Takumi Higaki, Natsumaro Kutsuna, Hasezawa Seiichiro (GSFS, The University of Tokyo)

CARTA-based object recognition in fluorescent microscopic images

This protocol showed step-by-step procedures to perform CARTA-based object recognition in fluorescent microscopic images, as described in our manuscript ("CARTA-based semi-automatic detection of stomatal regions on an Arabidopsis cotyledon surface" by Higaki T, Kutsuna N, Hasezawa S. *Plant Morphology*, accepted). Please see also our *Plant Morphology* paper for figure references.

Setup for ImageJ software

1. Install ImageJ software (http://rsbweb.nih.gov/ij/download.html). See also the ImageJ documentation (http://rsbweb.nih.gov/ij/docs/index.html).
2. Download and install the KBI ImageJ plug-ins (revision 1192) and Scala library (version 2.10.0). These are freely available at our website: http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/RegionSearch. The KBI plug-ins are developed and managed by Dr. Natsumaro Kutsuna in Hasezawa laboratory (The University of Tokyo).

Setting the randomly located region of interest (ROI)

1. Start the ImageJ software (http://rsbweb.nih.gov/ij/).
2. Open the target stack image using the ImageJ menu "File-Open" (http://rsbweb.nih.gov/ij/docs/guide/146-26.html#toc-Subsection-26.2).
3. Get the average-intensity-projection (AIP) image using the ImageJ menu "Image-Stacks-Z project…" (http://rsbweb.nih.gov/ij/docs/guide/146-28.html#toc-Subsection-28.6) (Fig. 1A).
4. Set the randomly located ROIs using the ImageJ menu "Plugins-kbi-Kbi_RoiUtil (mode: rndSmpl2d)" (Fig. 1B). In this example, the tiling parameters are tuned as follows. (4-1)-Enter "30" in the "tileSize" box in the dialog window for 30 pixels square ROI size. (4-2)-Enter "3000" in the "num1" box in the dialog window for 3000 ROI numbers.
5. Deselect a ROI by clicking the "Deselect" button on the ROI manager (http://rsbweb.nih.gov/ij/docs/guide/146-30.html#sub:ROI-Manager...) and save

the ROI files as a ZIP file using the ROI Manager menu “More>>-Save…-”.

Allocating the stomatal regions and iterative clustering

1. Start the ImageJ software (http://rsbweb.nih.gov/ij/).
2. Open the target AIP image and the randomly located ROI zip file using the ImageJ menu “File-Open”.
3. Run the plug-in using the ImageJ menu “Plugins-kbi-Kbi_ijTool (projects: carta)”.
4. Load the image feature CSV file by entering “ft-load” in the command line.
5. Load the clustering CSV file by entering “cl-load” in the command line.
6. Lasso the stomatal images on the SOM using the ImageJ tool bar “Rectangular Selection Tool” (http://rsbweb.nih.gov/ij/docs/guide/146-19.html#toc-Subsection-19.1). Multiple rectangular selections can be set by holding down the “Shift” key. In this example, 17 ROIs are selected (Fig. 2, light-blue boxes).
7. Enter “cl-to-roi” in the command line. You will see that the corresponding ROIs are selected in the ROI Manager.
8. Enter “grp-from-roi stomata” in the command line.
9. Save group information as a CSV file by entering “grp-file” in the command line.
10. Enter “step2-ga” in the command line. Image feature selection driven by a genetic algorithm (GA) will start [1] (Fig. 3). You can stop by pressing the OK button in the ‘Running GA…’ window. The last frame in the ‘cl-opt’ stack image is the improved SOM clustering result with selected image features. In the improved SOM clustering, the stomatal regions are mostly gathered (Fig. 4A).
11. Save the improved SOM clustering result as a CSV file by entering “cl-save” in the command line.
12. Load the improved SOM clustering result by entering “cl-load” in the command line.
13. Lasso the stomatal images on the improved SOM using the ImageJ tool bar “Rectangular Selection Tool” (http://rsbweb.nih.gov/ij/docs/guide/146-19.html#toc-Subsection-19.1). You can set the gathered stomatal region images (Fig. 4A, light-blue boxes).
14. Enter “cl-to-org-deep” in the command line. The ROIs allocated at the lassoed nodes are mapped on the original AIP image (Fig. 4B).