

# PTI Technical Note

## How to Select a Cooled CCD Camera for your Imaging system

Choosing an imaging camera for your applications can be a difficult task. Imaging cameras come in so many shapes, sizes, frame rates, sensitivity and interfaces. They can also be expensive, so making a wrong decision can really affect your ability to achieve publishable data. Before looking at all the different cameras, it is important to ask yourself the following questions and be honest with the answers.

### *How fast does the camera need to be? Myth and reality*

If you take a look at an example specification sheet that indicates frame rates of a given camera, you may notice that the frame rate is specified at zero exposure time.

		Region		
		1940 x 1460	970 x 730	646 x 486
Binning	1 x 1	6.3	11.8	16.8
	2 x 2	11.6	20.8	28.4
	3 x 3	16.2	27.8	36.5
	4 x 4	20.1	33.2	42.6

(Frames per second)

**Note: Frame rates are measured at 20 MHz with 0-millisecond exposure times.**

This specification describes how fast the camera can transfer data to the computer in frames per second. So a camera rated at 10 frames per second at zero exposure time will not work for an application that requires 10 frames per second. An exposure time is always required to integrate photons on the sensor and generate a usable image.

In order to get an image with a good signal to noise ratio, an exposure time of 100 or 200 ms may be required. Therefore the “real” frame rate may only be 5 frames per second. Another thing to consider is that the sensor quite often needs to be cleared before acquiring an image. These clear cycles can further reduce the camera frame rates from the specifications.

### *Real World example*

Let’s say the application is Cardiac Myocyte Calcium Imaging.

In order to reduce artifacts generated by cell path length, Photo bleaching and non uniformity a ratio imaging technique using Fura2 is selected for the protocol. Now, what camera requirements do we need to satisfy this experiment?

1. A fast camera is required of at least 30 frames per second
2. A High Quantum efficiency is required to allow short exposure times
3. A reasonable spatial resolution is required of approximately 512 x 512 pixels
4. A fast excitation switching device is required to synchronize with the camera

Camera choice could be a Photometrics Evolve 512. The Evolve has a frame rate specified at 30 frames per second in frame transfer mode. A QE of approximately 90%, 512 x 512 pixels and external inputs and outputs to synchronize with an excitation switching device. So in theory, it sounds like the perfect choice. How about reality? Let’s work through an example.

The Evolve is a frame transfer camera and outputs 30 frames per second at zero exposure time with a pre-sequence clear cycle. Sounds good? Not really. This is why...

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Since an exposure time is required, integration at each wavelength is necessary. Let's say an exposure for at least 30 ms at wavelength 340 nm, and then exposure at 380 nm for 30 ms before acquiring the 380 nm image, we need to clear the CCD of its charge at 340 nm. This requires a pre-exposure clear cycle. Once initiating a pre-exposure clear cycle, the camera's frame rate drops to 20 fps. This will yield 10 ratios per second. This is not satisfactory, as 30 frames per second are necessary for the experiment. What should one do?

Reduce the exposure time? Let's try to reduce the exposure time. When setting the exposure time to 10mS, do we see an increase in the frame rate? NO. Why not? The camera firmware is outputting the maximum frame rate, because this frame rate was specified at zero exposure time. OH Yeah, now what?

### *Increase the camera binning*

It is possible to set the camera binning to 2 x 2. This will bin vertical and horizontal pixels and decrease the image size to 256 x 256 pixels. Now, the frame rate should increase to 60 frames per second, right? NO. Why? We need to have the camera set to a pre-exposure clear cycle to clear the CCD charge. Depending on the camera, this will reduce the frame rate by 1/3 to about 40 frames per second. But we need to have an exposure. If we take 40 fps and take the reciprocal, we get 0.025 ms. 25 ms is the maximum exposure we can take without reducing the frame rate. In fact to be safe, we should only expose for 20mS, because a transfer of the image to the computer and movement of the light source is required. This takes a few milliseconds. So you see, we had to compromise the spatial resolution to achieve the temporal resolution. All cameras will be a compromise between spatial and temporal resolutions.

### *Quantum Efficiency*

In the above example, a camera with a QE of 90% was selected. This was necessary, since short exposure times of 20 ms are necessary. If a camera with a lower QE was selected, one would not have been able to lower the exposure time without compromising the image quality. In fact, an increase of the exposure time to double or even triple may be necessary. This increase in exposure time would have reduced the effective frame rate to 16 frames per second. So quantum efficiency is very important to allow short exposure time.

### *Resolution*

It was determined a resolution of 512 x 512 was originally necessary, however it was determined this was not possible, since camera binning reduced spatial resolution by 50%. What could have been done to retain spatial resolution? One should have chosen a different camera, the Evolve Delta. The Evolve Delta starts at a frame rate of 60 frames per second. With pre-exposure clear cycle the frame rate would have dropped to 40 frames per second. We could have exposed for 20mS and increased the camera electron multiplier to make up the gain. This would yield 40 frames per second.

### *What if I can't afford an expensive camera like the Evolve?*

Let's say one is on a budget, and cannot afford a high end camera. What camera to choose? This is a difficult decision, since there are so many cameras choices. The same principles holds true in lower priced cameras. Pick the fastest camera with the highest quantum efficiency. Let's work through an example.

Coolsnap MYO camera. This is a mid range workhorse. It outputs 6 frames per second in pre-sequence clear mode, has a QE of 75% and approximately 1940 x 1460 pixels. How to speed up this camera?

Binning the camera to increase the temporal resolution will be necessary. Let's work through the example. To get the MYO to speed up to the desired frame rate of 30 frames per second, a binning factor of 4 x 4 is required and a sub region of 646 x 486. This will give 42 fps. However, because a clear cycle is necessary, the effective frame rate will drop to 28 fps.

Twenty eight frames per second are not so bad. What exposure time is necessary? Oh yea, that frame rate was at zero exposure time. We need 30 ms to make that frame rate work. You may have to go even higher in the exposure time to get an image with a good signal to noise ratio, since now, the Quantum efficiency is less and NO electron multiplier is available. By reducing spatial resolution via binning and temporal resolution by increasing the exposure time, it is possible to overcome lower QE. We are basically pushing this camera to the max for this kinetic application.

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### Summary

Take into consideration your real world examples. It is important, first of all, to establish your required frame rate, and then select the highest QE and required spatial resolution.

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