Image processing methods for determination of downy mildews from light microscopy images

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ABSTRACT

In this paper we present image processing methods suitable for determination of downy mildews (fungi-like pathogens of crop plants) from light microscopy images of their conidiosporangia and conidiosporangiophores. The preprocessing of manually acquired microscopy images includes a multi-focus fusion algorithm robust to missing focal planes. The round-shaped conidiosporangia are segmented by means of thresholding and mathematical morphology and classified by their size and shape. The filamentous conidiosporangiophores are segmented by means of thresholding or matched filtering and classified by the structure of their branching pattern. The methods were tested on light microscopy images of species common in Central Europe.

Keywords: image processing, light microscopy, downy mildews, pathogen determination, image fusion, thresholding, morphological skeleton, matched filtering, pattern classification

1. INTRODUCTION

Plant pathogen diagnostics is currently based on a combination of host specificity, symptoms, microscopic characters (light microscopy), ultramicroscopic characters (scanning electron microscopy, SEM) and/or molecular markers [1]. Although the relevance of phenotypic characters has been discussed [2], microscopic investigation remains an indispensable method for determination of mycoses causal agents in phytopathological practice, particularly due to easy accessibility and low costs. Whereas, in comparison to a relatively objective analysis of molecular markers, it depends on systematic practice and experience of the plant pathologist, the other methods suffer from high costs, availability for a limited number of pathogens, and requirement of a pre-determination. In this work, we analyze image processing methods suitable for classification of downy mildews from light microscopy images of their asexual reproduction structures (conidiosporangiophores and conidiosporangia).

Downy mildews (Peronosporaceae, Chromista) are highly specific pathogens that cause severe infections of economically important crop plants. The diseases are easily spread in wet weather by microscopic conidiosporangia [3]. For the case study, species representing several genera with distinct morphomerical characteristics and frequent occurrence in Central Europe were selected: *Bremia lactucae* (lettuce downy mildew), *Plasmopara halstedii* (sunflower d.m.), *Peronospora destructor* (onion d.m.), *Pseudoperonospora cubensis* (d.m. of cucurbits), and *Phytophthora infestans* (potato late blight). The conidiosporangia are round objects characterized mainly by their shape (spherical / ovoid / ellipsoid, with / without papilla), as well as by their size and texture (see Fig. 1). The conidiosporangiophores are tree-shaped filamentous structures characterized particularly by their branching pattern (monopodial / sympodial / dichotomous / sparse).

2. METHODS AND RESULTS

The photographs were taken with the Olympus DP70 CCD digital camera attached to the Olympus BX60 light microscope with 200× and 400× magnification, respectively. In order to suppress degradations introduced during the acquisition process, several preprocessing steps are necessary.

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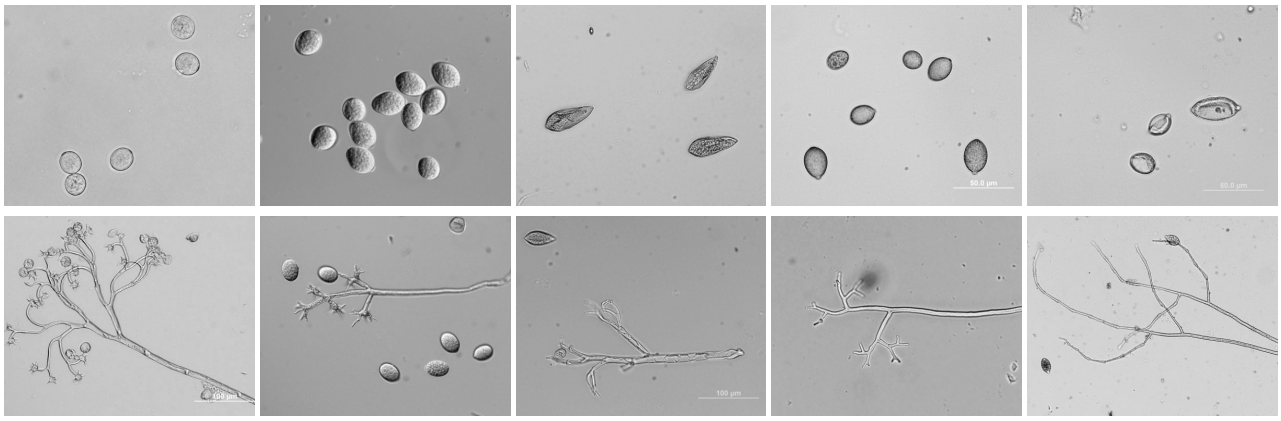

Fig. 1. Light microscopy images of conidiosporangia (top) and conidiosporangiophores (bottom) of *Bremia lactucae*, *Plasmopara halstedii*, *Peronospora destructor*, *Pseudoperonospora cubensis*, and *Phytophthora infestans* (from left to right).

The specimens are usually thicker than the attainable depth of field, and parts of them appear out of focus. Images acquired at different focal planes can be composed by means of multi-focus fusion [4] into one image with the whole specimen in focus. However, the step between documented focal planes is often so long that some parts of the specimen are not focused in any of the available images. The fusion algorithm has to be modified to deal with this problem. The value of each pixel in the fused image is computed as a weighted combination of its values in the images with the highest and the second-highest focus measure in a neighborhood of the pixel. The focus measure can be defined as a sum of gradient magnitudes over the neighborhood, for example. The resulting image is smoother and contains fewer artifacts than the classical fusion methods that use just the value from the image with the highest focus measure (see Fig. 2). The displacements between microscopic slides that might occur during manual shifting of focal plane have to be compensated for by image registration by means of a rigid-body transformation [5].

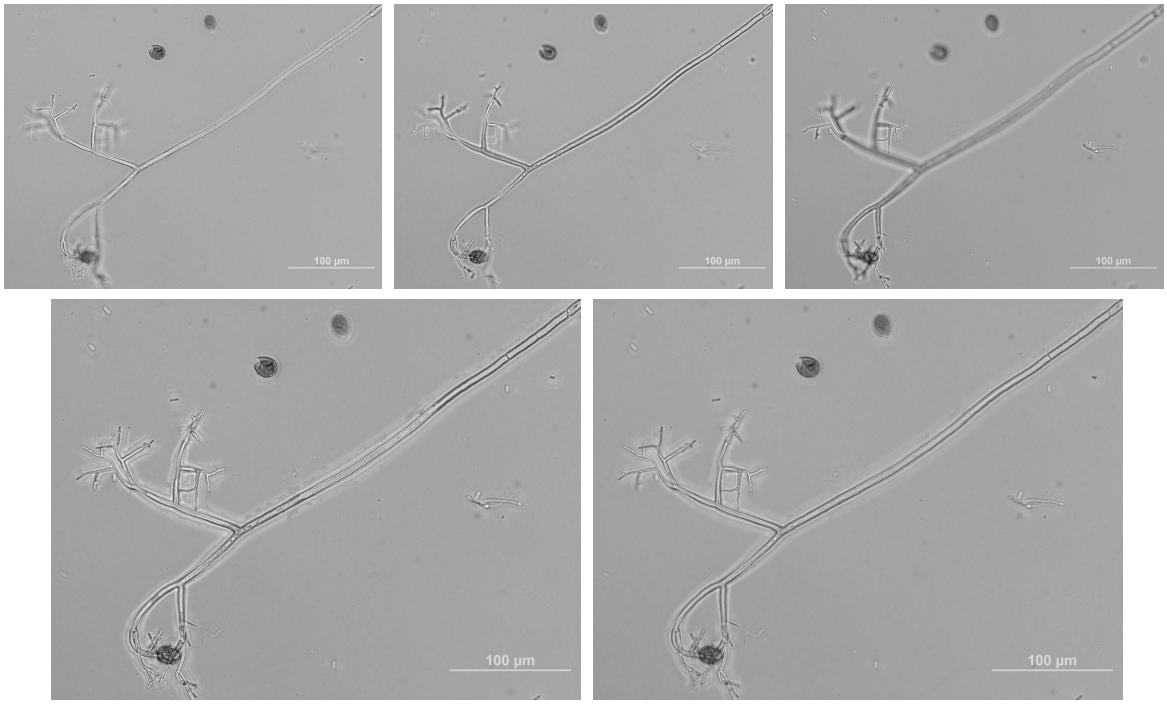

Fig 2. Light microscopy images from different focal planes (top). The results of multi-focus fusion by an algorithm that considers only the value from the image with the highest focus measure (bottom left) and one that combines values from the images with two highest focus measures (bottom right) weighted by the ratio of the focus measures (gradient magnitude).

The conidiosporangia are segmented from image background by means of a convenient segmentation method, e.g. adaptive thresholding. Mathematical morphology is employed to remove debris, fill in the objects, and rectify irregularities in shape. Objects that overlap each other or touch the border of the image are also removed. The remaining objects are labeled (see Fig. 3) and separately classified. For each object representing a conidiosporangium, convenient classification features are computed. The area of the segmented objects, their eccentricity, and Fourier descriptors [6], which describe the shape of the boundary by means of several Fourier coefficients of its 1D representation, proved best for this purpose. Color and/or texture can be used as well, but they are often affected by the imaging conditions. The features of all conidiosporangia in one image are used to classify the specimen. Unfortunately, due to high variability (which can be also influenced by environmental conditions) within some species (*Plasmopara halstedii*) and high similarity to other species (*Bremia lactucae*), some specimens may not be well determined only according to conidiosporangia. In such a case, a classification based on conidiosporangiophores is necessary.

Fig. 3. Segmentation and labeling of *Pseudoperonospora cubensis* conidiosporangia by means of thresholding and mathematical morphology.

The conidiosporangiophores are usually hyaline and their contour tends to blend with the background of the light microscopy image. As a result, it may be difficult to segment their shape simple thresholding. In such a case, a more sophisticated method, e.g. matched filtering [7], has to be employed. The matched filters are computed as average profiles of typical conidiosporangiophore segments of a particular width. The structure of a conidiosporangiophore can be represented by the morphological skeleton, a pixel-wide branching curve, which can be computed from the segmented image by means of a parallel thinning algorithm [8, Algorithm A1]. The skeleton consists of branches, i.e. linear segments corresponding to non-branching parts of the conidiosporangiophore. Due to common overlapping of branches, local features are used for the classification of conidiosporangiophores, namely the angle between branches and their curvature. They usually provide satisfactory level of discriminability among the species but may be difficult to compute due to common occlusions of branches.

Fig. 4. Segmentation and skeletonization of *Pseudoperonospora cubensis* conidiosporangiophore by means of thresholding and mathematical morphology.

To sum up, the computation of conidiosporangia features is relatively simple and the classification is robust to irregularities and outliers, as there are usually many conidiosporangia in one image. In some cases, however, it may not provide satisfactory discriminability. The classification based on conidiosporangiophores provides higher discriminability but lower robustness and the computation of features is more complex, particularly due to difficult segmentation and common occlusions. Combination of classifications based on both conidiosporangia and conidiosporangiophores is advisable where possible. The success ratio of the feature-based classification is, however, difficult to determine as it depends heavily on the input data, which may vary significantly, depending mainly on the cultivation environment and imaging conditions.

3. CONCLUSIONS

We have presented image processing methods convenient for the determination of downy mildews from light microscopy images of their conidiosporangia and conidiosporangiophores. These include a multi-focus fusion algorithm robust to incompleteness of documented focal planes and various segmentation methods. As a result, the selected species of downy mildews were mostly distinguishable by the properties of their asexual reproduction structures. Many future perspectives could be addressed, e.g. a combination with data from SEM and/or molecular analysis. Although the concept of biological taxons is in dynamic progress and the impact of morphological features for classification has decreased, the presented principles can be used in various biomedical applications.

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