
A novel method of illustrating microfungi

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Composite line drawings in most cases can represent the morphological characters of a fungus better than digital photographic images. Even so, the use of line drawings for illustrating microfungi in published literature has declined. A novel technique combining computer hardware and software to illustrate microfungi as line drawings is described in detail. This technique can be applied to drawing larger fungi and other disciplines such as entomology, where the effect of stippling is used.

Key words: drawing, morphology, taxonomy.

Introduction

Drawing has been used as a method to illustrate microfungi for more than 300 years, since the first published illustration by Hooke (1665). Since that time there have been some exquisite published illustrations of microfungi, including those by Tulasne and Tulasne (1863), Swart (1973; 1975; 1979a,b; 1981; 1982a,b,c,d; 1985; 1986a,b,c; 1987; 1988a,b), Delgado *et al.* (2005), Magyar *et al.* (2005), Hofmann and Pipenbring (2006), Hunter *et al.* (2006) and Nag Raj (1993), his book containing nearly 500 superb line drawings. In other publications line drawings have been provided alongside photomicrographs and provide additional data (Barber *et al.* 2005; Burgess *et al.* 2006; Carnegie *et al.* 2007). The importance of the line drawing should not be understated as there are numerous type specimens which consist of only a line drawing of the described organism. The detail that can be presented in a single composite drawing of a microfungus is rarely matched in a single photographic plate. Even so, it is becoming increasingly common to describe species of microfungi with only the presence of photographic plates (e.g. Ayala-Escobar *et al.*, 2006; Liu *et al.*, 2006; Raja *et al.*, 2006; Summerell *et al.*, 2006). This paper presents a novel method of illustrating microfungi whereby the traditional technique of drawing the specimen through a drawing tube is combined with a modern

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technique using computer hardware and software to produce a digital illustration.

Materials and Methods

Traditional technique

The traditional method of drawing requires the use of a drawing tube or camera lucida attached to a compound microscope. The specimen is drawn by tracing over the viewed image onto a sheet of paper. There are a variety of methods used from thereon. One commonly used method is to carefully trace over the original image onto tracing paper with a felt-tip pen. The final step of the illustration in most cases would require extensive stippling of the spores to give the effect of pigmentation, shape, ornamentation and other details of taxonomic importance. It is this process of stippling that is extremely time consuming as many hundreds of small dots may need to be completed and these must be carefully placed to ensure they do not have ‘tails’. The final product is then submitted for publication as an original, a photocopy or scanned copy in a digital format.

Novel technique

The technique described here includes the traditional technique of tracing an image of the viewed specimen onto a sheet of paper. This is completed in ‘grey-lead’ pencil to ensure mistakes can be altered. Notes are made on these sheets, and digital images are acquired of the specimen at the time of drawing to aid with the representation of the pigmentation and ornamentation of morphological structures. From hereon the method differs to the traditional technique.

These original images are scanned at medium resolution [approximately 300 dots per inch (d.p.i)] and saved in a suitable format (e.g. .bmp, .tiff, .jpeg or .pdf) ensuring that a scale bar is included in the drawing. A new document with a resolution of at least 600 d.p.i. is created in an illustrating software program such as Macromedia FreeHand (www.macromedia.com), but there are many others such as Adobe Illustrator (www.adobe.com) or Corel Draw (www.corel.com) that can be used. This will be the final resolution or ‘quality’ of the image created and is often the minimum resolution required for publication by scientific journals. The original drawing is then imported into the program and placed onto the new document as a new layer (Fig. 1a). The following steps in the method are far easier to achieve using a Graphics Pen

Tablet attached to the computer rather than a traditional mouse. The first author uses a Wacom Graphire tablet (www.wacom.com).

Within Macromedia FreeHand there are many drawing 'tools'. The 'Pen' tool is selected and used to trace over the top of the original image. The 'Zoom' tool allows the user to magnify the image to ensure the line is traced accurately, and the 'Hand' tool allows the user to move over the page while drawing. The pen tool has handles that can be dragged to alter the curvature of the line being drawn (Fig. 1b). These lines are completely traced over the original image (Fig. 1c) and once the original image is deleted, the final result shows a very sharp line drawing (Fig. 1d) that can be saved in a suitable vector format (e.g. .eps, .ai, .cdr). If a mistake is made it can easily be removed using the 'Undo' function. The final vector format of the line drawing can be exported to virtually any size and resolution, making it ideal for the production of large posters or displays. This is not possible using a more conventional scanned image of an original without a reduction in resultant image quality.

There is more than one way of compiling the final outline of the composite drawing from this point forward. Each drawing can be cut and pasted into the same document and modified to the same scale. Alternatively, a new document is created in an image editing software package such as Adobe Photoshop CS (Adobe, www.adobe.com), with a resolution of at least 600 d.p.i. and an 8-bit greyscale colour mode. The previously saved vector file is placed into the new document and transformed to the desired size using the 'Transform' function. It is important at this stage to ensure that the scale bar saved in the vector file is present prior to transformation, and it is also advisable to place all components of your figure, which may include a number of vector files, into the Photoshop document prior to transformation to ensure they are all drawn to the same scale.

The next step will ultimately determine the pattern of stippling that will be given to the final drawing, enabling the morphological structures to be accurately represented according to their pigmentation, ornamentation, shape and other features. This step can be disregarded if the morphological structures are hyaline. If the structures are pigmented, then the 'Magic Wand' tool is used to select the area within the outer cell walls. To give a uniform appearance to the structures, the 'Paint Bucket' tool can be selected and then a suitable shade of grey filled into the internal area. Alternatively, to give more body to the structures and varying degrees of pigmentation, the 'Brush' tool is selected and used to shade the internal areas of the morphological structures (Fig. 1e). The size, mode, opacity and flow of the brush can be altered to give the desired effect. Select using the 'Magic Wand' tool the internal areas of the structures previously brushed. A lower tolerance level in the settings of the 'Magic Wand'

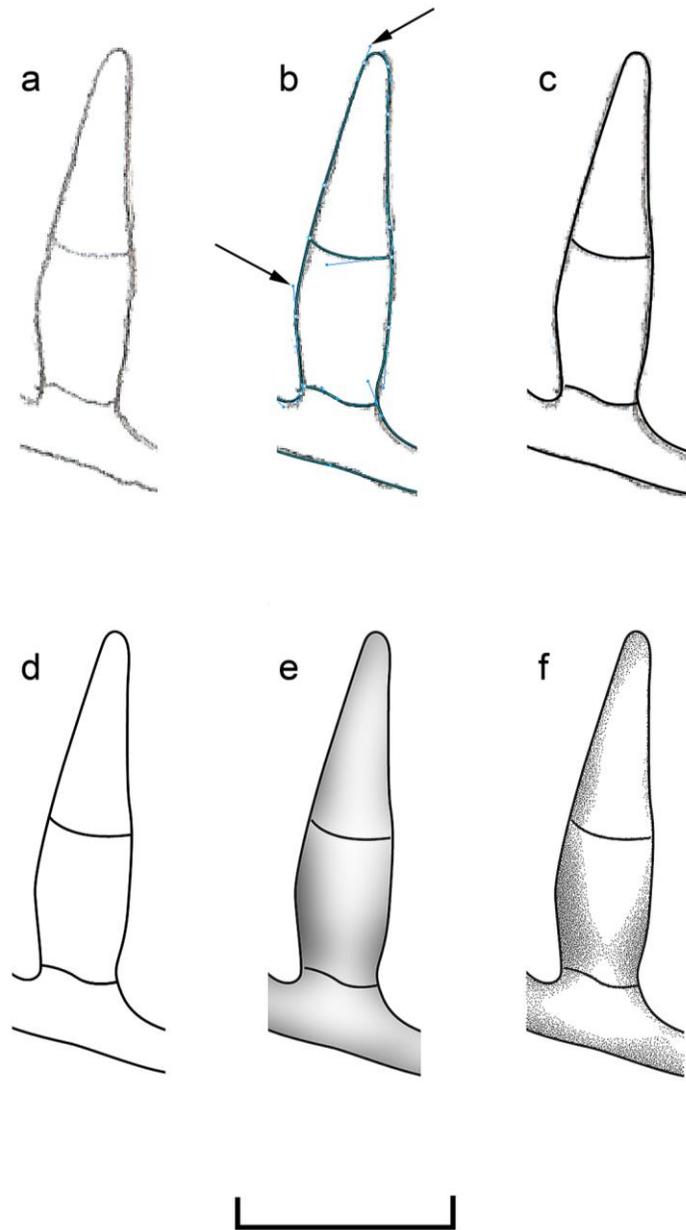


Fig. 1. Individual steps of drawing a conidium using the novel method. **a.** Scan of the original drawing through the drawing tube. **b.** Completed tracing over the original drawing using the 'Pen' tool. Handles of the 'Pen' tool arrowed. **c.** Completed tracing over the original drawing. **d.** Final tracing as imported in Adobe Photoshop after the original background image has been removed. **e.** Internal cells of the spore after the 'Brush' tool has been used. **f.** Final image after the Andromeda Screens 3 Filter has been applied to the brushed conidium. Scale bar indicates 10 μm .

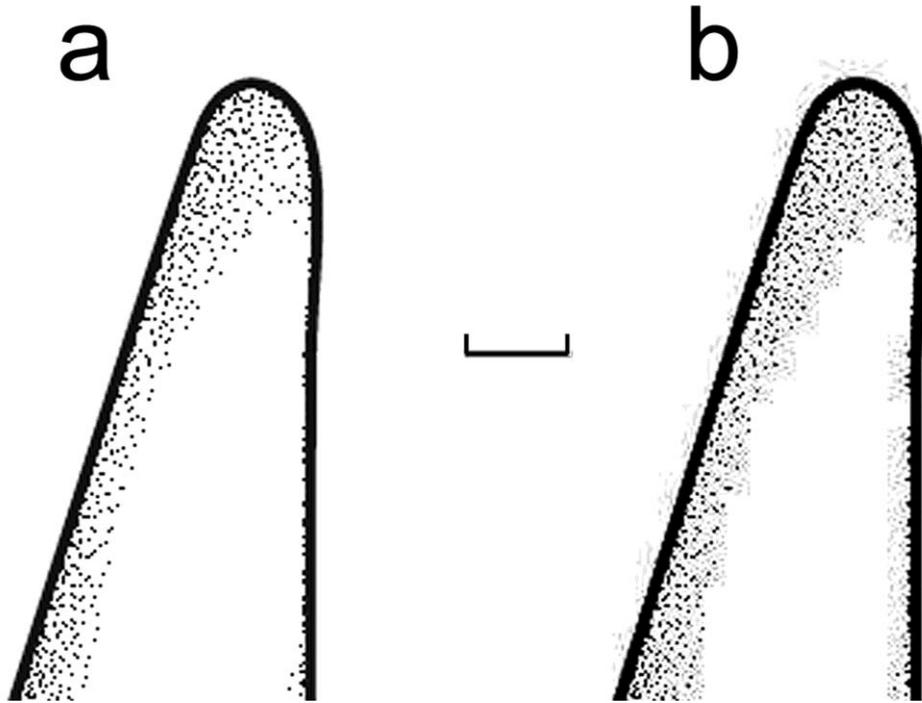


Fig. 2. Apical tip of the drawing represented in Fig. 1. **a.** Completed drawing showing the sharp, discrete stipples produced when the image is saved in an uncompressed tiff format. **b.** Completed drawing showing the artefacts that appear when the image is saved in a compressed jpeg format. Scale bar indicates 1 μm .

tool will almost certainly need to be selected to enable complete selection of the brushed zones. Finally, a Series 3 Screen Filter (Andromeda Software Inc., www.andromeda.com) available as a Plug-in for Adobe Photoshop is used to apply a stipple effect to the brushed drawing. The number of dots per inch can be adjusted within the filter and a range of different effects applied. It is also important here to mention that mistakes can be removed using the 'History' toolbar in Adobe Photoshop.

Upon completion of the drawing it is important to save the image in an uncompressed format such as a .tiff file (Fig. 2a). This ensures the individual dots representing the stippling effect are retained as discrete sharp dots. If the image is saved as a compressed .jpeg file at this stage artefacts appear in the image and the resultant image is of poorer quality (Fig. 2b).

The technique detailed here has been used to describe and illustrate a wide range of fungi of varying shapes, sizes, pigmentation and ornamentation (Fig. 3). The composite drawing of the conidiogenous cells and conidia of *Neofusicoccum macroclavatum* (T. Burgess, Barber & G. Hardy) T. Burgess,

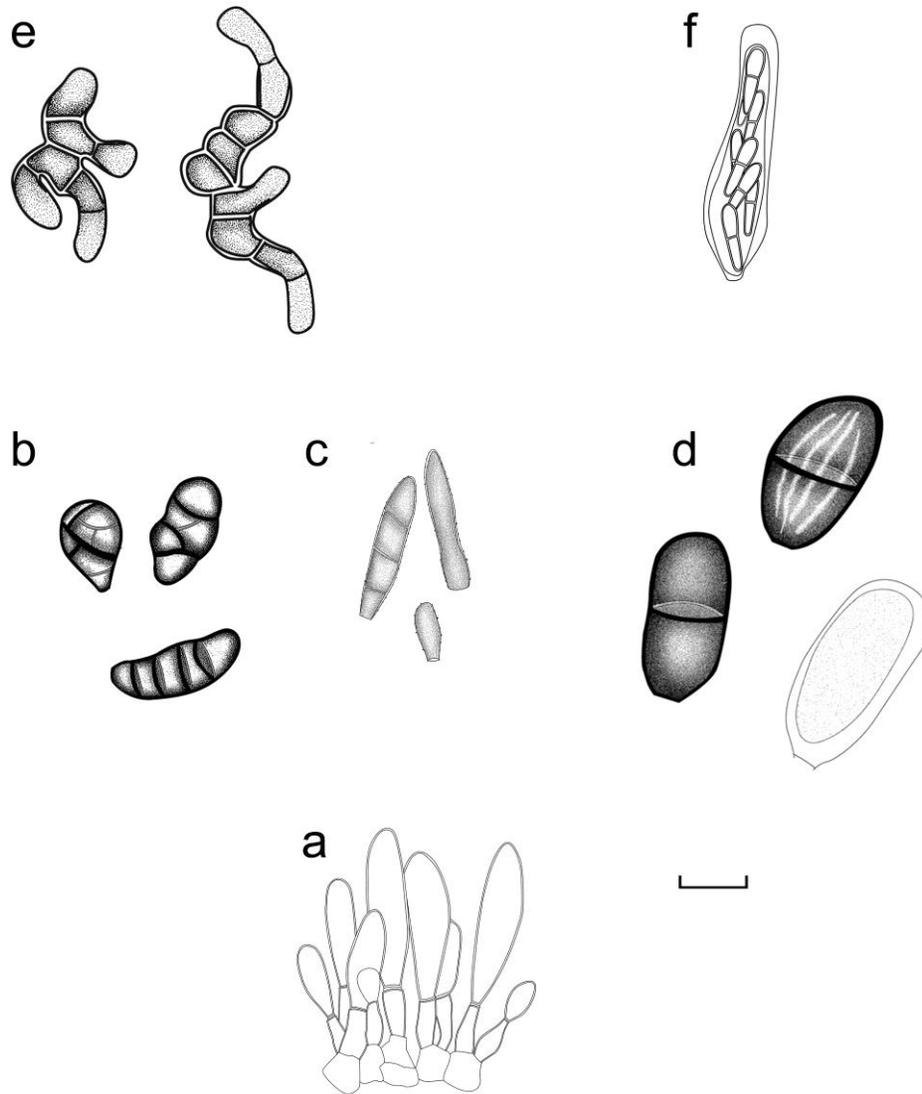


Fig. 3. Morphological structures of varying shapes, sizes, pigmentation and ornamentation illustrated using the novel method. **a.** Hyaline conidiogenous cells and conidia of *Neofusicoccum macroclavatum*. **b.** Muriform, pigmented conidia of the *Dichomera*-like synanamorph of *Neofusicoccum parvum*. **c.** Verruculose and pigmented conidia of *Kirramyces angophorae*. **d.** Subhyaline to pigmented conidia of *Lasiodiplodia rubropurpurea* with and without striations. **e.** Germinating and pigmented ascospores of *Mycosphaerella parva*. **f.** Hyaline ascospores of *Mycosphaerella yunnanensis* within an ascus. Scale bar indicates 10 μm .

Barber & G. Hardy, (Fig. 3a) was completed by moving in Macromedia FreeHand a background original drawing of a several individual drawings of conidiogenous cells and conidia and tracing over the top. Conidia can be drawn with complex features as shown for the muriform, pigmented conidia of the *Dichomera*-like synanamorph of *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (Fig. 3b). Conidia can also be given the appearance of being verruculose as shown for *K. angophorae* Andjic, Carnegie & Barber (Fig. 3c) by placing small dots on the outer wall of the spore using the 'Pen' tool in Adobe Photoshop. Pigmentation and ornamentation of conidia can vary greatly within a species as they mature, as shown for *Lasiodiplodia rubropurpurea* T. Burgess, Barber & Pegg (Fig. 3d). The effect of striations along the internal wall of the mature conidia was produced by creating a custom brush in Adobe Photoshop and applying this as individual striations post-stippling. Finally, the novel method can be easily applied to much smaller ascospores that can be illustrated as pigmented and germinating as in the case of *Mycosphaerella parva* R.F. Park & Keane (Fig. 3e) or hyaline and yet to be ejected from the ascus as shown for *M. yunnanensis* Barber & T. Burgess (Fig. 3f).

Discussion

The illustration of microfungi has become less common with the development of digital cameras that can be attached to microscopes, however, in the majority of cases more information about the morphology of these microfungi can be presented in a composite line drawing (e.g Barber *et al.* 2005; Burgess *et al.* 2006; Walker *et al.* 2006). The development of a novel technique using both computer hardware and software greatly reduces the time required to complete line drawings after they have been compiled through a drawing tube. This technique also has many other advantages including the ability to export the line drawing in virtually any resolution and the capacity to remove mistakes with the push of a button. As shown, this technique can be applied to fungi with varying morphological features and it has been recently used to describe and illustrate new and interesting microfungi (Barber *et al.*, 2005; Burgess *et al.*, 2006; Andjic *et al.*, 2007a,b; Barber *et al.*, 2007; Burgess *et al.*, 2007).

A substantially greater length of time is required to illustrate microfungi under the microscope in comparison to acquiring digital photographic images. This is perhaps the reason why line drawings have become less common in recent times. It can be argued that the effort required to complete drawings encourages the illustrator to be more observant of the morphological features of

the subject and therefore acquire a greater understanding and knowledge of the morphology of the fungus. It is for this reason that the author believes students of mycology and fungal taxonomy should be encouraged to line draw wherever the morphological features of the fungus are important for characterising a species, and in particular, where new species of fungi are being described. The increased focus on DNA-based studies (e.g. Cai *et al.*, 2005; Kodsueb *et al.*, 2006) has seen a dramatic reduction in the number of students with the skills to correctly use a microscope to gain a sound understanding of the morphology of fungi.

The technique described can be applied not only to microfungi but also to larger fungi and invertebrates. It would be most useful for drawings that require stippling and publications consisting of a large number of line drawings (e.g. a book, thesis or monograph). It is also ideal for images presented in posters and large displays.

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