Manual

1/7/2025

SIPEREA

Scalable Imaging Platform for AREA measurement, for vast and endless expansion

Version 1.1

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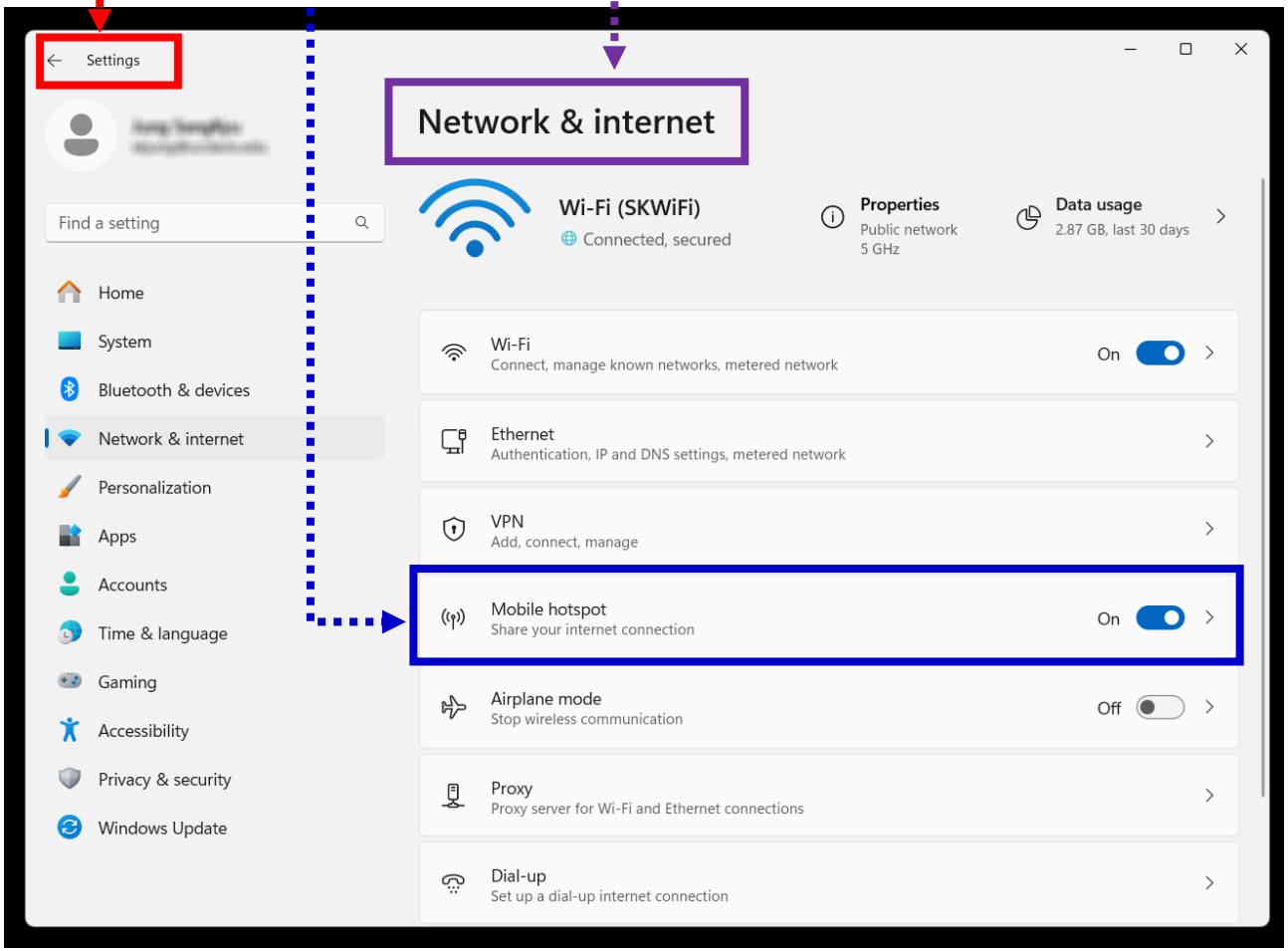
MOBILE HOTSPOT NETWORK SETTINGS

Set up a hotspot network on your PC

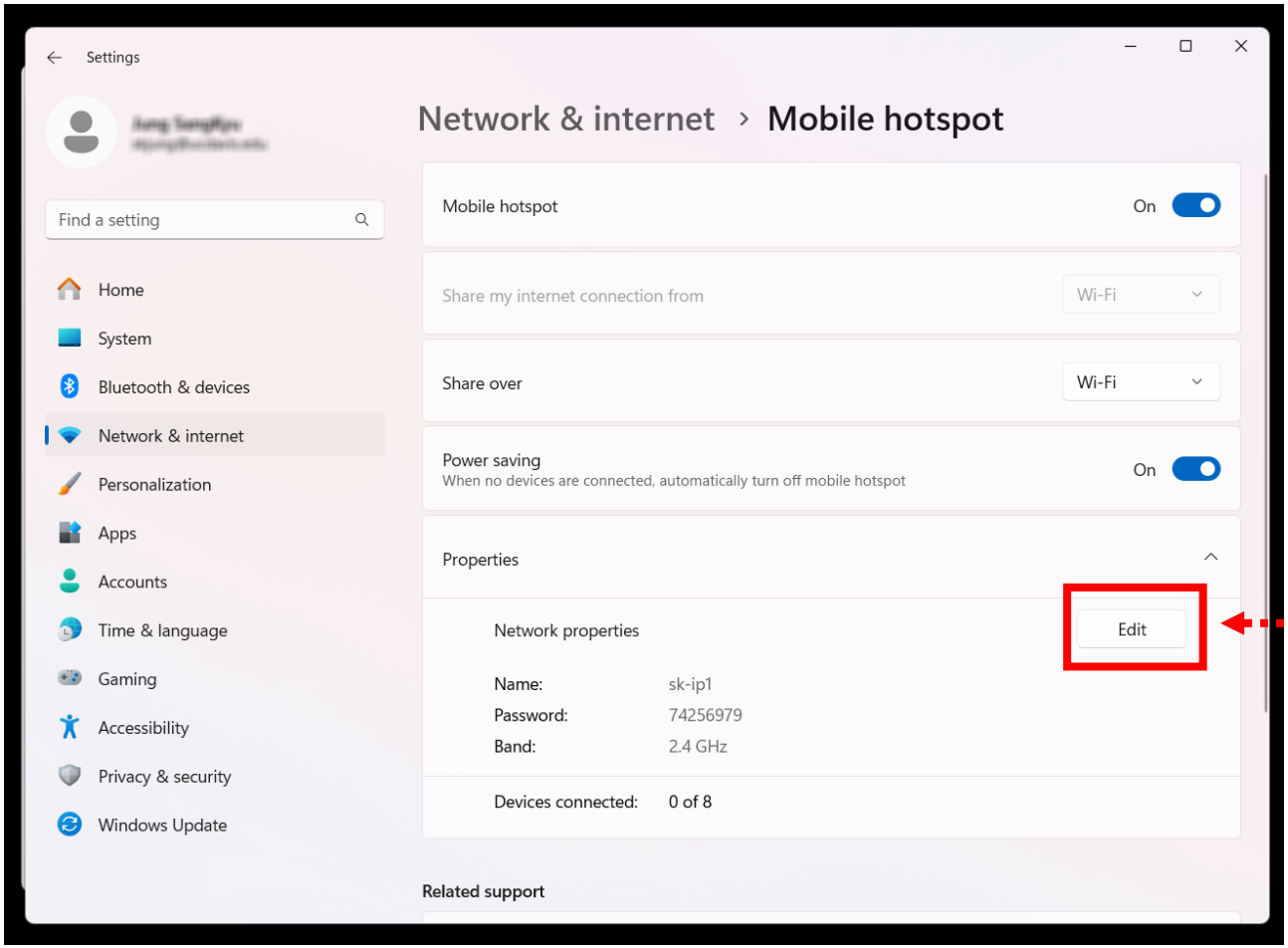
Step 1: Go to Settings and then “Network & internet”

Step 2: Enable Mobile hotspot



Set up a hotspot network on your PC

Step 3: Click the “Edit” button

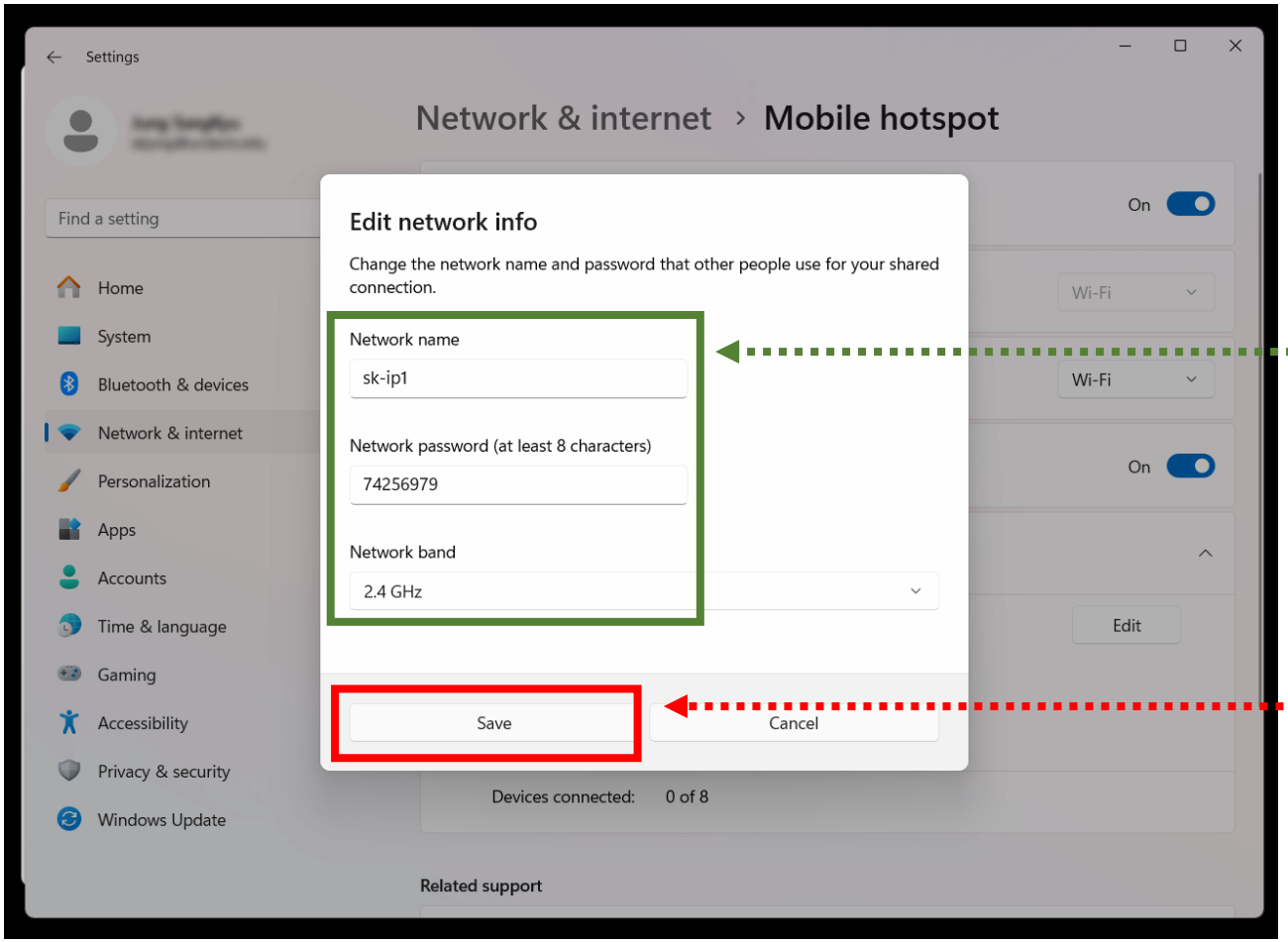


Set up a hotspot network on your PC

Step 4: Enter “sk-ip1”, “74256979”, and select “2.4GHz”

Step 5: Click the “Save” button

When hotspot is enabled, all ESP32-CAMs will connect to this hotspot network.



ESP32-CAM SETTINGS

Set up ESP32-CAM

ESP32-CAM

Shield module

Insert the ESP32-CAM into the shield module and connect the USB cable.

Set up ESP32-CAM

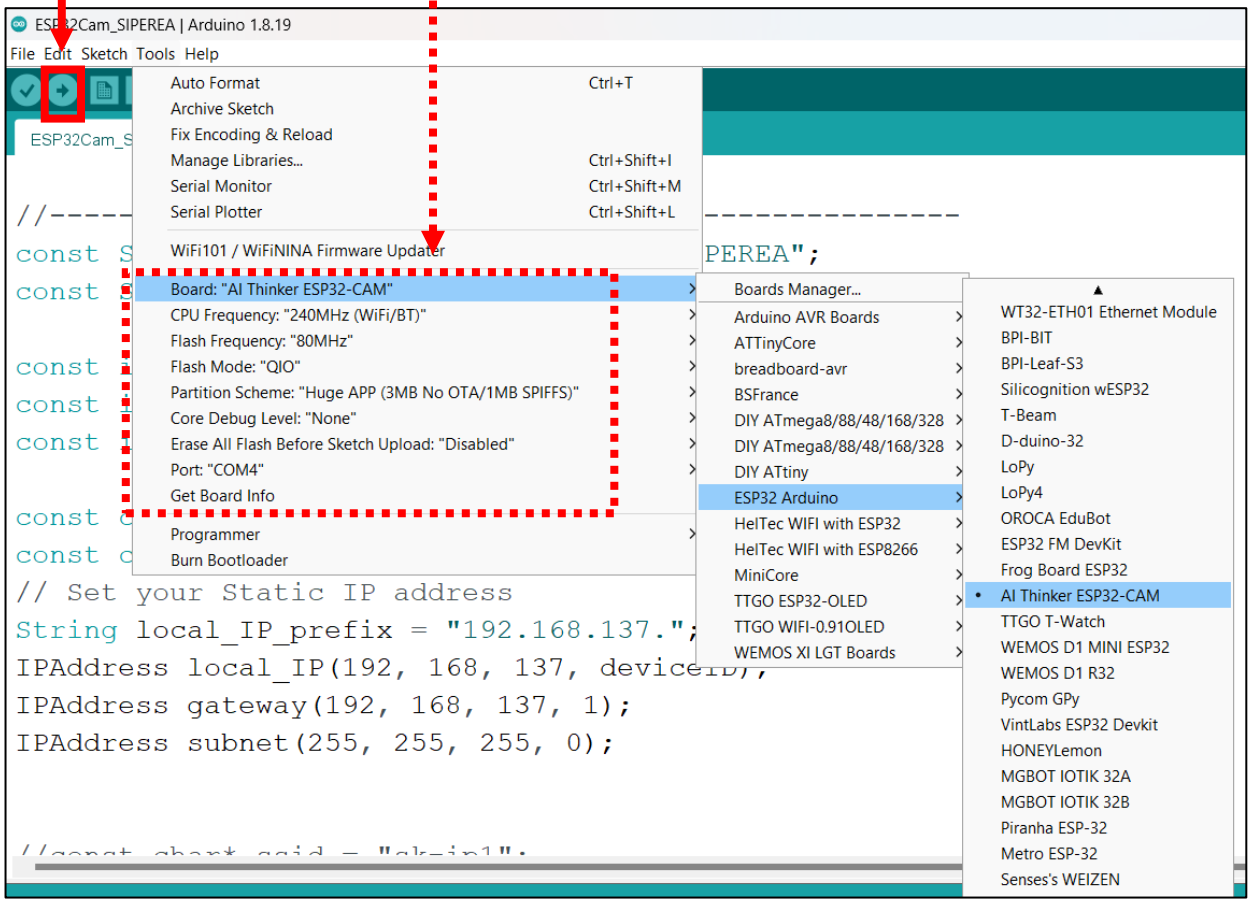
- Set up the Arduino IDE program environment for ESP32-CAM programming. Then open ESP32CAM_SIPEREA.ino and set the deviceID and IP address information as needed.

The static IP address of ESP32-CAM is **192.168.137.102**

local_IP_prefix deviceID

Set up ESP32-CAM

- Select the ESP32-CAM board, set the port number of the device, etc., then click the upload button to upload the program to ESP32-CAM.



Set up ESP32-CAM

- After the program upload to ESP32-CAM is completed, ESP32-CAM attempts to connect to the designated mobile hotspot.
- If the ESP32-CAM successfully connects to the mobile hotspot and receives the same address as the static IP address set, the ESP32-CAM's LED blinks once. If it fails, the LED blinks three times.

IMAGING HARDWARE SETTINGS

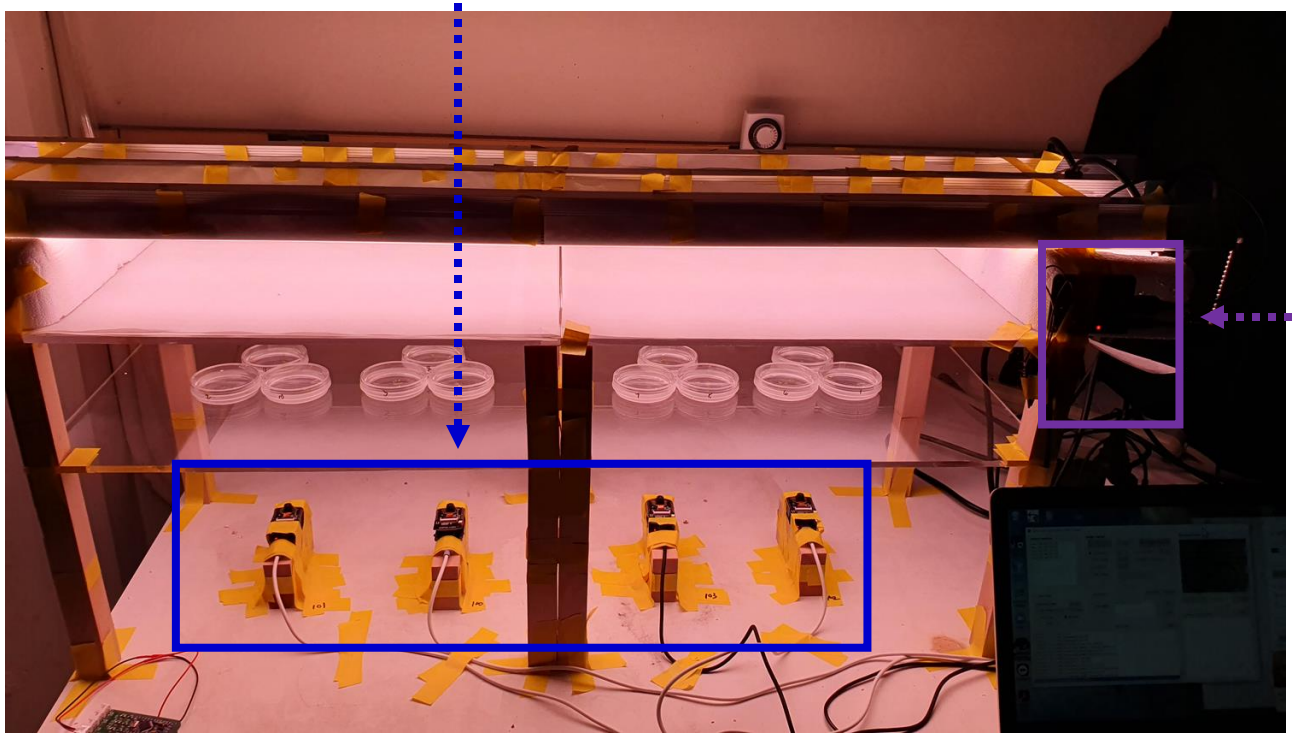
Set up imaging hardware

The four ESP32-CAMs for imaging have the following fixed IP addresses.

http://192.168.137.100
http://192.168.137.101
http://192.168.137.102
http://192.168.137.103

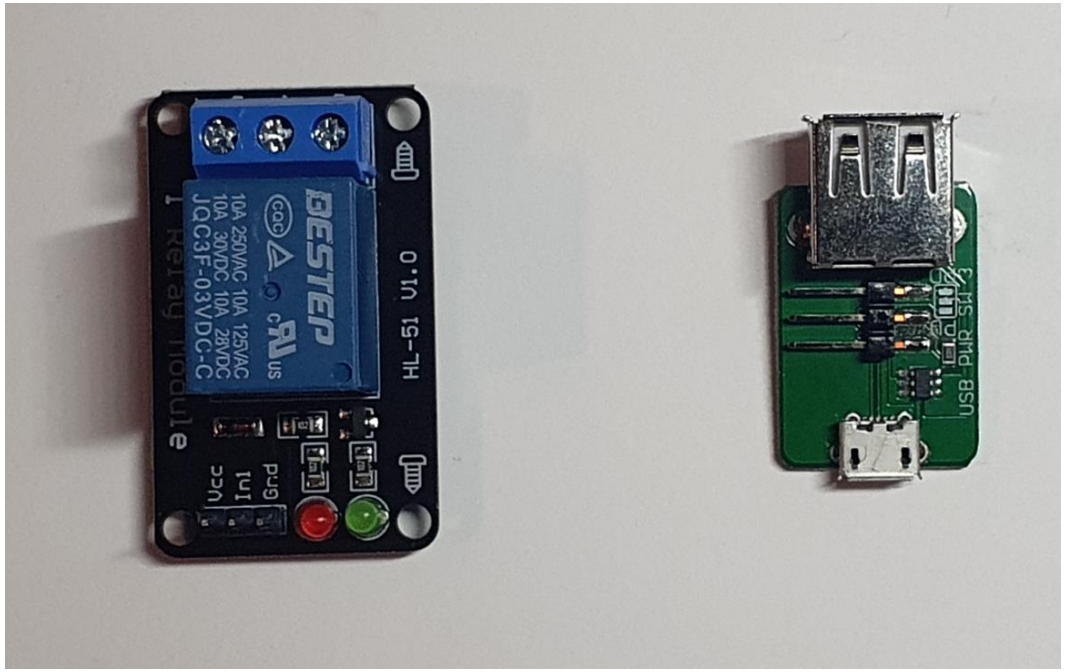
One ESP32-CAM is used to control an auxiliary light for dark imaging

http://192.168.137.104



Auxiliary LED light for dark imaging

- There are two optional modules to control the auxiliary USB LED strip light:

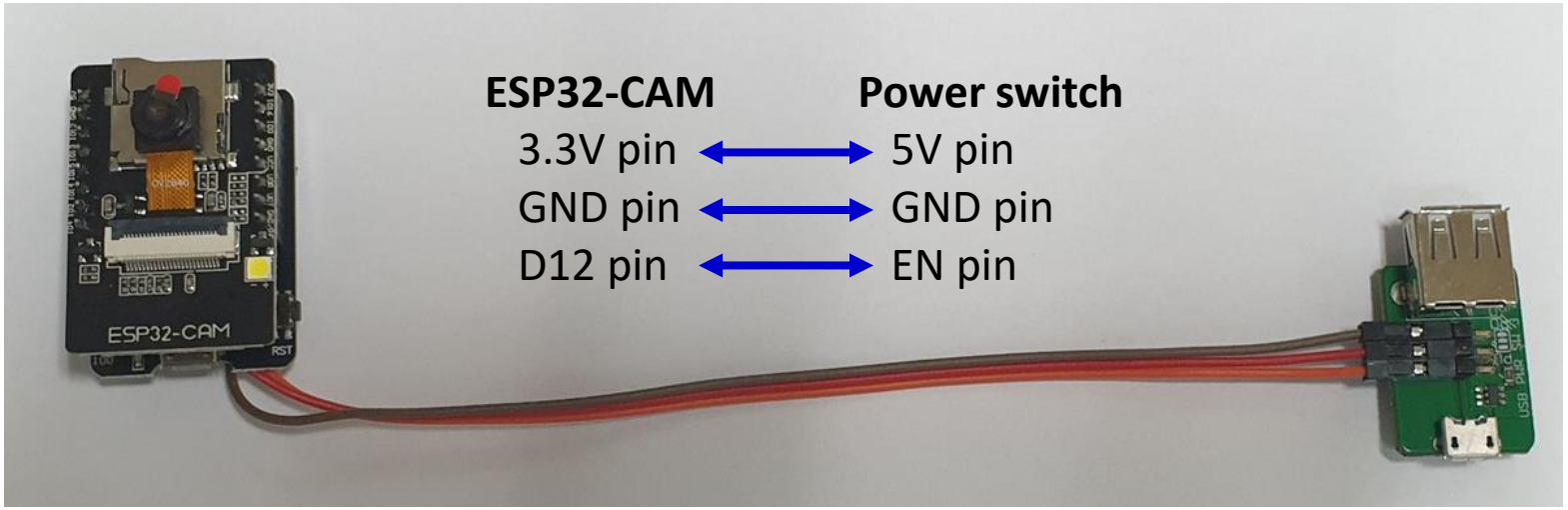


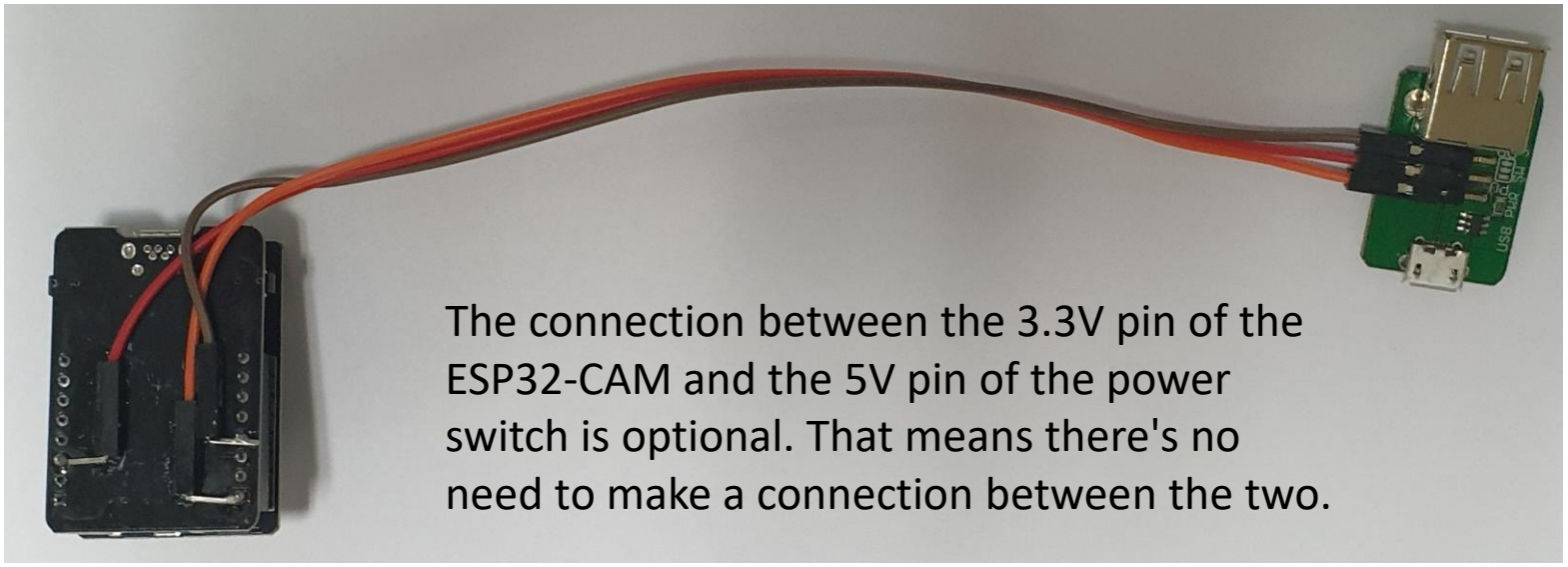
3.3V relay module

Power switch module (SKU: 648-1, Pi Shop Inc., DE, USA)

Auxiliary LED light for dark imaging

- Wiring connection between ESP32-CAM and power switch



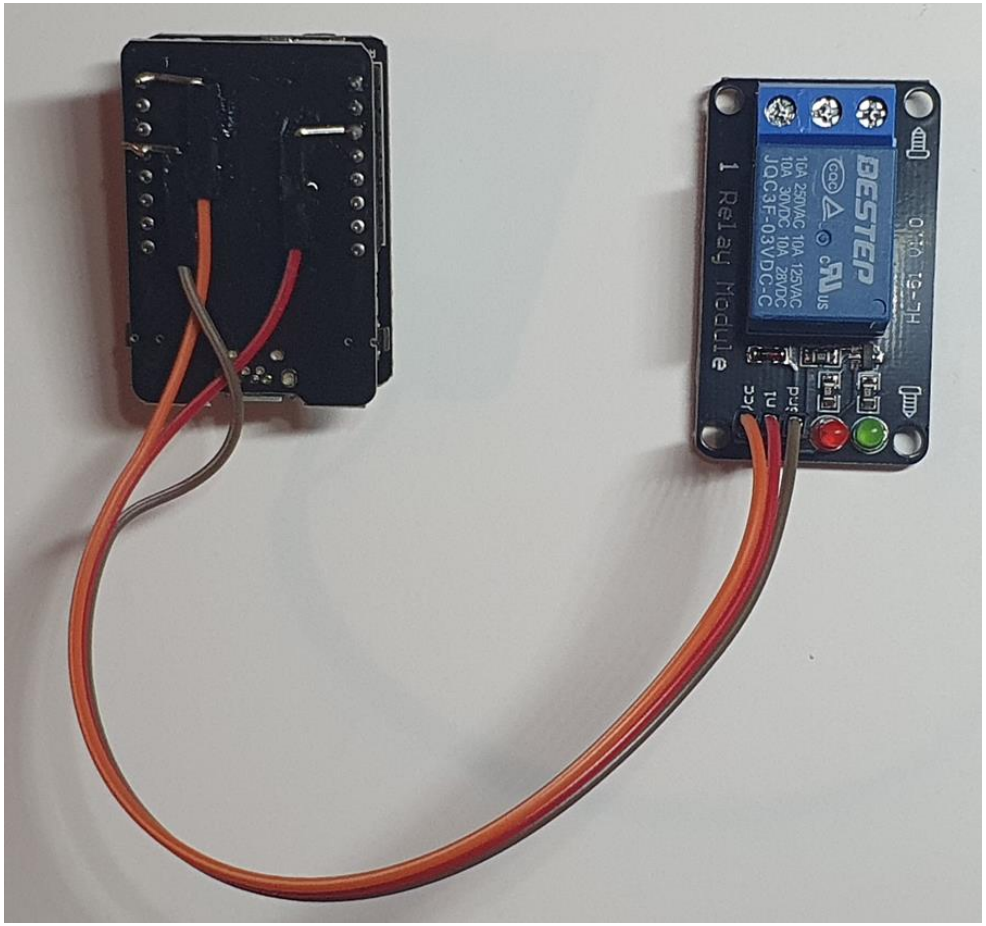


Auxiliary LED light for dark imaging

- Wiring connections between ESP32-CAM, power switch, and USB LED strip

Auxiliary LED light for dark imaging

- Wiring connection between ESP32-CAM and relay module



ESP32-CAM		Relay
3.3V pin	⟷	VCC pin
GND pin	⟷	GND pin
D12 pin	⟷	IN pin

The connection between the 3.3V pin of the ESP32-CAM and the VCC pin of the power switch is mandatory for the relay module.

IMAGE ACQUISITION PROGRAM SETTINGS

Image acquisition program

Step 1: Enter IP addresses of ESP32-CAMs for imaging and lighting (auxiliary LED light)

Step 2: Set destination folder for captured images

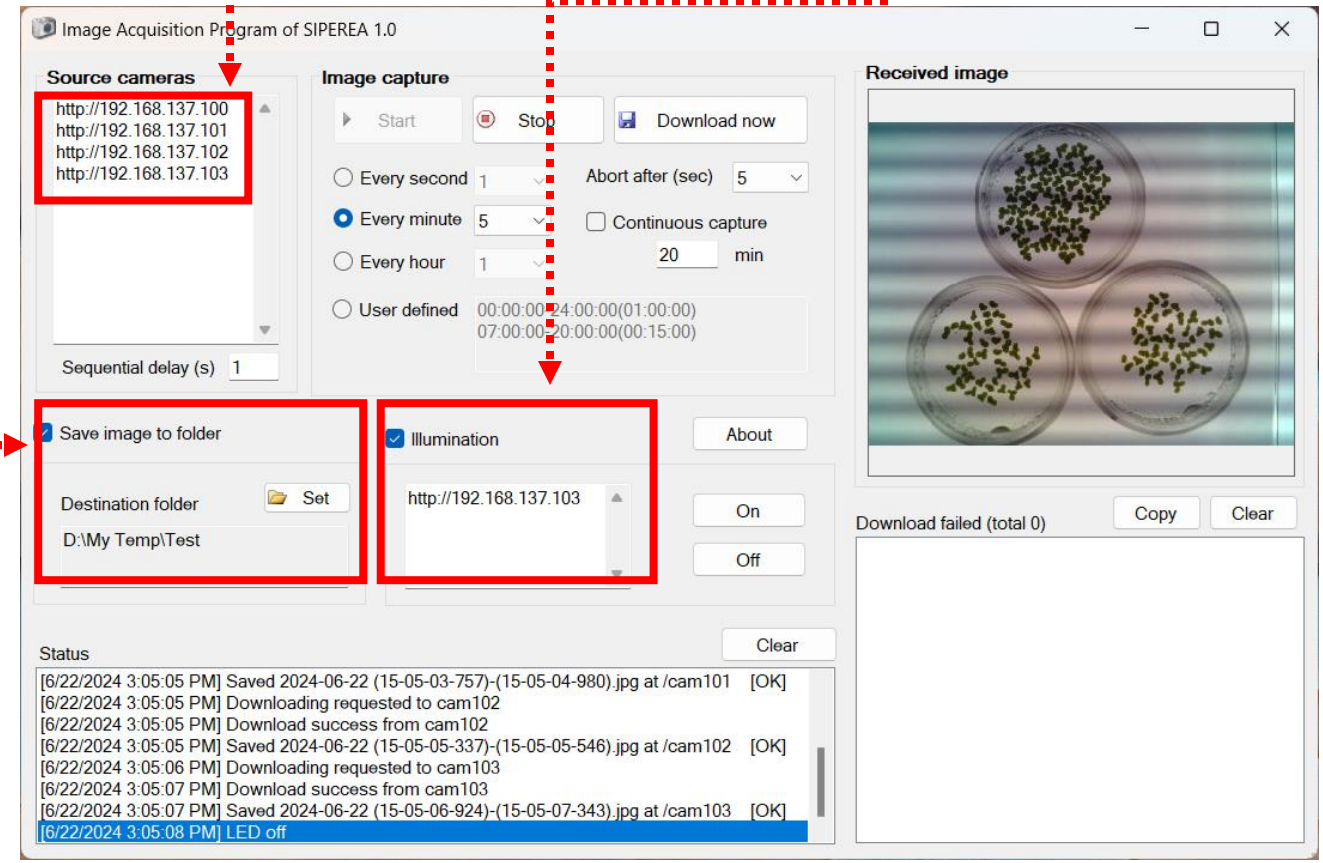


Image acquisition program

Step 3: Set imaging time interval

Step 4: Click the "Start" button to start imaging.

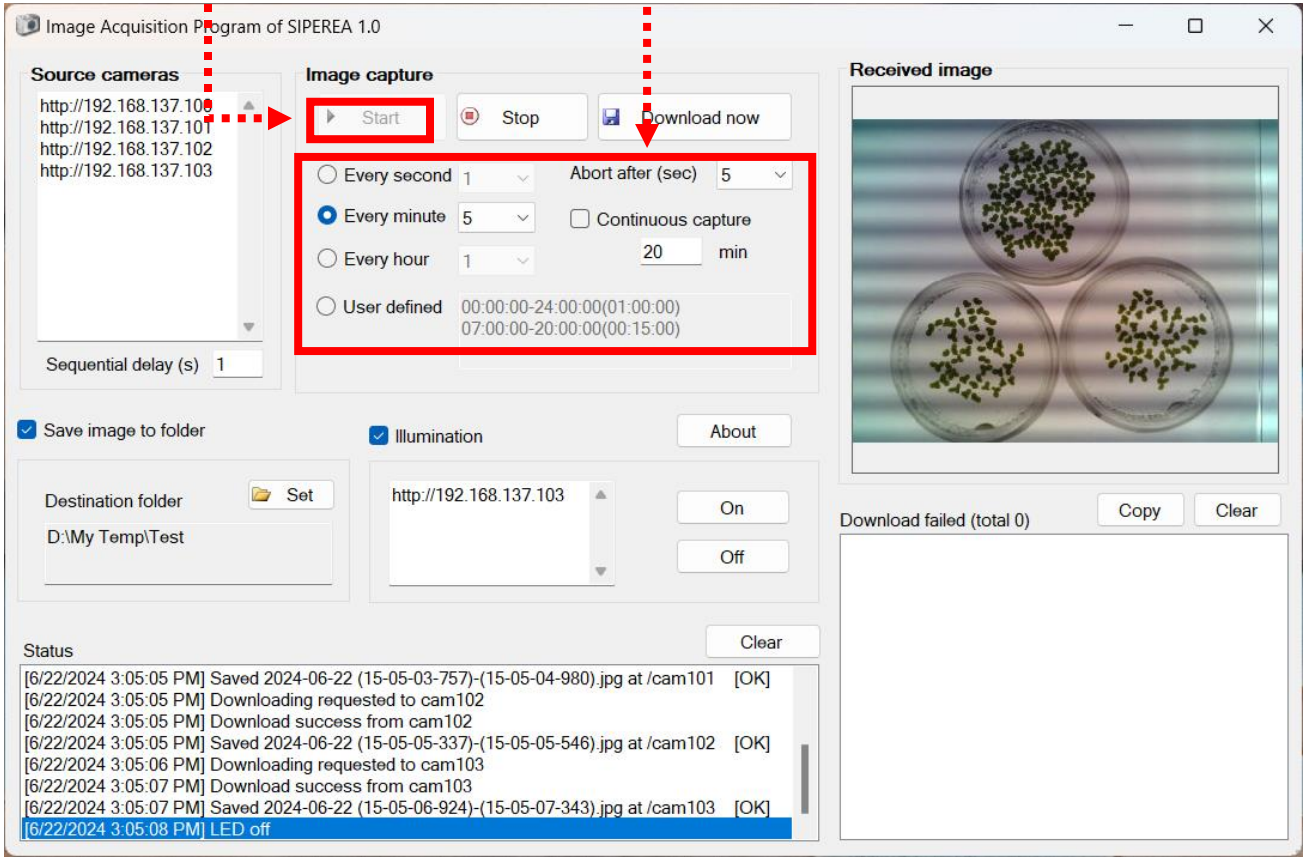


Image acquisition program

AutoImageCapture creates subfolders based on the last three digits of the ESP32-CAM's IP address. Users can view captured images in subfolders.

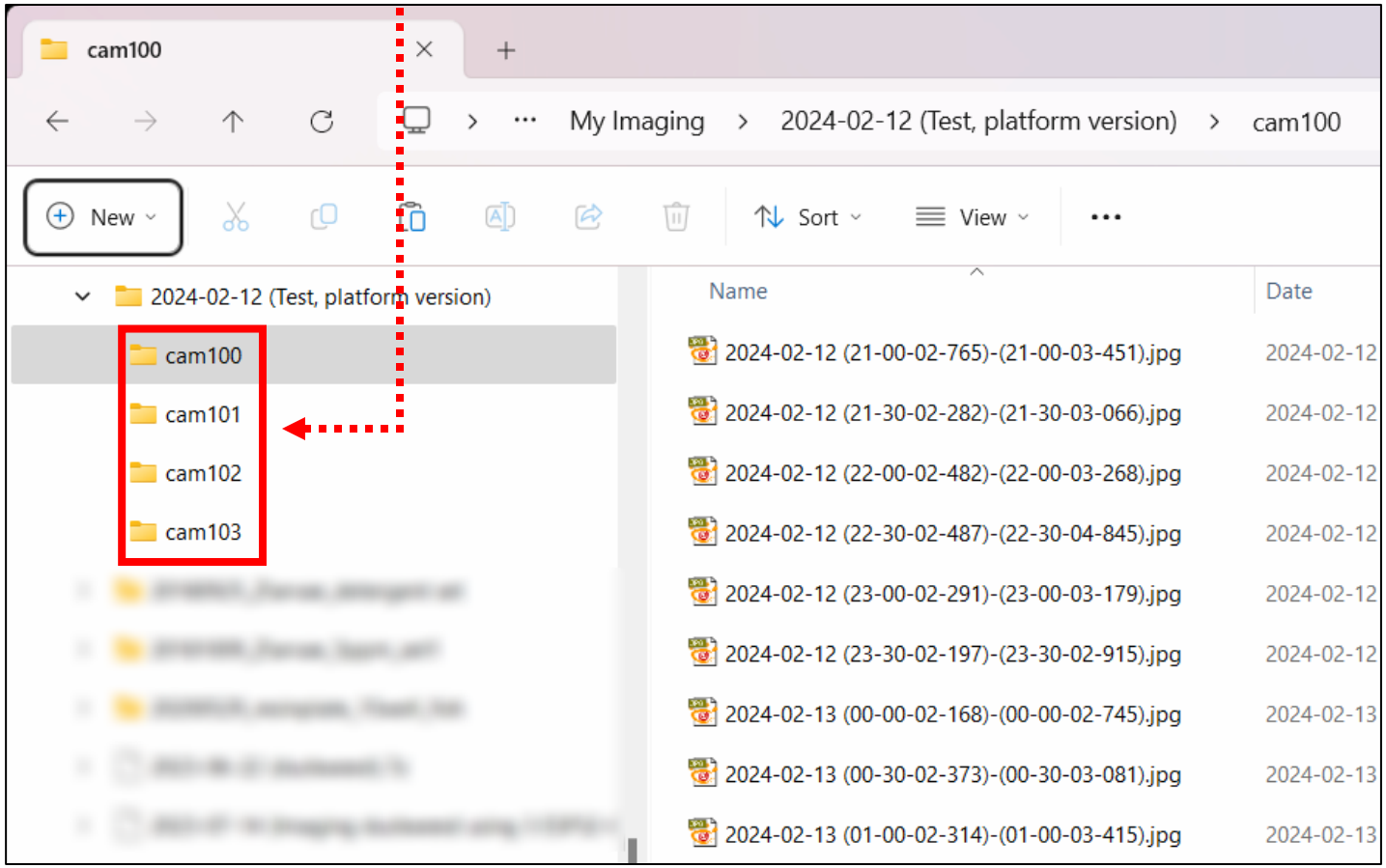
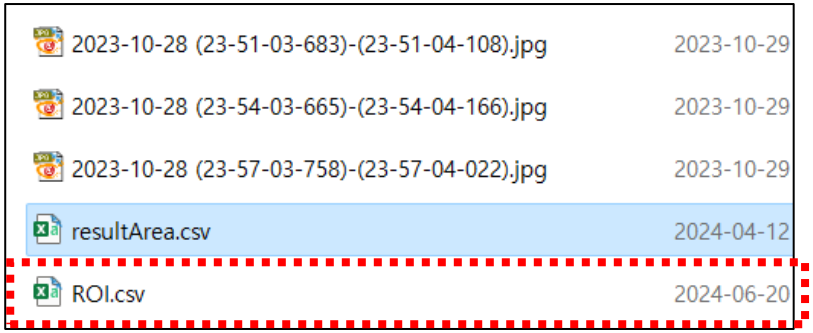


IMAGE ANALYSIS PROGRAM SETTINGS

Image analysis program

To analyze images with the image analysis program, the user must first create ROI.csv and place it in the source folder.

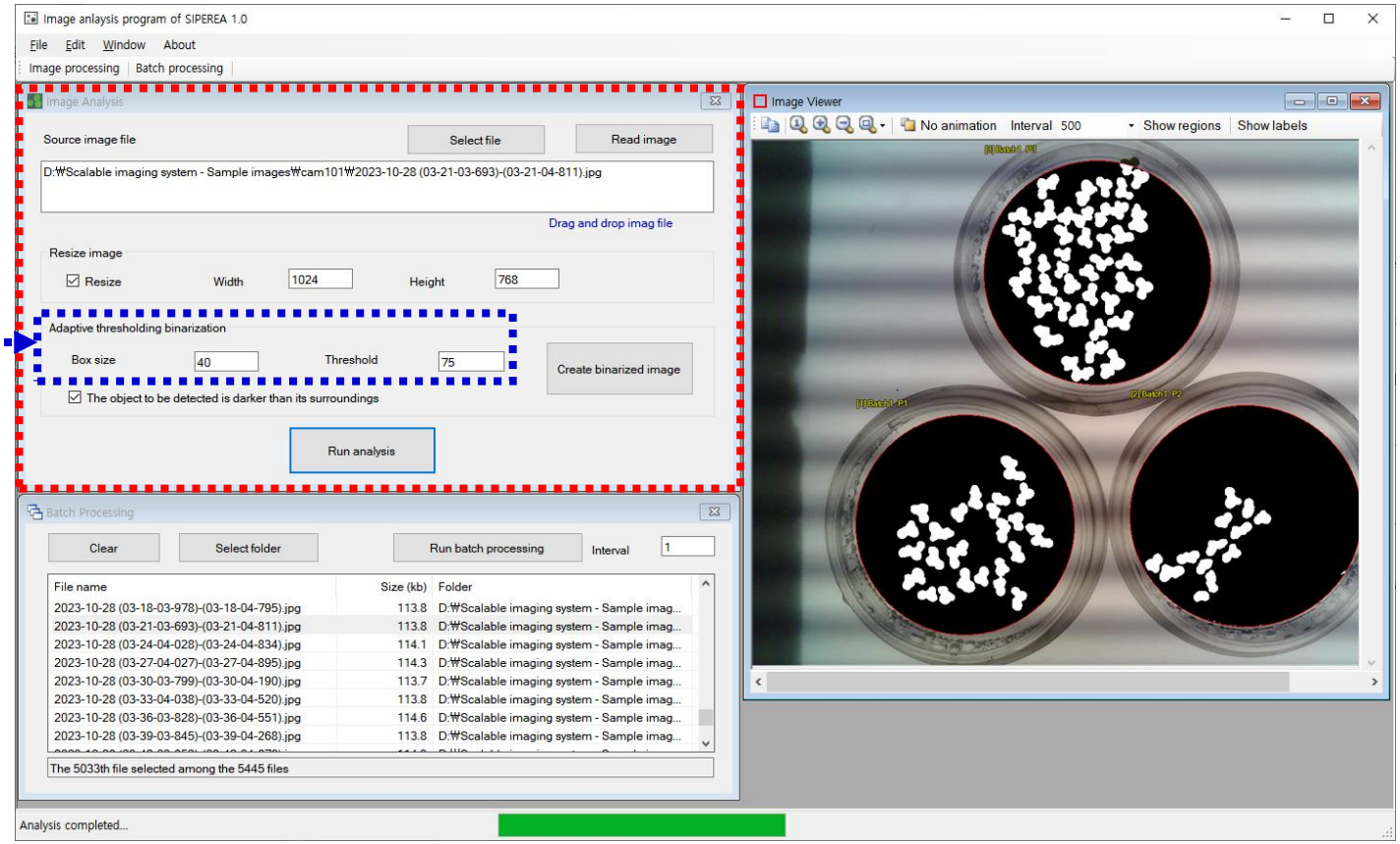


	A	B	C	D	E	F	G	H	I	J	K
1	Condition1	Condition2	Date	Plate#	Well#	Shape	X1	Y1	X2	Y2	
2	Batch1			1		circle	151	401	471	722	
3	Batch1			2		circle	553	387	892	710	
4	Batch1			3		circle	339	29	671	356	
5											
6											

Please refer to the next section for instructions on how to create ROI.csv.

Image analysis program

A window that loads and tests one image file to find optimal image processing parameters.

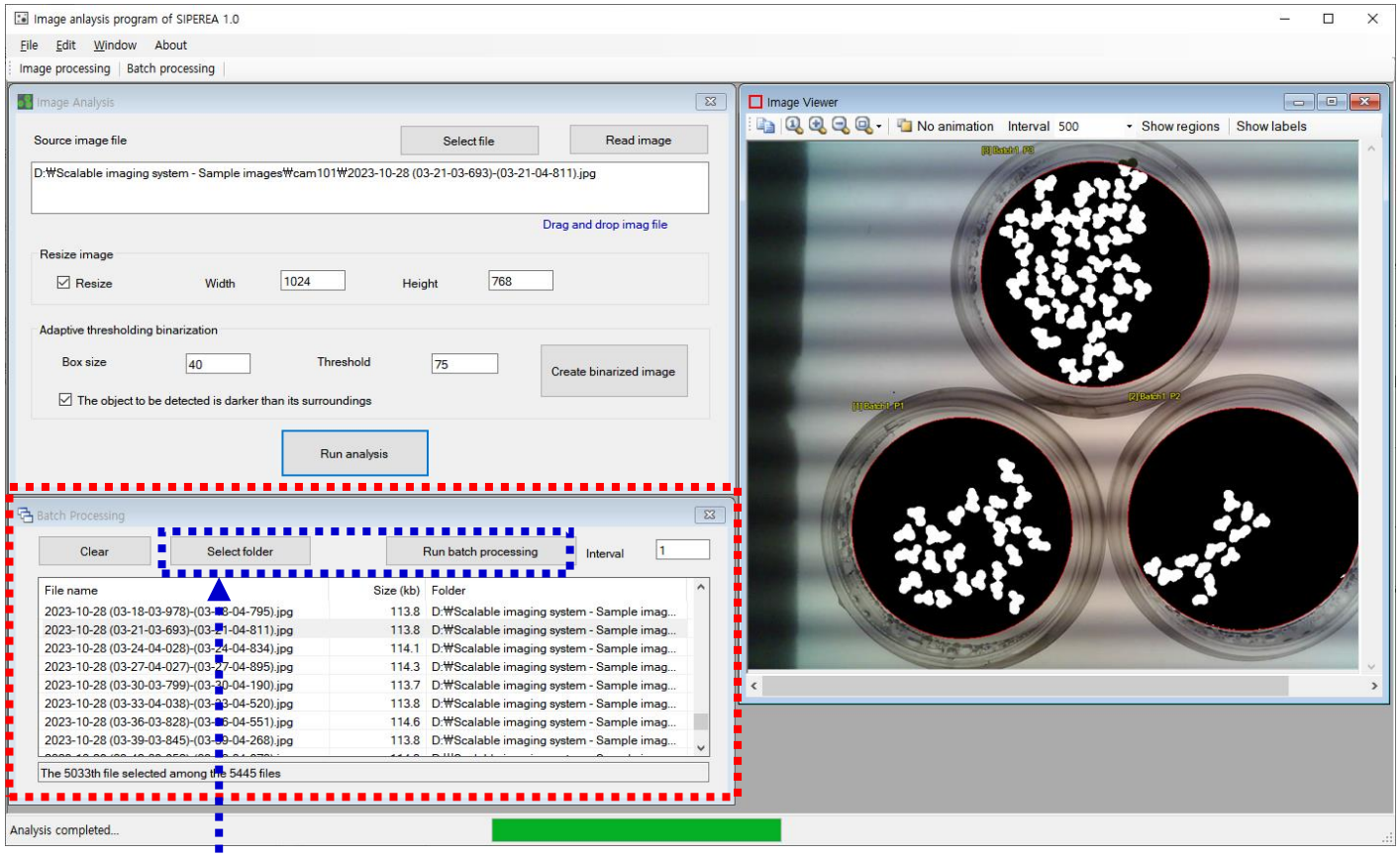


Box size: In general, just set it larger than the object you want to detect

Threshold: A value between 0.7 and 0.9 is optimal

Image analysis program

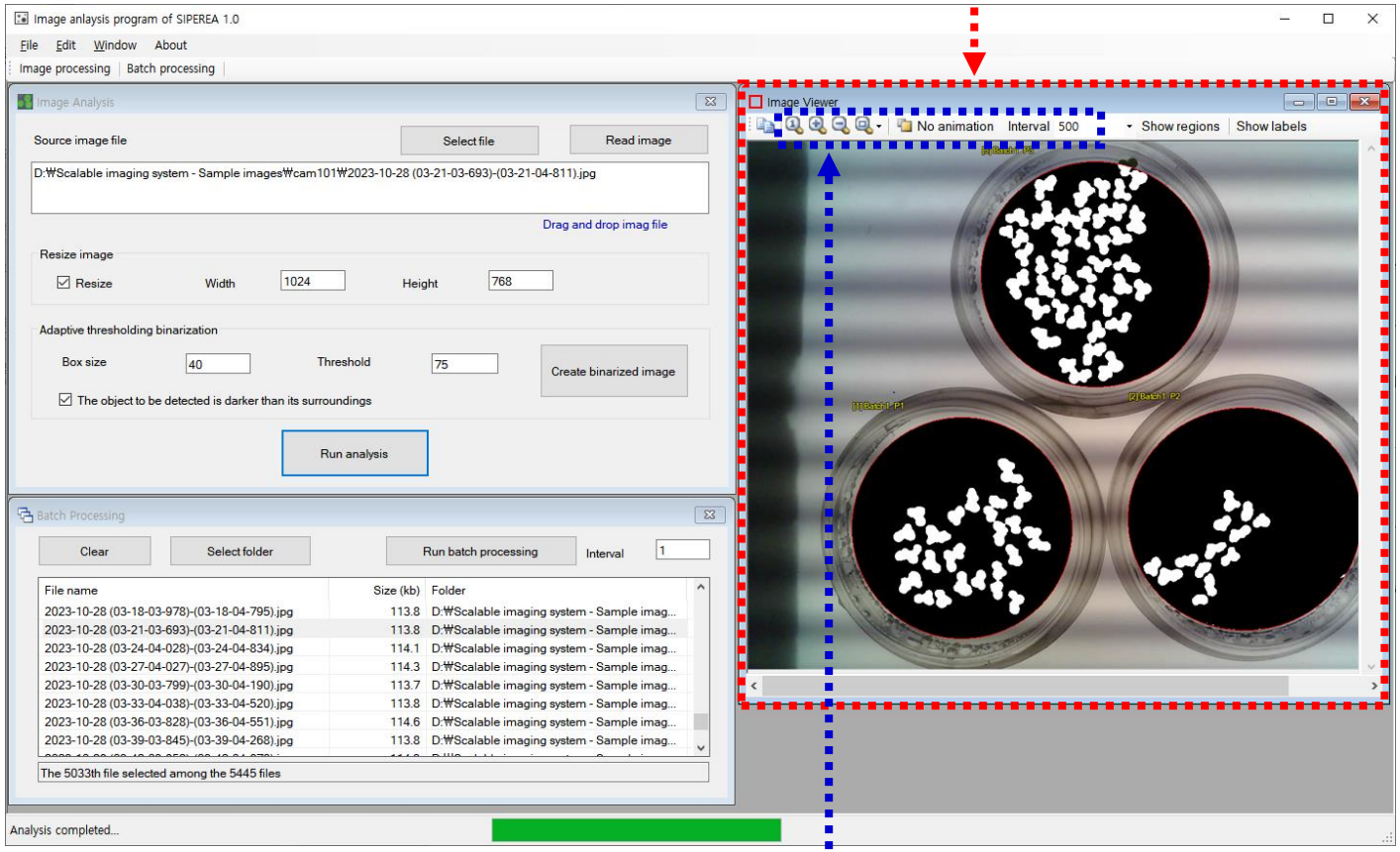
A batch processing window that adds multiple files and analyzes them continuously



First, click the ‘Select Folder’ button and then add files.
Then click the ‘Run Batch Process’ button to process all files.

Image analysis program

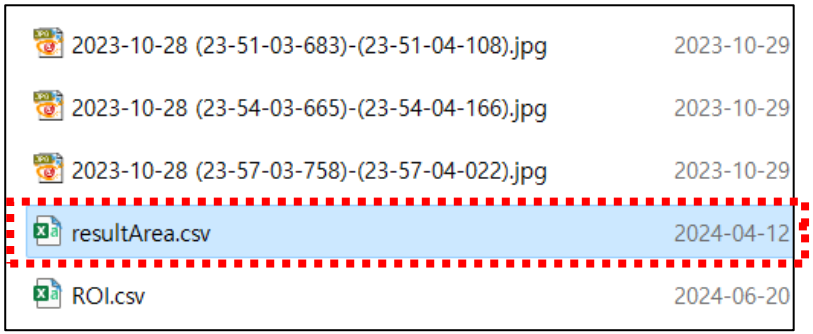
A window to check the original image and the image after processing



Buttons to enlarge or reduce the image
Image animation before and after image processing

Image analysis program

When batch processing is completed, a 'resultArea.csv' file is created in the image folder.

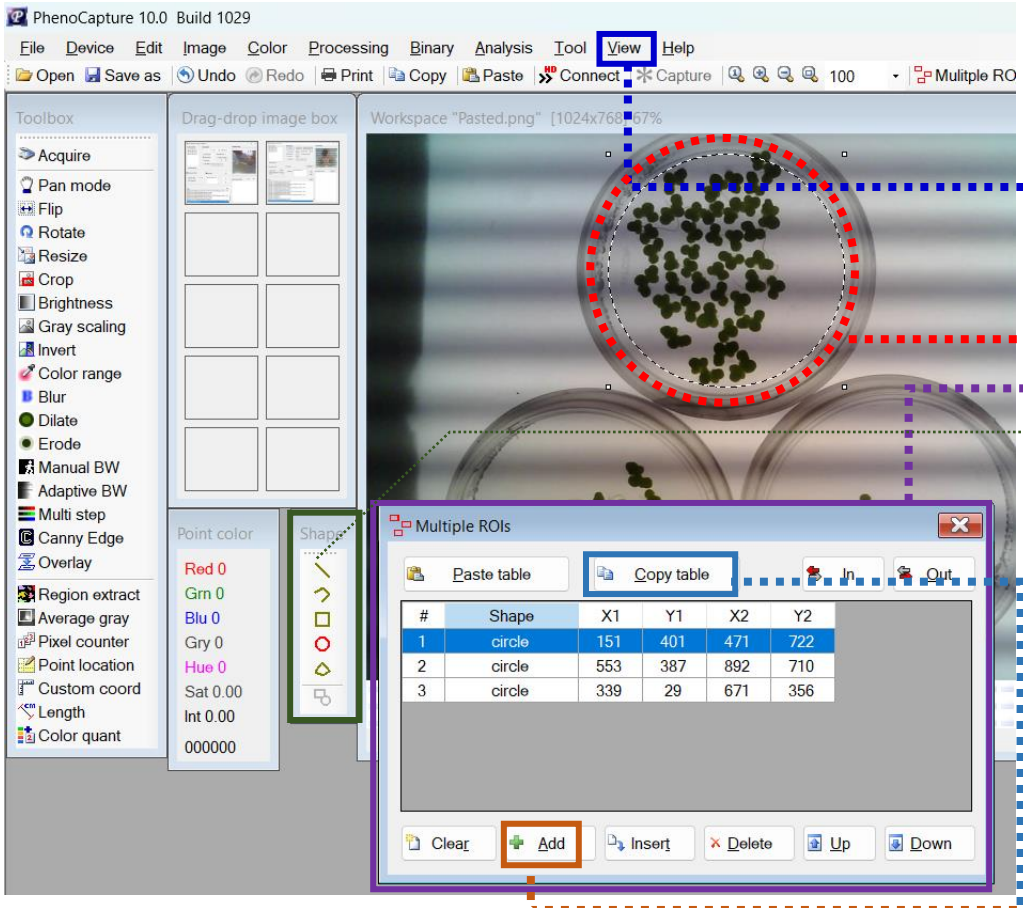


Area value by ROI (by well)

	A	B	C	D	E
1	File name	Time(day)	Batch1||P1	Batch1||P2	Batch1||P3
2	2023-10-16 (13-34-24-000)-(13-34-24-385).jpg	0	515	222	510
3	2023-10-16 (13-36-03-791)-(13-36-04-338).jpg	0.001	469	244	487
4	2023-10-16 (13-39-03-860)-(13-39-04-250).jpg	0.003	472	269	469
5	2023-10-16 (13-42-04-102)-(13-42-04-540).jpg	0.005	462	222	510
6	2023-10-16 (13-45-03-784)-(13-45-04-313).jpg	0.007	506	213	532
7	2023-10-16 (13-48-04-048)-(13-48-04-610).jpg	0.009	473	271	473
8	2023-10-16 (13-51-03-768)-(13-51-04-398).jpg	0.012	483	214	524
9	2023-10-16 (13-54-04-080)-(13-54-04-884).jpg	0.014	484	263	473
10	2023-10-16 (13-57-03-745)-(13-57-04-168).jpg	0.016	521	227	510
11	2023-10-16 (14-00-04-054)-(14-00-04-667).jpg	0.018	525	229	508
12	2023-10-16 (14-03-03-638)-(14-03-04-555).jpg	0.02	477	275	470
13	2023-10-16 (14-06-04-041)-(14-06-04-847).jpg	0.022	486	218	531
14	2023-10-16 (14-09-03-855)-(14-09-04-275).jpg	0.024	470	238	490

ETC.

ROI.csv file generation



To easily generate ROI files for AniWellTracker, users can use the free PhenoCapture program developed by the author of SIPEREA. (www.phenocapture.com)

1. Launch PhenoCapture.
2. Load one of the source images.
3. Select 'Multiple ROI' from the 'View' menu to open the 'Multiple ROI' window.
4. Select the circle or rectangle icon from the 'Shape' tool menu.
5. Drag the cursor to make a circular or rectangular selection.
6. Click the 'Add' button to insert the current selection into the table.
7. Move or recreate the circular or rectangular selection.
8. Click the 'Add' button again to add the current selection into the table.
9. Repeat steps 7 and 8 to select all wells.
10. Click the 'Copy table' to export the contents of the table to the clipboard.

ROI.csv file generation

	A	B	C	D	E	F	G	H	I	J
1	Condition1	Condition2	Date	Plate#	Well#	Shape	X1	Y1	X2	Y2
2	Test	Control	Aug-19	1	1	circle	318	70	444	196
3	Test	Control	Sep-19	1	2	circle	506	74	632	200
4	Test	Control	Oct-19	1	3	circle	695	76	821	202
5	Test	Control	Nov-19	1	4	circle	319	259	445	385
6	Test			1	5	circle	507	264	633	390
7	Test			1	6	circle	693	266	819	392
8	Test			1	7					72
9	Test			1	8					77
10	Test			1	9	circle	690	453	816	579
11	Test	Control	2019-17	1	10	circle	314	633	440	759
12	Test	Control	2019-18	1	11	circle	503	636	629	760
13	Test	Control	2019-19	1	12	circle	689	640	814	764
14	Test	Control	2019-20	1	13	circle	314	819	439	943
15	Test	Control	2019-21	1	14	circle	500	821	625	945
16	Test	Control	2019-22	1	15	circle	686	823	811	947

Additional information that users may enter

Pasted from clipboard

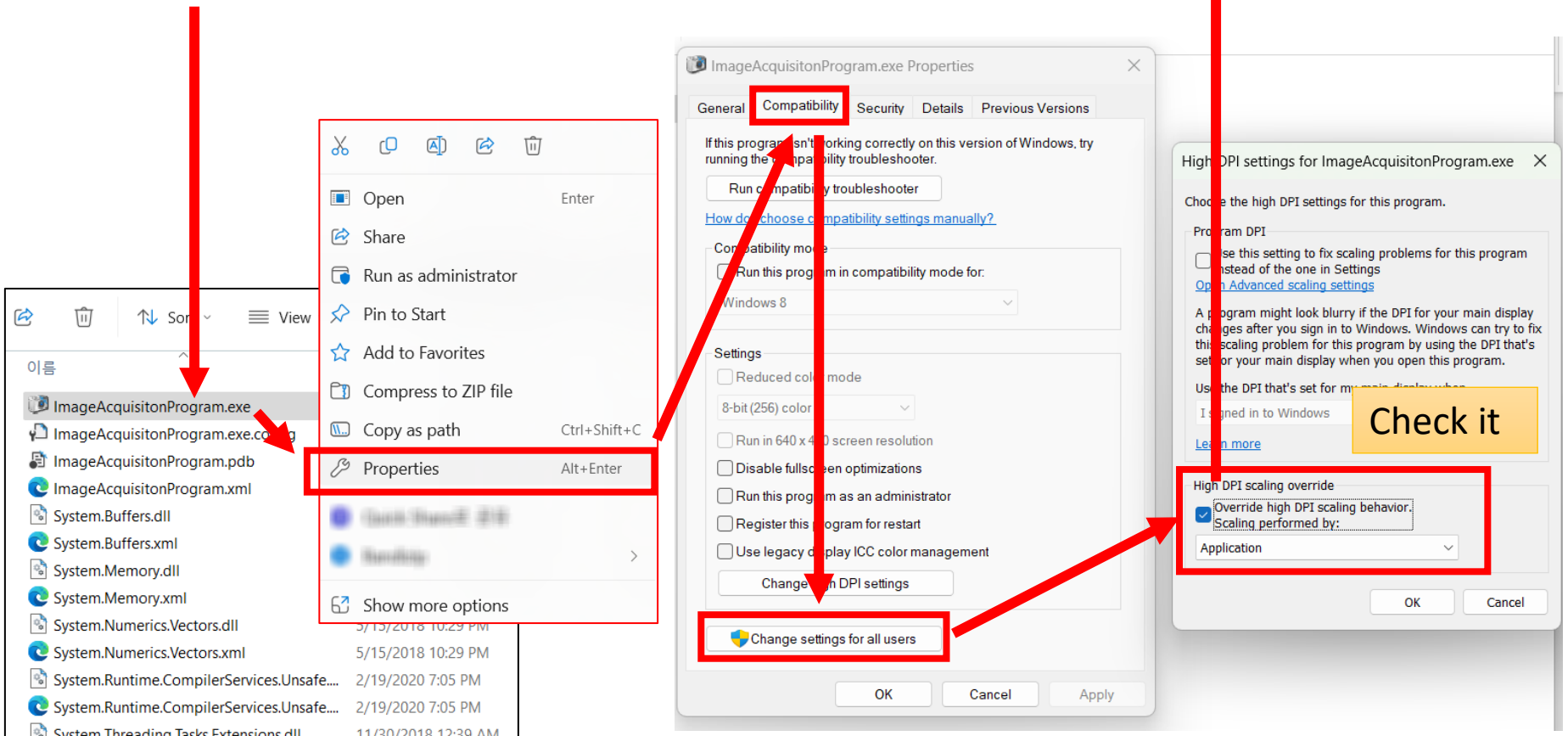
Users can use Excel program to create a ROI file manually.

1. Create 10 columns from A to J as shown.
2. Enter a title for each column. That is, 'Condition1', 'Condition2', 'Date', 'Plate#', 'Well#', 'Shape', 'X1', 'Y1', 'X2', and 'Y2'. Users can change the number of wells.
3. Paste the table contents to a specific location in the Excel worksheet. ROIs are designated as 'Shape', 'X1', Y1', 'X2' and 'Y2'.
4. Enter text in the cell indicated by the blue box. All of this text does not affect image analysis.
5. Save as 'ROI.csv' in the folder containing the source images.
   (CSV: comma-separated text file format)
6. All done!

How to make program sharp when it's blurry

In File Explorer, place the mouse cursor on the program and press the right button of the mouse

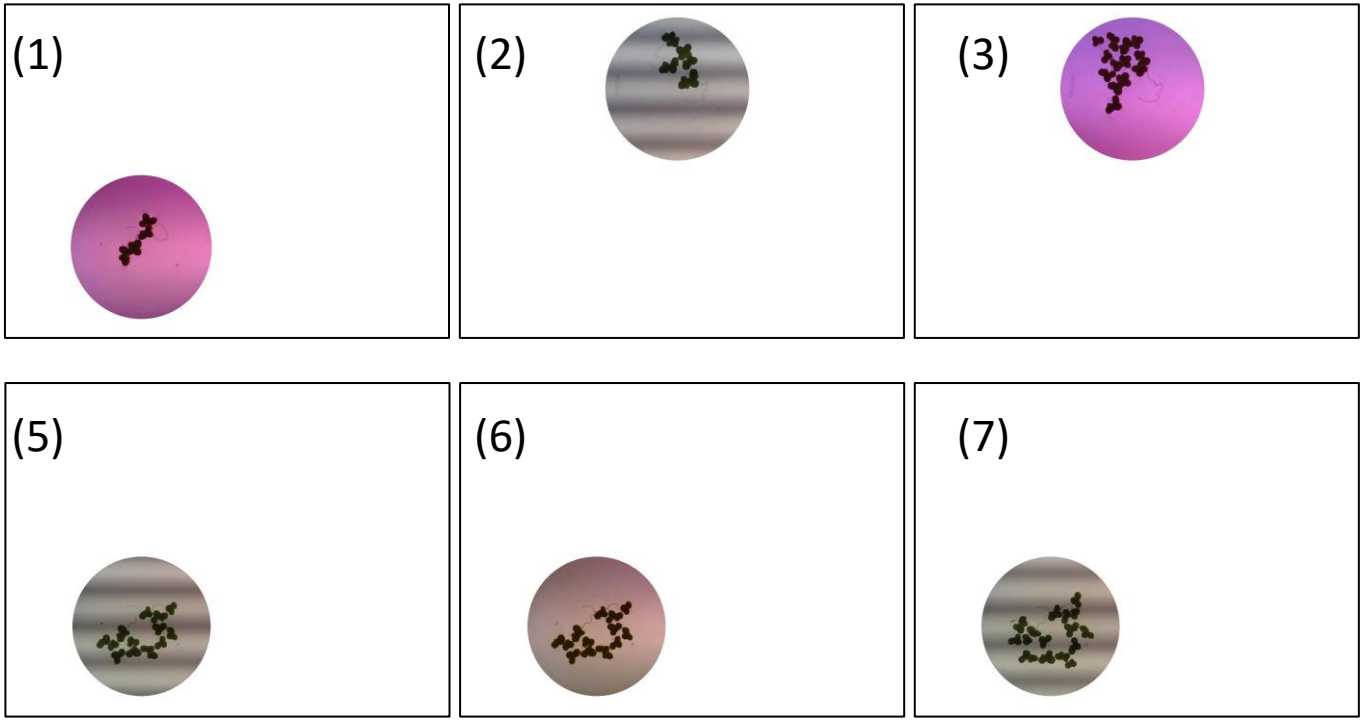
When you run the program again, the screen appears clearly.



Analysis of area calculation accuracy

The duckweed area calculated using SIPEREA's image analysis program was compared to the areas obtained using MATLAB and ImageJ to assess the margin of error. It is important to note that MATLAB and ImageJ do not calculate areas using the same algorithm implemented in the SIPEREA program. Instead, the analysis requires step-by-step manual processing, which may result in variations depending on the individual performing the analysis.

7 images used for analysis
(1600×1200 pixels)

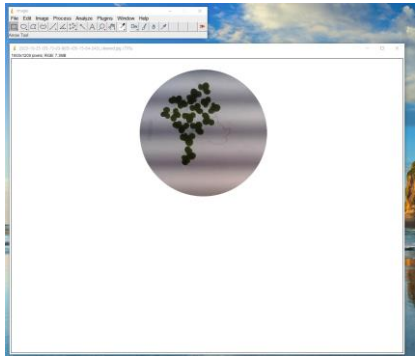


Analysis of area calculation accuracy

The methods used for analysis with MATLAB and ImageJ are briefly described below.

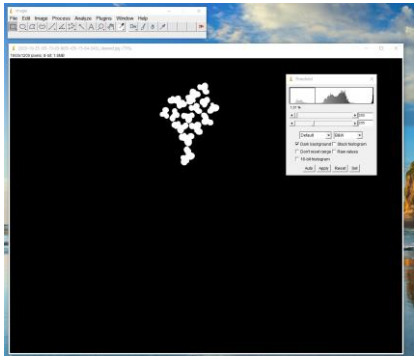
Image processing using ImageJ

<Original image>



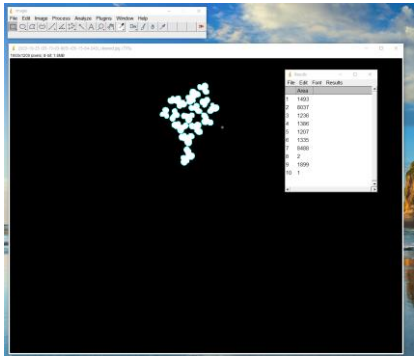
ImageJ

<Binarized image>



ImageJ Threshold

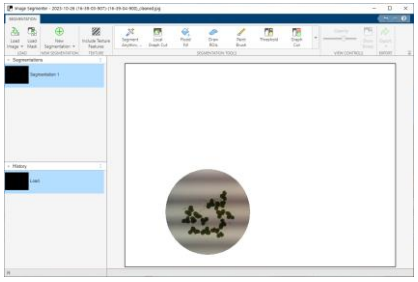
<Area measurement>



ImageJ Measurement (area)

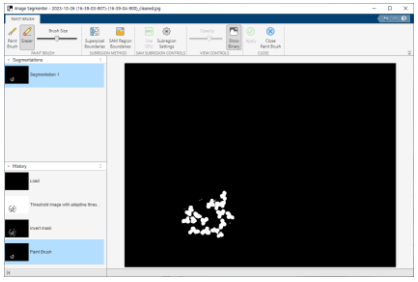
Image processing using MATLAB

<Original image>



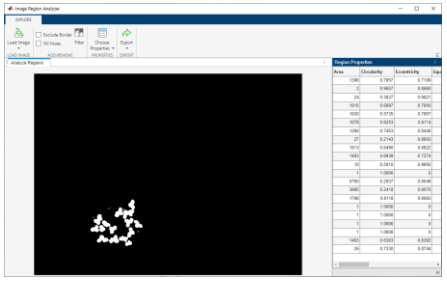
MATLAB Image Segmenter

<Binarized image>



MATLAB Image Segmenter

<Area measurement>



MATLAB Image Region Analyzer

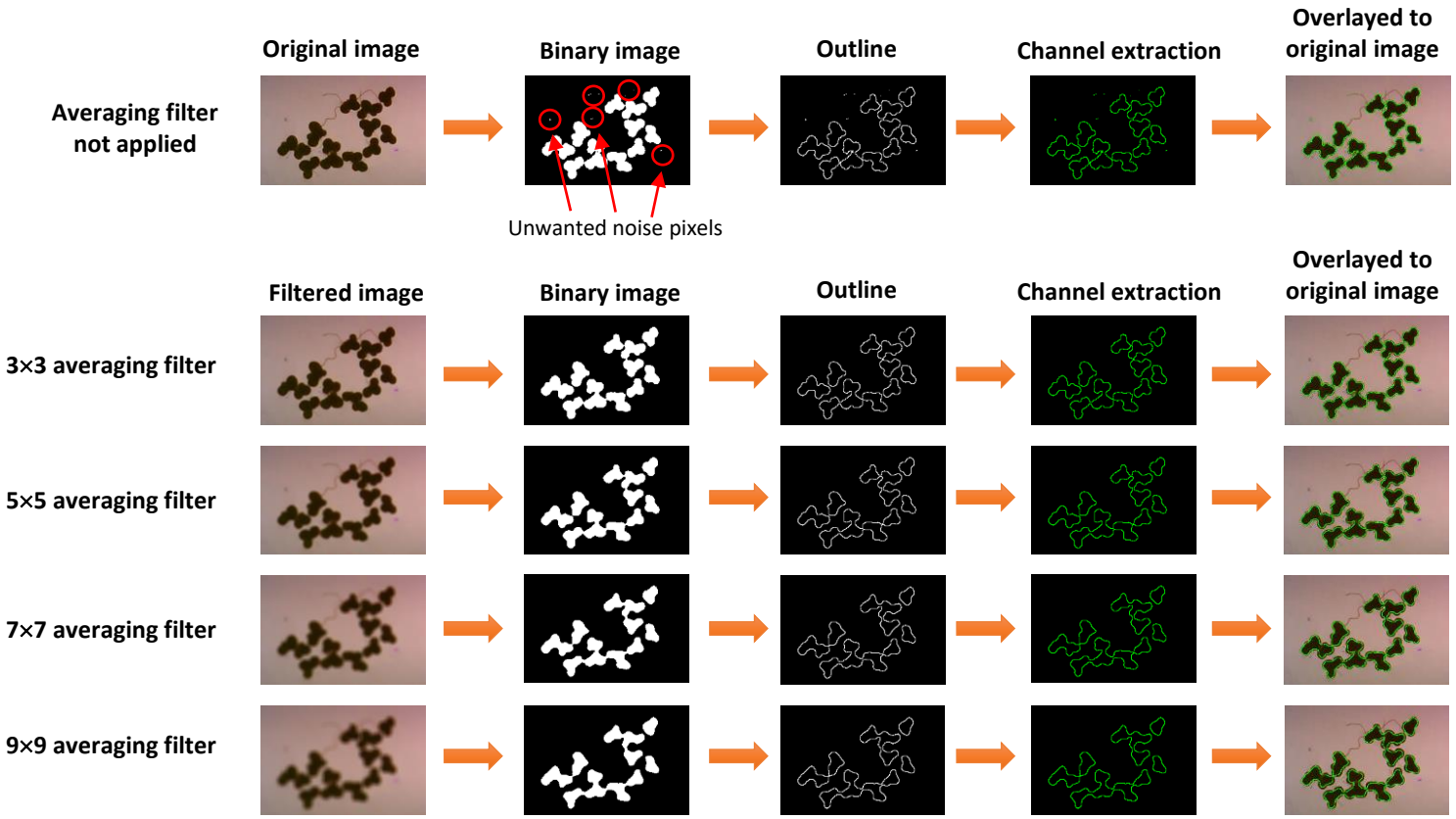
Analysis of area calculation accuracy

The results of area analysis using MATLAB, ImageJ, and SIPEREA are as follows. As shown in these results, the duckweed area analyzed using SIPEREA differs from the areas analyzed using MATLAB or ImageJ by less than 3%. Generally, this level of error is considered highly accurate.

Test image #	Area by MATLAB	Area by ImageJ	Area by SIPEREA	Percent Error Between MATLAB and SIPEREA =ABS(MATLAB-SIPEREA)/SIPEREA*100	Percent Error Between ImageJ and SIPEREA =ABS(ImageJ-SIPEREA)/SIPEREA*100
1	7716	8004	7967	3.15	0.46
2	12493	12336	11975	4.33	3.01
3	21914	21686	21703	0.97	0.08
4	24752	24537	23563	5.05	4.13
5	18868	18781	18710	0.84	0.38
6	18738	18995	18868	0.69	0.67
7	24089	23370	23246	3.63	0.53
				Average (%)	**Average (%)**
				2.66	**1.33**

Testing kernel size of averaging filter

SIPEREA's image analysis program uses a 3×3 averaging filter before applying an adaptive threshold. This slide serves as a reference to demonstrate the effect of the averaging filter's kernel size on image analysis. The 3×3 kernel effectively eliminates unwanted noise while accurately detecting duckweed regions. In general, the detected duckweed regions tend to decrease in size as the kernel size increases.



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