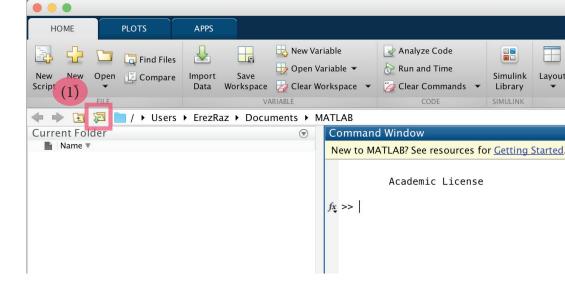
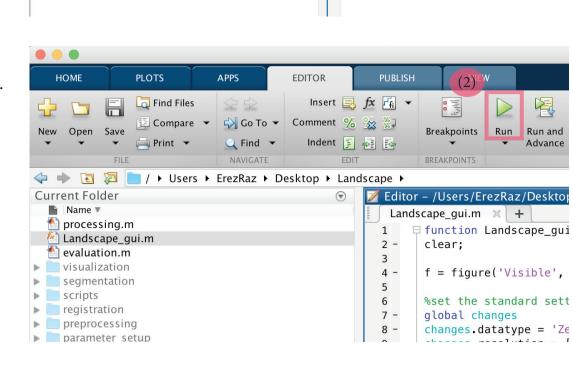
## Getting Started

To run *Landscape* MATLAB is required. Open MATLAB.

(1) In MATLAB, browse for the folder that contains the Landscape software.



Double click the file **Landscape\_gui.m** and click on **Run**.



**Settings**: includes information required to analyze

The main window of the software appears:

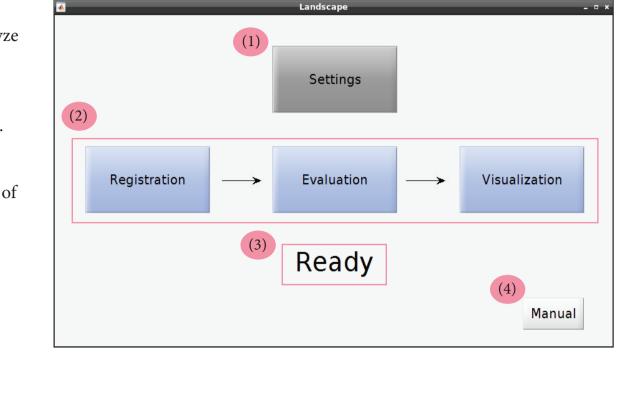
your imaging data. Workflow consisting of the three main steps: **Registration**: this step includes the 3D image registration of your imaging data.

**Evaluation**: this step allows to evaluate the registration results. **Visualization**: this step includes the generation of heatmaps.

(3) Status indicator: it informs about the current status of the analysis.

(4) Manual: it includes the present step-by-step instructions of how to run the software.

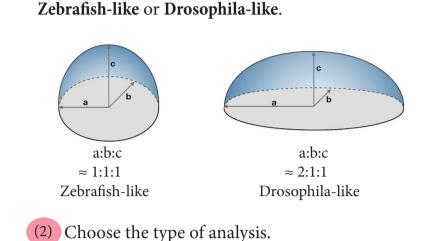
The *Settings* window will appear.



## Before you start working with your data make sure to check the settings. Click on **Settings**.

Settings

(1) Choose the type of your input data. The following image helps to determine if your data is



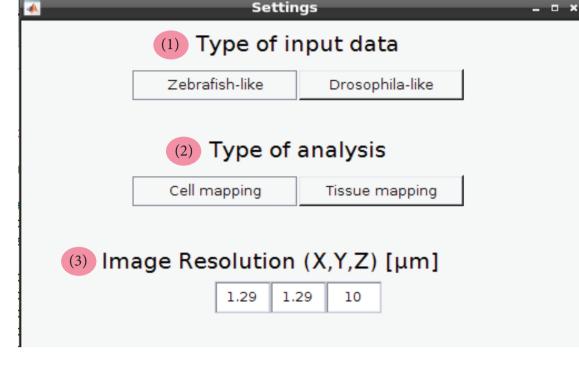
such as tissues, choose Tissue mapping. (3) Provide the resolution of your imaging data. Now, you can close the settings window.

If you want to analyze the distribution of cells choose

**Cell mapping.** If you want to analyze larger structures,

Registration

Now you can start processing your data. The registration step will use your *input data* and generate *registered data*.



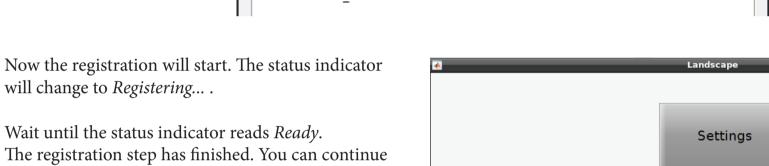
## Click on **Registration**. A window will appear. Select the folder that contains your *input data*.

Please select a folder with the data DemoData

Demo Results DemoData Cells



DemoData\_Cells



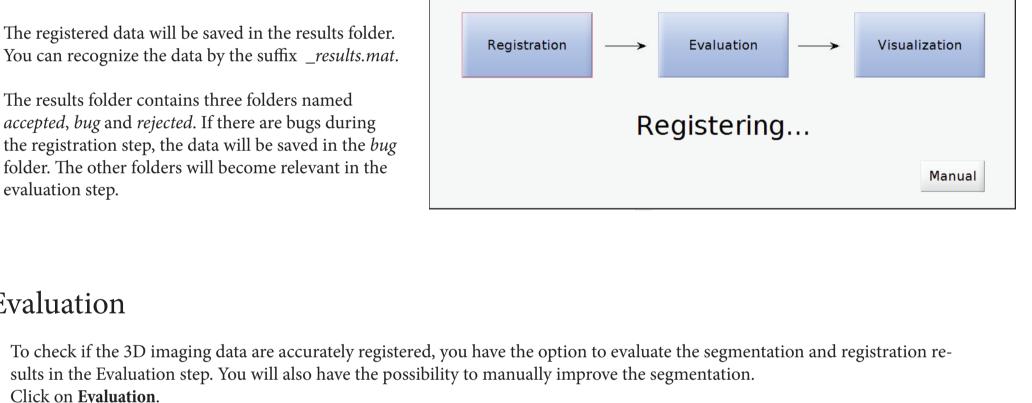
The registered data will be saved in the results folder. You can recognize the data by the suffix *\_results.mat*.

will change to Registering....

with the next step, the evaluation.

The results folder contains three folders named accepted, bug and rejected. If there are bugs during

Evaluation



Results for dataset: d\_227

Accept Improve Reject

Cells/Tissue of Interest

Click on Evaluation.

red data from all channels.

section "Improve function").

evaluation step.

DemoData\_Cells

After selecting the folder two windows will open. The large window will show a visualization of the input, segmented and registe-

Please select a results folder to evaluate

A window will appear. Select the folder that contains your *registered data* (see registration step).

DemoData

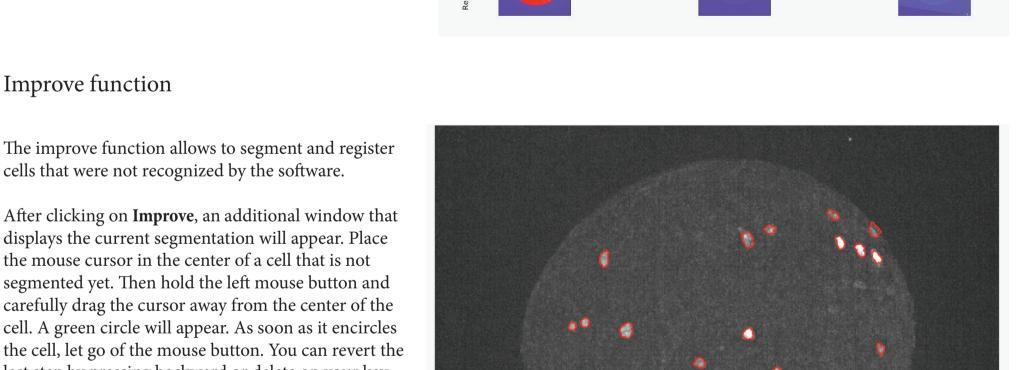
subfolder of your results folder. Otherwise click on the **Reject** button. The data will be transfered to the *rejected* subfolder. The rejected data will be ignored in the visualization step and not considered for the heatmaps.

You can check if the cells/tissue of interest are pro-

perly segmented (orange box). If not, you can click on the **Improve** button in the small window (See next

When your data are accurately registered, click on **Accept**. The data will be transferred to the *accepted* 

Improve function The improve function allows to segment and register cells that were not recognized by the software.



displays the current segmentation will appear. Place the mouse cursor in the center of a cell that is not segmented yet. Then hold the left mouse button and carefully drag the cursor away from the center of the cell. A green circle will appear. As soon as it encircles the cell, let go of the mouse button. You can revert the last step by pressing backward or delete on your keyboard. Additionally, you have the option to manually change

that were not recognized without having to be selected manually. Also, by adjusting the segmentation

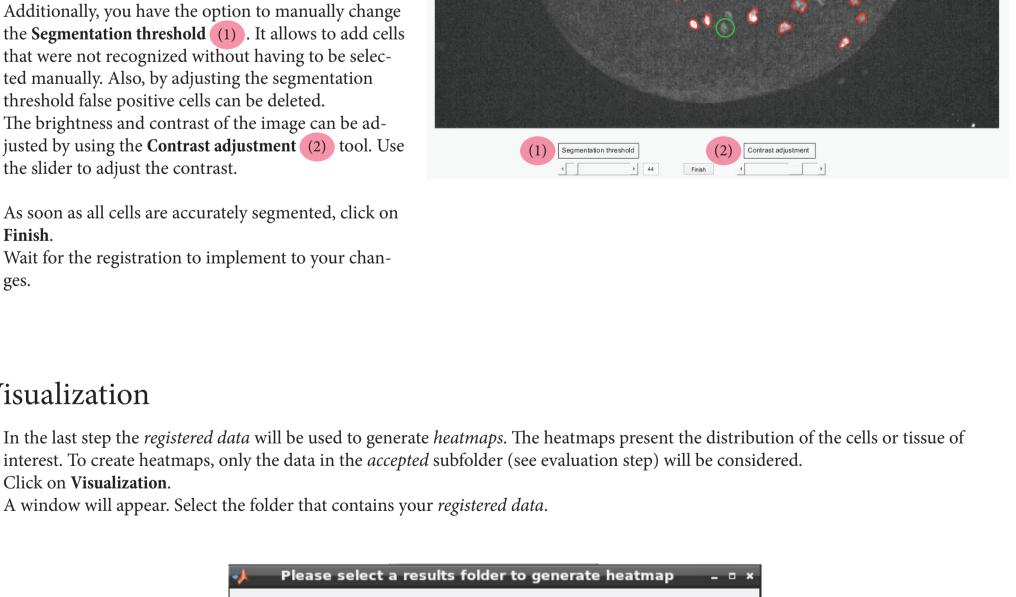
threshold false positive cells can be deleted.

Visualization

Click on Visualization.

The brightness and contrast of the image can be adjusted by using the **Contrast adjustment** (2) tool. Use the slider to adjust the contrast. As soon as all cells are accurately segmented, click on Finish. Wait for the registration to implement to your changes.

A window will appear. Select the folder that contains your registered data.



Settings

Evaluation

Accumulator.mat

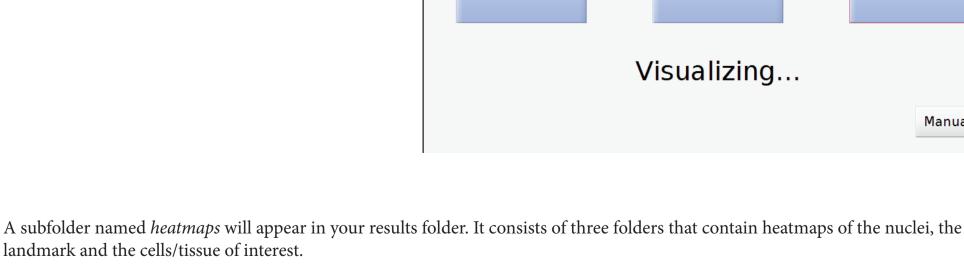
Visualization

Manual

Look <u>I</u>n: DemoData DemoData\_Cells

The visualization step will start. The status indicator

will change to Visualizing.... Wait until the status indicator reads *Ready*. The Visualization step has finished.

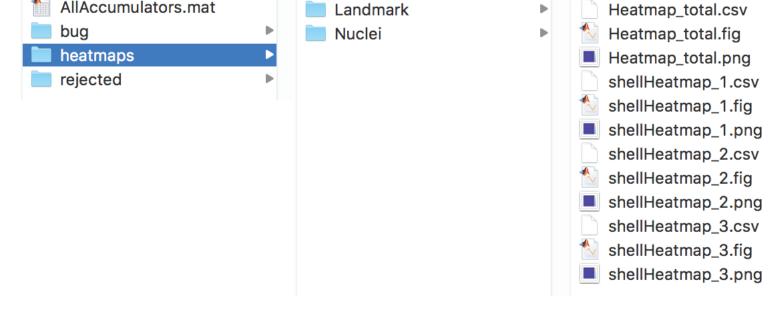


Registration

Specifically, the heatmap folders contain the following files: **Accumulator.mat** contains all relevant information for generating heatmaps.

**Heatmap\_total** shows the distribution of all cells within the entire embryo. **shellHeatmap\_1** shows the distribution of cells above the landmark shell. **shellHeatmap\_2** shows the distribution of cells at the level of the landmark shell.

**shellHeatmap\_3** shows the distribution of cells below the landmark shell. accepted CellsOfInterest AllAccumulators.mat Landmark



The heatmaps are provided as image files (.png) and matlab files (.fig). In addition, for the heatmaps of the nuclei and cells of interest corresponding .csv files are provided that contain the data depicted in the heatmaps in a tabular format.