DIGITAL PHOTOGRAPHY

Camera and image capture

The higher the number of pixels, the better the resolution. Your camera should be able to capture images of at least 1200 x 900 pixels which is equivalent to 1 million or 1 megapixel total. Most modern cameras can do better than this, often 3 or more megapixels.

Some cameras add pixels to the image by trying to match colour, brightness and contrast. This increases the resolution (more pixels in the image) but it does not increase the quality of the image. Check the size of images is actual optical resolution - i.e. physical number of pixels captured.

If the resolution is very high and the image complex, this can result in one image occupying 16mb of memory! Low compression and high resolution usually result in files of approximately 1.5 Mb.

Set your camera to maximum resolution (sometimes described as "Full", "Hi" or "Fine"). Set file saving without compression in TIFF format.

Saving and editing the image

Image dimensions can be seen by holding the mouse over a file and the total size by right clicking on a file and clicking on properties. An image of 1 Mb (1000000 bytes) will usually be of adequate resolution to publish at finished size.

To calculate the physical size of your image, divide the dimensions of the image in pixels by the image resolution set within the image editing programme.

Default saving of images is usually 72 or 96 ppi (pixels per inch). This is not adequate resolution for publication. The resolution must be at least 300 ppi (preferably 600 ppi) at the final printed size.

An image of 1200 x 900 pixels will print at 4" x 3" (1200 divided by 300 and 900 divided by 300). An image of 640 x 480 at a resolution of 300 ppi will only print at 640/300 by 480/300 inches (2.13" x 1.6"). For publication, working at a print size of 4" x 3" is usually adequate so an image of 1200 x 900 pixels is adequate but 640 x 480 pixels is not.

Images should be resized for printing in your editing software. Go into "Photo size" (resize, magnification or canvas size in some programmes) and set the width and height of the photograph with the box labelled "resolution" set at least 300 ppi.

Notes

The size of a file is set by the number of pixels in it. If it is compressed, it has less pixels in it and is therefore smaller. In general, large files may be printed at higher resolution.

Changing the image resolution does not change the size of the file. If just means that the pixels will be closer together or further apart when printed.

Changing the on-screen view does not alter the size of the image. Computer monitors display at approximately 96 ppi so that an image of 640 x 480 pixels is viewed at 6.7 inches by 5 inches (640 and 480 divided by 96).

Images displayed in slide shows (such as Powerpoint) and on the Web require far lower resolutions than those for publication (only 72 or 96 ppi).

TIFF and EPS files are 24-bit (24 bits per pixel or 16.7 million colour combination) files and can be saved without compression. JPEG files are compressed and therefore unsuitable for publication purposes *unless they have been saved in "lossless" format*. GIF files are only 8-bit (8 bits per pixel) so only have 256 colour combinations.

Printer resolution and image resolution are not the same.

Do not use the scale command in page set up of your printer settings to change the print size as this will lower the quality of the print.

For further information, visit www.shortcourses.com website.

Sending your photographs for publication

1. Save each photograph (preferably TIFF or EPS format) in a separate file with the figure number.

2. Also save each photograph in a small compressed JPEG format (100-300kb) file.

3. Try to upload these the large figure files with your manuscript. They will take some time to upload. If they do not load, send the JPEG figures instead with a note to the Editors stating that you have done this and also have larger, better resolution files.

CLINICAL PHOTOGRAPHS

These should be free of extraneous material, and if portions of the handler, for example fingers or hands are to be included, particularly adjacent to lesions, they must be gloved. In those cases where the original prints have extraneous material, this should be removed by a program such as Adobe Photoshop.

Backgrounds

The background, tabletops, or floors should be unobtrusive, preferably uncoloured and of a medium tone and without an obvious junction between wall and floor. This is easily done if the initial photograph is taken with the subject against a light grey non-textured background such as a continuous piece of carpet, which extends down the wall and is coved onto the floor or table.

Subject

It may be necessary for desirable to take a photograph of a wider area – the so-called “long shot” of the whole animal, or most of it, to illustrate the location and extent of lesions. Then a photograph of a smaller area, the so-called “ close up” should be taken to share detail in the actual lesion. Occasionally one photograph is able to show both, and in that case sufficient anatomic landmarks should be included to allow the viewer to orientate himself/herself. All extraneous material such as dressings should be removed

Orientation

The subject should always be in an anatomically correct position. This is no problem with standing animals, but animals in ventral or lateral recumbency should be taken from different points of view. The convention is that in ventral recumbency, the photograph is vertical, with the head to the top of the frame. In lateral recumbency, the photograph is horizontal and so is the subject.

Lighting

It is critical to remember that the human brain has been programmed so that the direction of shadows indicates which is the top or the bottom. Natural light always comes from above and thus in the various positions listed above, a light source should be from the top of the frame. This means that if a photograph is taken in an incorrect anatomic position, it may not be possible to rotate that photograph to the correct anatomic position without placing the shadows in what appears to the brain, an unnatural position.

HOW TO PREPARE COLOUR PHOTOMICROGRAPHS FOR PUBLICATION

Histological sections

In general, 5 micrometer sections are desirable for photomicrography using the 10x, 20x, and 40x objectives, and for the 100x objective with its limited depth of focus a 3 micrometer section is preferable. For very low power objectives such as the 4x, thicker sections (6-7 micrometers) may be necessary, if the tissue is not dense.

Optimal H&E staining ensures the differential eosinophilia of the tissue components which is critical for best results. With proper differentiation of the eosin, all components, e.g. edema, collagen, keratin and muscle, stain a different shade of pink or red. This differential staining occurs only with alcoholic solutions of eosin and if the eosin is differentiated in 95% ethanol (Luna, 1992). Differential eosin staining is not a matter of personal preference, but is essential. Undifferentiated eosin-stained tissues lose much of their clarity in the final photograph because all tissues record the same red or pink. Phloxine, which greatly reduces differential staining, should not be added to the eosin.

Haematoxylin should stain the nuclei as blue-black as possible, without blocking out the detail of the chromatin. Harris's haematoxylin is preferred as it produces a bluish-black, nuclear stain. Failure to properly differentiate the haematoxylin using acid-alcohol will result in inappropriate haematoxylin-staining of the cytoplasm (Luna, 1992).

Microscopy

The microscope must be set up for Koehler illumination for each objective, and this should be checked when taking each photograph. As the eye should be focused on infinity when looking down the microscope, looking away from the subject, focusing on a distant object, and then quickly viewing the microscope's image is helpful. The eye will accommodate, causing poor focus, if too long a time is taken. Ideally, photomicrography should be performed in the morning when the eyes are minimally fatigued.

Focus should be carefully adjusted for each photograph, if necessary, using a focusing telescope. If low power objectives (4x or 2x) are used, it is inadvisable to use a focussing telescope because the thickness of the specimen is greater than one cell. Use the COARSE focussing and move it up and down until the BEST APPEARANCE is there. Then play with the fine focus. The Coarse focus is best because it makes you look at the WHOLE slide-not one individual cell which may not be in the middle of the thickness of the specimen. Sometimes, take a couple of photographs with slightly different focussing planes and then pick the best one.

In general, the use of a 10x or 20x objective provides the best contrast and visibility of the subject in most sections. The use of the 4x objective should be used only when absolutely necessary to show the overall pattern, such as that of a neoplasm.

Non-digital Photomicrography
For 2 x2 slides, Fuji T64, Fuji D50 (Velvia), and Kodachrome 64 are recommended films, because they have a high “D Max”, and thus the ability to reproduce black, e.g. blue-black nuclei. Many of the other Ektachrome films cannot make a deep black.

For colour prints, a slower film such as 50 or 100 ISO films should be used. The correct ISO rating for the equipment will have to be found by running a test roll. Most colour negative (print) films do better with slight overexposure and so a series from at least half the rated ISO to the nominal rating should be run. The best negative can be selected by the darkroom technician or the photomicrographer should select the negative where there is no lost of detail in the dark structures such as the interiors of nuclei.

Digital photomicrography
See details above.

Reference: Luna, L.G. Histopathologic Methods and Colour Atlas of Special Stains and Tissue Artifacts. Gaithersburg, MD: American Histolabs Inc., 1992: 71-3.

HOW TO EVALUATE YOUR COLOUR PHOTOMICROGRAPHS

Colour Prints

Gross defects such as lack of sharp focus or histological artifacts such as microtome knife marks or over thick sections should be evaluated first. Any photomicrograph containing these obvious defects should be rejected .

1. Exposure of the print.
This is evaluated first by checking the microscope's clear background. Exposure in the enlarger controls the density of the background which should be a light grey. This described as- "pearl grey" or "snow on an overcast day". Evaluation is based on density and any colour cast should be ignored at this stage. The usual problem is that the background is darker than it should be.

2. Colour cast.
The microscope's clear background should have no colour. It should be an extremely light grey. Colour casts are a common problem and are unacceptable. They can be removed by a computer program such as Adobe Photoshop or at the time of printing.

3. Staining contrast and differentiation of eosin.
Ideally, haematoxylin stained nuclei should be very dark. This will usually be a dark blue or even blue black. Eosin stained structures should be differentiated. In other words, all eosin stained tissues depicted in the photograph PG collagen, smooth muscle, keratin, serum, blood cells and eosinophilic granules should be stained a different shade of red or pink. If the eosin is undifferentiated, these components will all be much the same colour and thus will not be easily identified in the photomicrograph. These photomicrograph should be returned to the author with the advice to use correctly differentiated haematoxylin and eosin stained sections.

4. Visibility of the lesion and the suitability of the magnification too high or too low, inclusion of adjacent landmarks so that the viewer may orient himself/herself.

5. Composition and anatomically correct orientation. The surface of the skin section should be horizontal with the surface to the top.

6. Most microscopes do not have suitable condensers for use with low-power objectives such as a 4x or 6.3x. The 10x has better contrast and the nuclei are clearly visible. As photomicrographs are often reduced to column width, they will also be very small and detail will be lost. The solution, on most occasions, is to take a photograph using a vertical format and a 10x objective. For many skins, this will cover the full thickness. The advantages of using this method are that the photograph will occupy only one column, and because of the higher magnification, detail will be more visible.

Colour transparencies

The slides should be evaluated on a light box, preferably illuminated by 5000 degree Kelvin bulbs which allow correct evaluation of colours. Projection does not allow adequate assessment of the image. It tends to be "forgiving" and the identification on colour casts is difficult. The same features listed above for colour prints should be evaluated in the transparencies.

Digital photographs
See above

MORPHOMETRICS

It is not acceptable to grade lesions subjectively, on the basis o + to +++++ and then evaluate the data statistically, as if it were normal in distribution, when in fact it may well be non-parametric. Authors will be required to indicate how they have controlled or accounted for fixation artefacts such as shrinkage, plane of section and repeatability of the data. The variance in the same sample, amongst different samples taken at the same site, and then later amongst the samples taken at different sites should be indicated in order to justify the repeatability and thus the reliability of the technique.

Joan Rest
16 January 2006

Acknowledgement

I am grateful to Professor M D McGavin for his assistance in producing these guidelines.