Welcome to:

LMBR

Imaging Workshop

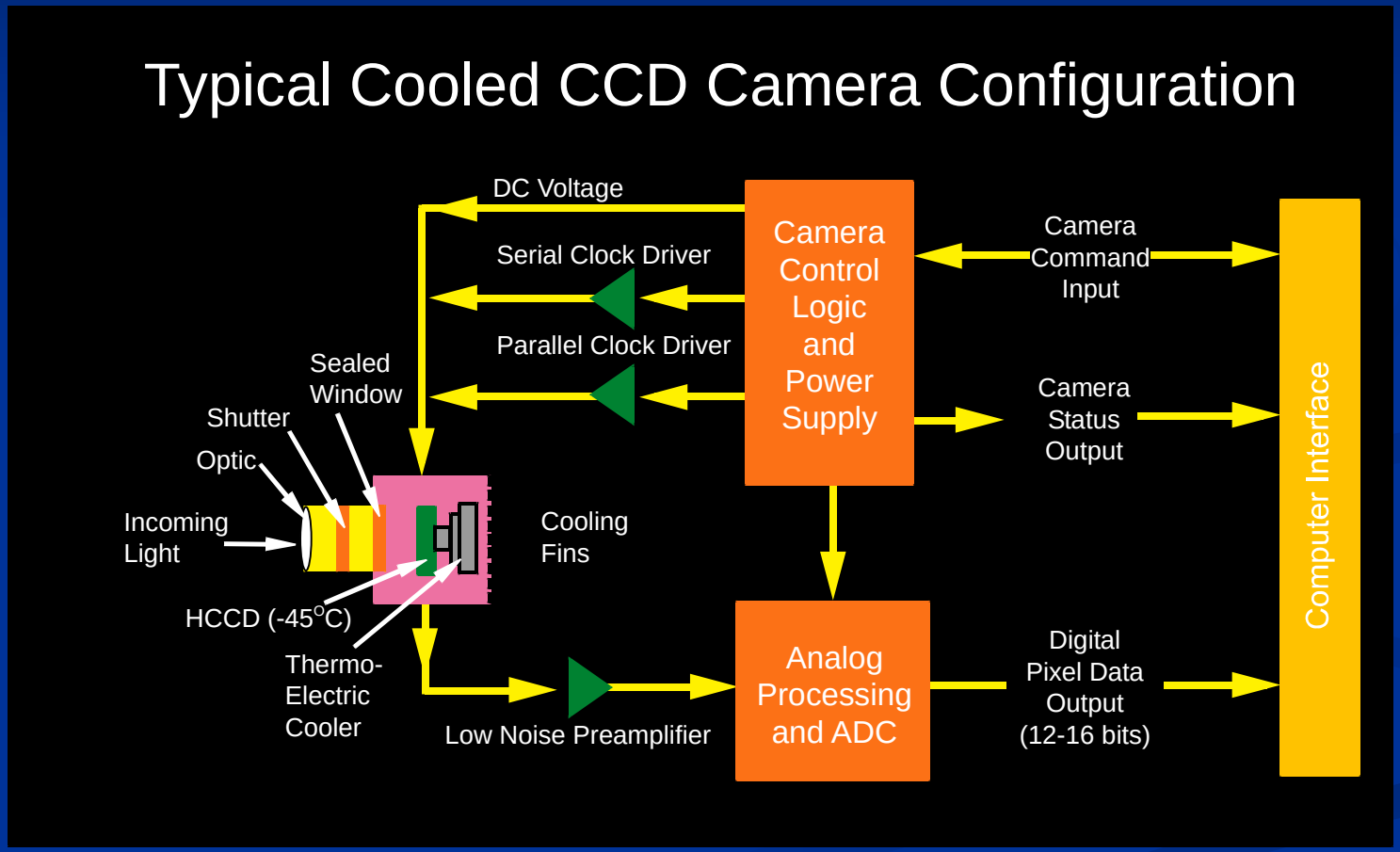
Imaging Fundamentals

Mike Meade, Photometrics

Introduction

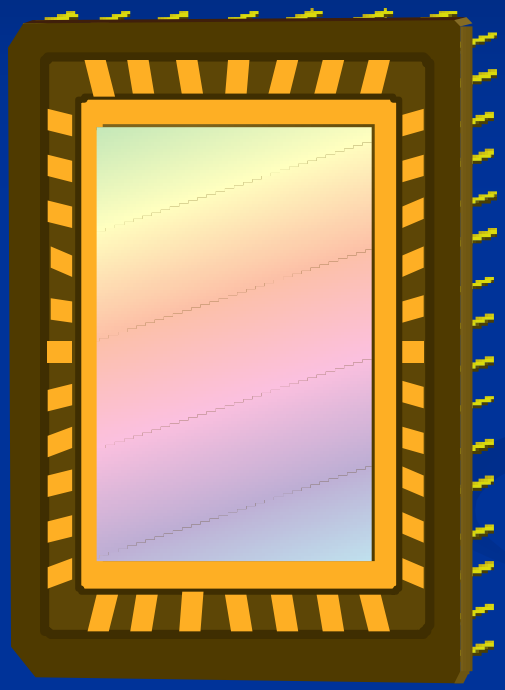
CCD Fundamentals

CCD Fundamentals



CCD Fundamentals

ROPER SCIENTIFIC ™



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)

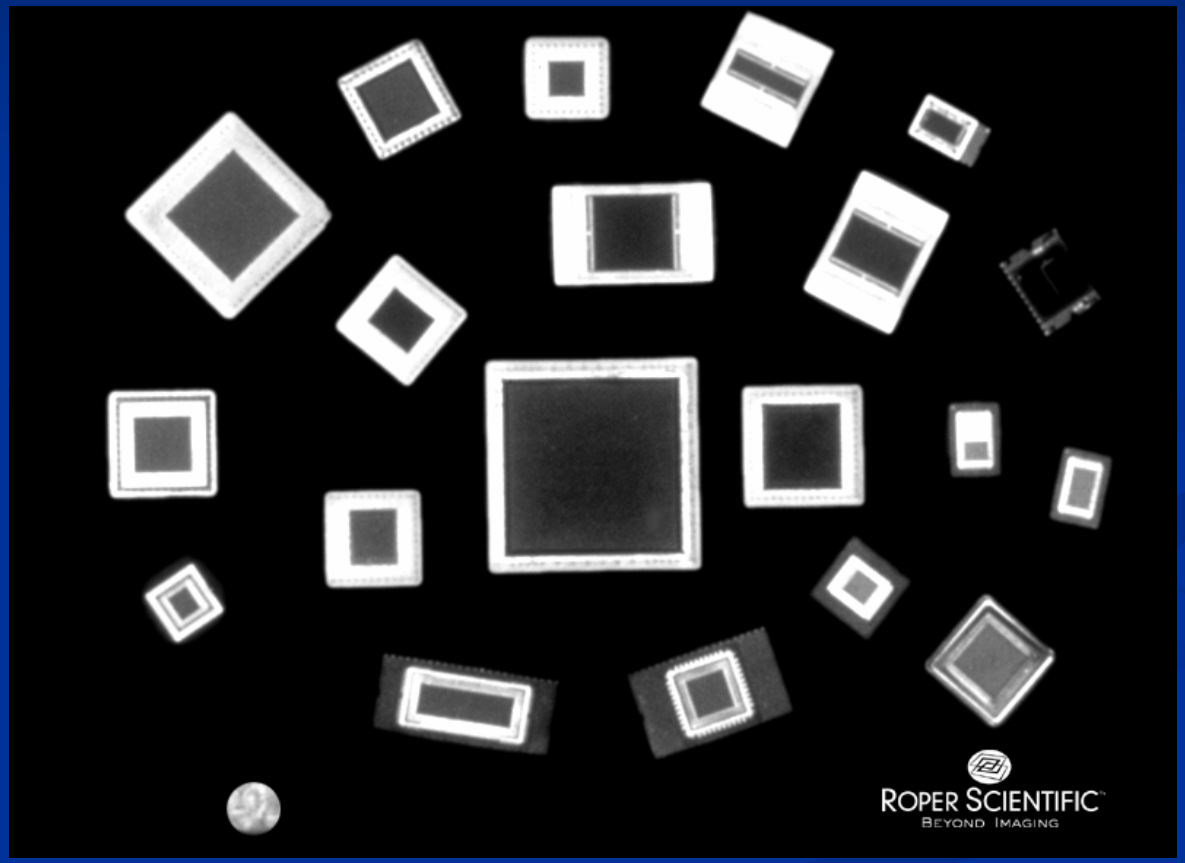
  Marconi (formerly EEV — England)

  SITe (Beaverton, OR)

  Sony

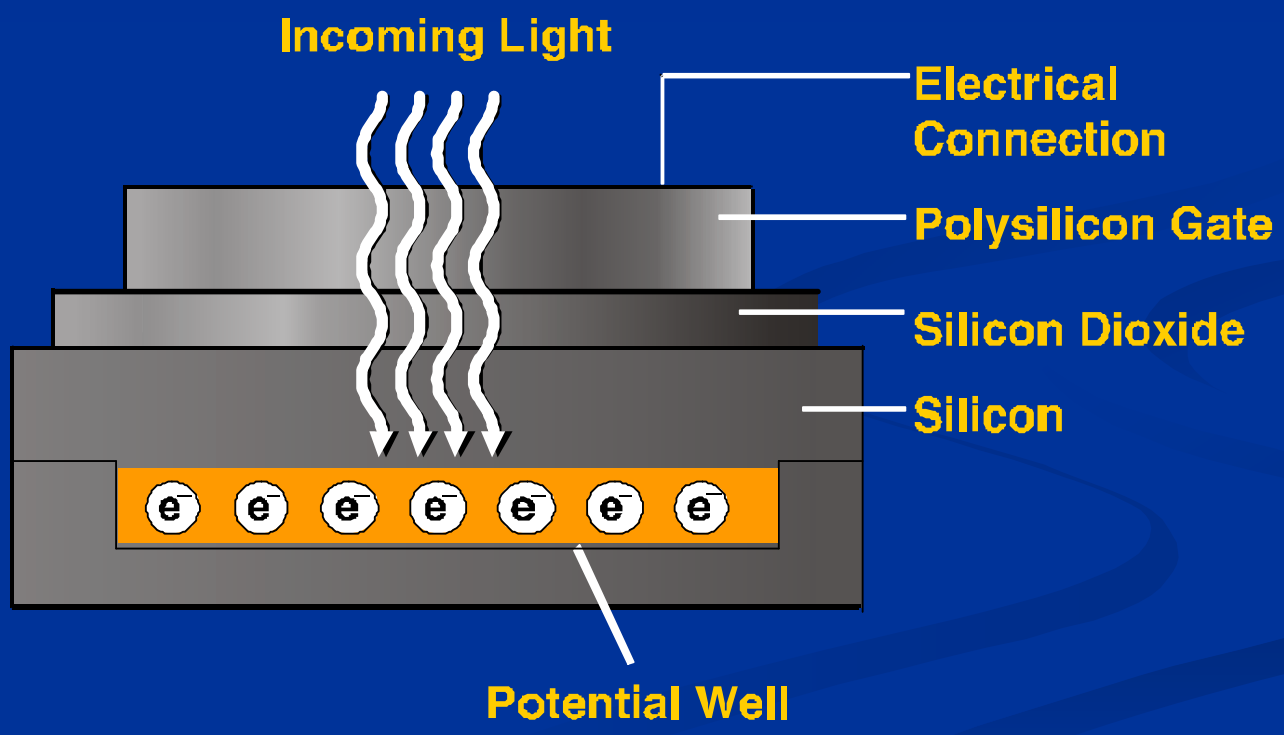
  Others

CCD Fundamentals



CCD Fundamentals

- MOS Photodetector

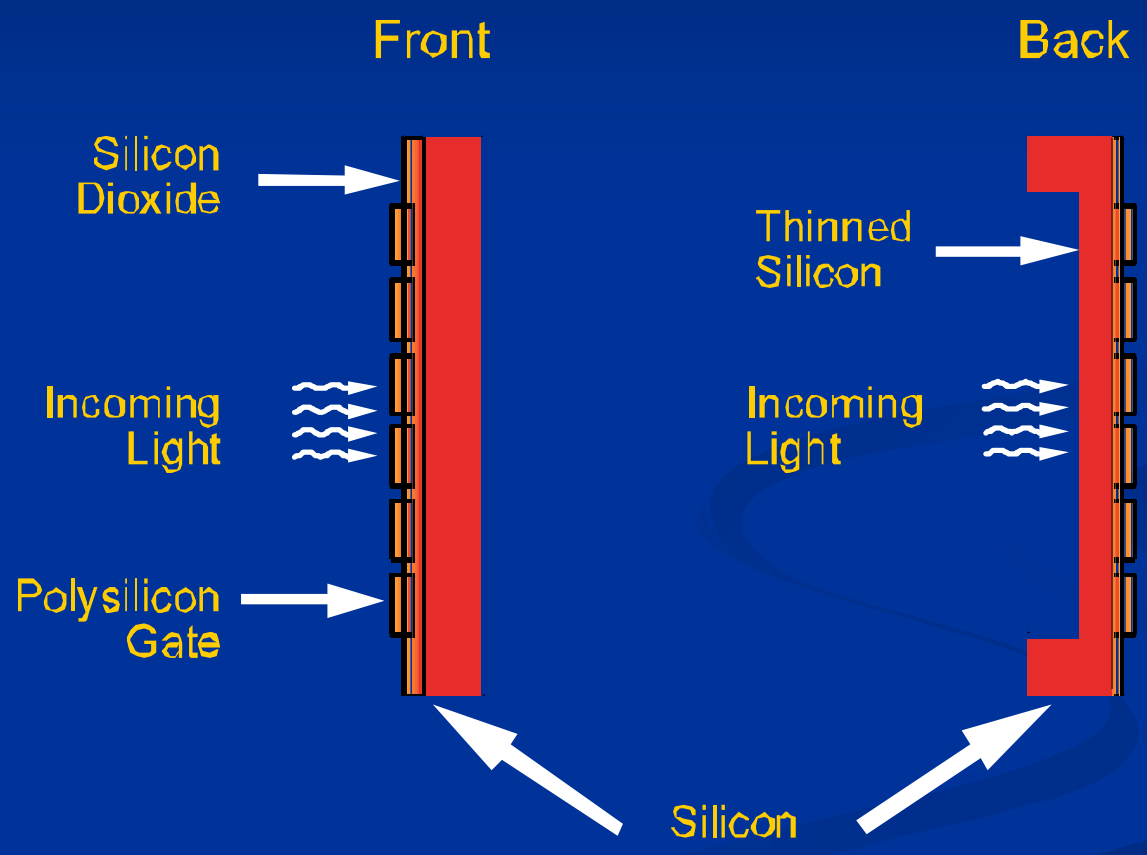


CCD Fundamentals

Quantum Efficiency

Ability of CCD to convert photons to electrons

CCD Fundamentals



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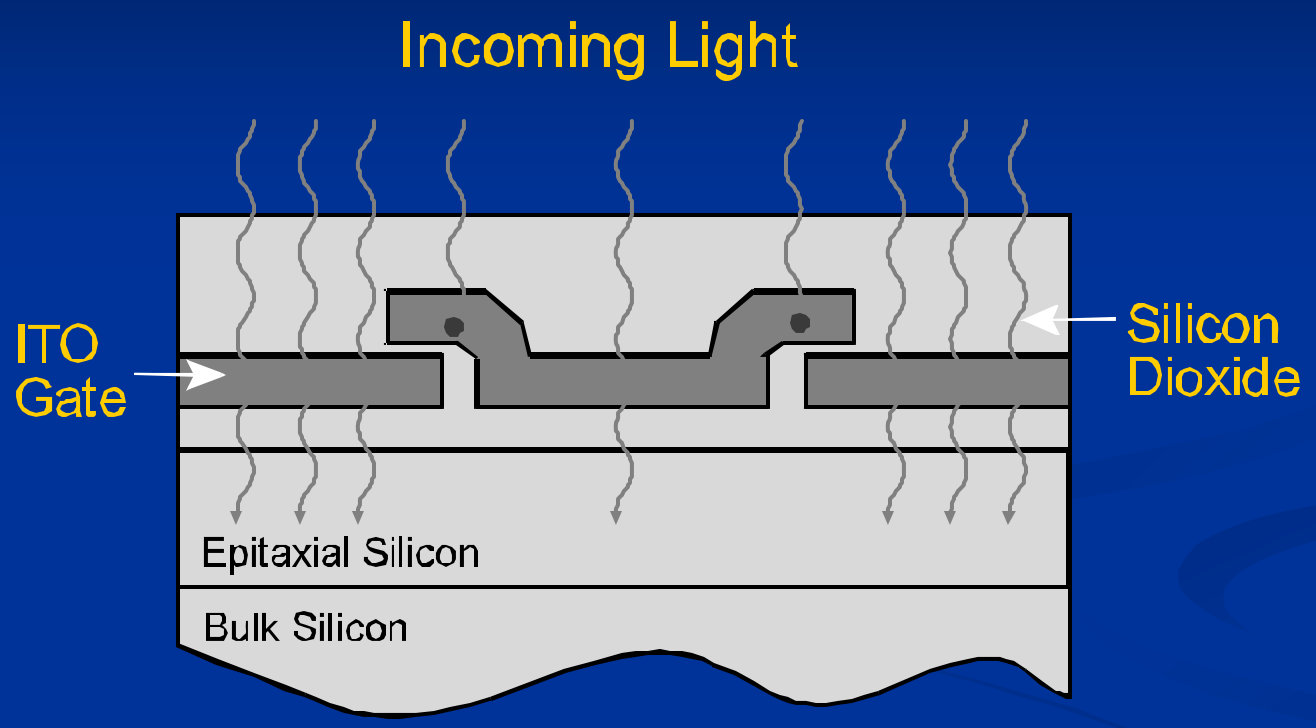

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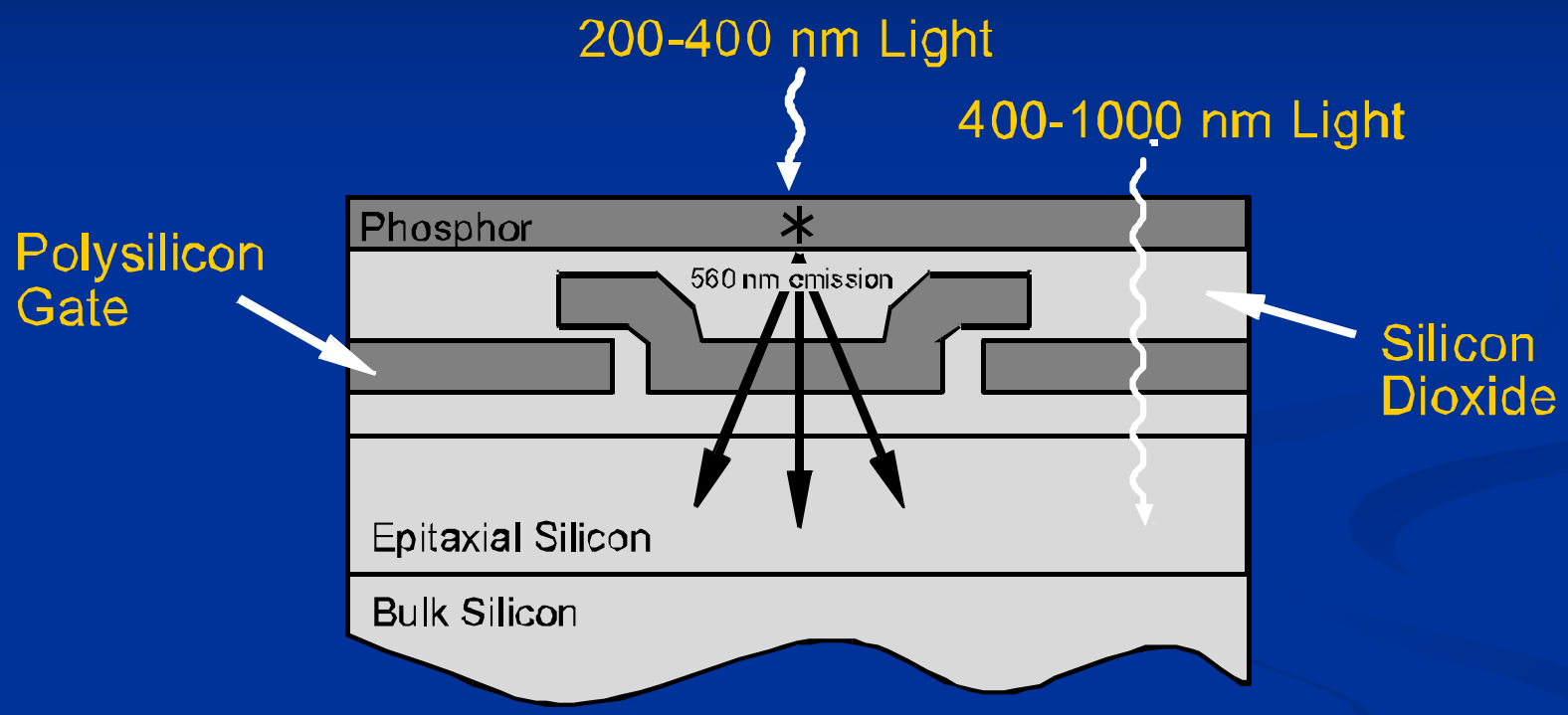

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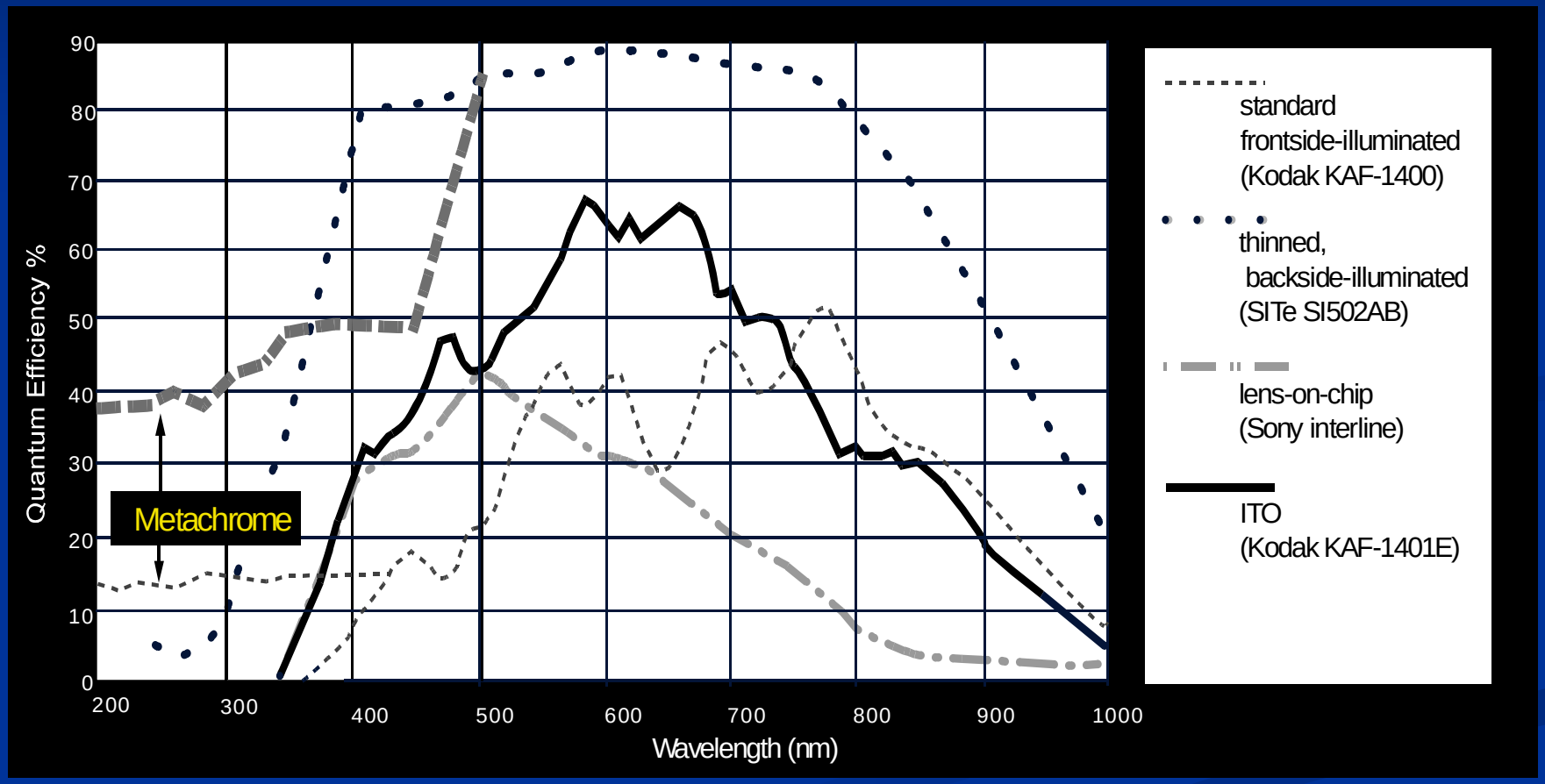

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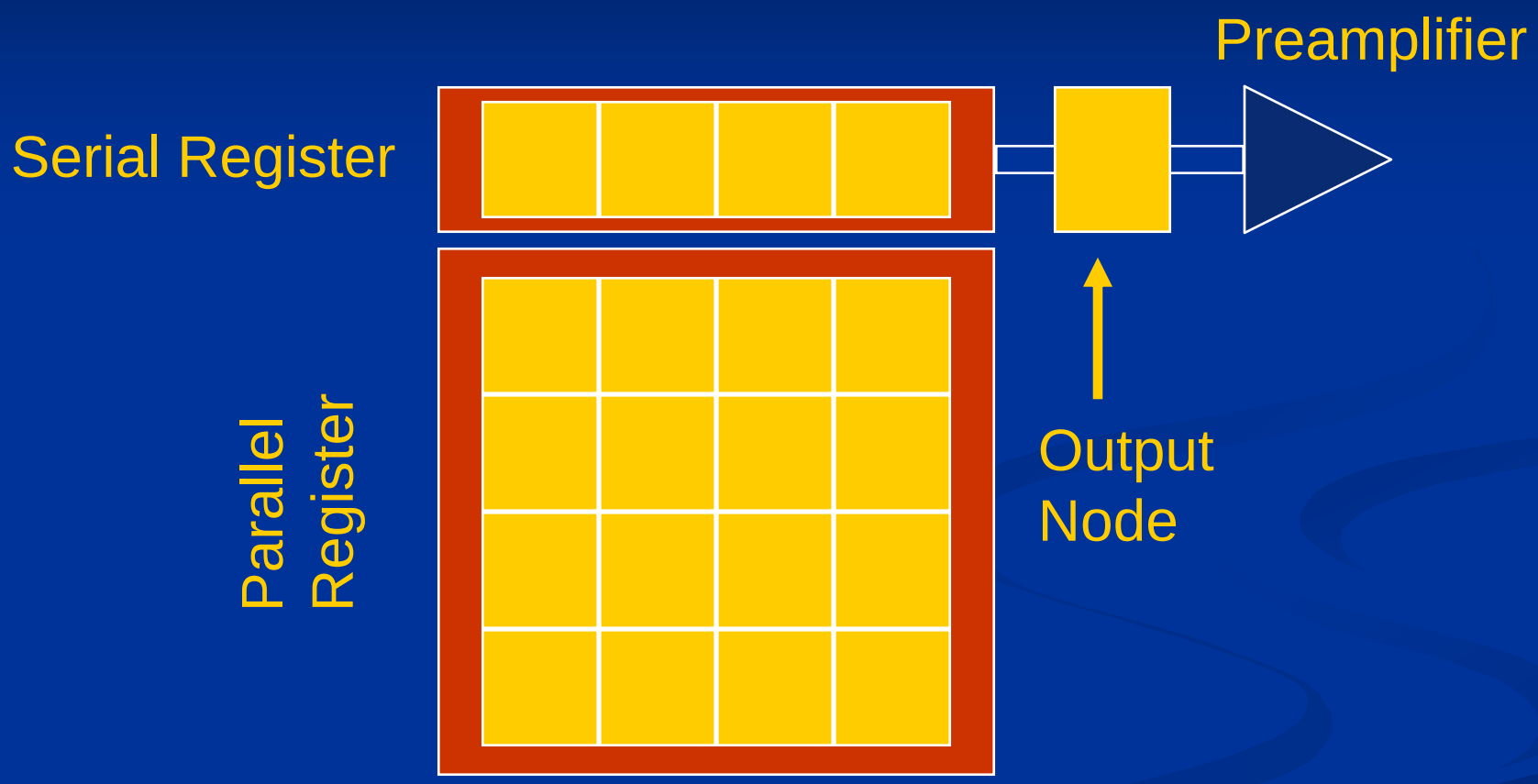

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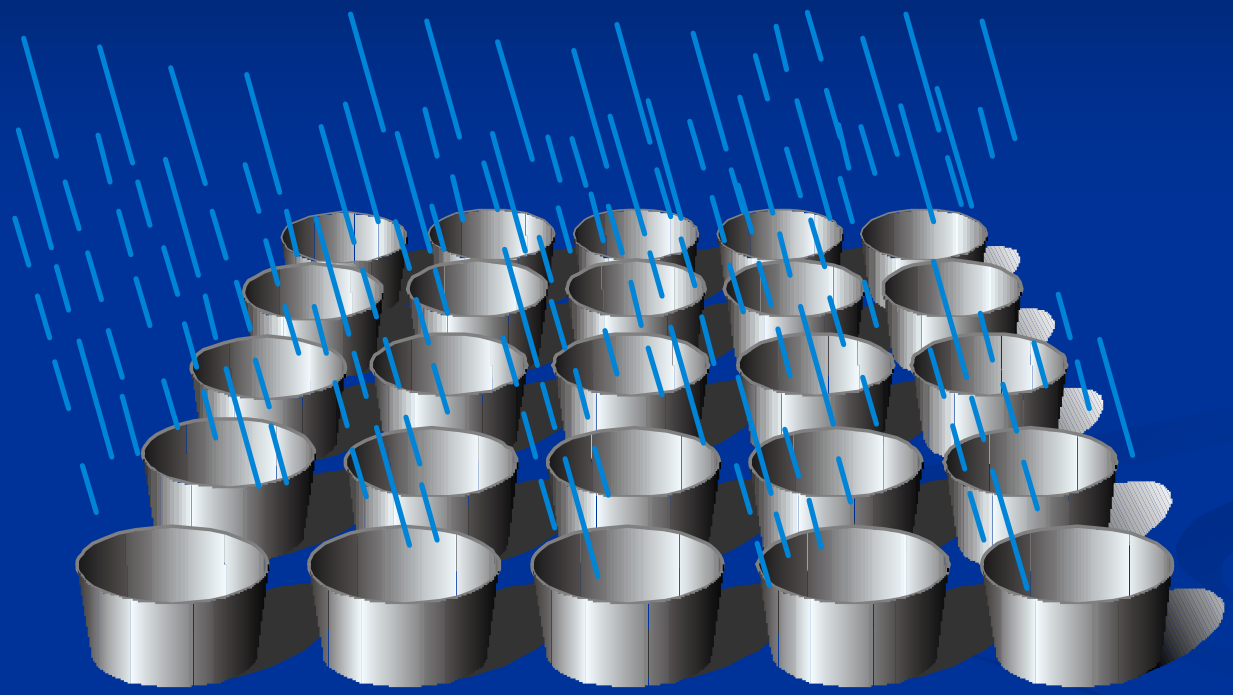
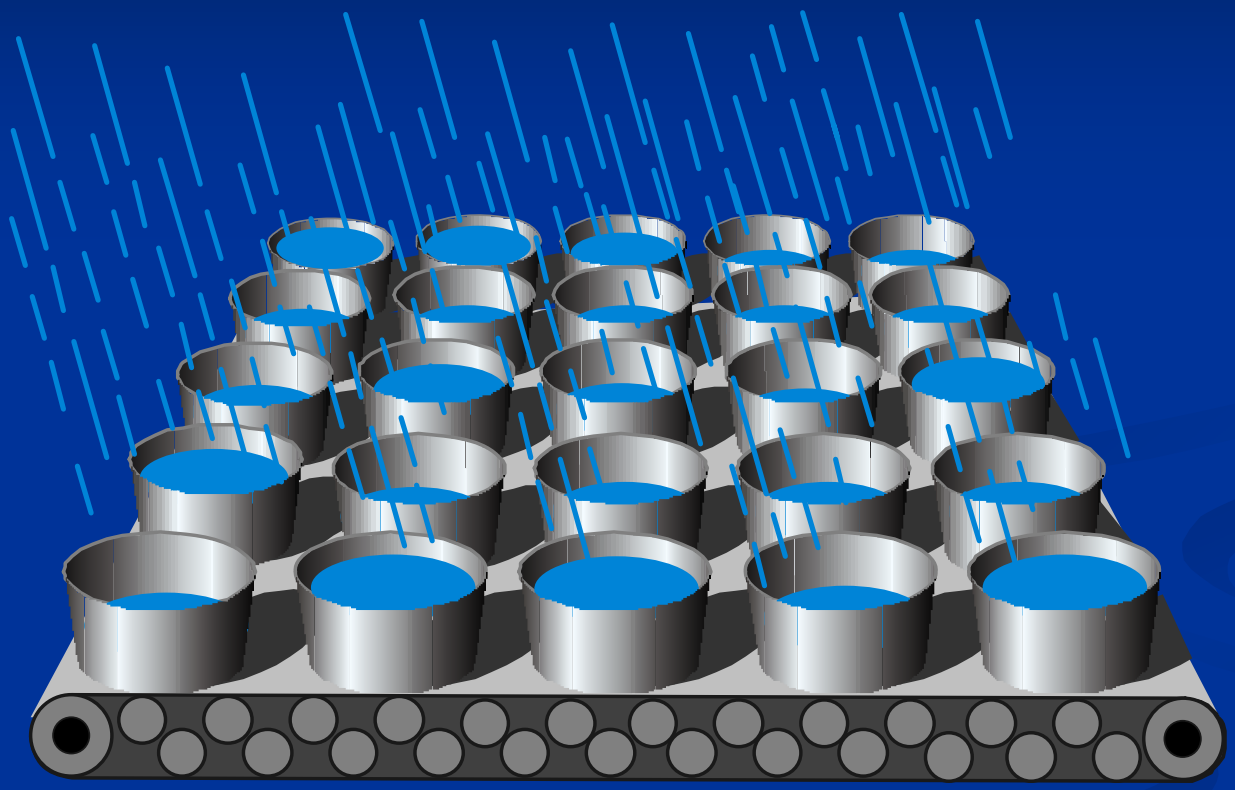


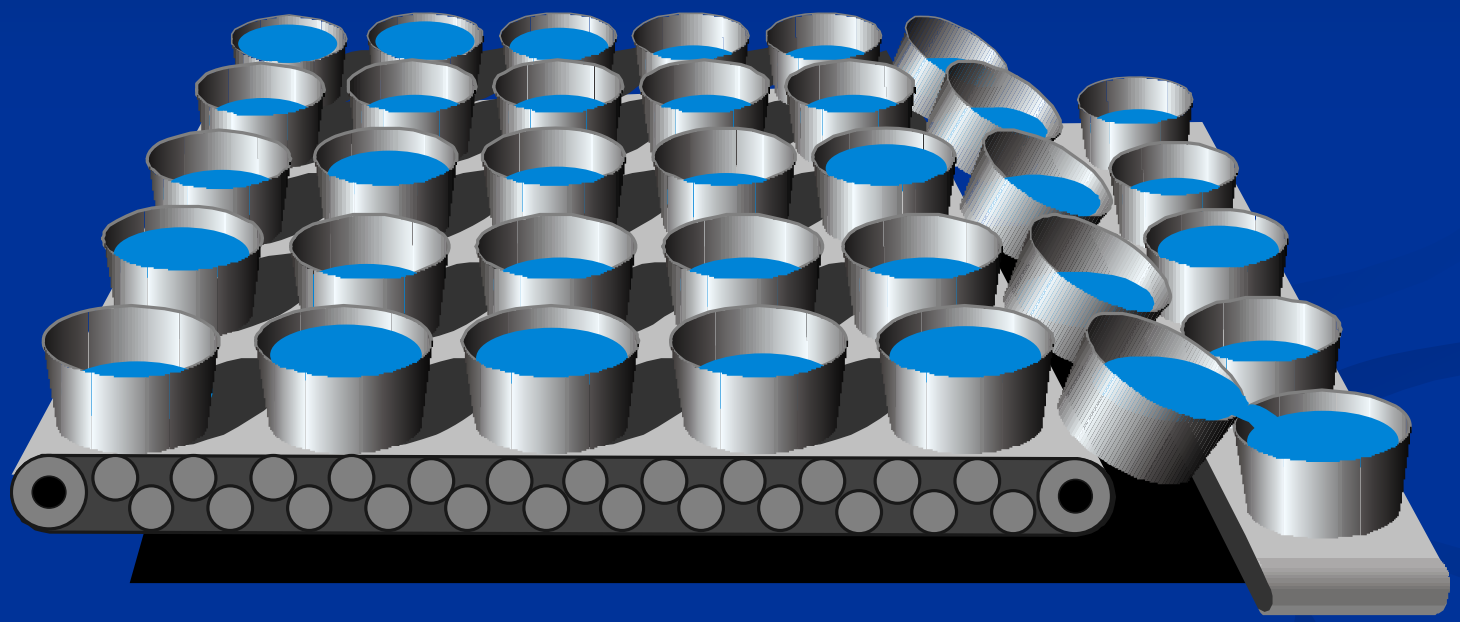
Array of Discrete Photodetectors

CCD Fundamentals



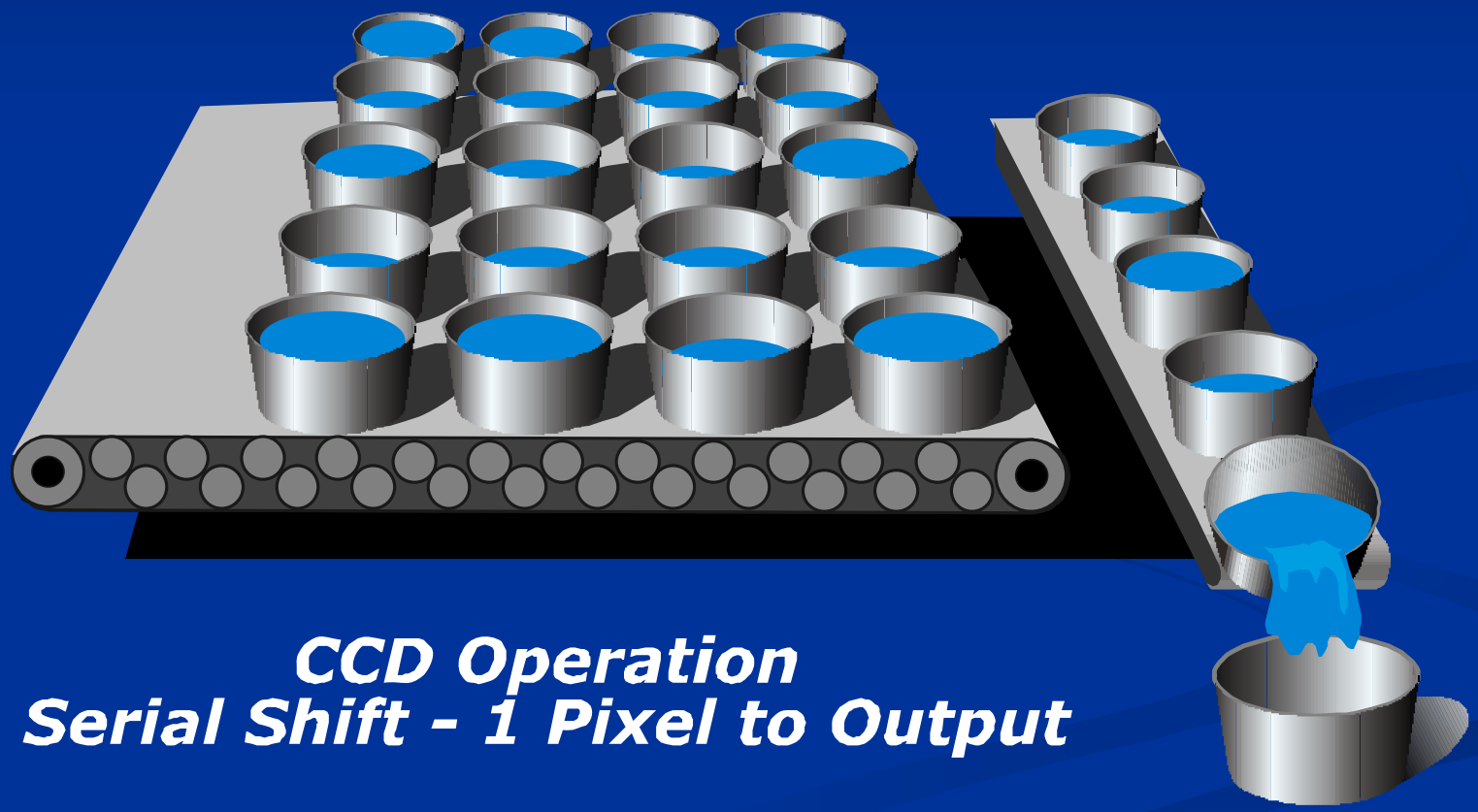
CCD Operation
Integration of Photo-Induced Charge

CCD Fundamentals



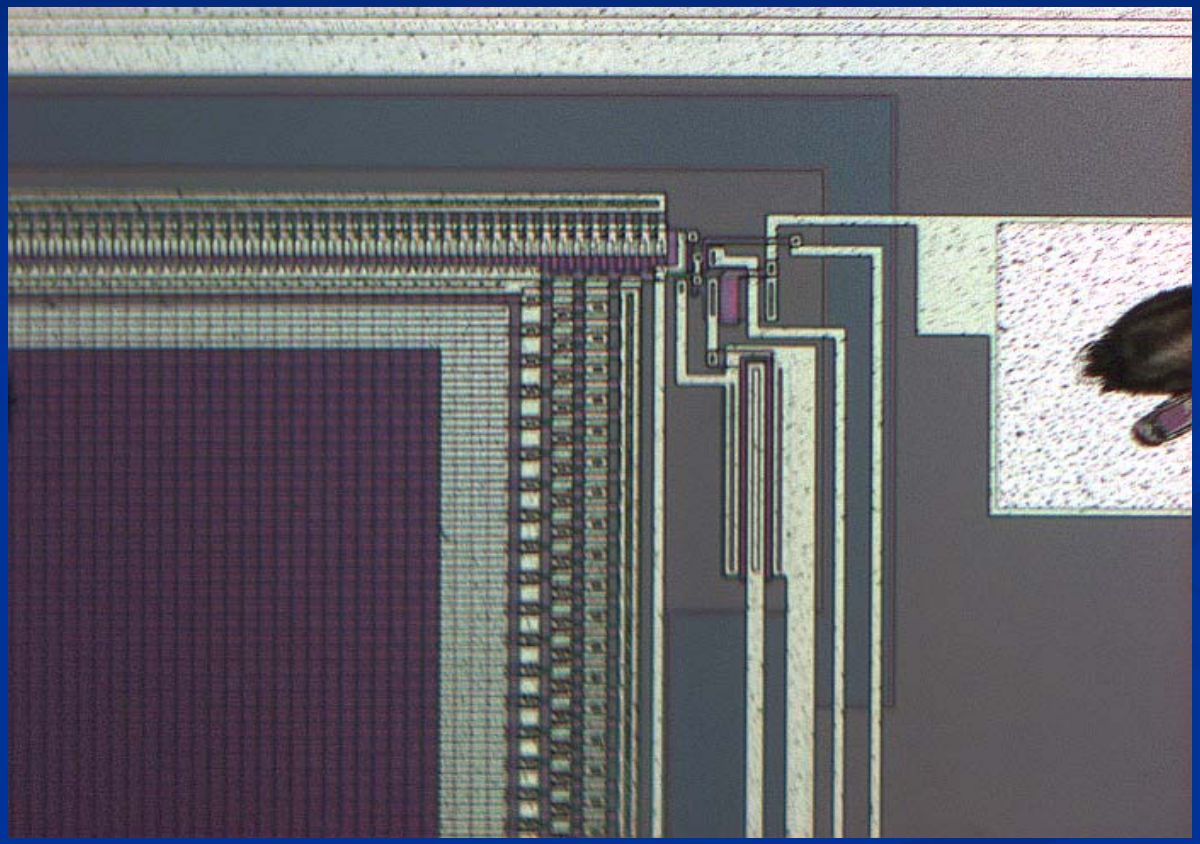
CCD Operation
Parallel Shift - 1 Row

CCD Fundamentals



CCD Operation
Serial Shift - 1 Pixel to Output

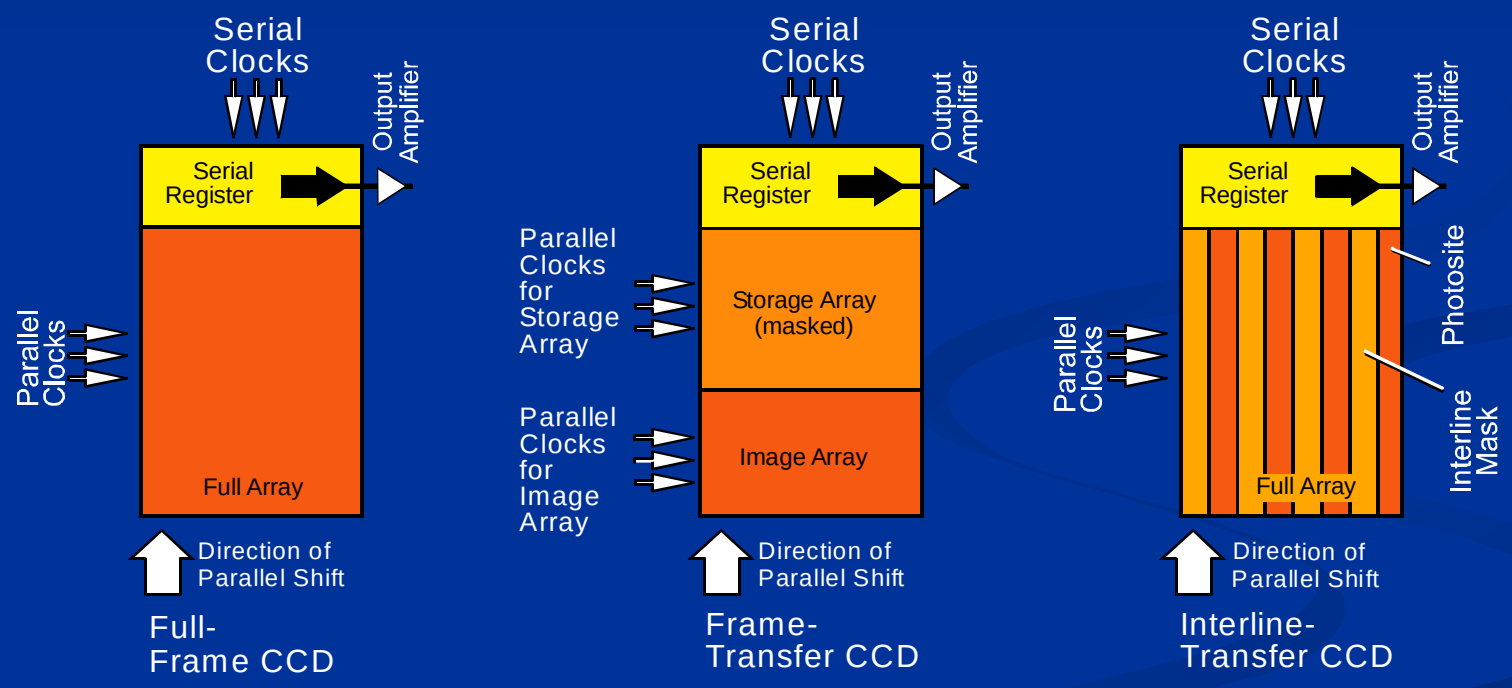
CCD Fundamentals



Introduction

CCD Architecture

CCD Fundamentals



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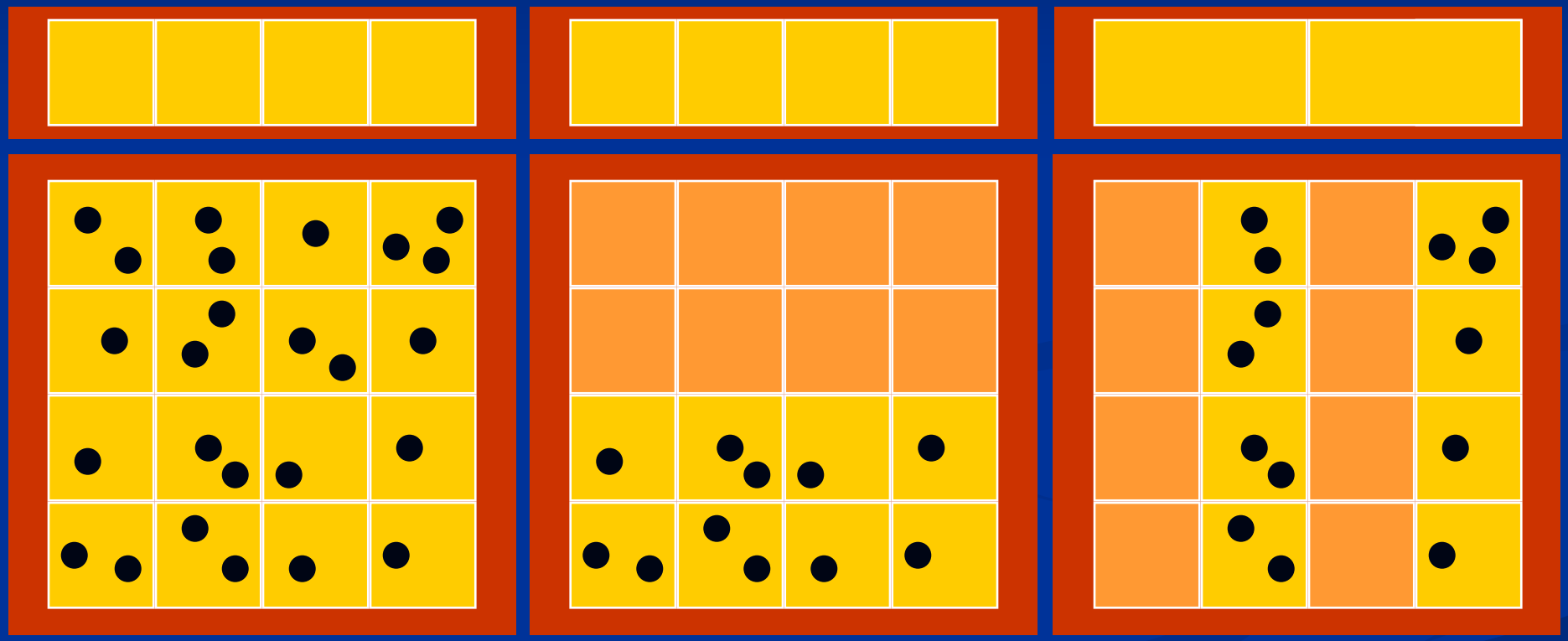
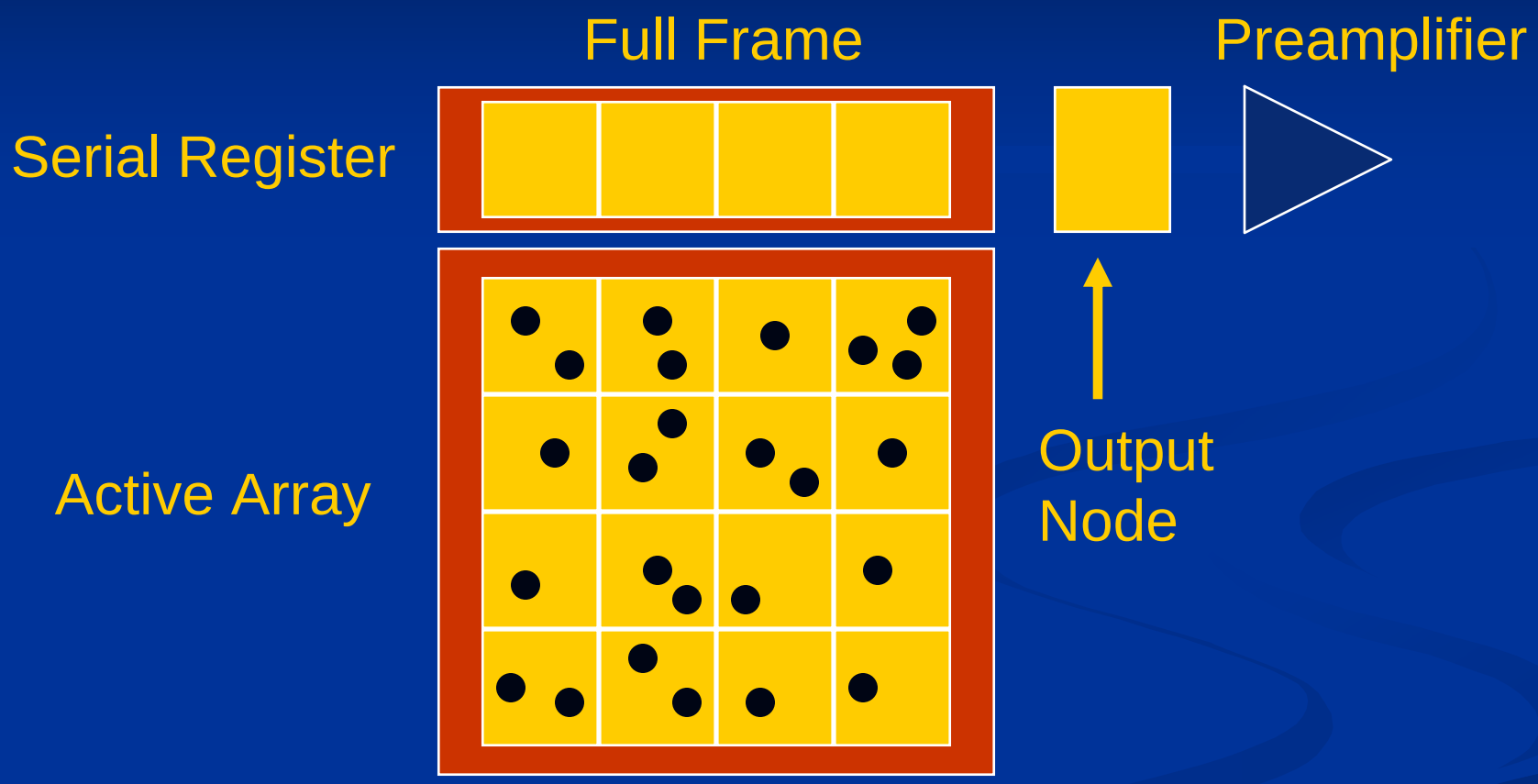


Full Frame

Frame Transfer (EMCCD)

Interline Transfer

CCD Fundamentals



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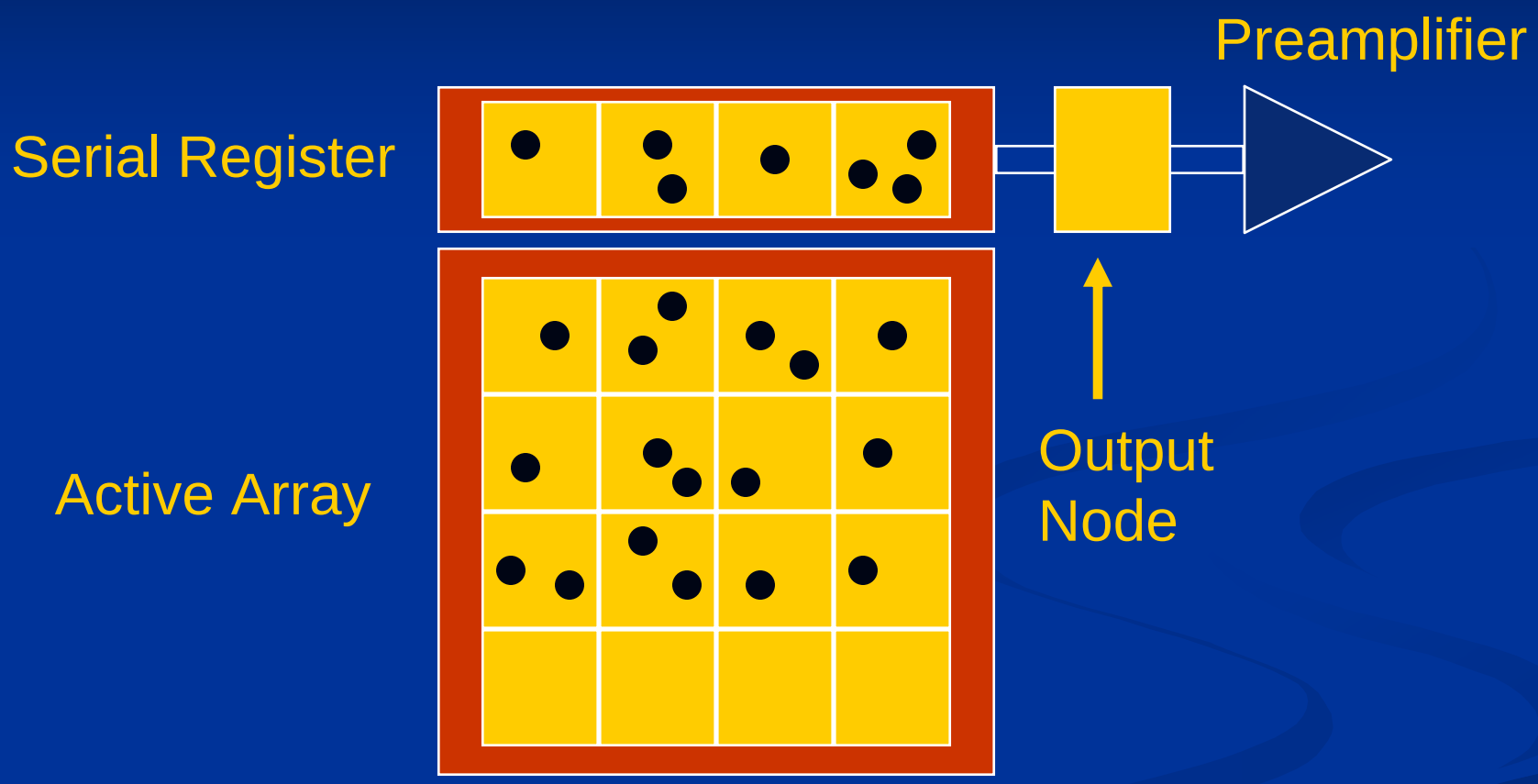

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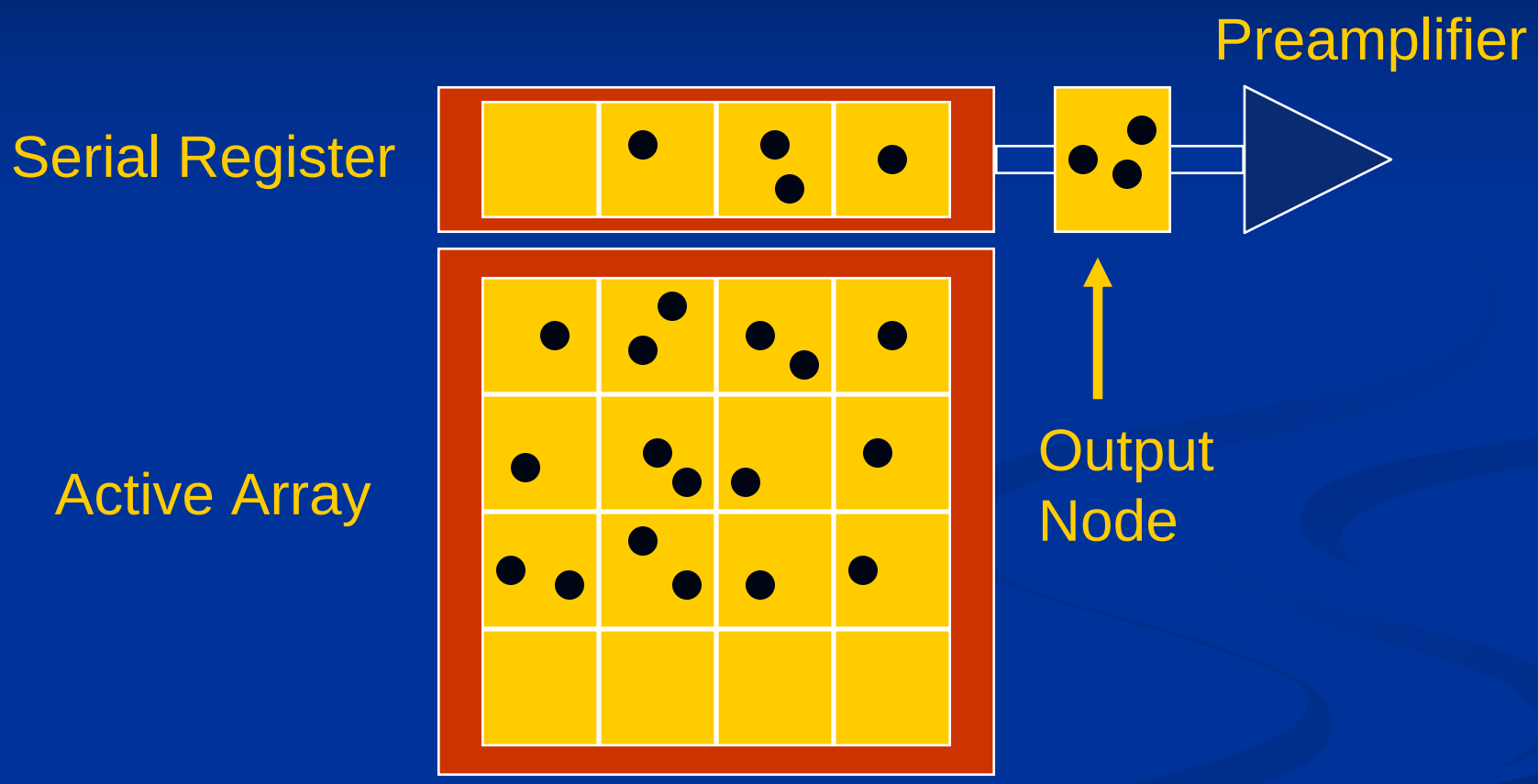

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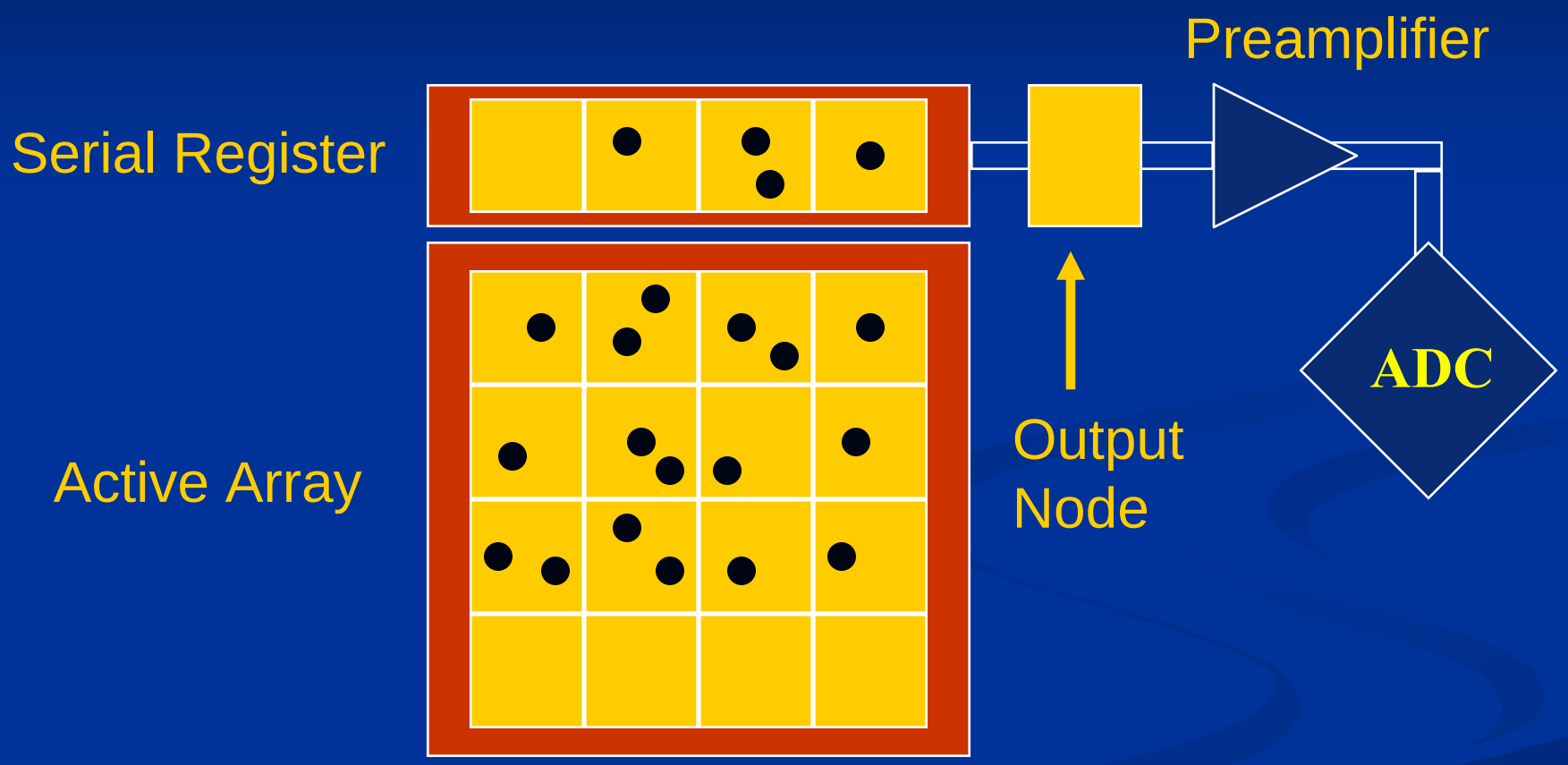

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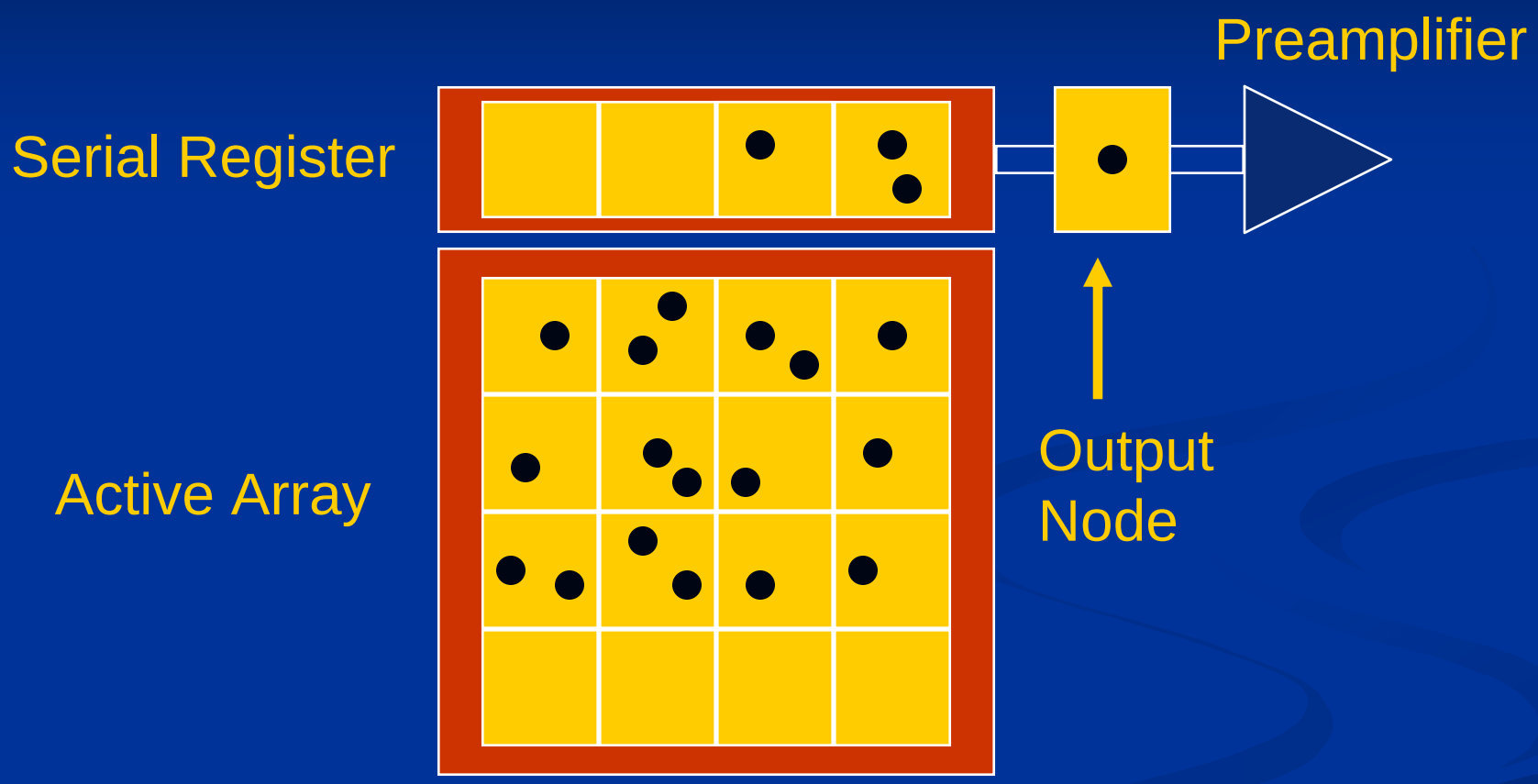

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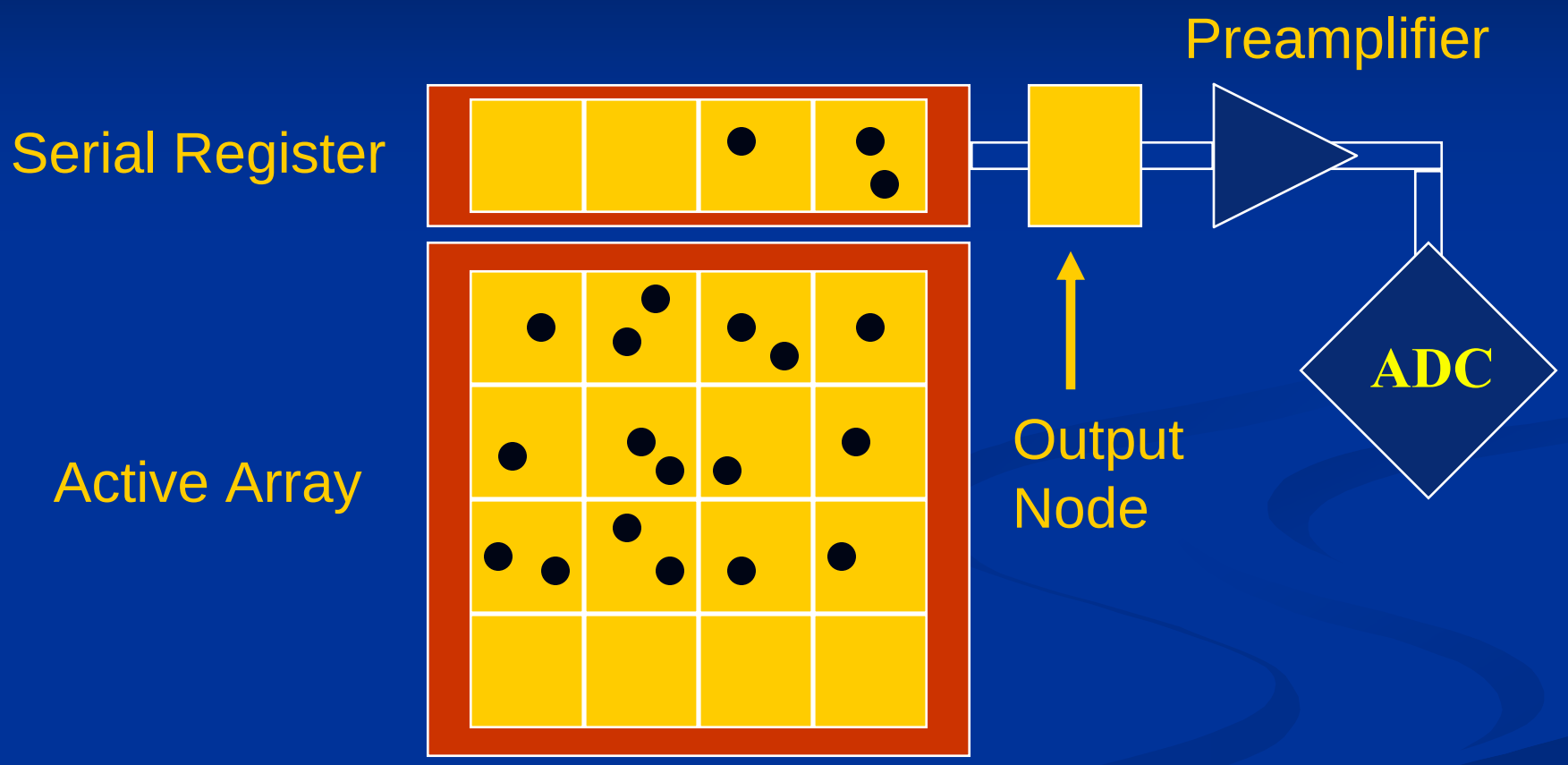

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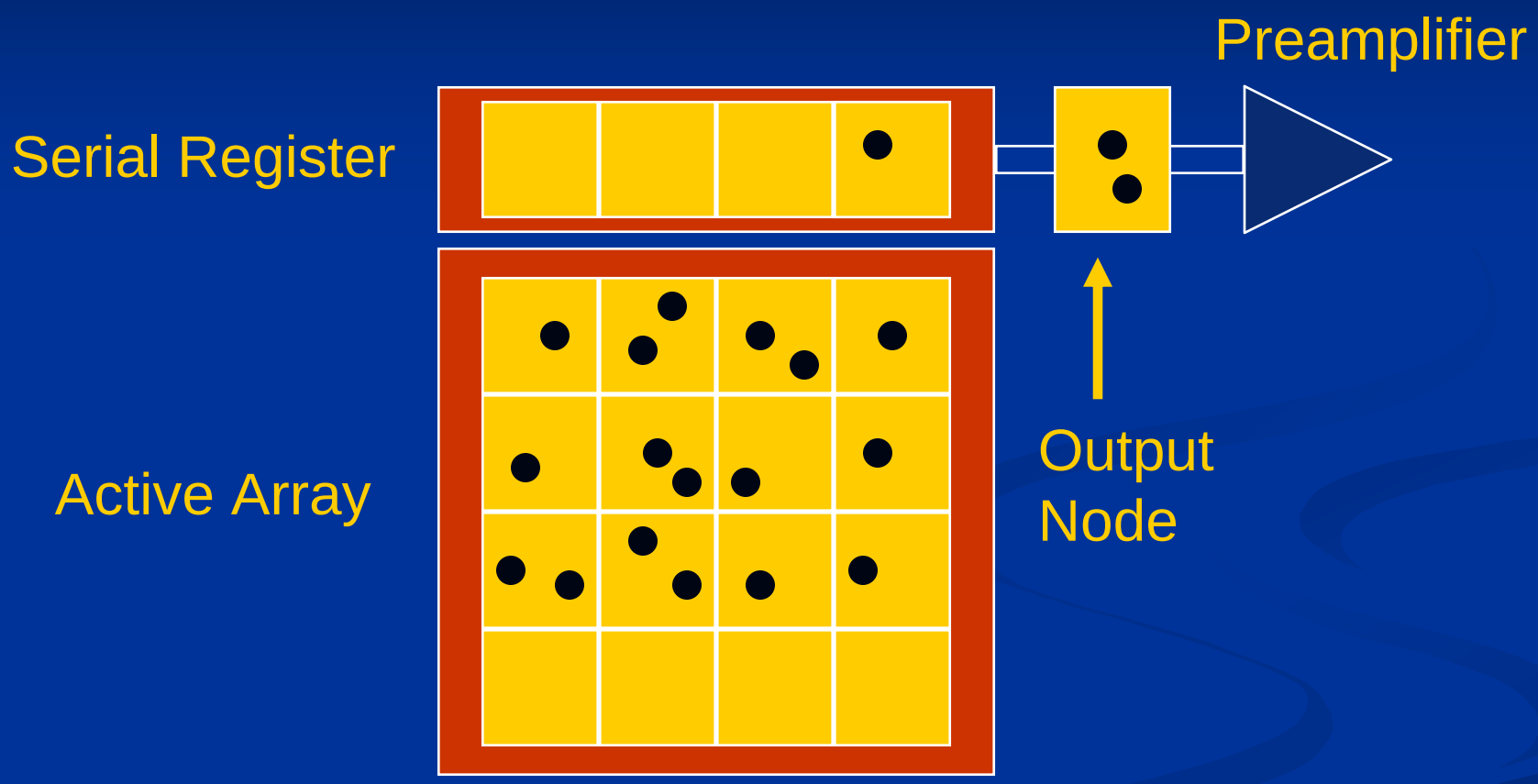

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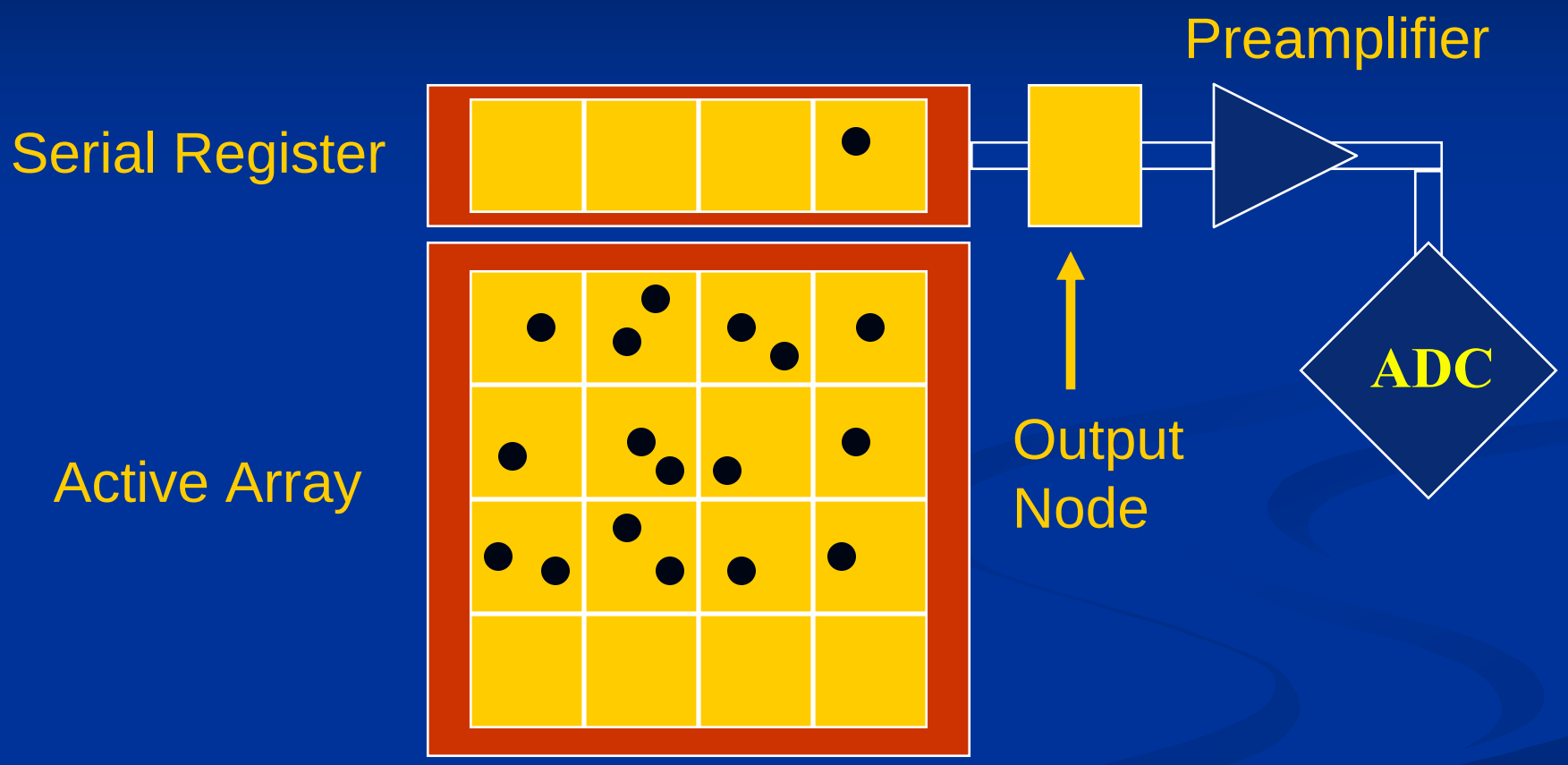

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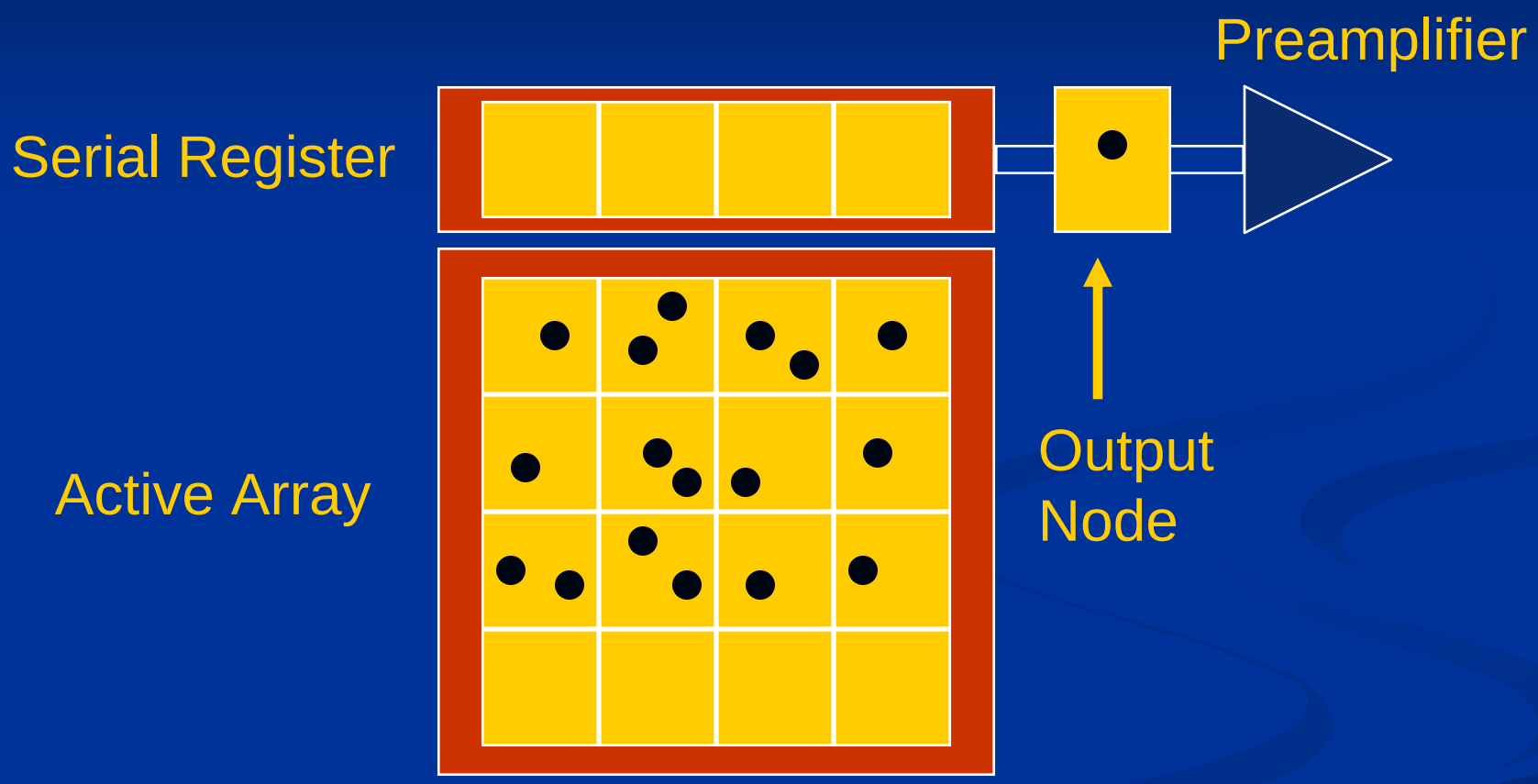

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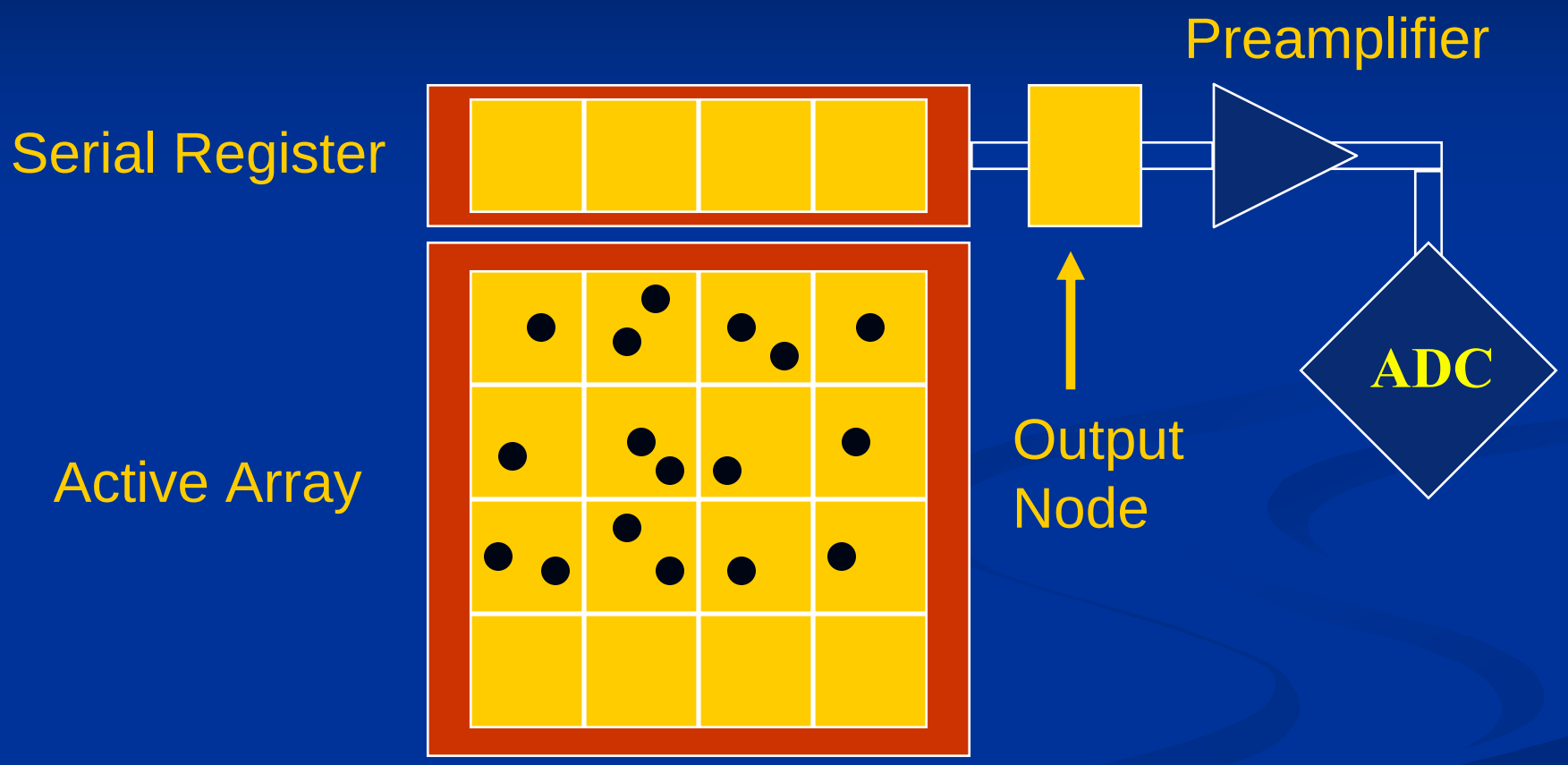

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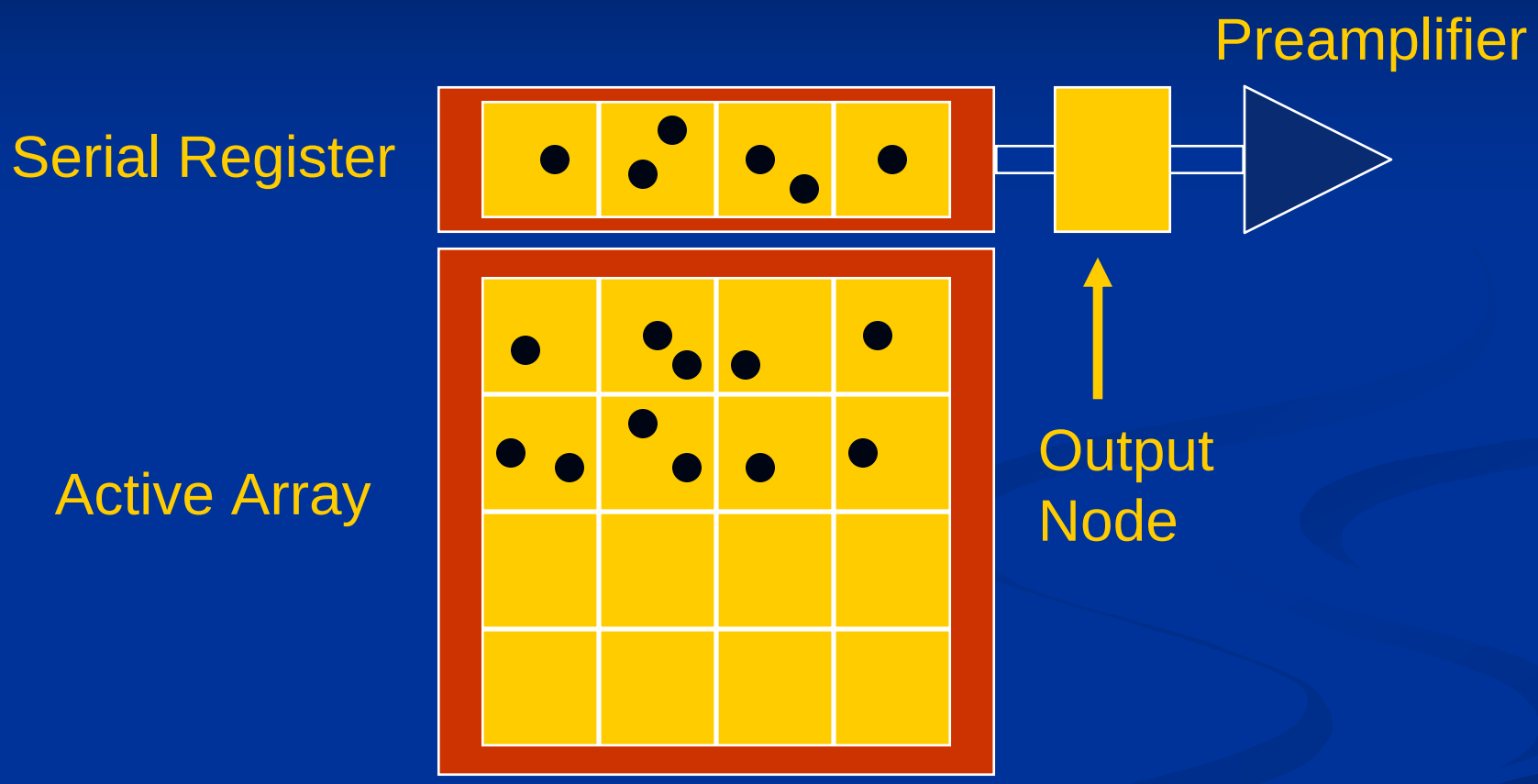

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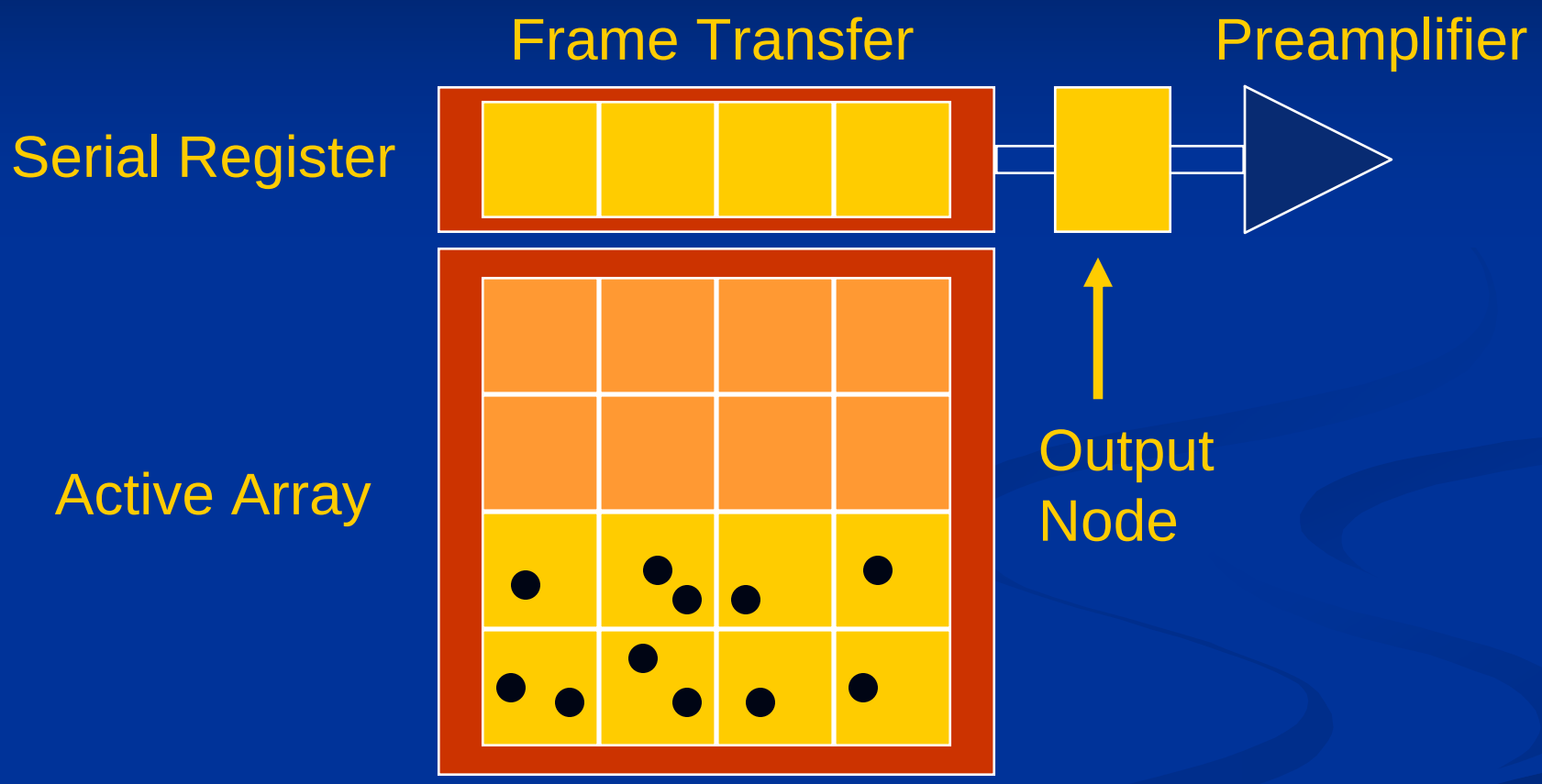

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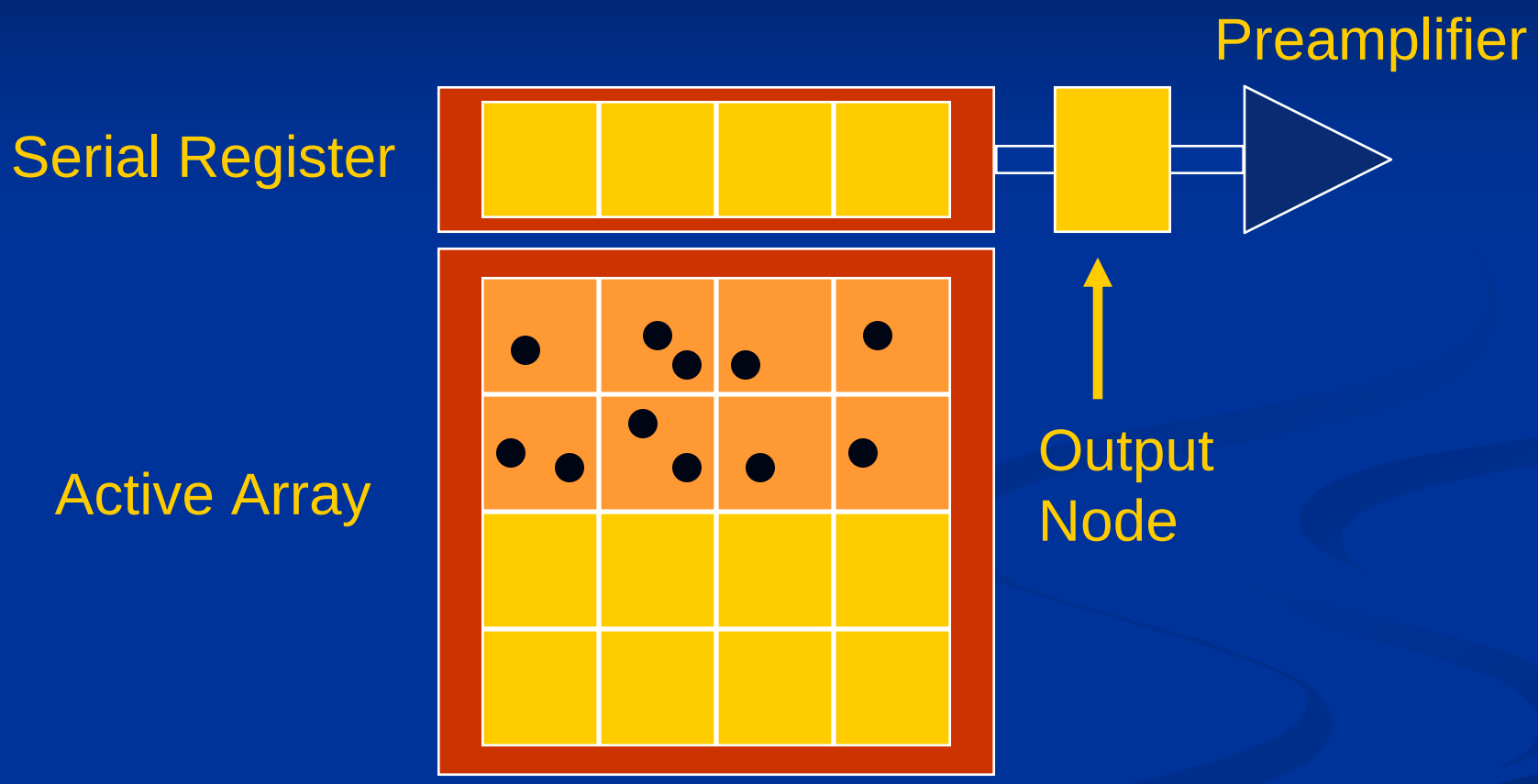

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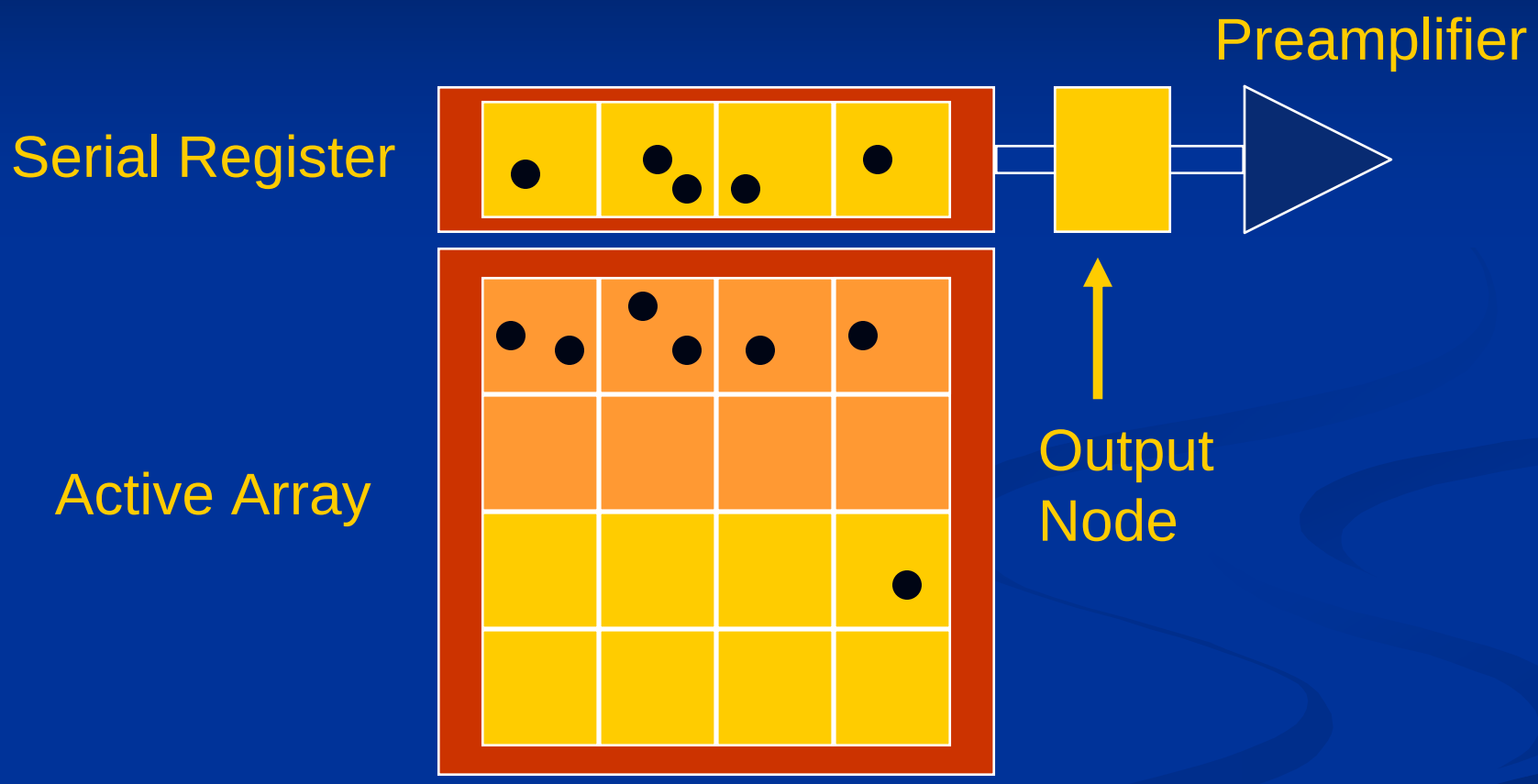

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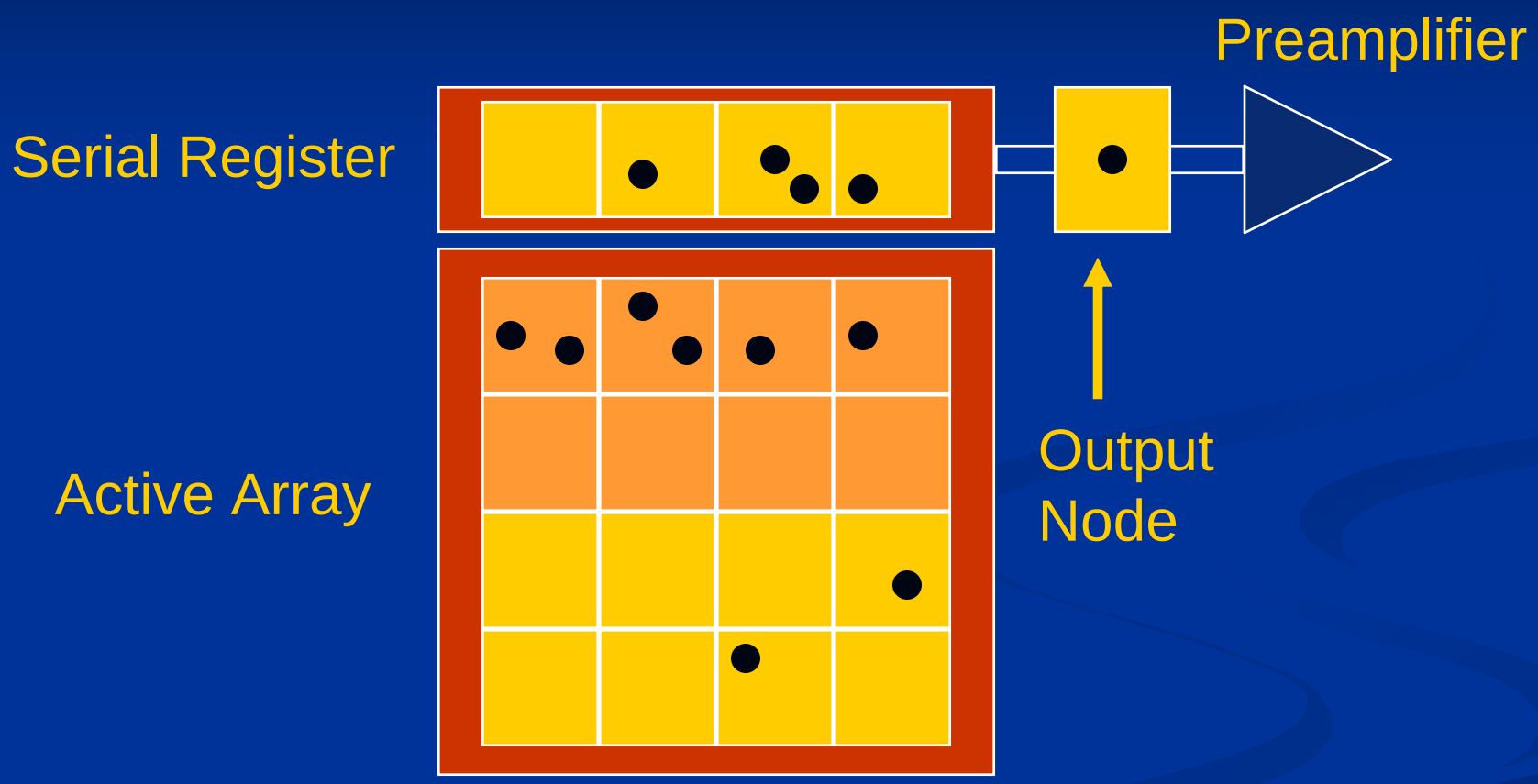

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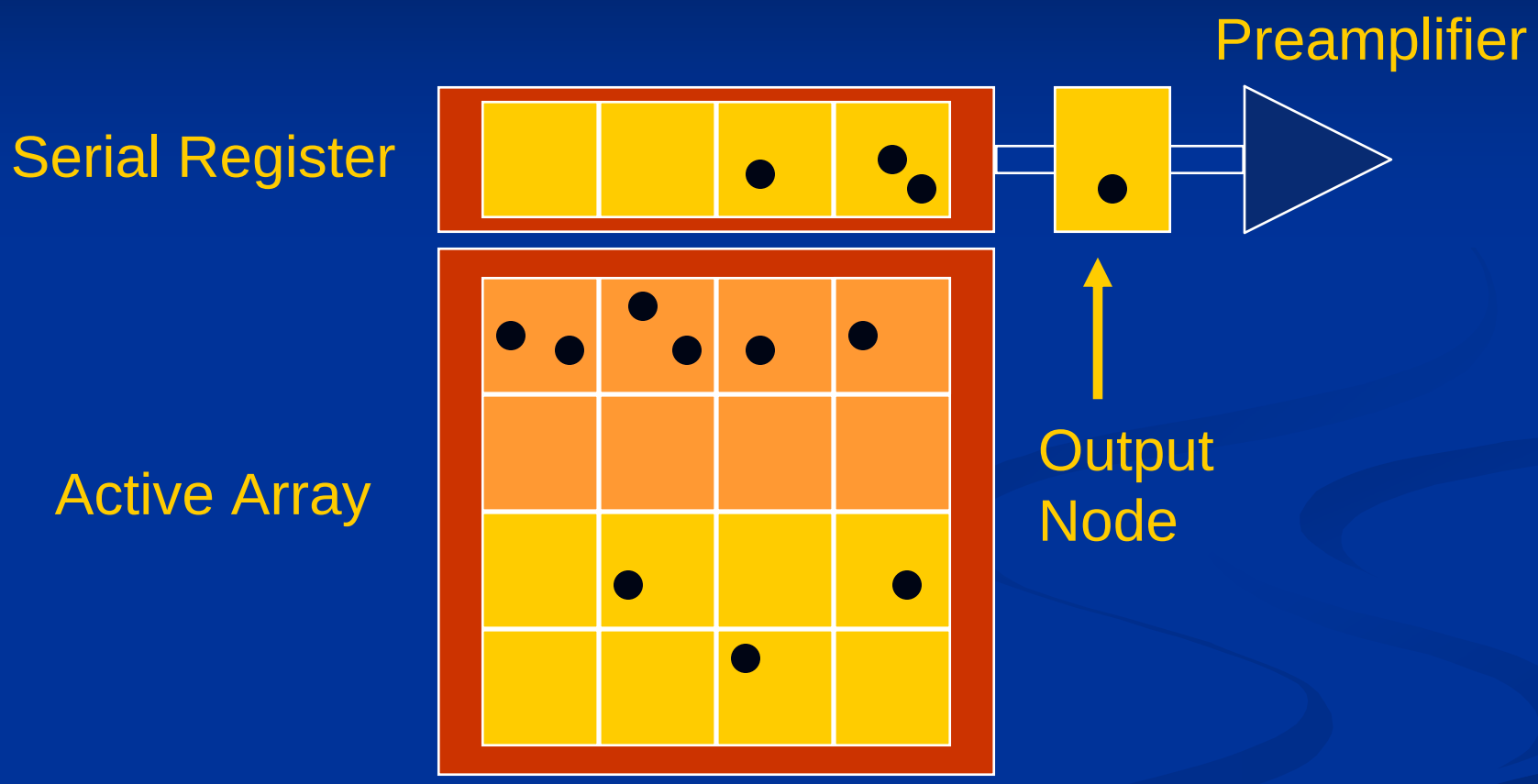

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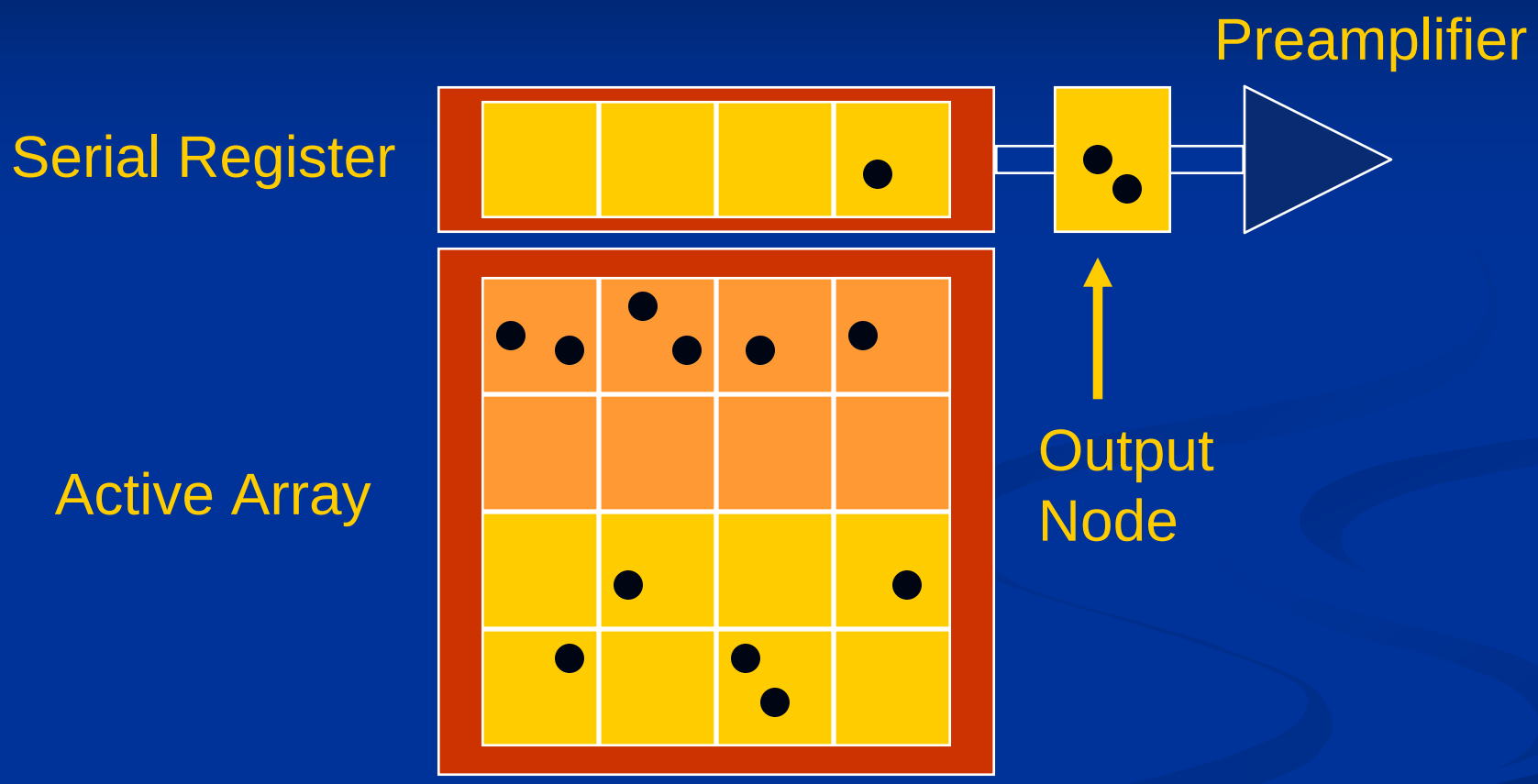

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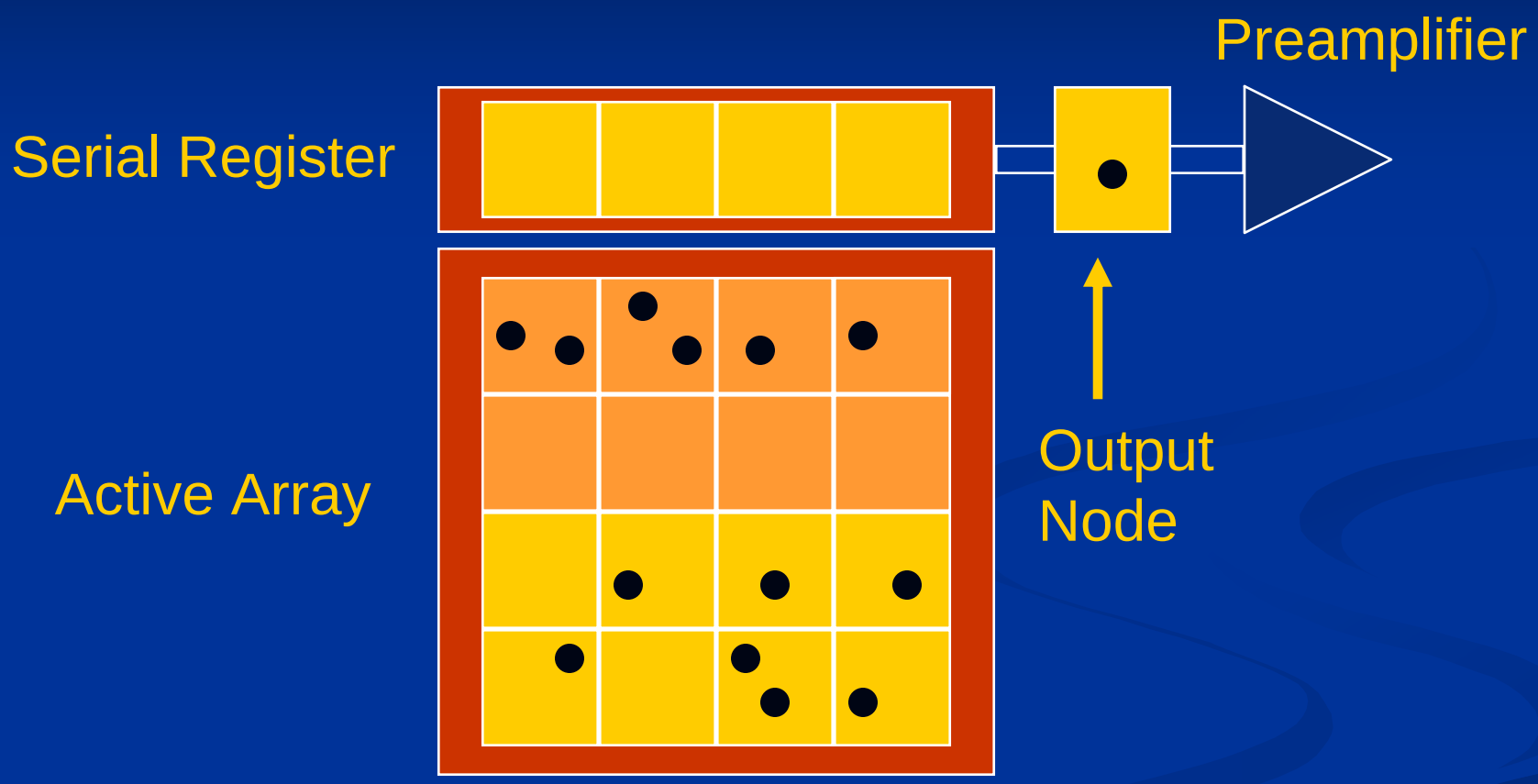

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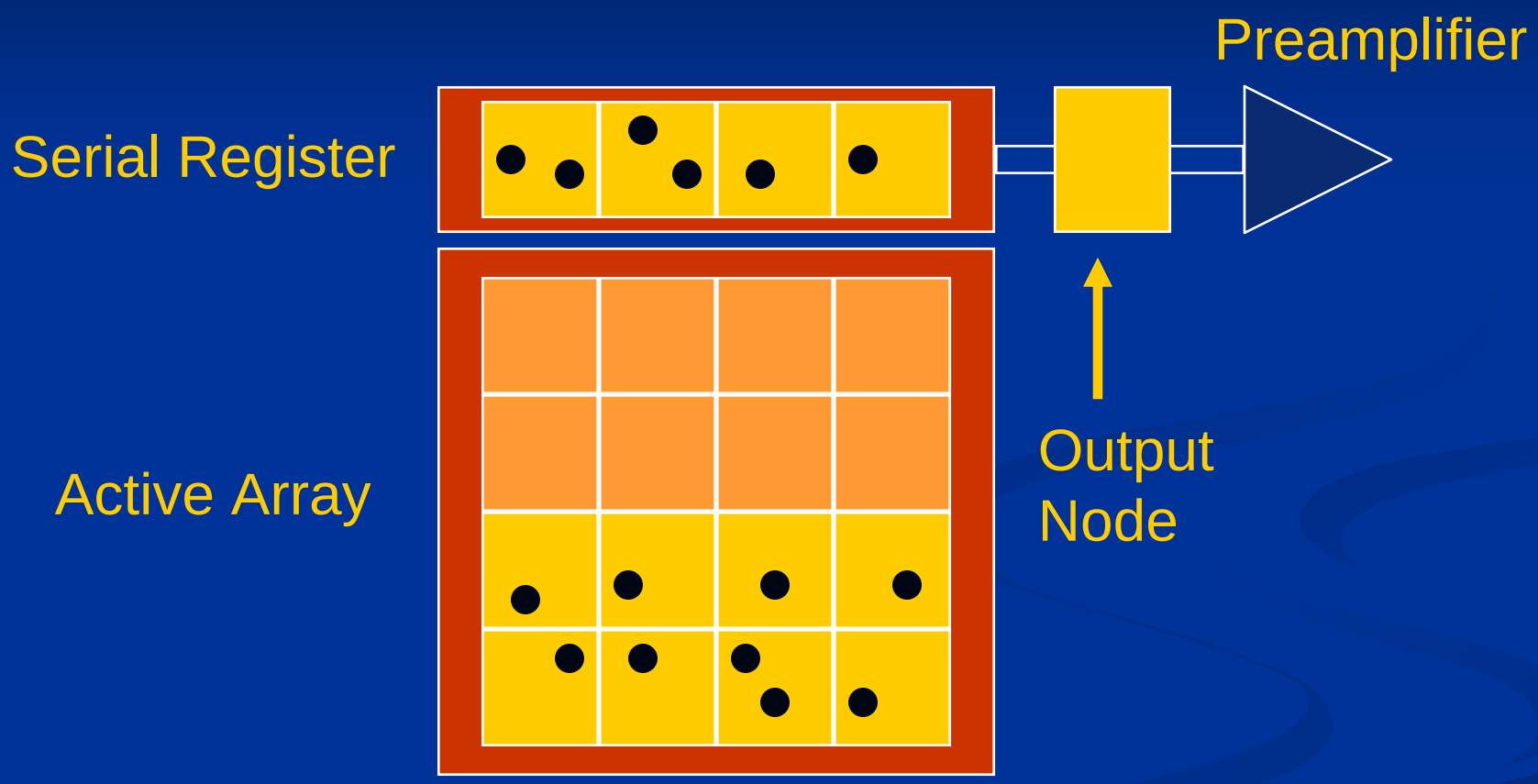

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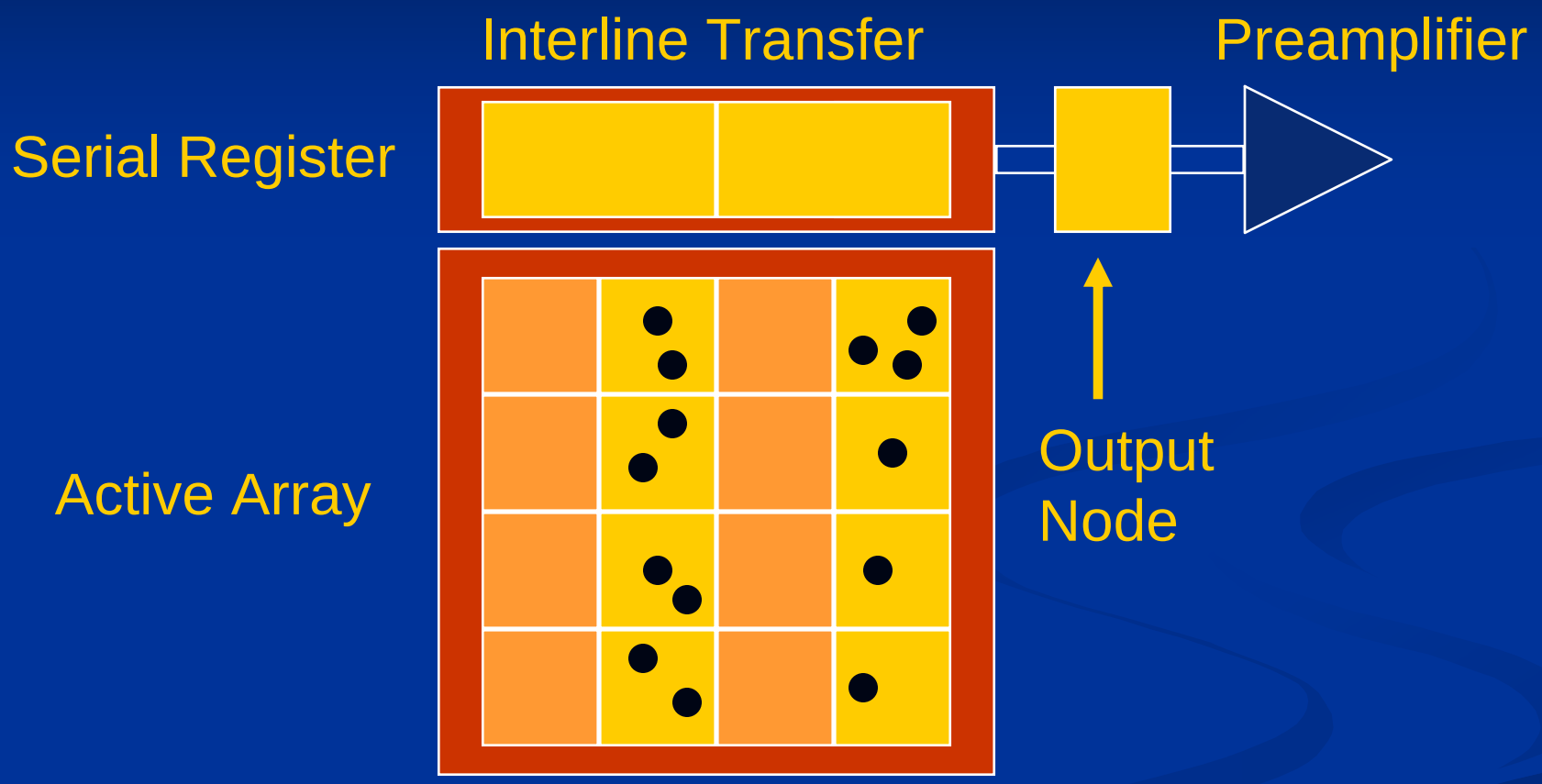

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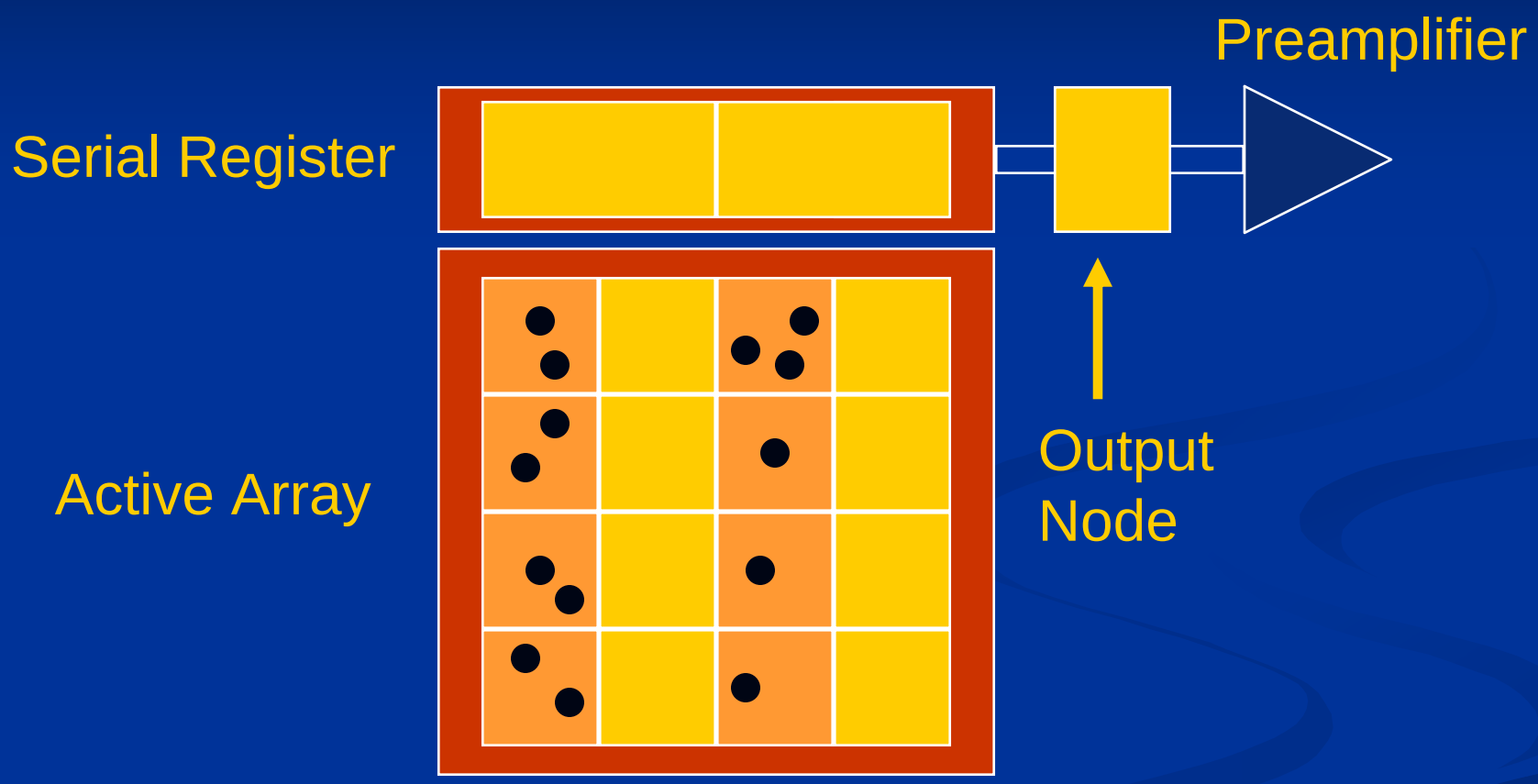

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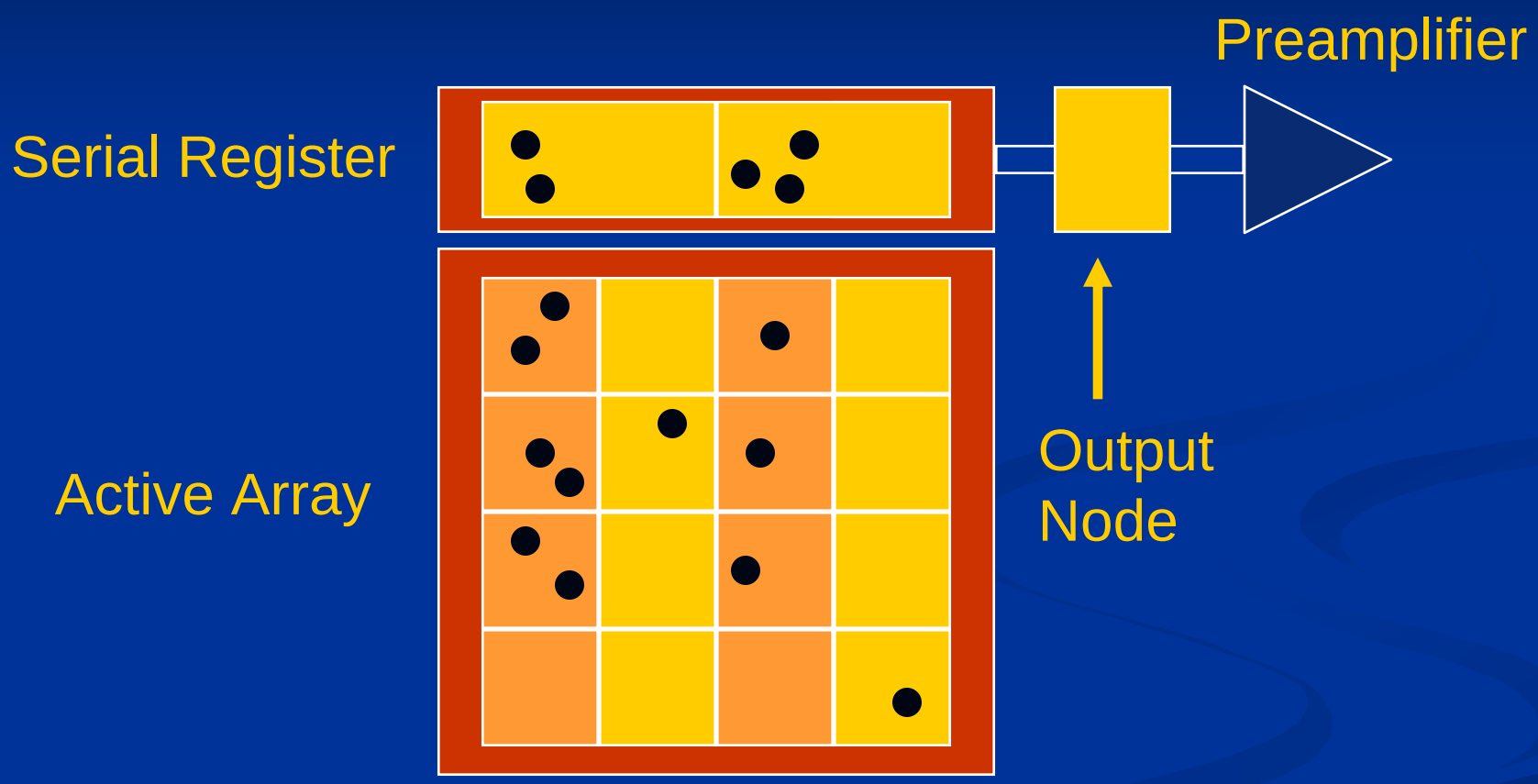

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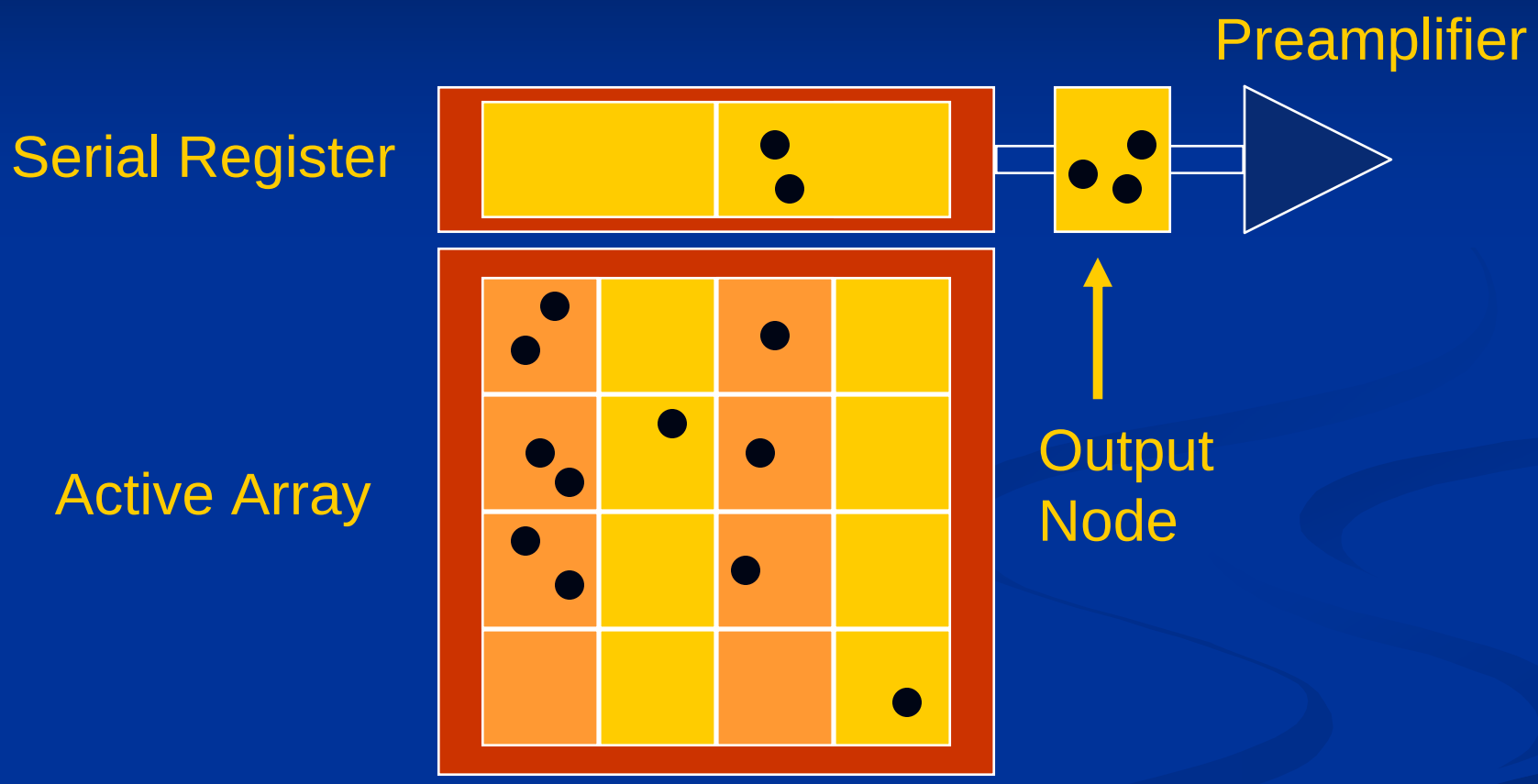

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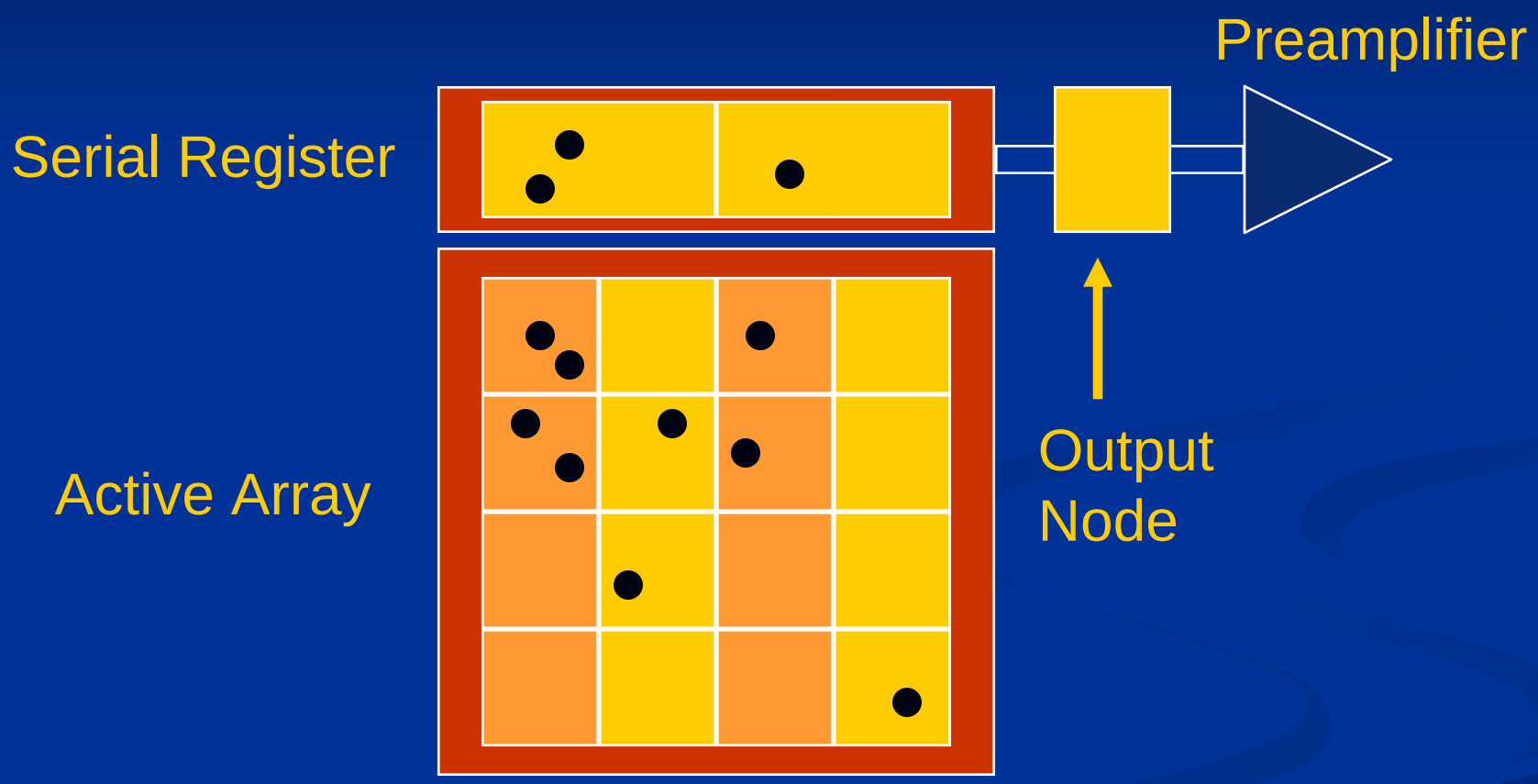

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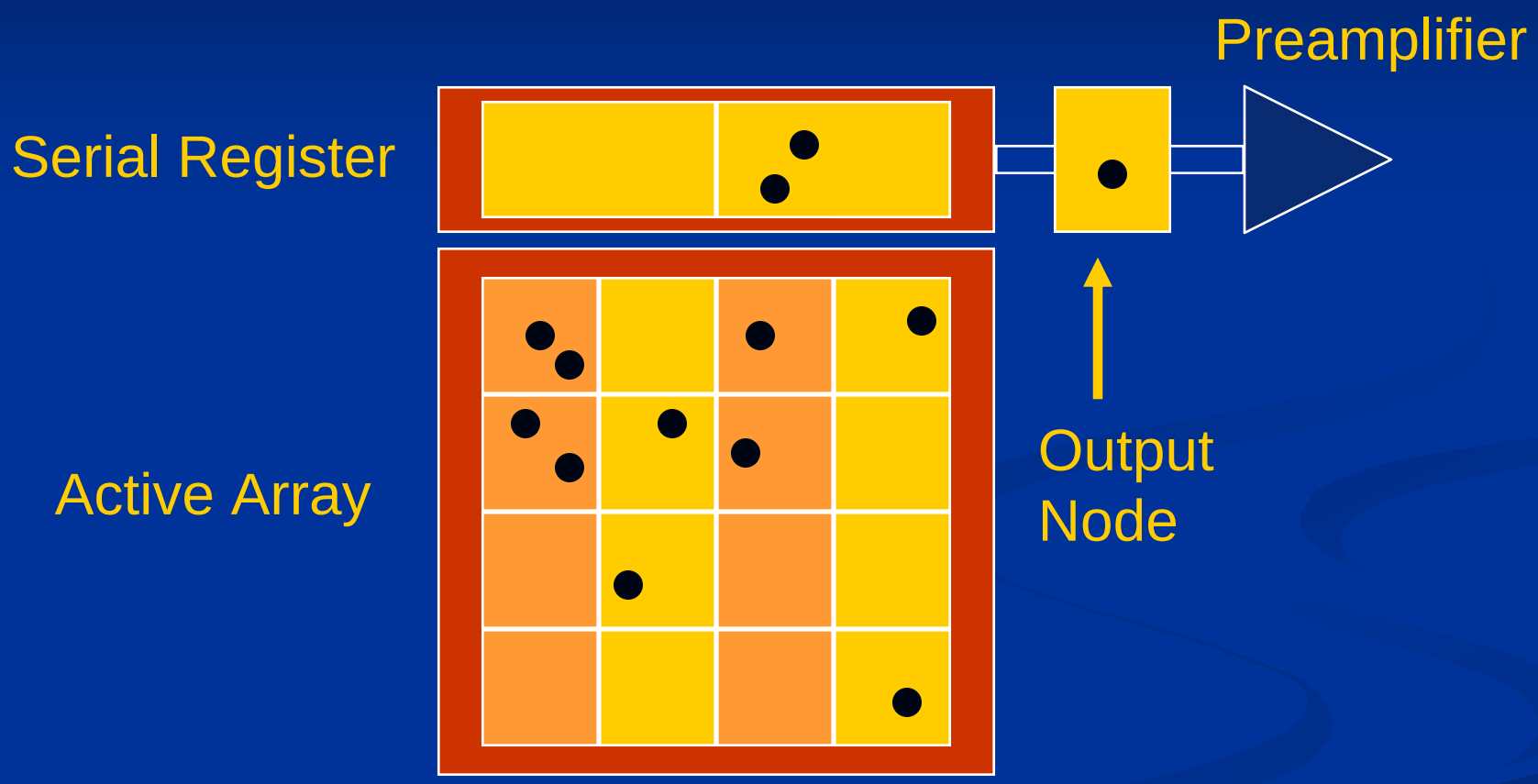

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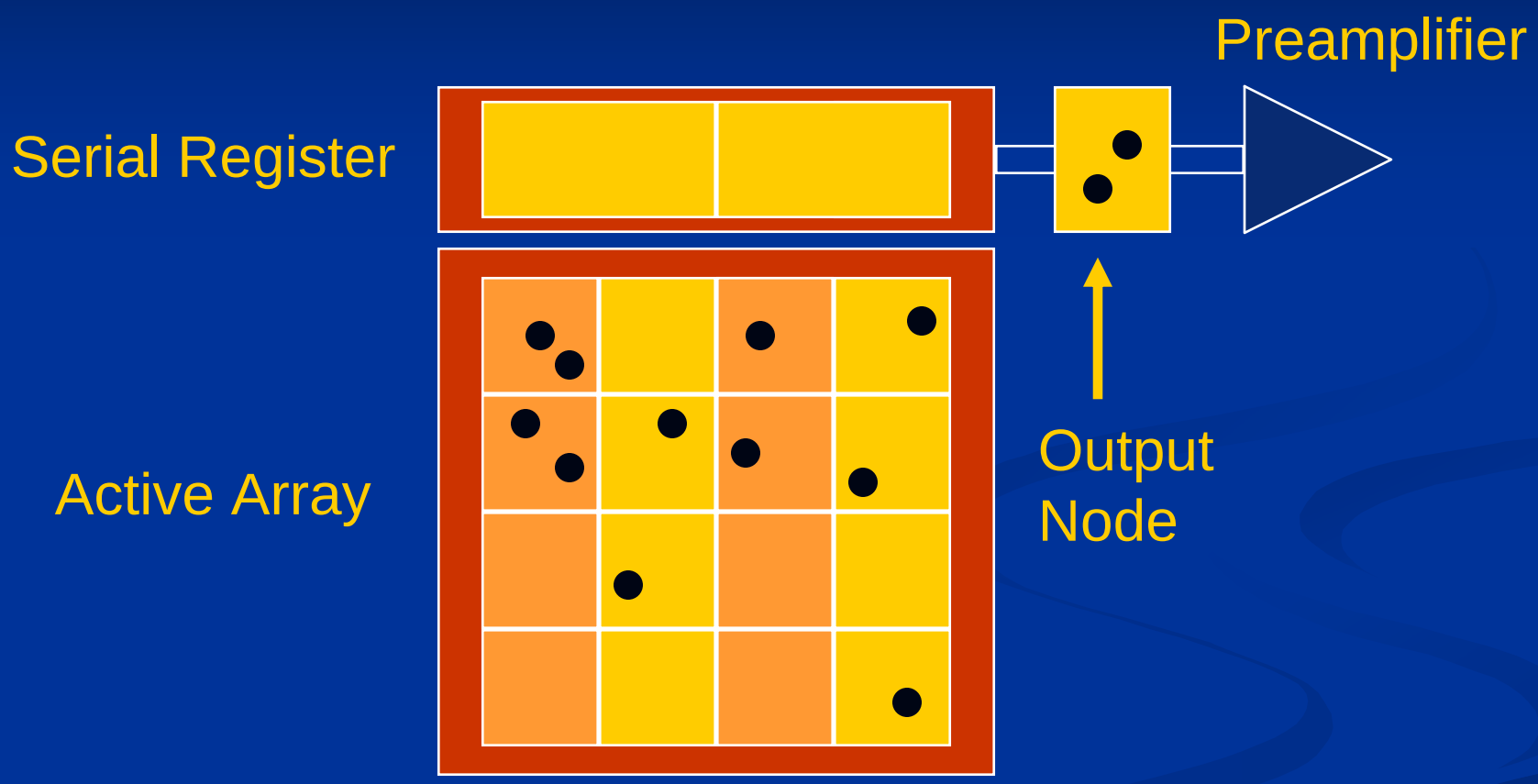

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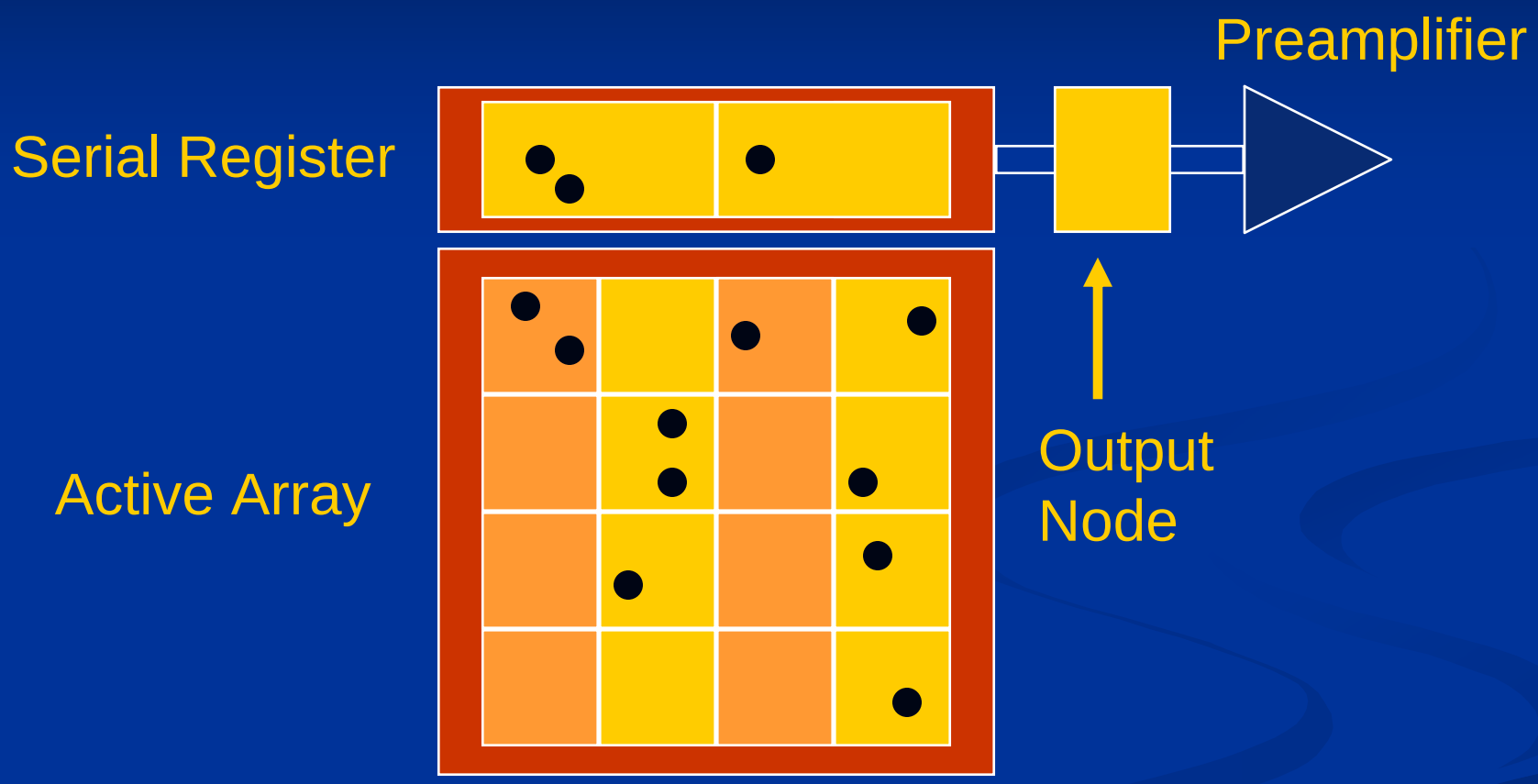

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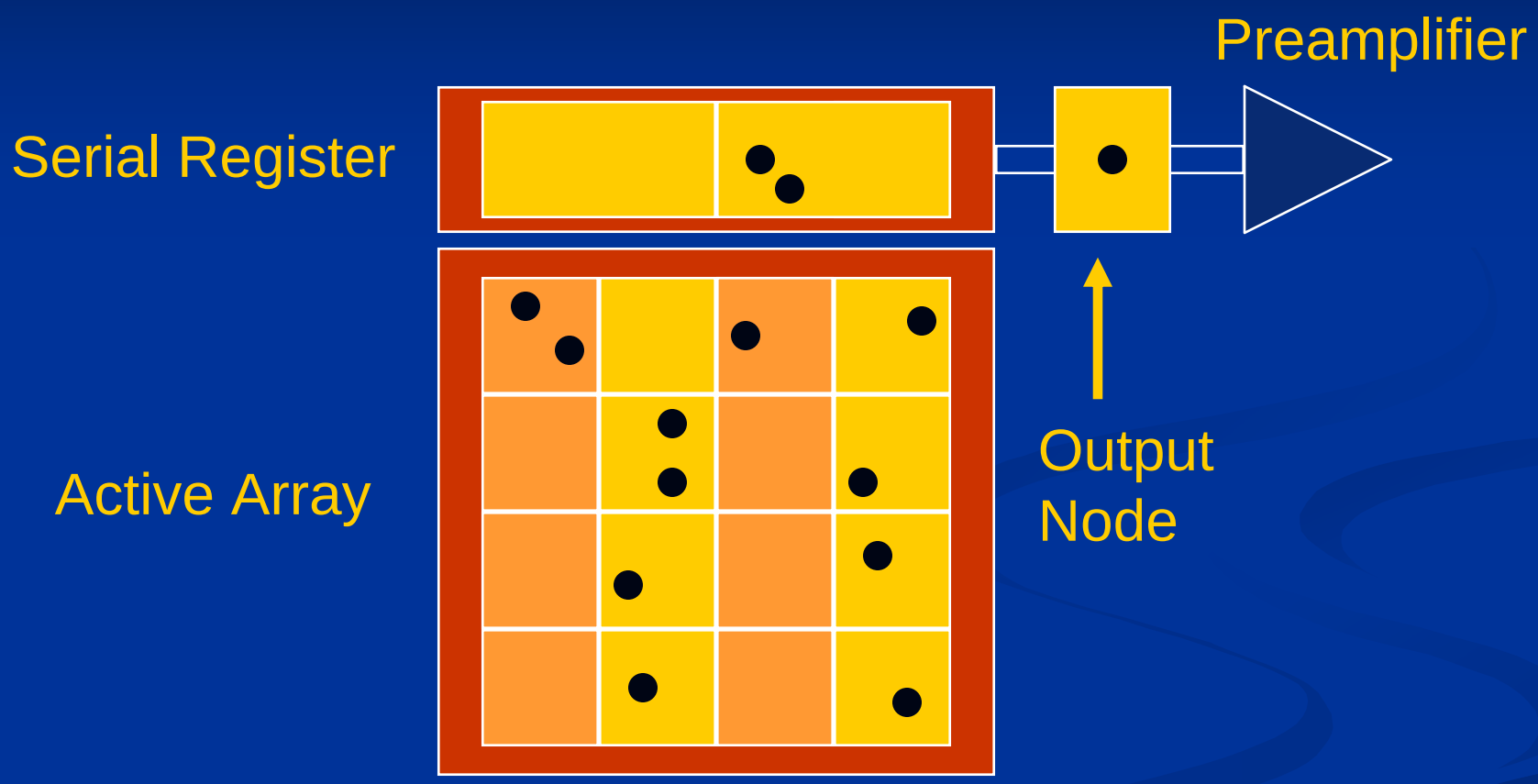


CCD Fundamentals

Full Frame	Frame Transfer	Interline Transfer
High Spatial Resolution	Integrate while Clocking	Integrate while Clocking
Large Number of Pixels	High-Speed Operation	High-Speed Operation (Video)
Greater Selection of CCD Formats	Well Suited for Complex Readout Schemes	Lens-on-Chip Technology
100% Fill Factor for Entire Array	100% Fill Factor in Active Array	

CCD Fundamentals

Ideal CCD Characteristics

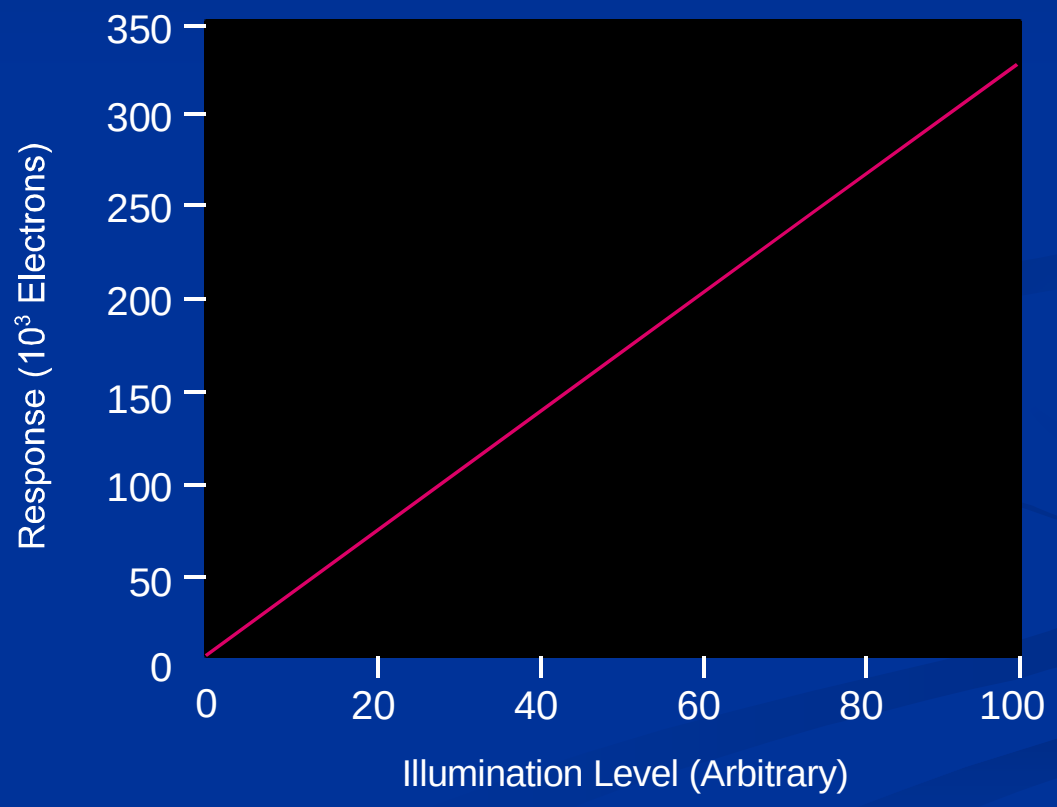
- High quantum efficiency
- Wide spectral response
- Low dark current
- Ability to integrate charge
- Sufficient resolution

CCD Fundamentals

Performance Considerations

CCD Fundamentals

CCD Linearity



CCD Fundamentals

Noise Sources in CCDs

- Photon-induced shot noise
- Readout noise
- Dark current noise
- kTC reset switch noise
- Spurious charge

- **Total System Noise = all noise sources added in quadrature**

CCD Fundamentals

Photon (Shot Noise)

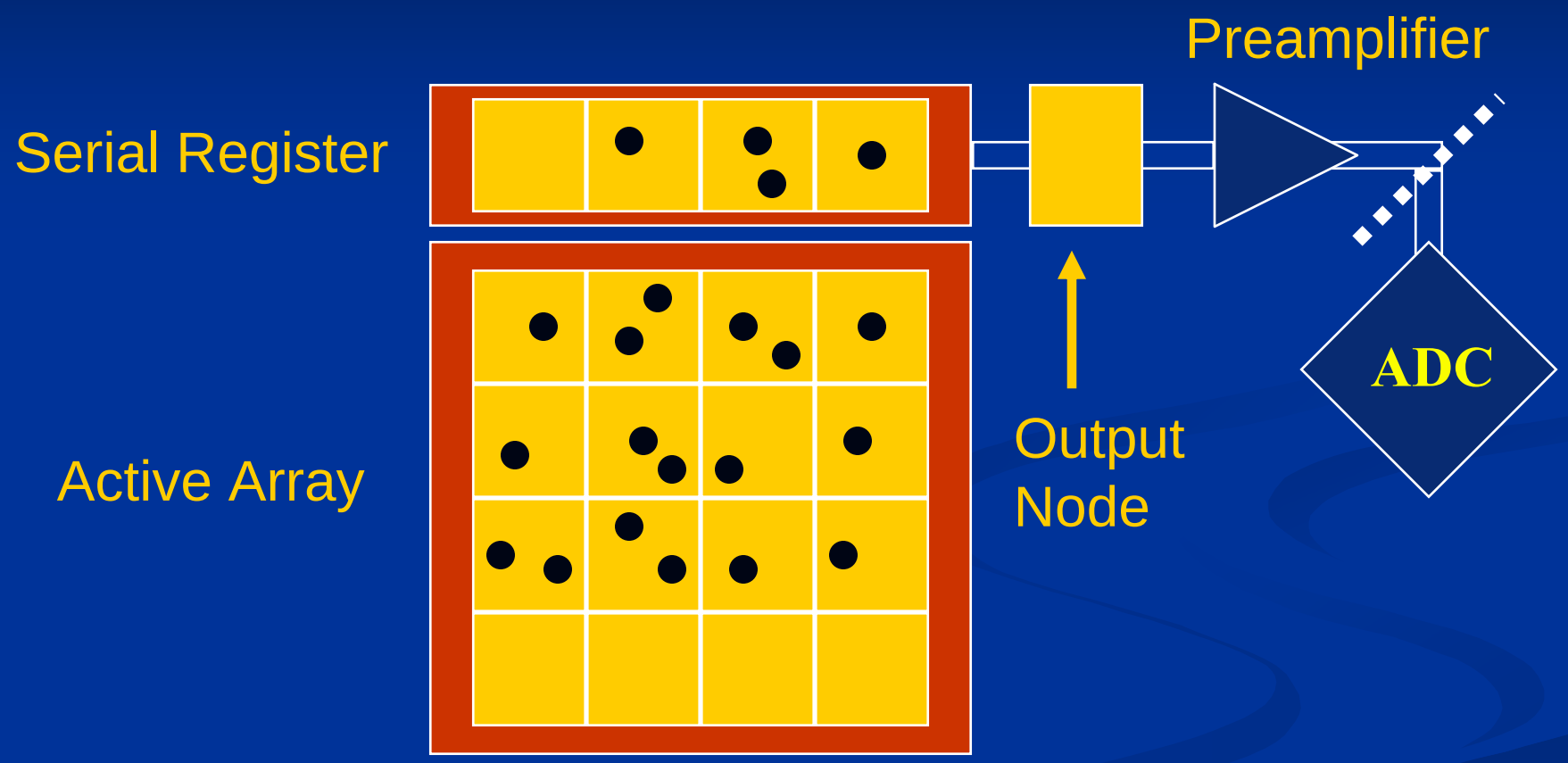
- Law of physics
- Square root relationship between signal and noise
  noise = square root of number of electrons
- Poisson distribution
- When photon noise exceeds system noise, data is photon (shot) noise limited

CCD Fundamentals

Read Noise (preamplifier 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 not as relevant in high-signal applications

Analog Gain


Preamplifier
Serial Register
ADC
Output
Node
Active Array

CCD Fundamentals

Dark Current

Electrons created by thermal emission

Increases with time and temperature

CCD Fundamentals

Dark current subtracts, dark current noise remains

- dark current noise = $\sqrt{\text{dark current}}$
- dark current (dark noise) can lower SNR

CCD Fundamentals

Reduced by cooling the CCD

- Dark current is cut in half as the CCD temperature drops approximately every 6.7° C

Reduced by utilizing multi-pinned-phase (MPP) technology

CCD Fundamentals

Limit of cooling effectiveness

- MPP CCDs already have low dark current
- Poor CTE (charge transfer efficiency) at <-120°C

CCD Fundamentals

Noise Reduction in CCDs

- Photon Noise - A law of physics!
- Readout Noise - Reduced by careful electronics design
- Dark Current Noise - Reduced by cooling and MPP
- kTC Noise - Reduced by using correlated double sampling
- Spurious Charge - Reduced by careful shaping of clock waveforms

Introduction

Dynamic Range

CCD Fundamentals

Dynamic Range = Full Well/Read Noise

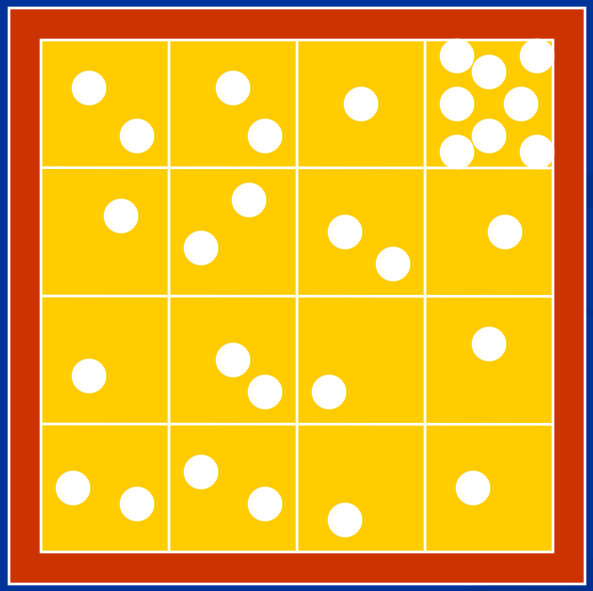
CCD Fundamentals

Dynamic Range of CCD is matched to A/D Converter

12, 14, 16 bit

CCD Fundamentals

Intrascene Dynamic Range



Introduction

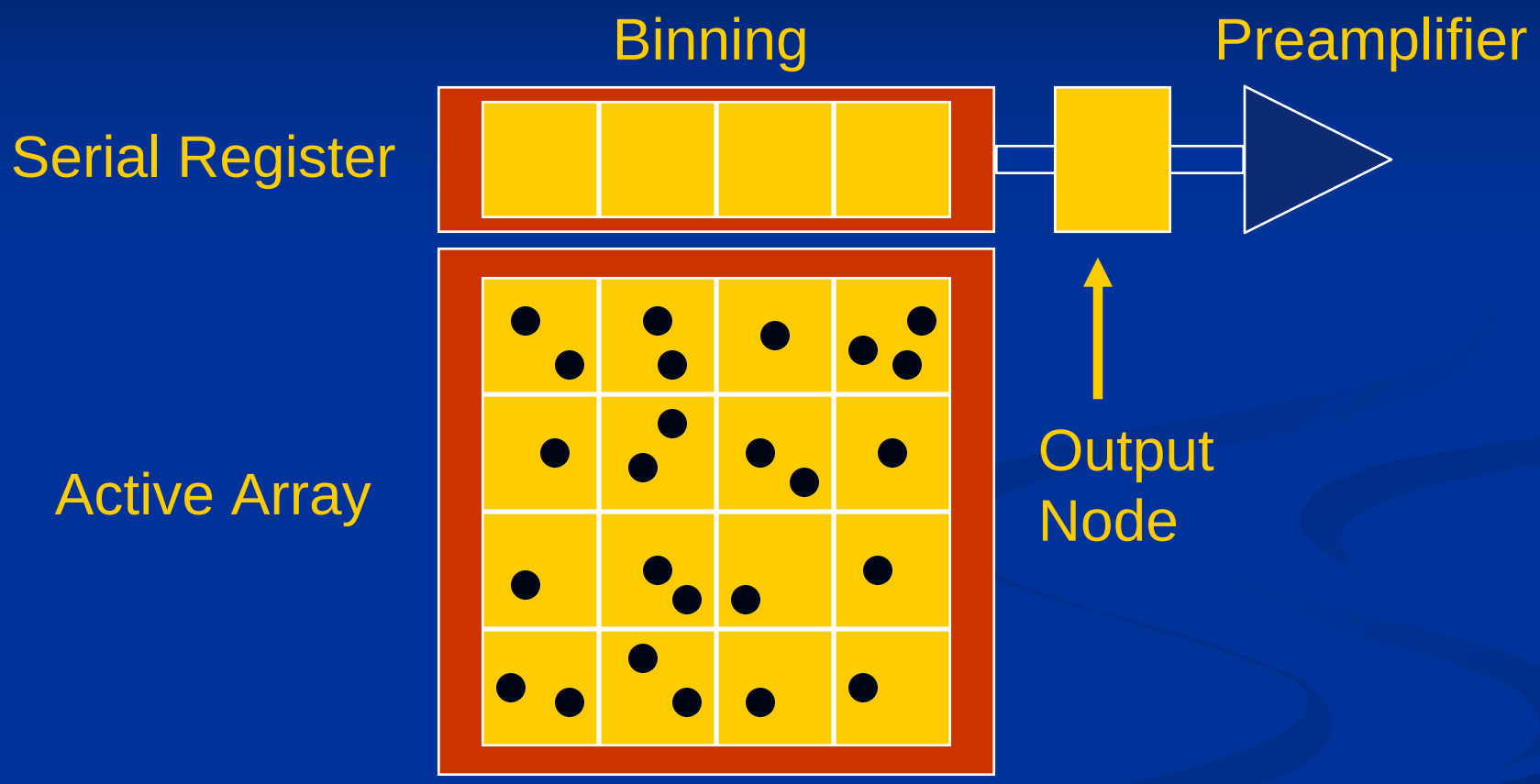
Binning

CCD Fundamentals

Binning

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

CCD Fundamentals



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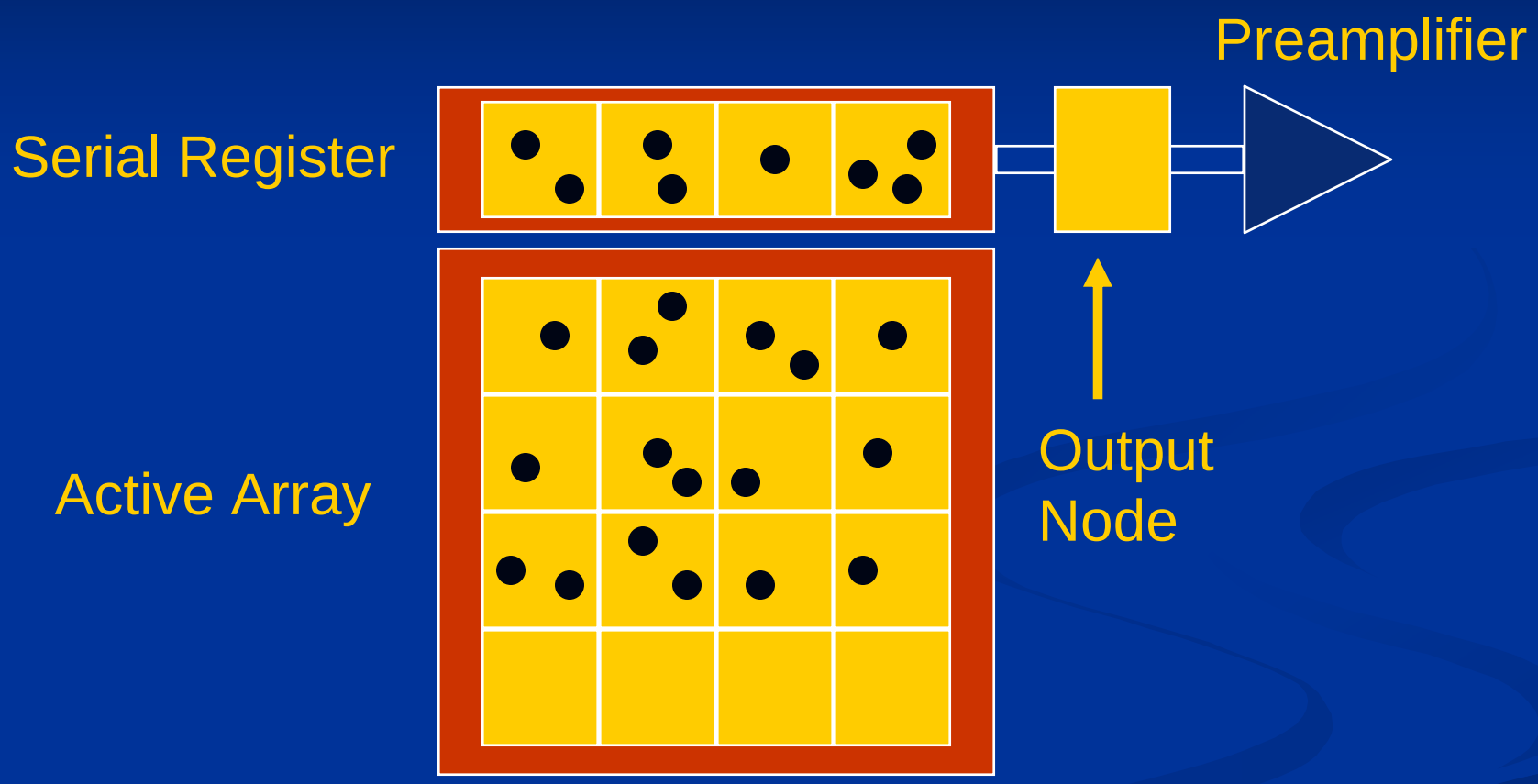

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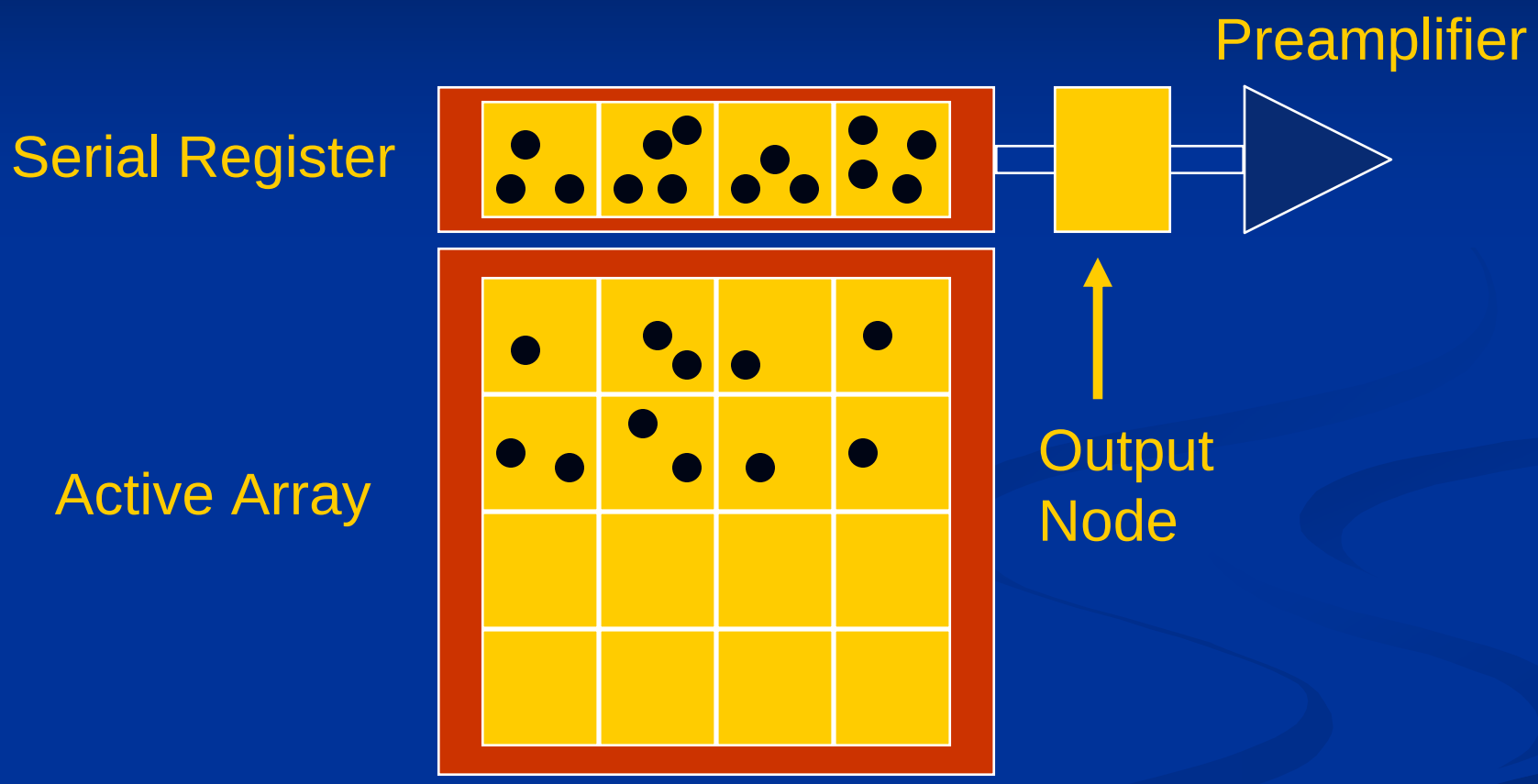

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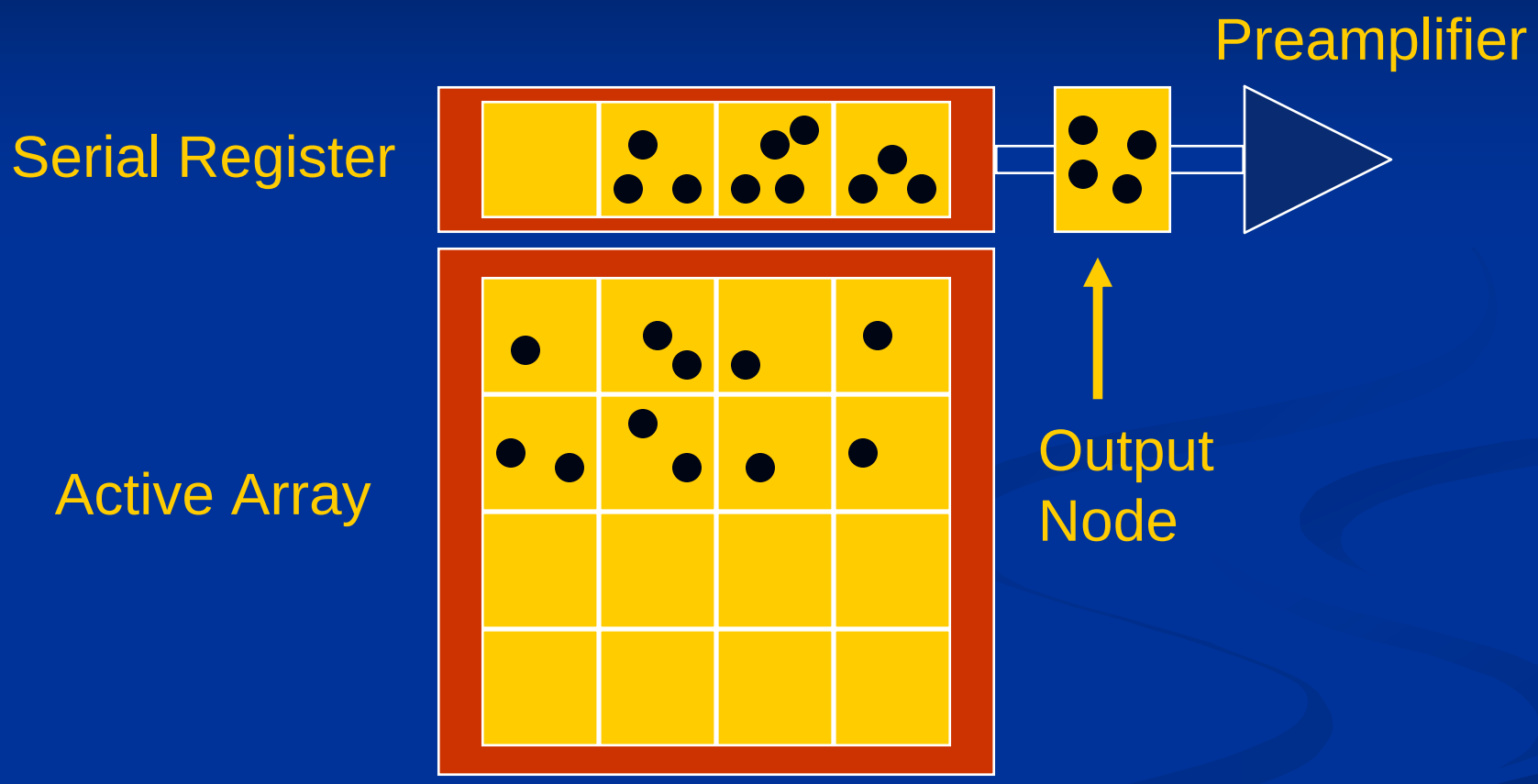

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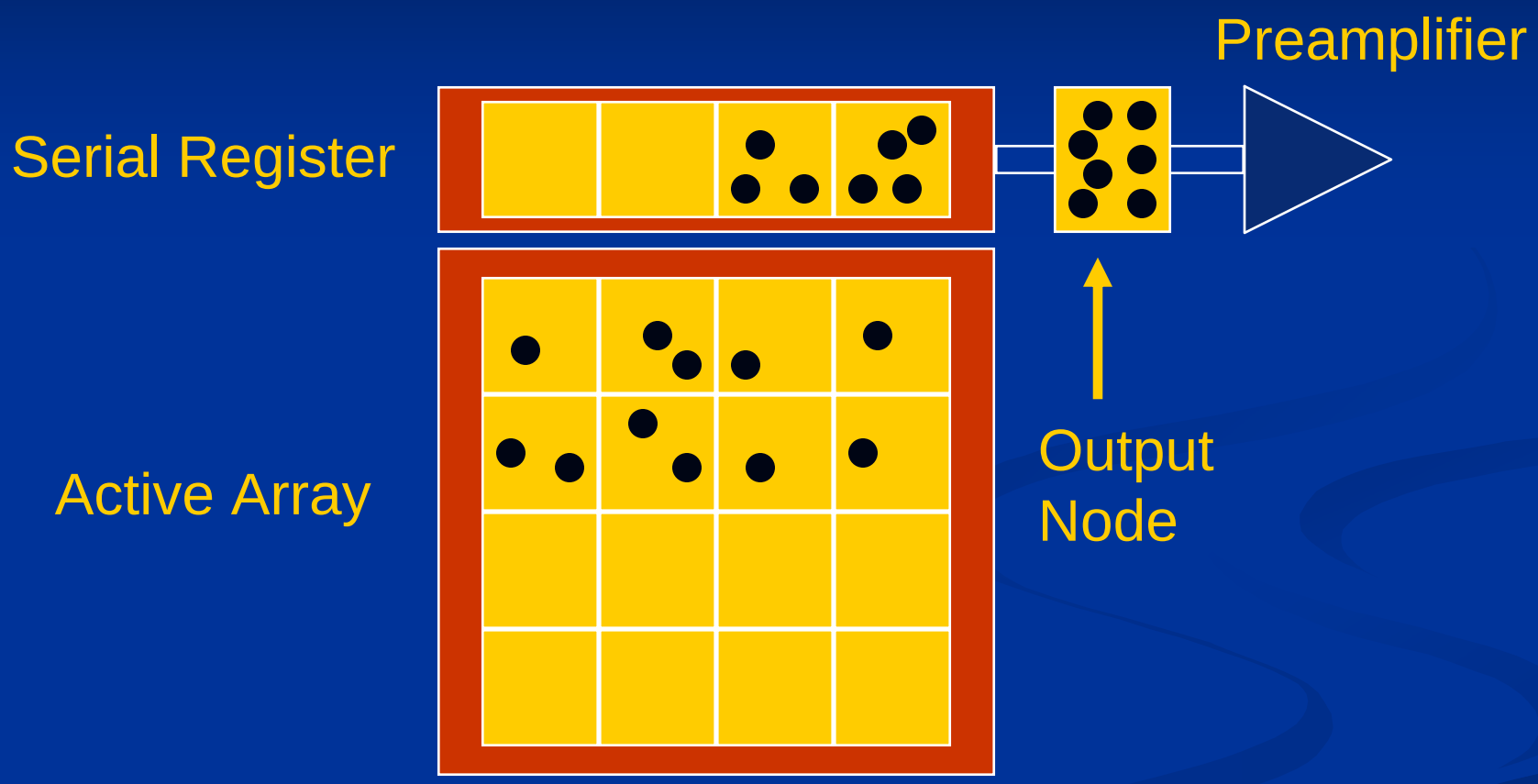
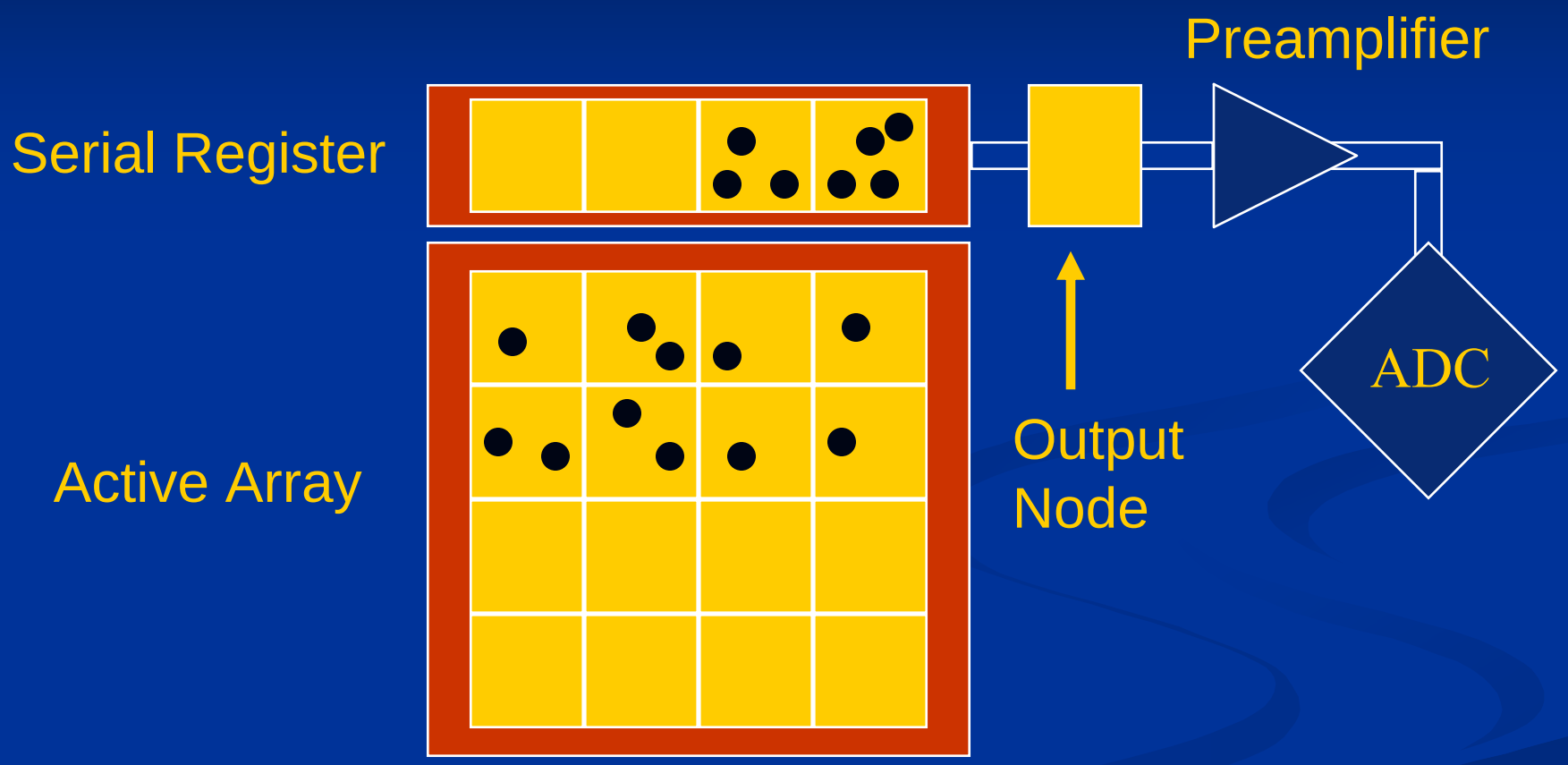
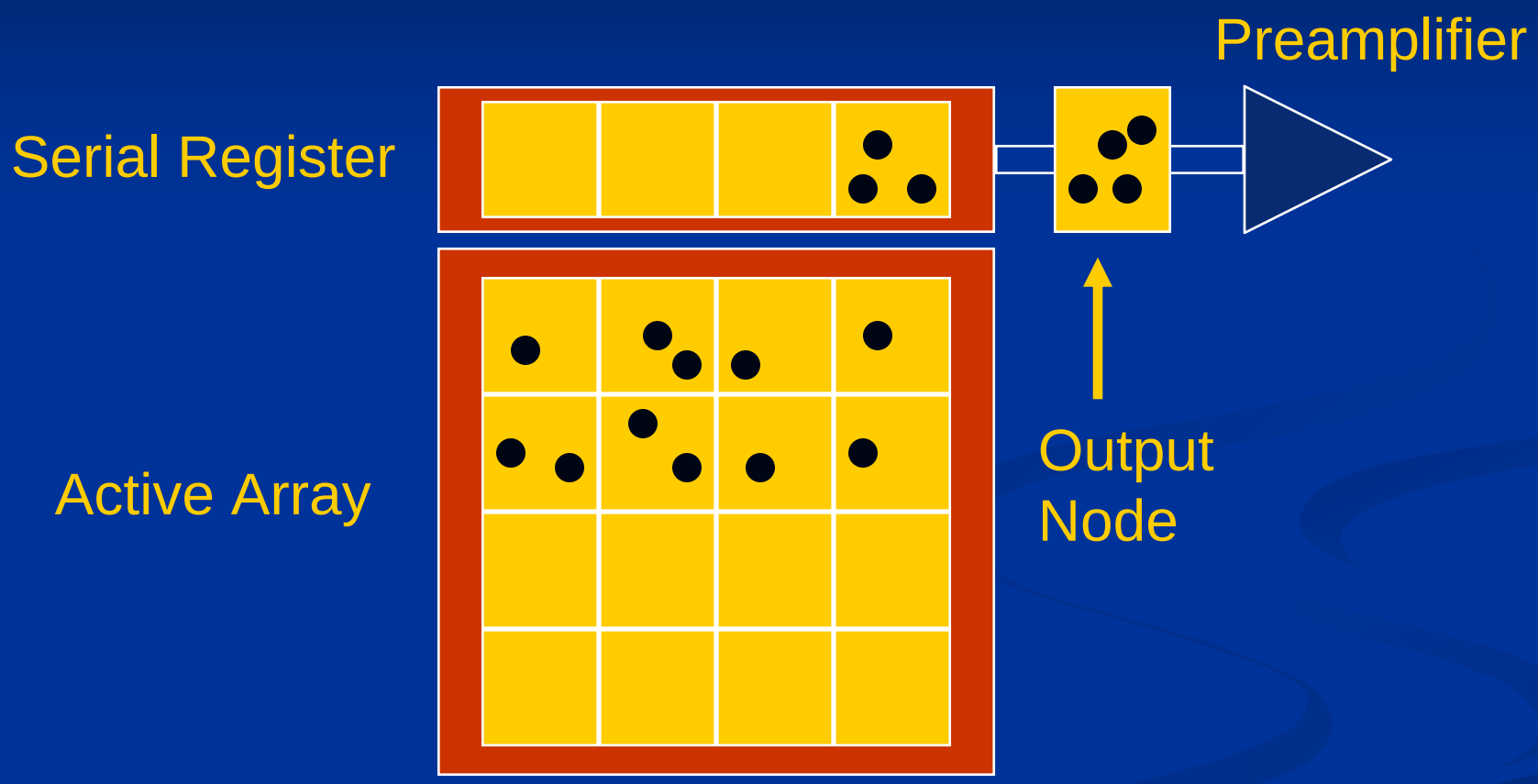


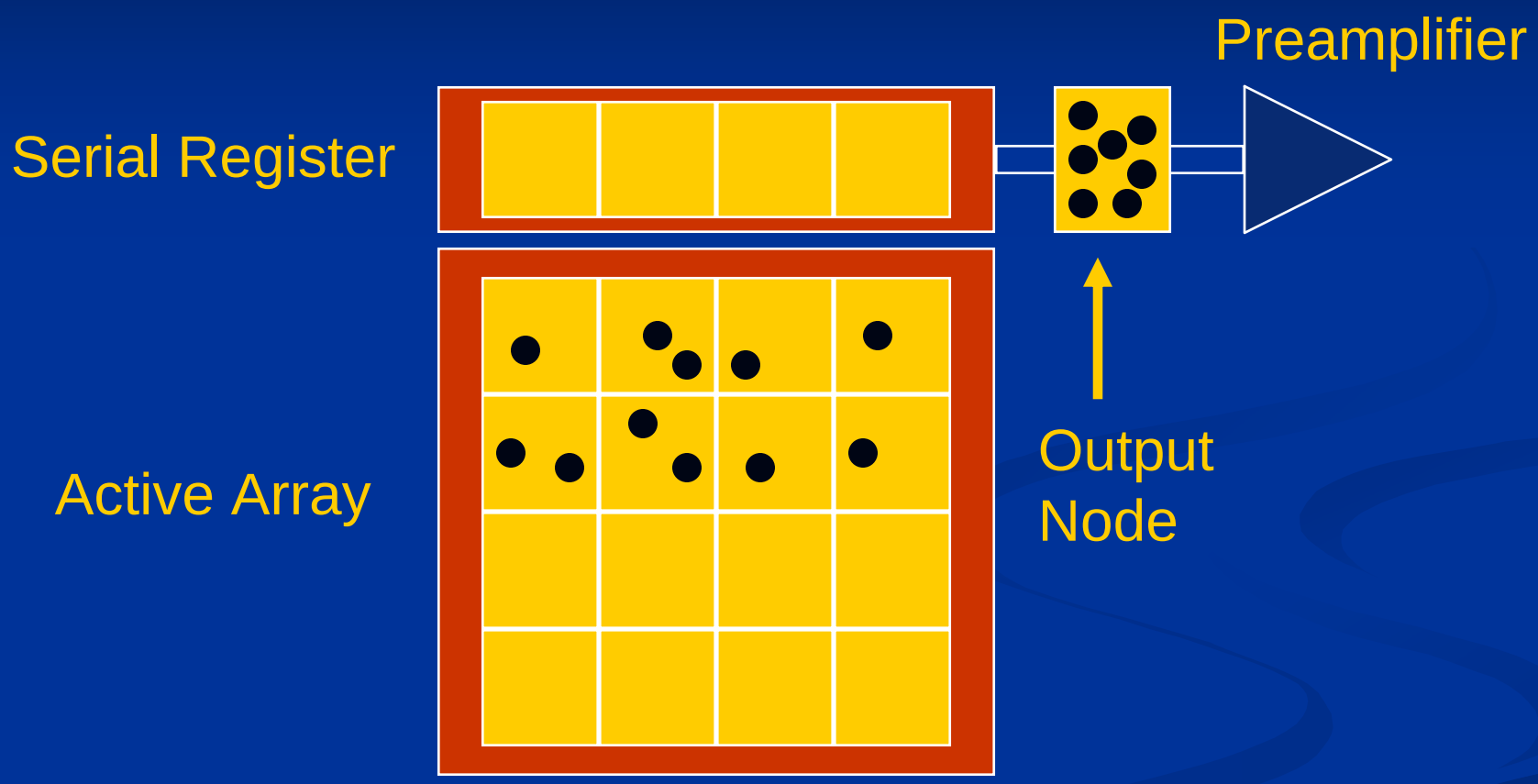
CCD Fundamentals



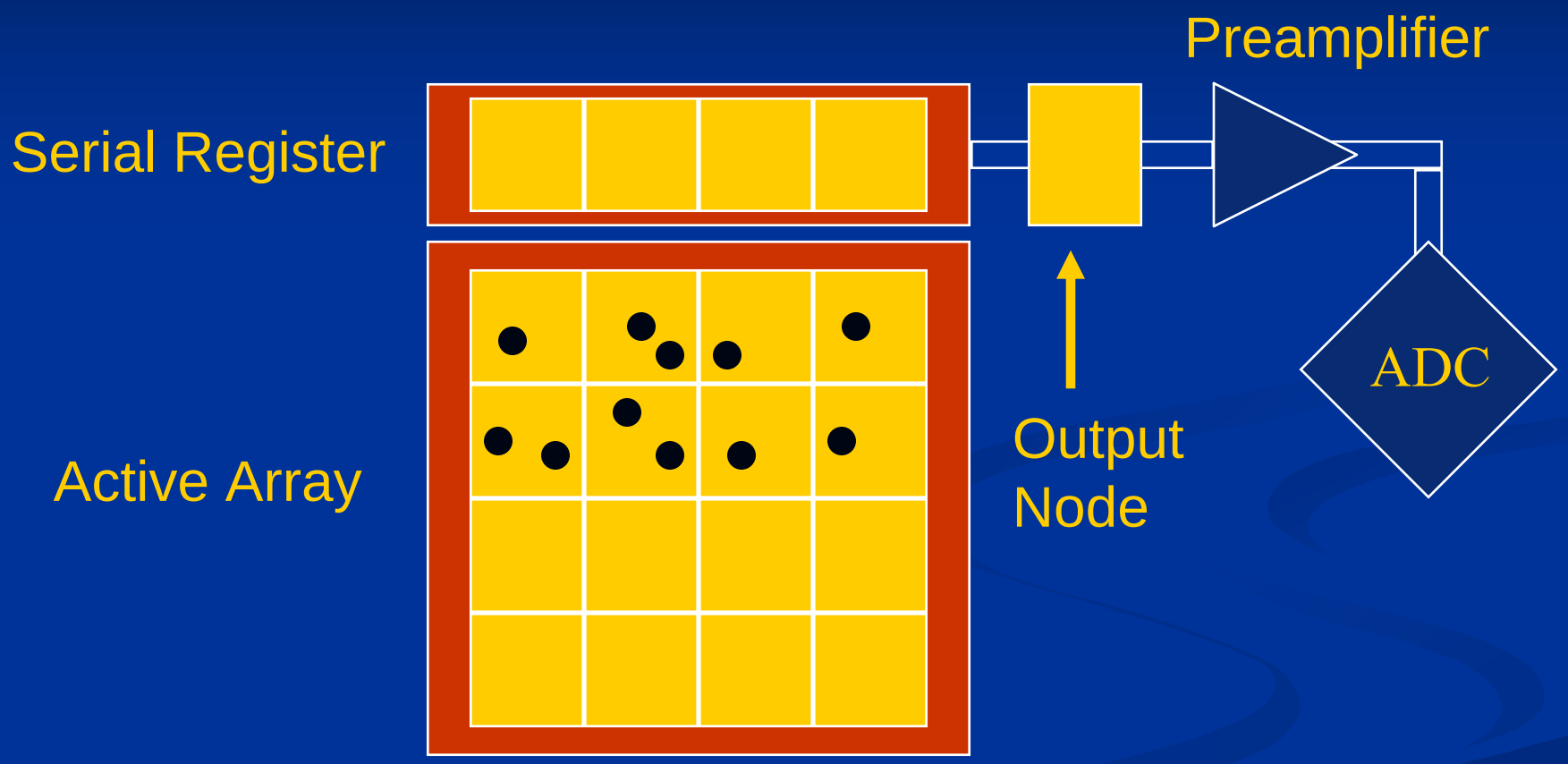
CCD Fundamentals



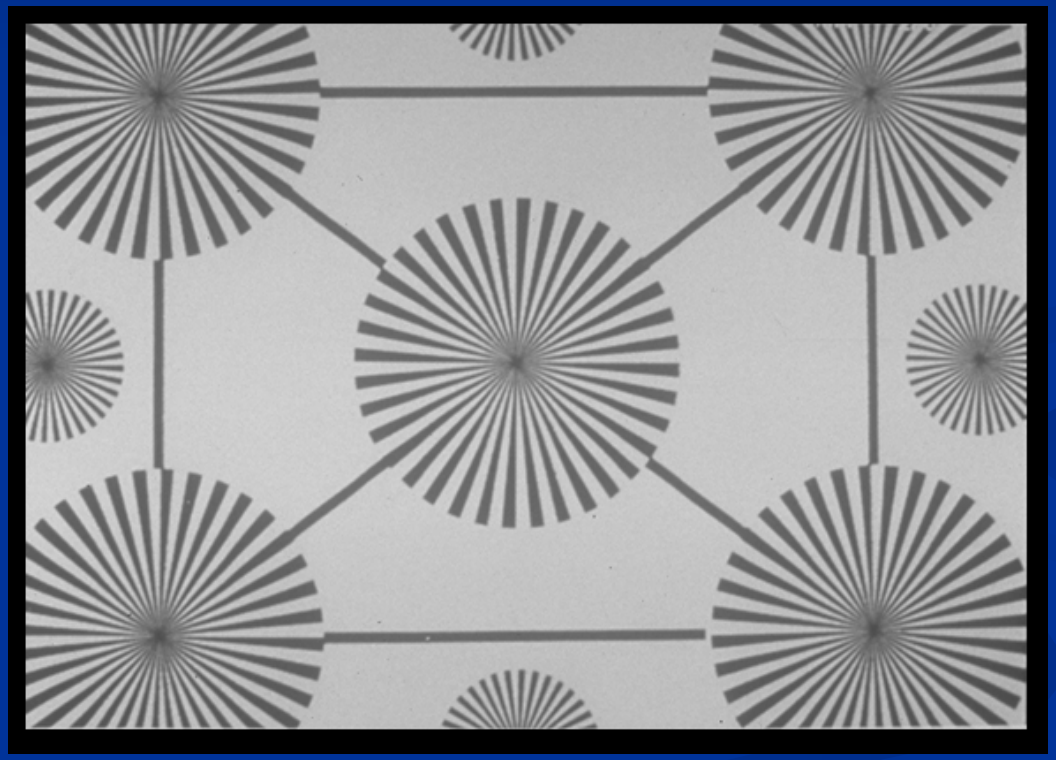
CCD Fundamentals



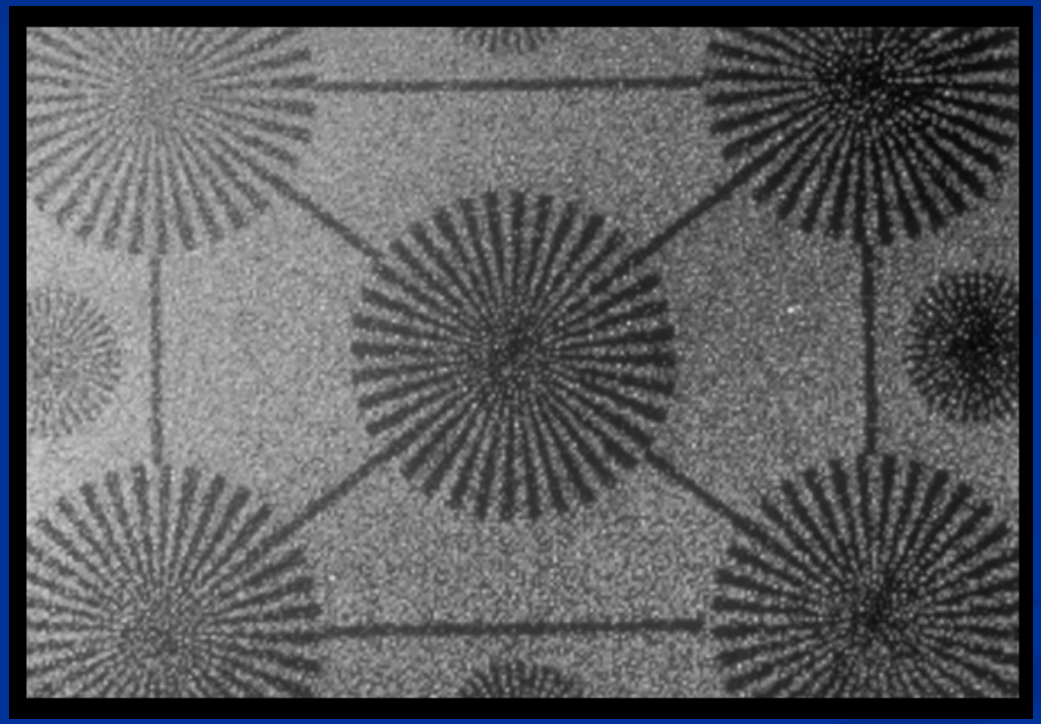
CCD Fundamentals



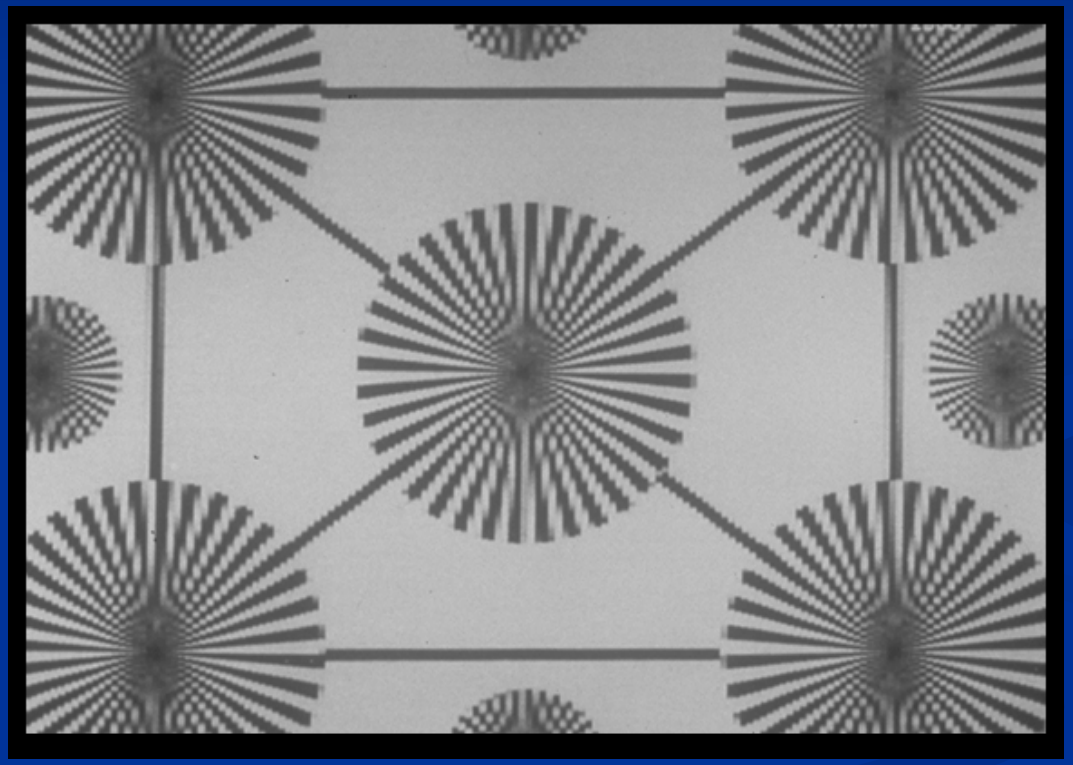
CCD Fundamentals



CCD Fundamentals



CCD Fundamentals



CCD Performance

What does your experiment require?

Performance Applications

Benefits of Cooled CCD Technology

- Low-light sensitivity (ASA 100,000)
- Ultra-low noise and dark current
- Real-time image capture with digital output
- Dynamic range up to 16 bits (65,000 gray levels)
- High quantum efficiency over wide spectral range
- Programmable readout modes
  - Binning
  - Subarray readout

Performance Applications

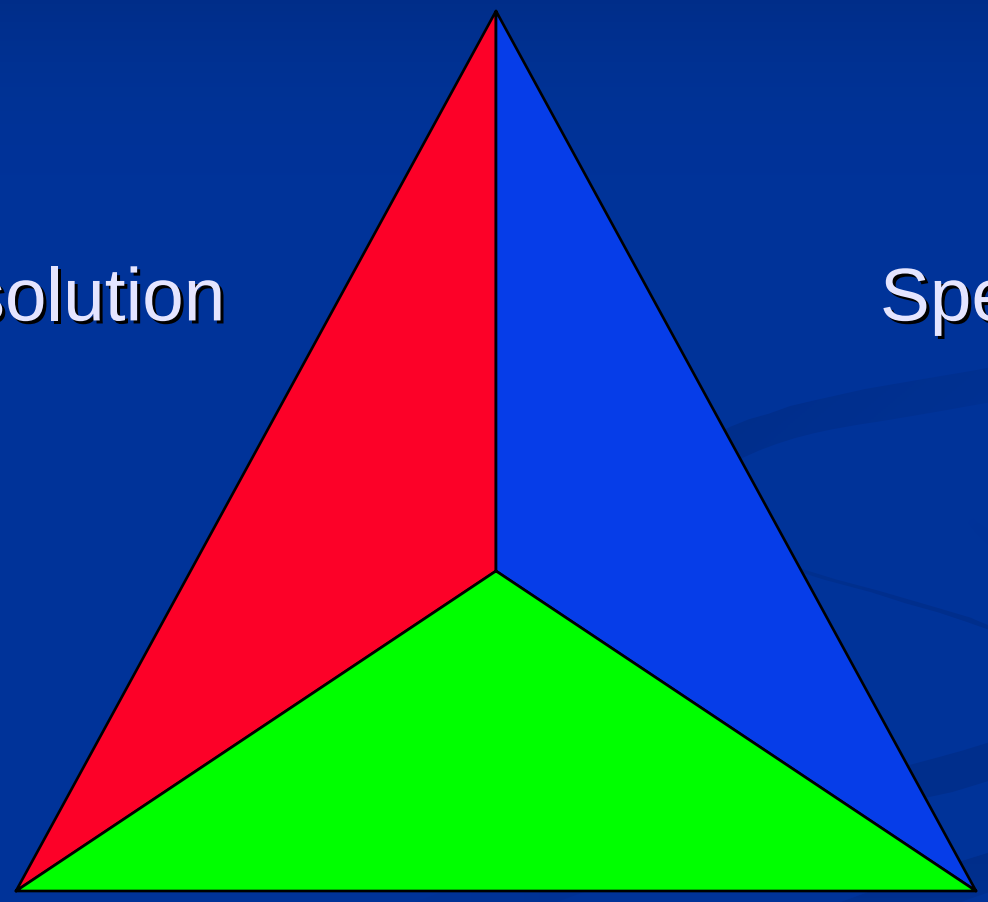
Matching Camera System Requirements to an Application

Camera parameters to consider
- Spatial resolution
- Dynamic range and linearity
- Temporal resolution
- Low-light sensitivity
- Adapting to instruments: lenses, microscopes, spectrometers, etc.
- Image analysis / application-specific software

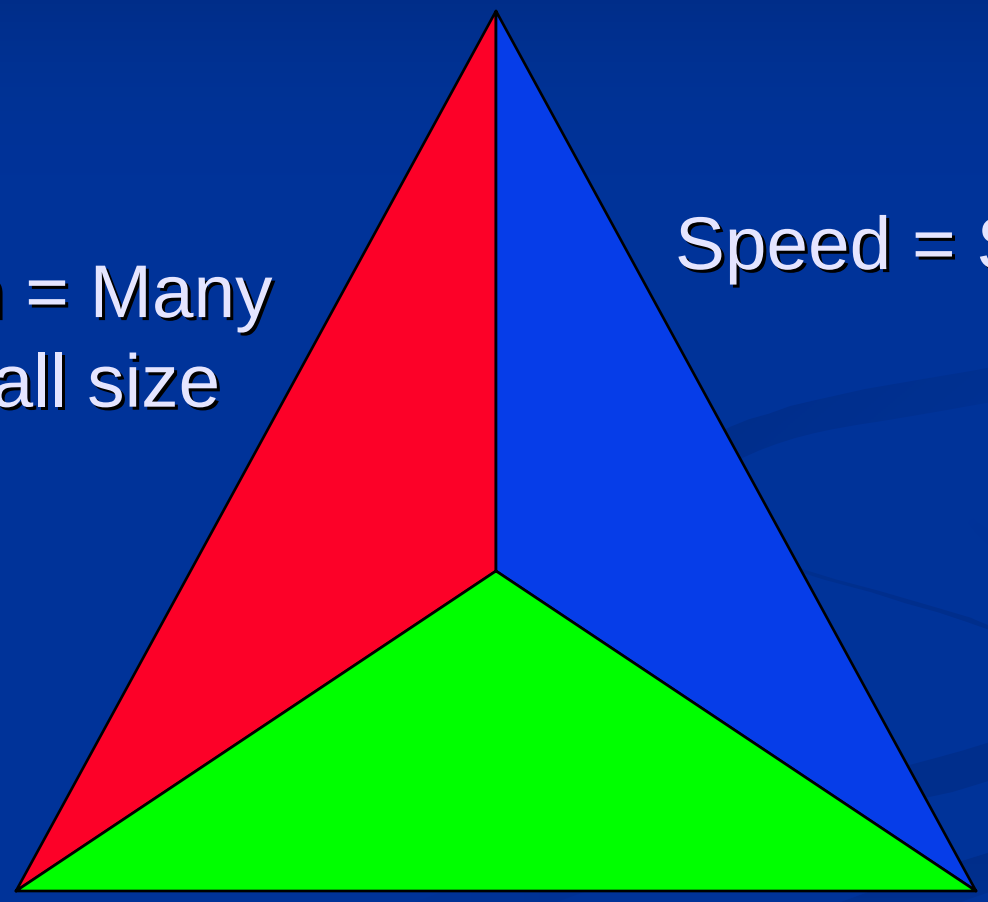
Common Applications

- Spinning Disk Confocal
- FRET
- Low Light Timelapse
- Deconvolution

Camera Performance Aspects



Performance Limitations



Spatial Resolution

- The maximum resolution required depends on the type of data analysis performed.
- Many applications do not require an "optically matched" (high) resolution.
  - Ratiometric Imaging (Fura/Indo)
  - Other calcium or isobestic indicators.
  - Motion tracking
- Fewer Digital conversions (less pixels) = Increase in frame rate.
- Larger Pixel Size = Higher Sensitivity

Speed

- Does speed refer to fast framerate or short exposure?
- Speed requires high sensitivity and fast readout (Light is the limit!)
- Is other hardware required to achieve the speed you want? (Dualview, piezo focus, AOTF)

Intensity (sensitivity)

- Light collection determines the length of integration.
- Some experiments demand high frame rates:
  - Live Cell Multi-Dimensional Acquisition (3-D)
  - Motion tracking
- What are the intensity requirements of the experiment in comparison to the temporal/spatial limitations?

Intensity (Dynamic Range)

- The dynamic range required depends on the experiment and sample.
- Dynamic Range 8-Bit (255) 12-Bit (4,095) 16-Bit (65,535)
- Many applications benefit from higher dynamic range.
  - FRET (Fluorescence Resonance Energy Transfer)
  - Ratiometric Imaging
  - FLIM (Fluorescence Lifetime Intensity Measurement)
  - Deconvolution
- Quiet Digitization makes best use of 16-Bit systems.

Common CCD Types

- Interline Transfer
  - High Resolution (7um per pixel)
  - Moderate Frame Rate
  - Moderate Sensitivity
  - 12 Bit System (4,095 Greylevels)

Interline

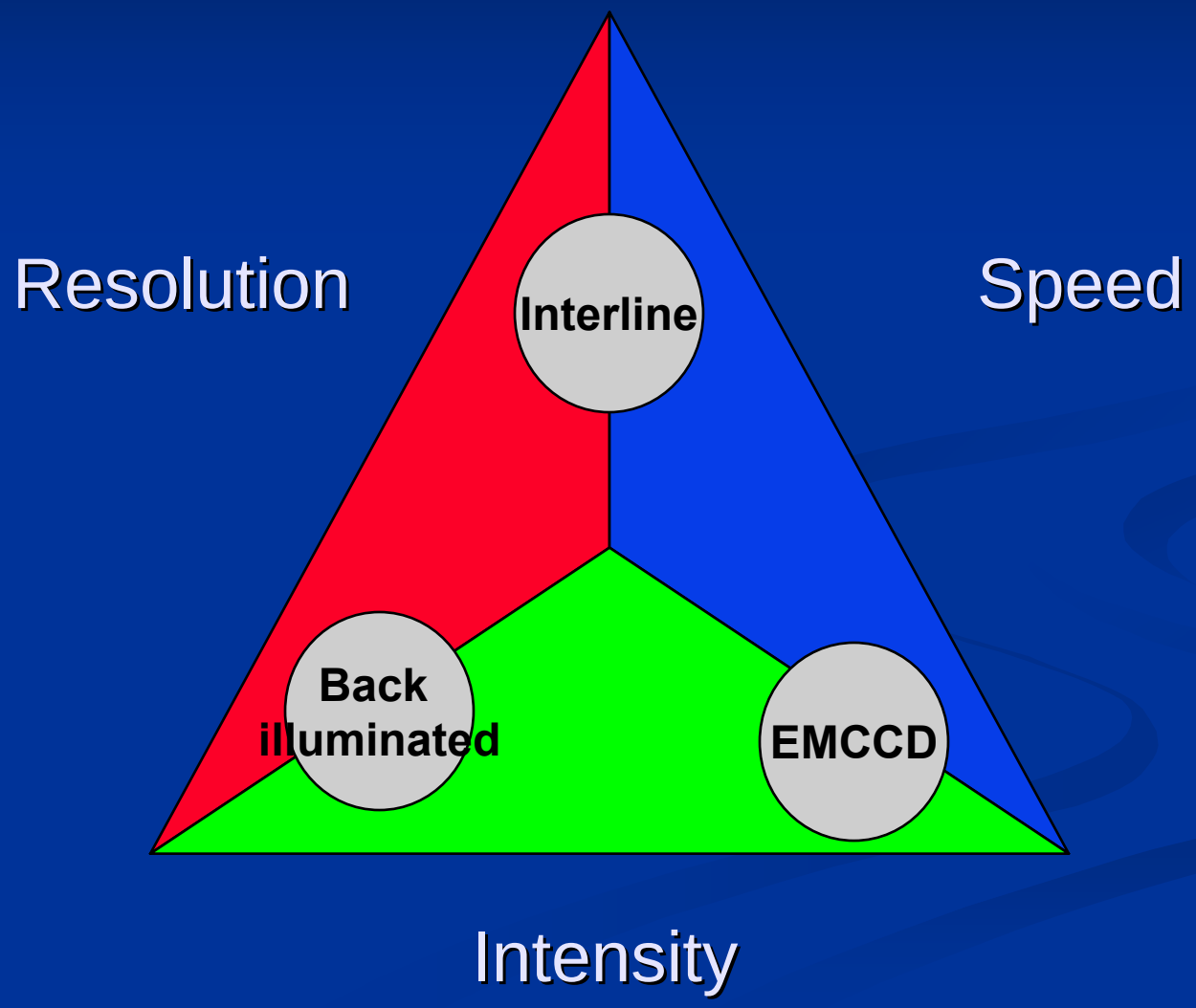
- Intensified (On-Chip Gain)
  - Moderate Resolution
  - High Speed and Sensitivity
  - 16 Bit System (65,535 Greylevels)

EMCCD's

- Short Exposure (BT Camera)
  - High Sensitivity
  - Slow, Quiet digitization of Data
  - Various Resolution Options
  - 16 Bit System (65,535 Greylevels)

Slow Scan Back Illuminated

Cameras


Resolution
Speed
Intensity
Interline
Back illuminated
EMCCD

Resolution and speed

Interline Transfer

Most versatile type of camera!

- High Resolution / Moderate Sensitivity.
- 10 Frames Per Second @ 6.45um Pixel Res.
- ~60% Light Collecting Ability
- Balanced System

Applications

Fixed Fluorescence – Timelapse Imaging – Colocalization – Live Cell Fluorescence

Resolution and Intensity
Back illuminated

- Various Resolution / Very High Sensitivity.
- High Sensitivity Systems
  - Dual or Quad view
- 95% Light Collecting Ability
- Very low read noise
- Deeply cooled

Applications

Long Term Timelapse – FRET – Co Localization – Bioluminescence

Intensified / On-Chip Gain

- **EMCCD**
  - Moderate Resolution / Very High Sensitivity.
  - High Speed System
  - 30 Frames Per Second @ 16um Pixel Res.
  - 92% Light Collecting Ability

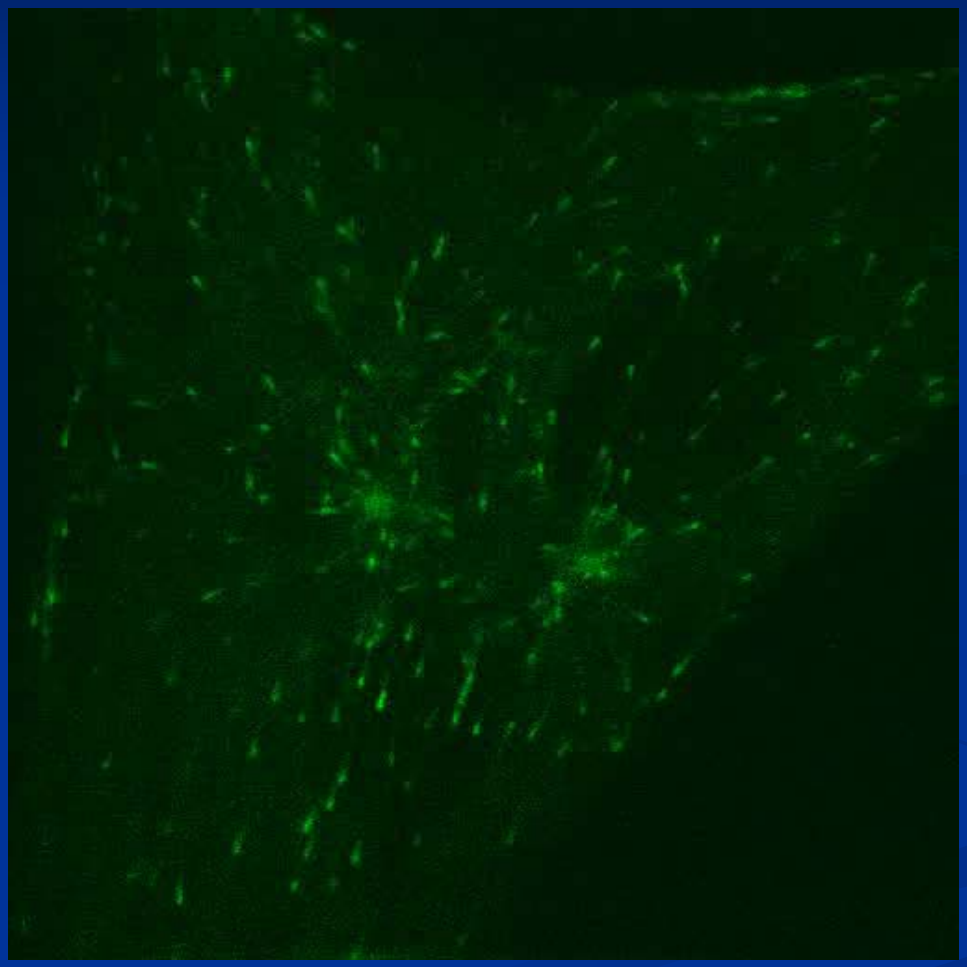
Applications

Confocal Imaging – TIRF – FRET – Calcium – Motion Tracking

Do I really need an EMCCD?

`Images courtesy of Michael Davidson; FSU

Is the application low light fast?



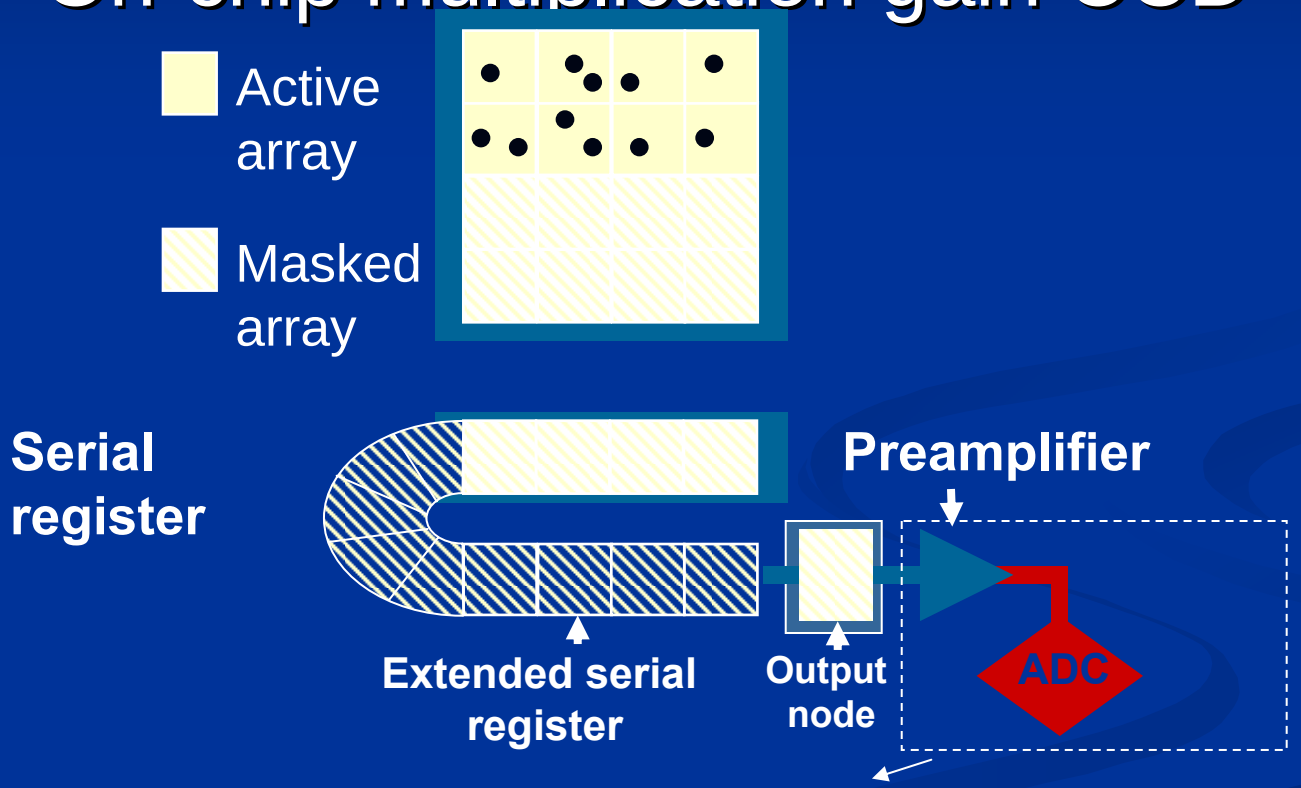
`Images courtesy of Michael Davidson; FSU

On-Chip Multiplication Gain Technology

- **Based on conventional CCD**
  - Frame Transfer - fast frame rates, no shutter
  - Back illuminated - highest Q.E.
  - Small pixel size - high spatial resolution
  - Dual Amplifier Capability – slow scan, low noise option
- **Solid State detector**
  - No damage due to bright light
- **Multiplication in the solid state domain**
  - Minimum excess noise
  - No need for external amplifier hardware (e.g., photocathode)
  - Easy to vary the multiplication level

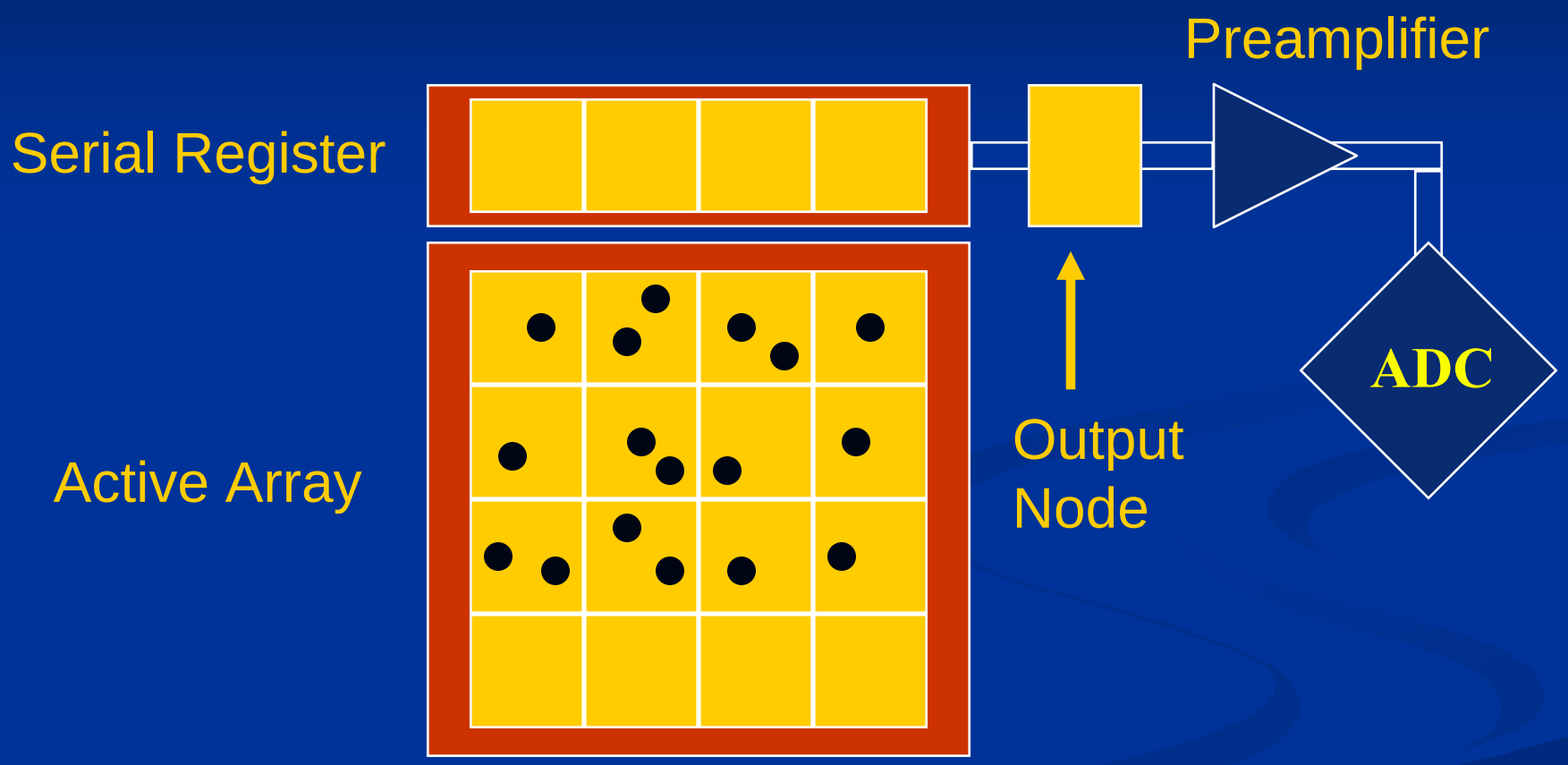
Theory of Operation

On-chip multiplication gain CCD



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

Analog Gain


Preamplifier
Serial Register
ADC
Output
Node
Active Array

Signal-to-Noise Ratio

Signal-to-Noise (SNR) is an important consideration in low-light level imaging applications

The Key Terms

- **Signal** Created when the incoming Photons (S) are detected as electrons.
- Don't forget QE! (Quantum Efficiency)

- **Noise (σ)** Three main sources

  #1: Light itself: **Photon Shot Noise $\sqrt{S}$**

  - Law of Physics
  - Given by square root of the signal

  #2: Detector and Electronics: **Read Noise**

  #3: Thermal Energy: **Dark Noise ($\sqrt{D}$)**

  -Modern CCD detectors are cooled

  -not the limiting factor at high frame rates

SNR: The Classic equation

Standard CCD SNR Equation:

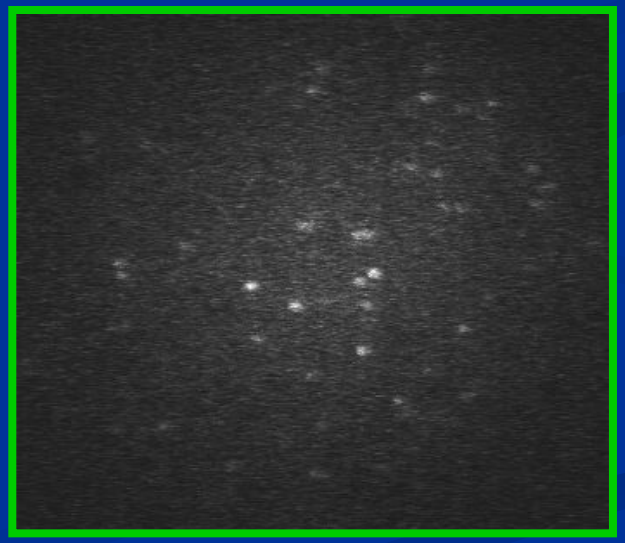
$$SNR = S \div \sqrt{[Sn2+Rn2+Dn2]}$$

High read noise is the limitation for low-light detection

The Read Noise limitation

- Low-light level applications were often read noise limited
  - i.e., signals below the read noise could not be detected

Read noise limited



Read noise minimized

- Nothing is free; EMCCD's are low light fast!!

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.

For more information, refer to the Technical Note:
On-chip multiplication gain

Acrobat Document

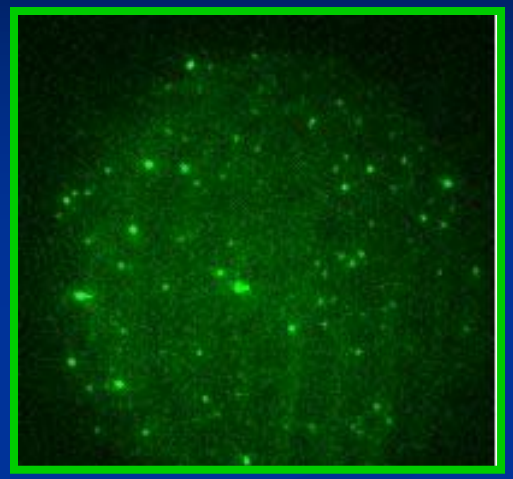
Excess Noise Factor

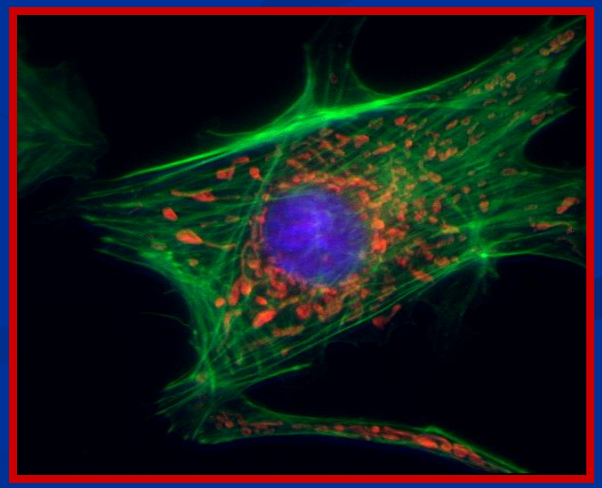
- Excess noise (F) is generated in the multiplication process
- Increase in the pulse height distribution of the input signal
- Measured to be between 1.0 and 1.4

Two-In-One Camera

Dual readout amplifiers

1. EM Gain amplifier for **high speed and low-light applications,** operates at 10 MHz and 5 MHz
2. Traditional amplifier for **slow scan, low-noise** applications, Operates at 5 MHz and 1 MHz





EMCCD Applications

- Single-molecule fluorescence
- High-speed motility studies
- High-speed FRET/Ion imaging
- Bio-Luminescence (NO WAY!)

When to use an EMCCD in Life Science Applications

- **Less excitation light reduces phototoxicity**
  - Live cell fluorescence
  - Fast bleaching dyes
- **Single Molecule Fluorescence**
- **Fast kinetics studies**
  - Motility
  - Ratio imaging for Calcium, pH, FRET

Common Applications (a recap)

- Spinning Disk Confocal
- FRET
- Low Light Timelapse
- Deconvolution

Spinning Disk Confocal

- High Frame Speed & Low Light
- Can be Synchronized with focusing hardware
- Dynamic Range helpful for decon/processing later.
- Operated at 60-100x Mag, large Pixel Appropriate.

FRET

- Low Light
- Large Dynamic Range key
- Could be Fast Framerate or slow.
- Varying Magnifications used

Low Light Timelapse

- Low Light
- No Framerate requirement
- Lowest camera noise possible
- Varying Magnifications used

Deconvolution

- Adequate Illumination
- Varying frame rate
- Maximum resolution

What is the right system for your lab?

- One Lab may have several different needs for an imaging system. Can one system perform all experiments well? Is a bundled system appropriate?
- What limitations do the microscope systems place on the imaging hardware?
- Will a dual-view, piezo or high speed excitation system change your camera choice?
- What system will provide the best performance for future experiments? As experiments develop demands change.

- Thank you!