

For both MAC and Windows users

Spa-RQ user manual

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Supplementary to original manuscript:

Spa-RQ: an Image Analysis Tool to Visualise and Quantify Spatial Phenotypes Applied to Non-Small Cell Lung Cancer

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[PAGE 1-13: HOW TO INSTALL MATLAB RUNTIME AND START SPA-RQ](#)

[PAGE 14-20: HOW TO OPERATE SPA-RQ](#)

How to install MATLAB Runtime and run Spa-R and Spa-Q tools

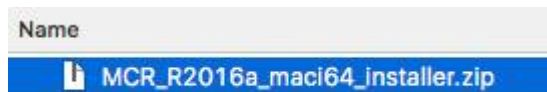
Download MATLAB Runtime (for every operating system)

1. Go to this website: <https://www.mathworks.com/products/compiler/matlab-runtime.html>
2. Download the MATLAB Runtime R2016a (v9.0.1) to your operating system

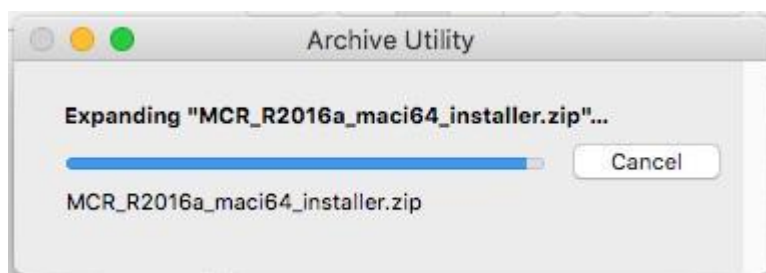
Release (MATLAB Runtime Version#)	Windows	Linux	Mac
R2018b (9.5)	64-bit	64-bit	Intel 64-bit
R2018a (9.4)	64-bit	64-bit	Intel 64-bit
R2017b (9.3)	64-bit	64-bit	Intel 64-bit
<p>! Apply Updates to R2016a-R2017a versions of MATLAB Runtime after installing the runtime</p> <p>Important security fixes are available for the R2016a, R2016b, and R2017a releases of the MATLAB Runtime. After installing the MATLAB Runtime for one of these releases, you should apply the latest Update by clicking on the appropriate Update link below. Note this applies only if your application uses MATLAB apps authored with MATLAB App Designer (.mlapp files). For more information see this bug report.</p>			
R2017a (9.2)	64-bit Update	64-bit Update	Intel 64-bit Update
R2016b (9.1)	64-bit Update	64-bit Update	Intel 64-bit Update
R2016a (9.0.1)^{1,2}	64-bit Update	64-bit Update	Intel 64-bit Update
R2015b (9.0) ^{1,2,3}	32-bit / 64-bit	64-bit	Intel 64-bit
R2015aSP1 (8.5.1) ¹	32-bit / 64-bit	64-bit	Intel 64-bit
R2015a (8.5) ¹	32-bit / 64-bit	64-bit	Intel 64-bit
R2014b (8.4) ¹	32-bit / 64-bit	64-bit	Intel 64-bit
R2014a (8.3) ¹	32-bit / 64-bit	64-bit	Intel 64-bit
R2013b (8.2)	32-bit / 64-bit	64-bit	Intel 64-bit
R2013a (8.1)	32-bit / 64-bit	64-bit	Intel 64-bit
R2012b (8.0)	32-bit / 64-bit	64-bit	Intel 64-bit
R2012a (7.17)	32-bit / 64-bit	32-bit / 64-bit	Intel 64-bit
R2011b and earlier ⁴	Open MATLAB and run the command <code>mcrinstaller</code> to locate MATLAB Runtime for redistribution with your compiled components.		

Install MATLAB Runtime on MAC

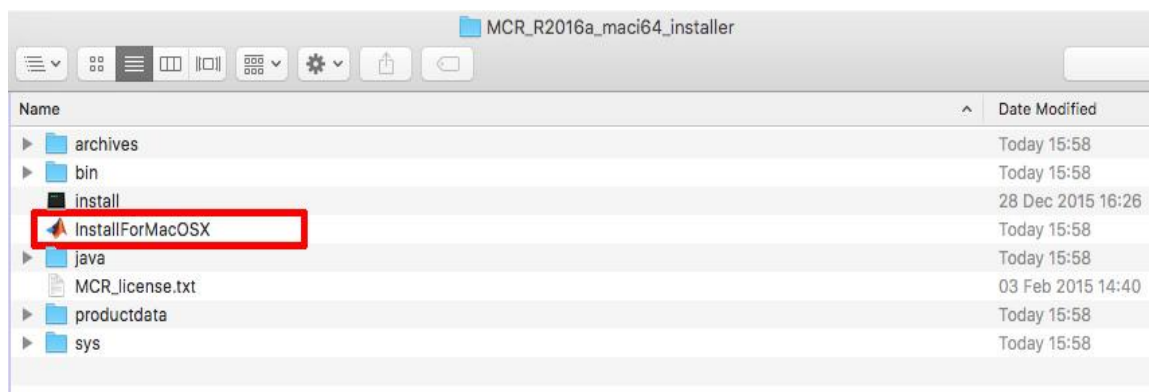
1. After the successful download browse the following file in *Finder*



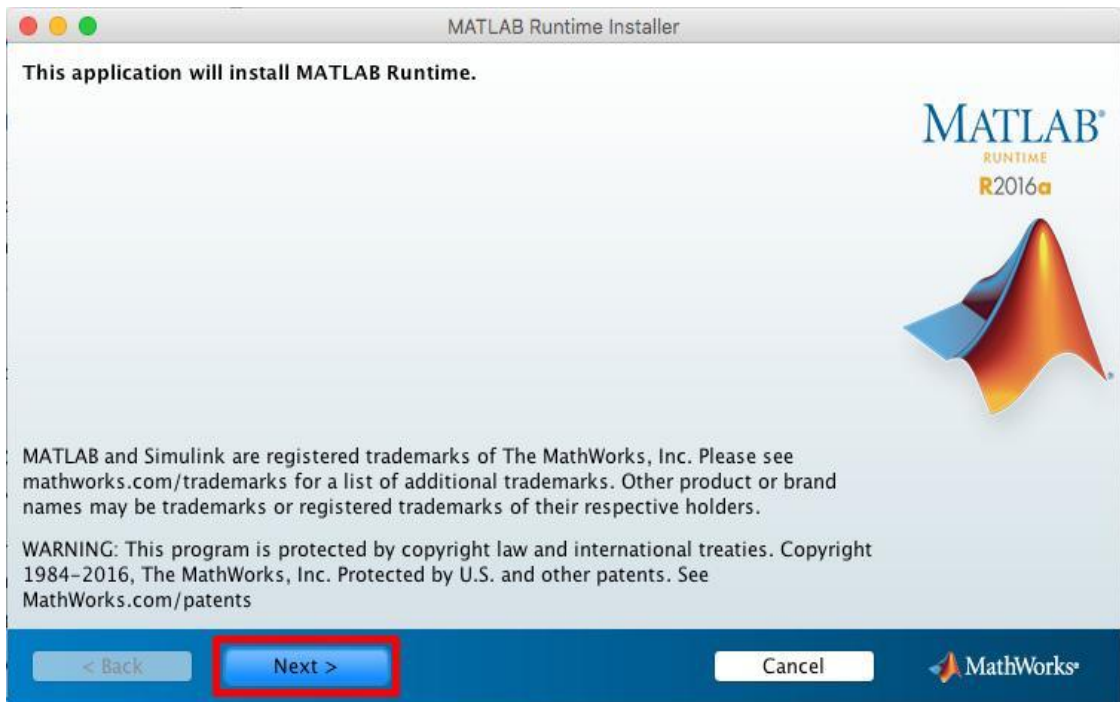
2. Unzip the MATLAB Runtime installer



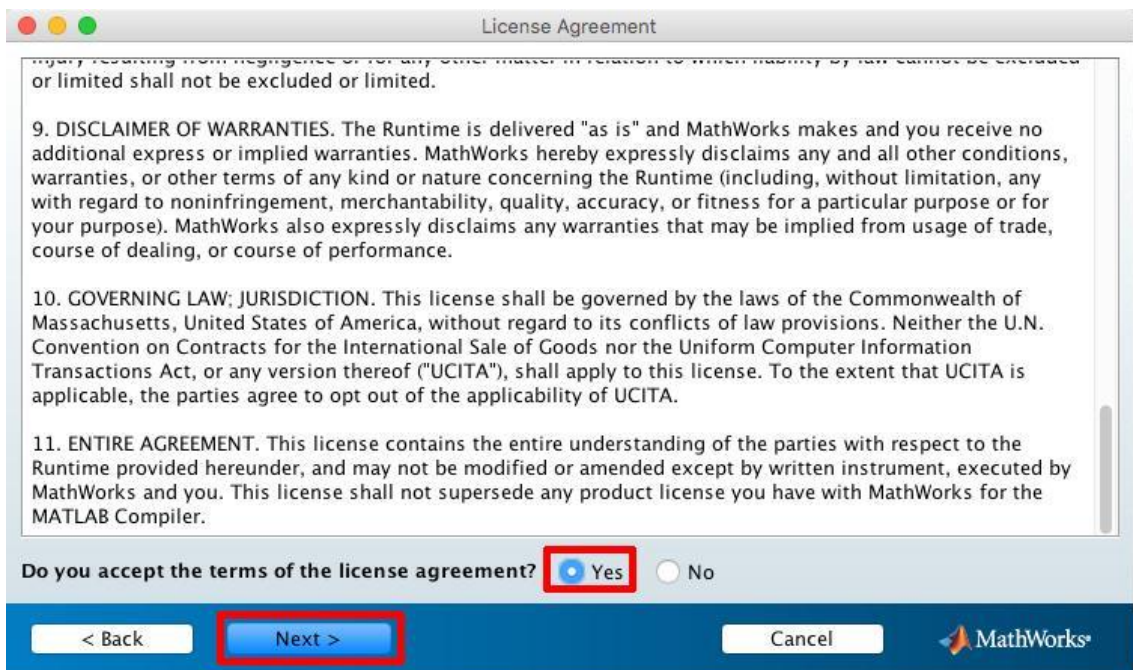
3. Go into the folder of the MATLAB Runtime installer and run *InstallForMacOSX*



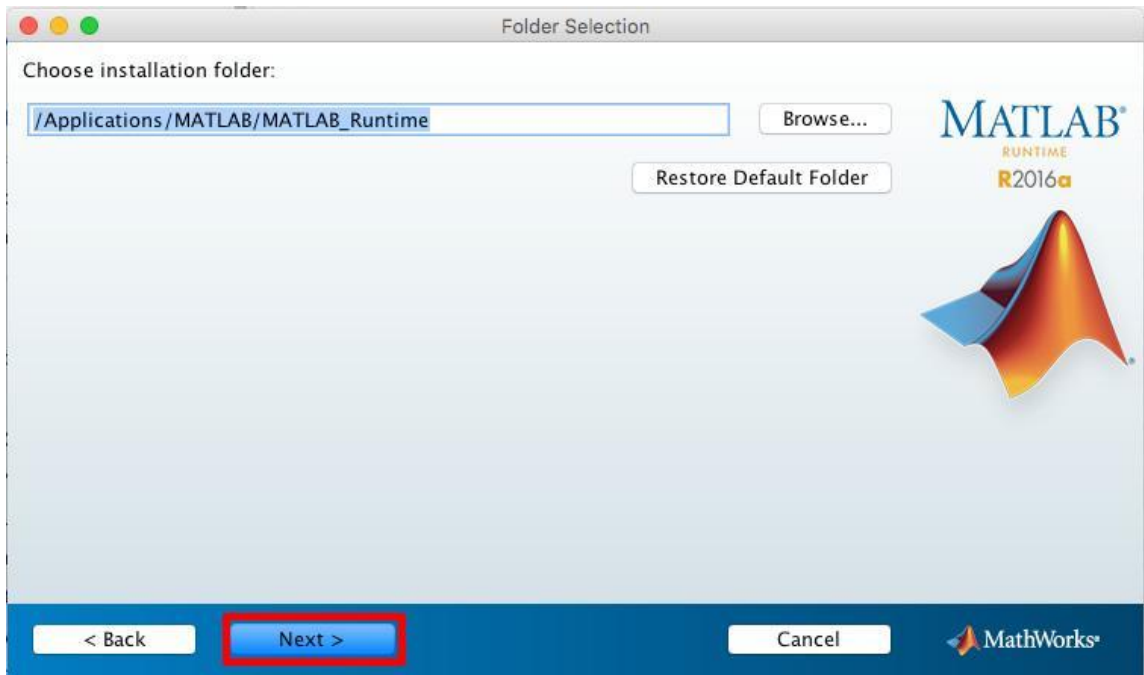
Start the install process by clicking '**Next**' in the installer window



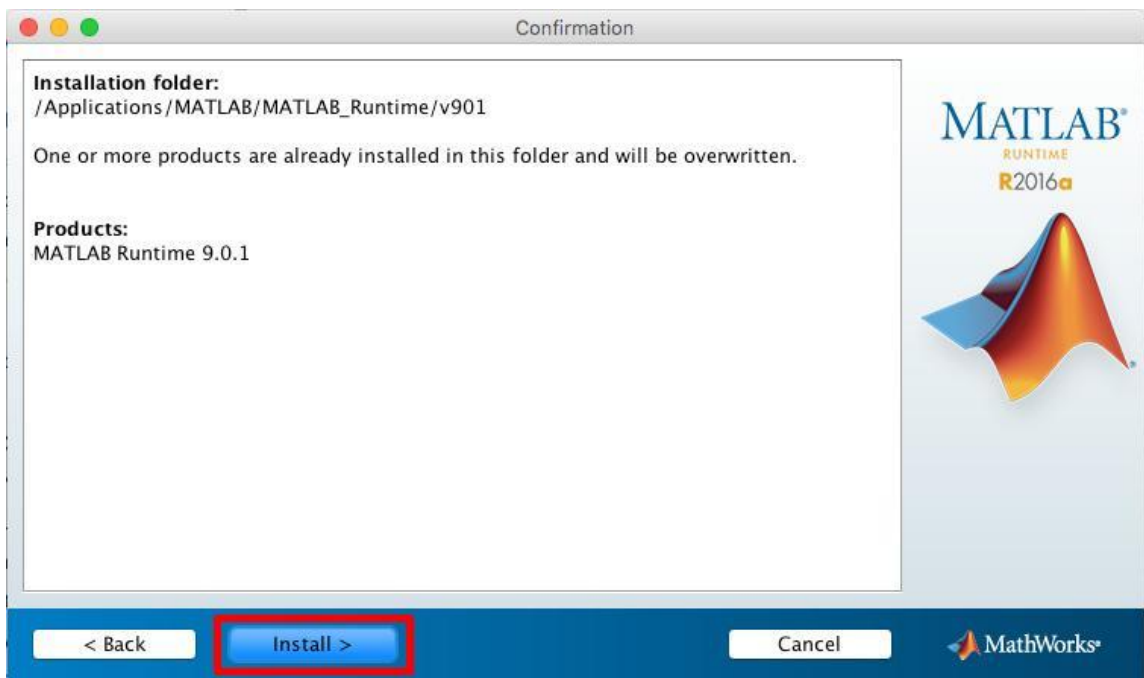
4. Read the license agreement and Accept it by clicking '**Yes**', followed by '**Next**'



5. Leave the MATLAB Runtime folder as default, and click **'Next'**



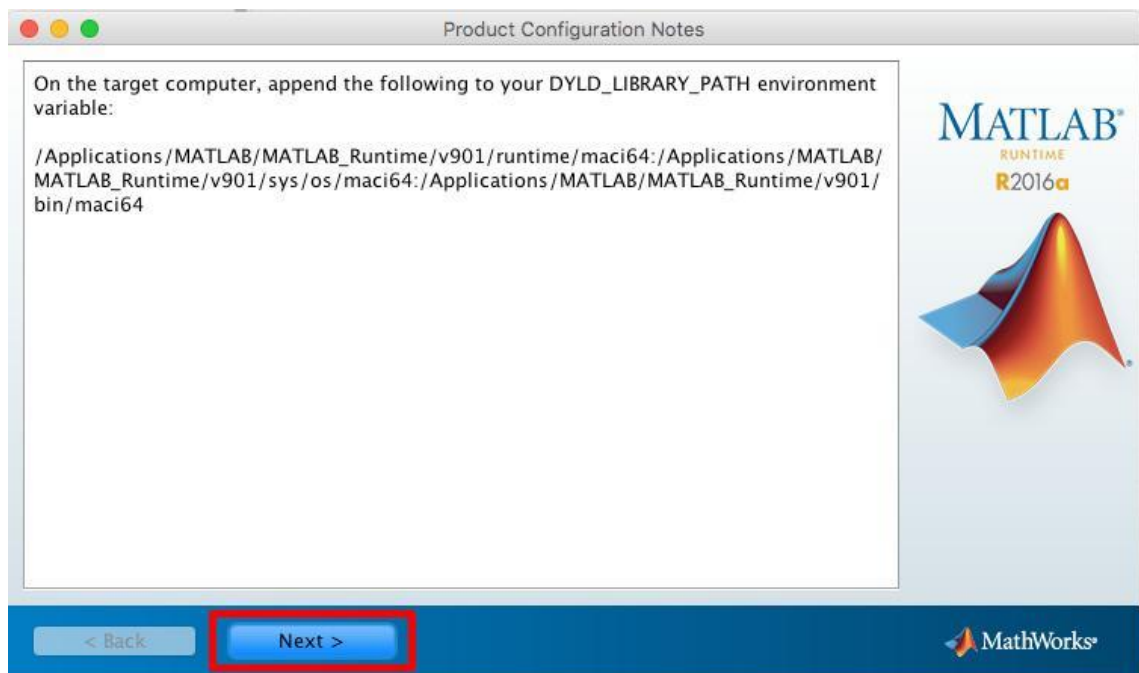
6. Click **'Install'**



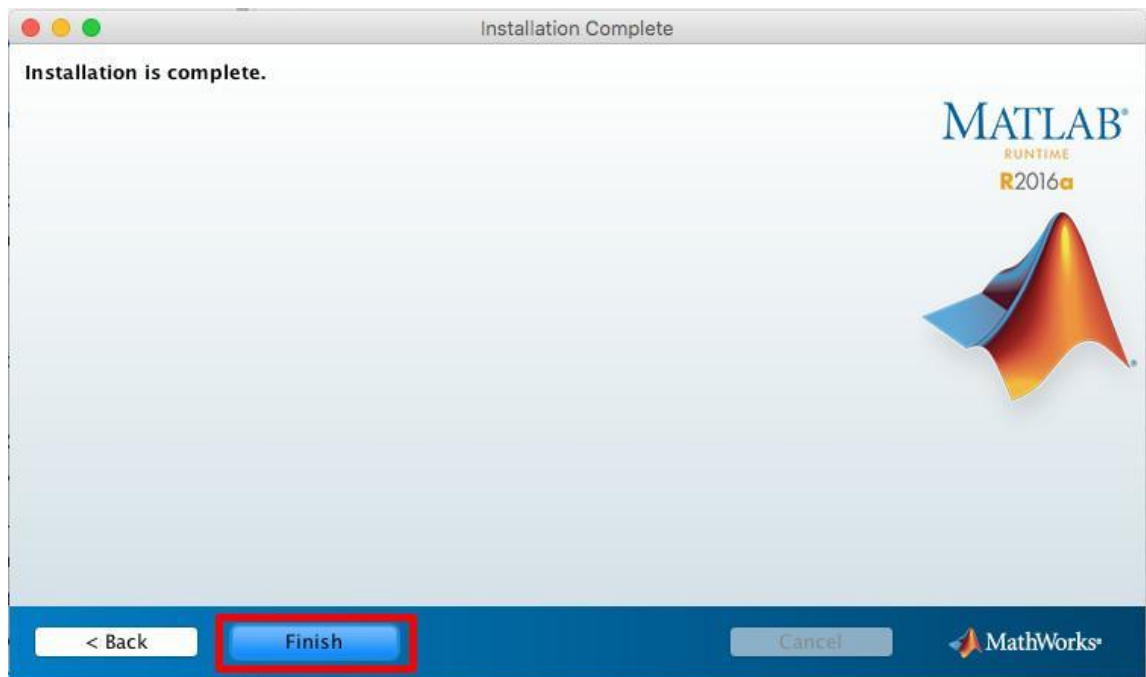
7. Wait until the installation is completed



8. Click '**Next**'



9. Click on '**Finish**'



Note: Now the *MATLAB Runtime* is installed to your computer.

Run Spa-R and Spa-Q on MAC

1. Go into the Spa-RQ folder in **Finder**
In the Spa-RQ folder, there are **Spa_Q** and **Spa_R** subfolders
The Spa_R folder contains a tool for microscopy image registration.
The Spa_Q folder contains a tool for quantification of staining overlap.

Name	Date Modified	Size	Kind
▶ Spa_Q	07/02/19 14:16	--	Folder
▶ Spa_R	07/02/19 14:16	--	Folder
Spa-RQ_user manual.pdf	Today 11:38	4,9 MB	PDF

2. Enable the launching of an **OS X Terminal Window** from a Specific Folder
 - Go to **System Preferences** and select **Keyboard > Shortcuts > Services**
 - Activate **New Terminal at Folder** in the settings



2. Run **Spa-R** tool:
 - Right click on **Spa_R** folder and select **Services > New Terminal at Folder**
 - Run **Spa-R** with the following command (copy the following to the Terminal):

```
sh run_Spa_R.sh /Applications/MATLAB/MATLAB_Runtime/v901/
```

Note: the following commands do the same thing

```
chmod +x run_Spa_R.sh (ENTER)
./run_Spa_R.sh /Applications/MATLAB/MATLAB_Runtime/v901/
```

3. Run **Spa-Q** tool:
 - Right click on **Spa_Q** folder and select **Services > New Terminal at Folder**
 - Run **Spa-Q** with the following command (copy the following to the Terminal):

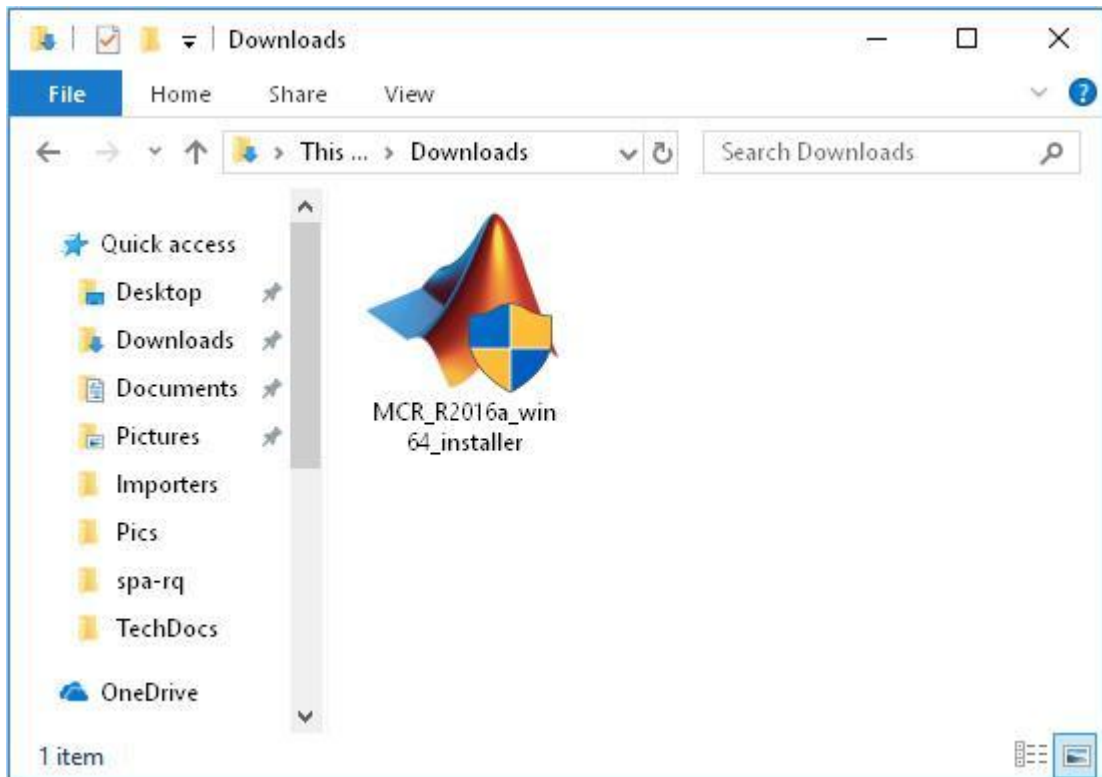
```
sh run_Spa_Q.sh /Applications/MATLAB/MATLAB_Runtime/v901/
```

Note: the following commands do the same thing

```
chmod +x run_Spa_Q.sh (ENTER)
./run_Spa_Q.sh /Applications/MATLAB/MATLAB_Runtime/v901/
```


Install MATLAB Runtime on Windows

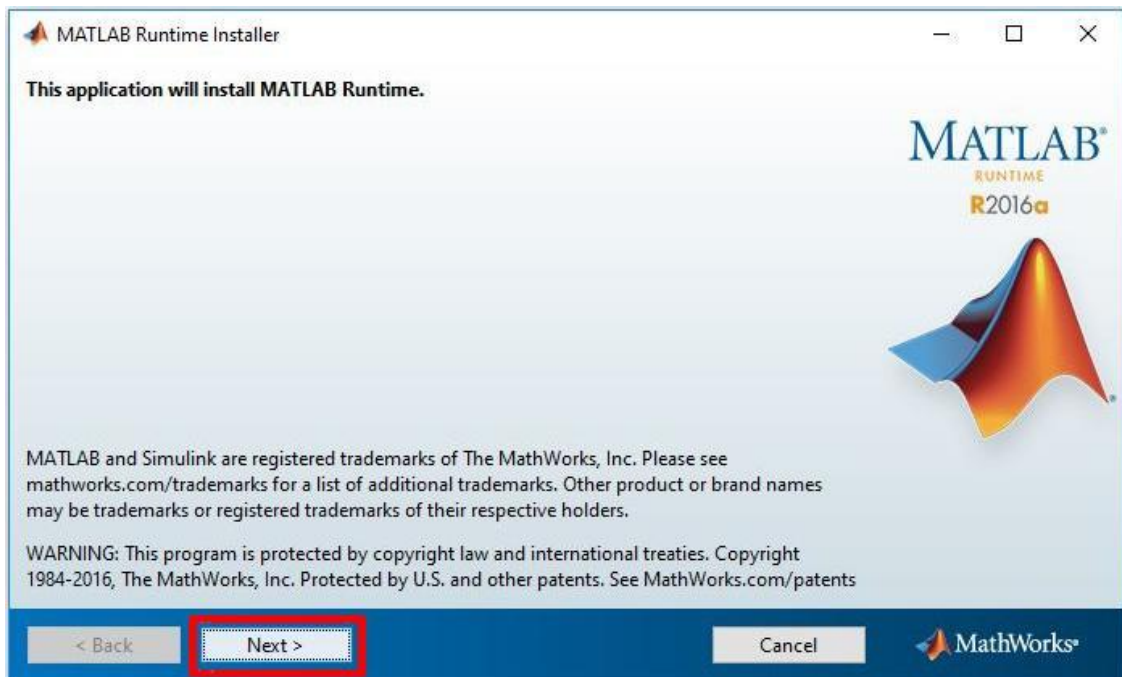
1. After the successful download, browse the following file in File Explorer



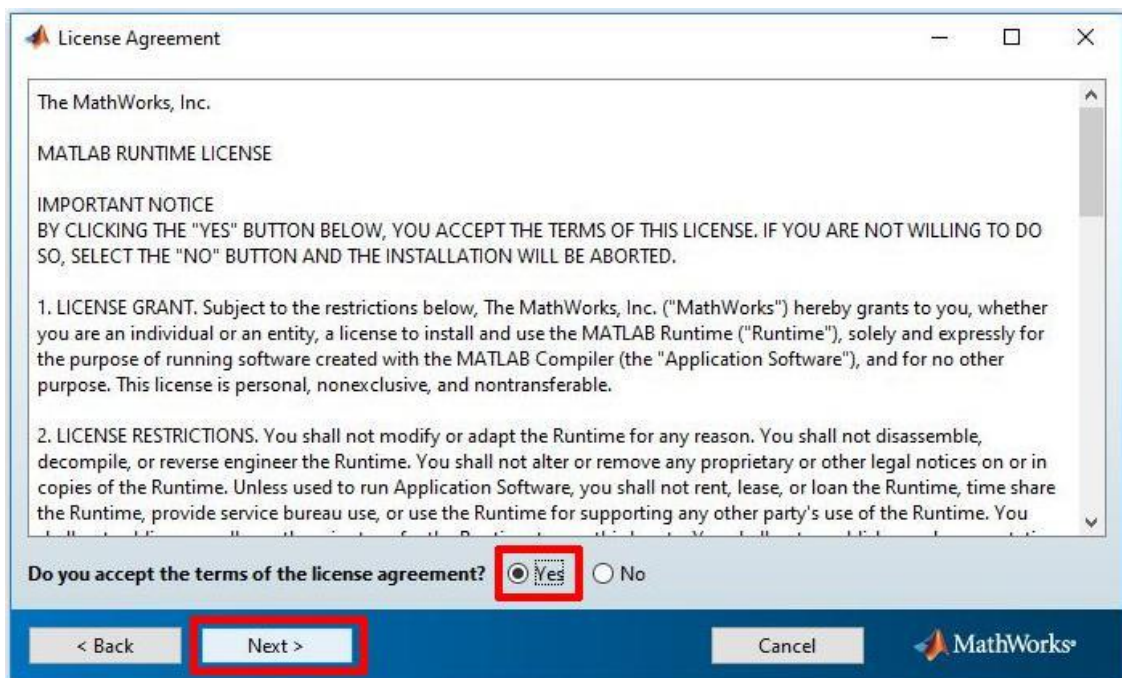
2. Start install by double clicking on the installer shown above; a file extraction panel will appear



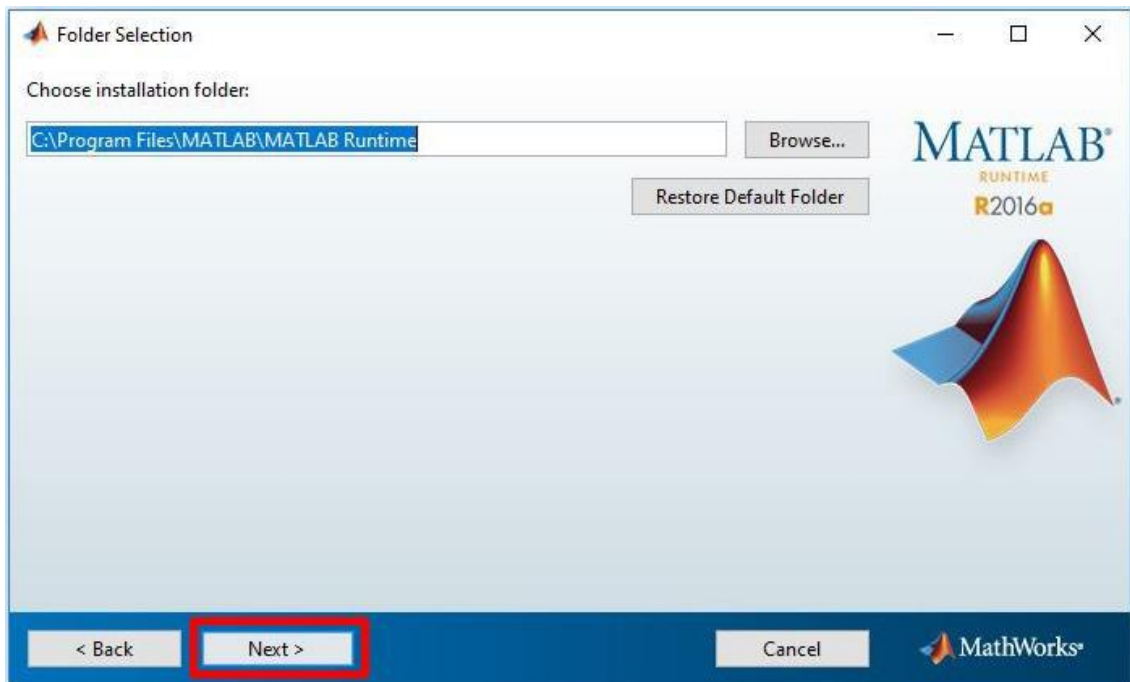
3. Start the install process by clicking **'Next'** in the installer window



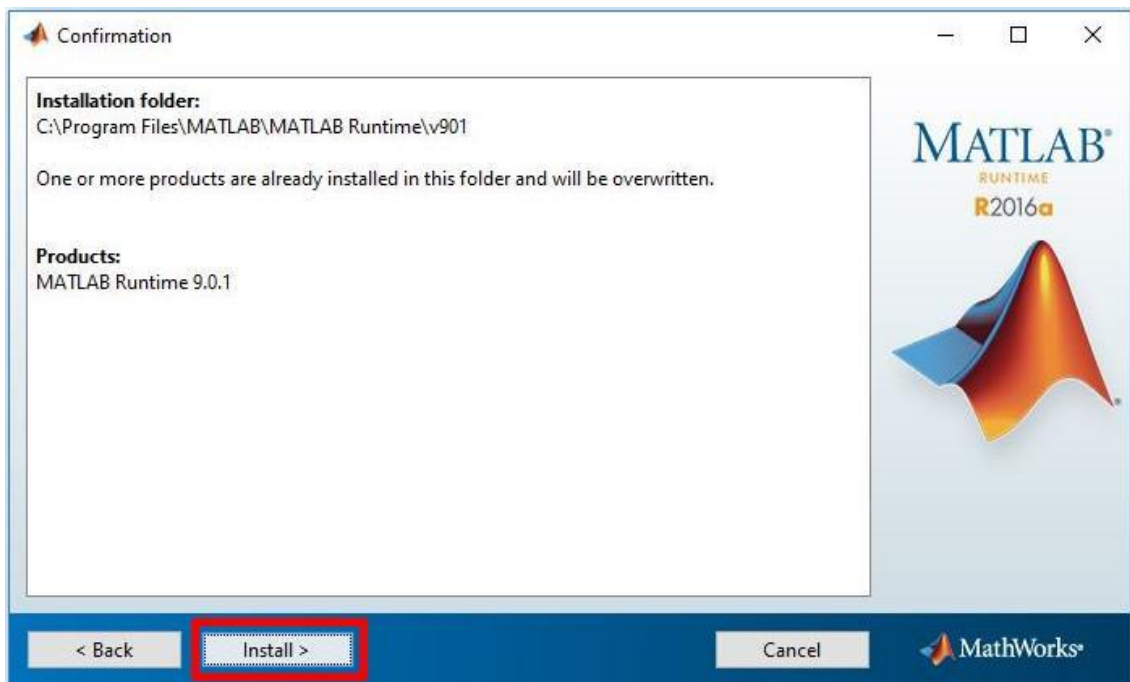
4. Read the license agreement and Accept it by selecting **'Yes'**, followed by **'Next'**



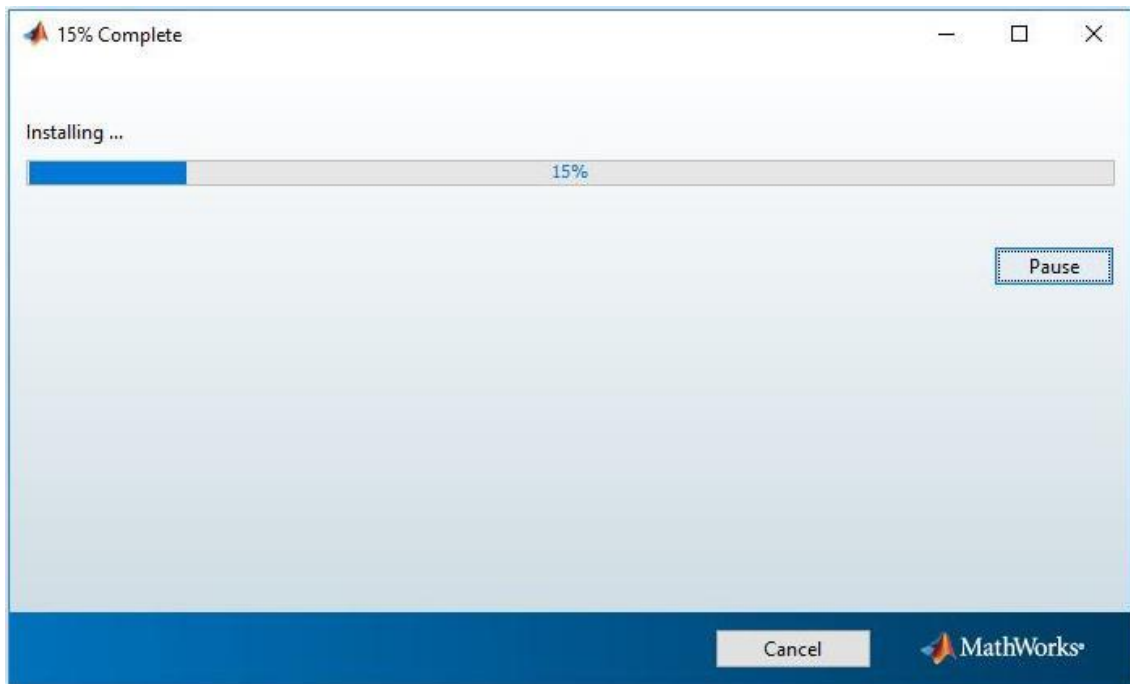
5. Leave the MATLAB Runtime folder as default, and click **'Next'**



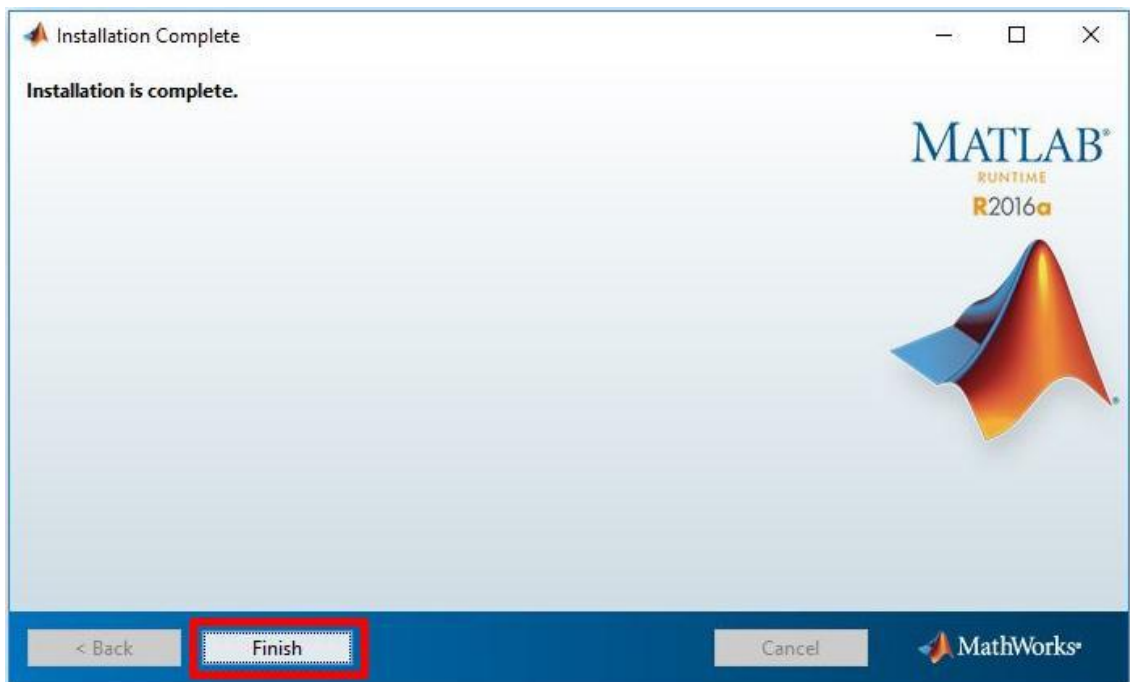
6. Click **'Install'**



7. Wait until the installation is completed



8. Click '**Finish**'



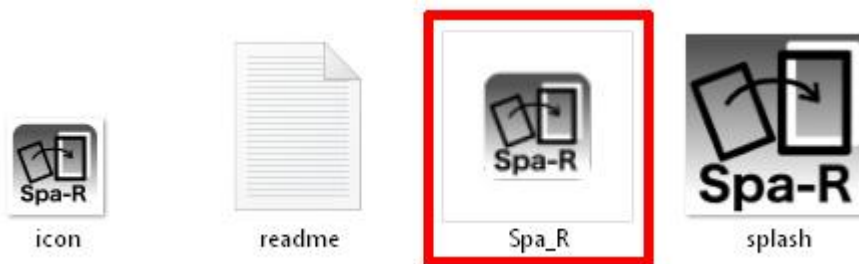
Note: Now the MATLAB Runtime is installed to your computer.

Run Spa-R and Spa-Q on Windows

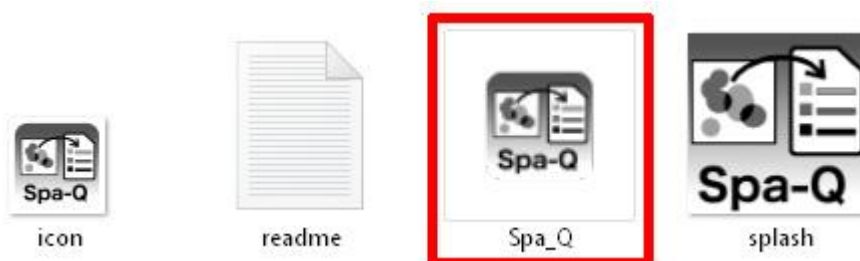
1. Go into the Spa-RQ folder in File Explorer.
In the Spa-RQ folder, there are **Spa_Q** and **Spa_R** subfolders.
The Spa_R folder contains a tool for microscopy image registration.
The Spa_Q folder contains a tool for quantification of staining overlap.

Name	Date modified	Type	Size
Spa_Q	2/11/2019 9:35 AM	File folder	
Spa_R	2/11/2019 9:35 AM	File folder	
Spa-RQ_user manual	2/11/2019 9:57 AM	PDF File	4,785 KB

2. Go in the **Spa_R** folder.
Run the **Spa-R** tool by double-clicking on the **Spa_R** executable icon.



3. Go in the **Spa_Q** folder.
Run the **Spa-Q** tool by double-clicking on the **Spa_Q** executable icon.



Note: For the first run, it will take some time for the tools to start.

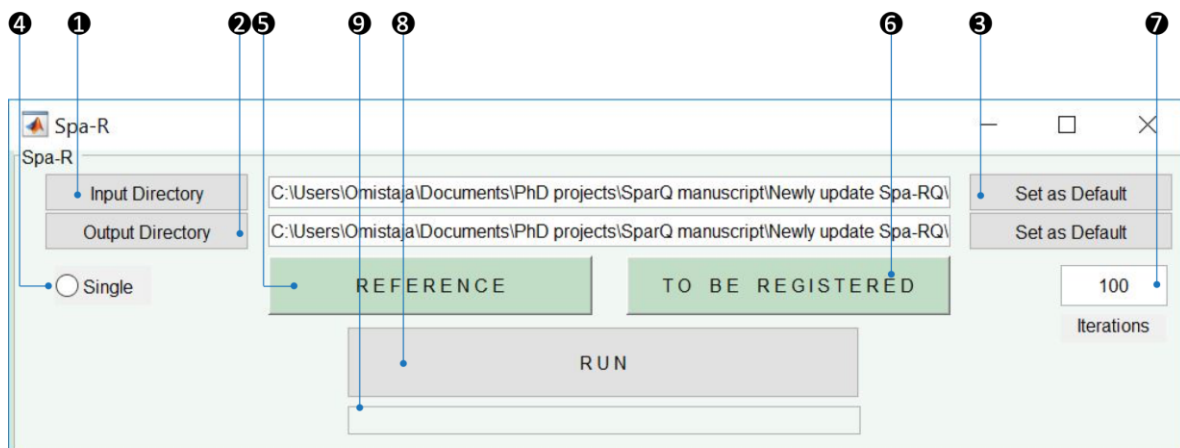
Spa-RQ tools user manual

Link to download the tools:

<https://bitbucket.org/MagoBitbucket/spa-rq-tools/downloads/>



Spa-R (Spa-Registration)



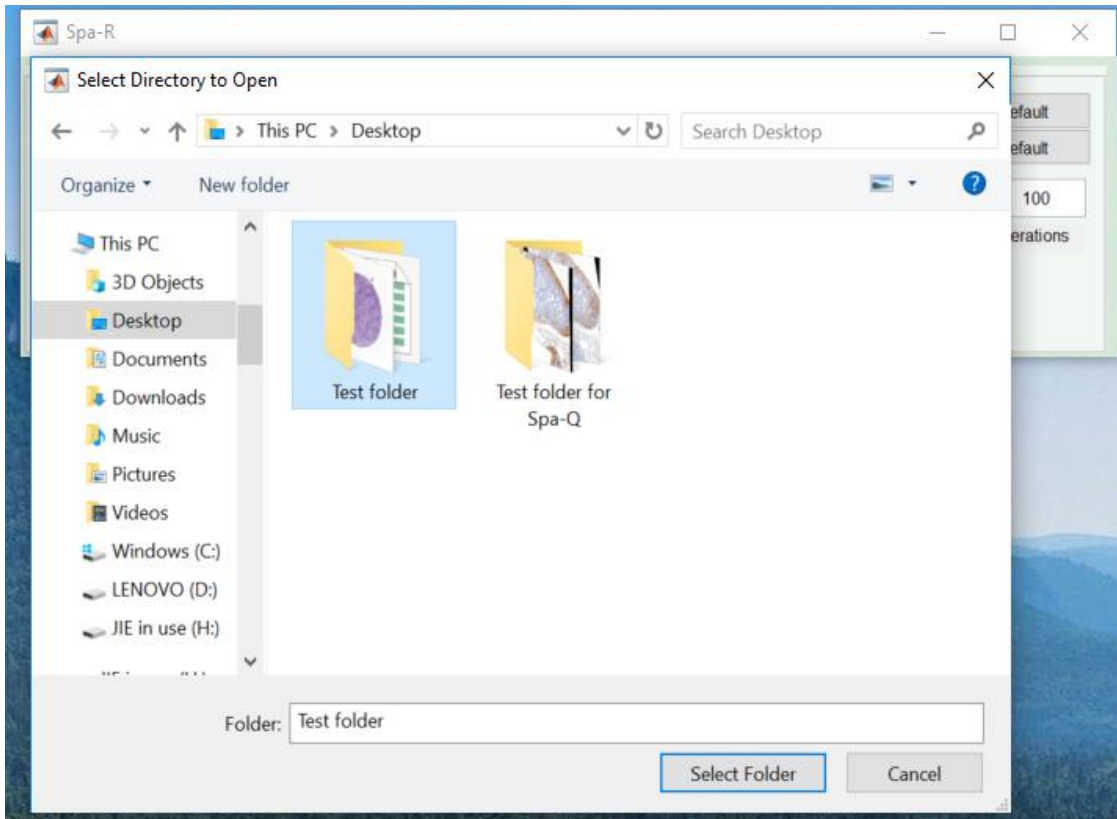
- ❶ Click to choose the folder where the images locate
- ❷ Click to choose the folder where registered images will be stored
- ❸ Click to fix default input and output folders for multiple Spa-R launches
- ❹ Click to choose the reference image from the input folder
- ❺ Click if only a single image is to be registered
- ❻ Click to choose a single or set of images to be registered
- ❼ Enter the desired number of Iterations. 100 is the default/recommend setting.
- ❽ Click to start the registration process.
- ❾ The progress bar will indicate the progress of registration.

Step-by-step instructions

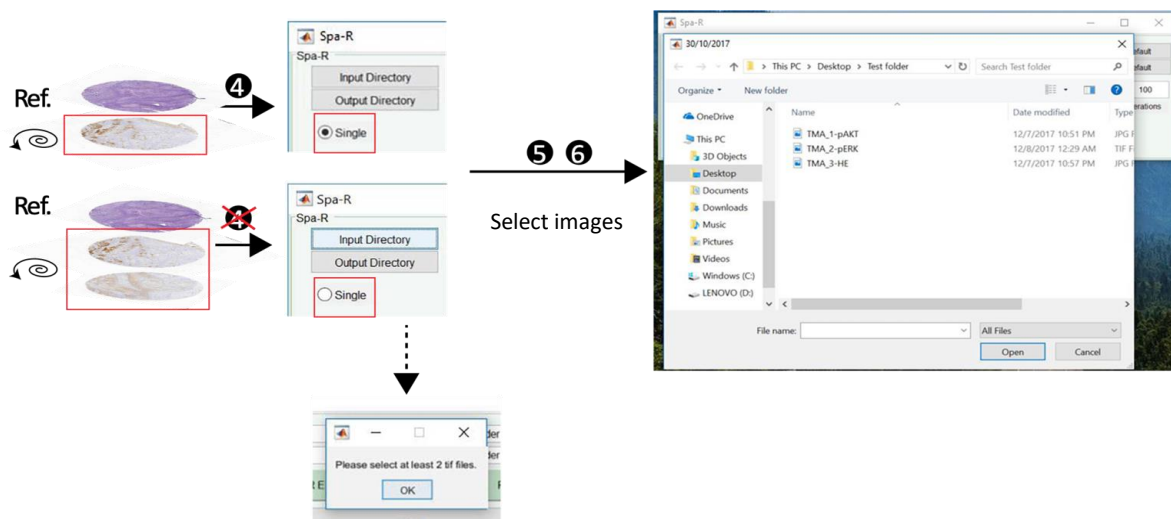
1. Name the input images with the same prefix (e.g. sample name) and a “_n-” extension. “n” indicates the series order, followed by the marker’s name. See the example below:



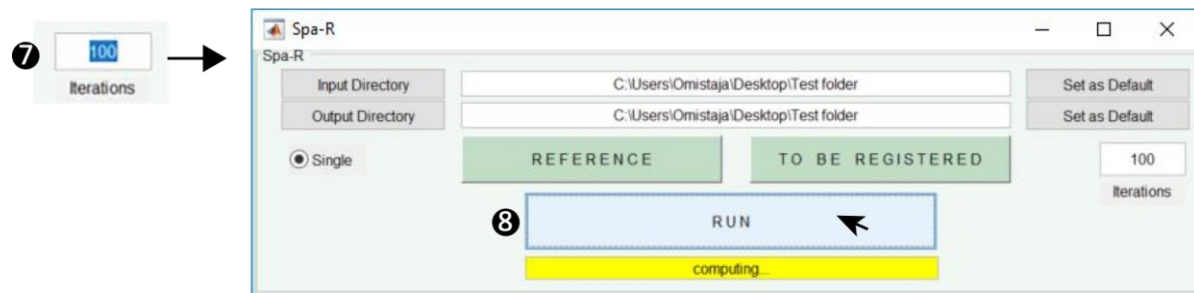
2. Set up the **input folder** ① and **output folder** ②. Optionally ③ can be selected to set the default folder directory whence re-launching Spa-R.



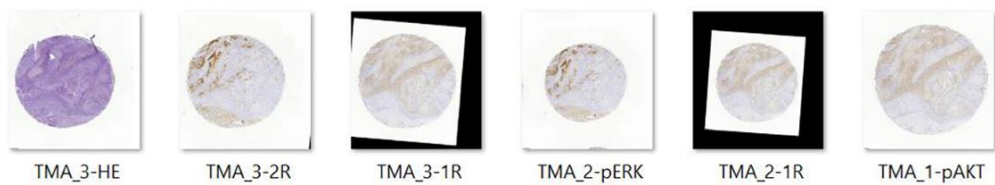
3. Select the **reference image** and **image(s) to be registered**



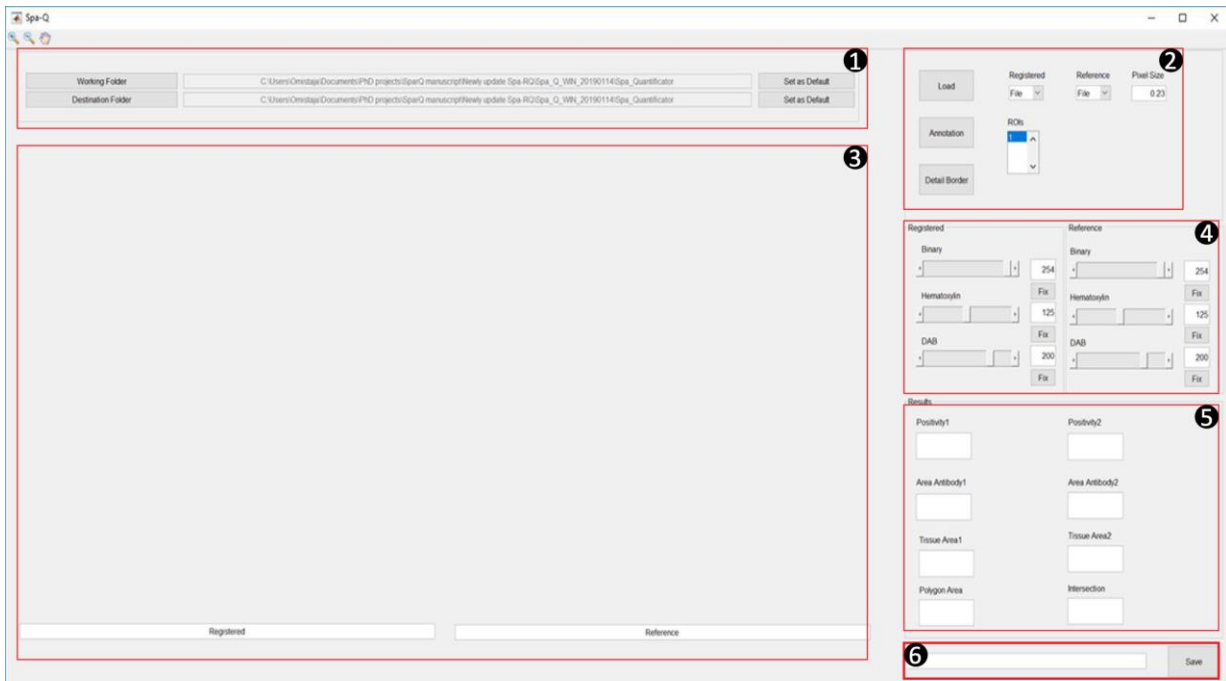
4. Determine the **iteration number** and start the registration. 100 is the default/recommended setting. However, users can test different iteration numbers and compare the required time and registration performance to fit this to their needed task.



5. Registered images will be generated and stored in the pre-selected **output folder**



Spa-Q (Spa_Quantification)



Modules

- ① Input & output settings
- ② Image and ROI selection
- ③ Images and ROIs display window
- ④ Parameter settings for image analysis
- ⑤ Quantitative results display
- ⑥ Progress indicator and "save" function

Step-by-step instructions

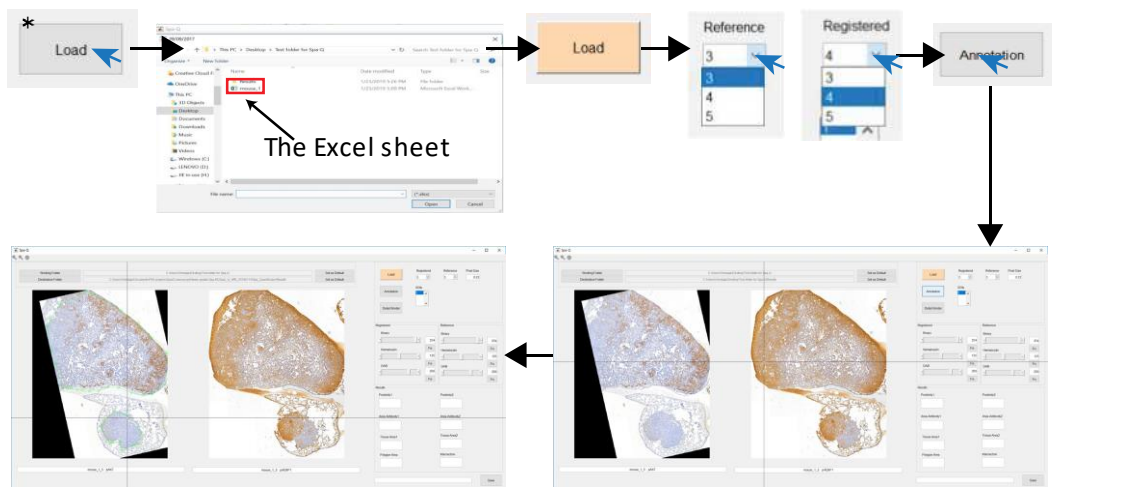
1. Generate an **Excel sheet** in the **input folder** following below format



	A	B	C	D
1	Date	SlideName	SlideNum	Antibody
2	1/1/2019	mouse_1	3	p4EBP1
3			4	pERK
4			5	pAKT
5				

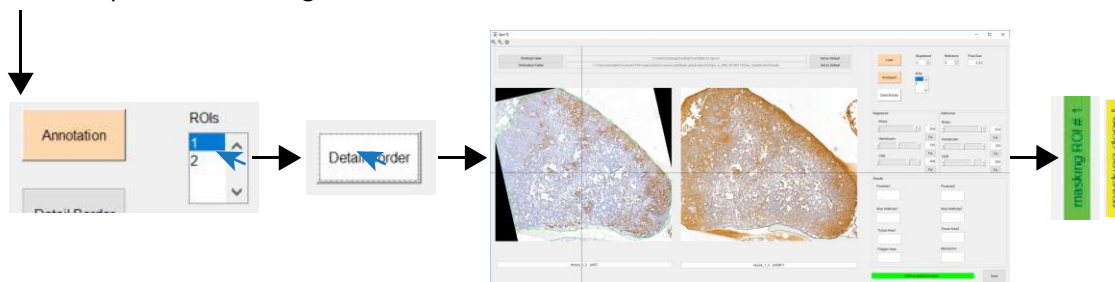
2. Pre-select the *input* and *output* folders (see above under Sap-R)

3. *Images* and *ROIs* selection



b. Annotate one of the images
 Left-click to draw ROI outline
 Right-click to start another ROI
 Double middle click* to confirm annotation done
 This step is to make rough outlines for ROIs

a. Polygon tool will appear automatically



c. Left-click to draw fine outline for the selected ROI
 Double middle click* to start image segmentation

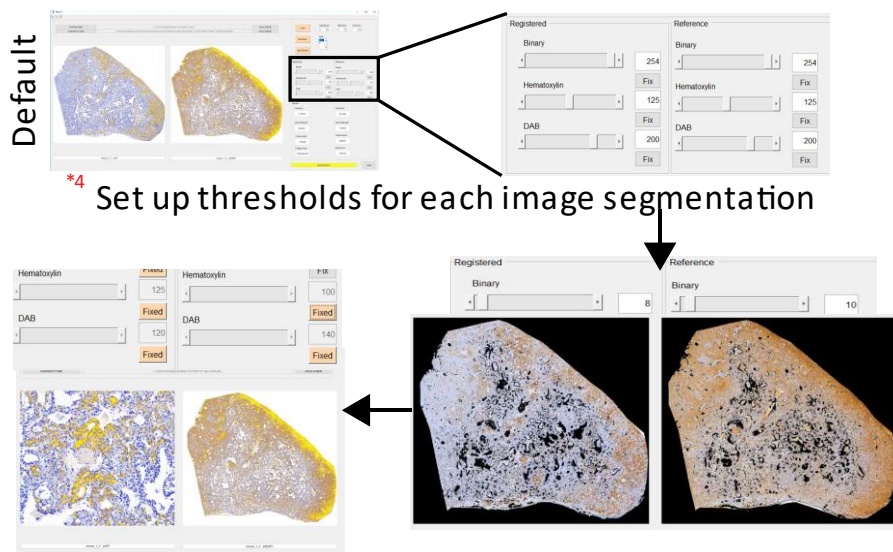
* The middle click is performed by clicking the scroll wheel.

3a: How to add a middle click to Windows Touchpad
 Go to **Settings > Device > Touchpad > Three-finger gestures > Taps**
 Stroll down **Taps** and choose **Middle mouse button**

3b: How to add a middle click to Mac
 Follow the instructions on <https://www.howtogeek.com/267782/how-to-add-a-middle-click-to-your-macs-trackpad/> to set up the middle click with trackpad.

4. Image segmentation by adjusting **binary**, **hematoxylin** and **DAB** thresholds for each of the images.

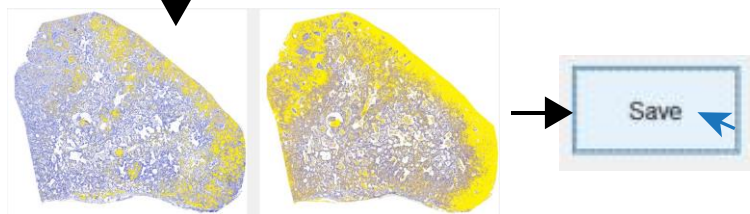
Users should set thresholds for each staining by testing the intensity parameters on multiple ROIs, and through inter-observer analysis, as described in the Methods. Once parameters are defined, they are entered for ROI 1 in Spa-Q and quantification is done by pressing 'save'; the same thresholds are automatically applied to next ROIs, and pressing 'save' completes the quantification on each image set.



Default

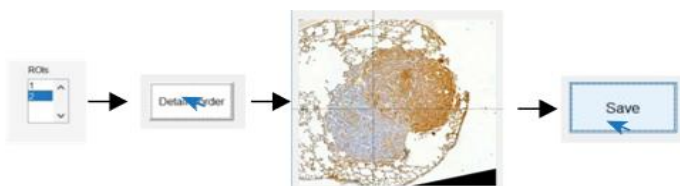
*4 Set up thresholds for each image segmentation

*5 Zoom in to check the thresholds



5. Quantification of another ROI

After saving the quantification results of the first ROI, go back to **ROIs**, select another ROI and refine its border, and press 'save' to obtain the results of the second ROI, and move to the next.



6. Results will be displayed on the screen, and saved in the pre-selected results folder

