Biophysics 210
Discussion Section 3: Fourier Optics and Cameras

Helpful Equations

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

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

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 your microscope was set up with a Zyla 4.2 sCMOS camera instead?

4. Explain briefly why one would switch from a CCD camera to a 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?

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 Minimum temperature 'vibration free mode' (fan off) @ 25°C ambient	-55°C -40°C			-20°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 @ 200 MHz: 1.2 [1.7] / 2.4 [2.7] @ 560 MHz: 1.45 [1.8] / 2.6 [2.9]	Rolling Shutter @ 216 MHz: 0.90 [1.4] @ 540 MHz: 1.10 [1.6]
Maximum Quantum Efficiency [*3]	60%	72%
Sensor Operating Temperature Air cooled Water cooled	 0°C (up to 30°C ambient) -10°C*	 0°C (up to 27°C ambient) -10°C*
Dark current, e^-/pixel/sec @ min temp [*4] Air cooled Water cooled	 0.14 0.04	 0.14 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