CCD Fundamentals

Typical Cooled CCD Camera Configuration

- DC Voltage
- Serial Clock Driver
- Parallel Clock Driver
- Low Noise Preamplifier
- Shutter
- Incoming Light
- Optic
- HCCD (-45°C)
- Thermo-Electric Cooler
- Cooling Fins
- Camera Control Logic and Power Supply
- Analog Processing and ADC
- Camera Command Input
- Camera Status Output
- Digital Pixel Data Output (12-16 bits)

- Computer Interface
CCD Fundamentals
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

Incoming Light

- Electrical Connection
- Polysilicon Gate
- Silicon Dioxide
- Silicon

Potential Well
CCD Fundamentals

Quantum Efficiency

Ability of CCD to convert photons to electrons
CCD Fundamentals

Front
- Silicon Dioxide
- Incoming Light
- Polysilicon Gate

Back
- Thinned Silicon
- Incoming Light

Silicon
CCD Fundamentals

Incoming Light

ITO Gate

Silicon Dioxide

Epitaxial Silicon

Bulk Silicon
CCD Fundamentals

Typical Quantum Efficiencies

- standard frontside-illuminated (Kodak KAF-1400)
- thinned, backside-illuminated (SITe Si502AB)
- lens-on-chip (Sony interline)
- ITO (Kodak KAF-1401E)

Quantum Efficiency %

Wavelength (nm)

Metachrome
CCD Fundamentals

Serial Register → Parallel Register → Preamplifier

Output Node
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

CCD Operation
Serial Shift - 1 Pixel to Output
CCD Fundamentals
Introduction

CCD Architecture
CCD Fundamentals

- **Serial Clocks**
- **Output Amplifier**

- **Parallel Clocks**
- **Direction of Parallel Shift**

- **Serial Register**

- **Full-Frame CCD**
- **Serial Clocks**
- **Frame-Transfer CCD**
- **Interline-Transfer CCD**

- **Serial Clocks**
- **Output Amplifier**

- **Parallel Clocks**
- **Direction of Parallel Shift**

- **Storage Array (masked)**

- **Image Array**

- **Parallel Clocks**
- **Direction of Parallel Shift**

- **Full Array**

- **Interline Mask**

- **Photosite**
CCD Fundamentals

- Full Frame
- Frame Transfer (EMCCD)
- Interline Transfer
CCD Fundamentals

Serial Register

Full Frame

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node

ADC
CCD Fundamentals

- Serial Register
  - Active Array
  - Output Node
  - Preamplifier
  - ADC
CCD Fundamentals

Serial Register

Active Array

Output Node

Preamplifier
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node

ADC
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Frame Transfer

Serial Register

Active Array

Output Node

Preamplifier
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
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CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Interline Transfer

Active Array

Output Node

Preamplifier
CCD Fundamentals

Serial Register

Preamplifier

Active Array

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

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Preamplifier

Output Node
CCD Fundamentals

Serial Register

Active Array

Output Node

Preamplifier
CCD Fundamentals

Serial Register

Active Array

Preamplifier
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
# CCD Fundamentals

## Full Frame
- High Spatial Resolution
- Large Number of Pixels
- Greater Selection of CCD Formats
- 100% Fill Factor for Entire Array

## Frame Transfer
- Integrate while Clocking
- High-Speed Operation
- Well Suited for Complex Readout Schemes
- 100% Fill Factor in Active Array

## Interline Transfer
- Integrate while Clocking
- High-Speed Operation (Video)
- Lens-on-Chip Technology
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

Response (10^9 Electrons) vs. Illumination Level (Arbitrary)
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
  \[ \text{noise} = \text{square root of number of electrons} \]
- Poisson distribution
- When photon noise exceeds system noise, data is photon (shot) noise limited
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

Serial Register

Active Array

Preamplifier

Output Node

ADC
CCD Fundamentals

Dark Current

Electrons created by thermal emission

Increases with time and temperature
Dark current subtracts, dark current noise remains

- dark current noise = $\sqrt{\text{dark current}}$
- dark current (dark noise) can lower SNR
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 $<-120^\circ C$
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
- $kT/C$ Noise - Reduced by using correlated double sampling
- Spurious Charge - Reduced by careful shaping of clock waveforms
Introduction

Dynamic Range
Dynamic Range = Full Well/Read Noise
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

Serial Register

Binning

Preamplifier

Active Array

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Preamplifier

Active Array

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node

ADC
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node
CCD Fundamentals

Serial Register → Active Array → Output Node → Preamplifier
CCD Fundamentals

Serial Register

Active Array

Preamplifier

Output Node

ADC
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

Resolution

Speed

Intensity
Performance Limitations

Resolution = Many Pixels, small size

Speed = Signal/noise?

Intensity
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)

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

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

Resolution  Speed

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

- Active array
- Masked array

Serial register

Extended serial register

Preamplifier

Output node

ADC

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

\[
\text{SNR} = \frac{S}{\sqrt{S_n^2 + R_n^2 + D_n^2}}
\]

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:

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

Note: $F$ is the excess noise factor.

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