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Application of a self-learning algorithm to analyse microscopic images of stomata

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Aim and relevance of research

The stomata on plant leaves are crucial for gas exchange and, when observed on (sub)fossil leaves, also **provide insight in historic plant growth conditions** such as atmospheric CO_2 and humidity. Current methods to **quantify stomatal traits** rely on manual **analysis of microscopic images**, which is labour intensive and requires expert knowledge.

Our study applies a **machine-learning approach** with **the goal to test the effectiveness** of two newly developed **automated detection methods for identifying and counting stomata**. We aim to expand this new method to **automatically measure stomatal features** (i.e. guard cells, epidermal cell properties) based on microscopic images of a broad phylogenetic sample of plant species. Our results compare automated methods developed by Jayakody et al. (2017; in prep.) with manual counts of stomatal densities of a **phylogenetically broad sample**, including angiosperms, gymnosperms and ferns (Fig. 1).

The use of computer-aided methods has the advantage to **increase efficiency in data acquisition** due to time-savings as the sample throughput is higher in a shorter time and thereby the potential of **more reproducible results**.

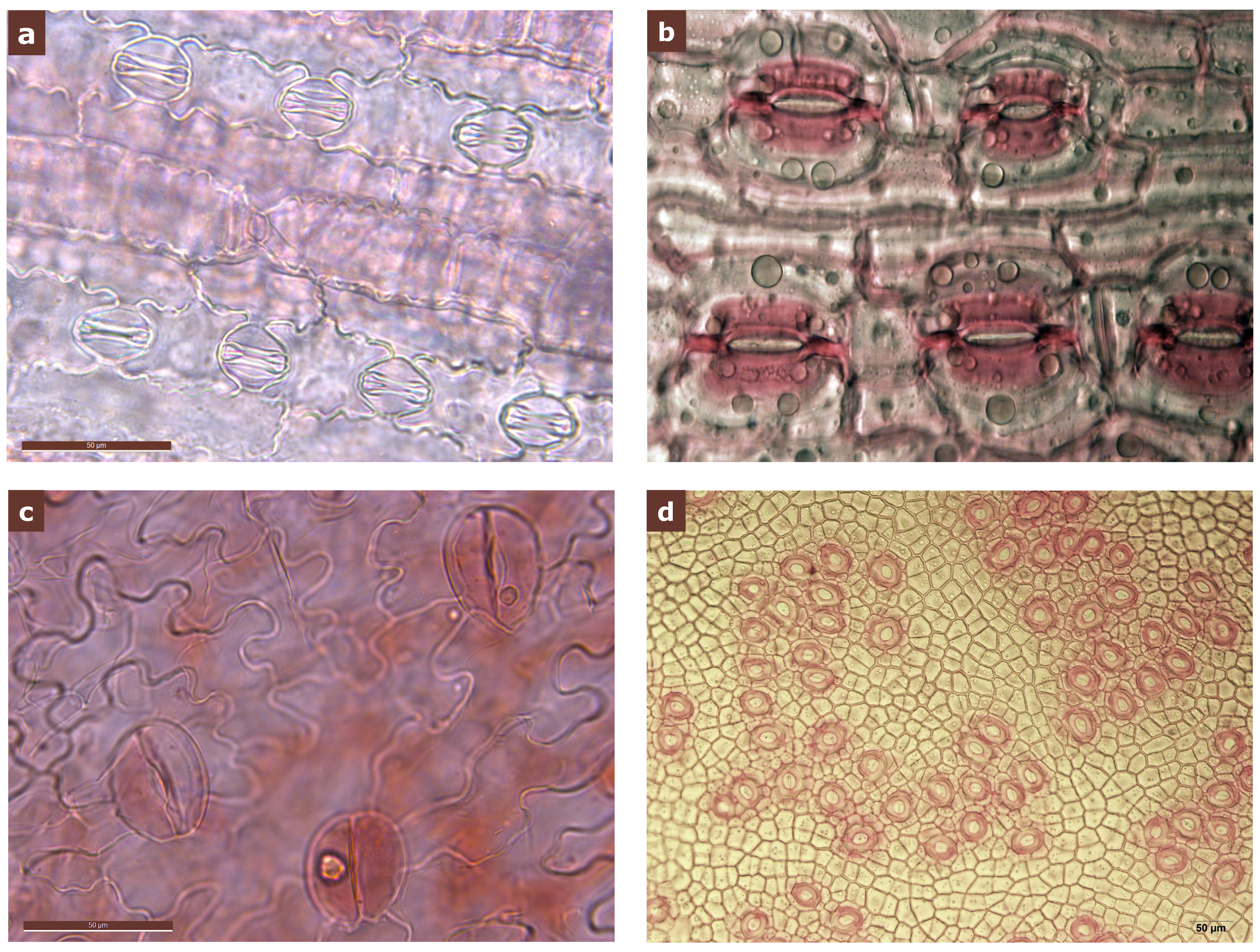

Fig.1 Microscopic images from each of the examined clades. (a) *S. bicolor* (angiosperm, grass); *(b) C. deodora* (gymnosperm); (c) *M. struthiopteris* (fern); (d) *E. wandoo* (angiosperm).

Methodology and results

- Using MATLAB® with a customized cascade object detection learning algorithm (Jayakody et al., 2017) to identify stomata based on Histogram of Oriented Gradients (HOG) which capture the overall shape of an object.
- By sliding a window over the image, the classifier decides whether an object of interest exists in that window based on provided positive (images containing stoma) and negative (no object of interest but backgrounds associated with the object) training sample images.

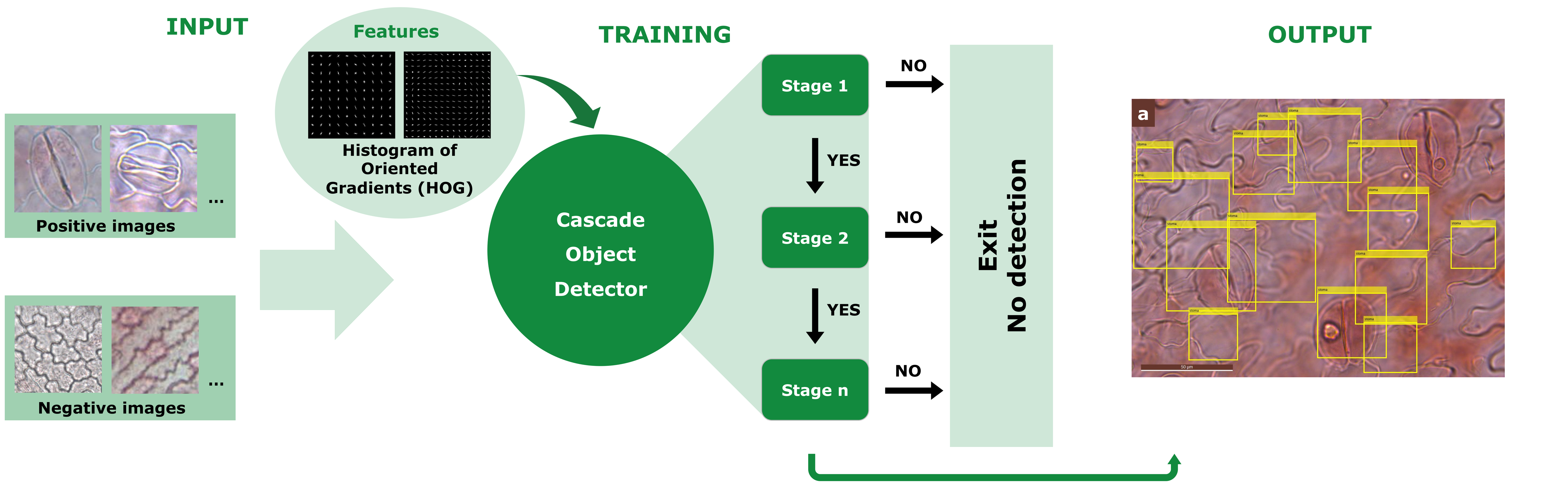

Fig. 2 Positive and negative training datasets serve together with the HOG feature descriptor as input for training the cascade object detector. The detector consists of stages which each apply binary classifiers. At each stage the classifier decides whether an object got detected or not (i.e. labelled as positive (YES) or negative (NO), respectively). Either the detection stops and the next region is classified, or the region is passed to the following stage. Only when the final stage classifies the region as positive, the detector reports an object. (a) Sample result of the automated method tested on microscopic images. The yellow bounding boxes show automatically detected regions of interest of Fig. 1c (precision 0.14).

Alternative test results: Artificial stomata

- Exploring the sensitivities of the detector to size, rotation, pore aperture and guard cell width ratio using simplified stomata with constant high image quality.
- Training with images of artificial stomata on a structured background while applying the same approach and object detector as above.

Results:

- Relatively high number of false detections and low detection precision like above (Fig. 2a).
- When forced to search for a certain size of the stomata, the results seem to improve (see Fig. 3b).

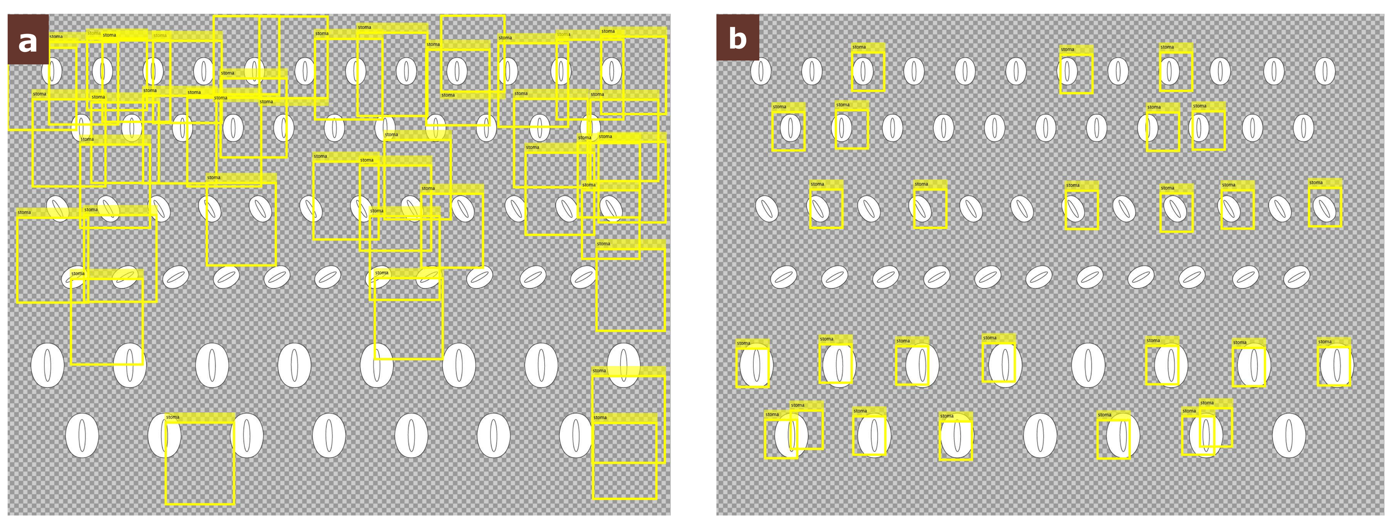

Fig. 3 Sample results of artificial stomata image with yellow bounding boxes showing automatically detected regions of interest (ROIs). (a) Automatic setting for object of interest (precision 0.05). (b) Forcing the algorithm to look for a pre-defined size of the object of interest (precision 0.88).

Findings and outlook

- The detector using HOG features is particularly **sensitive to size** and thus also to differing stomata from various plant types. Forcing the algorithm to detect for a certain size improves the results but makes it **necessary to know the object of interest size beforehand**.
- **Training on images of different magnifications is necessary**, which, however, is again time- and labour-intensive.
- To improve precision of detection, either **more data for training** the detector covering all sizes is required or **different detectors for different sizes (i.e. plant types)** will have to be trained.
- Cascade object detectors are not state of the art anymore (e.g. Dalal & Triggs, 2005) and **more advanced methods are being developed**.
- Testing the effectiveness of such new automated object detection methods (e.g. region based convolutional neural network (RCNN) method) is in preparation.
- In the future, object detection and counting can also be applied to **different fields, e.g. identifying pollen**.

References

Dalal, N., & Triggs, B. (2005, June). Histograms of oriented gradients for human detection. In 2005 IEEE computer society conference on computer vision and pattern recognition (CVPR'05) (Vol. 1, pp. 886-893). IEEE.
Jayakody, H., Liu, S., Whitty, M., Petrie, P. (2017). Microscope image based fully automated stomata detection and pore measurement method for grapevines. Plant Methods, 13(1), 94.