Re: How many pixels?

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- *From*: Aaron <nghy@xxxxxxxxxxx>
- *Date*: 21 Apr 2006 04:21:02 −0500

Hi,

I just entered this thread and had a few comments. First Zeiss has recearched this subject and included a table within their brouchure for the Axiocam HR that relates the camera resolution in pixels needed to capture all the information provided by various objectives (different magnification and NA). You can go to the Zeiss website (www.zeiss.com) and download the AxioCam brochure in pdf format. you may be surprised to learn that far more than 2 to 4 megapixels are needed for some common objectives.. The calculations show that lower power objectives require greater numbers of pixels than higher power objectives. And the use of a reducing lens to concentrat the output of the microscope onto the CCD increases that demand.

Since this result seemed counter−intuitive, as an excercise I computed the NA per unit magnification of various objectives that I own. I discovered that although the absolute NA of the objectives increases with magnification the opposite is true for the NA per unit of magnification.

Consider two examples of typical objectives

10X 0.25NA 0 .25/10 = .025 NA/1X mag

100X 1.3NA 1.3/100 = 0.013 NA/1X mag

The second point I wish to make is that size of the image output from the microscope has to be matched to the CCD size to avoid other subtle problems.. In most situations the image output from the microscope is too large compared to the CCD size.. So only the center part of the microscope output falls on the detector. As a practical matter, this reducing lens is tough to locate and expensive. It becomes the weak link that prevents the full potential of the microscope and the camera from being realized. .

While some OEM scope manufacturers and some independent suppliers (Diagnostic Instruments) provide reducing lenses, obtainig an exact match with optical qualities equivalent to the microscope itself is

very difficult and almost impossible for microscopes designed with compensating eyepieces.

If the image falling on the CCD is larger than the CCD itself, the resulting captured image will represent a subsection of the FOV as seen in the eyepieces. This process parallels cropping the center of a digital image and then enlarging the same. Normally this process leads to degredation of the image details.. The relay/reducing lens may introduce distortion itself, not provide compensation needed for the objectives or not match the image out put to the exact size of the CCD. The qualities of the reducing/relay lens is a very practical complication to obtaining the highest quality images from the microscope and camera.

Aaron.

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On Wed, 19 Apr 2006 17:08:17 GMT, "NoSpam" <NoSpam@xxxxxxxxxxx> wrote:

> Hi,
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> There have been a few postings recently which made me think about the maximum
> useful number of pixels in a microphotograph. I will present some numbers and my
> conclusion is that about 2 to 4 Megapixels in a digital camera will do justice to
> the capabilities of any light microscope.
>
> It is possible that I have overlooked something and that my conclusions are incorrect.
> Please let me know what you think.
>
> The smallest separation observable with a light microscope is given by wavelength di−
> vided by the product of 2 times the NA. For a wavelength of 0.5 microns and a NA of
> about 1.0 the minimum resolvable distance would therefore be 0.25 microns. Objectives
> with a NA of about 1.0 magnify about 100 times.A resolution element of 0.25 microns
> in the object plane will therefore appear with a 25 micron linear size at the exit pupil
> of the objective. A typical value for the diameter of this pupil is 20mm or about
> 20 000 microns.There will therefore be 20,000microns/25microns or 800 resolution

alements along a given diameter in this pupil. If we square this number we get the approximate number of resolved pixels in the exit pupil as 0.64 Megapixels. These are all the resolved picture elements which a camera has to record. Further magnification or reduction in size of the exit pupil will not change this fundamental requirement for taking a picture at 100x linear magnification with a NA of 1.0.

If instead of a 100x objective with a NA of 1.0 we use a 10x objective with a NA of 0.25, then the linear size of a resolved object element in the exit pupil decreases by a factor of [(100/10)x(0.25/1.0)] or by 2.5. The total number of pixel in the exit pupil will then be 2.5x2.5=6.25 as large as the above number of 0.64 Megapixels and about 4 Megapixels will be required.

If these consideration reflect reality then cameras with more than 4 Megapixels will not be needed for microphotography. For high magnification half that number may be all that is needed.

I am interested in your comments.

G.R.

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