Using Additional Optics to Adjust Pixel Size

Introduction

Additional optics such as magnification couplers (also known as camera mount adaptors) can be used to optically adjust the effective pixel size and field of view of scientific cameras to better match the microscope resolution and field of view, respectively. Couplers are placed just before the camera on the camera port of the microscope and are available in several magnification or demagnification values, the most common of which being 0.5x, 0.66x, 1x, 1.5x and 2x.

The way couplers function can be described quite simply; demagnification increases the effective pixel size and magnification decreases the effective pixel size at the sample. As Table 1 shows, an 11µm pixel demagnified with a 0.5x coupler would have an effective pixel size of 22 µm but when magnified with a 2x coupler it would have an effective pixel size of 5.5 µm. These two couplers therefore represent a doubling or halving of the initial pixel size.

11 µm Pixel	Magnification Coupler				
	0.5x	0.66x	1x	1.5x	2x
Effective Pixel size	22.0	16.7	11.0	7.3	5.5

Table 1: The effect of demagnifying and magnifying couplers on the effective pixel size of an 11 µm pixel camera with a 1.4NA objective

Changing pixel size with magnification couplers allows the user to trade off sampling at the appropriate resolution for field of view or vice-versa. Using a demagnification coupler increases field of view at the expense of a larger effective pixel whereas using a magnification coupler decreases effective pixel size at the expense of a smaller field of view.

The ideal magnification coupler is almost always 1x, which contains no lens and allows light to travel from the microscope to the camera without any additional correction. This avoids the other issues inherent with magnification couplers such as a reduction in the number of photons (each additional lens in the light path reduces the number of photons by 3-4%), reduced image quality (couplers can contain lower quality lenses than other microscope optics) and uneven illumination of the camera sensor.

Magnification Means Pixels Cover a Smaller Sample Area

One of the most common uses of magnification couplers is to map the pixel across a smaller sample area. If the pixel is too large, the image cannot be sampled at the microscopes limiting resolution. Nyquist sampling is often desired in microscopy applications because it ensures the finest sample features possible are captured in the image while maximizing the sensors field of view.

A full explanation of Nyquist sampling and diffraction limited resolution can be found in our *Resolution Technical Note*.

As Table 2 shows, to achieve Nyquist sampling at 100x/1.4NA the largest effective pixel size that can be used is 11 µm. However, to achieve Nyquist sampling at 60x/1.4NA, a pixel size of 6.6 µm is required so an 11 µm pixel camera will not achieve Nyquist sampling. By cross-referencing with Table 1, the 11 µm pixel camera can be made to reach a minimum effective pixel size of 6.6 µm with a 2x magnification coupler to give an effective 5.5 µm pixel. A 1.5x coupler only reaches 7.3 µm which unfortunately isn't enough – using this would give equivalent resolution to a 60x/1.2NA objective.

Magnification	Pixel size for Nyquist (µm)
40x 1.3 NA	4.8
60x 1.4 NA	6.6
100x 1.4 NA	11

Table 2: Pixel size required for Nyquist at 40x, 60x and 100x assuming an emission wavelength of 510 nm (GFP)

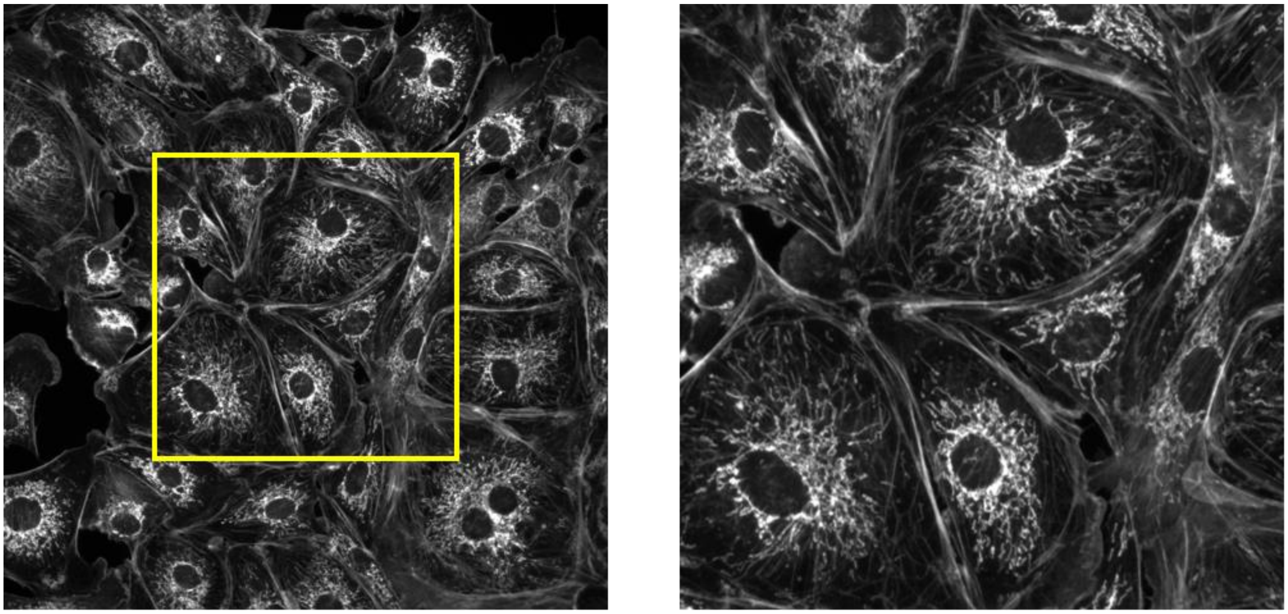
However, using a magnification coupler with an 11 µm pixel camera to reach the required pixel size for Nyquist sampling will have a significant effect on field of view. As Table 3 shows, using a 60x objective with a 2x magnification coupler means the final magnification will be 120x which results in a 50% reduction in field of view. The more the sample is magnified, the less sample area can be seen.

Magnification	Coupler Magnification	Actual Magnification	Field of View
40x	1x 1.5x 2x	40x 60x 80x	100% 75% 50%
60x	1x 1.5x 2x	60x 90x 120x	100% 75% 50%
100x	1x 1.5x 2x	100x 150x 200x	100% 75% 50%

Table 3: The difference in objective magnification and actual magnification when applying 1x, 1.5x and 2x magnification couplers.

The difference in field of view when comparing 60x magnification with 60x magnification with a 2x coupler is illustrated in Figure 1. The sacrifice in field of view when using an 11 µm pixel camera to match Nyquist at 60x is quite considerable.

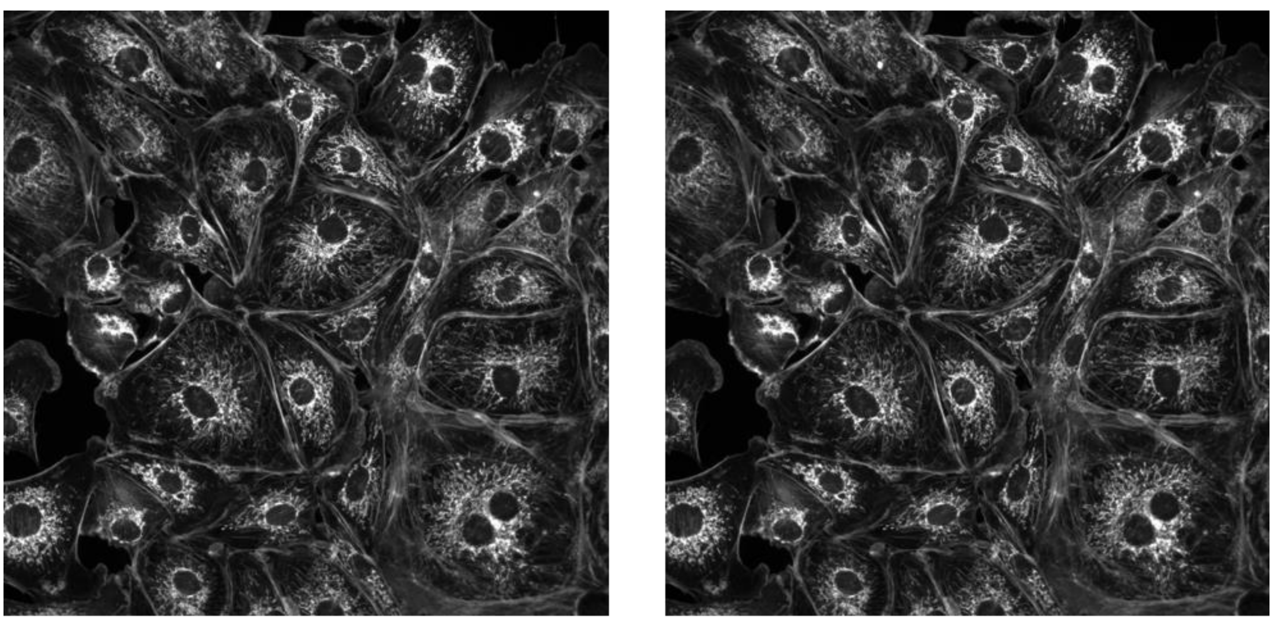
A more favorable solution that would allow for Nyquist sampling at 60x whilst maintaining the field of view would be to use a scientific camera with a 6.5 µm pixel size. This would also result in a more sensitive camera because a larger pixel has a larger area for photon collection. A 6.5 µm pixel has a 42.25 μm^2 pixel area whereas a 5.5 µm pixel has a 30.25 μm^2 pixel area, which gives ~30% reduction in sensitivity. The field of view comparison between an 11 µm pixel camera and a 6.5 µm pixel camera at 60x magnification is shown in Figure 2.



11 µm pixel camera at 60x

11 µm pixel camera at 120x (60x objective with 2x magnification coupler)

Figure 1: Field of view comparison of an 11 µm pixel camera at 60x magnification (left) with an 11 µm pixel camera at 60x magnification with a 2x magnification coupler resulting in an actual magnification of 120x (right). The yellow box highlights the field of view from the image right



11 µm pixel camera at 60x

6.5 µm pixel camera at 60x

Figure 2: Field of view comparison of an 11 µm pixel camera at 60x magnification (left) with a 6.5 µm pixel camera at 60x magnification (right).

Image Quality is Only as Good as The Worst Lens

One of the major issues when using magnification couplers is that it introduces another lens into the system. This may be undesirable for two reasons; firstly, every additional lens in the light path loses 3-4% of the photons through reflection, and secondly, the quality of the image is only as good as the worst lens in the system.

The quality of the lenses in a microscope determine how well the system performs. Objective lenses are among the most expensive part of the microscope and for a good reason, they are extremely high-quality lenses with multiple corrections to ensure high quality light transmission.

Apochromatic objectives, for example, are chromatically corrected for red, blue, and yellow. They also provide spherical aberration correction for two to three wavelengths and generally have a high numerical aperture (NA) and long working distance. Plan, also known as planar, semi-plan, semi-planar, or microplan, objectives correct for field curvature. Field curvature is a type of aberration present when the off-axis image cannot be brought to focus in a flat image plane, resulting in a blurred image as it deviates from the optical axis. Plan objectives display better than 90% of the field flat and in focus.

Magnification couplers designed by the microscope manufacturers should use the same high-quality lenses expected of their objective lenses however third-party magnification couplers may use cheaper lenses which don't have the same correction quality as the rest of the lenses in the system.

Maximizing Optical Efficiency

To best way to maximize optical efficiency is to use the largest pixel camera possible that matches Nyquist with the objective being used. This allows for sensitivity to be maximized by using the largest pixel area possible and resolution to be maximized by matching Nyquist sampling with the objective. This also avoids using extra, unnecessary lenses and losing light through reflection.

When choosing between cameras that satisfy these conditions, the next step is to match the camera field of view (via pixel count) to the maximum field of view possible with the microscope. This is usually done by choosing a camera with a 19 mm, 22 mm or 25 mm field of view depending on the microscope used.

Conclusion

Magnification couplers are often used to change the pixel size or field of view of a scientific camera, either to match Nyquist sampling or to match the field of view of the camera sensor to the microscope. However, there are trade-offs when using magnification couplers such as incorporating a lower quality lens into the system and having uneven illumination of the sensor due to using a sensor larger than the microscope was designed for.

Photometrics does not recommend using a magnification coupler if it can be avoided for low light imaging. When choosing a camera for a microscope system, it is far better to select a camera with the right pixel size that matches the objective used for the application and a field of view that matches the maximum output of the microscope.

For this reason, Photometrics offers cameras with a range of pixel sizes; 4.25 µm to match 40x, 6.5 µm to match 60x and 11 µm to match 100x, as well as cameras with multiple field of view options to optimally fit 19 mm, 22 mm and 25 mm microscopes.