A Few Thoughts About Image File Storage

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A recent thread on the MSA list server about problems with image formats (in this case TIF or TIFF) showed, that there is a bit of confusion in the microscopy community about the best file format for digital images. I will try to shed a bit of light onto this problem.

Digital images are at the core a large array of numbers. One number per pixel for b/w images, 3 numbers per pixel for color images. The simplest file format consists of 2 numbers that define the width and height of the image, and then just a listing of numbers for each pixel. By using the right conventions, the image can be recreated from a data file like this. In essence, this is the format of a bitmap image (BMP), and other formats derived from it. It is immediately obvious, that this is a reasonable format for vacation pictures, but it is not sufficient for scientific images. The minimum information we need to keep with the file is a calibration value, that tells us what the real world size of each pixel is, which is not the case for a simple file like this.

As most file formats were developed without a microscopist in mind, they simply do not have an option to store this information within the file itself. Some manufacturers have found a workaround by providing an additional text file where all this information is kept. Others are using more complex file types, which do provide methods for keeping the information. One of those file formats is TIF (or TIFF). This acronym stands for Tagged Image File Format and has grown to be the *de-facto* standard for imaging, mostly due to its flexibility.

The TIF file format defines a number of standard "tags"in the so-called header part of the TIF file, such as width, height, and other properties of the image. It also provides a method for anybody to incorporate their own, "private" tags. However, these private tags are usually not accessible or even known to the general public, so they only provide a consistent way of storing information for files from those manufacturers who share that information. TIF specifies, that unknown tags be ignored by the software that can't identify them. So, if company A stored magnification in a private tag, Software from company B will not read or correctly interpret this unless the information about it is shared between the companies.

Other uses of the TIF format lead to misinterpretation. For example, tag number 282 is the "Xresolution". While this seems to be a natural tag to store the image resolution or calibration, it leads to unsuspected results. Software, such as WORD or other programs use this value, together with the number of pixels in *x* and *y* to calculate the actual size of the image on paper. If a TEM image is acquired with, for example a 1 nm pixel resolution and an image width of 1000 pixels, and the information "1 nm" is stored in tag 282, it will be printed on paper at a size of 1nm x 1000 = 1 micron, definitely too small for any publication. Another issue can be the way 12 or 14 bit b/w images are interpreted. As there is no 12 or 14 bit file format, the images need to be stored as 16-bit files. This can then lead to black images (for example in Photoshop), because the application displays the full 16-bit. The 12 bits of information only fill the darker 6% (1/16th) of this range, and the image appears black. This can usually be fixed easily with an adjustment of the display levels. There is a new standard under consideration (EXIF), which combines features found in JPEG with TIFF features, and it remains to be seen if this new standard addresses these issues. The good news is, that all TIFF files should be compatible, as long as the writer and reader software adhere to the TIFF standards.

To avoid cumbersome re-calibration of images when they are transferred from one application to another, I suggest, that the Microscopy Society of America, as a representative organization of microscopists, develop a minimum set of parameters that need to be stored with an image. A subset of "MSA tags" in TIF files could then be defined to share information across applications. These tags should also be submitted to the body that defines the standard TIF tags and other standard setting authorities for incorporation into general standards.

Compression is also an issue that is often misunderstood. In short, there are two types of compression: lossy and loss-less. It is important to understand the differences. A "loss-less" compression, such as the one employed in the popular "ZIP" utilities, can restore a file to the exact form it was before. The compression ratios depend on the contents of the file, but are typically 2:1. A "lossy" compression, such as JPG, can result in much higher compression ratios (10:1 and higher), but it will change the content of the files. The choice is therefore to either preserve all the information in an image, or create a small file size. Both have their places: For publications or displays of images one can probably accept minor changes to the content, therefore JPG would be a reasonable choice. If the image needs to be processed further, any changes to the file could potentially introduce artifacts, which make the choice of loss-less compression obvious. In fact, MSA recently addressed the problem with a statement regarding ethical digital imaging (*Microscopy Today*, Vol 11, Number 6, page 61): "Ethical imaging requires that the original uncompressed image file be stored on archival media ...".

To demonstrate the losses, Fig.1 shows a test image in its original form. The uncompressed file size of this image is about 863 KB. Using loss-less compression, the file size can be reduced to about 140 KB. Using JPG compression and a "quality factor" of 50%, the file size can be reduced further to about 23 KB, which is almost a factor 40 smaller than the original image. However, the compression leads to artifacts and losses in the image. Fig. 2 shows the compressed image, and Fig. 3 shows the difference between the original and the compressed image. The contrast of this difference image has been increased to demonstrate the effect. The image shows the information that is lost by the compression, and it is immediately clear, that some of the information lost may prevent further image analysis.

So, what is the best file format to store images? Although there is no general answer to this question, TIF seems to be the best all-around format at this point, even with the shortcomings explained above. ■

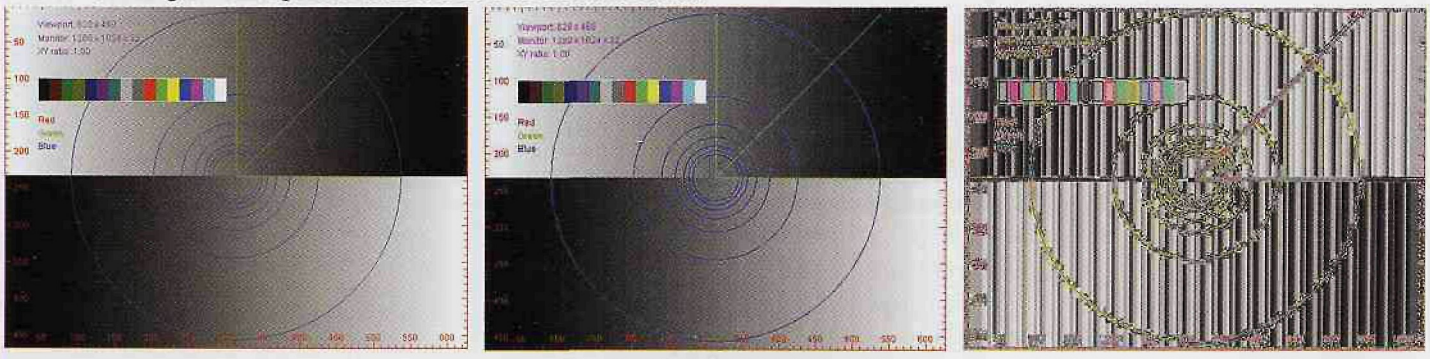

Fig.1 (Left) Test image . Fig.2 (Center) JPEG compressed image, quality factor 50%. Fig.3 (Right) Losses to Image in Fig 1 due to compression (contrast equalized to show details)

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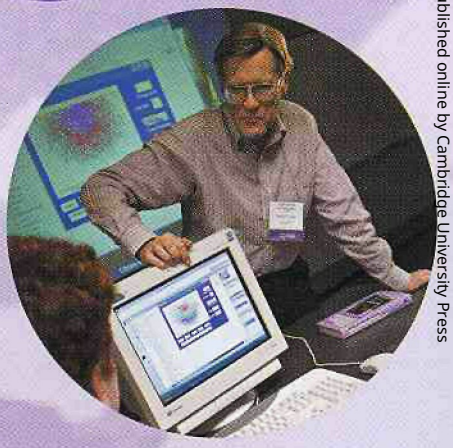

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This course provides an understanding of the concepts, instrumentation, and applications of the rapidly expanding field of scanned probe microscopes (SPM). AFM and STM will be covered extensively, while other SPM techniques will be covered in varying depths depending on the interest of students. The theory of operation for both imaging and spectroscopy will be addressed, with attention paid to instrumental artifacts and methods to avoid them.