A Perfect Match: Choosing the Right Digital Camera for your Microscopy Application

Capturing and documenting a true-to-life representation of your sample is an important aspect of microscopy, achieved using the digital camera. Digital cameras specialized for microscopy come in all shapes and sizes, and selecting the best camera for your system can be a daunting task. It can be of help to consider four main features that essentially dictate camera performance: color reproduction, sensitivity, live image quality and resolution. Being aware of what these parameters really mean for your chosen application will guide you to a camera to complete your microscope system and truly enhance your experience.

Color Reproduction

Color is important for Bright-field Imaging Stereomicroscopy. Color cameras are able to measure each pixel hue by having a RGB (Red, Green, Blue) filter in front of the sensor. This filter reduces the amount of captured light, making it difficult for color cameras to properly image low light samples, for example in fluorescent applications. These cameras are therefore highly specialized for color imaging, essential when considering the importance of color, as different hues and intensities allow different structures in material or biological samples to be distinguished. If the color is imposed on the sample by specific staining, it can lead to diagnostic indications, and it is then vital to reproduce the exact same color profile on the monitor as one would view through the oculars. This has remained a challenge for some time, until recent developments. A new technology known as color profiling maximizes the accuracy of color mixing.

Cameras containing this kind of technology will ensure a faithful color reproduction, to the extent allowed by the connected monitor. Some models now even feature native support of the Adobe RGB color space, which allows the camera to interpret a greater range of colors and display them on compatible professional-grade monitors.

Camera Chip Technology - CCD or CMOS?

Digital imaging for wide-field microscopy is achieved using imaging chips formed
from a number of densely packed light-sensitive pixels. The two primary chip technologies are the charge coupled device (CCD) and the complementary metal-oxide semiconductor (CMOS).

CCDs tend to be more expensive than the CMOS but generally introduce less "noise" in the image.

In contrast, CMOS chips can usually pack more megapixels at a lower cost, while generally sacrificing sensitivity.

Both technologies have also evolved into specialized cameras for research applications. EMCCD chips are now available to greatly enhance the camera sensitivity. Furthermore, new sCMOS chips achieve both a high pixel count and faster speeds than CCD chips, while having the same low level of noise.
Sensitivity

Sensitivity is important for fluorescence and low-light imaging. High sensitivity is the number one priority for low light and fluorescence applications. Color is not required for these techniques, meaning that superior sensitivity can be achieved by first removing the RGB color filters covering the chip. Furthermore, a dedicated monochrome chip will have a higher sensitivity by simply having larger pixels, enabling the capture of a greater number of photons (fig. 1).

In highly sensitive chips, however, the image noise can be more evident, since the measure signal is so low. The most notorious contributor of noise is thermally derived, and this can be counteracted by active cooling of the camera chip, either with a Peltier-effect plate of with forced air or even a water flow. In addition, the simple act of reading the value of a pixel can introduce noise, but to overcome this latter source of noise requires more sophisticated electronics, driving up the cost of the camera.

Color or Monochrome?

So it seems that a choice must always be made between having a color or a monochrome camera. Presenting an innovative solution to this constraint, dual-chip cameras are now arriving on the market. For example, the Olympus dual-chip DP80 camera houses both a dedicated color and a monochrome chip, allowing rapid and automatic switching between the two. Versatility is incredibly valuable in many labs, but this camera also presents unique opportunities for overlaying color and fluorescent images with single pixel precision (fig. 2).

Resolving the Details

Resolution is important for low magnification and large field of view. Resolution is perhaps the best known feature of the digital camera. Defined by pixel size (smaller pixels capture finer details), resolution is often indicated by the total number of pixels, measured in Mega-pixels (MP): the finer the pixel size the more of them can fit onto a chip. But is higher resolution always better?

It is however a common misconception that higher magnifications require higher camera resolution. In fact, the opposite is true and higher camera resolution is only effective when working at low magnifications. For example, when using a 100X objective a resolution of just 1 MP will be generally sufficient: any additional resolution will merely increase data volume without providing any extra information. An image instead captured at 4X magnification might require more...
than 10 MP to capture the details from the large field of view. The digital zoom can later be used on this high-resolution image, allowing the sample to be analyzed retrospectively and facilitating the transition towards digital sample libraries (fig. 3).

Increased resolution from a finer pixel size tends to lower camera sensitivity, but if both resolution and sensitivity are important for the user, the versatility of a CCD featuring "pixel binning" should be considered. This function allows the user to choose whether to group adjacent pixels to increase the camera’s sensitivity, albeit at the expense of a lower total resolution.

When it comes to maximizing resolution, pixel-shifting technology allows a drastic increase without compromising on sensitivity. To create an image, the sensor is precisely shifted up and across by a tiny amount, and this new position is measured again, to create "virtual pixel": the only compromise is that it takes a few seconds for the camera to acquire and combine all the intermediate images.

**Live Image Quality**

The live image is the first step in using a camera and its speed is the first important parameter, enabling the user to quickly find and focus on the region of interest. There are, however, situations where the camera’s main purpose is not grabbing a snapshot but instead displaying the real-time image of the sample, for example when evaluating a sample on-screen or when presenting and discussing a sample with an audience. In these situations not only the speed, but the quality of the live image becomes important, and cameras can exhibit unnatural moving images, with striping and color-ghosting (fig. 4), creating a stressful experience for the user. However, if a technology known as "progressive readout" is present, a fast and artifact-free image will be presented, reducing user stress during extended sessions and dramatically improving audience understanding during presentations and discussion sessions.

**The Perfect Camera**

Although color, sensitivity, live image quality and resolution are interlinked, we have seen that different cameras prioritize each feature depending on the designated application. It might seem complex, but the following decisions will help in matching the right camera to the application:

- If working with fluorescence, select a monochrome camera and check noise values when working with low-emitting dyes
- For color imaging, ensure the camera includes color profiling technologies
• When imaging large sample areas at low magnifications opt for a higher resolution
• If mainly checking samples on-screen (for presentations or discussions) prioritize live image quality and speed

Last but not least, remember that nowadays the camera capabilities are greatly influenced by software: it is often possible to enhance image quality by simply improving the driving software, so remember to update it from time to time!

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