

Multispectral macroscopy for mycology

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Introduction

During the recent years vision technology has become widespread for industrial applications, mainly for quality control.

Studies carried out on *Penicillium* species have shown that it is possible to classify species, even on the clone level, on the basis of descriptors (summarizing geometry and color) obtained from carefully calibrated color images (Dörge *et al.*, 2000; Hansen *et al.*, 2002a). These descriptors may potentially be used for generating databases allowing the comparison of fungal cultures grown in different laboratories.

These previous studies were carried out on traditional RGB images, which may not contain sufficient information to classify certain species. In such a case technology based on light emitting diodes (LEDs) may be useful. One of the major advantages of this technology is that it allows the user to design a wavelength set-up that is optimal for a given application. Furthermore, for technical reasons a higher image resolution becomes possible, which is essential in mycology where small structures may be important for the description of a given species. Near-infrared (NIR) wavelengths are of particular interest, as they may reveal information in highly pigmented regions, which are usually regarded as black. The result is a multispectral image (in this case a combination of ten images measured at ten different wavelengths).

Multivariate data analysis (Martens and Næs, 1992) applied to image descriptors is a powerful tool for revealing otherwise hidden data structures, which is useful, e.g. for automatic identification of different strains of fungi (Hansen *et al.*, 2002b). The related field of multivariate image analysis (Geladi and Grahn, 1996) is useful for dealing with multispectral images, reducing the data to simpler structures putting emphasis on the major variation in the objects under observation.

The purpose of this presentation is to show the potential of vision technology when multispectral images consisting of ten different wavelengths are analyzed using multivariate image analysis.

Materials and methods

The following species were chosen for the study: *Aspergillus flavus* (grown on a YES and a CYA medium (Samson *et al.*, 2000)) and *Stachybotrys chartarum* (grown on a V8 medium (Simmons, 1992)). The growing colonies were transferred onto their respective media using a 5 mm bore. The plates were incubated at 25 °C for 8 days.

Images were captured using a VideometerLab instrument (Videometer A/S, Denmark) equipped LEDs emitting light centered at the following wavelengths: 370 (UV), 428, 472, 503, 515, 592, 612, 630, 875 (NIR), and 940 (NIR) nm.

Image-PCA (Principal Component Analysis) was applied to the obtained multispectral images, producing so-called score images describing the main variation in each individual image. Image-PCA ranks the scores according to importance, the first score being the most important.

Image-PCA examples

An example showing how image-PCA works is given in Fig. 1 where a multispectral image consisting of five different wavelengths of a fungal culture (*Aspergillus flavus* grown on a YES medium) was obtained. The signals at the first four (visible) wavelengths are similar, while the image obtained at the NIR wavelength is very different, the latter revealing more information on the layers under the surface of the culture.

When image-PCA is applied to this data set, a new set of three images is obtained, accounting for almost all information contained in the five original images. The first score image accounts for 91 % of the total variance, and is very similar to the first four images. The second score image accounts for 8 % of the total variance, and is similar to the NIR image. The last score image accounts for only 1 % of the total variance and, as a result, also contains more noise than the first two score images, which is also visible. Thus, in the present example 99 % of the information in the five original images could be compressed into only two principal images, which are easier to handle in the subsequent data analysis and/or classification steps.

As a final step the three score images may be combined into a traditional RGB image with score images 1, 2, and 3 in the red, green, and blue channels, respectively. This results in a so-called pseudo-image focusing on the main sources of variation in the original multispectral image. Fig. 2 shows the original RGB image (a) as well as the pseudo-image (b) obtained in the example presented above. Due to the information from the NIR wavelength the pseudo-image contains structures from the base of the culture, usually only visible when the dish is turned upside down. Furthermore, more emphasis is put onto the yellow "grains" which are also observed in the RGB image.

Two further examples are shown in Figs. 3 and 4, where corresponding RGB and pseudo-images (obtained from image-PCAs) are presented.

In Fig. 3, *Aspergillus flavus* grown on a CYA medium is shown. The RGB image (Fig. 3a) is merely black-and-white due to strong pigmentation of the center region. The pseudo-image (Fig. 3b) reveals the structure of the base of the culture while preserving information on the amount of pigmentation.

In Fig. 4, *Stachybotrys chartarum* grown on an V8 medium is shown. In this case, the pseudo-image (Fig. 4b) clearly separates the image into growth medium and fungal culture. Furthermore, the culture (appearing black and white in the RGB image (Fig. 4a)) is separated into three very distinct components, revealing a new and otherwise undetectable structure.

Conclusions

From this preliminary study of a small number of fungal cultures it can be concluded that:

- The use of multispectral vision technology allows the user to fine-tune the image acquisition to use only the most informative wavebands for measuring specific fungal cultures.
- The extension of the ordinary visible wavelength range into the near-infrared reveals new useful information when images are captured. Structures that are usually hidden due to strong pigmentation can be seen.
- Multivariate image processing tools, such as image-PCA, allows for simple interpretation of otherwise complex multispectral images of fungi.

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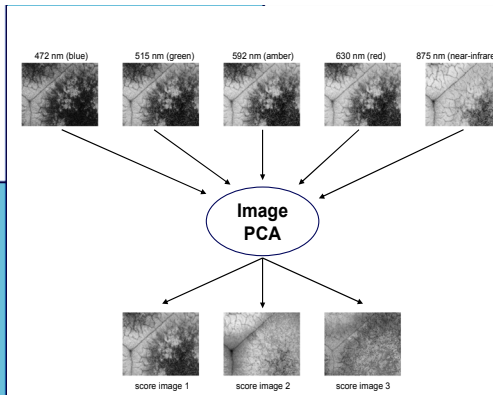


Fig. 1. Schematic representation of an image-PCA carried out on a multispectral image consisting of five wavelengths in the visible and near-infrared (NIR) range. The result is shown as three score images representing the first three major variations in the original image. The object is *Aspergillus flavus* grown on a YES medium.

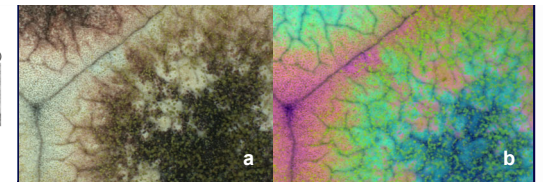


Fig. 2. Traditional RGB image (Fig. 2a) and pseudo-image (Fig. 2b) obtained as a result of the image-PCA carried out in Fig. 1. Structures from the base of the culture, as well as the yellow "grains" in the RGB image show up much clearer in the pseudo-image.

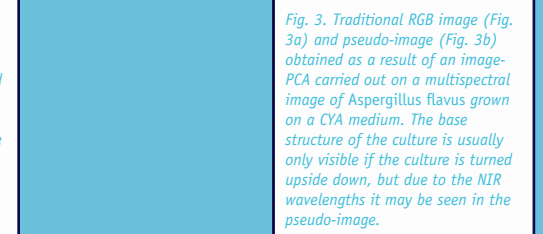


Fig. 3. Traditional RGB image (Fig. 3a) and pseudo-image (Fig. 3b) obtained as a result of an image-PCA carried out on a multispectral image of *Aspergillus flavus* grown on a CYA medium. The base structure of the culture is usually only visible if the culture is turned upside down, but due to the NIR wavelengths it may be seen in the pseudo-image.

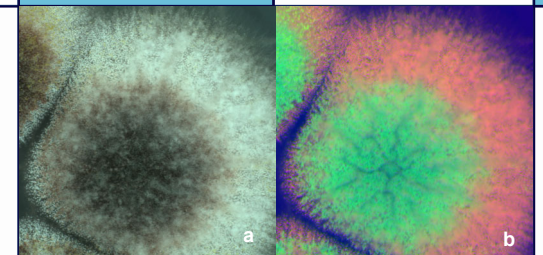


Fig. 4. Traditional RGB image (Fig. 4a) and pseudo-image (Fig. 4b) obtained as a result of an image-PCA carried out on a multispectral image of *Stachybotrys chartarum* grown on a V8 medium. In the pseudo-image the culture is separated into three very distinct components, revealing a new and otherwise undetectable structure.



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