ARE LARGE IMAGE SENSORS A PERFECT FIT FOR LARGE FIELD OF VIEW MICROSCOPE APPLICATIONS?

In the last years nearly all microscope manufacturers used image circles with 18 mm diameter to image their field of view to cameras connected to their microscopes. Only recently some microscope manufacturers increased their field of view to offer more information to their customers. However, this also resulted in larger image circles to be covered by cameras with their image sensors. Therefore, a run for cameras with appropriate image sensors started, and soon large image sensors were advertised, such that the questions arose: are larger image sensors a perfect fit for large field of view (FOV) microscope applications. Before the question can be answered, we should have a look to the relationship between resolution, magnification, spectral range and pixel size of image sensors.

Image Sensors and Cameras

Starting with the image sensor in a camera system, the modulation transfer function (MTF) describes the ability of the camera system to resolve fine structures. It is a variant of the optical transfer function[1] (OTF) which mathematically describes how the system handles the optical information, or contrast of the scene or the sample, and transfers it onto the image sensor and then into a digital format for processing via computer. The resolution ability depends on both the number and also the size of the pixel.

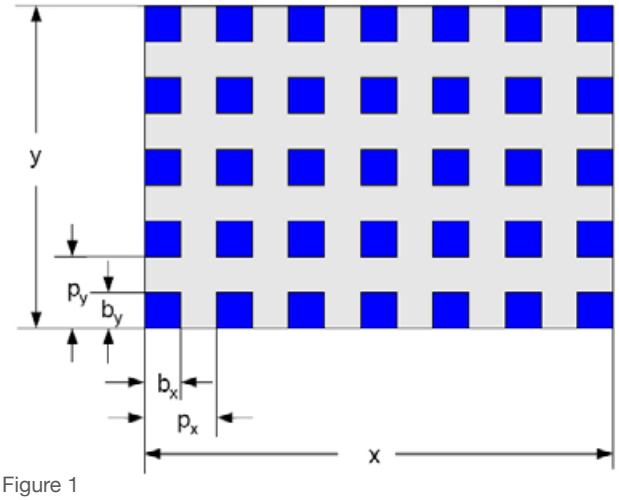

Figure 1
Illustration of a digital image sensor with characteristic geometrical parameters: x, y - horizontal, vertical dimensions, p_x, p_y - horizontal, vertical pixel pitch, b_x, b_y - horizontal, vertical pixel dimensions

The maximum spatial resolution is described as the ability to separate patterns of black and white lines and it is given in line pairs per millimeter [lp/mm]. The theoretical limit is given in the literature and defines the maximum resolution achieved if one black line is imaged on one pixel while one white line is imaged to the neighbor pixel. Assuming square pixels with $b_x = b_y = b$ and $p_x = p_y = p$ (see fig. 1 pixel schematic) then the maximum possible axial R_{axial} and diagonal $R_{diagonal}$ resolution ability is given by the pixel dimensions:

$$R_{axial} = \frac{1}{2 \cdot p} \qquad R_{diagonal} = \frac{1}{\sqrt{2} \cdot 2 \cdot p}$$

The following table depicts the maximum resolution ability values for image sensors with various pixel sizes.

Table 1: Maximum Theoretical MTF Data Of Selected Image Sensors

item	image sensor	pixel pitch [µm]	R_{axial} [lp/mm]	$R_{diagonal}$ [lp/mm]
GSENSE0505	sCMOS	2.5	200	141.4
ICX285AL	CCD	6.45	77.5	54.8
MT9M413	CMOS	12	41.7	29.5

The contrast which is transferred through the optical system consisting of camera and imaging optics is defined as contrast or modulation M, with the intensity I [count] or [DN[2]] in an image:

$$M = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

The modulation depends on the spatial frequencies, which means that M is a function of the resolution R: M = M(R). The quality of the imaging process is described by the modulation transfer function, MTF. Thus, both parameters, the resolution and the contrast, define the quality of an image. Increasing resolution improves the sharpness of an image while increasing contrast adds to the “brilliance”.

Keep in mind the above equation represents only the maximum possible MTF and requires the measuring pattern to be optimally positioned, if the line pair pattern is shifted by half a pixel, nothing could be seen, as shown in figure 3. This is illustrated by three different use cases. Let us assume the structure to be resolved is given by these black and white line pairs. Figure 2 shows what happens if the pixel of an image sensor has the same pitch like the width of one line pair.

[1] https://en.wikipedia.org/wiki/Optical_transfer_function
[2] DN=digital number, like count

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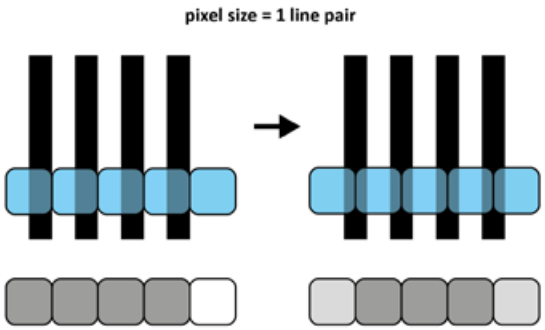

Figure 2
Illustration of a line pair structured optimally imaged to one row of pixels which have a pitch, similar to the width of the line pair. Left: the structure is imaged in a way that each pixel "sees" a line pair. The pixel row below shows the resulting measured light signal of the corresponding pixel. Right: the structure is shifted compared to the pixel row and the pixel row below shows the resulting measured light signal of the corresponding pixel above.

In this case the structure could never be resolved, even if it is moved, the resulting light information (see fig.2 pixel rows below) is not able to give enough information about the structure. If now the theoretical maximum MTF is assumed, we come to the illustration in figure 3.

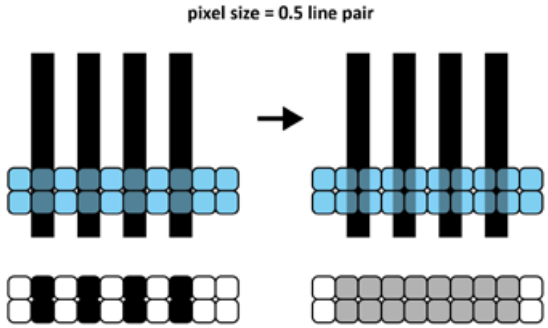

Figure 3
Illustration of a line pair structured optimally imaged to one row of pixel which have a pitch, similar to half the width of the line pair. Left: the structure is imaged in a way that each pixel "sees" either a black or a white line. The pixel row below shows the resulting measured light signal of the corresponding pixel. Right: the structure is shifted compared to the pixel row, now the pixel always registers half white and half black, with the pixel row below showing the resulting measured light signal of the corresponding pixel above.

Only in the case that the structure is imaged in a way that each pixel "sees" either black or white, the maximum MTF can be reached. In case the structure is shifted by half a pixel all the information is gone, and nothing can be resolved. Therefore, the maximum theoretical MTF value is a nice start in case the user has to estimate some starting values for the imaging optics used with a camera system. A more practical case and condition is shown in figure 4.

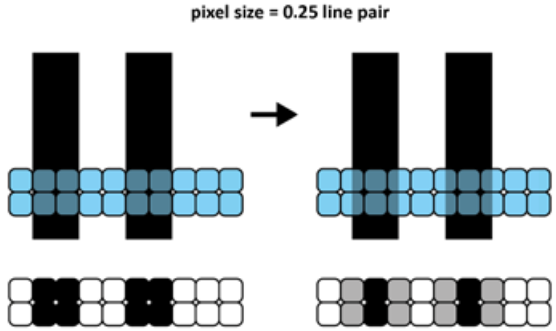

Figure 4
Illustration of a line pair structured optimally imaged to one row of pixel which have a pitch, similar to the quarter of the width of the line pair. Left: the structure is fully resolved by the pixel. The pixel row below shows the resulting measured light signal of the corresponding pixel. Right: the structure is shifted compared to the pixel row, still the structure can be resolved with a little bit less sharpness compared to the left image. Again the pixel row below shows the resulting measured light signal of the corresponding pixel above.

Now the pixel pitch corresponds to the quarter of the line pair width (see fig. 4). In this case the structure can always be resolved with more or less sharpness, even if the structure is not optimally positioned on the pixel row. Therefore it is important to match for each imaging application the required imaging optics and pixel size of the image sensor to the structures which have to be resolved.

Imaging Optics – Microscope Objective – Rayleigh Criterion

Defining resolution becomes more complex in microscopy, since there are typically no MTF charts available and multiple lenses or objectives are involved in the image reaching the camera. But there are characteristic parameters and physical relationships that help to determine the best possible resolution.

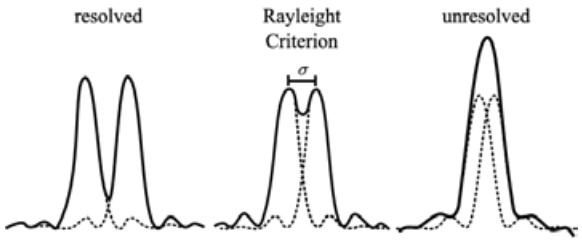

Figure 5
Schematic to show the Rayleigh criterion, if two point signals (dotted curves) which can be resolved (left graph, solid line is the impression of the optical system) approach each other, they reach a minimum distance, in which they still can be resolved (middle graph, Rayleigh criterion, solid line is the impression of the optical system). If the distance is further decreased, both signals cannot be resolved and they are perceived as one signal (right graph, unresolved, solid line is the impression of the optical system)[4].

[3] https://en.wikipedia.org/wiki/Angular_resolution

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In microscopy, a function termed the "Rayleigh-Criterion"[3] (see fig. 5) describes the minimum distance between two objects and the ability to separate them as a function of the numerical aperture (NA) of the objective and the spectral wavelength of the light that should be detected. In a simplified way it is given by:

$$d = \frac{0.61 \cdot \lambda}{NA}$$

(with distance d = width of line pair, λ wavelength and numerical aperture NA of the objective). The major parameters of each microscope objective are the magnification Mag_{obj} and the numerical aperture NA.

Table 2: Parameters of microscope objectives

objective	Mag_{obj}	NA
CFI plan apochromat lambda 4X	x 4	0.2
CFI plan apochromat lambda 40XC	x 40	0.95
CFI plan apochromat lambda 60X Oil	x 60	1.4
objective fluar 5x/0.25	x5	0.25
objective clr plan-neofluar 20x/1.0	x 20	1.0
objective i plan-apochromat 63x/1.4 Oil	x 63	1.4

The total magnification of the object on the microscope stage is defined as magnification of the microscope objective multiplied by the magnification of the so called TV- or camera-adapter, which consists of a lens with a c-mount and a mount to the microscope which serves as "ocular" for the camera. Therefore, the total magnification Mag to be considered is:

$$Mag = Mag_{obj} \cdot Mag_{CamAd}$$

From the chapter before it was concluded that the optimum pixel size or pitch should be equal to a quarter of the line pair width which corresponds to the minimum resolvable distance.

$$pixel\ pitch_{opt} = 0.25 \cdot width\ of\ line\ pair = 0.25 \cdot d$$

If now the Rayleigh criterion is inserted for d and the total magnification of the optical path in the microscope is included, the pixel $pitch_{opt}$ can be expressed as follows:

$$pixel\ pitch_{opt} = \left(\frac{0.25 \cdot 0.61 \cdot \lambda}{NA}\right) \times \left(Mag_{obj} \cdot Mag_{CamAd}\right)$$

To illustrate the consequences, let's take an example: an objective with Mag_{obj} = 60 and NA = 1.4, the camera adapter has a Mag_{CamAd} = 1.0 and blue-green fluorescence with λ = 514 nm should be observed:

$$pixel\ pitch_{opt} = \left(\frac{0.25 \cdot 0.61 \cdot 0.514}{1.4}\right) \times (60.0 \cdot 1.0)[\mu m] = 3.4\ [\mu m]$$

This means a relatively small pixel pitch. If an objective would be used with a smaller NA, for example like NA = 0.93, the resulting optimum pixel pitch would be 5.2 µm. The result is similar sensitive towards the correct chosen magnification of the camera adapter, if it is for example smaller like Mag_{CamAd} = 0.5, the optimum pixel pitch would be 1.7 µm. As well if we just apply the theoretical limit of 0.5 times the width of the line pair, it would result in 6.8 µm.

Or it is possible to take an existing pixel pitch, which is popular for emCCD and some new sCMOS image sensors like 11 µm, and ask what the optimum magnification Mag_{obj} of an objective is, if we assume an NA around 1.

$$Mag_{obj\ opt} = \frac{pixel\ pitch \cdot NA}{0.25 \cdot 0.61 \cdot \lambda} \times \frac{1}{Mag_{CamAd}}$$

With a pixel pitch = 11 µm, NA = 1.0, Mag_{CamAd} = 0.7 and the same wavelength like before λ = 514 nm we would get:

$$Mag_{\mathrm{obj}\ opt} = \frac{11 \cdot 1.0}{0.25 \cdot 0.61 \cdot 0.514} \times \frac{1}{0.7} = 200.5$$

This is well above the largest common magnifications of 150 for microscope objectives. The value could be optimized by a larger magnification of the camera adapter, but this would reduce the imaged area compared to the area as seen through the oculars.

For the different combinations of microscope objectives with their magnification and numerical aperture plus the additional possible magnification at the camera adapter or port there is a very nice application software available designed by Dr. Andrew Barlow[5] which enables the input of all the parameters like pixel size, microscope magnification and camera adapter magnification.

As output, a variety of derived parameters is interactively shown, for example by selecting the wavelength the "Nyquist Status" is given, which means, if for the given combination the probe would be sampled properly (Nyquist Status = OK or oversampled) or if it the resolution is not good enough (Nyquist Status = undersampled).

[4] Schematic taken from: https://www.researchgate.net/publication/298070739_Tomographic_reconstruction_of_combined_tilt-_and_focal_series_in_scanning_transmission_electron_microscopy

[5] https://appadvice.com/app/microscope-resolution/1229786939

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Figure 6
Screen shot of the "Resolution" App designed by Dr. Andrew Barlow.

The quantity of information is related to the area of the sample which can be seen and imaged. As a consequence, the larger the image circle of the microscope becomes, the more information of the sample can be accessed at one glance or in one image.

Field of View and Image Circle

As mentioned at the beginning, some microscope manufacturers released and advertised microscopes with a larger field of view resulting in larger image circles. The circle diameter was increased from the standard 18 mm to 25 mm. Therefore, more information can be obtained in one image which maybe enables the use of microscope objectives with less magnification.

Assuming a large biological sample at medium microscope magnification, 10x or 20x, then figure 7 shows a variety of different image circles which ranges from 32 mm image diameter down to 18 mm. The latter has been the typical image circle diameter of microscopes with camera ports. Usually, the area, which can be seen in the oculars, is a bit larger. For example if the microscope has an image circle of 18 mm, the area in the oculars is about 22 mm (see figure 7 [c] & [d]).

Figure 8 shows the different images in a microscope camera set-up. Starting from the left with an area of 22 mm in the oculars, a part of that FOV is imaged to the camera port (see fig. 8 middle) and out of that a sCMOS camera with 4.2 MPixel and 6.5 µm pitch detects the remaining image (see fig. 8 right). This area size ratio between the different images stays the same independently of the magnification of the microscope objective.
Without touching the magnification of the camera port, which was assumed to be x1.0 in the example, figure 8 shows that the combination of microscope and camera was appropriate. The image sensor nearly fills the image circle such that the amount of information lost is at minimum.

This illustrates that the combination of microscope and camera can be tuned and optimized to measure the best possible information in terms of quality and quantity. The quality is accessed by the signal-to-noise and a proper resolution, which should prevent any kind of under-sampling.
Recently some microscope manufacturers have released microscopes with a larger FOV and image circle of 25 mm (see figure 7 [b]). This also supports scanning processes like in digital pathology, where whole samples have to be measured patch by patch. While the presented image sensor (see fig.8) with 2048 x 2048 pixels and a 6.5 µm pixel pitch fits perfectly for the 18 mm image circle, they are too small for the new one as the comparison in figure 9 illustrates.

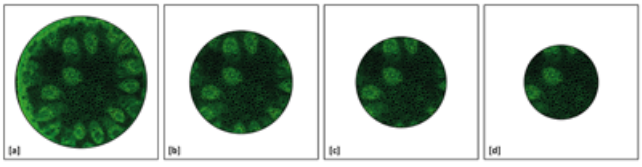

Figure 7
Comparison of different image circle diameters from left to right: [a] 32 mm, [b] 25 mm, [c] 22 mm and [d] 18 mm with the same microscope and camera adapter magnification.

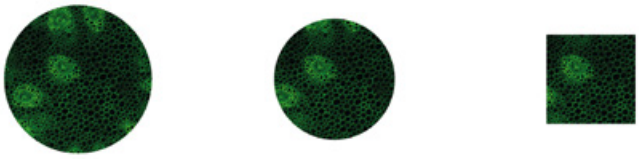

Figure 8
Images in a microscope camera set-up, from left to right: the image seen through the oculars - the image at the camera port with magnification x 1.0 - the image recorded by a camera with 2048 x 2048 pixels and a 6.5 µm x 6.5 µm pixel pitch.

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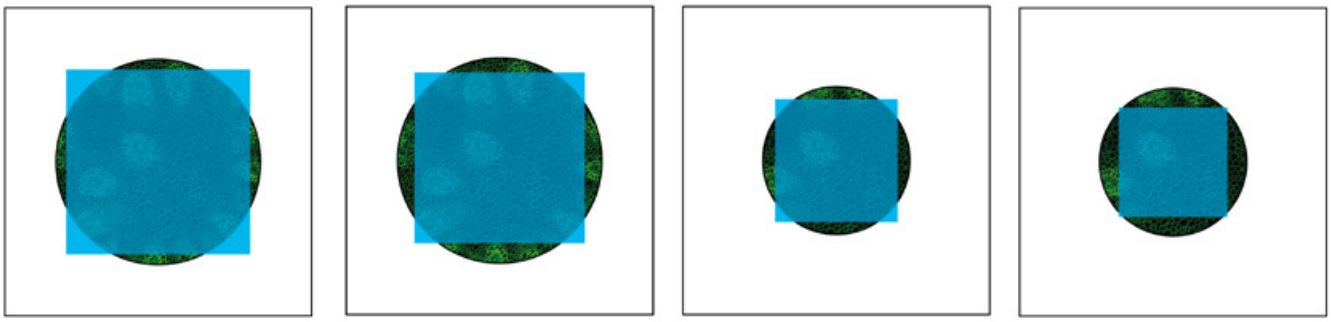

Figure 9
Image circles of a microscope with different sCMOS image sensors on top to show how good they fit to the image circles. From left to right: 25 mm image circle and an image sensor with 2048 x 2048 pixels and 11 µm pixel pitch (e.g. GSENSE400BSI), 25 mm image circle an image sensor with 3200 x 3200 pixels and 6.5 µm pixel pitch, 18 mm image circle and the same image sensor with 2304 x 2304 pixels and 6.5 µm pixel pitch and 18 mm image circle and an image sensor with 2048 x 2048 pixels and 6.5 µm pixel pitch (e.g. CIS2020A or GSENSE2020BSI).

From figure 9 it looks like the best fit in terms of size are the first and the last combination. Both combinations in the middle are a little too large with respect to the image sensor, but fill out the image circle. Because of that for example the image sensor GSENSE400BSI and the cameras, in which the image sensor is integrated, are assumed to be a perfect fit for the microscopes with the large 25 mm FOV and image circle. At a first glance, if the geometrical conditions are taken (see figure 9 left), it looks pretty good. Let's check, if the quality requirements are met. For that purpose the following table shows some results obtained with the software designed (see figure 6) by Dr. Andrew Barlow.

Table 3[6]: Comparison of Microscope objectives, camera adapter magnification, pixel pitch and spectral response

Microscope Objektive	NA	Medium	Camera Adapter Magnification		Pixel Pitch [µm]	Wavelength [nm]				
						405.0	488.0	525.0	620.0	800.0
x20	0.75	air	x1		2.50	oversampled	oversampled	oversampled	oversampled	oversampled
					4.65	undersampled	undersampled	undersampled	OK	oversampled
					6.50	undersampled	undersampled	undersampled	undersampled	undersampled
					11.00	undersampled	undersampled	undersampled	undersampled	undersampled
					24.00	undersampled	undersampled	undersampled	undersampled	undersampled
			x2		2.50	oversampled	oversampled	oversampled	oversampled	oversampled
					4.65	oversampled	oversampled	oversampled	oversampled	oversampled
					6.50	undersampled	OK	oversampled	oversampled	oversampled
					11.00	undersampled	undersampled	undersampled	undersampled	OK
					24.00	undersampled	undersampled	undersampled	undersampled	undersampled
x40	0.95	air	x1		2.50	oversampled	oversampled	oversampled	oversampled	oversampled
					4.65	OK	oversampled	oversampled	oversampled	oversampled
					6.50	undersampled	undersampled	undersampled	OK	oversampled
					11.00	undersampled	undersampled	undersampled	undersampled	undersampled
					24.00	undersampled	undersampled	undersampled	undersampled	undersampled
			x2		2.50	oversampled	oversampled	oversampled	oversampled	oversampled
					4.65	oversampled	oversampled	oversampled	oversampled	oversampled
					6.50	oversampled	oversampled	oversampled	oversampled	oversampled
					11.00	undersampled	OK	OK	oversampled	oversampled
					24.00	undersampled	undersampled	undersampled	undersampled	undersampled
x60	1.40	oil	x1		2.50	oversampled	oversampled	oversampled	oversampled	oversampled
					4.65	OK	oversampled	oversampled	oversampled	oversampled
					6.50	undersampled	undersampled	undersampled	OK	oversampled
					11.00	undersampled	undersampled	undersampled	undersampled	undersampled
					24.00	undersampled	undersampled	undersampled	undersampled	undersampled
			x2		2.50	oversampled	oversampled	oversampled	oversampled	oversampled
					4.65	oversampled	oversampled	oversampled	oversampled	oversampled
					6.50	oversampled	oversampled	oversampled	oversampled	oversampled
					11.00	undersampled	OK	OK	oversampled	oversampled
					24.00	undersampled	undersampled	undersampled	undersampled	undersampled
x100	1.40	oil	x1		2.50	oversampled	oversampled	oversampled	oversampled	oversampled
					4.65	oversampled	oversampled	oversampled	oversampled	oversampled
					6.50	oversampled	oversampled	oversampled	oversampled	oversampled
					11.00	undersampled	undersampled	undersampled	OK	oversampled
					24.00	undersampled	undersampled	undersampled	undersampled	undersampled
			x2		2.50	oversampled	oversampled	oversampled	oversampled	oversampled
					4.65	oversampled	oversampled	oversampled	oversampled	oversampled
					6.50	oversampled	oversampled	oversampled	oversampled	oversampled
					11.00	oversampled	oversampled	oversampled	oversampled	oversampled
					24.00	undersampled	undersampled	undersampled	OK	oversampled

[6] (the results of the readout at the various wavelengths show the Nyquist Status of the combination of microscope objective + camera adapter magnification + pixel pitch as function of the applied wavelength. For faster access, the results the OK and oversampled results have been color-filled with green while the results where the resolution was not good enough (undersampled) were color-filled with red

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The selected microscope objectives are a reasonable standard range as well as the range of different pixel sizes from 2.5 µm – 4.65 µm – 6.5 µm – 11.0 µm and 24 µm (for which image sensors and scientific cameras exist). It is interesting to note that for a 20x objective the 18 mm image circle good fit of the 6.5 µm pitch image sensors (e.g. CIS2020A, GSENSE2020e, GSENSE2020BSI) only works properly with an additional camera adapter magnification of x2. This means for a proper sampling the FOV has to be reduced. Only from x40 to x100 it is possible to use this combination with a camera adapter magnification of x1 with full FOV. But let's go back to the question if the new 25 mm image circle and the 11 µm pitch image sensor (e.g. GSENSE400BSI) are a good fit. In most objective combinations (except x100 and larger) it is required to always use a camera adapter magnification of x2 to have a possibility for a proper sampling, only with x100 for wavelengths larger than 620 nm it is possible to use this combination with proper Rayleigh resolution. What does that mean for an application? Let's have a look to some use case examples.

Use Case 1:
Camera adapter magnification x1 & 11 µm pitch - 2048 x 2048 pixel image sensor

In this case the larger image sensor area successfully covers the whole 25 image circle at the same number of pixels like the 18 mm image circle cameras with smaller pitch.
In figure 10 the 2048 x 2048 pixel images are shown, which are on the left side achieved by a 11 µm pitch and 2048 x 2048 pixel image sensor and on the right side by a 6.5 µm pitch image sensor with the same amount of pixels. Obviously, since the covered sample area of the larger sensor is imaged by the same amount of pixels, the left image has less detail resolution. In this case the larger pixel image sensor will collect more photons resulting in a better signal-to-noise ration but less resolution and less details in the image.

Use Case 2:
Camera adapter magnification x2 & 11 µm pitch - 2048 x 2048 pixel image sensor

From the table it can be seen that with a camera adapter magnification of x2 the pixel pitch of 11 µm can be applied with high magnification objectives. Figure 11 shows the difference in the images between the two camera adapter magnifications.

With the additional magnification at the camera port, the imaged field of view for the camera is reduced. Now it is possible to compare the image results between the 25 mm image circle application with the large pixel sensor and the 18 mm image circle application and the smaller pixel sensor again.

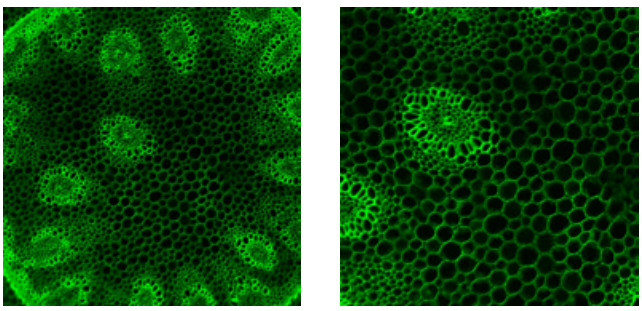

Figure 10
Comparison of the same 2048 x 2048 pixel images from the microscope sample image with a x1 camera adapter magnification: Left: 25 mm image circle and a 11 µm pitch image sensor with 2048 x 2048 pixel resolution – right: 18 mm image circle and a 6.5 µm pitch image sensor with 2048 x 2048 pixel resolution.

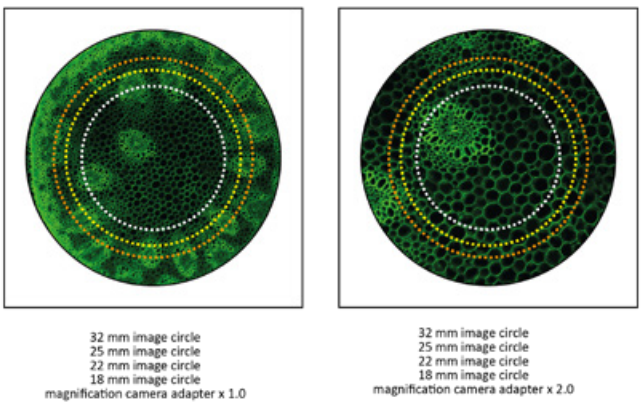

Figure 11
Comparison of different image circles and the resulting image if the magnification of the camera adapter is changed: left – camera adapter magnification = x1 – right: camera adapter magnification = x2.

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The results for the two cases are shown in figure 12. The left image shows the resulting image if in a 25 mm image circle a large pixel pitch 11 µm image sensor with 2048 x 2048 pixel is used with a camera adapter magnification of x2 compared to the older situation with an 18 mm image circle and a smaller pixel pitch 6.5 µm image sensor (see fig. 12 middle). If the smaller image is zoomed up to the same size like the larger image, it is clear that both images nearly show the same image details and area. Since both images get the same amount of light (number of photons) the signal-to-noise ratio is the same.

Are Large Image Sensors a perfect fit for Large Field of View Microscope Applications?

Well, it depends, how a large image sensor is achieved. If the same amount of pixels is used but having with a larger pixel area or pitch, then the advantage of the large field of view cannot be exploited. If you look to Use Case 1, where a camera adapter magnification of x1 is chosen, the resulting image is simply under-sampled. In case a camera adapter magnification of x2 is used, the advantage of more information due to the large field of view is gone, and there is no difference to the former 18 mm image circle with a 6.5 µm pitch image sensor and 2048 x 2048 pixel resolution, except the higher dark current and high readout noise due to the larger pixels will result in a lower signal-to-noise ratio. If a “large” image sensor is made of smaller and more pixels, the more information of the larger field of view could be efficiently used on cost of a smaller amount of light per pixel. However, if there is enough light available it would be a real improvement and “perfect” fit, since more details can be resolved. The useful application of large pixel image sensors is restricted to large magnification and long wavelength microscope applications.

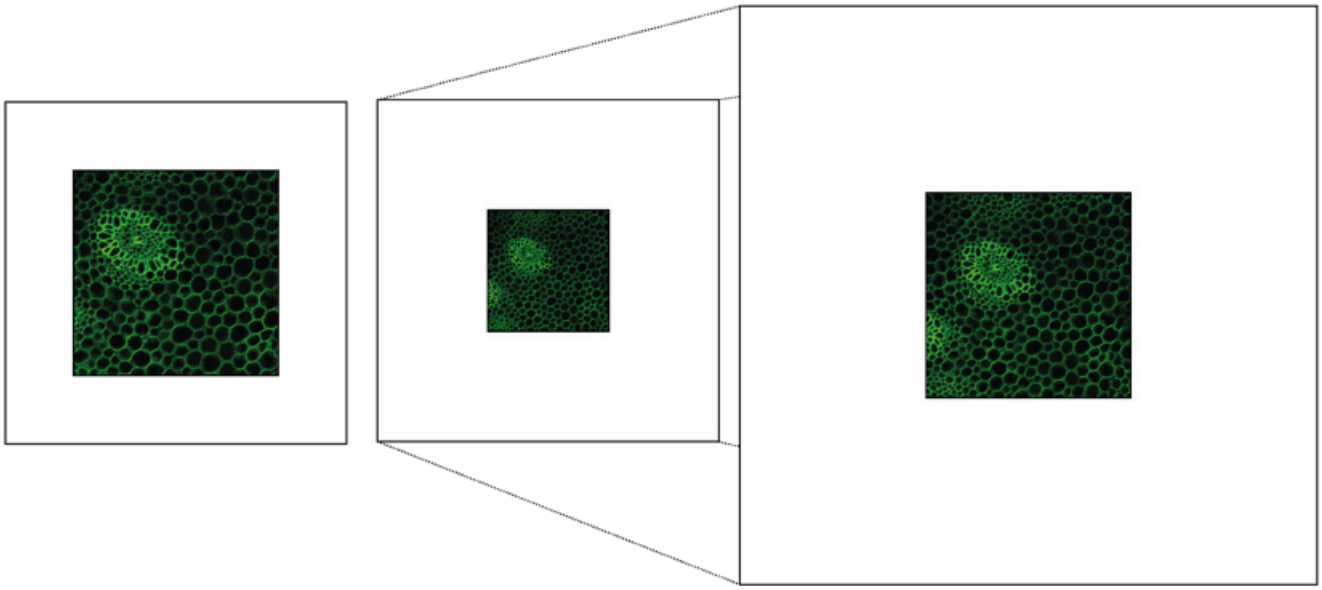

Figure 12
Comparison of different image circles, different pixel pitch and different magnification of the camera adapter: left – image circle 25 mm, pixel pitch 11 µm (2048 x 2048 pixel) and camera adapter magnification = x2 – middle: image circle 18 mm, pixel pitch 6.5 µm (2048 x 2048 pixel) and camera adapter magnification = x1, right: same like middle only zoomed to the same image size like left.

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