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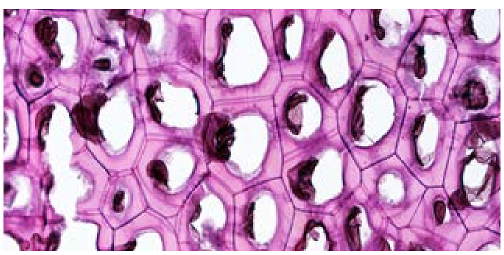
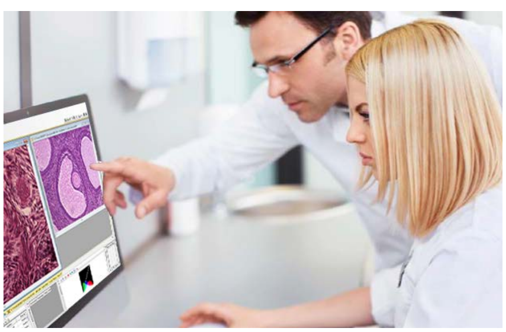




# DIGITAL PATHOLOGY:
## A Primer for Microscope Camera Selection

### WHAT'S INSIDE

- Introduction
- Importance of Color Reproduction in Pathology Images
- Understanding Camera Sensitivity, Noise and Resolution
- Types of Camera Interfaces and Image Analysis Software
- About Lumenera's INFINITY Microscopy Cameras for Pathology

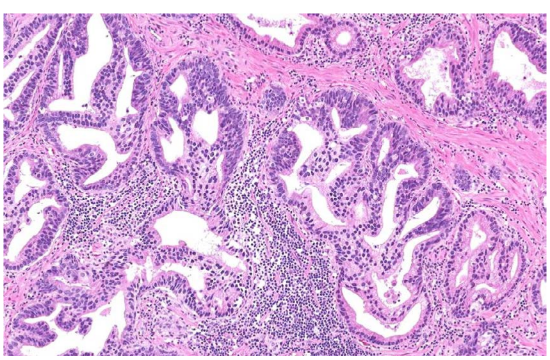
# INTRODUCTION

Most pathologists today use digital systems within their practices and research projects to communicate, store data, and analyze images. Whether it's within clinical, forensic, surgical, or other branches of pathology, communication and consultation among specialists is greatly improved by the use of digital systems and enables faster diagnosis for patients.

Selecting the proper imaging equipment is one of the most important elements a pathologist needs to take into consideration when using a digital system.  To produce a high quality pathology image, one must have a digital camera that can dependably and accurately reproduce what a pathologist sees in the eye piece of their microscope. Excellent color, high dynamic range and high resolution are the key elements that comprise a high quality pathology image. It is also important that pathologists can detect features in dark areas *(which means the camera must have high sensitivity)*, that the camera doesn't add unwanted artifacts to the image *(has low noise)*, and can be easily connected to a computer *(via a USB interface, for example)*.

This paper will review the fundamentals of imaging within the field of pathology and what should be taken into consideration when selecting a digital microscopy camera.

# COLOR REPRODUCTION



Sample Image Above: Prostate Carcinoma
Taken with Lumenera's INFINITY*2-2* Microscopy Camera

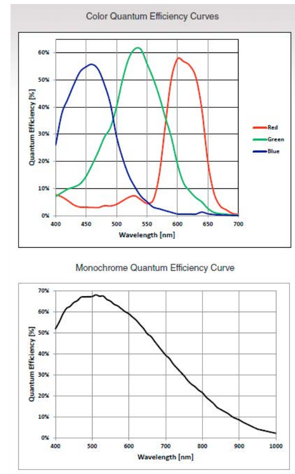
When selecting a camera for pathology, accurate color reproduction is of the utmost importance. Since the captured digital image contains data that can result in a patient's diagnosis, it is important that the camera displays color that matches exactly what the pathologist sees when looking into the eye piece of their microscope. Most consumer-grade camera models automatically post-process their images to increase the saturation of certain colors in order to make the image more visually appealing, thereby not accurating capturing the original colors. It is important to select a camera that is designed specifically for scientific use— producing images that are properly calibrated via white balancing and gamma. A properly calibrated computer monitor is also important when assessing the color of digital pathology images.

**Did You Know?** Lumenera's INFINITY microscopy research-grade cameras are renowned for their exceptional color reproduction capabilities. You can view sample images demonstrating this in their online image gallery:
www.lumenera.com/resources/sample-image-video/microscopy/application.html

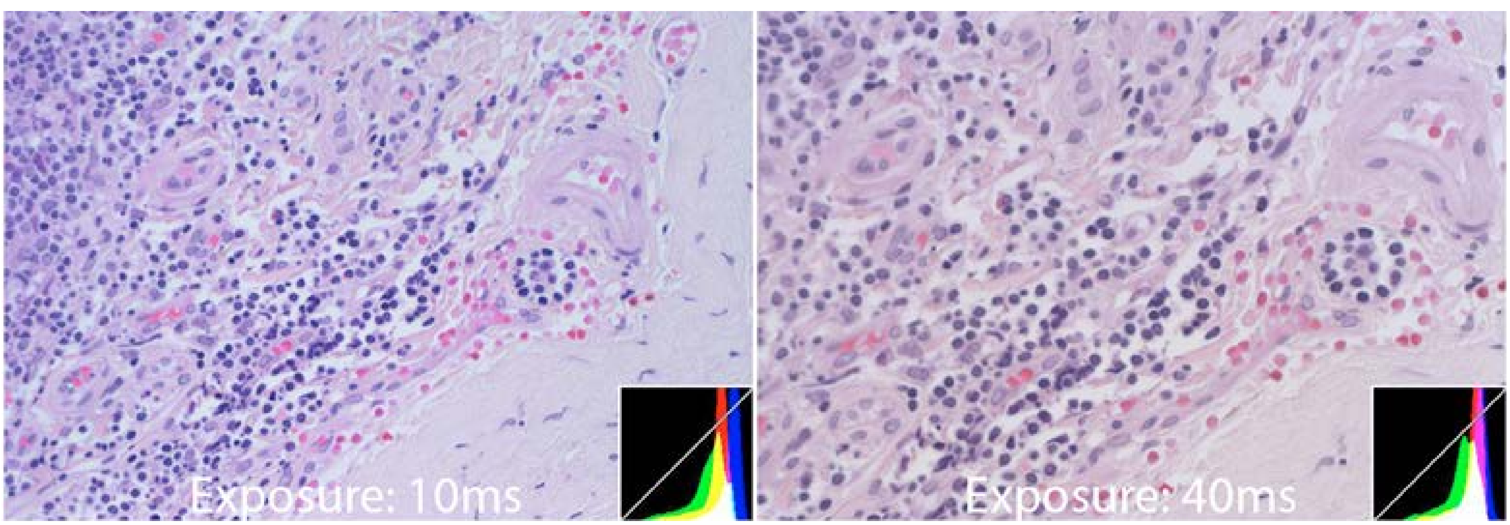
# CAMERA SENSITIVITY and NOISE

When selecting a camera, image quality is the outcome of the camera's sensitivity and noise levels. The camera's sensitivity involves a combination of its quantum efficiency (QE), pixel size, and charge conversion efficiency. Higher efficiencies and pixel sizes are vital to increasing sensitivity, but must be considered in conjunction with the camera's noise specifications. There are two main noise sources that impact pathology applications: read noise and dark current noise. These values should be as low as possible while maintaining high sensitivity.

QUANTUM EFFICIENCY (QE): QE is defined as the camera's ability to convert incident photons into usable signal electrons. This operation is performed by the camera's image sensor and is dependent on the wavelength of the light. For this reason, camera datasheets typically include QE curves, demonstrating the camera's quantum efficiency over a range of wavelengths, usually starting from the edge of ultra-violet and into NIR (near infrared). The figure on the right shows example QE curves taken from Lumenera's INFINITY*3-3*UR microscopy camera datasheet. Pixel size also plays a significant role in the camera's sensitivity. The larger the pixels, the more sensitive the camera's sensor will be to incoming light as there is a larger surface area to collect the incident light.

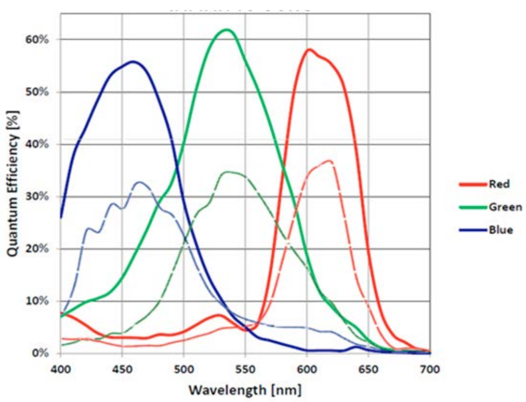



To help demonstrate the impact that Quantum Efficiency and Pixel Size have on an image, take a look at the image comparison below. This comparison was done using two INFINITY microscopy cameras that have different quantum efficiencies and pixel sizes. Both of these images were captured on a microscope in the same environment, using the same light source and lighting conditions. At first glance the images do not appear to be that different in terms of brightness. However, in order to achieve this, the camera with a lower QE and smaller pixels *(image shown on the right)* required an exposure time of 4 times longer. The difference in exposure time illustrates how one camera is more sensitive than the other.




*Comparison images between the INFINITY3-3URC (left) and INFINITY1-3C (right), captured with identical gain and gamma settings*

CAMERA SENSITIVITY and NOISE *(Continued from Previous Page)*




*Graph Comparing Quantum Efficiency Curves Between:*
*INFINITY3-3URC (solid lines)*
*INFINITY1-3C (dashed lines)*

The difference in required exposure time is further highlighted when the QE curves for each camera are compared side by side as shown in the graph on the left.

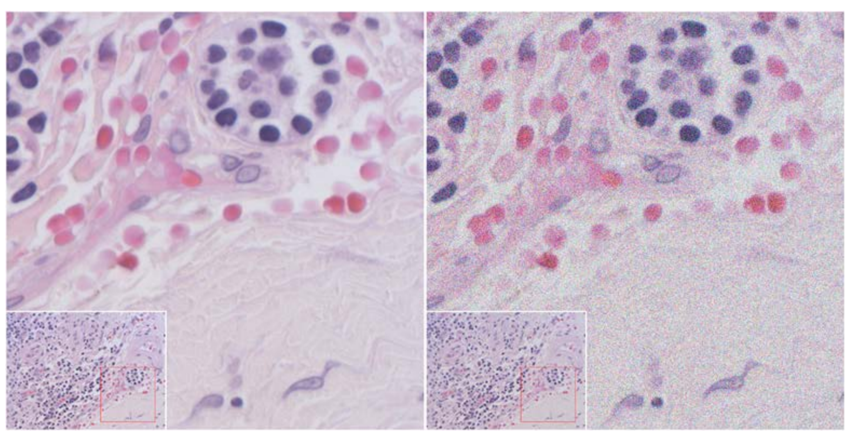
As the graph shows, the maximum QE for the INFINITY*3-3*URC is close to 50% greater than the highest value for the INFINITY*1-3*C, resulting in a more sensitive camera.

Furthermore, by increasing the size of each pixel, the overall sensitivity of the sensor also increases. This is because the incident light is grouped into larger areas on the sensor, meaning that less light is required to hit these bigger targets. In the previous example, the pixel size of the INFINITY*3-3*URC is of 4.54x4.54 microns whereas the pixels on the INFINITY*1-3*C are of 3.2x3.2 microns each. Pixel size and quantum efficiency are customarily found on the camera's datasheet.

The difference in exposure time illustrates how one camera can be more sensitive than the other. One benefit to having a camera with high sensitivity is that it requires less light to illuminate a slide, as in the case where specific optical filters may be used or where the maximum illumination is limited due to the lamp wattage of the light source. Another benefit of a more light-sensitive camera is that shorter exposure times are possible, which has a direct impact on frame rate and therefore the refresh rate of the image on screen during framing and focusing operations. In other words, the camera will operate at maximum frame rate in lower lighting conditions.

NOISE: Lowering the noise levels of the camera through its design is a crucial element in producing high quality images. Read noise establishes the minimum noise value for the camera, rendering anything beneath this minimum threshold unusable. This form of noise is measured in electrons (e-) and is most relevant for short exposure times. Dark current noise is a time and temperature dependant noise value caused by heat inside the camera. Expressed in electrons per second (e-/s), this noise will increase over time and at higher temperatures. For long exposures, such as with fluorescence confocal microscopy, dark current noise will make the read noise value less prevalent.

The appearance of noise in an image is best understood through use of an example. To the right is an enlarged portion of the image from the first example taken with Lumenera's INFINITY*1-3*C. There is a low noise level in the left image and the detail in the bottom right corner is visible and sharp. The image on the right has simulated noise added making the shapes and patterns in the tissue indistinguishable.



*Featured Above:*
*Comparison of an image with a normal amount of noise (left) vs. an image with a lot of simulated noise (right). Both taken with the INFINITY1-3C.*

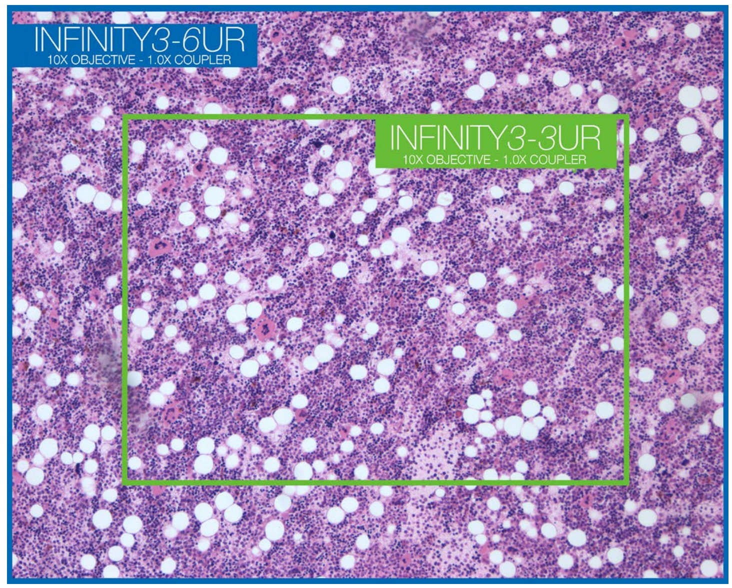
CAMERA SENSITIVITY and NOISE (Continued from Previous Page)

Best results are achieved when the signal-to-noise ratio is as high as possible, with its upper bound defined by the camera's dynamic range. The dynamic range is the ratio between the maximum amount of electrons that can be generated by a pixel and its noise floor. It illustrates the camera's ability to generate usable data in dimly and brightly lit areas of the image simultaneously.

To achieve a high level of sensitivity in a camera, the noise floor must be as low as possible so that a small amount of incident light can be detected by the sensor. In addition, having a high QE will convert more photons to electrons, thus providing a stronger signal. The combination of low noise and high QE will translate to a camera with a high signal-to-noise ratio, providing superior image quality.

# RESOLUTION

To image a large portion of a slide in a single frame, while maintaining clarity and detail of the specimen, it is important to select a camera with sufficient resolution. However, a balance between resolution and sensitivity must be achieved since they are codependent. For sensors of equal dimensions, one with a higher pixel count will necessarily have smaller pixels, rendering them less sensitive to light. It is, therefore, crucial to select a camera with sufficient resolution, but not too much, to maintain a high level of sensitivity. The selected resolution should be high enough to distinguish the smallest amount of detail expected to be encountered in the application. This will ensure that nothing is missed due to lack of clarity in the image. In the following example, the pixel size between the INFINITY*3-3*URC and the INFINITY*3-6*URC is identical, but the sensor size of the INIFINTY*3-6*URC is larger (1" vs. 2/3") and therefore has a higher number of pixels.




*Field of view comparison between Lumenera's INFINITY3-3URC and INFINITY3-6URC microscopy cameras.*

The selected objective for the microscope will also impact the image resolution. As the numerical aperture (*also known as 'NA')* is increased, the objective's ability to resolve two small areas of the specimen which are close to one another is also increased. This is because objectives with smaller numerical apertures become diffraction limited when attempting to resolve small details in the image. Lower NA objectives allow less light to reach the camera sensor, but will increase the depth of field, allowing the observer to focus on a thicker portion of the specimen at one time. Using an objective with a higher magnification will also help resolve the smaller detail, but will greatly reduce the field of view. This is why a large numerical aperture is vital when viewing fine detail of the specimen, while maintaining a large field of view.

# CAMERA INTERFACE

An often overlooked, yet equally important feature of the camera is the interface – how it connects to the computing environment. In a lab setting, when working within a budget and trying to keep costs down as well as simplifying the equipment set up, it is important to select a camera that does not require a custom peripheral or interface card to communicate with the computer.

Using a standard interface such as USB will help keep additional spending to a minimum as computers already come equipped with this technology. This also offers ease of integration by providing a more user-friendly, hassle-free, plug-and-play set up. If you are looking to produce higher resolution images in larger bit modes that will result in a larger digital file size, you'll need to select a USB interface that can easily and quickly transfer large amounts of data without compromising on image quality or frame rate. Once resolution and bit-depth are taken into account, you may want to consider which version of USB is suitable for your application. With recent developments in USB 3.0, it has become the new standard for moving large data payloads at high transfer rates, and is roughly 10 times faster than its predecessor, USB 2.0.

## IMAGE ANALYSIS SOFTWARE

You will want to ensure that your camera comes with image analysis software, or works with third party software products. Lumenera's INFINITY cameras come complete with user-friendly software at no additional charge, and are also integrated with leading software technology partners such as Media Cybernetics (Image Pro Premier), Molecular Devices (Metamorph), MicroManager (ImageJ) and National Instruments (LabVIEW) to name a few.

For a full list of Lumenera's microscopy software technology partners please visit: www.lumenera.com/partners/technology-partners.html

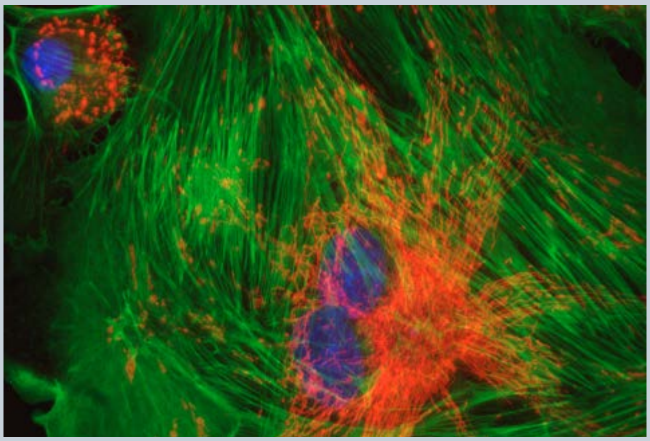
# TOTAL COST OF OWNERSHIP

When shopping for any new product, price always plays a part in product selection. When it comes to an imaging solution, aspects such as ease of integration, image analysis software, image quality, product support, and warranty should be factored into the overall cost of the imaging solution. Pathologists can easily address all of these imaging and budgetary factors with Lumenera's wide range of USB 2.0 and USB 3.0 INFINITY cameras, all of which include a 4-year warranty.

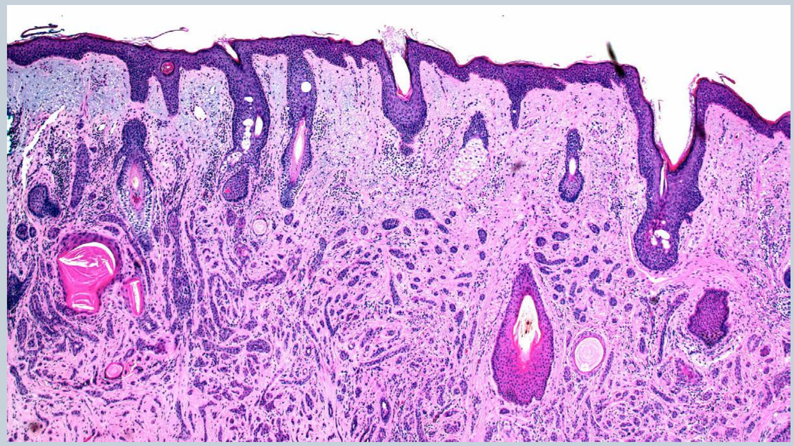
## About Lumenera's
# INFINITY MICROSCOPY CAMERAS

Lumenera has been providing imaging solutions to pathologists for 15+ years.
Contact a Lumenera Sales Representative to discuss your imaging requirements and for help selecting the right camera for your pathology application.

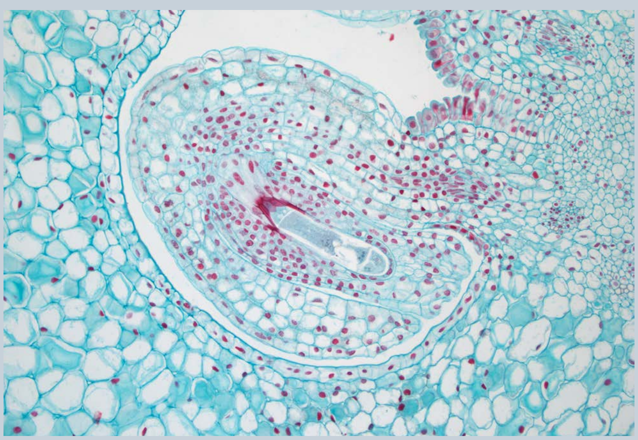
## SAMPLE PATHOLOGY IMAGES



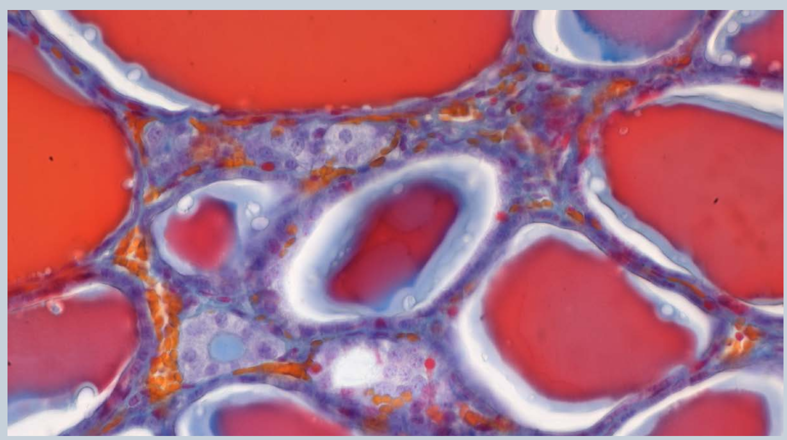
BPAE Fixed Cell taken with the INFINITY*3-3*UR



Micro Cystic Adnexal Carcinoma taken with the INFINITY*2-5*



Ilium Brownii taken with the INFINITY*3-3*UR



Thyroid Gland of a Sheep taken with the INFINITY*3-6*UR

View more sample images in the online gallery: www.lumenera.com/resources/sample-image-video/microscopy.html

## WHY CHOOSE INFINITY?

- Incredibly easy to install
- Compatible with any microscope with a c-mount
- User friendly, intuitive software included, for both PC and Mac
- Free software updates from Lumenera's website
- Simple USB interface with no need for extra cards
- Industry leading 4-year warranty

**Would you like help** selecting the best INFINITY camera to meet your application and budget requirements? Contact an INFINITY microscopy camera sales representative: info@lumenera.com