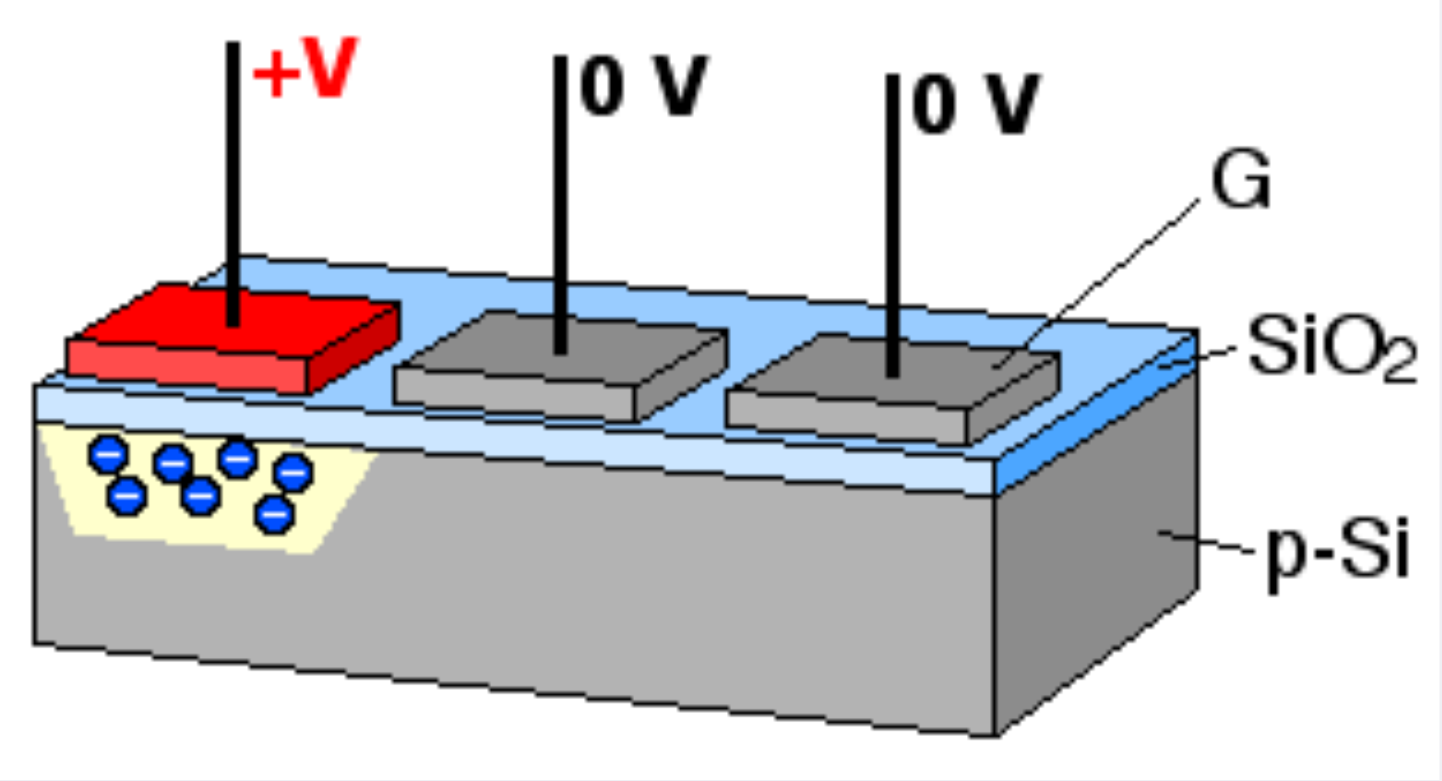
CCD Operation
Parallel Shift - 1 Row

CCD Fundamentals

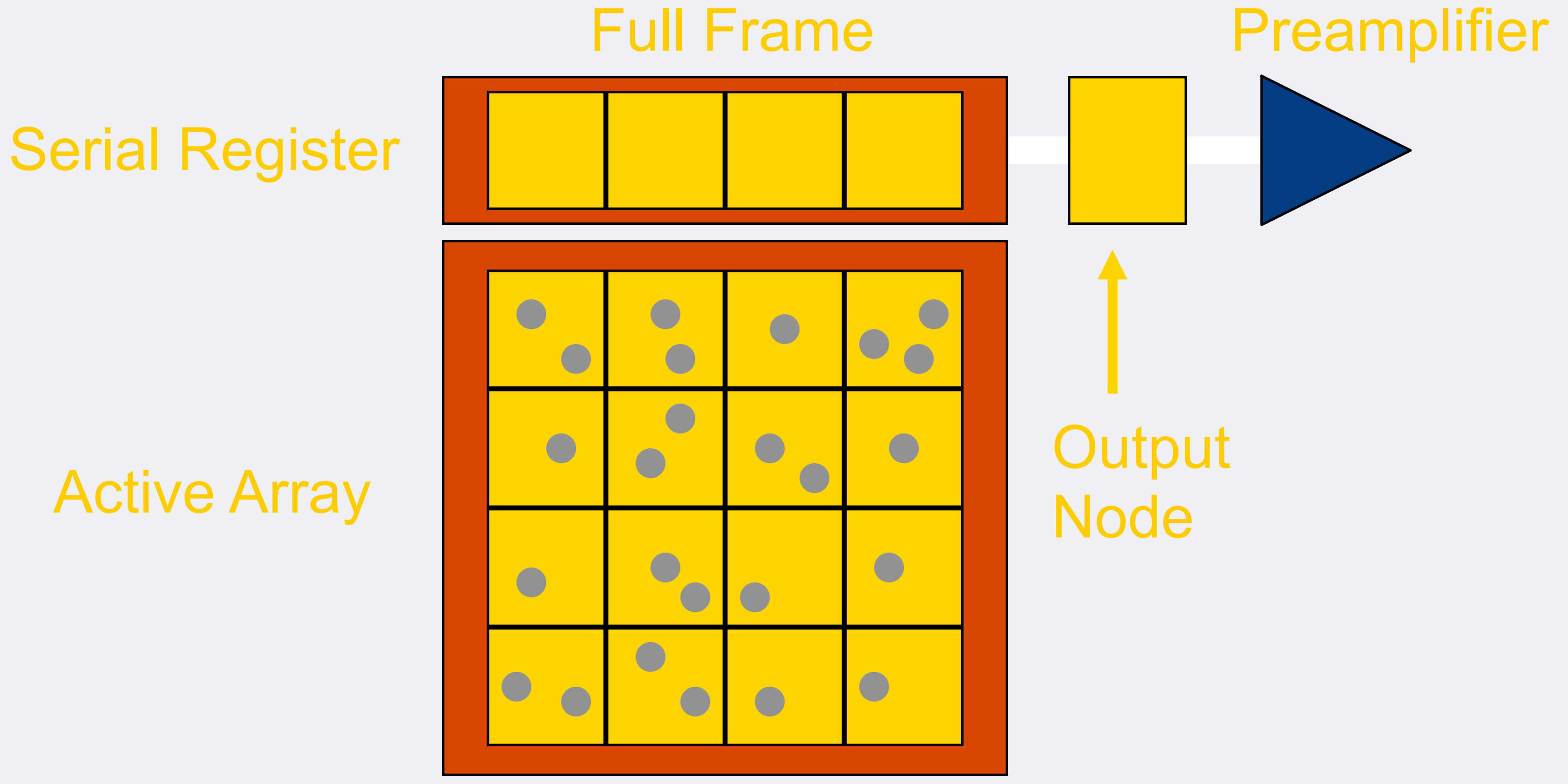


How do we get charge to move

- Pixels



CCD Fundamentals



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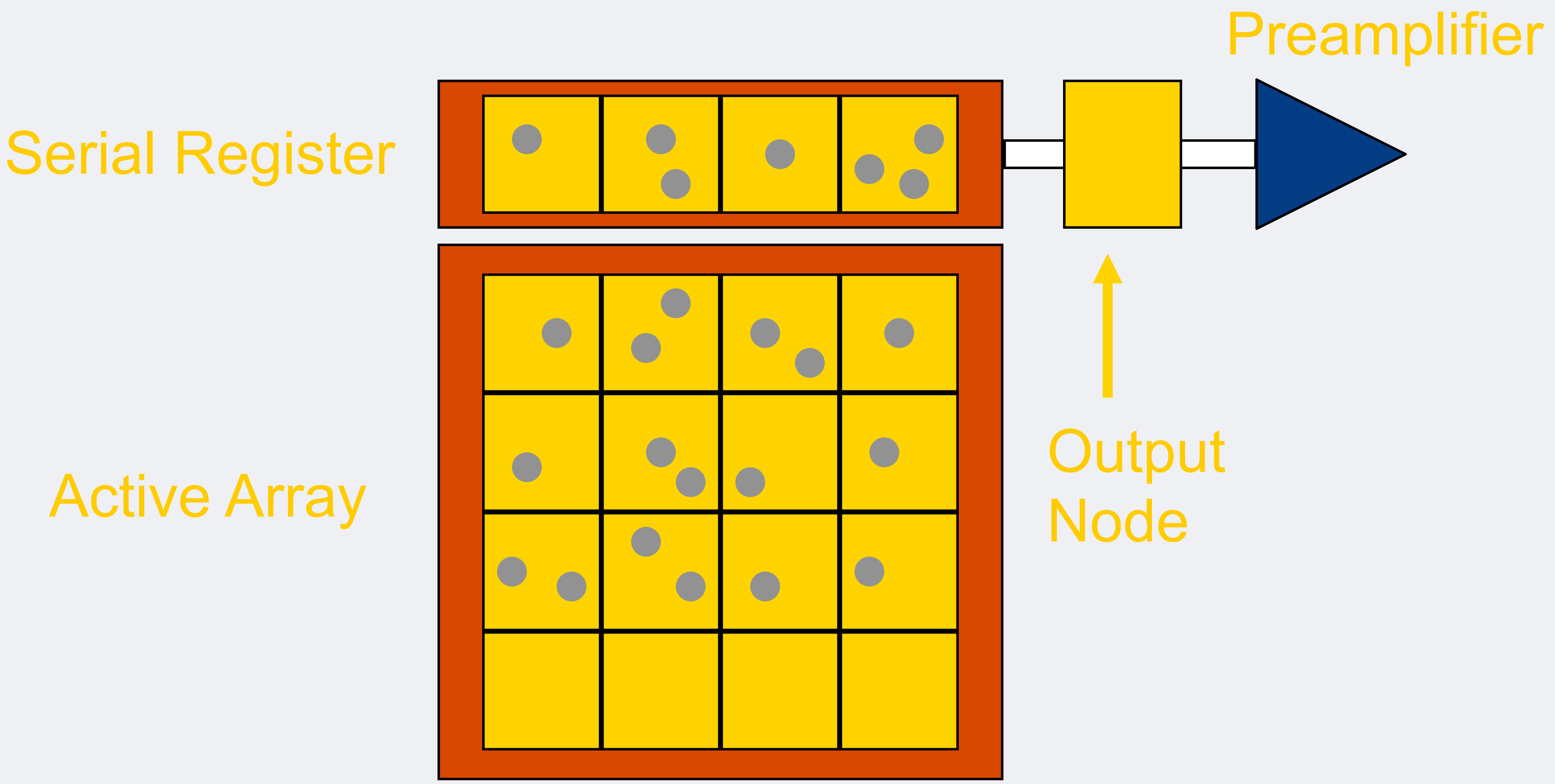

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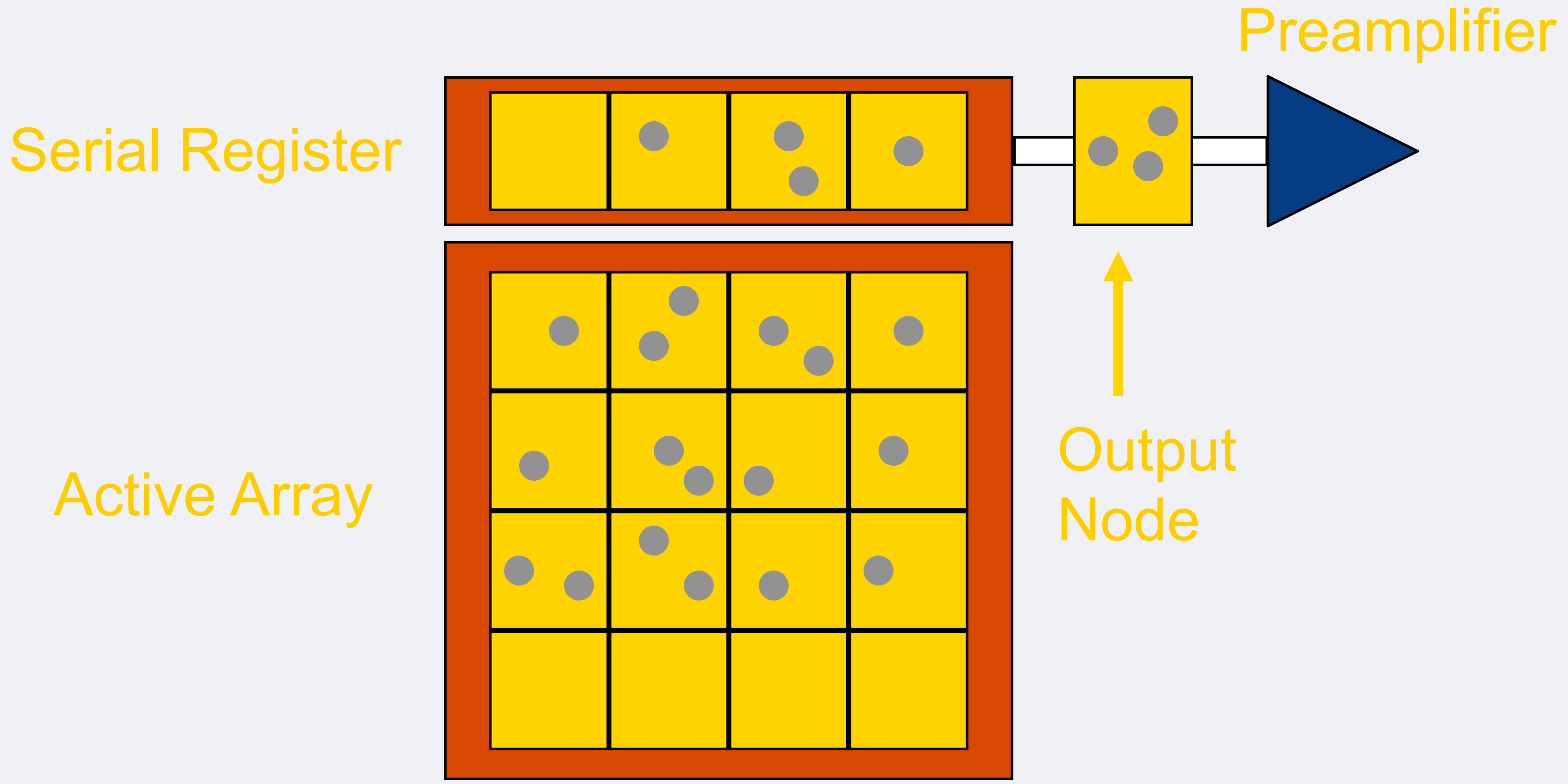

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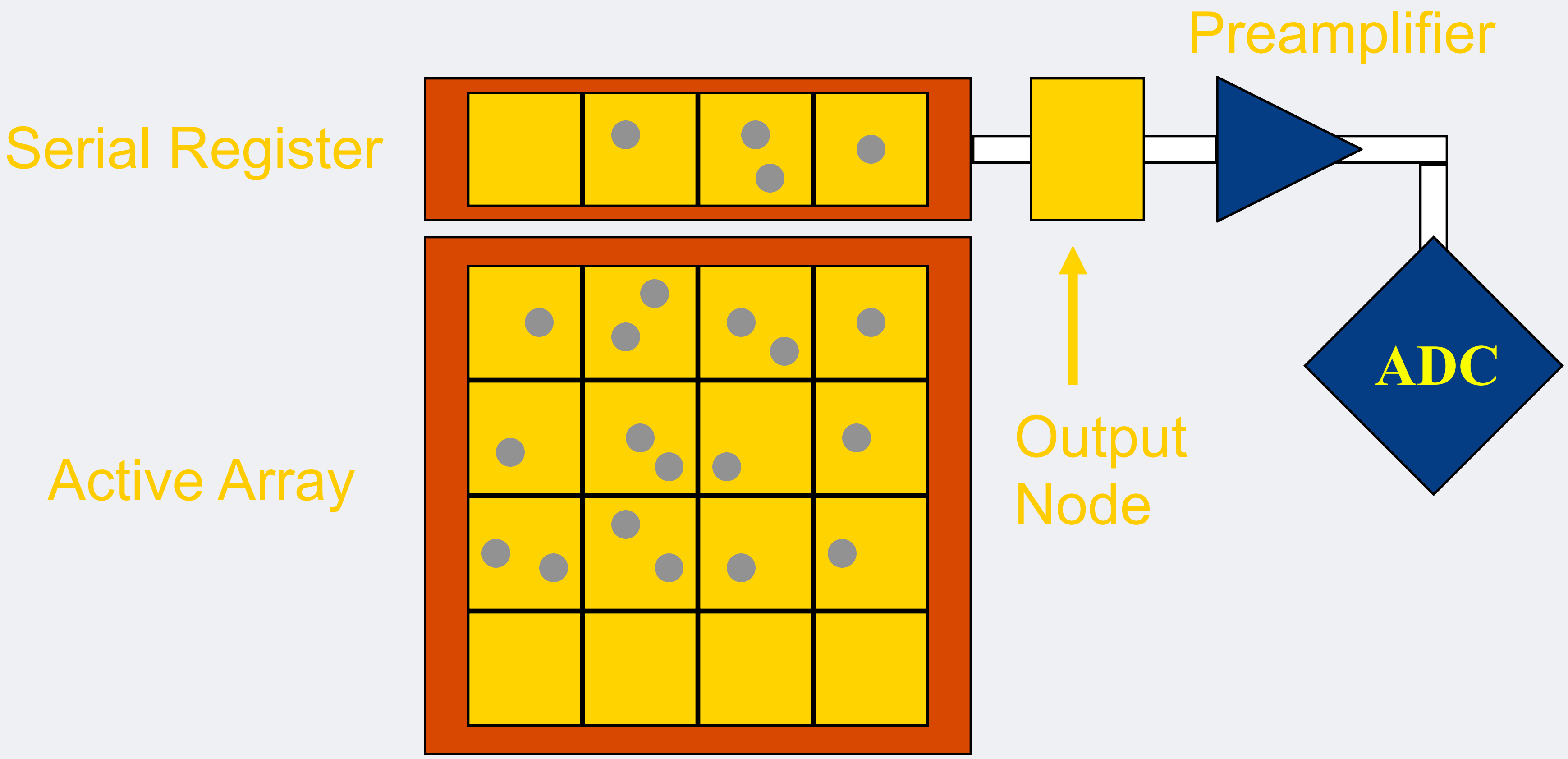

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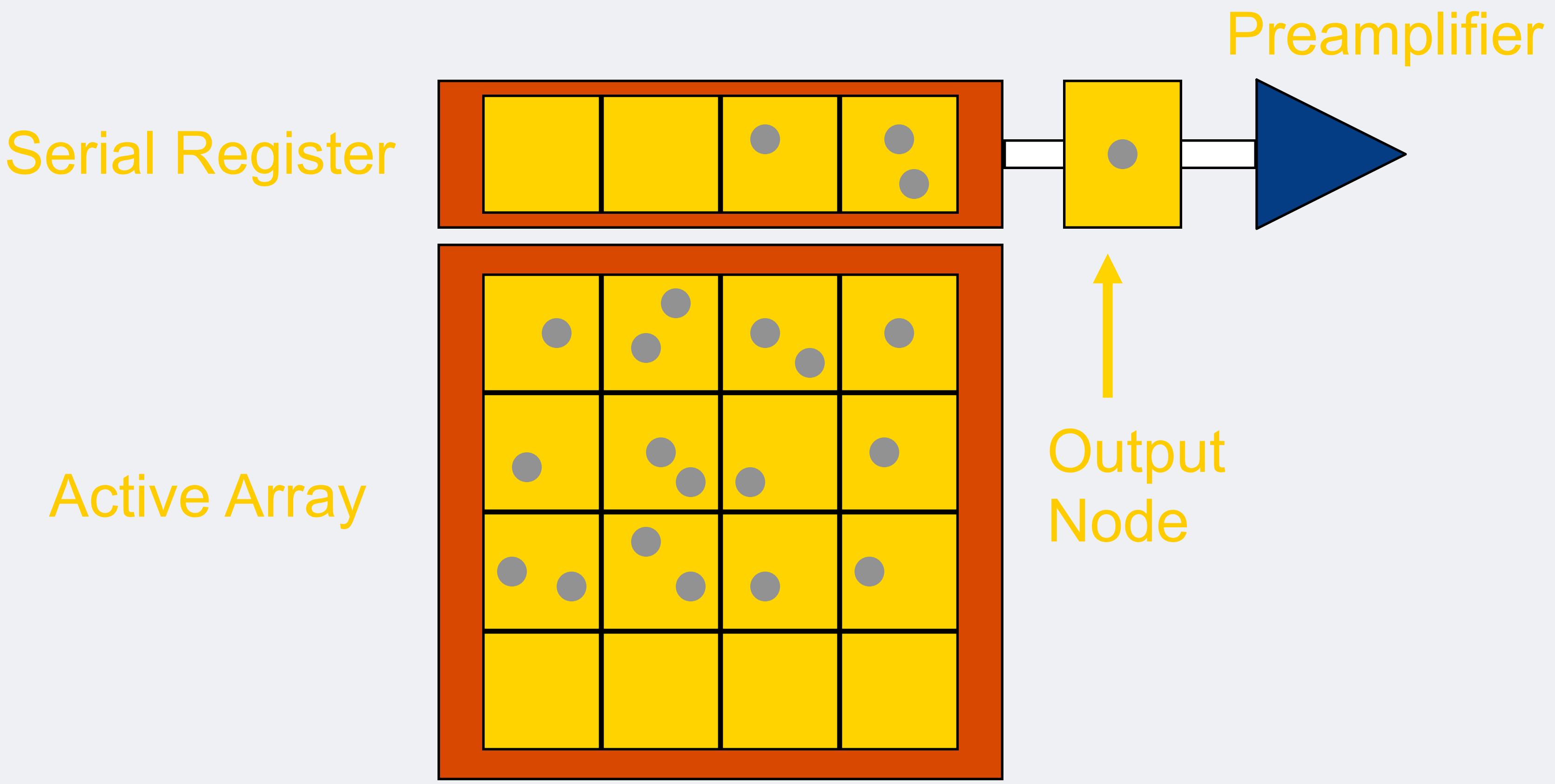

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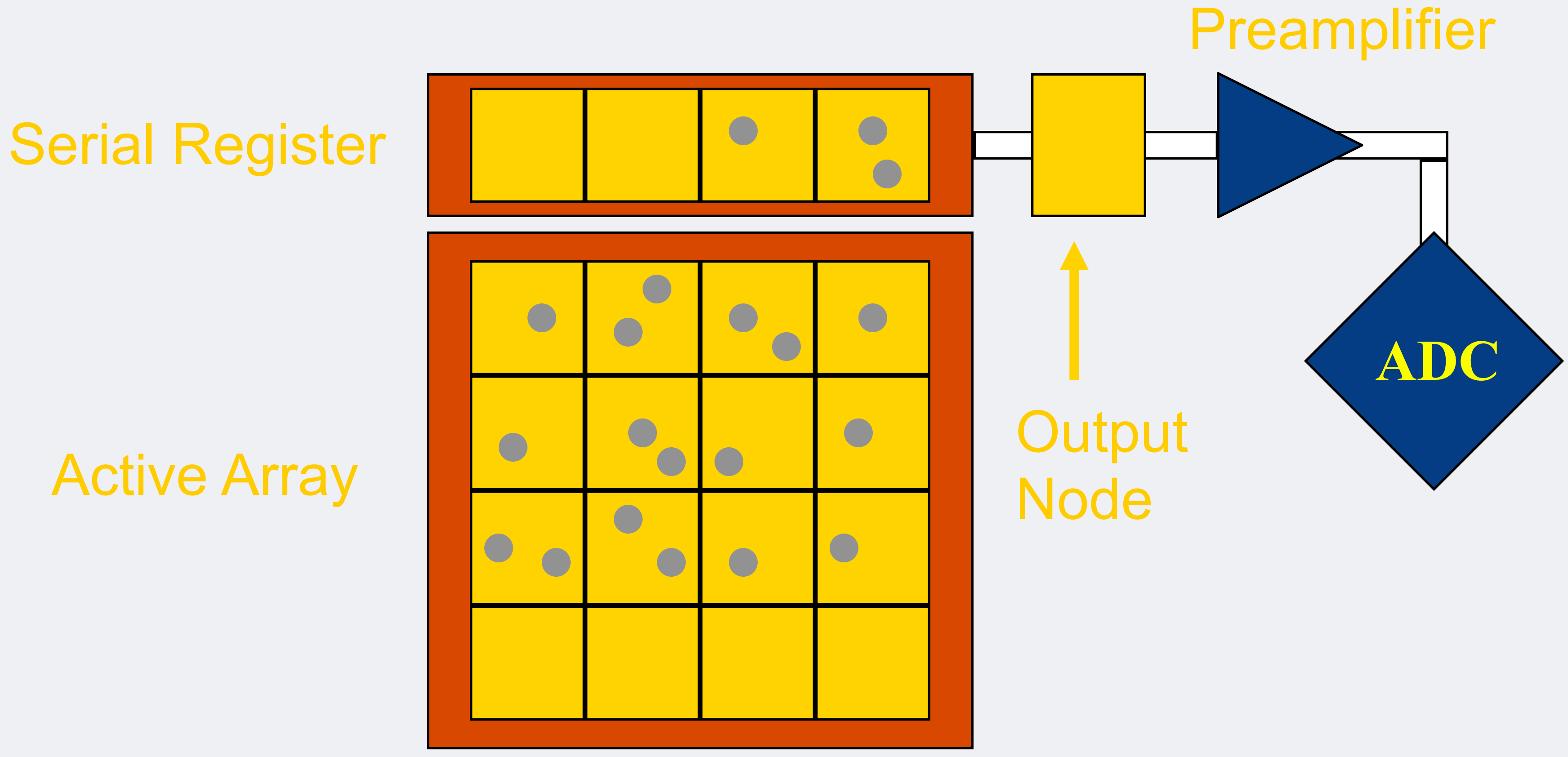
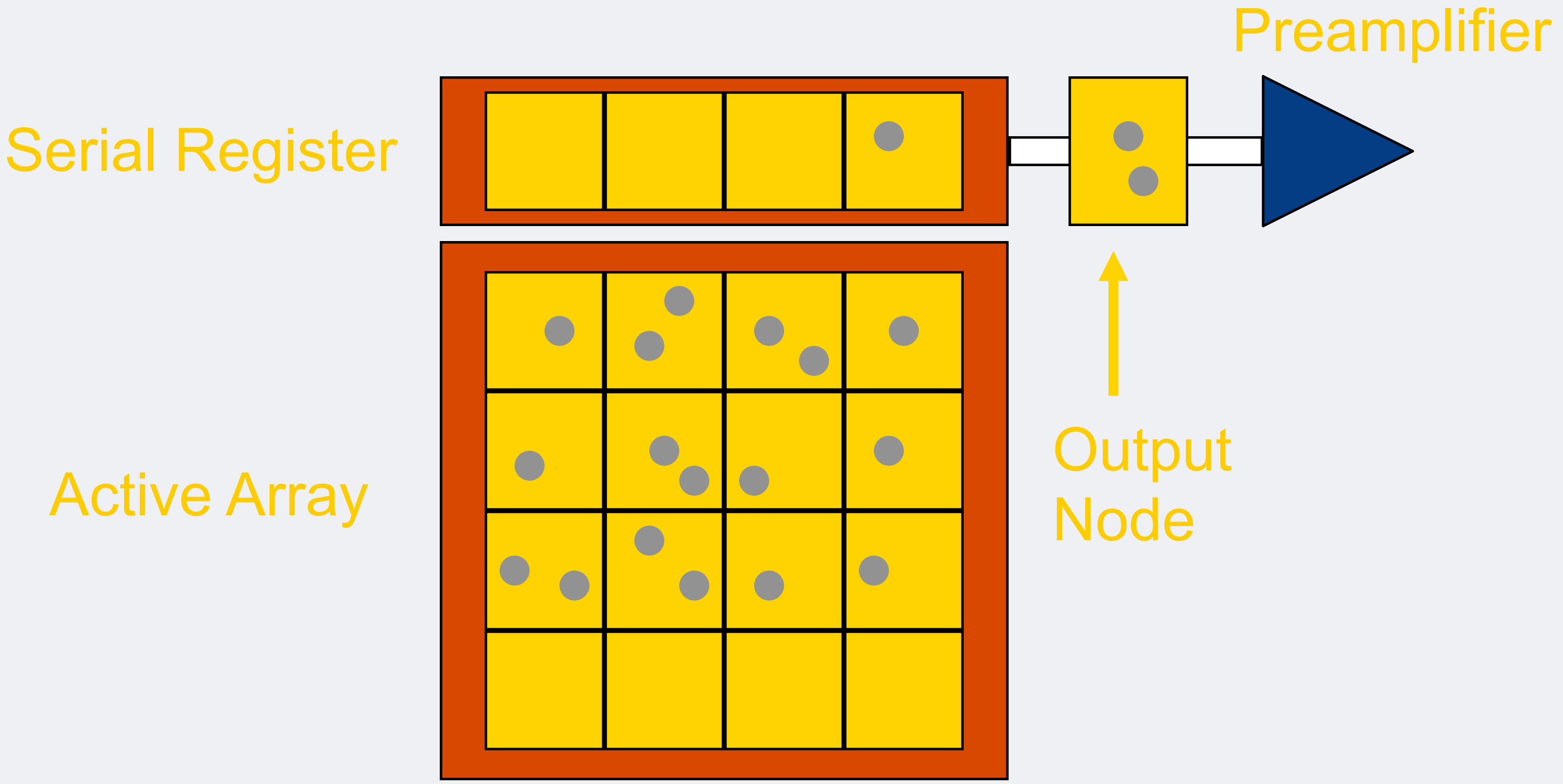
CCD Fundamentals



CCD Fundamentals



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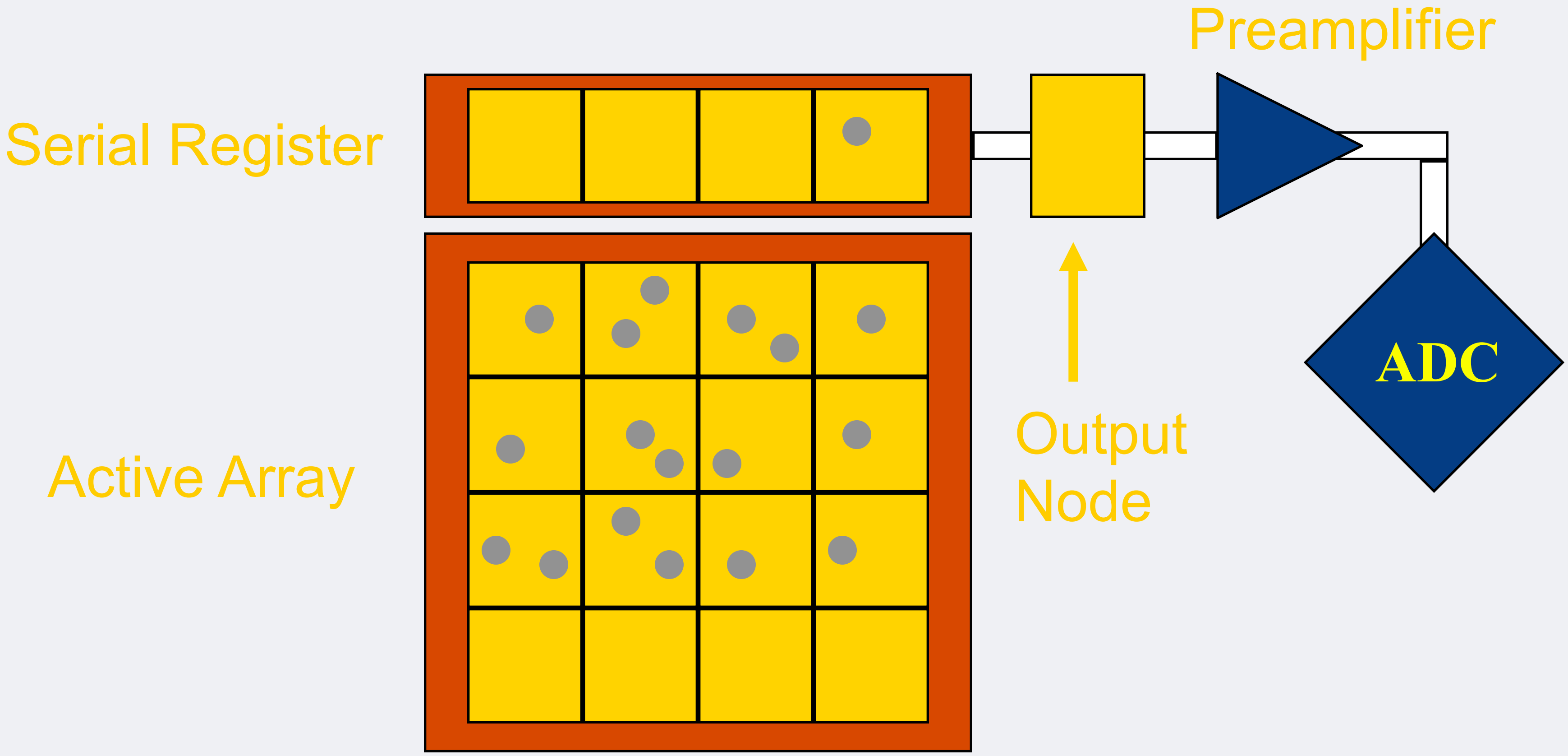

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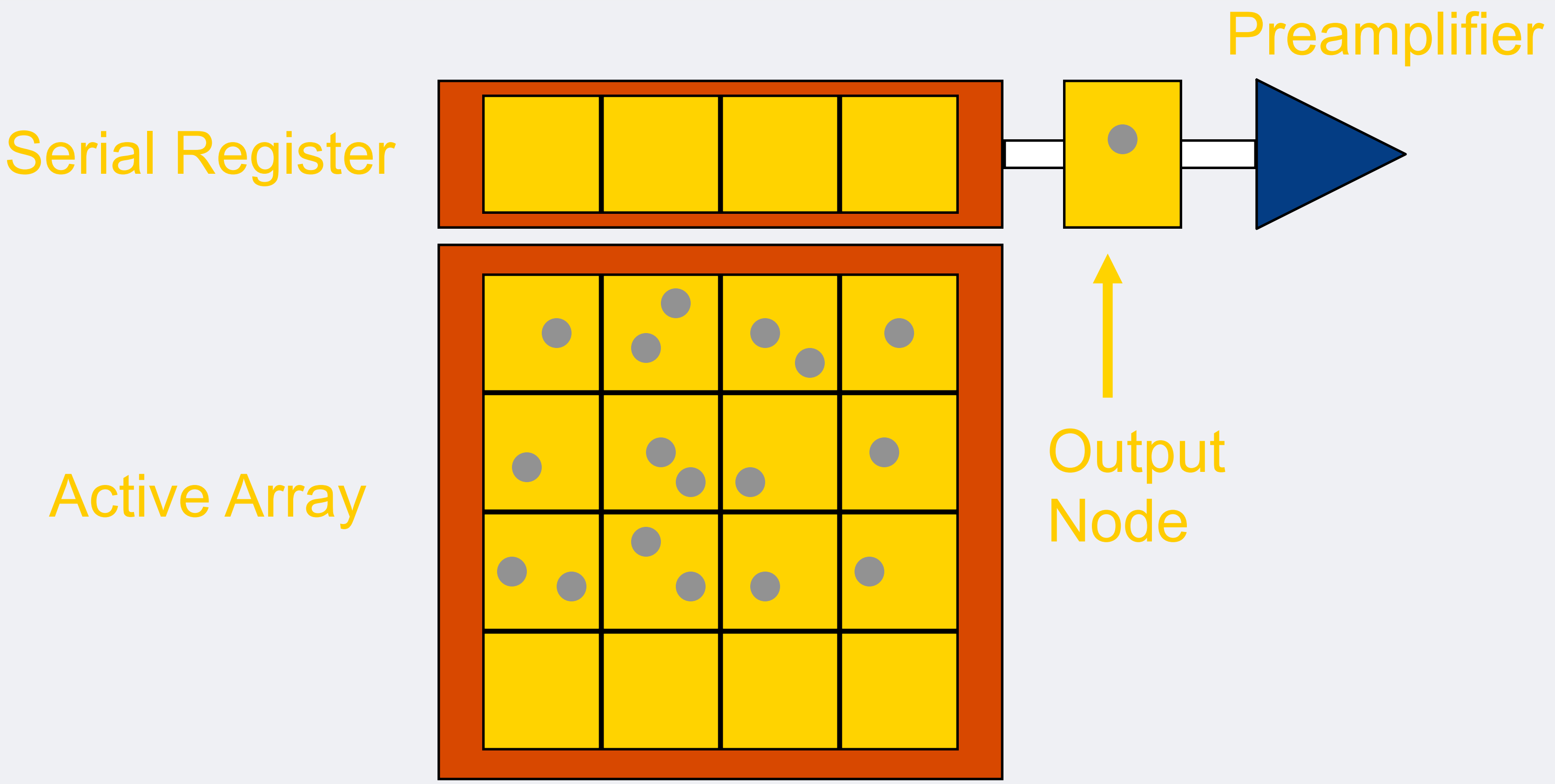

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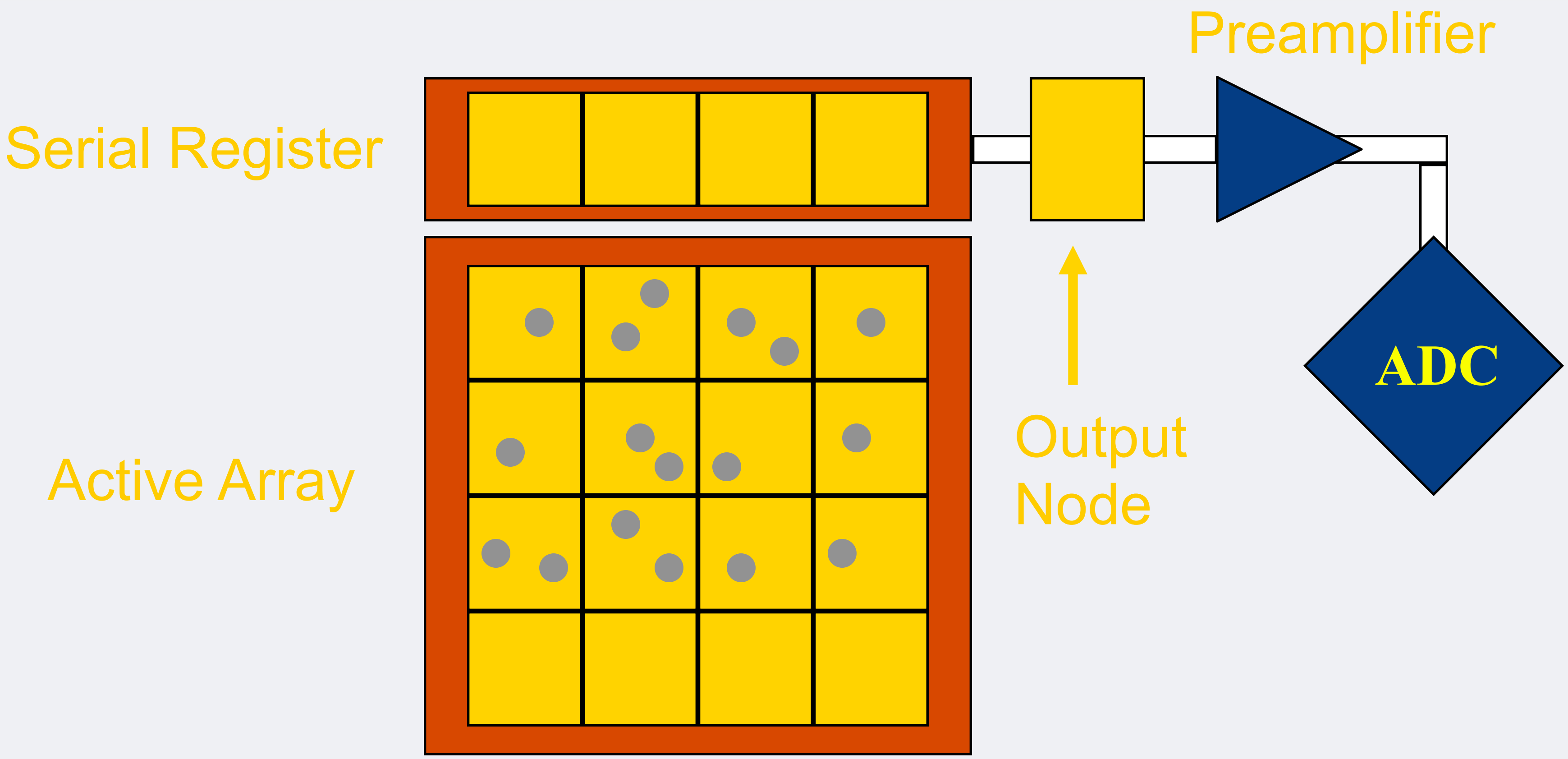

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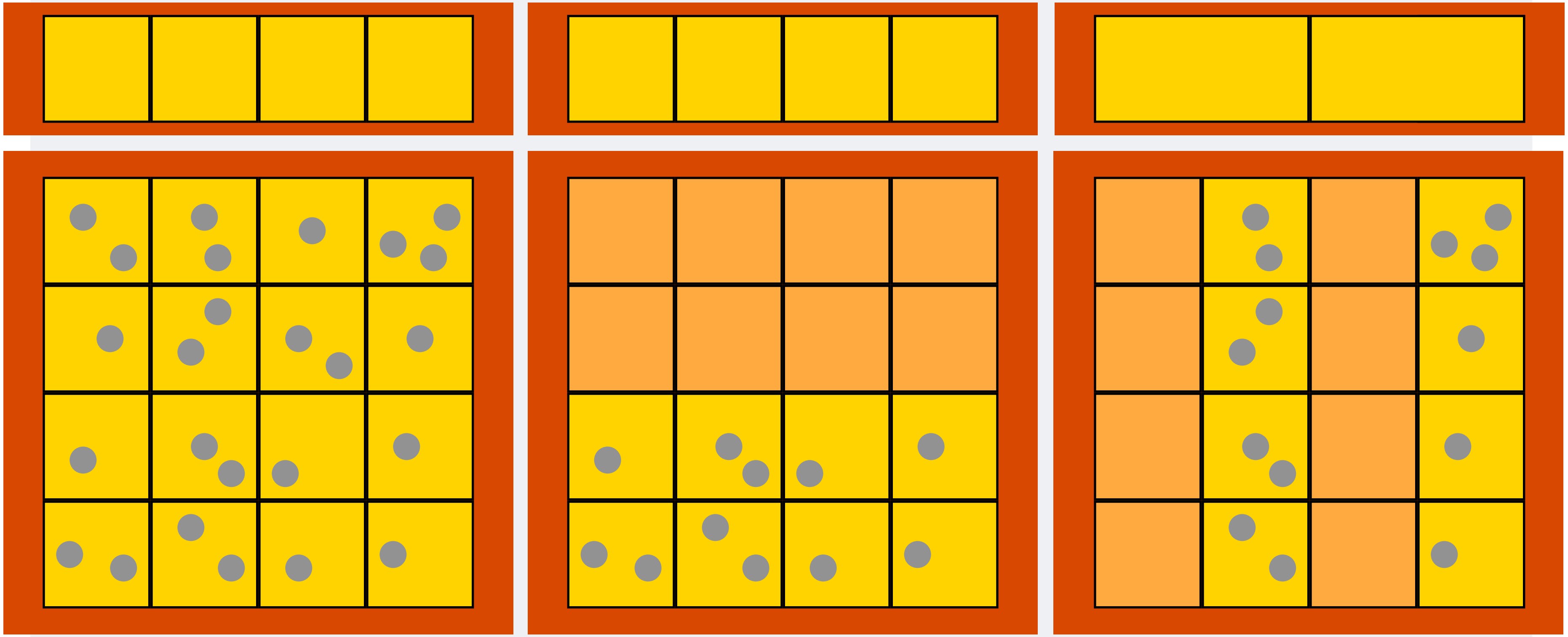

Full Frame

Frame Transfer (EMCCD)

Interline Transfer

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Before EMCCD and SCMOS

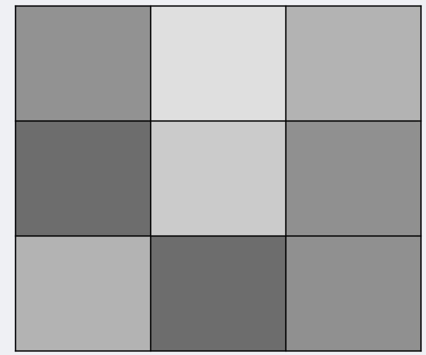
Lets look at camera noise

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

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

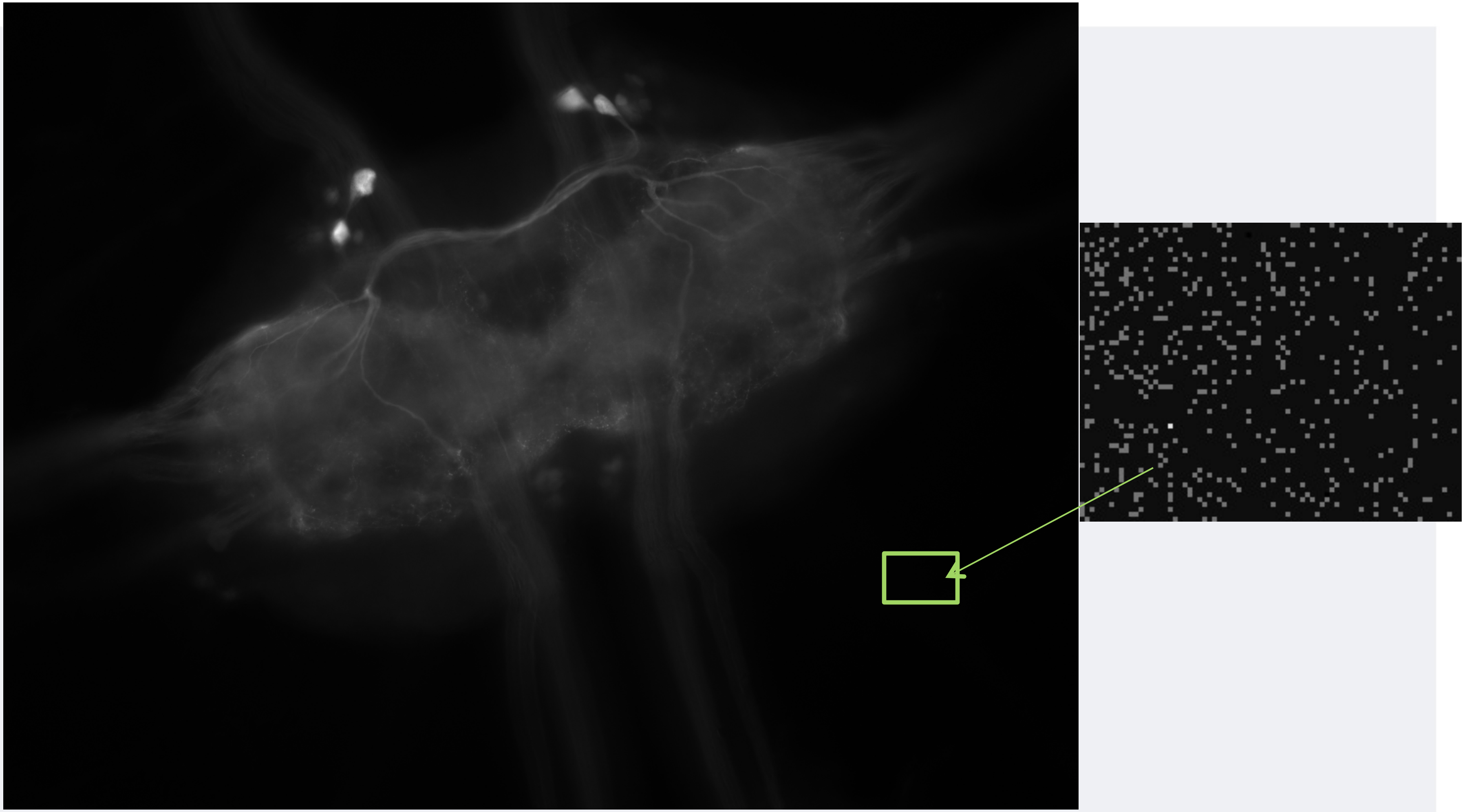
3. ***Photon Shot*** – square route of signal - signal dependent

Other Noises

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

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.

Read Noise

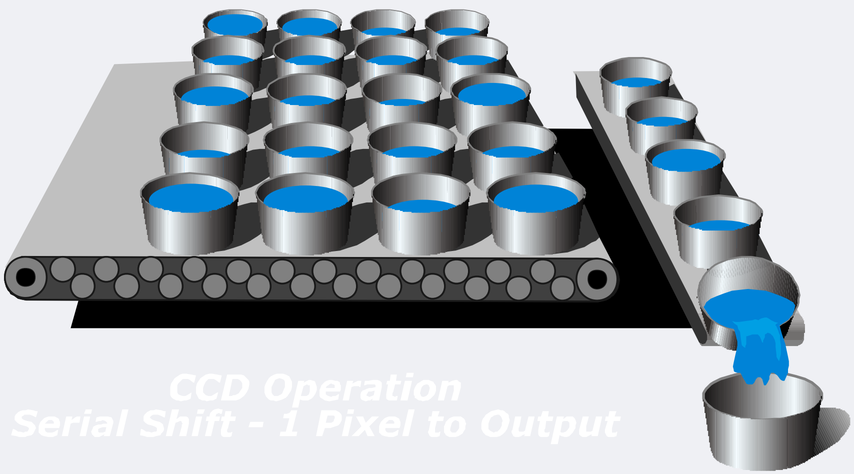
- Minimized by careful electronic design

- Under low-light/low-signal conditions where read noise exceeds photon noise, data is read noise limited

- Read noise is not as significant in high-signal applications

- Read noise = std* system gain* 0.707

  (std of subtracted bias images)



Reading all the buckets - what's my Error?

Dark Current

- Dark Current is created by heat and cosmic noise and can be reduced by cooling
- Dark Current builds over time unlike read noise
- Dark current reduction is sensor dependent
- For example, some sensors will halve dark current for every 7 degrees of cooling; some require more cooling
- Other technologies can be applied which reduce the cooling required

Retiga SRV (cooled to -30) Dark Current 0.15 e/p/s
Exi Blue (cooled to zero) Dark Current 0.005 e/p/s

Photon Shot Noise

- Law of physics
- Square root relationship between signal and noise
- Noise = square root of number of electrons
- Poisson distribution
- When photon noise exceeds system noise, data is photon (shot) noise limited

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

EMCCD

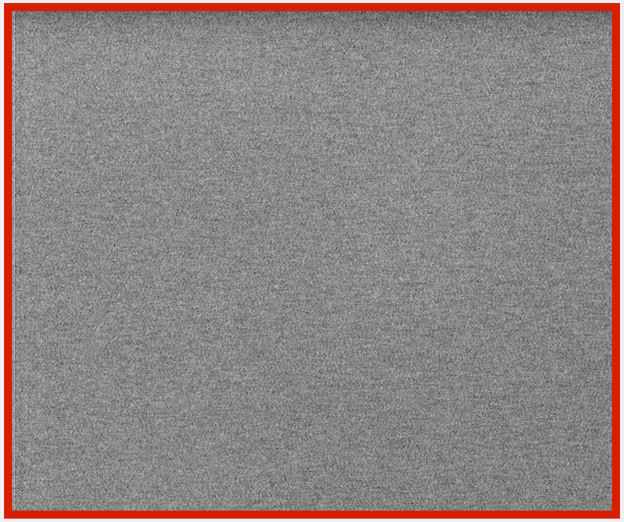
EM CCD – Electron Multiplied CCD sensors have been in place for over 10 years ago and are used for scientific, military and surveillance applications

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

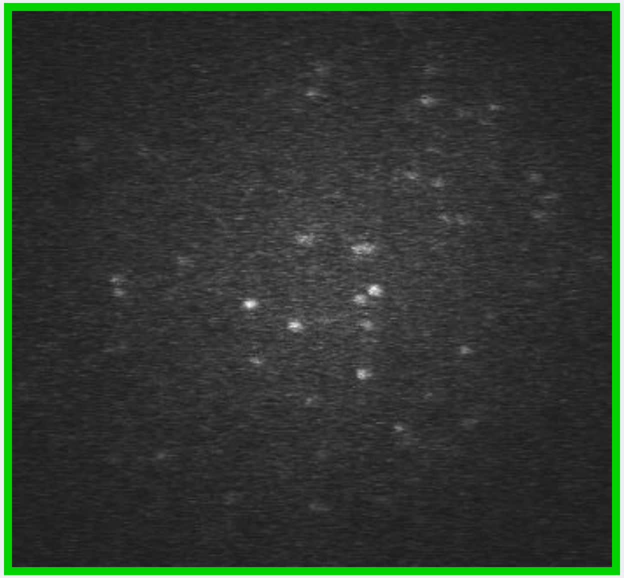
Based on CCD technology, the advancement comes from the addition of an Electron Multiplication register enabling higher signals to be achieved relative to the fixed camera noise - Read Noise

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

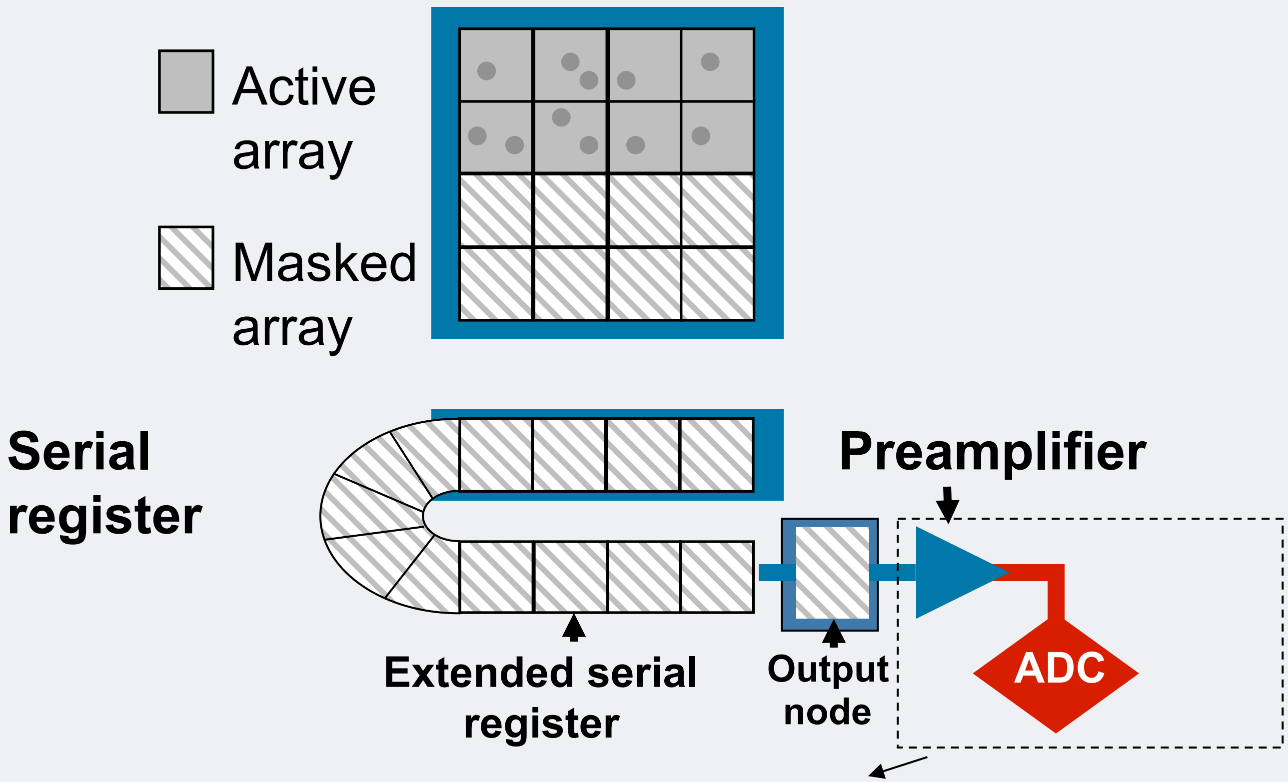


By minimizing the read noise

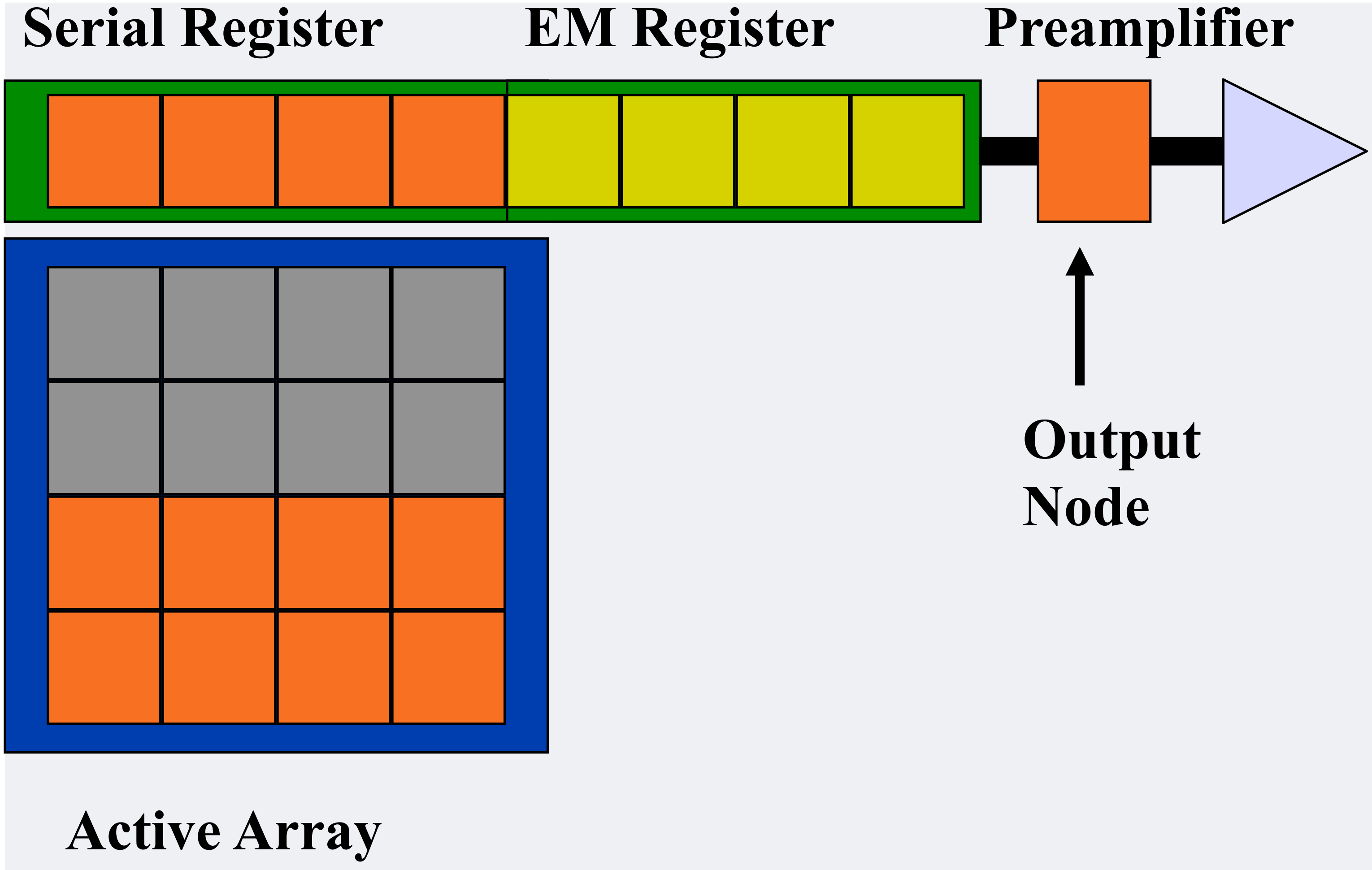
Example: single molecule fluorescence

Theory of Operation

On-chip multiplication gain CCD



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



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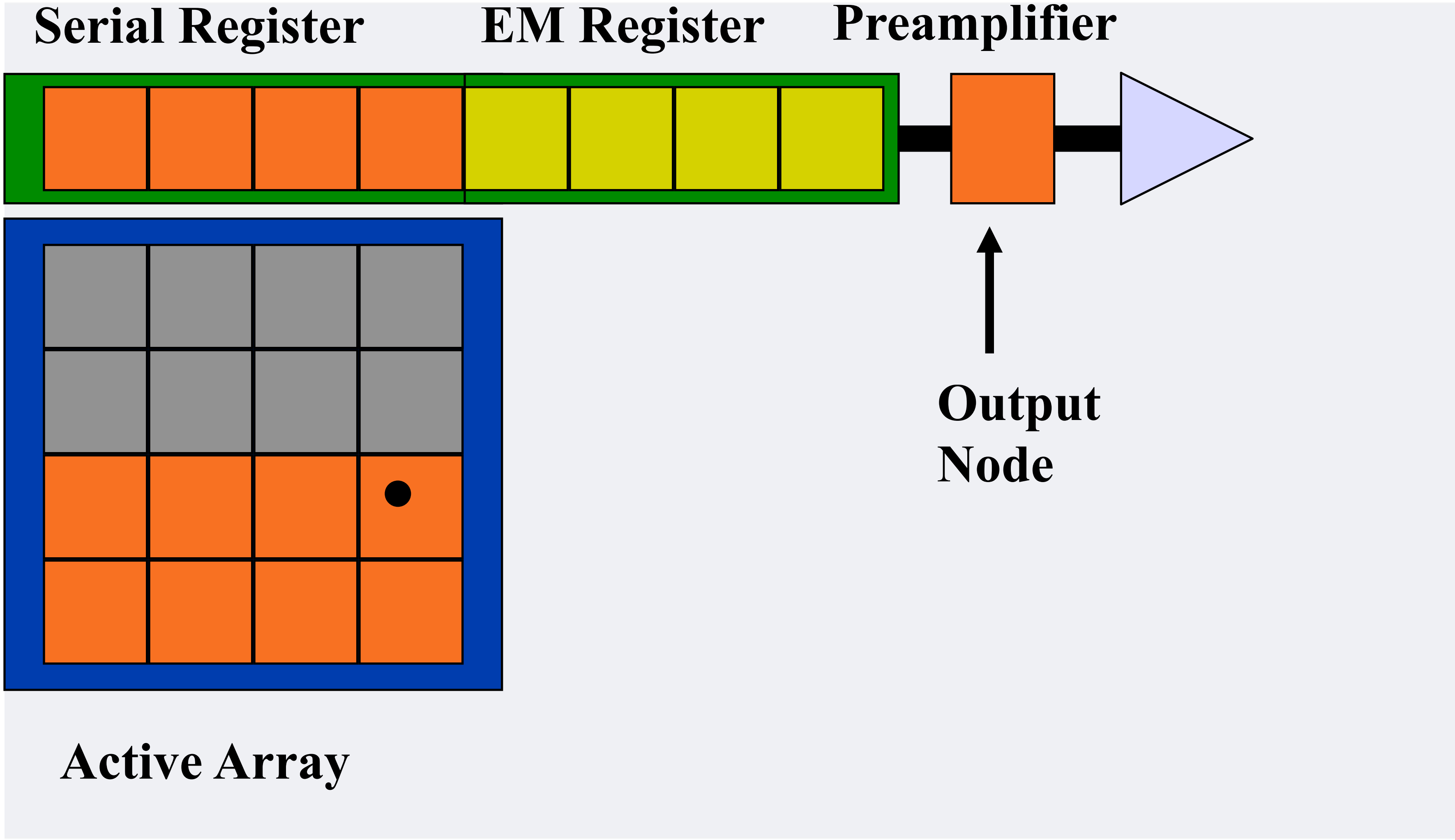
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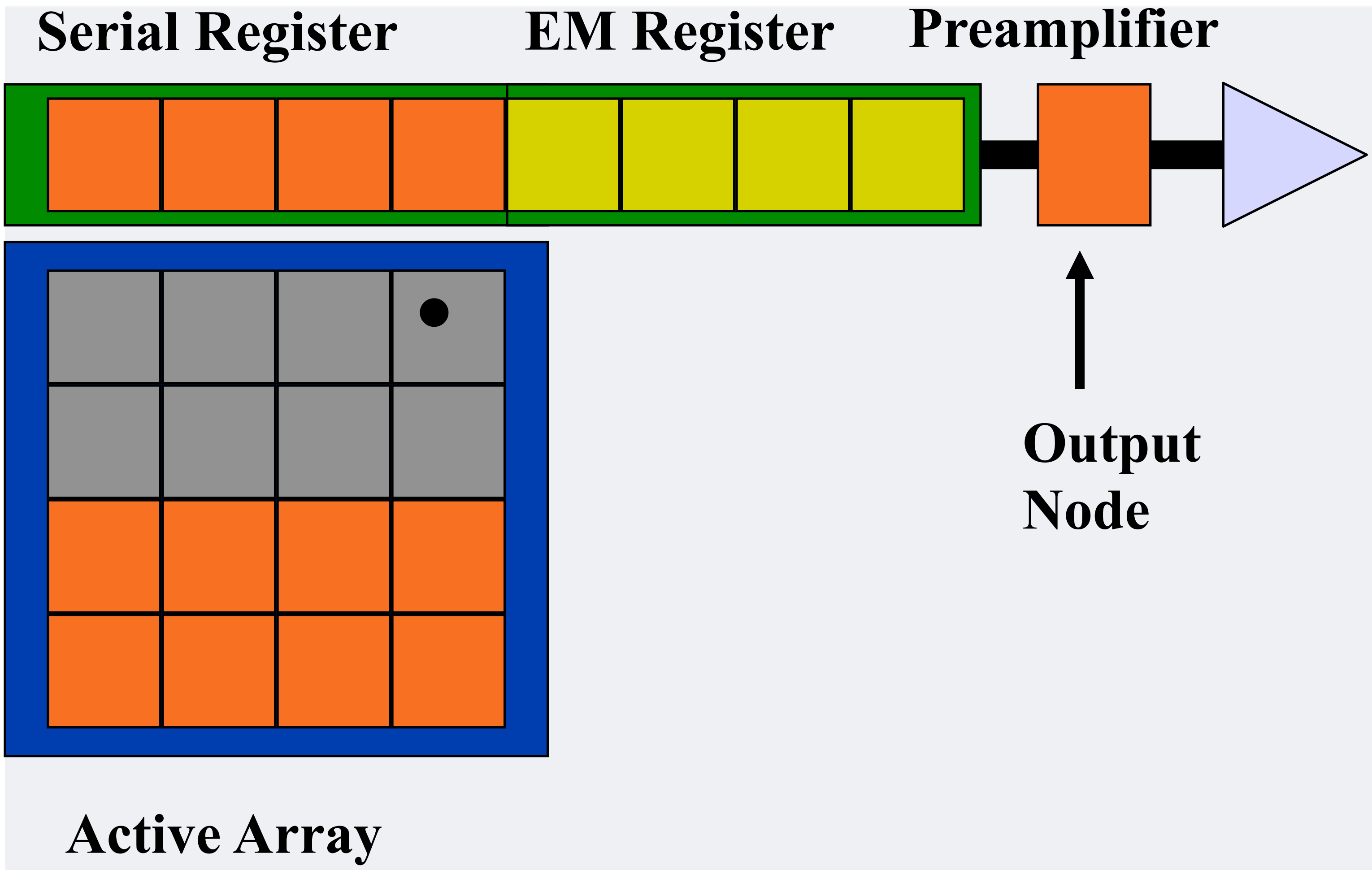
EM Register
Preamplifier
Output
Node
Active Array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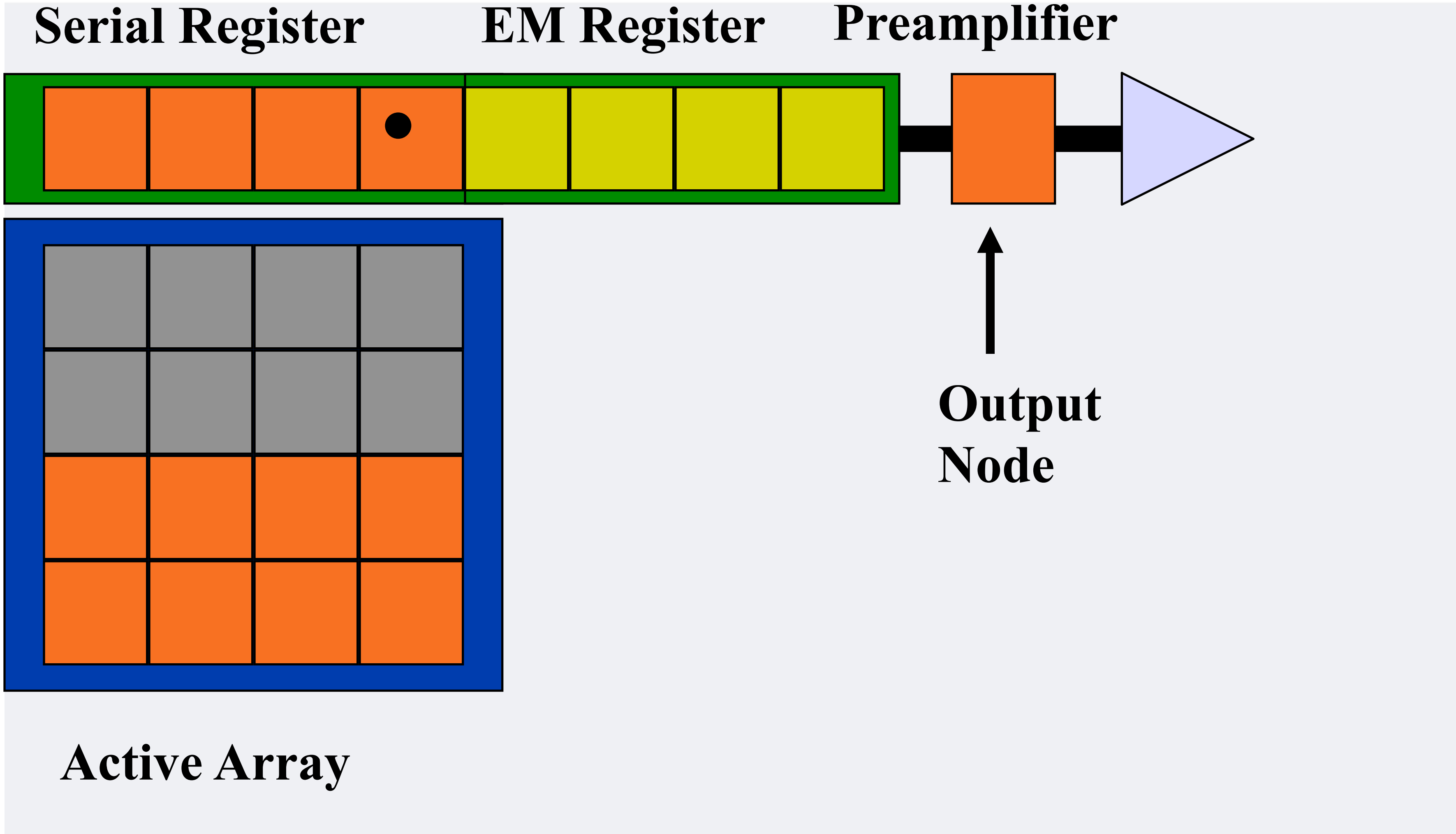
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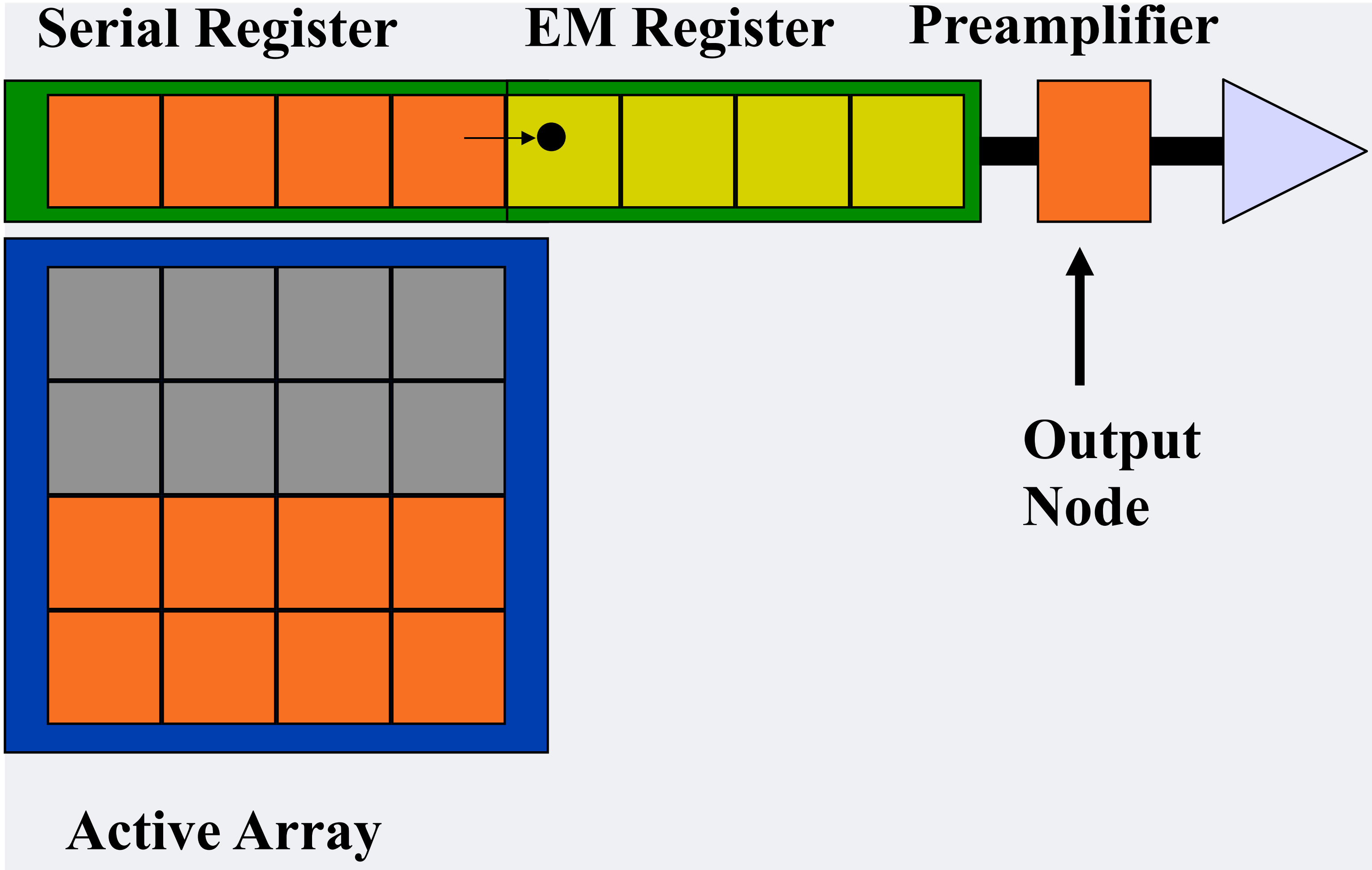
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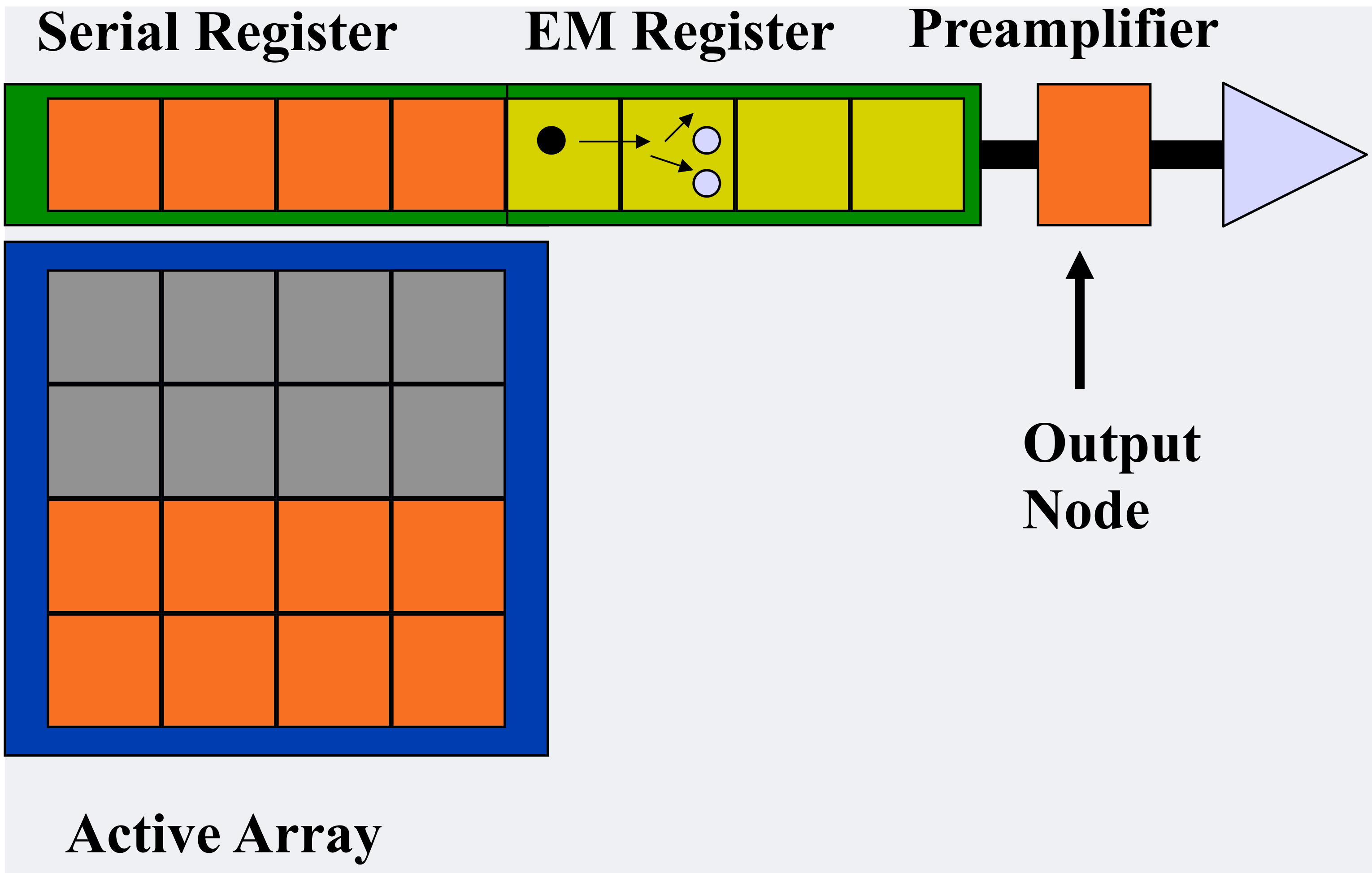

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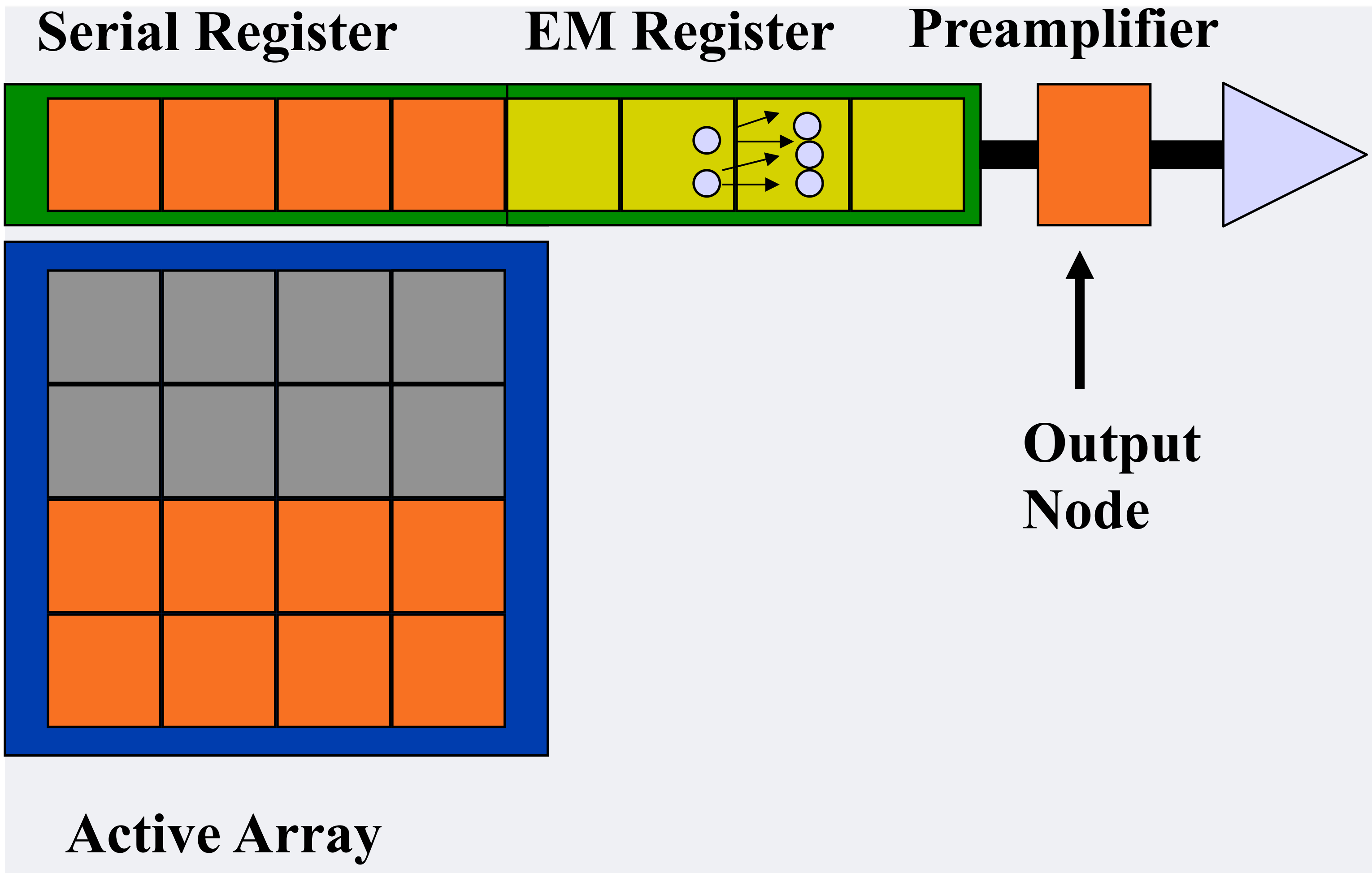
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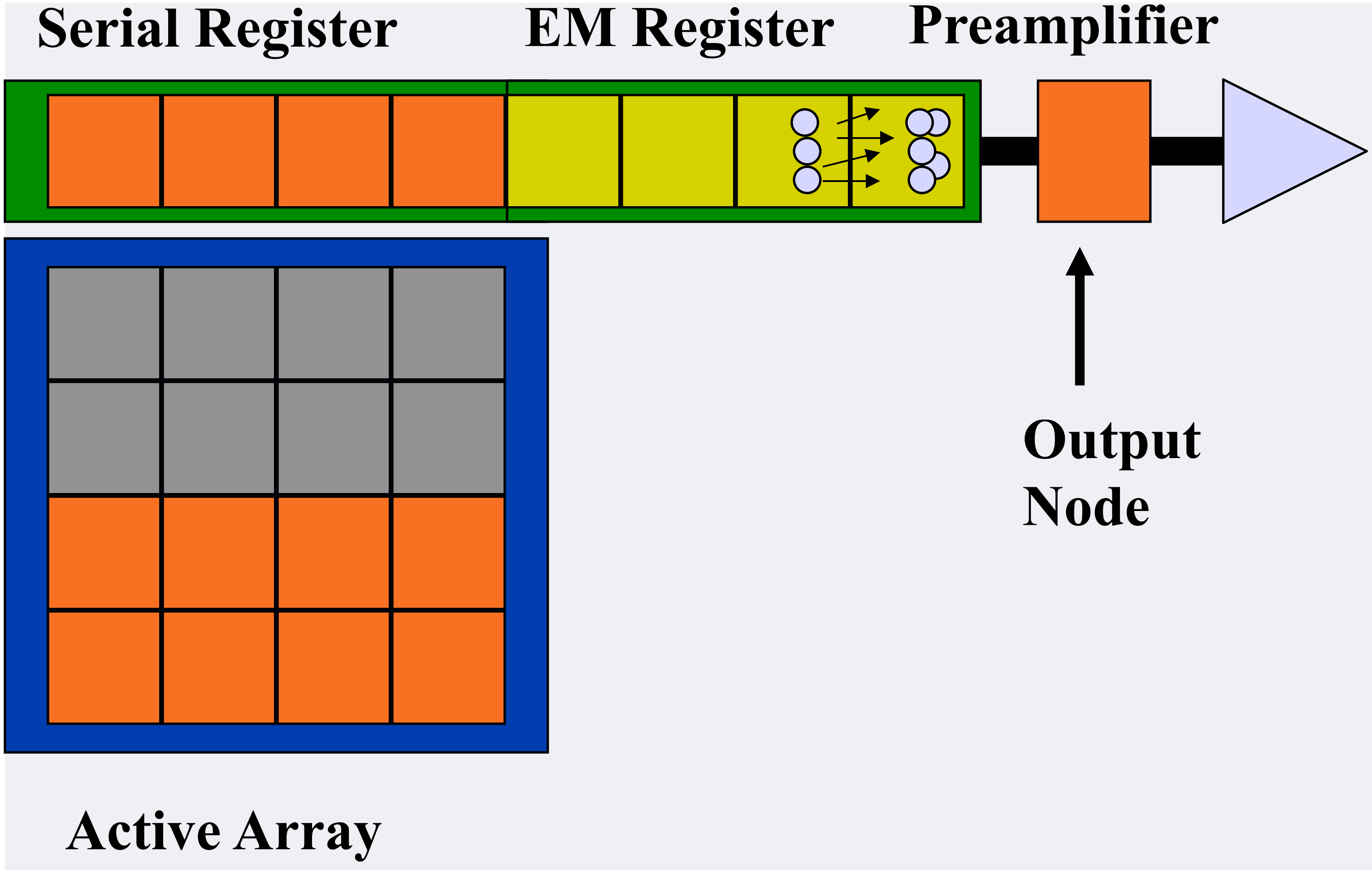
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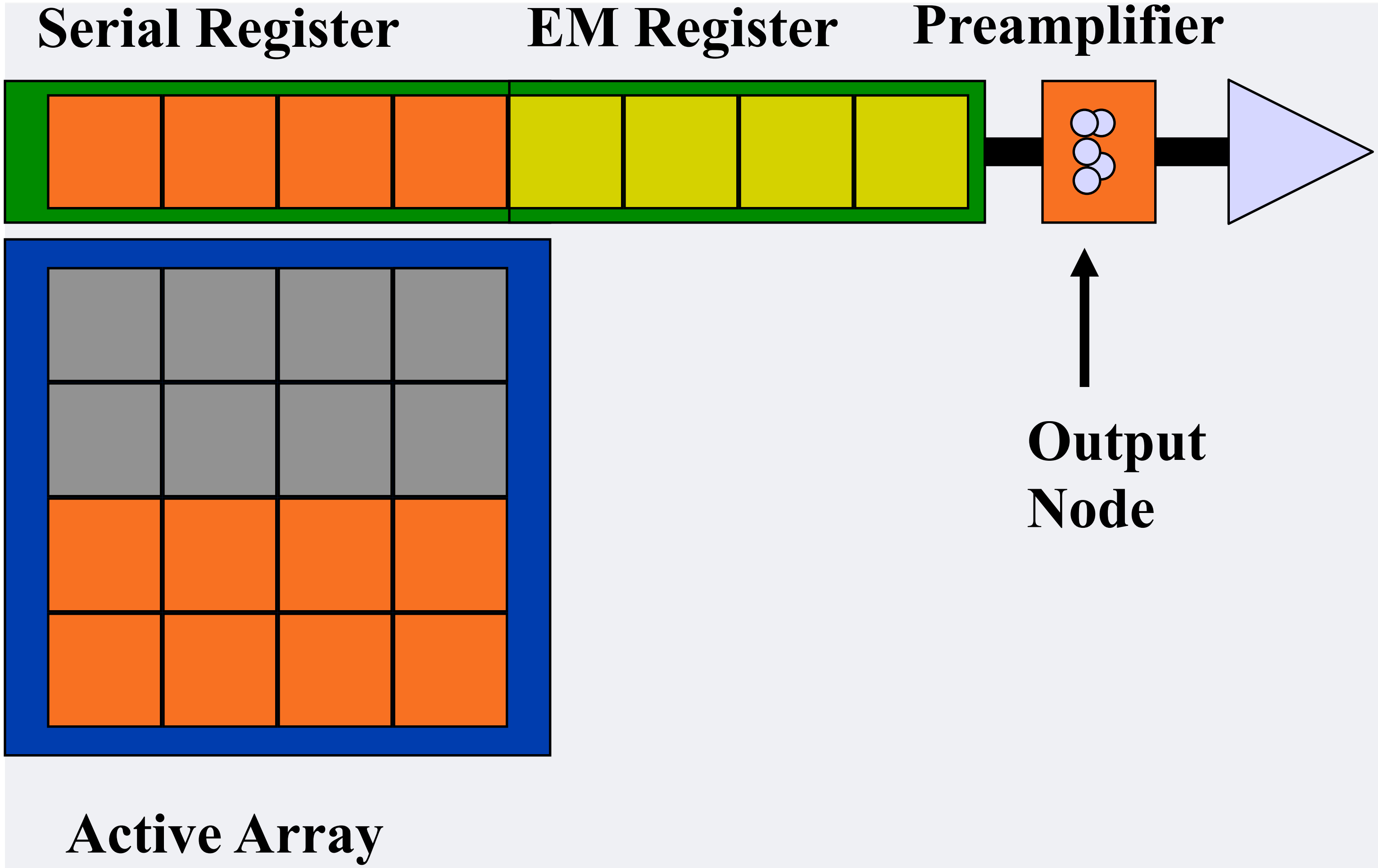
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EM Register
Preamplifier
Output
Node
Active Array

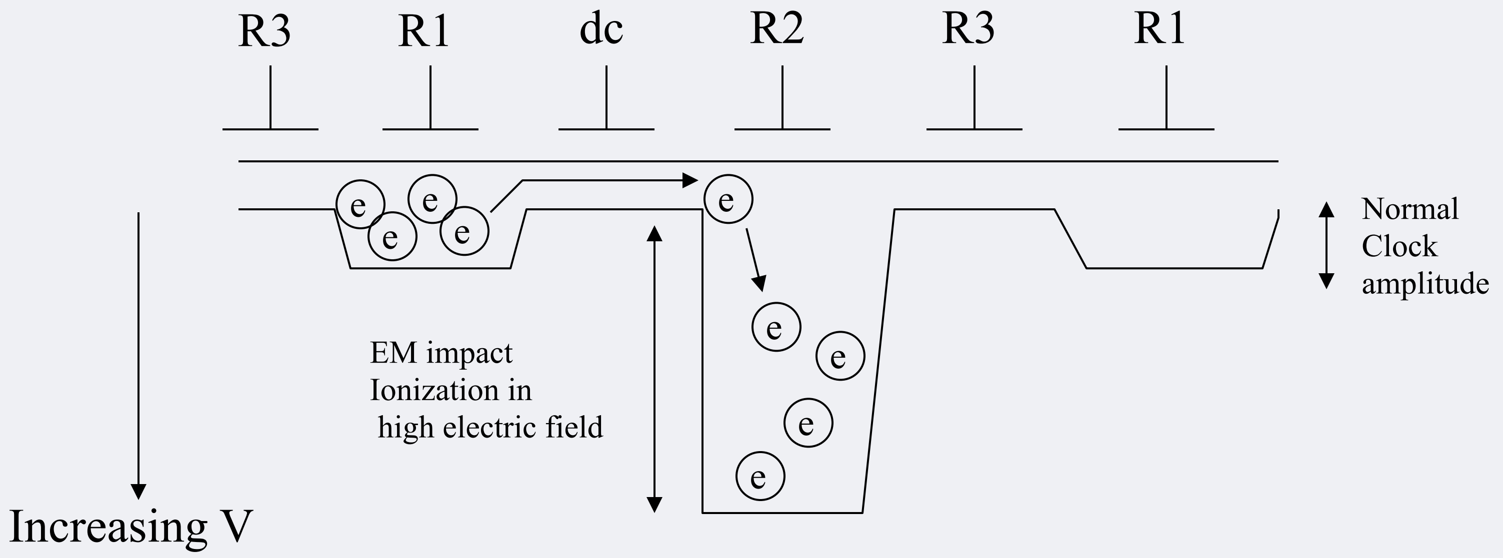
More detailed look at EMCCD's

- They contain a 'gain register' between the usual serial shift register and the output amplifier.

- Similar to serial register except for R2 phase of the clock cycle which has 2 electrodes
  - 1st held at fixed potential
  - 2nd which is clocked at a much higher voltage amplitude (40-50V) than is required for charge transfer alone.
  - Intense electric field between them causes transferring electrons to cause ***impact ionization***

- Note that EMCCDs are subject to aging and that over time the voltage applied will give reducing amounts of ionization.

Electron multiplication / Impact ionization



Multiplication per transfer quite small (x1.01 to x1.016). Executed over a large number of transfers leads to significant EM Gain!

Mathematically: $G = (1+g)^N$

G= EM gain, g=probability of secondary electron generation

$1.015^{536} = 2923$X EM Gain

Issues with EMCCD cameras

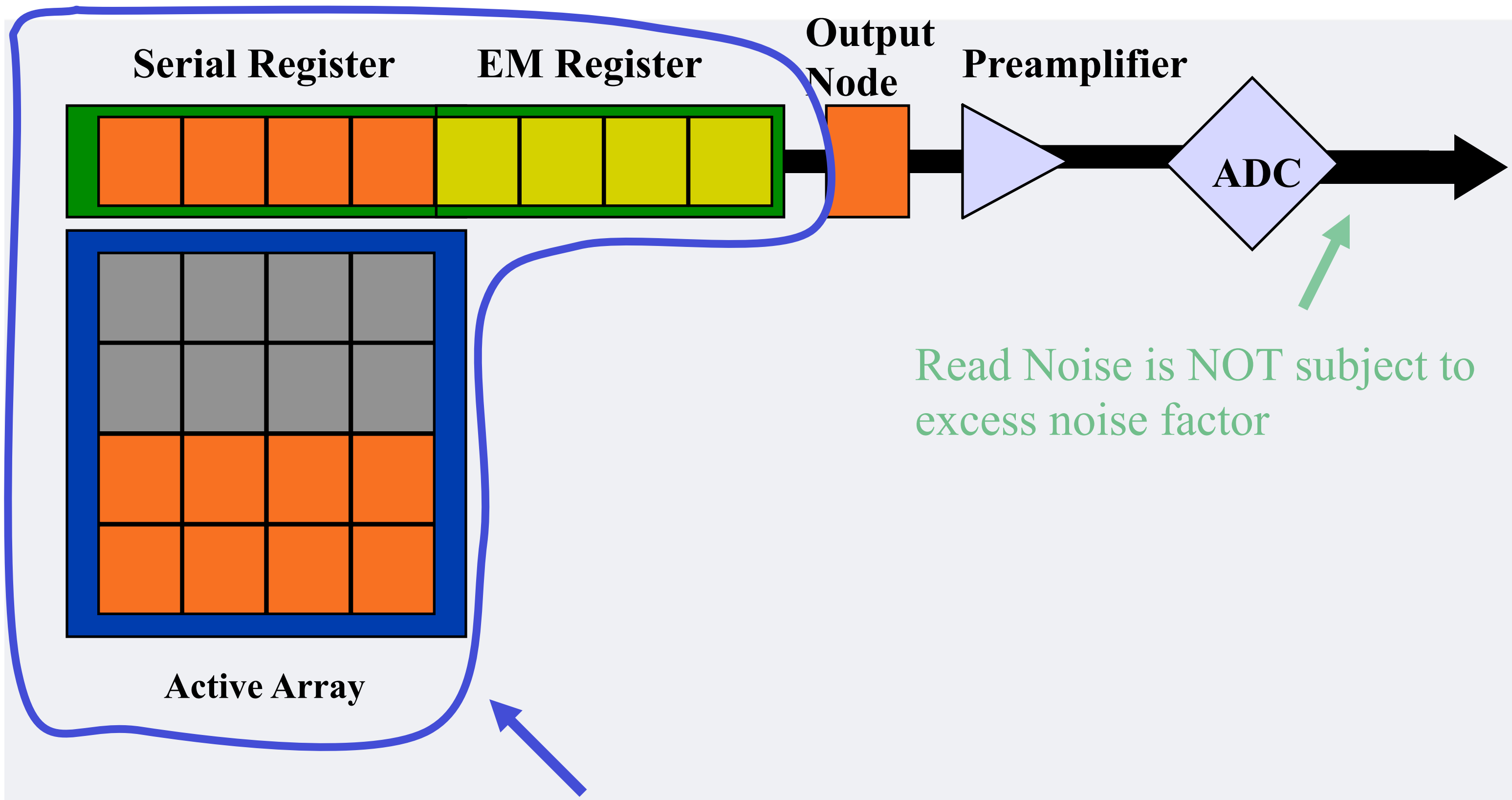
- EM Gain decay
- Bias Stability
- EM Gain Stability
- Back ground events – CIC and dark current

- Excess Noise Factor

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.

Basic concepts



Read Noise is NOT subject to excess noise factor

Anything that occurs here is subject to excess noise factor!

e.g. Shot noise, Dark Noise, CIC

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

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

SNR: The new equation

On-Chip Multiplication Gain CCD SNR:

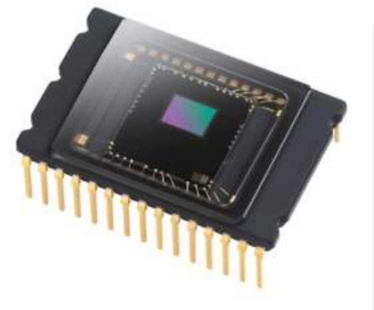
$$SNR = [S*QE] \div \sqrt{[S*QE*F^2 + D*F^2 + (\sigma_R/G)^2]}$$

Note: F is the excess noise factor.

Available sensors

- E2V 997 – 512 by 512 Back illuminated 16 micron pixels
- E2V 201 – 1024 by 1024 Back illuminated 13 micron pixels
- E2V CCD60 128 by 128 Back illuminated 24 micron pixels
- Texas Instruments TC 285 – 1004 by 1002 Front illuminated 8 micron pixel

CMOS



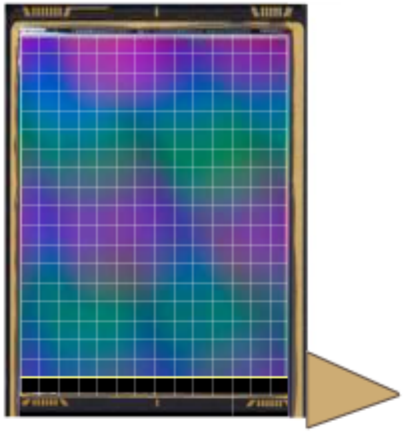
- CMOS as a technology is as old as CCD but was not considered as a sensor capable of light detection until 1992 by Dr. Eric Fossum, a scientist at NASA’s Jet Propulsion Laboratory
- CMOS technology, as CCD, uses an array of light sensitive pixels to collect full area image
- CMOS technology differs by completing all digitisation at the pixel point rather than needing to read the signal and then digitise
- CMOS sensors also, by nature, require around 100x less power than CCD making them the perfect choice for camera phone sensors
- As sensors are mass produced for mobile phone imaging and also for non-imaging applications, the pricing has been driven low by the market

CMOS Architecture

Introduction

Charge Coupled Device (CCD) detectors come in three major architectures, Full Frame (FF), Frame Transfer (FT) and Interline (IL). The animation shows the different CCD types and readout modes.

- Full Frame CCD
- Frame Transfer CCD
- Interline CCD
- EMCCD
- CMOS Readout
- CMOS Global
- CMOS Rolling



CMOS – Any downsides?

- Each photo site in the CMOS sensor has three or more transistors ,which has its benefits and its draw backs
- The transistors allow for processing to be done right at the photo site, and each pixel/photo site can be accessed independently
- Because the transistors occupy space on the array, some of the incoming light hits the transistors and not the photo sites, which leads to picture noise
- CMOS sensors also function at a very low gain which may contribute to noise.
- Small pixel sizes often lead to small full well capacities and low dynamic range
- Rolling Shutter mode not accepted and can lead to

Rolling Shutter

Rolling Shutter

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Where is CMOS currently used?

- Brightfield Microscopy
- Industrial Inspection
- High Speed applications

Where is CMOS Heading ?

- High Speed Fluorescence Microscopy for dynamic studies and observation

Making CMOS Scientific

- July 2009 3 camera manufacturers launched a white paper on a new type of camera technology called SCMOS (scientific CMOS) – Camera became available for mass market March-June 2011

- June 2010 – Another manufacturer launches a Scientific CMOS camera

- But what is Scientific SCMOS?

- SMCOS is a trade mark owned by Fairchild imaging
- Scientific CMOS is open and can be freely interpreted by camera companies

What is Scientific CMOS (not SCMOS)

- Its undefined

We think it means

- Faster than normal CCD equivalent cameras
- Lower Read noise than CCD cameras
- Linear Response to light

Note

SCMOS = Fairchild sensor 6.5 micron pixel
Scientific CMOS = Sony 3.5 micron pixels

SCMOS offers

- SCMOS offers 4 or 5 Million pixels at 100 frames per second with 1.3(ish) Electrons of read noise, 55%+ QE

- The technology is fantastic allowing for the advancement of many high speed applications

- However the technology is new and has some issues and so still makes the cameras application specific – pattern noise, linearity, Random Telegraph signal, split in image from 2 read outs, large amounts of data, triggering

- Over the next few years this market will establish for many new high speed experiments such as SPIM and high speed tracking

FIN