

Spa-RQ user manual

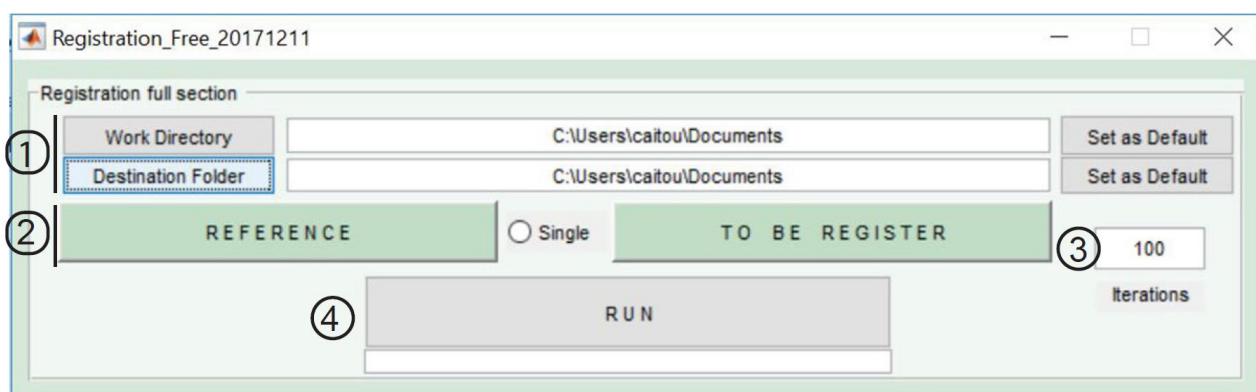
a. Start the software

- Download MATLAB runtime 2015a for free from:
<https://se.mathworks.com/products/compiler/matlab-runtime.html>
- Double-click on Spa-R or Spa-Q logo to initiate each tool domain.

b. Name format of input images

- Image names should be appended with a “_n-” extension, where “n” indicates the order of the section stained with an antibody, followed by the marker’s name. For example, if adjacent sectioning strategy is applied, where the 6th tissue section is stained with p4EBP1 and the 10th section is stained with pERK, the acquired image should contain _6-p4EBP1 and _10-pERK respectively.

c. Spa-R user interface

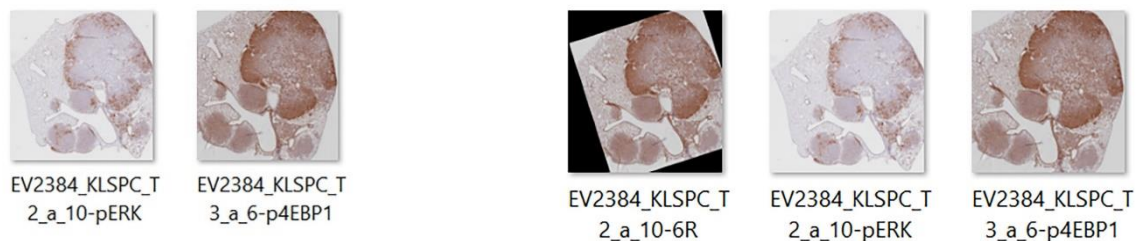


Modules: ① Input and output folder settings; ② Uploading of reference and target images; ③ Adjustment of Iteration rate; ④ “Run” command and the progress bar below it.

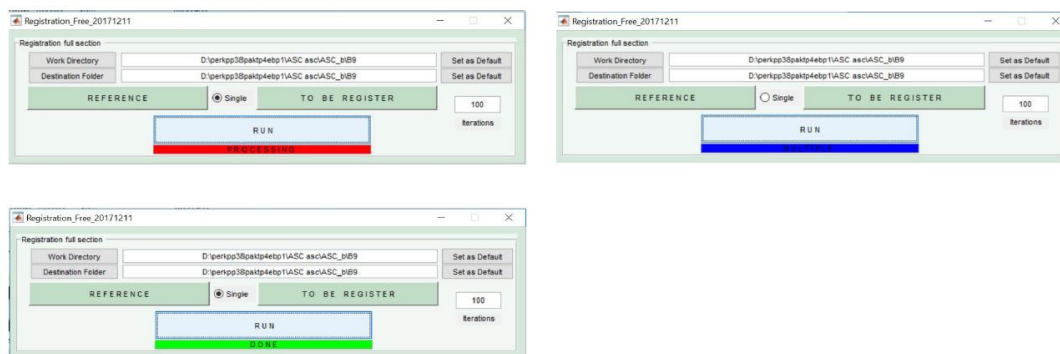
Steps:

1. Click on “Work Directory” and “Destination Folder” to set up the input folder and output folder (which can be the same). If “Set as Default” button is clicked, the same folder setting will appear next time when Spa-R is launched.
2. Click on “Reference” to choose one reference image from the input folder. Click on the “To be register” button to choose target images. If only one image is to be registered, “Single” need to be chosen.
3. Enter a desired iteration number. 100 is shown as default, but users can change it according to their own requirement.
4. Press “Run” to start image registration.

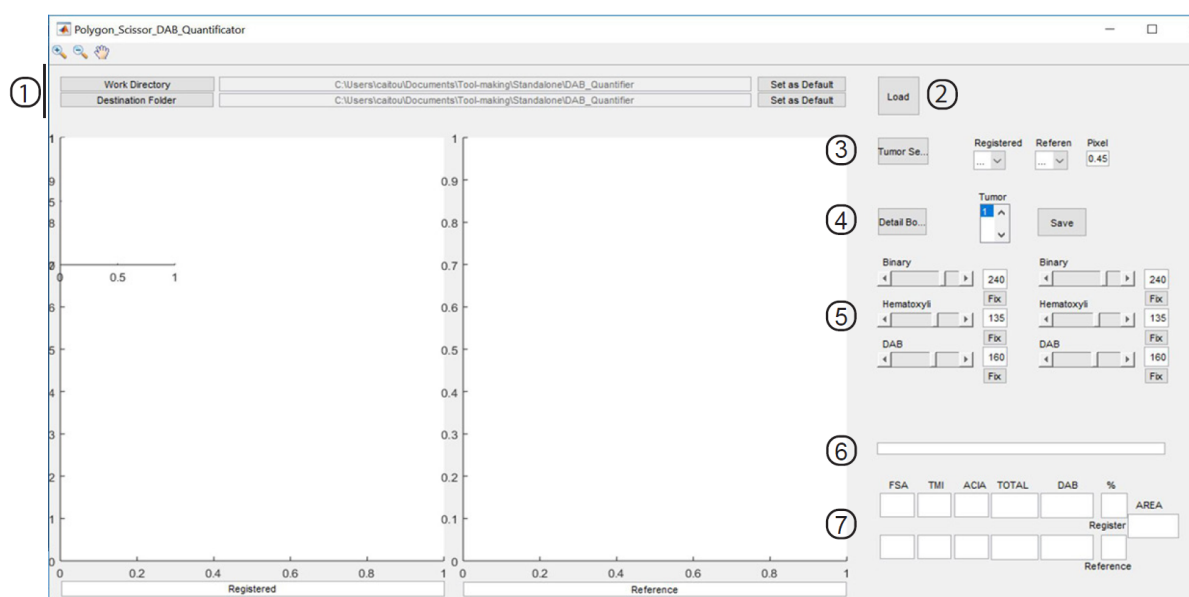
- e.g. Source images in the input folder (left) and registered image in the output image (right)



The processing stages of Spa-R are indicated by a bar with changing colour. Upper left: when a single image is under registration. Upper right: when multiple images are in the process. Lower: when the registration is finished.



d. Spa-Q user interface



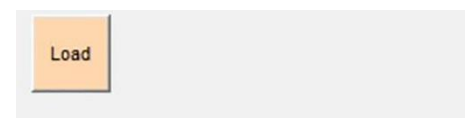
Modules: ① Input and output folder settings; ② Uploading of Excel sheet; ③ Selecting images and ROIs; ④ Selecting a ROI for quantitation and refine the ROI outline; ⑤ Threshold settings; ⑥ Progress bar; ⑦ Result panel (% area)

Steps:

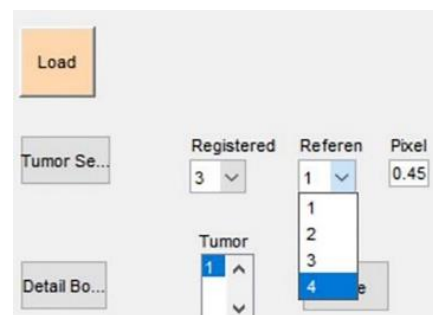
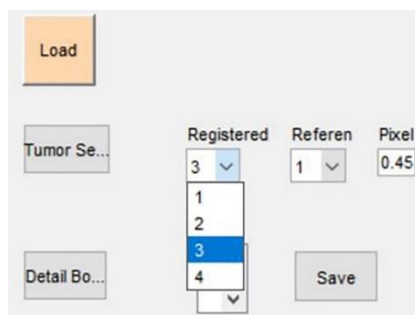
1. Set-up the input and output folders, similar to Spa-R
2. Create an Excel sheet in the input folder and click on “Load Excel” to upload it. The colour change of the button indicates the Excel sheet information has been successfully loaded.

Left: format of the Excel sheet. Right: the “Load” button will change colour when the excel information has been uploaded.

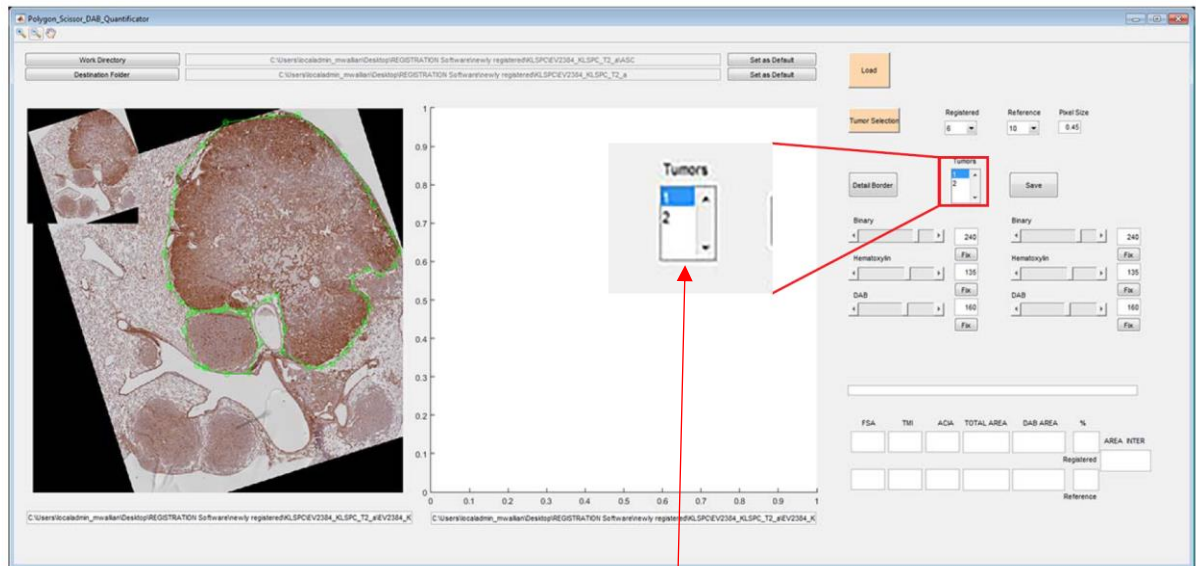
Date	SlideName	SlideNumber	Antibody
21/12/2017	ASC	1	p4EBP1
		2	pAKT
		3	pERK
		4	pp38



3. Select the images to be analysed (stroll down “Registered” and “Reference” sections to choose one from each, according to the registration scheme) and click on “Tumor se” to upload images.



4. Region-of-interest selection: A polygon selection tool will automatically appear when clicking on the image (only one image of the registered image appears at this stage). Once polygon is made, double left-click the mouse to confirm the selection of a ROI, which will appear on the ROI panel, indicated by the number. Multiple ROIs to be quantified can be manually selected and stored. Once all the ROIs are selected, double click the mouse to end this step.

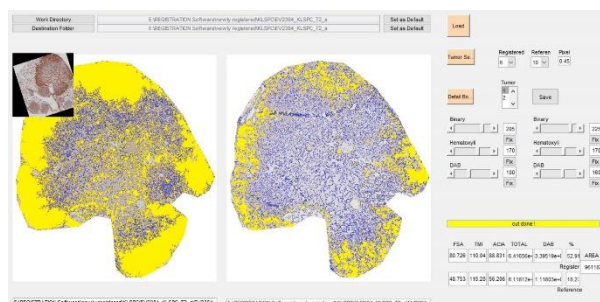


5. Select the ROI to be analysed (shown in blue highlight in above image) and click on “Detail border” to again use polygon tool to the refine the border of this ROI. Double-click the mouse and then Spa-Q will automatically enter a masking step to segment DAB staining on both images (indicated in yellow), based on default threshold settings.

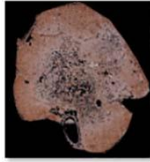


6. Set up the suitable thresholds for binary, hematoxylin, and DAB separately for each of the paired image, and press “Save” to obtain the results.

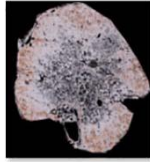
Below: staining segmentation (shown in yellow). The thresholding parameters were set by the user.



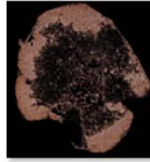
7. Open the output folder to view all the masks for segmentations and the result sheet.



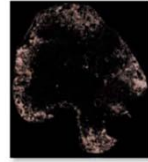
Binary_ASC_6-P4
ebp1



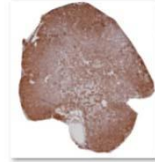
Binary_ASC_10-p
ERK



DAB_ASC_6-p4E
BP1



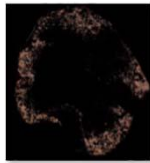
DAB_ASC_10-pE
RK_T1



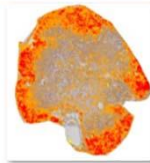
Original_ASC_6-p
4EBP1



Original_ASC_10-
pERK



Overlap_ASC_a_6
-p4EBP1_10-pER
K



OverlapLandscap
e_ASC_6-p4EBP1
_10-pERK



STATS_EV2384_K
LSPC_T2_a_10-6R
_T1