

Choosing a Digital Camera for your Microscope



When selecting a digital camera, remember to consider these four things to ensure a successful pairing:

- » Compatibility
- » Coupler or Adapter
- » Pixel Pitch
- » Resolution



Camera Compatibility With the Microscope

Make sure the camera sensor format is compatible with the microscope objectives.

Microscope objectives are comprised of a multi-lens assembly that focuses light waves from the specimen onto the sensor to form an image. Objectives allow microscopes to provide magnified, real images. The most common objectives range in magnification from 4X, 10X, 40X to 100X range in magnification from 4X, 10X, 40X to 100X.

Most microscope objectives are circular components whilst camera sensors are typically rectangular in shape. Therefore, if the camera sensor size is too big, the objectives will focus light on only a small circular part of the camera sensor resulting in vignetting. Vignetting is the reproduction of the optical edge of the microscope's light path which manifests as dark edges on your image.

To avoid vignetting and maximize field of view, the entire camera sensor needs to be illuminated- meaning the camera sensor format must be equal or smaller than the microscope objectives.

What Coupler or Adapter Should You Choose?

Couplers are used to maximize the camera's FOV without compromising image quality.

The golden rule of selecting a coupler is that the higher the magnification on the coupler, the lower the FOV acquired. On the other hand, the lower the magnification on the coupler, the higher the FOV. If the magnification is too low, it may lead to vignetting and if the magnification is too high, image loss may occur.

Below is a quick guide on which coupler magnification to choose for different sensor chips.

Sensor Format	Coupler Magnification
1/3"	0.35X
1/2"	0.5X
2/3"	0.66X
1"	1X

Pixel Pitch/Size

Pixel size is one of the most important factors when it comes to selecting a digital camera for your application. To determine the right pixel size:

- Determine the resolution of your microscope
- Calculate your sampling frequency
- Calculate required pixel size

a. Determine the resolution of your microscope:

To determine the resolution of your microscope, Ernst Abbe's diffraction formula for lateral (i.e. XY) resolution can be used as follows: $d = \lambda / 2 \text{ NA}$ where:

- λ = wavelength of light used to image a specimen
- d = minimum resolvable distance between 2 objects
- NA = Numerical Aperture of your objective

For example, using white light at λ 550nm, at 4X/0.2 NA: $d = 550\text{nm} / (2 \times 0.20) = 1375 \text{ nm}$

b. Calculate sampling frequency f_s using Nyquist sampling theorem, according to the formula below. *The Nyquist Theorem states that to adequately reproduce a signal it should be periodically sampled at a rate that is 2X the highest frequency you wish to record.*

Sampling frequency $f_s = d/2$

From above example: $1375/2 = 687.5$

c. Calculate your required camera pixel size = sampling frequency f_s * magnification *The caveat here is that there are several sources of magnification: the objectives, the tube lens and the camera mount/coupler.* To get total magnification, multiply all the sources of magnification.

Therefore: **The required pixel size** = f_s (687.5nm) X Objective magnification (4) X Tube lens magnification (1) X camera mount magnification (1) = $2750\text{nm}/1000 = 2.75\mu\text{m}$

Objective/ Numerical aperture	Resolution Limit (μm)	Required pixel size (μm)
4X (0.20)	1.5	2.9
10X (0.45)	0.64	3.2
20X (0.75)	0.39	3.9
40X (1.30)	0.22	4.5

Resolution: How Many Megapixels Should You Get?

Resolution refers to the number of pixels on a sensor for instance; 2 MP- 2 million pixels, 4 MP - 4 million pixels, etc.

Generally, image sensors with higher resolution will have smaller pixels. However, microscope optical limitations mean a higher number of pixels or smaller pixel pitch does not always provide higher resolution. On the other hand, larger pixels collect more light, which plays a role in camera sensitivity. Therefore a trade off between resolution and sensitivity is required.

The key to achieving better resolution is to select the proper pixel pitch in relation to the numerical aperture (NA), the total magnification of the optical system, and the sample's frequency as illustrated above. If the choice is between two identical sensor sizes, then err on the side of smaller pixels.

Pixelink vision compliant cameras come with free Pixelink Image Capture software and are compatible with third party image acquisition software like WinROOF2018, LabVIEW, NI MAX, MATLAB, and Halcon.