



DeepPhagy Manual

A deep learning framework for quantitatively measuring autophagy
activity in *Saccharomyces cerevisiae*

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The latest version of DeepPhagy is available from
<http://deepphagy.biocuckoo.org/>

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Index

Statement.....	3
Introduction.....	4
Download & Installation	5
The Work environment.....	7
➤ What cells could be recognized accurately?	7
➤ How to load an image?	7
➤ How to zoom the picture?.....	8
➤ How to count cells of a picture?.....	9
➤ How to label cells?.....	9
➤ How to change the marker's size or shape?	11
➤ How to save the label results?.....	12
➤ How to tune the classification threshold ?.....	12
➤ How to draw scales?	13
➤ How to save numbers on the image?	15
➤ How to batch count?	15

Statement

1. **Implementation.** The softwares of the CUCKOO Workgroup are implemented in JAVA (J2SE8).
2. **Availability.** Our softwares are freely available for academic researches. For non-profit users, you can copy, distribute and use the softwares for your scientific studies. Our softwares are not free for commercial usage.
3. **Usage.** Our softwares are designed in an easy-to-use manner. Also, we invite you to read the manual before using the softwares.
4. **Updation.** Our softwares will be updated routinely based on users' suggestions and advices. Thus, your feedback is greatly important for our future updation. Please do not hesitate to contact with us if you have any concerns.
5. **Citation.** Usually, the latest published articles will be shown on the software websites. We wish you could cite the article if the software has been helpful for your work.

Introduction

Autophagy is an evolutionarily conserved pathway from yeast to human for degrading and recycling cellular components and exerts in both bulk/nonselective and selective manners. The autophagic process is dynamically orchestrated by protein products of AuTophagy-related (*ATG*) genes and autophagy regulators, where the dysfunction of autophagy has been implicated in a wide range of human diseases including cancer, diabetes and neurodegenerative disorders. Accurately monitoring and measuring autophagic activity are fundamental for understanding the functional importance and regulatory roles of autophagy under physiological and pathological conditions.

In this work, we introduce the DeepPhagy (Deep learning for autoPhagy, version 1.0), the first software program specifically designed for fully automatic prediction of autophagic phenotypes from fluorescent images of yeast. Three modes including “Count”, “Batch” and “Advanced” modes are supported. The DeepPhagy 1.0 user interface (Figure 1) is shown below.

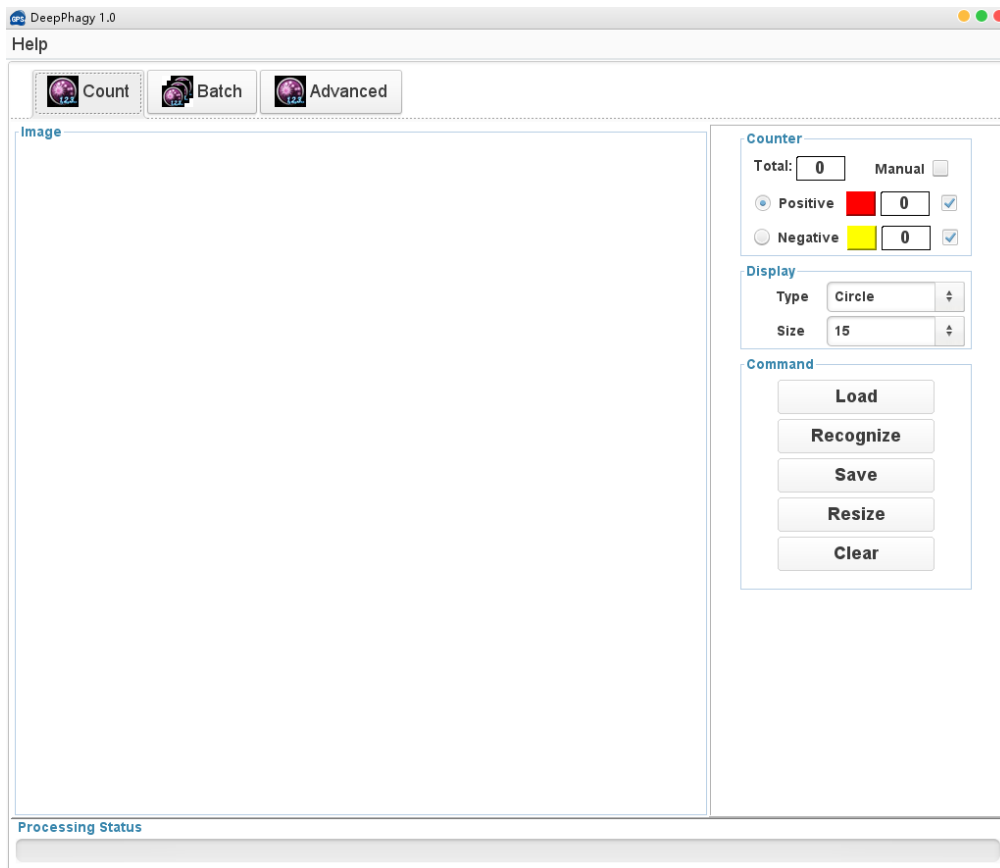


Figure 1

Download & Installation

The DeepPhagy 1.0 software was implemented in JAVA 1.8 (J2SE 8.0), and could be installed on three major Operating Systems (OS), including Windows, Linux/Unix or Mac OS X systems. The local stand-alone packages of DeepPhagy 1.0 can be freely available at <http://deepphagy.biocuckoo.org/down.php>. We recommend that users could download the latest release.

Please choose the proper package to download. After downloading, please double-click on the software package to begin installation, following the user prompts through the installation. And snapshots of the setup program under windows 10 are shown below:

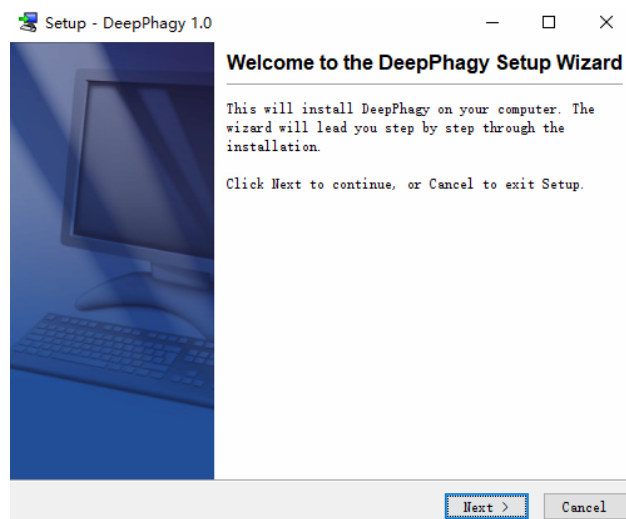


Figure 2

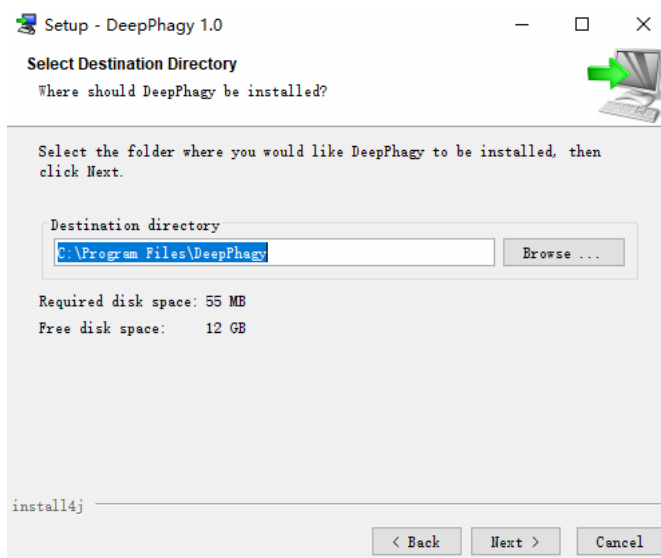


Figure 3

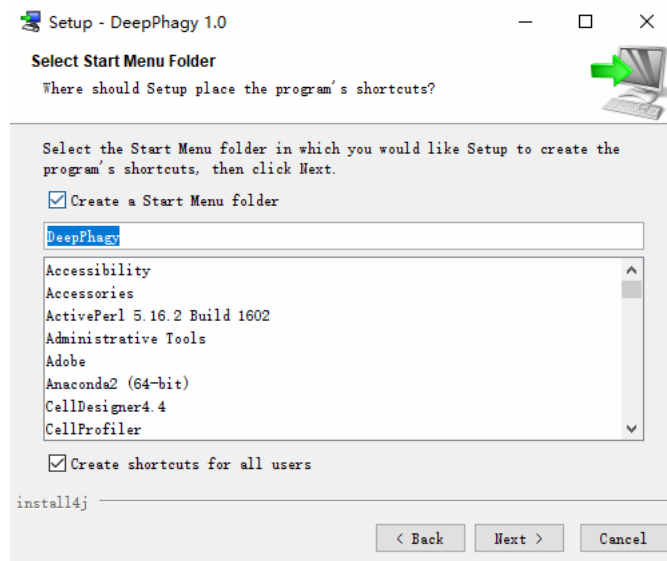


Figure 4

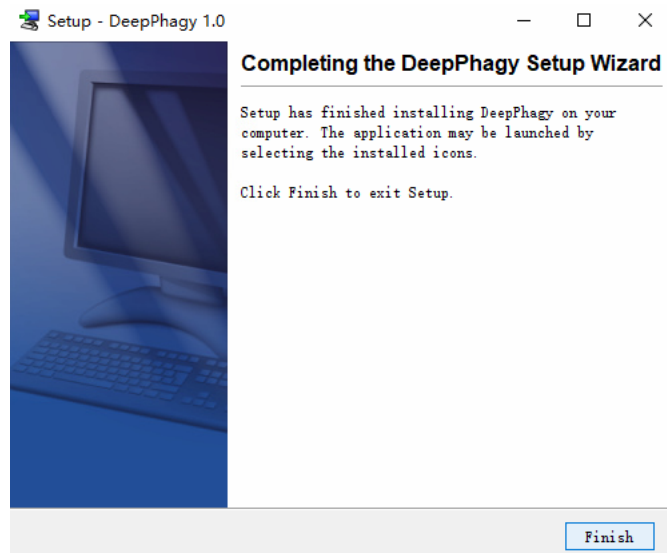


Figure 5

Finally, please click on the “Finish” button to complete the setup program.

The Work environment

➤ *What cells could be recognized accurately?*

Some regular round and oval cells, such as *Saccharomyces cerevisiae* cells, were more accurately recognized.

➤ *How to load an image?*

On the main interface “Count” or “Advanced”, click of the button “Load”.

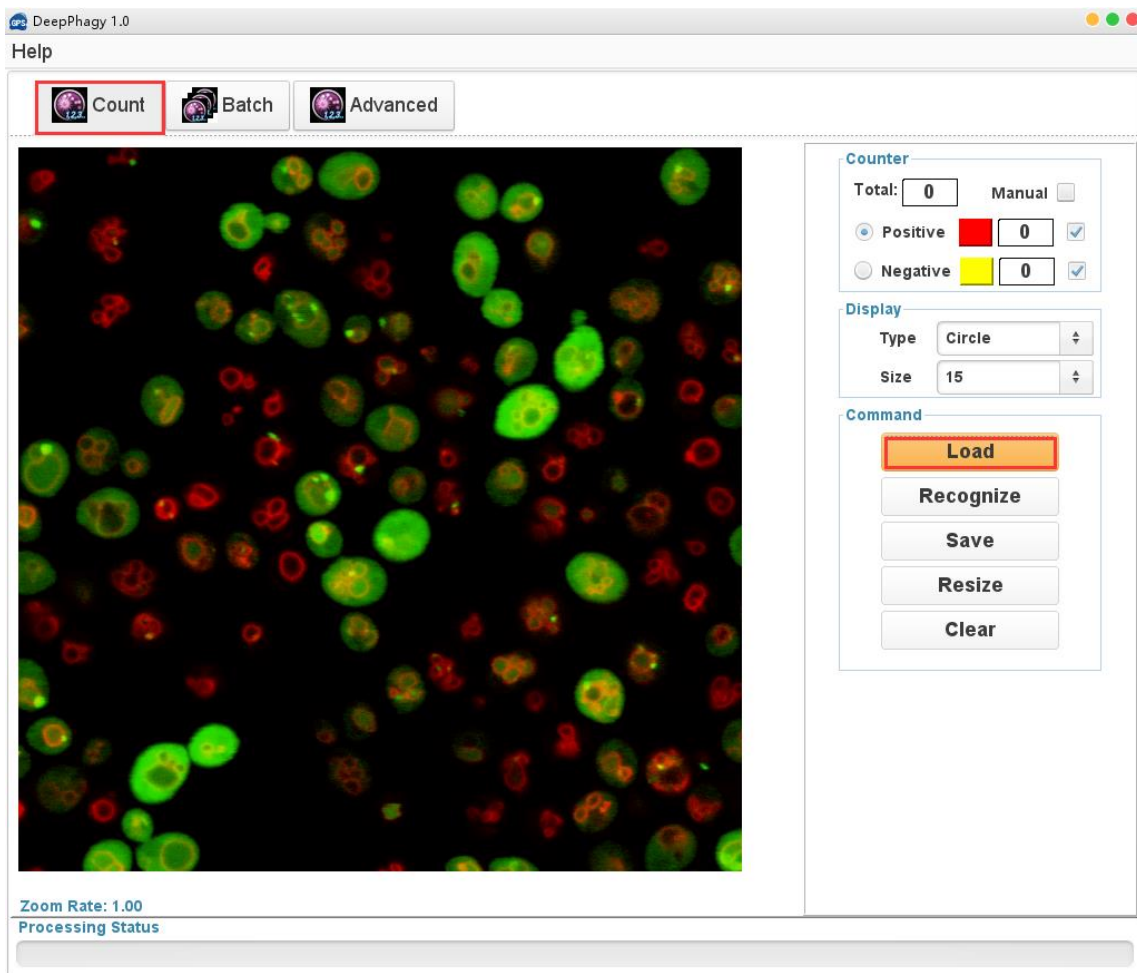


Figure 6

In the file browser, select an image file.

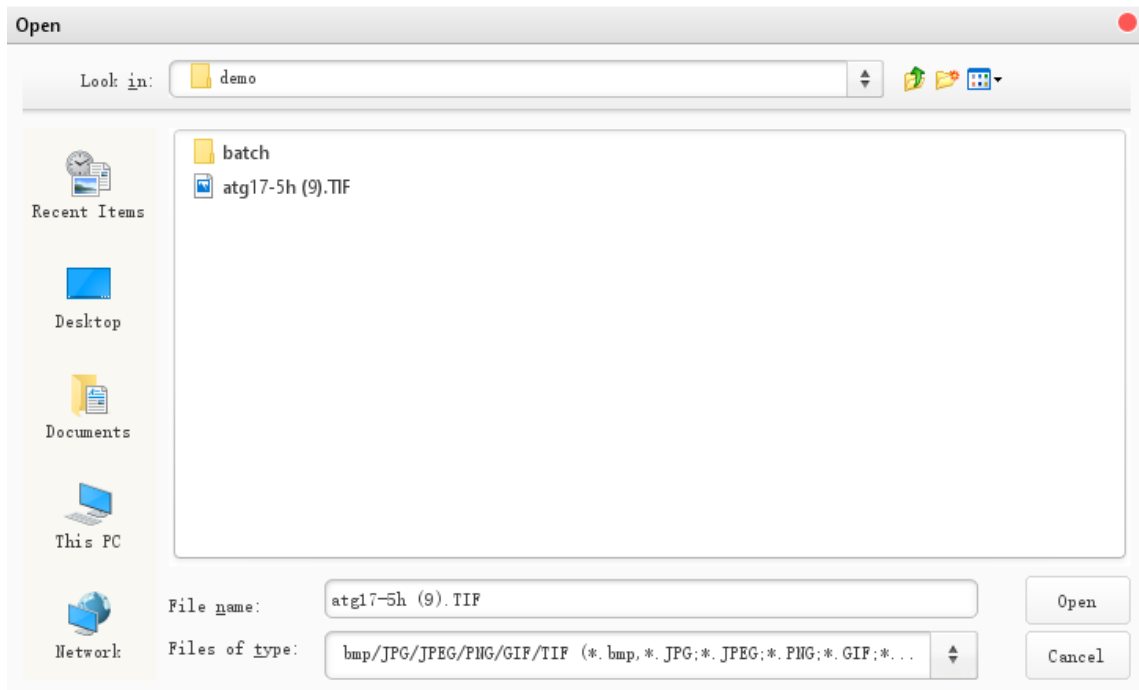


Figure 7

The supported image formats include BMP, JPG, PNG, GIF and TIF. The original image should be larger than 650 * 650 pixels, because inputted pictures will be downsampled into 650 * 650 pixels for better computing performance.

➤ ***How to zoom the picture?***

Scroll up or down the mouse wheel on the main interface to display the picture in a smaller or magnified view. The button “Resize” displays the image in a normal size (Figure 8).

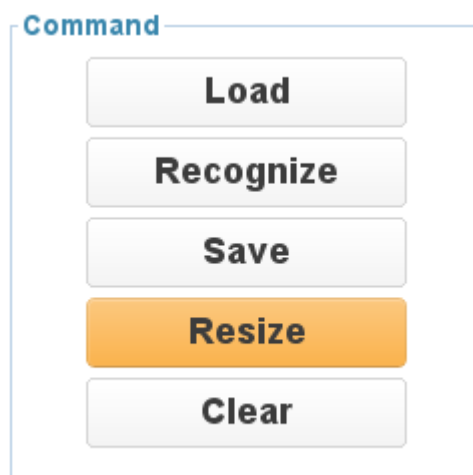


Figure 8

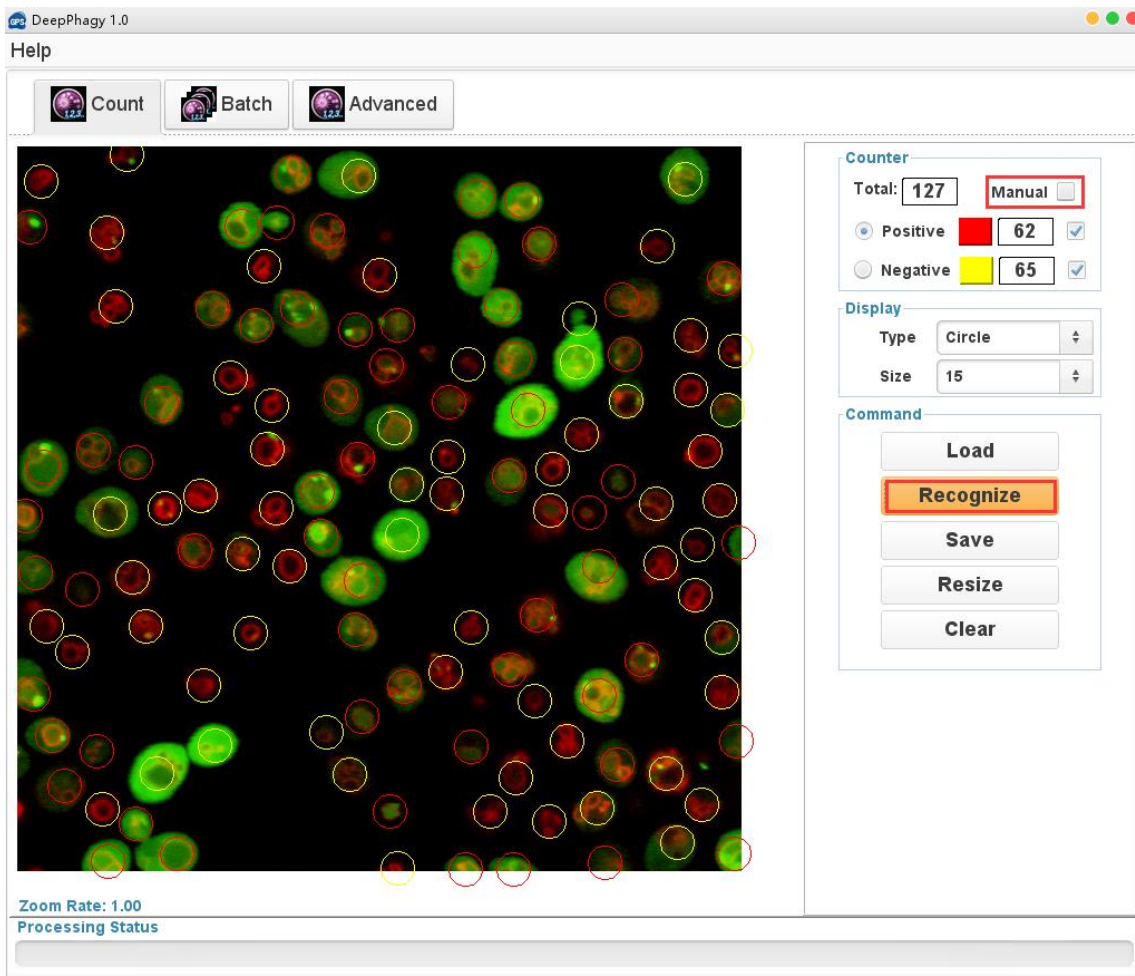
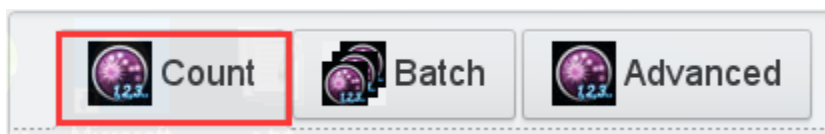


Figure 9

➤ *How to count cells of a picture?*

Step 1. Select group tab “Count”.



Step 2. Load a picture by the button “Load” on the panel “Command”.

Step 3. Click the button “Recognize” on the panel “Command” (Figure 9).

Step 4. The auto-recognition results can be further fine-tuned manually by checking the “Manual” box in “Counter” panel.

➤ *How to label cells?*

Step 1. Checking the “Manual” box in “Counter” panel to enable the label function, otherwise disenabled.

Counter

Total: Manual ☒

☒ Positive ☒

☐ Negative ☒

Step 2. Marking positive cells: press radio button “positive” to enable the function.

Counter

Total: Manual ☒

☒ Positive ☒

☐ Negative ☒

Positive color is red by default. Click the color panel behind the radio button “positive” to change the marker's color.z

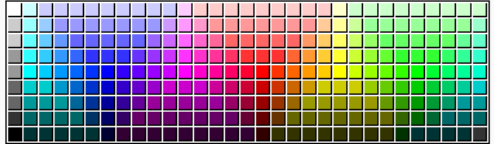
Counter

Total: Manual ☒

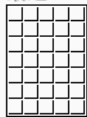
☒ Positive ☒

☐ Negative ☒

Color Choose



最近:



预览

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Step 3. Marking negative cells. Please refer to step 2 similarly.

Counter

Total: Manual ☒

☐ Positive ■ ☒

☒ Negative ■ ☒

Note: single labeling marker can be cancelled by right-click the mouse, all the labeling results can be cleared by clicking the “Clear” button.

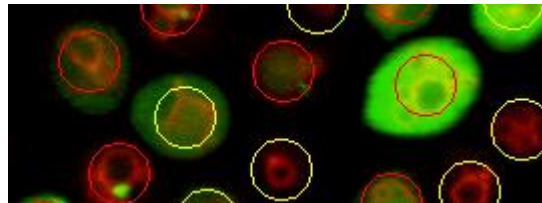
➤ ***How to change the marker's size or shape?***

The default shape is the “Circle” and default size is 15.

Display

Type

Size



By clicking on the drop-down box “Size” on the panel “Display”, from 15 to 25, then resizes the marker's size.

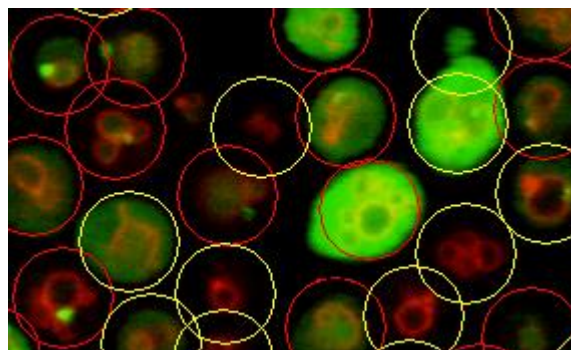
Display

Type

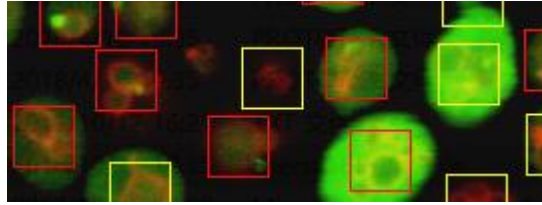
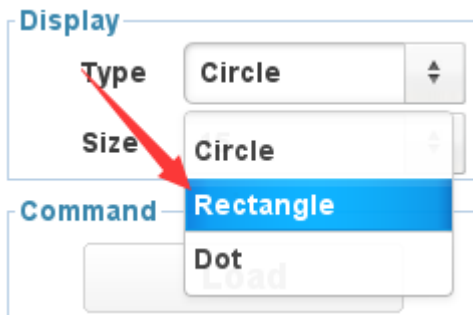
Size

Command

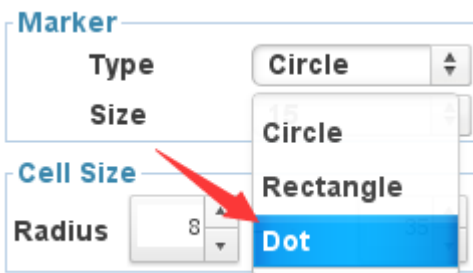
15
20
25
30
35
40
45
50



By clicking on the drop-down box “Type”, and select the “Rectangle”:

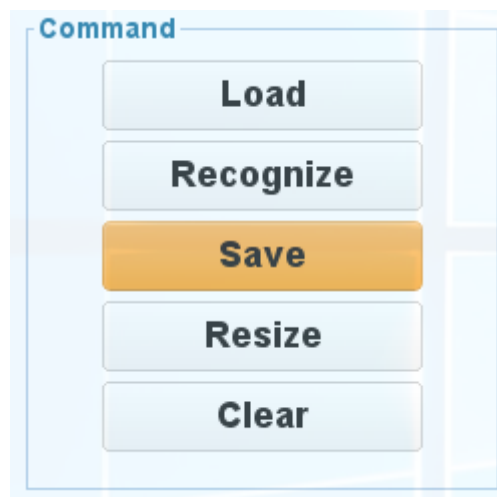


The “Dot”:



➤ *How to save the label results?*

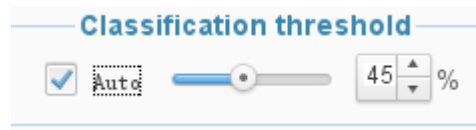
Click the button “Save” to save the label results.



The labeled image can be exported into multiple formats by clicking on the “Save” button.

➤ *How to tune the classification threshold?*

In “Advanced” interface, the default mode of the classification threshold is automatic when the checkbox “Auto” is selected.



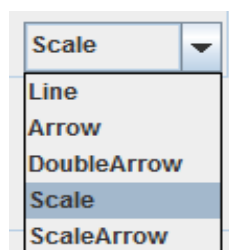
If the “Auto” check box is not selected, the threshold can be modified by dragging the slider or adjusting the spinner behind the slider.

➤ *How to draw scales?*

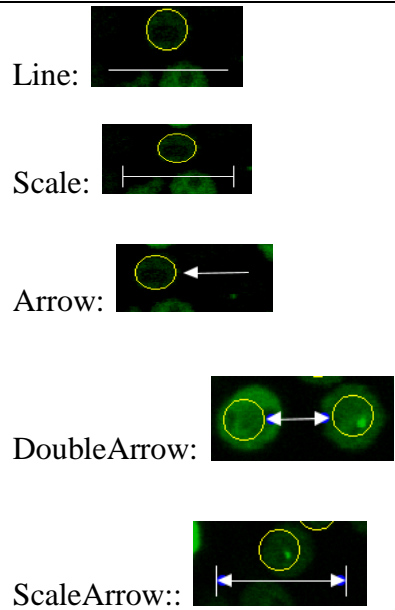
Step 1. In “Advanced” interface, Click the button “scale”  on the panel “Scale”.

Step 2. The configuration interface of a scale is as follows:

Figure 10



It provides five styles:



Note: the “font” of the computer must support “ μm ” or other characters, otherwise the scale may not be displayed normally.

Step 3. The text is located at the center of the scale and the default coordinate of the scale is in the lower-right corner.

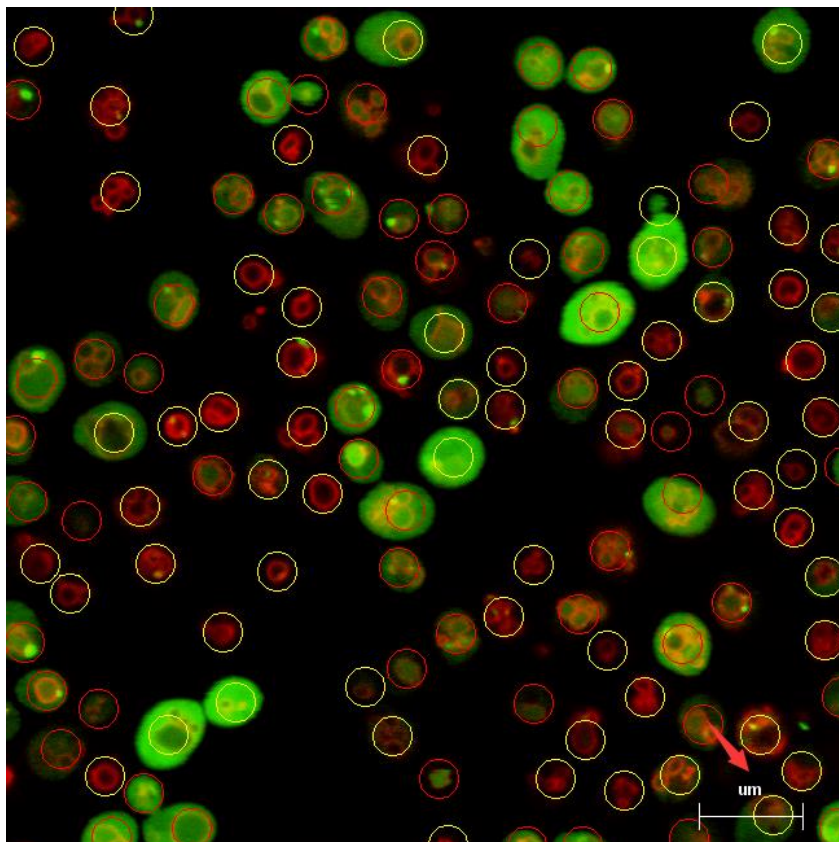


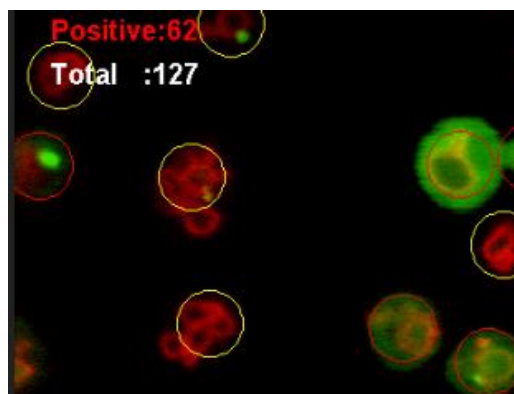
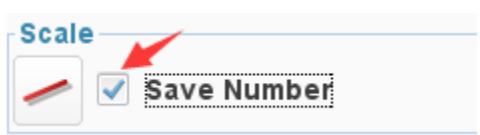
Figure 11

The line and text could be rotated or moved by dragging the mouse. They can be cancelled by

pressing the key “Delete”.

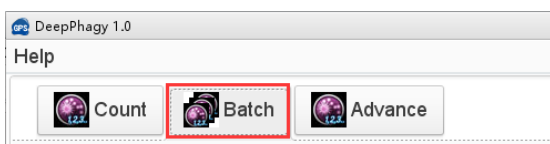
How to save numbers on the image?

The checkbox “Save Number” can be selected to draw the count results on the top left corner of an image.



➤ *How to batch count?*

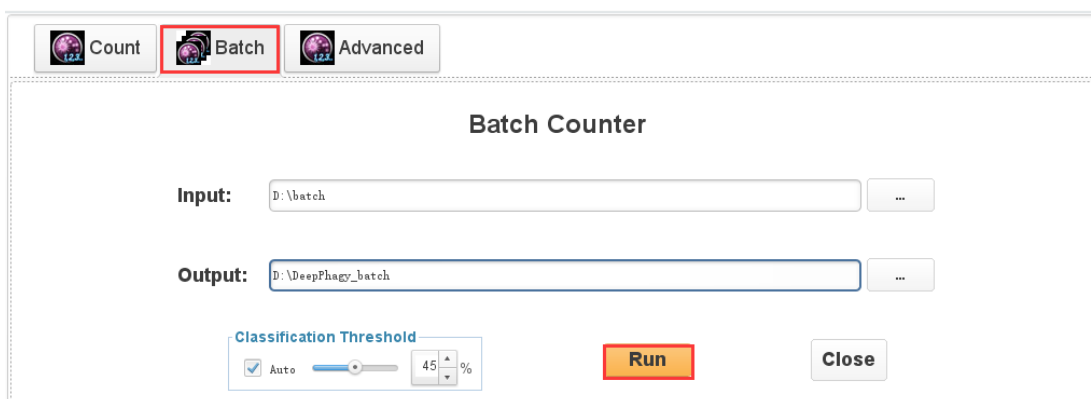
Step 1. Select the group tab “Batch”.



Step 2. The dialog of “Batch”:

Textbox “Input”: the folder of image files.

Textbox “Output”: the save path of batch count results.



The other parameter values are the same as the “Advanced” configurations.

Step 3. After clicking the button “Run”, the auto-recognition images with a txt file recording the counting results are saved in the output path.