wpwupingwp/pyGUS: quantification for GUS stain images

wpwupingwp

pyGUS

Quick start

Download the package, unzip, and then double-click `pyGUS.exe` or `pyGUS`.

OR

Make sure you have Python (3.8 or higher) installed.

Open terminal, run

```
# Install, using pip (recommended)
pip install pyGUS --user
# Or, use conda
conda install -c wpwupingwp pyGUS
# Run
# Windows
python -m pyGUS
# Linux and MacOS
python3 -m pyGUS
```

Table of Contents

Feature

✔ Quantify GUS stain images automatically.

✔ Use negative and positive reference to calibrate expression value.

✔ Support Macbeth color checker to calibrate color bias.

Prerequisite

Hardware

The program could run in normal computers and have no extra requirements for memory, CPU, et al.

Currently, macOS, Linux and Microsoft Windows systems were supported.

Software

For the portable version, nothing needs to be installed manually.

For installing from pip, Python is required. Notice that the python version should be **3.8** or higher.

✅ All third-party dependencies will be automatically installed with the Internet, including `opencv-contrib-python`, `numpy`, `matplotlib`, `coloredlogs` (python packages).

Installation

Portable

Download from the link, unpack and run.

Install with pip

1. Install Python. *3.8 or newer* is required.
2. Open the command line, run

```
pip3 install novowrap --user
```

Usage

Desktop

If installed with pip,

```
# Windows
python -m pyGUS
# Linux and MacOS
```

```
python3 -m pyGUS
```

If you use the portable version, double-click the `pyGUS.exe`. Then click the button to choose which mode to run.

Command line

❗ In Linux and macOS, Python2 is `python` and Python3 is `python3`. However, in Windows, Python3 is called `python`, too. Please notice the difference and make sure you use `Python 3` instead of `Python 2`.

- Show help information

```
# Windows
python -m pyGUS -h
# Linux and MacOS
python3 -m pyGUS -h
```

- Run

```
# Windows
#   mode 1
python -m pyGUS -mode 1 -ref1 [file1] -ref2 [file2] -images [files3] [file4] ...
#   mode 1 with automatically detect object
python -m pyGUS -mode 1 -auto_ref -ref1 [file1] -ref2 [file2] -images [files3] [
#   mode 2
python -m pyGUS -mode 2 -ref1 [file1] -images [file2] [file3] ...
#   mode 2 with automatically detect object
python -m pyGUS -mode 2 -auto_ref -ref1 [file1] -images [file2] [file3] ...
#   mode 3
python -m pyGUS -mode 3 -ref1 [file1] -ref2 [file2] -images [files3] [file4] ...
#   mode 3 with automatically detect object
python -m pyGUS -mode 3 -auto_ref -ref1 [file1] -ref2 [file2] -images [files3] [
#   mode 4
python -m pyGUS -mode 4 -images [file1] [file2] ...
# Linux and macOS
#   mode 1
python3 -m pyGUS -mode 1 -ref1 [file1] -ref2 [file2] -images [files3] [file4] ..
#   mode 1 with automatically detect object
python3 -m pyGUS -mode 1 -auto_ref -ref1 [file1] -ref2 [file2] -images [files3]
#   mode 2
python3 -m pyGUS -mode 2 -ref1 [file1] -images [file2] [file3] ...
#   mode 2 with automatically detect object
python3 -m pyGUS -mode 2 -auto_ref -ref1 [file1] -images [file2] [file3] ...
#   mode 3
python3 -m pyGUS -mode 3 -ref1 [file1] -ref2 [file2] -images [files3] [file4] ..
#   mode 3 with automatically detect object
python3 -m pyGUS -mode 3 -auto_ref -ref1 [file1] -ref2 [file2] -images [files3]
#   mode 4
python3 -m pyGUS -mode 4 -images [file1] [file2] ...
```

Options

`auto_ref`

If without `-auto_ref` option, the program require the user to manually select target region with mouse. Left click to add dots and draw lines. Right click to link the first dot and last dot to draw a close region.

If the image has extremely low contrast or the edge of the object is blur, such

selection is also required even with `-auto_ref` option opened.

convex

Use convex hull to fix break edges of the object in low contrast image. If set, the total area of the object may be overestimated. However, it has no effect for counting the expression region area.

Photo tips

Equipment

Cameras and stereo microscopes are recommended. Smartphones with camera functions are not recommended, especially models with built-in color grading. To reduce the effects of perspective distortion, lens with medium focal length (e.g. 50 mm in full-frame) is recommended. Make sure lens and camera sensor are free of stains.

Background

White board or **white** paper with no debris, no wrinkles, no obvious reflection

Lighting

It is recommended to use the bottom lighting of the whiteboard. When using indoor lighting, the international standard color rendering index (Ra index) of the bulb is required to be no less than 85, and the color temperature is between 4000 k to 7000 k; when using sunlight, avoid morning and evening. Close overhead lighting can cause significant reflections and is not recommended

Focus

The whole subject is not out of focus. Narrow down the aperture when shooting with a camera; adjust focus and magnification when using a stereo microscope to ensure that the edges of the subject are sharp

White balance

The recommended color temperature is around 6500k, with a minimum of 4000k and a maximum of 7000k. When the color temperature cannot be adjusted, adjust other parameters until the background is displayed in white without obvious color cast.

Exposure compensation

When using camera, the exposure compensation can be set to +1EV or +2EV to reduce the color cast of the paper. It could also reduce the influence of the transmittance of color cards or plants

Layout

Based on the size of the plant, adjust the lens-to-plant distance to make the plant cover the frame more than 50%. The main body does not cross the border, and the distance is not less than 10% of the screen width.

When there is only a single subject, place it in the center of the frame. When two subjects (two plants or plants with color card), place them on the left and the right, with interval between them not less than one-third of the width of the plant. They do not intersect, and they are not surrounded by each other (especially one with the long roots); there is no breakage, and the leaves do not overlap as much as possible.

File

No toning processing. The recommended minimum resolution is 500*500 px. When shooting with the camera, vignetting and distortion compensation can be turned on, and other adjustments (including white point, black point, contrast, brightness, style, etc.) should be turned off.

Input

All images should use white background.

Negative reference

One plant (whole or partial) with low expression or no expression.

Positive reference

One plant (whole or partial) with high expression.

Mode 1

The `mode 1` requires *negative reference* (`-ref1`), *positive reference* (`-ref2`) and target images (`-images`, one or more).

Mode 2

The `mode 2` requires one reference image (`-ref1`) with *negative reference on left and positive reference on right*. In each target images (`-images`, one or more), target plants are on left, positive references are on right separately.

Mode 3

The `mode 3` requires *negative reference* (`-ref1`), *positive reference* (`-ref2`) and target images (`-images`, one or more). In each image, the plant is on left and a Macbetch color checker is on right.

Mode 4

The `mode 4` requires one or more target images (`-images`). Users select two reference and target regions by mouse and press any key to continue to next image or to start running.

Output

`.csv` files: **csv** format table, with expression information of each image.

`-fill.png` files: Images filled with different colors. Blue means target, red means background, green means darker regions inner target.

`-calibrate.png` files: Images calibrated with color checker.

Performance

It depends on hardware. Normally one image cost seconds. The program does not require much memory or disk space.

Citation

Unpublished.

License

Q&A

Please submit your questions in the Issue page 😀

- Q: I can't see the full UI, some part was missing.

  A: Please try to drag the corner of the window to enlarge it. We got reports that some users in macOS have this issue.

- Q: I got the error message that I don't have `tkinter` module installed.

  A: If you want to run GUI on Linux or macOS, this error may happen because the Python you used did not include tkinter as default package (kind of weird). Run

  ```
  # Debian and Ubuntu
  sudo apt install python3-tk
  # CentOS
  sudo yum install python3-tk
  ```

  may help.

  For macOS users or linux users without root privilege, please try to install the newest version of Python or to use conda, see conda and Python for details.

- Q: It says my input is invalid, but I'm sure it's OK!

  A: Please check your files' path. The `space` or Chinese characters in the folder name or filename may cause this error.