Biophysics 210: Biological Light Microscopy
Discussion Section 3: Fourier Optics and Cameras
Tuesdays 1-2:30pm
Location: Genentech Hall Rm N114

Helpful Equations

Noise of camera = $\sqrt{(Shot\ Noise)^2\ +\ (Read\ Noise)^2\ +\ (Dark\ Current\ Noise)^2}$

Shot Noise = $\sqrt{\#\, of\ \text{photons}}$

SNR = $N\,/\sqrt{(Read\ Noise^2 + N}$; $\text{N} = \#\text{ of photons}$

Optical Resolution = 0.61λ / NA

1. For the following experiment examples, give one's best guess for which type of detector(s) discussed in the online lectures would likely be coupled to the microscope used.

   A. You have fixed, 20 micron sections from adult mouse brain prepared on histology slides. You have immunostained a few slides with an anti-Tuj1 antibody in order to highlight the microtubule network in neurons and costained with a nuclear dye (such as DAPI). You decide to image your stained slides using a point scanning confocal microscope. What type of detector is likely to be used for this experiment?

   B. You want to study endosome trafficking in live cells. As a first pass experiment, you generate a HeLa cell line that stably expresses an endosomal fluorescent reporter. You decide to image your HeLa cell line using a spinning disk confocal microscope. What detector(s) might be used for this experiment?

2. You have a widefield fluorescent microscope set up with a Clara model CCD camera with the specifications shown below. Assume that you have on average 100 photons incident on each pixel per second, that the quantum efficiency of the detector for this incident wavelength is ~55%, and a photon leads to the excitation of a single electron. What is your signal-to-noise ratio (SNR) for a 600 ms exposure under these conditions when the camera is run at 10 MHz? (Ignore dark current noise for simplicity)

3. What are three ways that the SNR found in previous question could be improved without modifying the biological sample and the light intensity illuminating the sample? Determine your new SNR for one of these methods? Which of these methods reduces the read noise of your CCD camera? What if the microscope was set up with a Zyla 4.2 sCMOS camera instead?

4. Briefly explain why you would switch from a CCD camera to a sCMOS camera. Why would you switch to an EMCCD camera? What camera specification are EMCCDs designed to minimize and how is this achieved?

5. When samples are bright (i.e. large # of photons striking the camera), which aspect of noise is most important? What does this imply about the importance of camera choice for bright samples?

6. You are doing fluorescence microscopy with a 100x 1.3 NA objective. What is the maximum pixel size that still allows you to achieve Nyquist sampling for 530 nm light? What about sampling 3 pixels per resolvable distance? Does the Clara model CCD from question 4 allow for Nyquist sampling? Would this change if it utilized an Interline Transfer architecture?
7. You now switch to a 20x 0.75 NA objective. What is the maximum pixel size that still allows you to achieve Nyquist sampling for 530 nm light? Does the Clara model CCD from question 4 allow for Nyquist sampling? What is the effective resolution of the 20x objective when imaged using the Clara CCD?
8. For objectives in question #6 and #7 which of the following camera choices allow for Nyquist sampling?

Camera	Camera pixel size	100x 1.3 NA objective	20x 0.75 NA objective
Zyla 4.2 sCMOS	6.5 um		
DU-897 EMCCD	16 um		
Prime 95B sCMOS	11 um		
Iris 15 sCMOS	4.25 um		

Model	Clara			Clara E		
Active pixels [W x H]	1392 x 1040					
Pixel size	6.45 x 6.45 µm					
Image area [W x H]	8.98 x 6.71 mm					
Pixel readout rate (MHz)	20, 10, 1					
Read noise (e-) *3	1 MHz	10 MHz	20 MHz	1 MHz	10 MHz	20 MHz
Typical	2.4	5	6.5	3.0	5	6.5
Minimum temperature air cooled (fan on) @ 25°C ambient	-55°C			-20°C		
Minimum temperature 'vibration free mode' (fan off) @ 25°C ambient	-40°C			Mode not available		
Dark current, e-/pixel/sec @ minimum temperature *4	0.0003			0.0015		
Maximum frame rate	11 frames per second @ 20 MHz					
Pixel well depth (typical)	18,000 e-					
Well depth with binning (typical)	30,000 e-					
Maximum dynamic range	> 6,500:1 @ 1MHz; 12,500 with binning					
Linearity *5	Better than 99%					
Dual digitization	16 bit @ 1 MHz; 14-bit @ 10 MHz & 20 MHz					
Baseline (bias) offset clamp	Yes					
Timestamp accuracy	12.5 ns					
System window type	UV-grade fused silica 'Broadband VUV-NIR', unwedged					
Interface *6	USB 2.0					
Lens mount	C-mount					

MODEL SPECIFIC SPECIFICATIONS*1

Model	Zyla 5.5			Zyla 4.2	
Sensor type	Front Illuminated Scientific CMOS			Front Illuminated Scientific CMOS	
Active pixels (W x H)	2560 x 2160 (5.5 Megapixel)			2048 x 2048 (4.2 Megapixel)	
Sensor size	16.6 x 14.0 mm 21.8 mm diagonal			13.3 x 13.3 mm 18.8 mm diagonal	
Pixel readout rate (MHz)	200 (100 MHz x 2 sensor halves) 560 (280 MHz x 2 sensor halves)			Slow Read 216 (108 MHz x 2 sensor halves) Fast Read 540 (270 MHz x 2 sensor halves)	
Read noise (e-) Median [rms] *2		Rolling Shutter	Global Shutter		Rolling Shutter
	@ 200 MHz	1.2 [1.7]	2.4 [2.7]	@ 216 MHz	0.90 [1.4]
	@ 560 MHz	1.45 [1.8]	2.6 [2.9]	@ 540 MHz	1.10 [1.6]
Maximum Quantum Efficiency *3	60%			72%	
Sensor Operating Temperature					
Air cooled	0°C (up to 30°C ambient)			0°C (up to 27°C ambient)	
Water cooled	-10°C*			-10°C*	
Dark current, e-/pixel/sec @ min temp *4					
Air cooled	0.14			0.14	
Water cooled	0.04			0.04	
Readout modes	Rolling Shutter and True Global Shutter (Snapshot)			Rolling Shutter and Global Clear *8	
Maximum dynamic range	25,000:1			33,000:1	
Photon Response Non-Uniformity (PRNU)	< 0.5%			< 0.1%	
Pre-defined Region of Interest (ROI)	2048 x 2048, 1920 x 1080, 1392 x 1040, 512 x 512, 128 x 128			1920 x 1080, 1392 x 1040, 512 x 512, 128 x 128	
User defined ROI granularity	1 pixel **				
Data range	12-bit and 16-bit			12-bit and 16-bit	
Interface options	USB 3.0 *9 Camera Link 3-tap Camera Link 10-tap			USB 3.0 *9 Camera Link 10-tap	

System Specifications [*2]

	Ultra 888		Ultra 897	
Sensor QE options	#BV: Back Illuminated, standard AR coated BVF: Back Illuminated, standard AR coated with fringe suppression UVB: Back Illuminated, standard AR with additional lumogen coating #EX: Back illuminated, dual AR coated EXF: Back illuminated, dual AR coated with fringe suppression			
Fringe Suppression	Available on EX2 and BV sensor options			
Active pixels	1024 x 1024		512 x 512	
Pixel size	13 x 13 µm		16 x 16 µm	
Image area	13.3 x 13.3 mm with 100% fill factor		8.2 x 8.2 mm with 100% fill factor	
Pixel Readout Rate	10 MHz	30 MHz [*3]	10 MHz	17 MHz
Minimum temperature, air cooled, ambient 20°C	-80°C	-60°C	-80°C	-80°C
Chiller liquid cooling, coolant @ 10°C, >0.75l/min	-95°C	-75°C	-100°C	-100°C
Thermostatic Precision	± 0.01°C			
Triggering	Internal, External, External Start, External Exposure, Software Trigger			
System window type	#BV and BVF: UV-grade fused silica, Broadband Visible-Near Infrared, 0.5 degree wedge UVB, #EX, EXF: UV-grade fused silica, Broadband Vacuum Ultraviolet-Near Infrared, 0.5 degree wedge			
Blemish specification	Grade 1 sensor from supplier. Camera blemishes as defined by Andor Grade A www.andor.com/learning-academy/ccd-blemishes-and-non-uniformities-black-pixels-and-hot-pixels-on-a-ccd-sensor			
Digitization	16-bit (at all speeds)			
PC Interface	USB 3.0 [*12]		USB 2.0	
Lens Mount	C-mount			
Direct Data Access	Camera Link 3-tap output			

Advanced Performance Specifications [*2]

	Ultra 888	Ultra 897
Dark current and background events [*4,5]		
Dark current (e^-/pixel/sec) @ -80°C	0.00025	0.00030
Dark current (e^-/pixel/sec) @ max cooling	0.00011	0.00015
Spurious background (events/pix) @ 1000x gain / -85°C	0.005	0.0018
Active area pixel well depth	80,000 e^-	180,000 e^-
Gain register pixel well depth [*6,7]	730,000 e^-	800,000 e^-
Pixel readout rates	EM Amplifier: 30, 20, 10 & 1 MHz Conventional Amplifier: 1 & 0.1 MHz	EM Amplifier: 17, 10, 5 & 1 MHz Conventional Amplifier: 3, 1 & 0.08 MHz

Read noise (e^-) [*7]	Ultra 888 EMCCD Amplifier				Ultra 888 Conventional Amplifier		Ultra 897 EMCCD Amplifier				Ultra 897 Conventional Amplifier		
MHz	30	20	10	1	1	0.1	17	10	5	1	3	1	0.08
Without Electron Multiplication	130	80	40	12	6	3.5	89	65	37	15	9.6	5.3	2.7
With Electron Multiplication	< 1	< 1	< 1	< 1	-	-	< 1	< 1	< 1	< 1	-	-	-

Linear absolute Electron Multiplier gain	1 - 1000 times via RealGain™ (calibration stable at all cooling temperatures)

95% Quantum Efficiency

Prime 95B™ Scientific CMOS Camera Datasheet

Specifications	Camera Performance
Sensor	GPixel GSense 144 BSI CMOS Gen IV, Grade 1 in imaging area
Active Array Size	1200 x 1200 pixels (1.44 Megapixel)
Pixel Area	11µm x 11µm (121µm²)
Sensor Area	13.2mm x 13.2mm 18.7mm diagonal
Peak QE%	>95%
Read Noise	1.6e- (Median) 1.8e- (RMS)
Full-Well Capacity	80,000e- (Combined Gain) 10,000e- (High Gain)
Dynamic Range	50,000:1 (Combined Gain)
Bit Depth	16-bit (Combined Gain) 12-bit (High Gain)
Readout Mode	Rolling Shutter Effective Global Shutter
Binning	2x2 (on FPGA)

Cooling Performance	Sensor Temperature	Dark Current
Air Cooled	-10°C @ 30°C Ambient	2.9e-/pixel/second
Liquid Cooled	-25°C @ 30°C Ambient	0.7e-/pixel/second

Look at the Big Picture

Iris 15™ Scientific CMOS Camera Datasheet

Specifications	Camera Performance
Sensor	GPixel GSense 5130 Scientific CMOS sensor
Active Array Size	5056 x 2968 (15 Megapixel)
Pixel Area	4.25µm x 4.25µm (18.06µm²)
Sensor Area	21.49mm x 12.61mm 24.9mm diagonal
Peak QE%	>73%
Read Noise	1.5e-
Full-Well Capacity	16,000e-
Bit Depth	16-bit
Readout Mode	Rolling Shutter Effective Global Shutter
Binning	2x2 (on FPGA)

Cooling Performance	Sensor Temperature	Dark Current
Air Cooled	0°C @ 30°C Ambient	0.5e-/pixel/second