FLU/VIS registration approach to automated segmentation of VIS plant images

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With the introduction of high-throughput multi-sensory imaging platforms, automated analysis of multi-modal image data turned into focus of quantitative plant research. Two commonly used modalities in greenhouse plant phenotyping are, in particular, visible light (VIS) and fluorescence (FLU) images. The first step towards quantitative analysis of FLU/VIS images is segmentation of plant structures which typically relies on calculation of distance measures between plant and reference (background) images. Due to a number of technical and natural reasons, background regions of VIS plant images do not always represent an optimal reference to plant structures. Naturally occurring shadows and reflections in the background walls may contain similar colors as the relevant plant regions. In contrast, FLU images exhibit significantly higher contrast between chlorophyll-rich plant and chlorophyll-free background regions which makes them to a natural reference for detection and segmentation plan structures. Once aligned, the binary mask of segmented FLU images can be applied for extraction of plant regions in structurally more heterogeneous VIS images. To efficiently perform registration of thousands of FLU/VIS images, robust algorithmic solutions for unsupervised finding of correspondences between two structurally similar but, in general, non-identical images are required. For establishment of image correspondences different algorithmic approaches based on different image features have been proposed in the past. Here, we investigate three common techniques for image registration that rely on finding correspondences between (i) feature-points (FP), (ii) frequency-domain features (FD), and (iii) image intensity (i.e. Mattes mutual) information (INT) [1-5]. We apply these methods for alignment of time-series of FLU/VIS images of developing arabidopsis, wheat and maize shoots and quantify their performance in terms of robustness and accuracy measured by a direct comparison with manually segmented (ground truth) data, s. Fig. 1. Our experimental results indicate that INT-based approach shows a superior performance in comparison to FP and FD. However, all three techniques turn out to be sensitive to structural image distortions and require additional pre-processing steps including adaptive filtering, structural enhancement and characteristic scale selection. To overcome the limitations of conventional approaches, we develop an iterative algorithmic scheme which integrates the results of single-step registrations using different methods and pre-processing conditions into one single (integrated) segmentation mask. Our experimental results show that this approach allows to detect not only affine but also slightly non-rigid image transformations such as non-uniformly moving leaves which otherwise require application of computationally more expensive registration approaches. On the basis of insights gained in this study, we conclude that combination of different registration techniques, different scaling levels, different image representations (color-edge, grayscale) and filters enables significantly more robust and accurate results of image registration and segmentation compared to a single-step image alignment using one particular method and/or image pre-processing strategy.

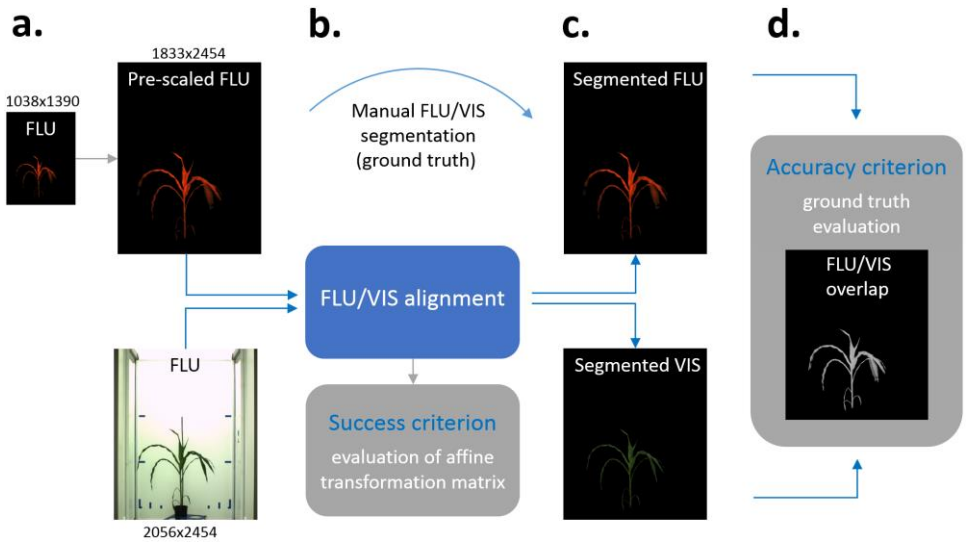

Figure 1. Scheme of evaluation of FLU/VIS image registration. **a**. FLU images are pre-scaled to the height of VIS images in order to improve robustness of subsequent affine registration. **b**. Registration of pre-scaled FLU and VIS images is performed and the resulting affine transformation matrix is evaluated w.r.t. admissible values of image translation, scaling and rotation. **c**. Affine transformation is applied to register manually segmented FLU and VIS images. **d**. To evaluate the accuracy of image registration, the overlap area between manually segmented VIS and registered FLU images is compared with the total area of manually segmented VIS images.

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