QuantGUV Quick Guide

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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
```

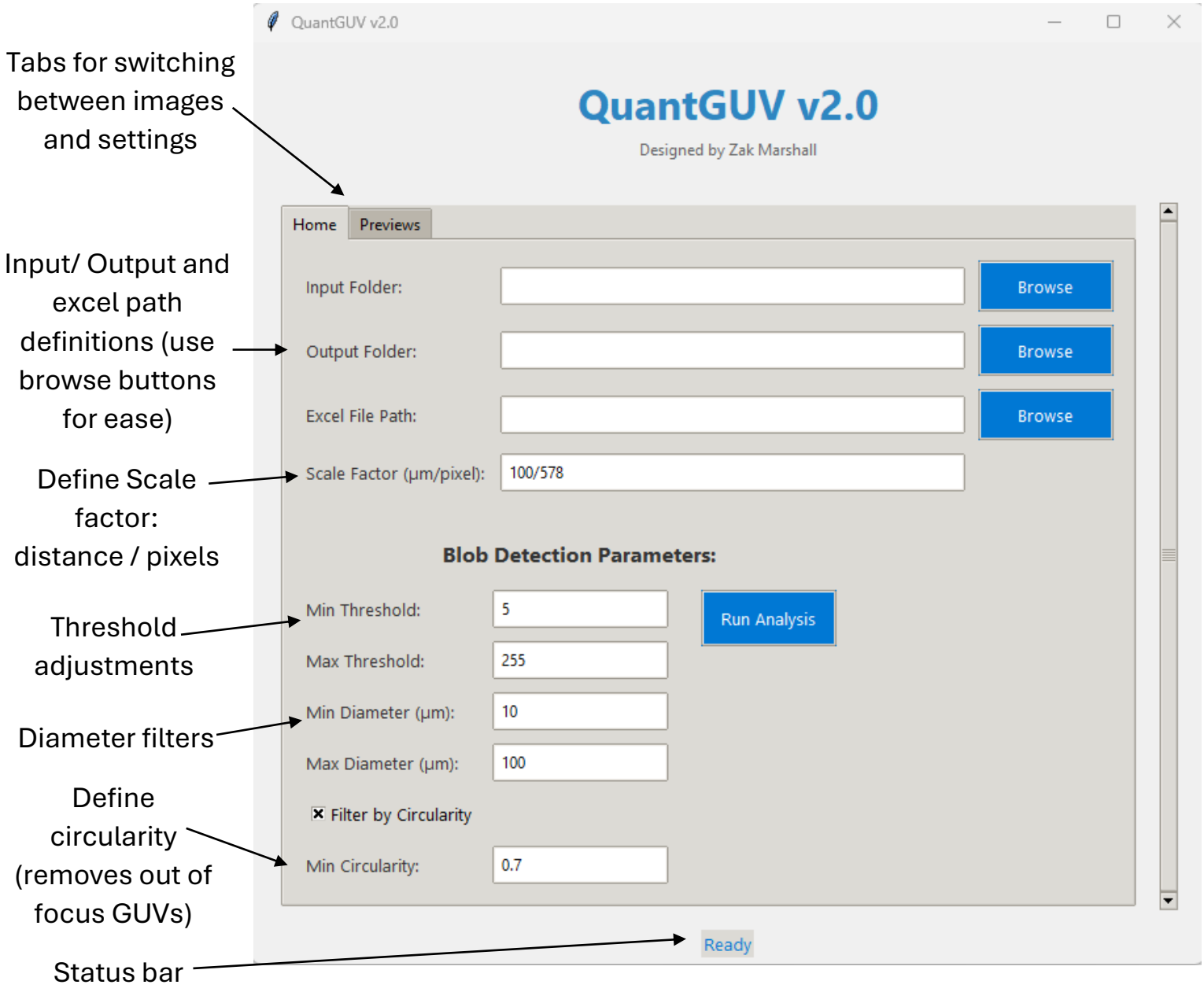
7. Run the code (F5)
8. This will give you the background signal (Bulk dye) and the GUV signal (Noise) subtract the noise from the background signal for each concentration to get a I_Blank value
9. Now using the equation below substitute in the I_Bulk value as I_Total and I_Blank as I_Outside to calculate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

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

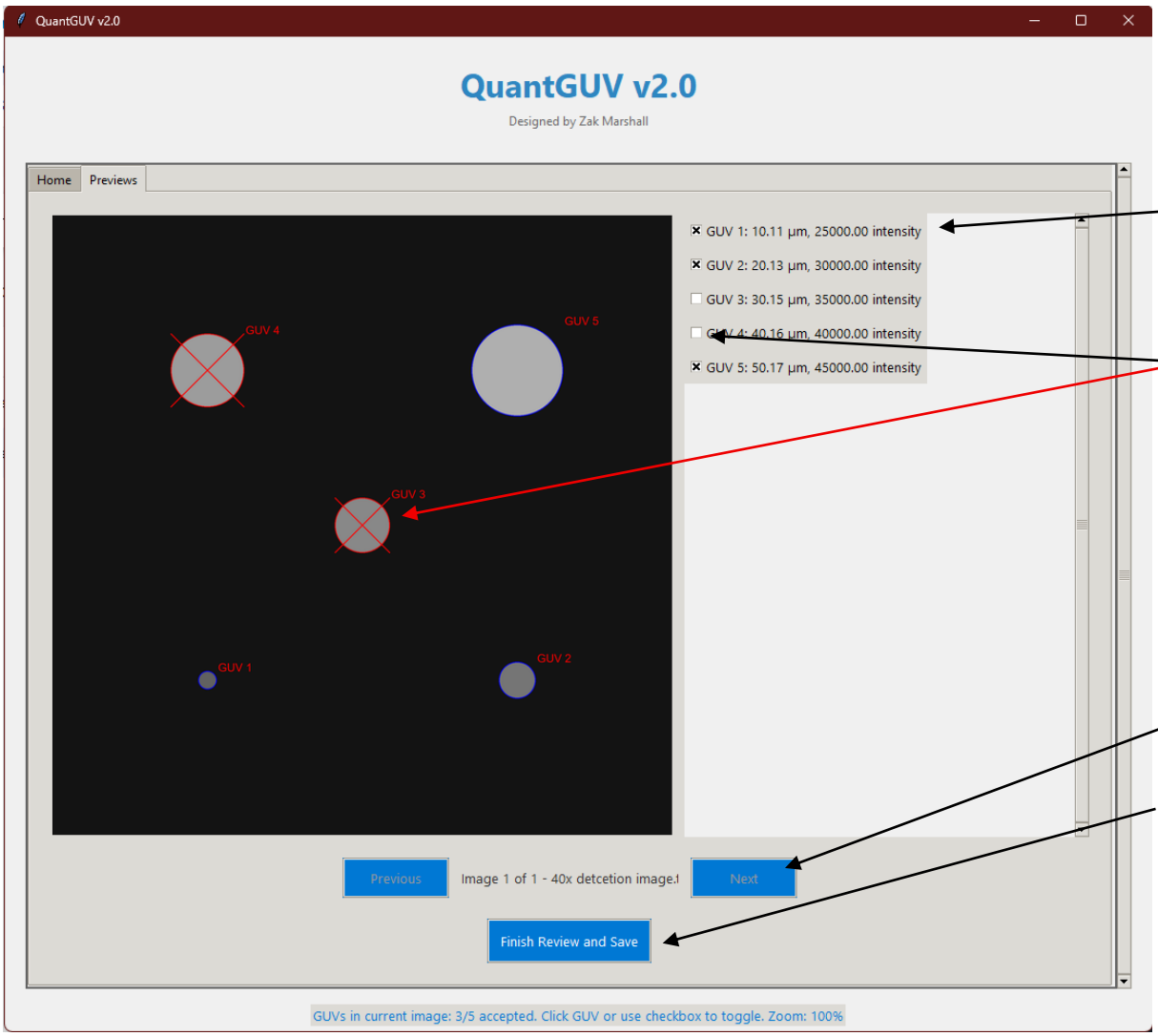
10. Use this to make your standard curve

QuantGUV Usage:

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



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



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 detcetion image.tif	GUV 1	10.11	2679.78	25000	20000
40x detcetion image.tif	GUV 2	20.13	10637.43	30000	25000
40x detcetion 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