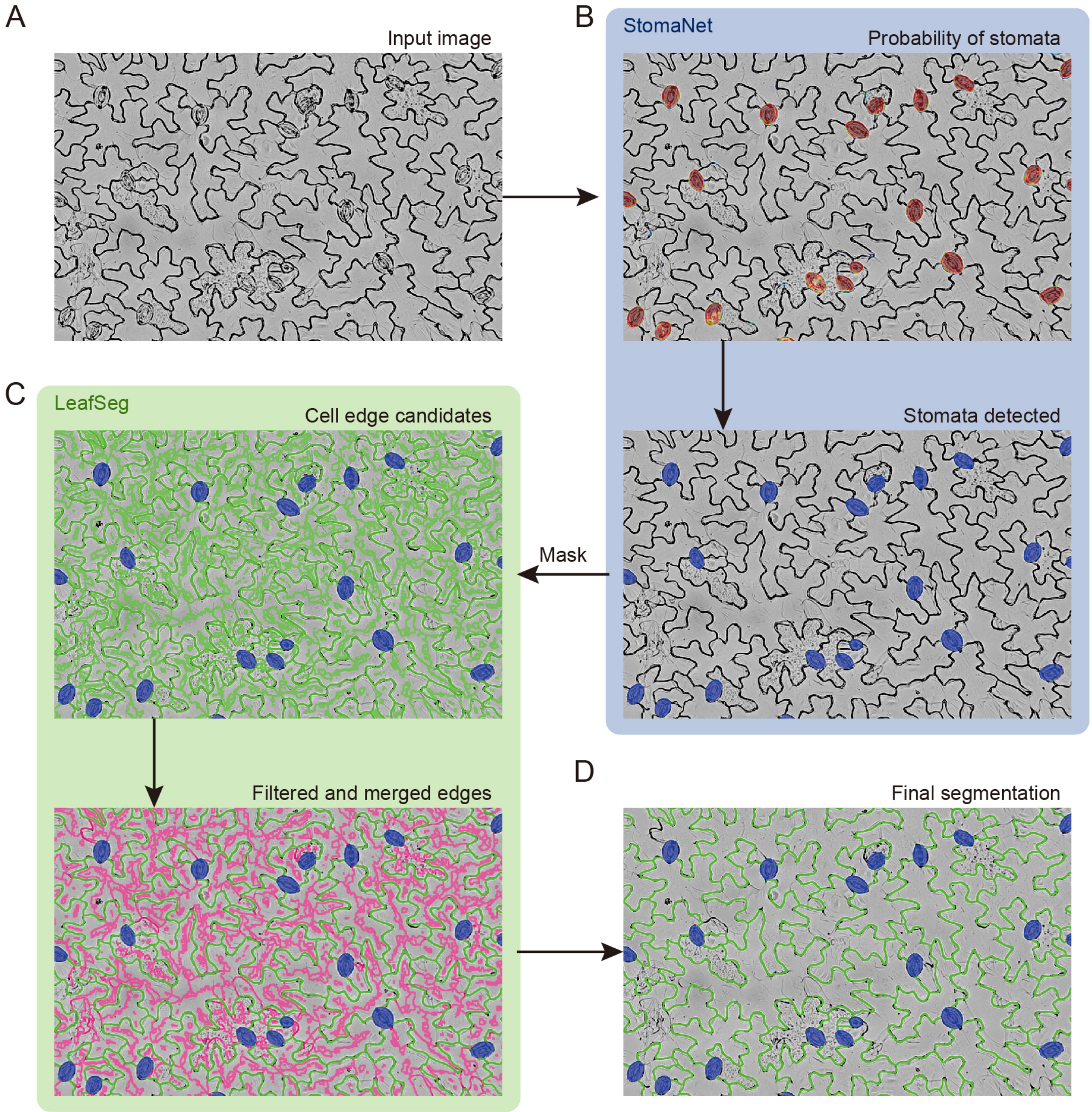
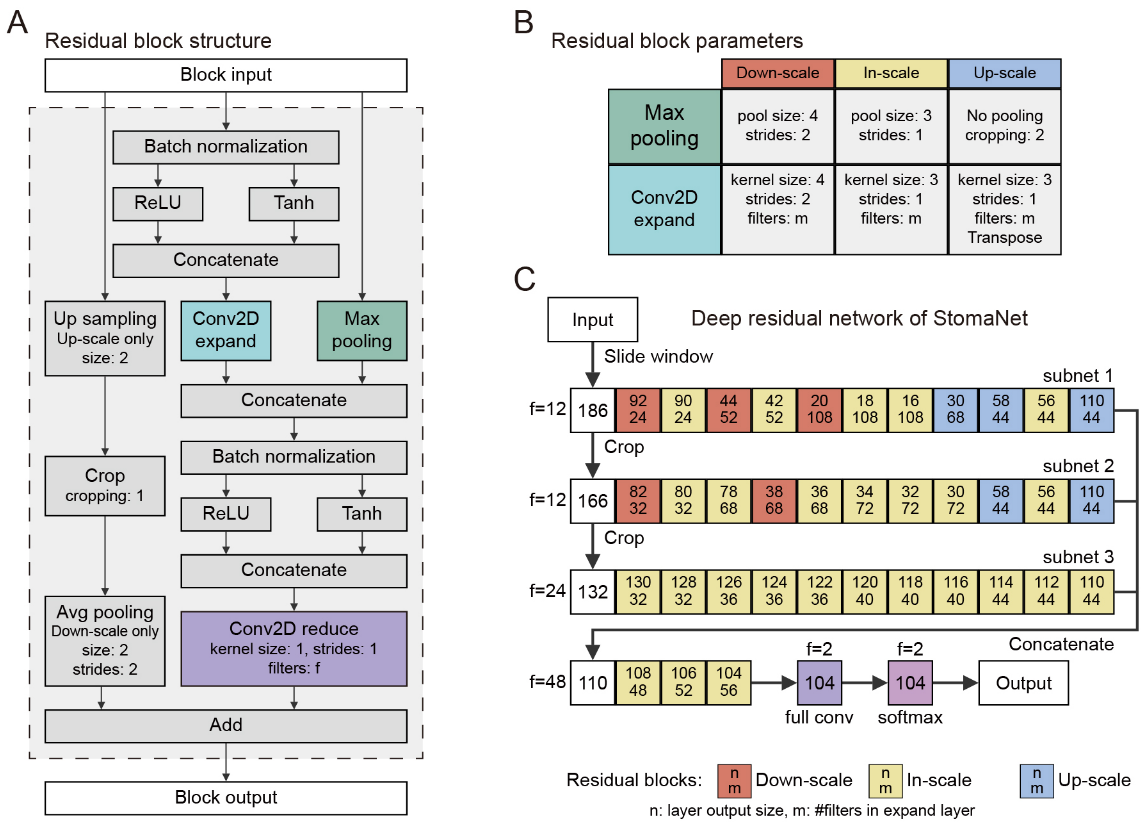
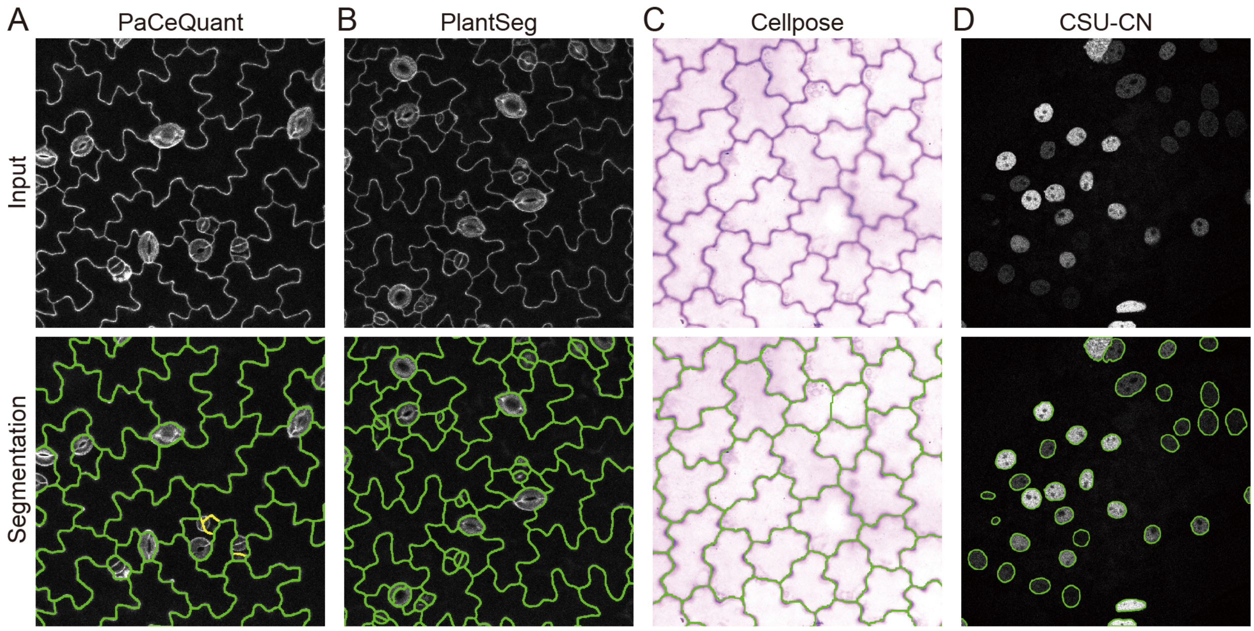


Supplemental Figure S1. Graphical representation of the LeafNet workflow (Supports Figure 1).

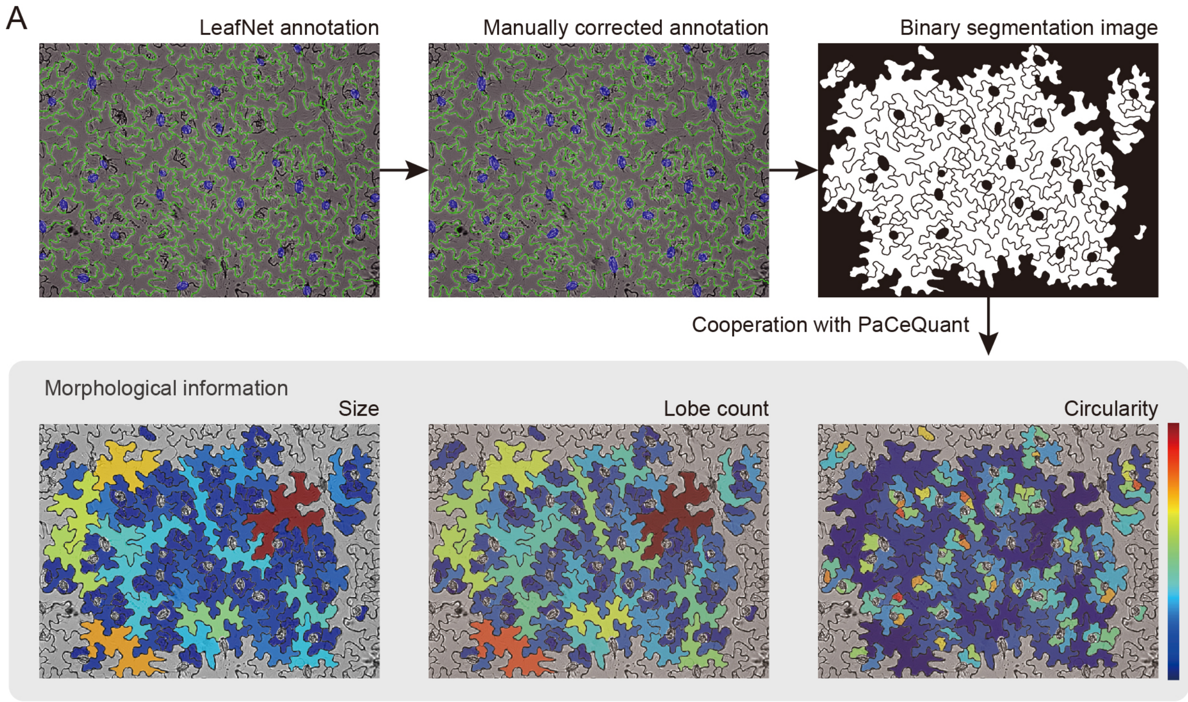
(**A**) Representative input image. (**B**) The StomaNet module for generating the probability heatmap of stomata (top) and for identifying reliable stomata (bottom). The identified stomata are masked in the input image for the next step. (**C**) The LeafSeg module for identifying edge candidates of pavement cells (top) and for detecting reliable edges (bottom, green). (**D**) Combined segmentation of stomata and pavement cells using LeafNet.



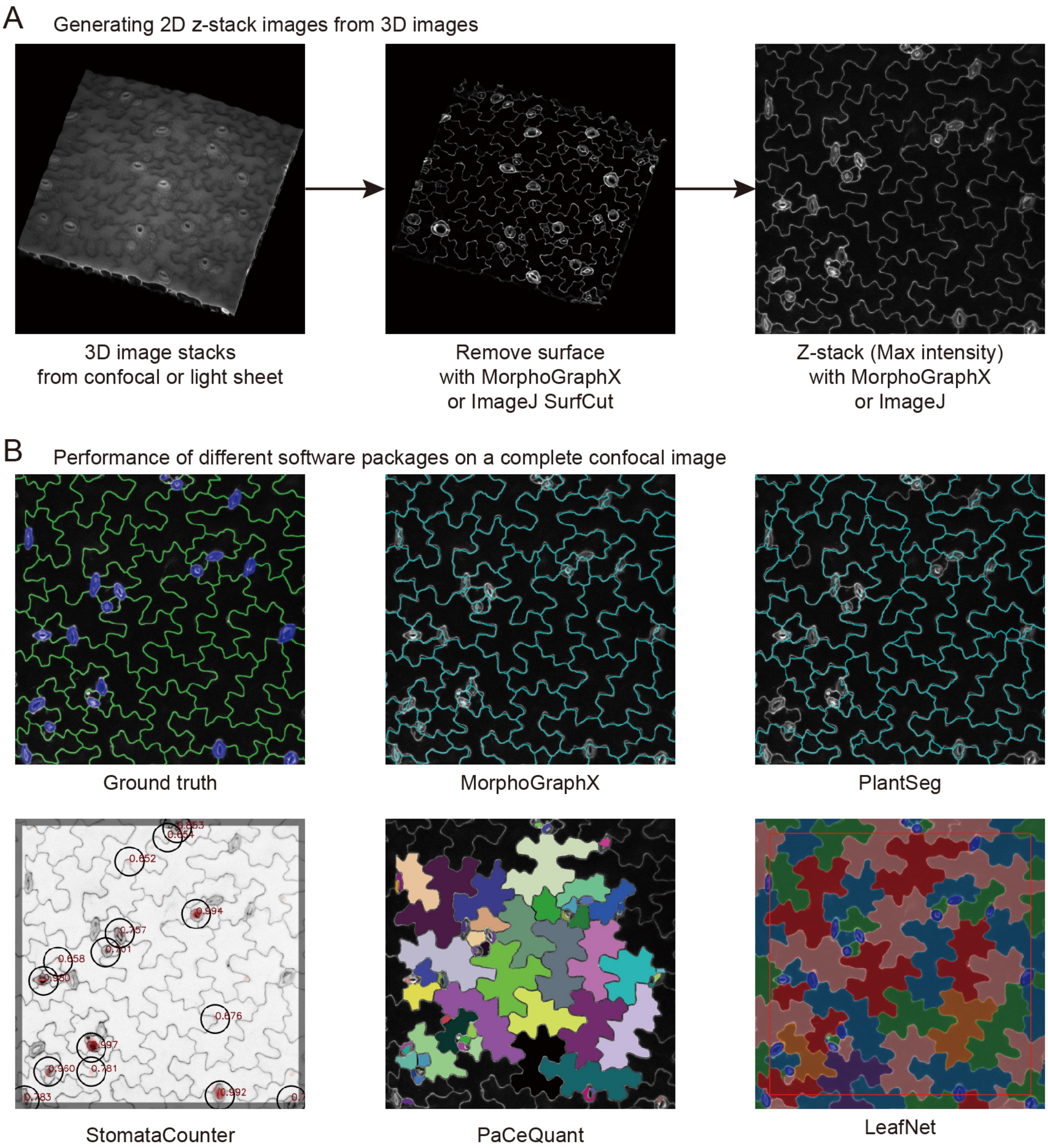
Supplemental Figure S2. Detailed structure of StomaNet (Supports Figure 1).
(**A**) The structure and parameters of a basic residual block. Input tensors are transformed with an expanding convolution (increasing filters), and a reducing convolution (decreasing filters). Max pooling and cropping layers are used as a tunnel for the information to be passed through directly, in speeding up the training process. Three types of residual blocks with this structure are used in the network to play different roles: Down-scale block for image size reduction and information extraction, In-scale block for information processing, and Up-scale block for recovering image size and generating pixel-wise segmentation. (**B**) The parameters of 'Max pooling' and 'Conv2D expand' in the three types of blocks. Other layers have the same parameters for the three types of blocks. (**C**) Detailed structure of the StomaNet module.



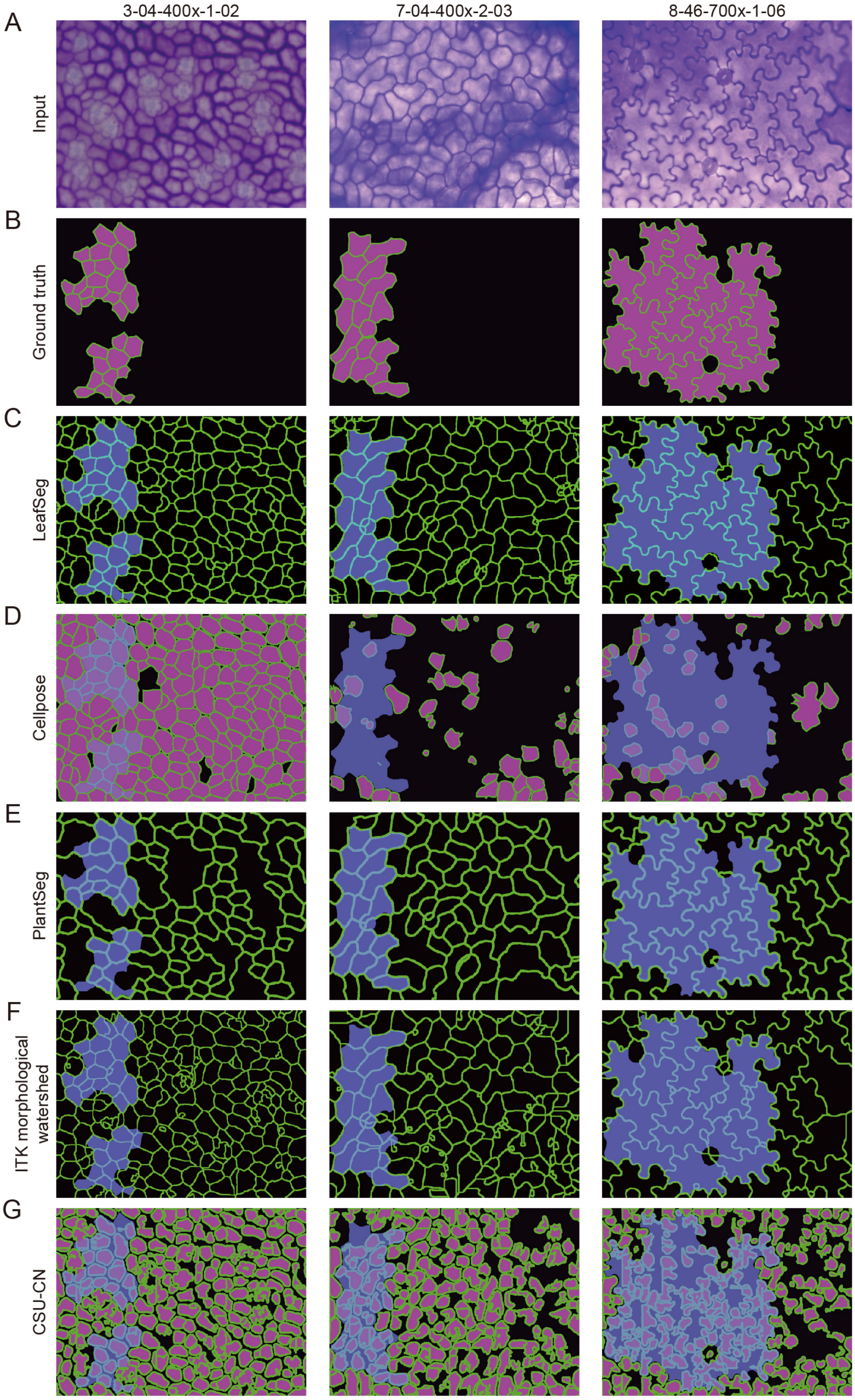
Supplemental Figure S3. Representative examples of segmentation results from four programs using their preferred images (Supports Figure 3).
(**A-B**) Input confocal image with maximum intensity z-projection (top) and the segmentation results (bottom) using PaCeQuant (**A**) and PlantSeg (**B**). (**C**) Input bright-field image with regularly shaped cells (top) and the segmentation results (bottom) using Cellpose. (**D**) Input image from the testing dataset of Fluo-N2DH-GOWT1 (top) and the segmentation results (bottom) using CSU-CN.



Supplemental Figure S4. Integration of LeafNet with PaCeQuant (Supports Figure 3).
(**A**) The procedure of integrating LeafNet and PaCeQuant for collecting morphological parameters. The annotation image generated by LeafNet with or without manual correction is used to generate a binary segmentation image for PaCeQuant. Morphological parameters generated by PaCeQuant are collected in LeafNet for integrated visualization.



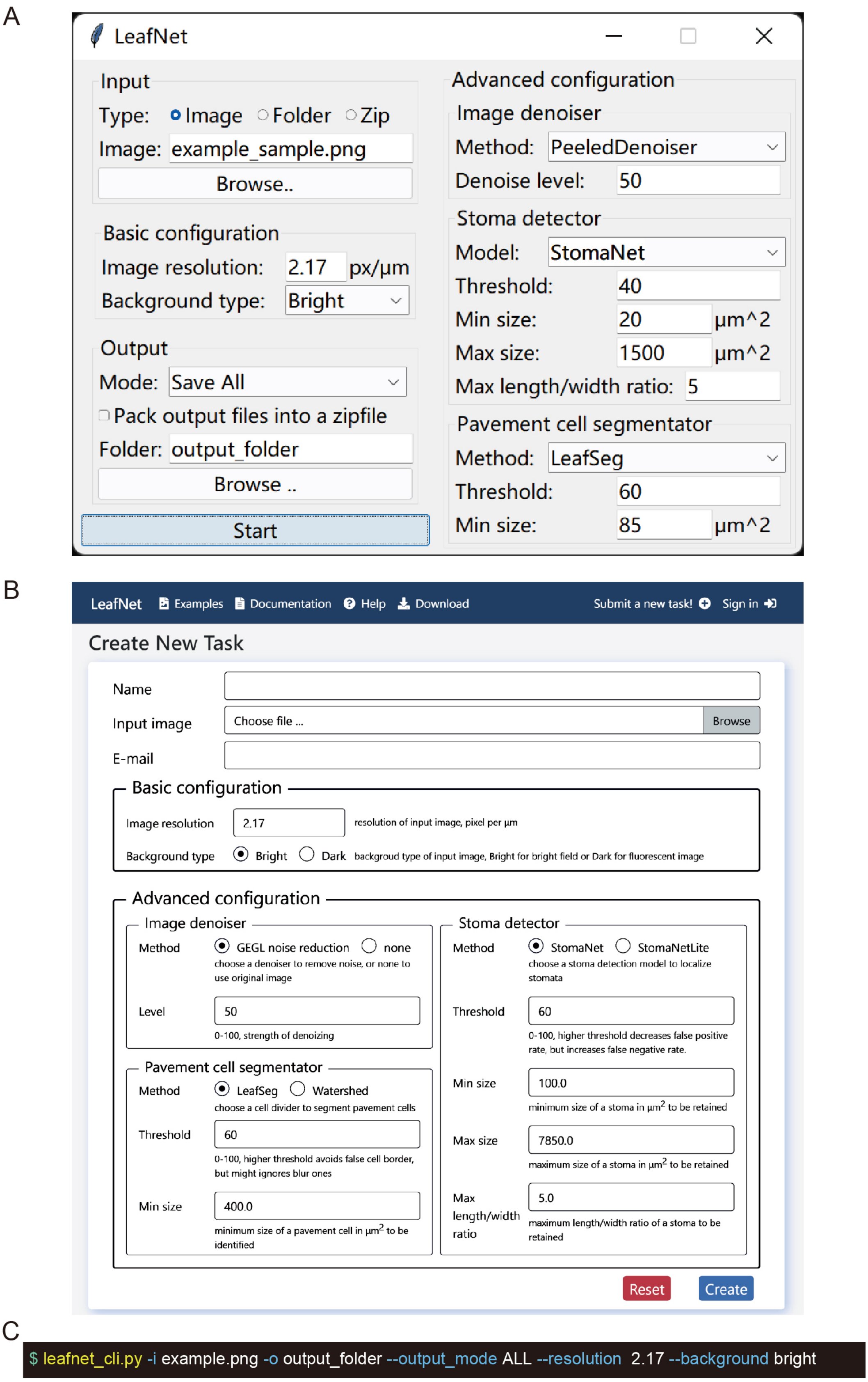
Supplemental Figure S5. Pre-processing of a 3D image and representative results from five programs (Supports Figure 5).
(**A**) The procedure of converting 3D image stacks from confocal or light sheet microscopes into 2D z-projection images. (**B**) The results of using five different software packages for a representative confocal image. The ground truth image is manually labeled with stomata in blue and pavement cell borders in green. StomataCounter only detects stomata with high probabilities. MorphoGraphX and PlantSeg only segment pavement cells with borders in light blue. PaCeQuant only segments complete pavement cells not adjacent to image edges. LeafNet simultaneously detects stomata in blue and generates segmented pavement cells in different colors.



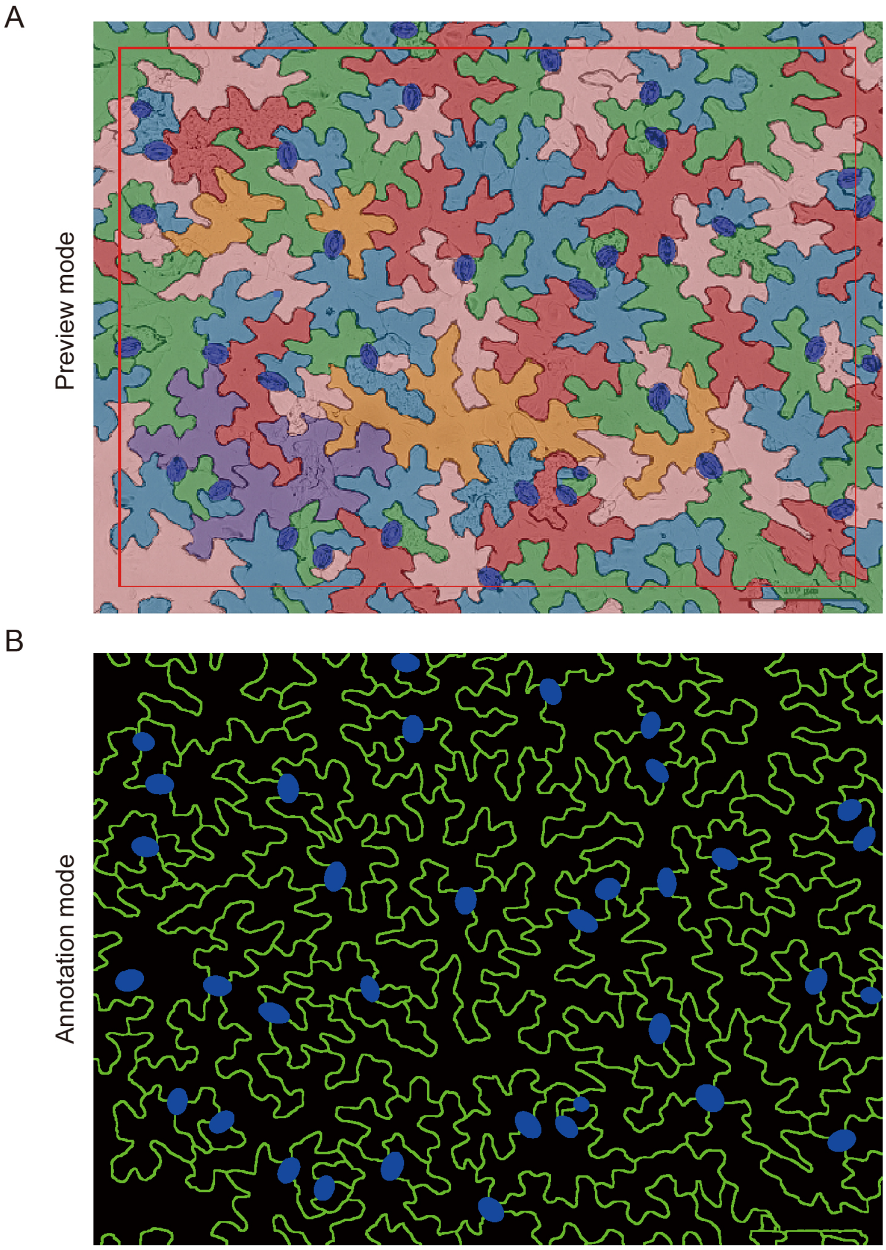
Supplemental Figure S6. Representative examples of segmentation results from five tools using complex datasets (Supports Figure 6).

(**A**) Three representative input images, including 3-04-400x-1-02 with regularly shaped cells and stomata, 7-04-400x-2-03 with regularly shaped cells and uneven lightening, and 8-46-700x-1-06 with puzzle-shaped cells. (**B**) The ground truth segmentation of pavement cells in Vőfély dataset. (**C-G**) The segmentation results of five different tools, including LeafSeg (**C**), Cellpose (**D**), PlantSeg (**E**), ITK morphological watershed (**F**), and CSU-CN (**G**) for the three input images in **A**.

Supplemental Data. Li et al. (2022). LeafNet: A Tool for Segmenting and Quantifying Stomata and Pavement Cells. Plant Cell.



Supplemental Figure S7. User interfaces for LeafNet (Supports Figure 7).
(**A**) Graphical interface of LeafNet standalone program. (**B**) Web interface of LeafNet web server. (**C**) Command-line interface of LeafNet program.

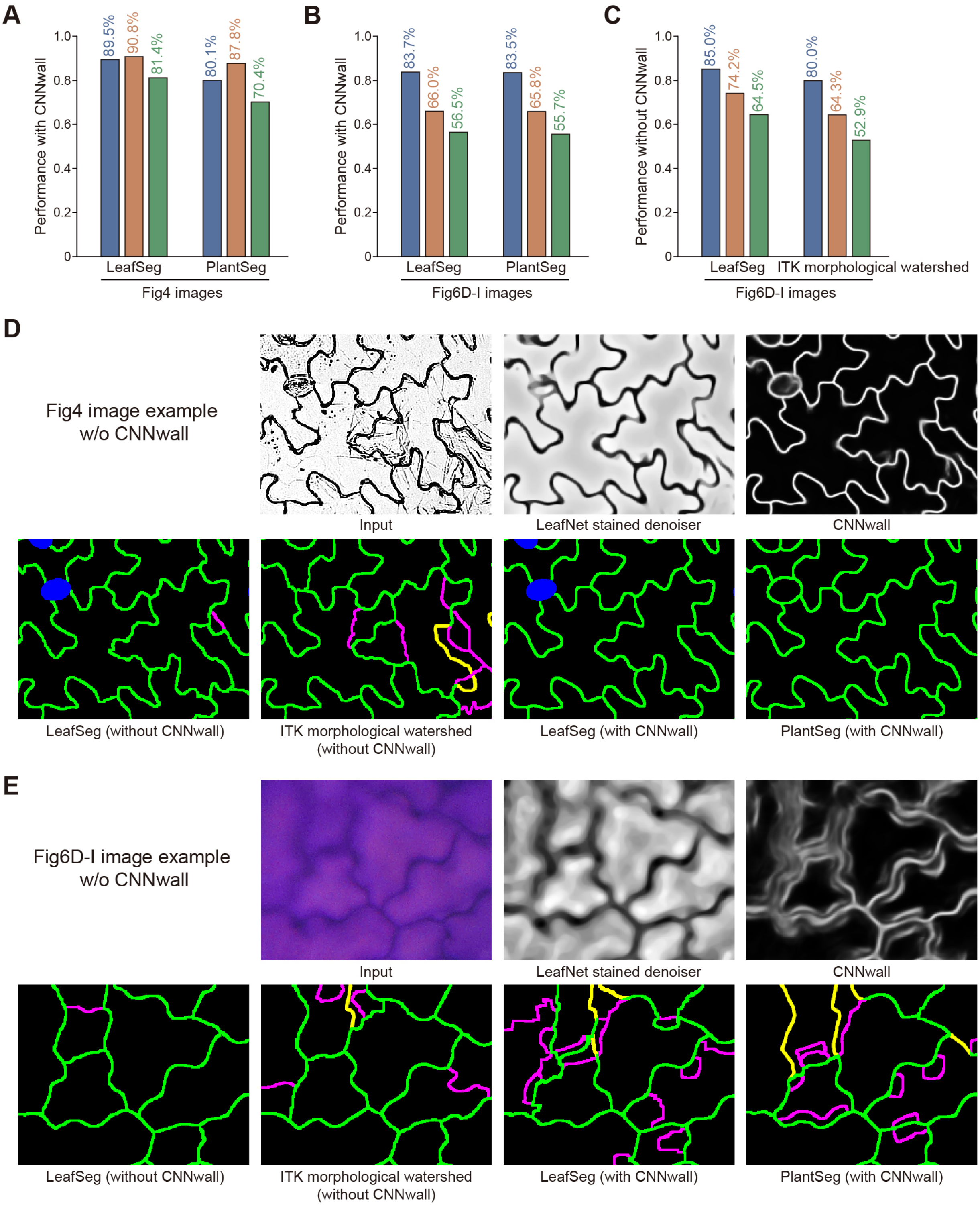


C

Statistics of morphological features

Image name	example.png
Resolution (px/μm)	2.17
Image size (μm²)	257911.61
Stoma size (μm²)	8189.39
Cell size (μm²)	249722.23
Stoma count	32
Stoma length/width ratio	1.38, 1.28, ...
Stoma sizes (μm²)	234.84, 276.20, ...
Pavement cell count	76.5
Pavement cell sizes (μm²)	3301.2, 2665.2, ...
Lobe count	17, 15, ...
Circularity	0.169, 0.178, ...
......	......

Supplemental Figure S8. Sample output for LeafNet (Supports Figure 7).
(**A**) Preview image, with stomata and pavement cells colored for visualization. (**B**) Annotation image, with pavement cells segmented by green lines and stomata labeled in blue. (**C**) Morphological information.



Supplemental Figure S9. Performance of CNNwall enhancement for pavement cell segmentation (Supports Figure 4 and 6).
(**A-B**) Performance of pavement cell segmentation using LeafSeg and PlantSeg with retrained CNNwall model for Figure 4 images (**A**) and Figure 6D-I images (**B**). (**C**) Performance of LeafSeg and ITK morphological watershed without CNNwall enhancement for Figure 6D-I images. (**D-E**) Segmentation of LeafSeg, ITK morphological watershed, and PlantSeg with or without CNNwall enhancement for Figure 4 exemplar image (**D**) and Figure 6D-I exemplar image (**E**).