How to take and process digital images for publication

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Abstract

The ability to take and process digital images is important in histopathology, cytopathology and pathology-centric research. In particular publishers demand that digital images are presented in a certain format. Since recording, processing and preparing digital images are often in the hands of the researcher, we should know how to handle digital images and prepare them for publication. This paper outlines the basic principles of digital imaging, what defines image resolution and how to prepare digital images for publication. Common pitfalls in preparing digital illustrations are reviewed together with the ethical constraints around the processing of scientific images.

Keywords cameras; digital images; ethics; image processing; image quality; microscopy; photomicroscopy

Introduction

Images are central to our understanding in pathological research and practice. They therefore form an essential medium by which we communicate histological and cytological phenomenon associated with disease and explore new biomarkers of diagnosis, prognosis and response to therapy. Pathological journals and books, since their inception over 100 years ago, have used images to convey important findings, evolving from line drawings of cells and tissues to photomicrographs. Attaching a digital camera to the microscope has revolutionized the ease with which we can record, store, modify, print, and incorporate images within our published works. While digital images certainly make life easier, there are still issues that need to be considered in how they are taken, how we process them and how they can be best presented for publication.

Back to basics: what are digital images?

Digital images are composed of pixels (derived from the term **PIC**ture **EL**ements). These pixels are a digital representation of the electronic (analogue) signal that a camera generates when detecting light. Using CCD technology (used across almost all digital cameras) the analogue light signals are sampled at discrete intervals, each of which is allocated a number which records the density and colour of the image at a specific point. This number dictates how the image is digitally stored and informs the computer screen/printer what grey level or colour to display/print at that point. An individual picture is composed of many millions of pixels, allowing images to be recreated on-screen or printed on a printer at a resolution that closely matches the original image. A digital image is therefore really only a matrix of numbers (Figure 1).

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What determines the quality of a digital image?

The quality or resolution of a digital image is determined by three principle factors: (1) Bit depth (ii) Image size (iii) Pixel density.

(i) Bit depth

A bit is the smallest unit of data used to describe an image. It can be a 0 or a 1, black or white. The bit depth determines how many grey values or colours are used to represent an image.

A one bit image only has two tones: black and white. Pixel values therefore are either 0 or 1 (Figure 2). In a two bit image (Figure 3), there are four (2^2) possible combinations with pixel values either being 00, 01, 10 or 11. The most commonly used configuration are 8 bit images which comprise 256 (2^8) possible grey values to represent information in the original image (Figure 4).

Colour images are usually represented by three images representing the RED, GREEN and BLUE components of the image. By mixing the primary colours, RED, GREEN and BLUE, to various degrees it is possible to represent the variations in colour that one would see in a typical image. Each of the individual RED, GREEN and BLUE components of a colour image is represented by a separate 8 bit image (Figure 5) with 256 possible values available for red channel, 256 for green and 256 for blue. In combination therefore, a colour digital photograph is represented by a 24 bit image (i.e. 8 bits $\times$ 3 images) with a possibility of 16.7 million (2^{24}) colour values (Figure 5).

Most cameras today record colour images in 24 bit and this would be typical of the cameras that would be used for photomicroscopy. However, it is now possible to capture colour images in 36 bit (12 bit $\times$ 3), and 48 bit (16 bit $\times$ 3) offering literally billions of colours to represent the image. Higher bit images of this type are more common for very high end photography and not commonly used for standard microscopy for publication.

(ii) Image size

Image size is the number of pixels that makes up an image and is primarily determined by the size of the sensor on the camera. This is commonly measured in "megapixels" where "mega" means a million. A 3 megapixel image is approximately 2000 pixels wide and 1500 pixels high (2000 $\times$ 1500 = 3,000,000 = 3 megapixels). A 6 megapixel image is approximately 3000 pixels wide and 2000 pixels high (3000 $\times$ 2000 = 6,000,000 = 6 megapixels). The more megapixels your camera has does not necessarily define the quality of the image but does determine how much your can enlarge that image without loosing quality. This is tied closely to display and printing quality which is determined by the **pixel density** in the next section, and highlights the dependency of these factors when discussing image quality. Depending on your requirements, the more megapixels your images have, the more flexibility you have when it comes to printing and publication. For example, at 300 dots per inch a 2 megapixel image can only produce a printout of 4 inches $\times$ 6 inches, whereas a 6 megapixel image can be printed out up to 8 inches $\times$ 12 inches at the same quality (more of this later…).

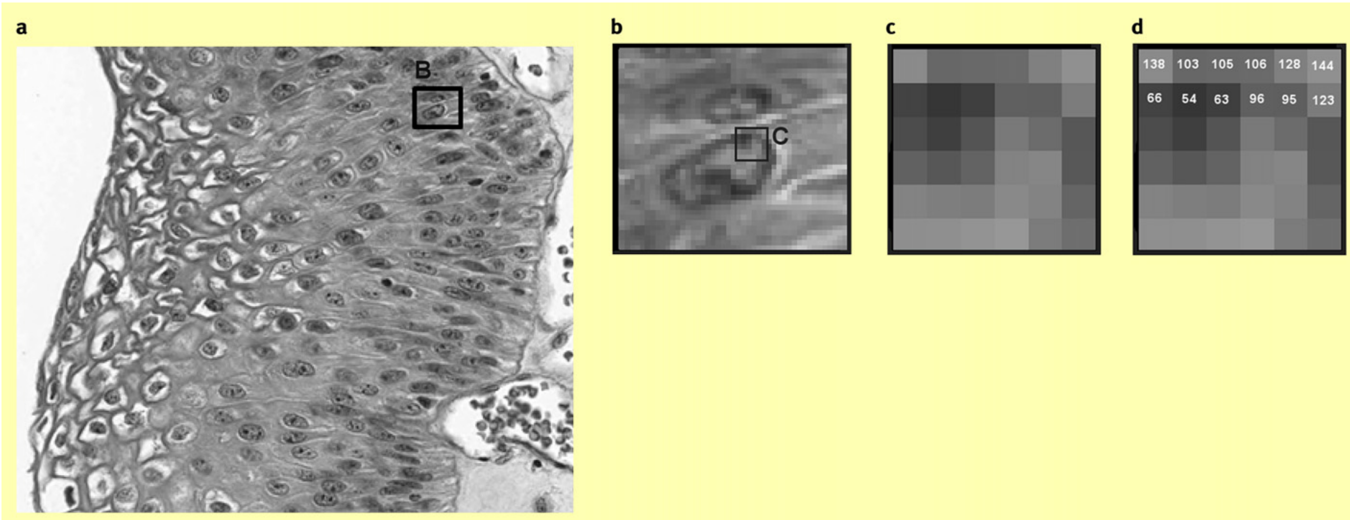

Figure 1 Grey level image **a** showing selected region **b** enlarged to a point where individual pixels can be seen **c**. Each pixel has a defined grey value from 0 to 255 **d** and it is these numbers which allow a digital representation of the original image.

Most pathologists take digital images using a digital camera attached to their microscope offering images in the 1—12 megapixel range. It is now possible however to scan entire microscopic slides at high resolution using dedicated scanning hardware from companies such as Zeiss, Hamamatsu, Aperio, Bioimagene and Olympus. Since the entire slide is scanned, this can result in image sizes in the order of 110,000 × 220,000 pixels. This equates to a 24 **Giga**pixel (i.e. 24,000 megapixel image) image! Many pathologists who have this facility often use this to record the entire slide and subsequently extract images from the virtual slide for publication. The resolution of virtual slides is sufficient to allow this for most journal print requirements.

(iii) Pixel density

While the previous two features (bit depth and image size) determine the inherent quality of a digital image, we also have to display the image on a computer screen or print the image out. This introduces a third factor called pixel density. Pixel density defines the number of pixels that are packed into a defined area on the screen for display or on paper when printed. Pixel density is generally described as dots per inch (dpi) for printers or pixels per inch (ppi) for screens, where the more dots/pizels per inch, the better the image quality.

In Figure 6, we see three images of the same bit depth and image size but which have different pixel densities (dpi). You will see that the image with the highest pixel density has the best quality.

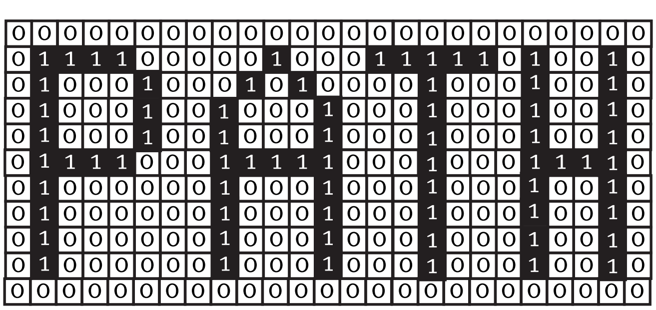

Figure 2 One bit image is bitonal: 0 or 1.

Preparing an image for publication

In preparing an image for printing or publication, there is one more feature that needs to be considered: the **print size**. The larger the required print size, the more pixels that need to be present in order to fill the space. There is therefore a close relationship between **print size**, **image size** and **pixel density** and these are the three most important factors that need to be manipulated when preparing an image for publication (Figure 7).

Once a digital image is recorded and stored, you can use a variety of software packages to “play” with resolution features outlined above and modify these at the touch of a button. This applies to generating images to print yourself (perhaps as part of a report or thesis) or for the submission of a manuscript to a journal.

Most journals specify the criteria for digital images to be reproduced in their printed media. For example, the Journal of Pathology requests that colour images are 300 dpi [*pixel density*] with a single column width of 83 mm (3.3 inches) [*print size*]. Most image editing computer programs allow you to take an image (recorded from your camera) and adjust these factors to match that required by the publisher. Figure 8 shows an example of this in Paint Shop Pro.

However, what *image size* do you need to meet these *pixel density* and *print size* requirements? Since the requirement is 300 dpi (dots per inch) and 3.3 inches in print size, then you need approximately 1000 pixels (300 × 3.3 = 990) available for the width of the illustration. Presuming that the image is square then a 1 megapixel image (1000 × 1000 pixels) is sufficient. The requirements for this journal, Diagnostic Histopathology, are

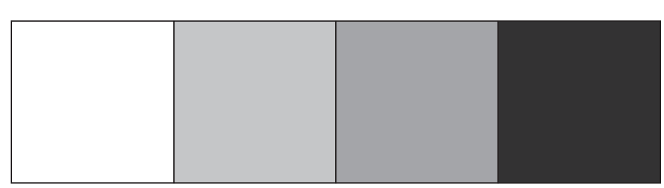

Figure 3 A two bit image represented by four grey values.

