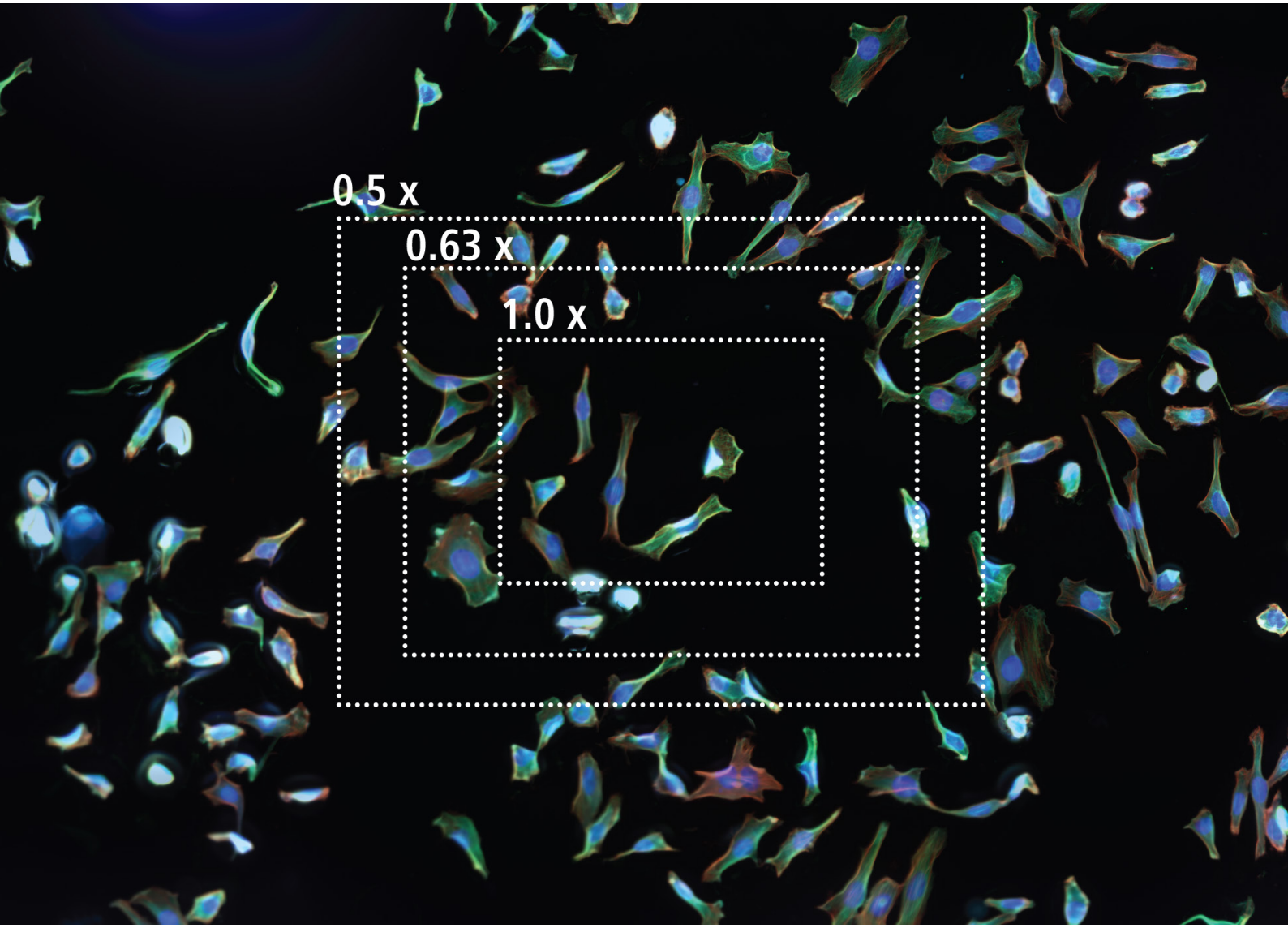


# ZEISS Axiocam Family

Collect More Photons By Choosing the
Right Camera Adapter for Your Application.

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## Collect More Photons By Choosing the Right Camera Adapter for Your Application.

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Different types of experiments may require different types of microscope cameras. This paper explains how choosing the right camera adapter for your widefield fluorescence microscope can increase sensitivity and enlarge the field of view at the same time.

**Abstract**

Biological imaging experiments require sufficient signal-to-noise ratio to reveal minute specimen details, allow robust image analysis and eventually lead to conclusive and reliable experimental results. The issue of signal and noise is especially important in case of dim and sensitive fluorescent specimens, where insight is often limited by the image noise rather than by the microscope's resolution. There are a few well-known methods to increase the signal-to-noise ratio, such as prolonging the camera exposure time or increasing the illumination intensity. Unfortunately, these methods also lead to slower experiments, and to increased specimen damage, photobleaching and phototoxicity. However, there is also a less known method that has recently become attractive due to the advances in the high-resolution microscope cameras, specifically the increasing sensor sizes and pixel counts. This method uses demagnifying camera adapters to match the field of view of the microscope with the size of the camera's image sensor. Demagnifying adapter allows the sensor to capture more of the light collected by the microscope's objective lens and thus increase the sensitivity of your microscope system. Additionally, the adapter enlarges the microscope's field of view and enables the user to capture a larger part of the specimen with a single image. Consequently, the adapter also dramatically accelerates tiling experiments.

**Introduction**

Microscopic imaging, as every other scientific experiment, is subject to different sources of noise. The most fundamental of them is the Shot noise, sometimes referred to as Poisson noise or photon noise. This noise is a consequence of the

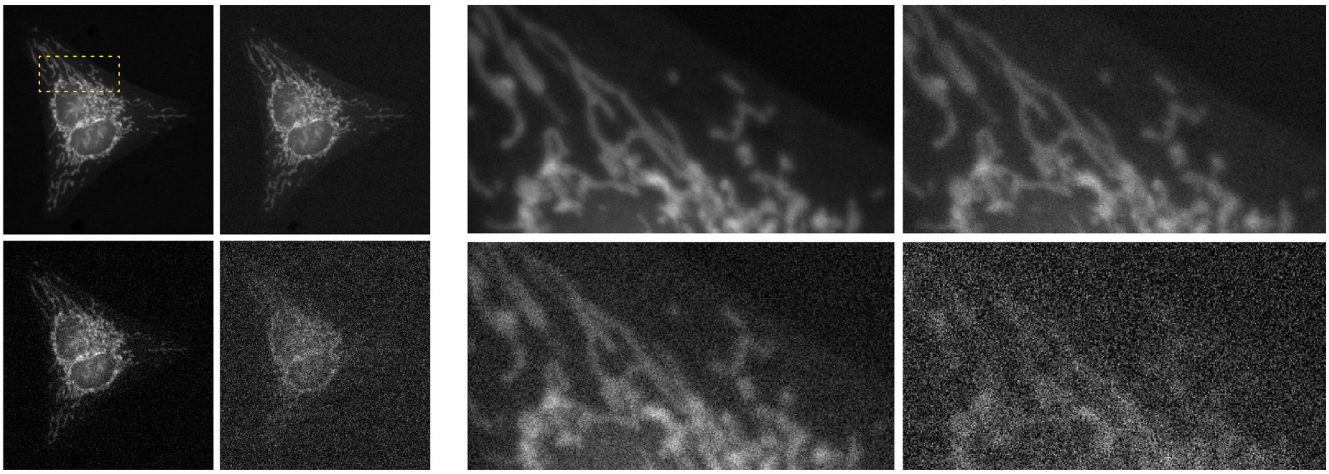

***Figure 1*** *Effect of the SNR on an image.* ***left*** *Fluorescence images of mitochondria acquired at four different imaging conditions with decreasing SNR. Compared to the first image, subsequent images have 2×, 4× and 8× lower SNR.* ***right*** *Magnified region of the images on the left indicated by the yellow rectangle. While all four images were recorded with identical microscope resolution, low SNR in the nosier images severely limits the ability to resolve small details.*

particle nature of the light; it cannot be circumvented and is ubiquitous in every image. Small amount of additional noise is generated by the microscope system in a form of sensor readout noise and dark current.

Noise is typically measured relative to the level of the correct signal in the images. This quantity is referred to as signal-to-noise ratio (SNR) and it determines whether the microscopic objects can be reliably detected and analyzed – or not. If the SNR is too low, the signal of the microscopic objects drowns in a sea of background noise, making analysis difficult or even impossible (see Figure 1).

The SNR is a ratio and can be improved in two different ways: either by decreasing the noise level or by increasing the level of the usable signal.

While the shot noise is ubiquitous and cannot be removed, camera noise can be diminished by employing high-end image sensors. Most notably, modern CMOS sensors with extremely low readout noise have seen a rapid development in the past decade, making them the tool-of-choice for widefield microscopy and the lifesciences research.

Image noise also increases strongly[1] with an increasing temperature. Most high-end microscope cameras, for example the Axiocam family from ZEISS, therefore further reduce the image noise by cooling the camera sensor.

Once the imaging noise has been reduced as low as practically achievable, SNR can only be further improved by increasing the usable signal, i.e. collecting more light per every pixel of the image. This requires the cameras to convert more than 70% of the absorbed photons into electric signal and consequently into an image. Back-illuminated sensors offer sensitivity of over 90%, however they are still rather expensive compared to the modest improvement in the SNR they deliver. Since some simpler and cheaper methods deliver a higher performance increase relative to the additional investment, they should be considered first, before more money is spent on a new microscope camera. One such efficient and affordable method, based on demagnifying camera adapters, is discussed in this paper.

**It's all about light**

The signal-to-noise ratio of an image can often be practically increased only by collecting more photons per every pixel of the image. There are several ways to do that. For example, one can extract more light from the specimen by increasing the illumination intensity or prolonging the exposure time (see Figure 2). Both expose the specimen to an increased illumination light dosage, which in turn leads to photodamage and photobleaching. Furthermore, long exposure time limits the maximum imaging speed. This is especially problematic in case of dynamic living specimens. But even with fixed and photostable specimens, long exposure times might make an acquisition of thick Z-stacks or large tile regions (mosaics) unpractically slow. Photobleaching and imaging speed often present a practical limit to how much light one can extract from the specimen.

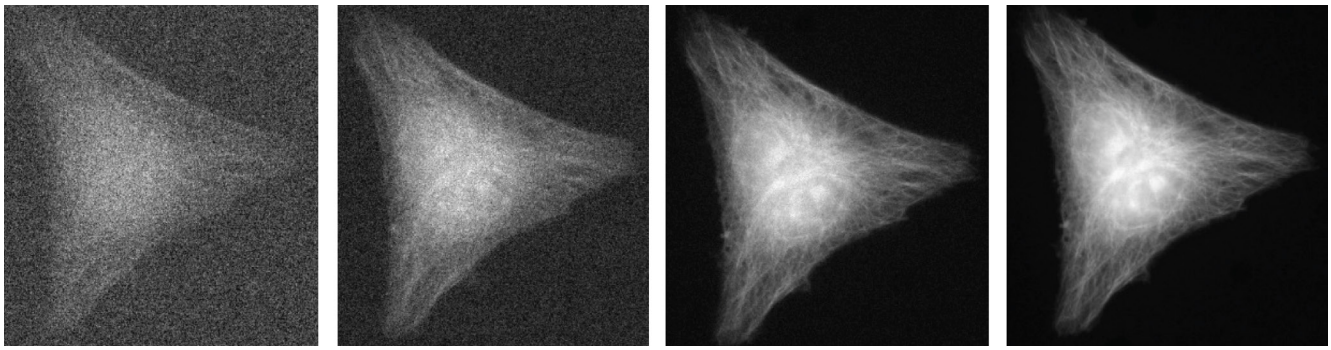

***Figure 2*** *A series of increasing exposure times recorded by a CMOS camera (ZEISS Axiocam 702 mono). Exposure times from left to right: 0.375 ms, 1.5 ms, 10 ms and 80 ms. Longer exposure times deliver substantially better image quality, but they take longer to record and expose the specimen to higher illumination light dosage. Specimen: LLC-Pk1 cells stained with anti-tubulin-Cy2.*

[1] Readout noise doubles with approximately every 6 °C increase of the sensor's temperature.

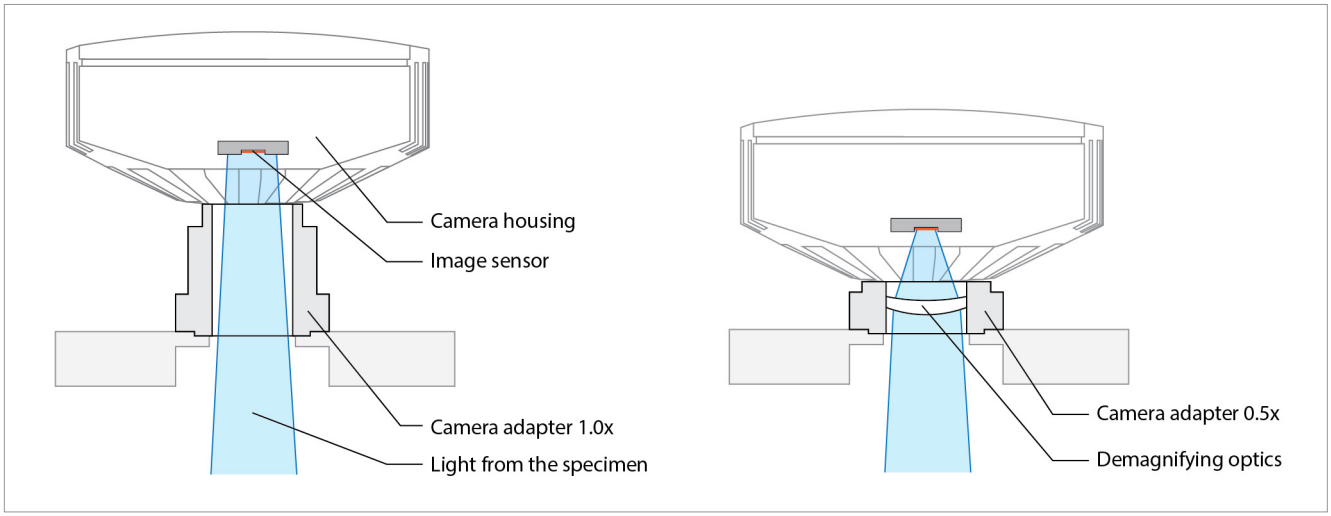


***Figure 3*** *Diagram of image formation with 1.0× (left) and 0.5× adapter (right). De-magnifying optics in the 0.5× camera adapter reduces the size of the image on the image sensor and thus increases the light flux collected by the pixels.*

**Pixel size matters**

The amount of light per pixel can also be increased by enlarging the image pixels. Large image pixels collect the light that would otherwise be split among many small pixels, thus increasing the signal per pixel, and consequently improving the SNR of the image.

Pixel binning is a method of combining adjacent sensor pixels, for example 2×2 or more, into larger image pixels. Combined pixels collect more signal than the individual small pixels and therefore deliver a better SNR. Binning is convenient as it does not require any changes to the microscope optics; it can be configured electronically, directly from the imaging software. As pixel binning reduces the total number of pixels in an image, it also reduces the data size, improves the data flow, reduces storage requirements and increases the maximum imaging speed.

The method is especially popular in combination with CCD cameras, where binning also reduces the camera readout noise. While this is not the case with most CMOS cameras on the market, some cameras of the latest CMOS generation, such as Axiocam 705, can reduce readout noise through pixel binning.

Unfortunately, sensor pixel binning sacrifices the full performance provided by the camera; binned image will always contain less image pixels than are provided by the sensor. Additionally, binning might reduce spatial resolution of the microscope system. Larger image pixels are less efficient in capturing minute specimen details, especially if the effective pixels are larger than the microscope's resolution. This is typically an issue with medium-magnification/high-NA objective lenses (e.g. 20×/0.8, 40×/1.3 or similar), while high-magnification objective lenses (63× and 100×) can typically tolerate moderate binning without any loss of resolution.

**Optical binning with camera adapters**

Pixel binning is not the only way to increase the effective pixel size and consequently the image SNR. Pixels can also be increased by reducing the microscope's magnification, for example by employing a low-magnification objective lens. However, low-magnification lenses typically have a lower numerical aperture (NA) and are therefore less efficient in collecting the light, which again negatively impacts the SNR. To improve the SNR, one would need to reduce the microscope's magnification while keeping the NA high! This can be realized using a demagnifying camera adapter.

A camera adapter is an optomechanical element that connects the microscope camera with the microscope's body. The simplest version of the adapter is just a hollow, threaded metal tube (see Figure 3). Such an adapter does not change the magnification of the microscope system. The magnification factor of this adapter is therefore 1x and the adapter is referred to as 1.0× adapter.

On the contrary, a 0.5× adapter includes a lens system that reduces the effective magnification of the microscope system twofold (see Figure 3). This in turn reduces the size of the image on the camera sensor. Effective size of the image pixels therefore increases twofold and the area of each image pixel increases fourfold. Each of the pixels now collects 4× more light on average, resulting in a significantly increased SNR. The effect of the 0.5× camera adapter is similar to 2×2 sensor binning.

However, as the camera adapter provides binning by purely optical means, it has several benefits over the sensor pixel binning. Most importantly, optical binning does not reduce the number of image pixels and the user can still enjoy the advantage of the full pixel count delivered by his camera. Since image pixels are now effectively larger, demagnifying adapter also allows the microscope to image a larger region of the specimen with a single shot (see Figure 4 and Figure 6). For example, a 0.5× camera adapter allows to record with a single image an area equivalent to four images recorded with a 1.0x adapter.
This enables the user to record a larger specimen without tiling. In case of even bigger specimens, optical binning with a 0.5× adapter reduces the number of required tiles, and thus the imaging time, for a factor of four! A demagnifying camera adapter, on top of increasing the sensitivity and the field-of-view, therefore also improves the imaging speed.

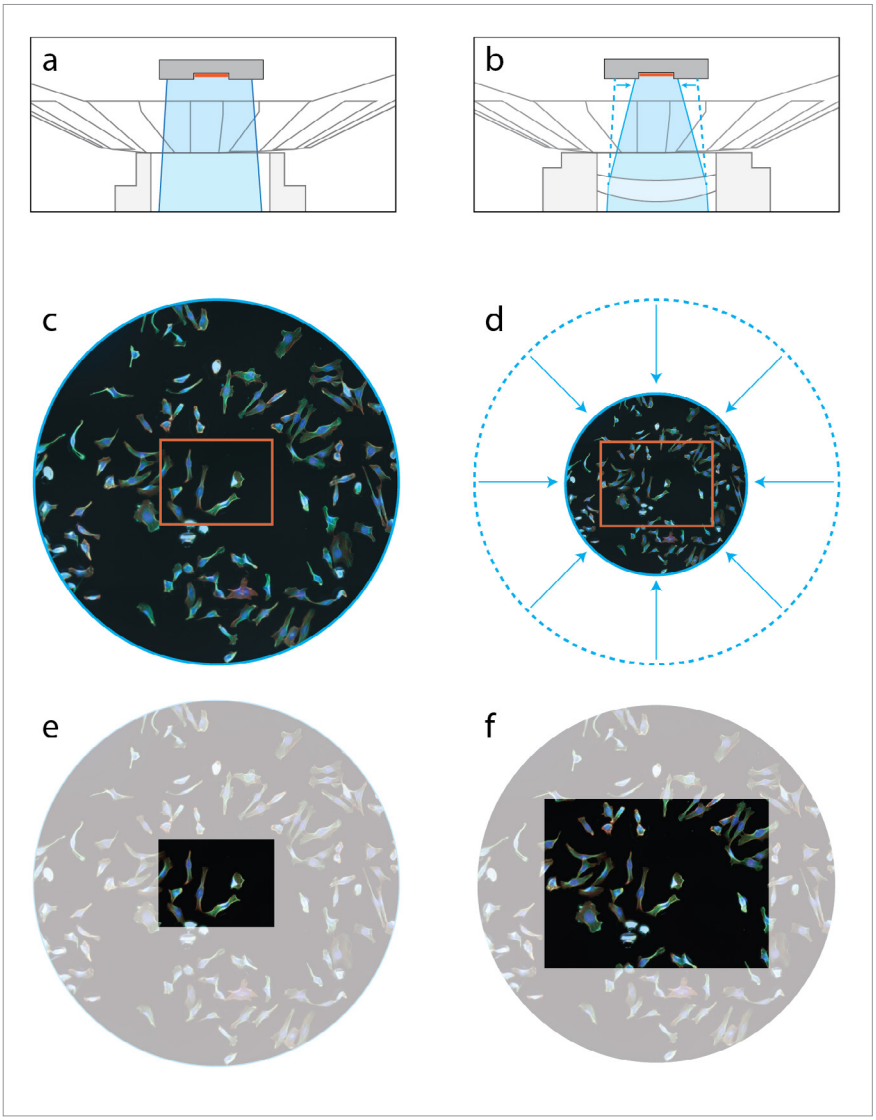


***Figure 4*** *Effect of the 1.0× (a) and 0.5× (b) camera adapter on the magnification (c–d) and on the field of view (e–f).* ***a)*** *1.0× camera adapter is optically inert and introduces no additional magnification* ***b)*** *0.5× camera adapter contains a de-magnifying lenses that reduce the image size and condense the light flux on the image sensor (red surface).* ***c)*** *A microscope's field of view (blue circle) is often considerably larger than the image sensor (red rectangle).* ***d)*** *By reducing the effective magnification, a 0.5× camera adapter reduces the image and condenses the incoming light flux onto the image sensor.* ***e)*** *As the sensor with the 1.0× adapter is considerably smaller than the field of view of the microscope, only a small fraction of the light collected by the microscope actually forms a digital image, while most of the light falls outside the sensor's active area and is irreversibly lost (gray area).* ***f)*** *A demagnifying camera adapter effectively increases the field-of-view of the image sensor and therefore allows it to record a bigger part of the specimen. Moreover, less of the collected light is now lost to detection (gray area).*

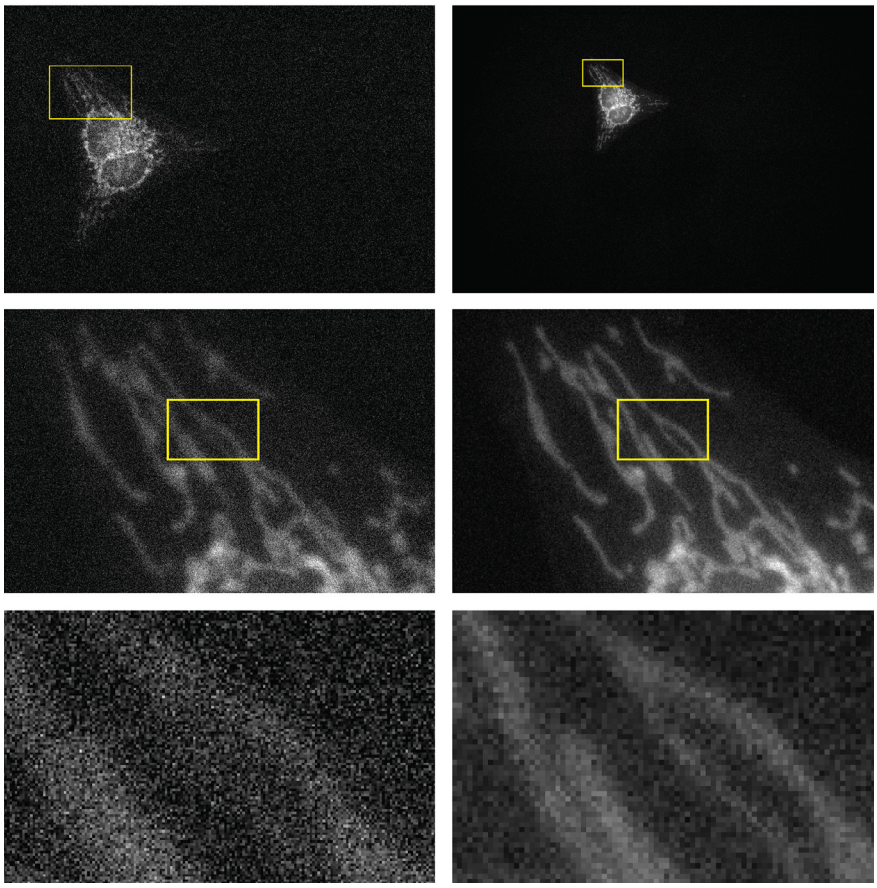

***Figure 5*** *Images of mitochondria acquired with identical imaging conditions and two different camera adapters.* ***left*** *ZEISS Axiocam 512 mono with 1.0× adapter, image size 1.3 mm × 0.9 mm,* ***right*** *ZEISS Axiocam 512 mono with with 0.5× camera adapter, image size 2.6 mm × 1.8 mm.* ***top row*** *Full sensor image. Note that 0.5× adapter records 4× larger area than the 1.0× adapter.* ***center and bottom row*** *Magnified regions indicated by the yellow rectangle.*

Like sensor pixel binning, optical pixel binning increases the effective image pixel size, which might lead to a reduced image resolution. Fortunately, this is typically not an issue with high-magnification lenses, where the resolution is especially vital. On the other hand, many medium-magnification / high-NA lenses can resolve details smaller than the effective pixel size. Optical binning can in such cases lead to some resolution loss. However, binning could even then be beneficial if an excellent sensitivity is more important than the image resolution, for example in case of extremely dim fluorescent specimens.

Demagnifying camera adapters introduce an additional lens system into the optical train. Untreated air-glass interfaces normally reflect around 4 % of the incoming light and introduction of an additional glass element can lead to a slight reduction in the overall sensitivity of the microscope system. However, this reduction pales in comparison with the improvement delivered by the optical binning: 0.63× adapter increases the average signal per pixel by a factor of 2.5× (i.e. 150 % improvement) and a 0.5× adapter by a factor of 4× (i.e. 300 % improvement). Moreover, glass interfaces of a camera adapter, as all other interfaces in a high-end microscope system, are treated with anti-reflection coatings, reducing their reflectivity to 1 % or less. In terms of sensitivity, the benefit of optical binning therefore vastly overweighs any downside of introducing an additional lens system into the microscope.

**It does not end here**

There is a number of different camera adapters for a variety of different purposes. Demagnifying adapters, which improve the SNR and increase the camera's field-of-view, are offered in different version with various magnification factors. Typical magnifications are 0.5×, 0.63× and 1.0×, which allow the user to match his camera's sensor and pixel size to his microscope's field-of-view and resolution, and most importantly, to his experiment (Figure 7, top row).

Same camera adapters additionally allow a precise adjustment of the camera's orientation or position. For example, an adjustable camera adapter (Figure 7, bottom-left) allows the camera to be precisely positioned along its X, Y and Z axes and rotated around its optical axis using a set of micrometer screws. Rotating camera adapter (Figure 7, bottom-right) provides a magnification factor of 0.63× and allows very precise rotation of the camera around its optical axis.

## Conclusion

Microscope camera adapters are an affordable and easy-to-use tool to adjust a widefield microscope setup and optimize resolution, sensitivity and field-of-view for different imaging applications. In this paper we have shown how a demagnifying camera adapter can help to improve signal-to-noise ratio (SNR) by optical binning, enlarge the camera's field-of-view and thus also increase imaging speed. As long as the camera pixels are not bigger than the microscope's resolution, the increased SNR and field-of-view come at little or no cost in form of a reduced image resolution. Camera adapters are an effective, flexible and affordable tool in the microscopist's toolbox.

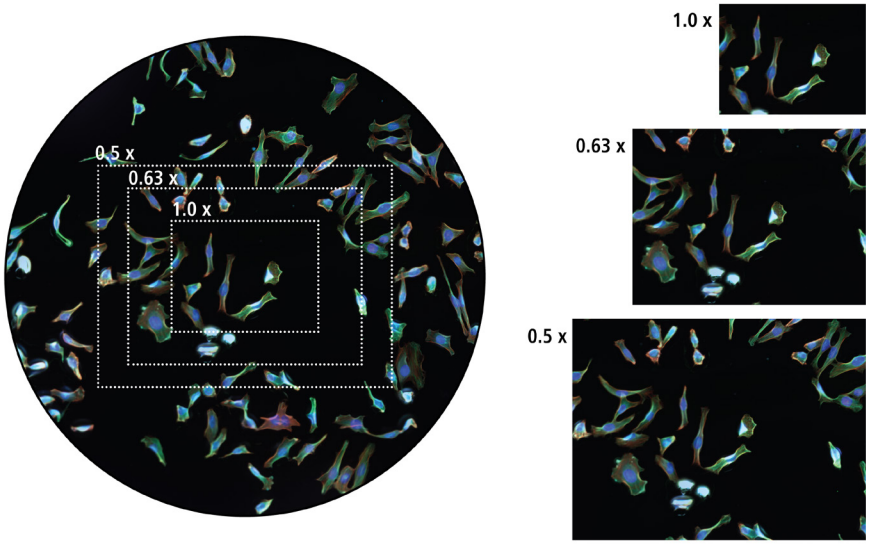


***Figure 6*** *Camera adapter and field of view.* ***left*** *Microscope's entire field of view (circle) compared to a camera sensor size in combination with three different camera adapters (rectangles).* ***right*** *Images of the same specimen acquired with three different camera adapters. Note that 0.5× adapter allows the camera to acquire 4× larger area than the 1.0× adapter.*


***Figure 7*** *ZEISS Camera adapters.* ***top row*** *camera adapters with magnifications 1.0×, 0.63× and 0.5×* ***bottom row*** *adjusting (x, y, z, rotation) camera adapter with magnification 1.0× (left), rotating fine-adjustable camera adapter with magnification 0.63× (right).*

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