

QuantGUV Quick Guide

By Zak Marshall

Standard Curve Generation:

1. Download the "I_Bulk QuantGUV.py" and open
2. Input folder locations of the Bulk dye TIFs on lines 7 and 8

```
6     # Folder paths
7     input_folder = r"INSERT_FILE_PATH_HERE"
8     output_folder = r"INSERT_FILE_PATH_HERE"
9     # Define the Excel file path
10    excel_path = os.path.join(output_folder, "I_Bulk.xlsx")
```

3. Run the code (F5)
4. Download the "I_Blank QuantGUV.py" and open
5. Input the folder locations of the Blank GUVs TIFs on lines 7 and 8

```
7     #Define Folders
8     input_folder = r"INSERT_FILE_PATH_HERE"
9     output_folder = r"INSERT_FILE_PATH_HERE"
10    excel_path = r"INSERT_FILE_PATH_HERE"
```

6. Define your magnification on line 24 and change if needed the scale ratio

```
23    #Define Magnification (these values are for a citation c10 confocal)
24    Magnification = "40x"
25    if Magnification == "20x":
26        scale_factor = 200 / 578 # distance in um / distance in pixels
27
28    elif Magnification == "40x":
29        scale_factor = 100 / 578 # distance in um / distance in pixels
```

7. Run the code (F5)
8. This will give you the background signal (Bulk dye) and the GUV signal (Noise) subtract the noise from the background signal for each concentration to get a I_Blank value
9. Now using the equation below substitute in the I_Bulk value as I_Total and I_Blank as I_Outside to calculate the I_Inside for each concentration of dye

$$I_{Inside} = I_{Total} - I_{Outside}$$

10. Use this to make your standard curve

QuantGUV Usage:

1. QuantGUV can be ran straight from the script and the GUI will open up

Tabs for switching
between images
and settings

Input/ Output and
excel path
definitions (use
browse buttons
for ease)

Define Scale
factor:
distance / pixels

Threshold
adjustments

Diameter filters

Define
circularity
(removes out of
focus GUVs)

Status bar

The screenshot shows the QuantGUV v2.0 GUI window. At the top, it says "QuantGUV v2.0" and "Designed by Zak Marshall". Below this are two tabs: "Home" and "Previews". The "Home" tab is active. It contains several input fields and buttons:

- Input Folder:** A text input field with a "Browse" button to its right.
- Output Folder:** A text input field with a "Browse" button to its right.
- Excel File Path:** A text input field with a "Browse" button to its right.
- Scale Factor (µm/pixel):** A text input field containing the value "100/578".

Below these is a section titled "Blob Detection Parameters:" which includes:

- Min Threshold:** A text input field containing the value "5".
- Max Threshold:** A text input field containing the value "255".
- Min Diameter (µm):** A text input field containing the value "10".
- Max Diameter (µm):** A text input field containing the value "100".
- Filter by Circularity:** A checkbox that is checked (indicated by an 'x' in the box).
- Min Circularity:** A text input field containing the value "0.7".

A "Run Analysis" button is located to the right of the threshold and diameter fields. At the bottom right of the window, there is a "Ready" status bar.

Annotations with arrows point to the following elements:

- The "Home" and "Previews" tabs.
- The "Browse" buttons for Input Folder, Output Folder, and Excel File Path.
- The "Scale Factor (µm/pixel)" input field.
- The "Min Threshold" input field.
- The "Min Diameter (µm)" input field.
- The "Filter by Circularity" checkbox.
- The "Run Analysis" button.
- The "Ready" status bar.

2. When parameters have been set up, click “Run Analysis”

The screenshot shows the QuantGUV v2.0 software interface. The main window displays a list of detected GUVs on the right and a preview of the image on the left. The list of detected GUVs includes:

- ☒ GUV 1: 10.11 μm , 25000.00 intensity
- ☒ GUV 2: 20.13 μm , 30000.00 intensity
- ☐ GUV 3: 30.15 μm , 35000.00 intensity
- ☐ GUV 4: 40.16 μm , 40000.00 intensity
- ☒ GUV 5: 50.17 μm , 45000.00 intensity

The preview image shows five GUVs labeled GUV 1 through GUV 5. GUV 3 and GUV 4 are marked with a red 'X', indicating they are deselected. GUV 1, GUV 2, and GUV 5 are marked with a blue circle, indicating they are selected. The interface also includes buttons for 'Previous', 'Next', and 'Finish Review and Save'. A status bar at the bottom indicates 'GUVs in current image: 3/5 accepted. Click GUV or use checkbox to toggle. Zoom: 100%'.

Annotations:

- List of detected GUVs
- Deselect GUV by clicking on them or the check box (deselected GUVs go red)
- Use Next and Previous buttons to review detections
- Press finish Review and Save to export detected GUV data to excel

3. Open the excel file to analyse the data using your standard curve

Image Name	Description	Diameter (μm)	Area (pixels)	Intensity	Background Normalized
40x detction image.tif	GUV 1	10.11	2679.78	25000	20000
40x detction image.tif	GUV 2	20.13	10637.43	30000	25000
40x detction image.tif	GUV 5	50.17	66051.99	45000	40000

Filename GUV
was detected in

GUV identifier in
image

GUV size in μm

GUV Intensity after
background
normalisation