## Manual 1/7/2025

## **SIPEREA**

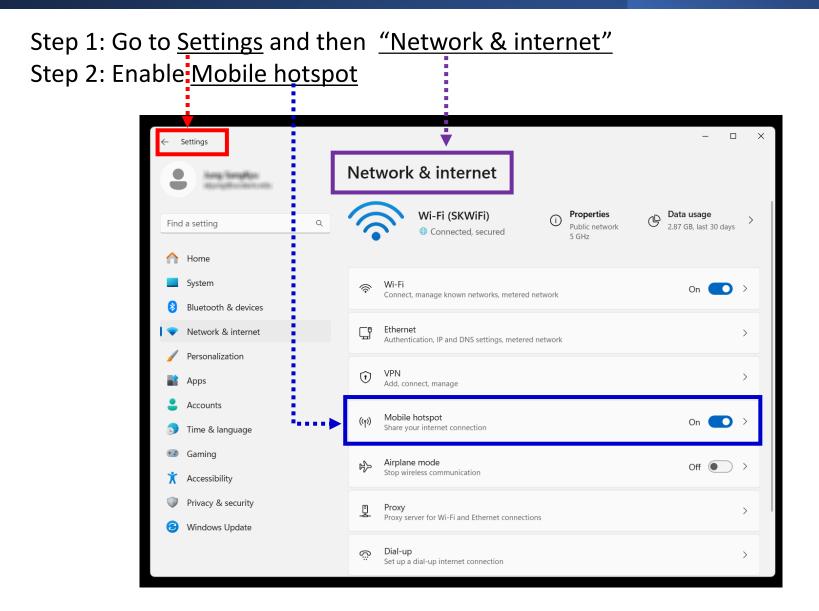
Scalable Imaging Platform for AREA measurement, for vast and endless expansion

#### Version 1.1

Contact: Sang-Kyu Jung (<u>skjung@hongik.ac.kr</u>) at Hongik University (Sejong, Korea)

# MOBILE HOTSPOT NETWORK SETTINGS

### Set up a hotspot network on your PC



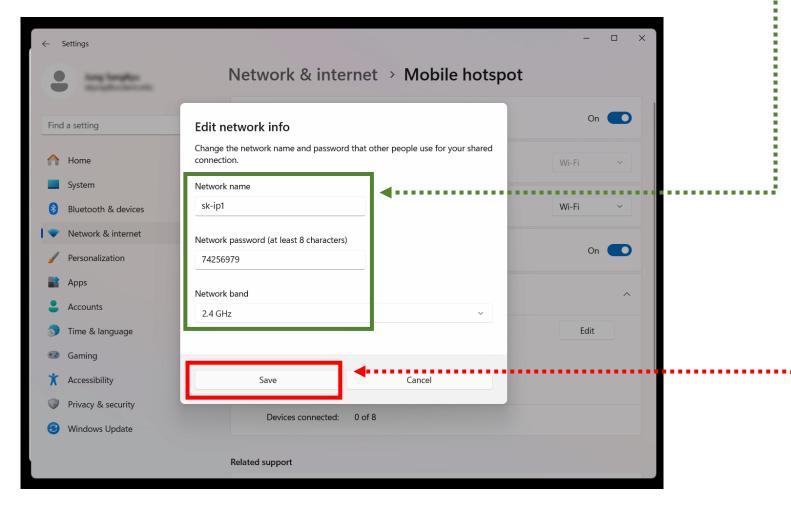
### Set up a hotspot network on your PC

Step 3: Click the <u>"Edit" button</u> ••

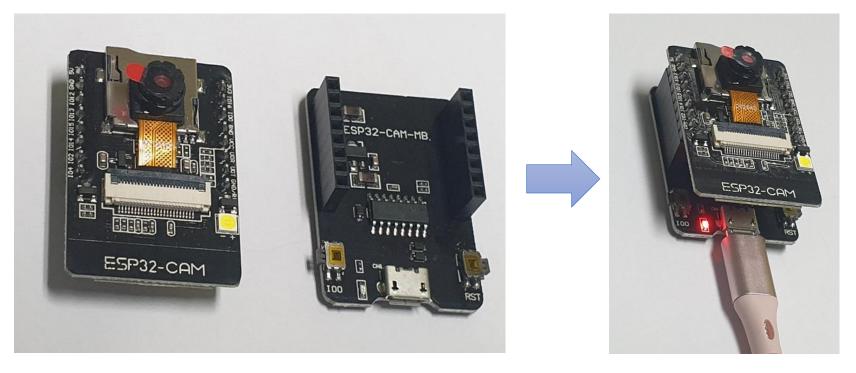
- Settings	- • × Network & internet > Mobile hotspot								
Find a setting Q	Mobile hotspot	On							
A Home	Share my internet connection	on from	Wi-Fi	~					
<ul><li>System</li><li>Bluetooth &amp; devices</li></ul>	Share over	Share over							
Network & internet     Personalization	Power saving When no devices are connected	, automatically turn off mobile hotspot	On						
Apps Accounts	Properties								
<ul> <li>Time &amp; language</li> </ul>	Network properties		Edit	- •					
<ul> <li>Gaming</li> <li>Accessibility</li> </ul>	Name: Password:	sk-ip1 74256979 2.4 GHz		-					
<ul> <li>Privacy &amp; security</li> </ul>	Band: Devices connected:								
Windows Update	Related support								

### Set up a hotspot network on your PC

Step 4: Enter <u>"sk-ip1", "74256979",</u> and select <u>"2.4GHz"</u> Step 5: Click the <u>"Save" button</u> When hotspot is enabled, all ESP32-CAMs will connect to this hotspot network.



## ESP32-CAM SETTINGS

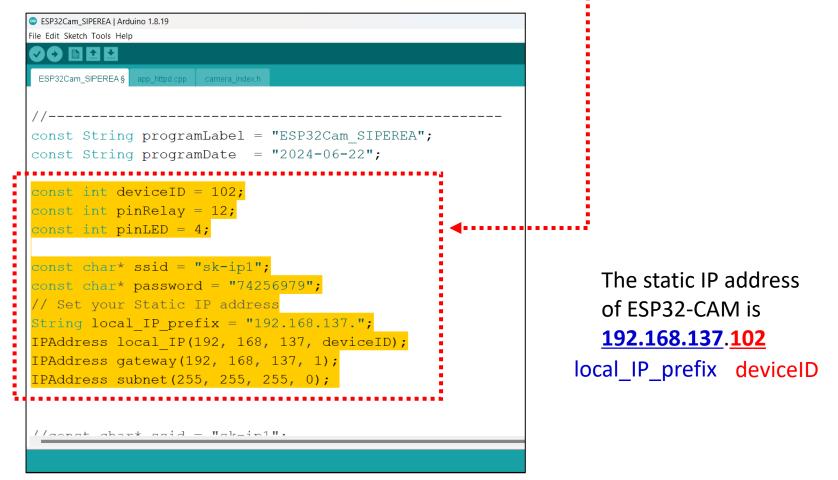


ESP32-CAM

#### Shield module

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

 Set up the Arduino IDE program environment for ESP32-CAM programming. Then open ESP32CAM\_SIPEREA.ino and set the <u>deviceID and IP address</u> information as needed.



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

ESE 2Cam_SIPE	REA   Arduino 1.8.19			
File Edit Sketch To	ools Help			
	Auto Format	Ctrl+T		
	Archive Sketch			
ESP32Cam_S	Fix Encoding & Reload			
	Manage Libraries	Ctrl+Shift+I		
	Serial Monitor	Ctrl+Shift+M		
//	Serial Plotter	Ctrl+Shift+L		
const S	WiFi101 / WiFiNINA Firmware Updater		PEREA";	
const 💈	Board: "AI Thinker ESP32-CAM"	>	Boards Manager	<b>A</b>
	CPU Frequency: "240MHz (WiFi/BT)"	>	Arduino AVR Boards	WT32-ETH01 Ethernet Module
	Flash Frequency: "80MHz"	>	ATTinyCore >	BPI-BIT
const i	Flash Mode: "QIO"	>	breadboard-avr >	BPI-Leaf-S3
const 🛓	Partition Scheme: "Huge APP (3MB No OTA/1MB SPIFFS)"		BSFrance >	Silicognition wESP32 T-Beam
const 1	Core Debug Level: "None" Erase All Flash Before Sketch Upload: "Disabled"		DIY ATmega8/88/48/168/328 >	D-duino-32
	Port: "COM4"	: 1	DIY ATmega8/88/48/168/328 >	LoPv
	Get Board Info		DIY ATtiny > ESP32 Arduino >	LoPv4
const 🗗		•••	HelTec WIFI with ESP32	OROCA EduBot
const c	Programmer	>	HelTec WIFI with ESP8266 >	ESP32 FM DevKit
	Burn Bootloader		MiniCore >	Frog Board ESP32
// Set y	your Static IP address		TTGO ESP32-OLED >	Al Thinker ESP32-CAM
String 1	ocal IP prefix = "192.168	3.137.";	TTGO WIFI-0.910LED	TTGO T-Watch
IPAddres	WEMOS D1 MINI ESP32 WEMOS D1 R32			
IPAddres	Pycom GPy			
	VintLabs ESP32 Devkit			
IPAddres	HONEYLemon			
				MGBOT IOTIK 32A
				MGBOT IOTIK 32B
				Piranha ESP-32
/ acnot	abort agid - "ak-in1".			Metro ESP-32
				Senses's WEIZEN

- 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.

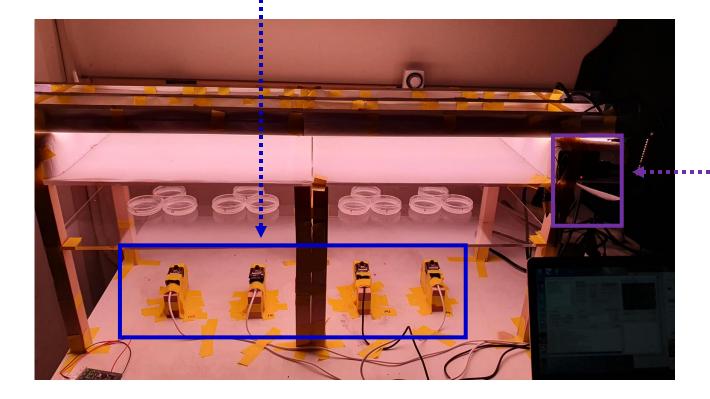
## IMAGING HARDWARE SETTINGS

## Set up imaging hardware

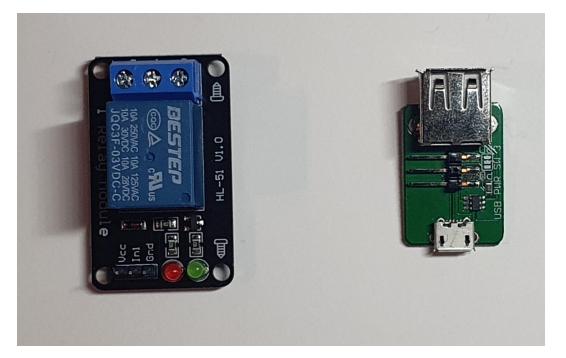
The four ESP32-CAMs for <u>imaging</u> have the following fixed IP addresses.

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



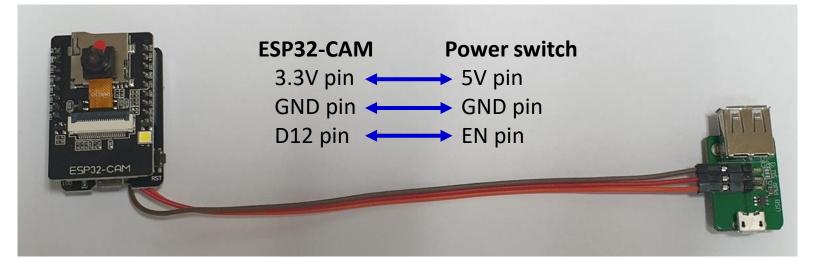
• There are two optional modules to control the auxiliary USB LED strip light:

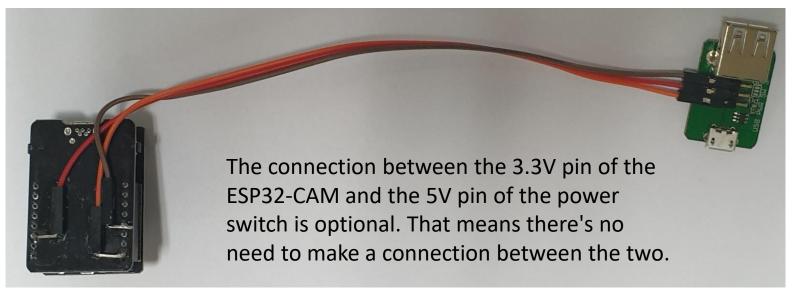


3.3V relay module

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

• Wiring connection between ESP32-CAM and power switch

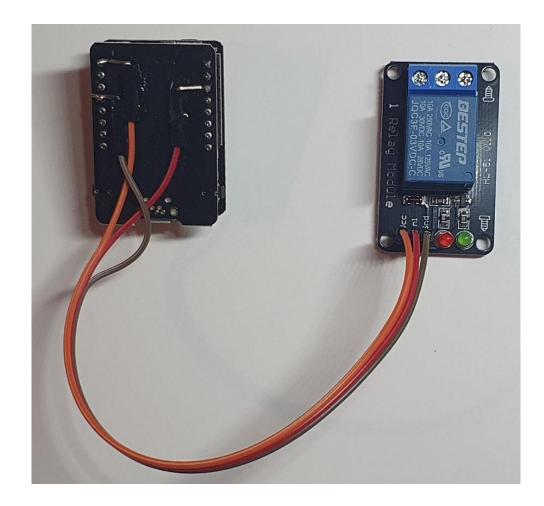




• Wiring connections between ESP32-CAM, power switch, and USB LED strip



• Wiring connection between ESP32-CAM and relay module

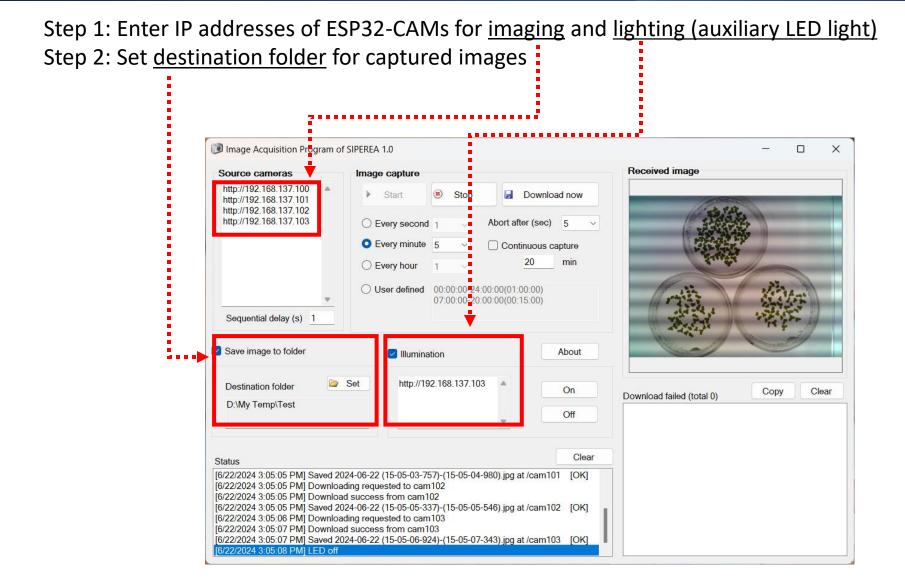




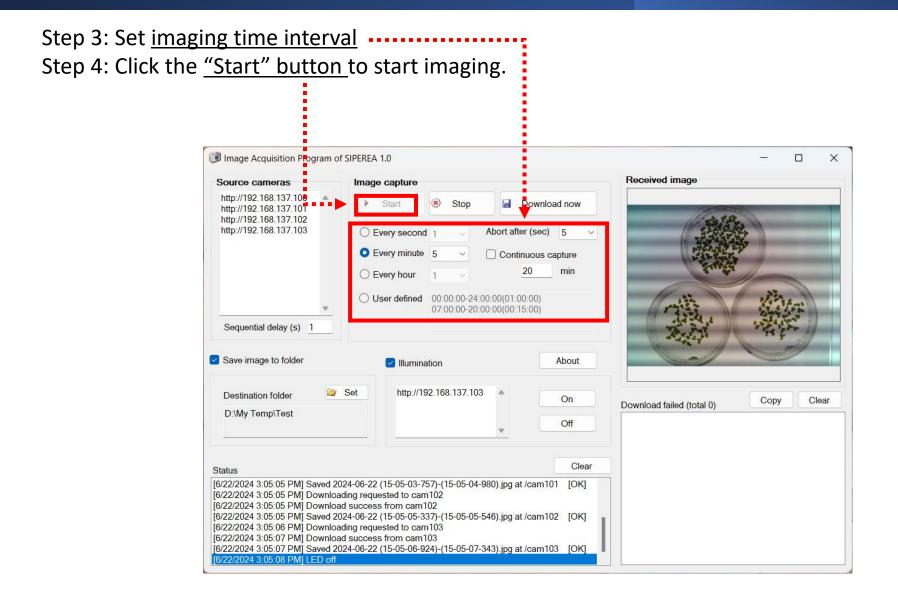
The connection between the 3.3V pin of the ESP32-CAM and the VCC pin of the power switch is mandatory for the relay module.

## IMAGE ACQUISITION PROGRAM SETTINGS

## Image acquisition program

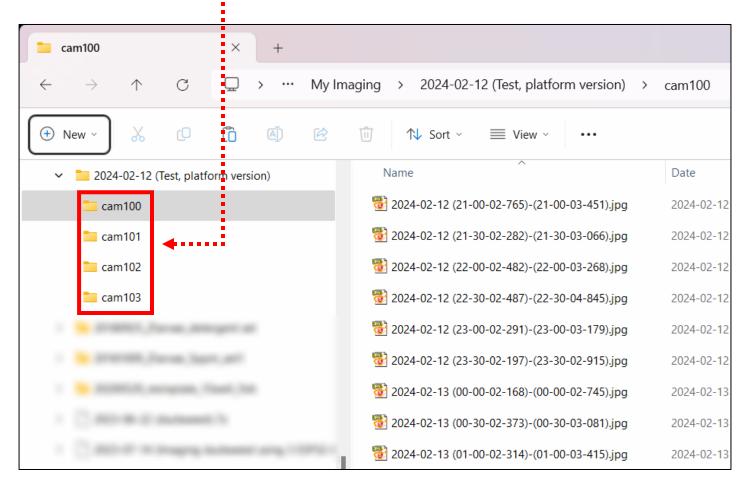


## Image acquisition program



## Image acquisition program

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



## IMAGE ANALYSIS PROGRAM SETTINGS

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



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3	Batch1			2		circle	553	387	892	710	
4	Batch1			3		circle	339	29	671	356	
5											
6											
7											

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

A <u>window</u> that loads and tests one image file to find optimal image processing parameters.

image anlaysis program of SIPEREA 1.0		-
<u>File Edit Window About</u>		
Image processing Batch processing		
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		🔹 🍓 No animation Interval 500 🔹 Show regions Show labe
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Vidui 1024	Theight 100	
Adaptive thresholding binarization		
Box size 40 Thresho	old 75 Create binarized image	
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	gs (D@n	
Run an	alysis	
Batch Processing		
Clear Select folder		
Clear Select folder	Run batch processing Interval 1	
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2023-10-28 (03-21-03-693)-(03-21-04-811).jpg	113.8 D:\Scalable imaging system - Sample imag	Contraction of the second
2023-10-28 (03-24-04-028)-(03-24-04-834).jpg	114.1 D:₩Scalable imaging system - Sample imag	Carly March
2023-10-28 (03-27-04-027)-(03-27-04-895).jpg	114.3 D:\Scalable imaging system - Sample imag	
2023-10-28 (03-30-03-799)-(03-30-04-190).jpg	113.7 D:\#Scalable imaging system - Sample imag <	
2023-10-28 (03-33-04-038)-(03-33-04-520).jpg	113.8 D:\Scalable imaging system - Sample imag	
2023-10-28 (03-36-03-828)-(03-36-04-551).jpg	114.6 D:\Scalable imaging system - Sample imag	
	113.8 D:₩Scalable imaging system - Sample imag	
2023-10-28 (03-30-03-825)-(03-30-04-351),jpg 2023-10-28 (03-39-03-845)-(03-39-04-368),jpg		
2023-10-28 (03-39-03-845)-(03-39-04-268).jpg		

<u>.</u>...

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

#### A <u>batch processing window</u> that adds multiple files and analyzes them continuously

Image anlaysis program of SIPEREA 1.0     Elie Edit Window About     Image processing Batch processing			_
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Adaptive thresholding binarization			
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Box size 40 Three	shold 75 Create binarized image		
The object to be detected is darker than its surround	dings		2 Basti P2
	ingo.	(I BID) (P	
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2023-10-28 (03-24-04-028)-(03-24-04-834).jpg	114.1 D:₩Scalable imaging system - Sample imag		
2023-10-28 (03-27-04-027)-(03-27-04-895).jpg	114.3 D:\Scalable imaging system - Sample imag		
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	113.8 D:₩Scalable imaging system - Sample imag		
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2023-10-28 (03-36-03-828)-(03-26-04-551).jpg	113.8 D:\Scalable imaging system - Sample imag		

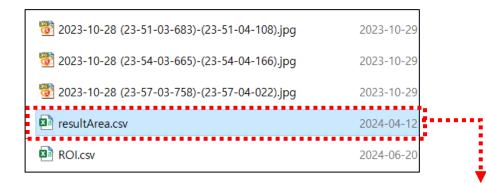
First, click the 'Select Folder' button and then add files. Then click the 'Run Batch Process' button to process all files.

#### A <u>window</u> to check the original image and the image after processing

jile <u>E</u> dit <u>Window About</u> mage processing <u>Batch processing</u> Source image <u>Analysis</u> Source image file <u>Select file</u> <u>Read image</u> D'#Scalable imaging system - Sample images₩cam 101₩2023-10-28 (03-21-03-693)-(03-21-04-811).jpg Drag and drop imag file Resize image Resize Width 1024 Height 768 Adaptive thresholding binarization Box size <u>40</u> Threshold <u>75</u> Create binarized image Run analysis Batch Processing Clear Select folder Run batch processing Interval File name 2023-10-28 (03-18-03-978)-(03-18-04-795).jpg 113.8 D:#Scalable imaging system - Sample imag	Select file Read image   33-10-28 (03-21-03-693)-(03-21-04-811),jpg   Drag and drop imag file   Height 78   (Create binarized image   wis    (Create binarized image   Name   (Create binarized image   Name   (Create binarized image   Name   (Size (M)   Folder   (Name   113.   DrScalable imaging system   Sample image   113.   DrScalable imaging system   Sample image   113.   DrScalable imaging system   Sample image   113.   DrScalable imaging system   114.   DrScalable imaging system   115.   DrScalable imaging system   114.				- c
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The 5033th file selected among the 5445 files		Clear         Select folder           File name         2023-10-28 (03-18-03-978)-(03-18-04-795) jpg           2023-10-28 (03-21-03-693)-(03-21-04-811) jpg         2023-10-28 (03-24-04-028)-(03-24-04-834) jpg           2023-10-28 (03-27-04-027)-(03-27-04-895) jpg         2023-10-28 (03-27-04-027)-(03-27-04-895) jpg           2023-10-28 (03-30-03-799)-(03-30-04-190) jpg         2023-10-28 (03-30-03-799)-(03-30-04-510) jpg           2023-10-28 (03-30-04-382)-(03-36-04-551) jpg         2023-10-28 (03-30-03-828)-(03-30-04-561) jpg           2023-10-28 (03-30-03-828)-(03-30-04-561) jpg         2023-10-28 (03-30-03-828)-(03-30-04-561) jpg	Size (kb) Folder 1138 D:\#Scalable imaging s 1134 D:\#Scalable imaging s 114.1 D:\#Scalable imaging s 114.3 D:\#Scalable imaging s 1137 D:\#Scalable imaging s 1136 D:\#Scalable imaging s	Interval 1	Image: state
The 5033th file selected among the 5445 files		Clear         Select folder           File name         2023-10-28 (03-18-03-978)-(03-18-04-795) jpg           2023-10-28 (03-21-03-693)-(03-21-04-811) jpg         2023-10-28 (03-24-04-028)-(03-24-04-834) jpg           2023-10-28 (03-27-04-027)-(03-27-04-895) jpg         2023-10-28 (03-27-04-027)-(03-27-04-895) jpg           2023-10-28 (03-30-03-799)-(03-30-04-190) jpg         2023-10-28 (03-30-03-799)-(03-30-04-510) jpg           2023-10-28 (03-30-04-382)-(03-36-04-551) jpg         2023-10-28 (03-30-03-828)-(03-30-04-561) jpg           2023-10-28 (03-30-03-828)-(03-30-04-561) jpg         2023-10-28 (03-30-03-828)-(03-30-04-561) jpg	Size (kb) Folder 1138 D:\#Scalable imaging s 1134 D:\#Scalable imaging s 114.1 D:\#Scalable imaging s 114.3 D:\#Scalable imaging s 1137 D:\#Scalable imaging s 1136 D:\#Scalable imaging s	Interval 1	

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

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

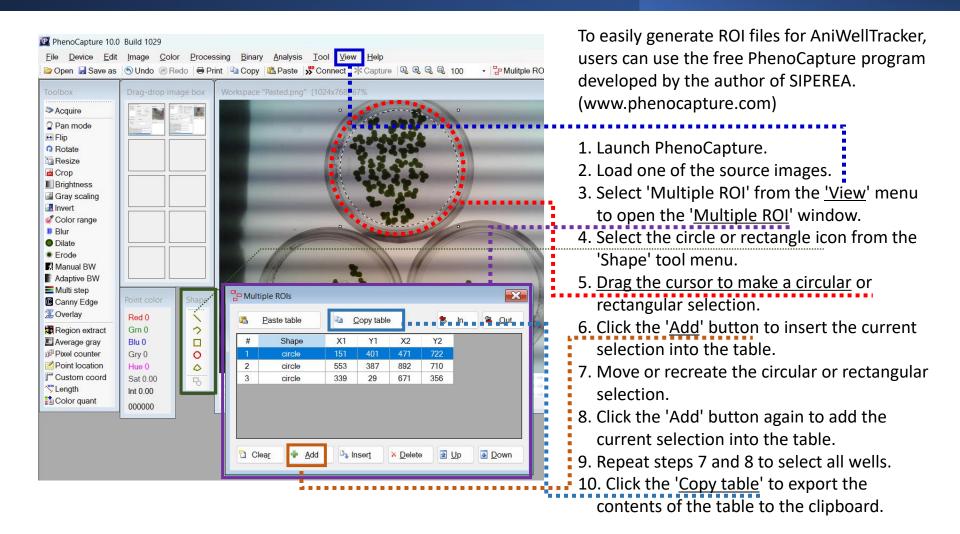


#### Area value by ROI (by well)

	Α	В	c 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



#### **ROI.csv file generation**



#### **ROI.csv file generation**

	А	В	С		D		Е	F	G	Н	I.	J
1	Condition1	Condition2	Date	Pla	ate#	W	/ell#	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	Control Doo 4			1		5	circle	507	264	633	390
7	Test	Additional			1		6	circle	693	266	819	392
8	Test inf	information that			1		7	Pas	sted fr	om cli	inhoar	'd <sup>72</sup>
9	<sup>Test</sup> us	ers may e	nter		1		8	1 43		erri en	poca	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
17												

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

- 1. Create 10 columns from A to J as shown.
- 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.

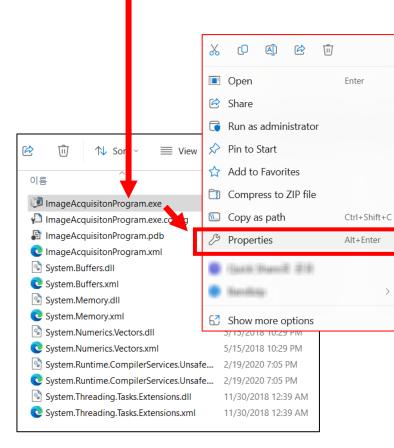
(CSV: comma-separated text file format)

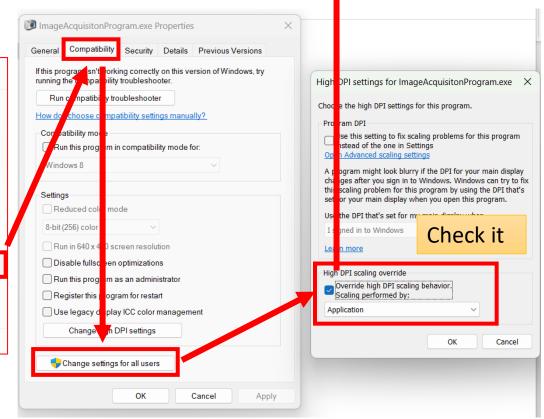
6. All done!

#### 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

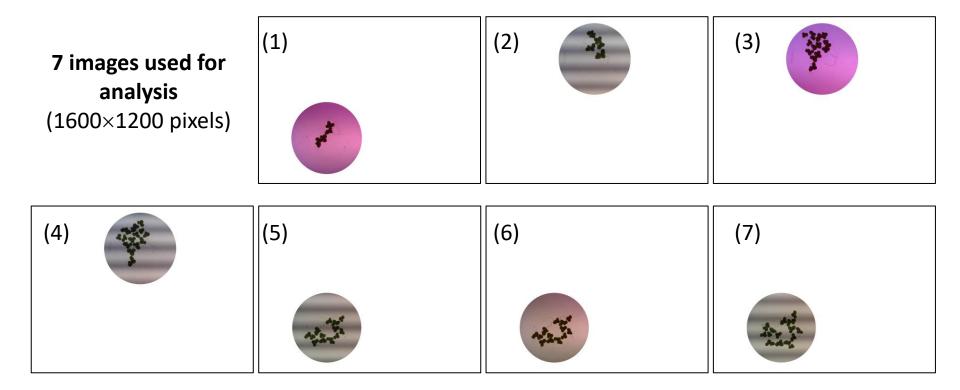
### When you run the program again, the screen appears clearly.





#### 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.



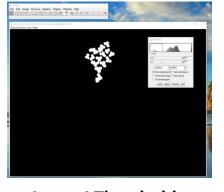
#### Analysis of area calculation accuracy

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



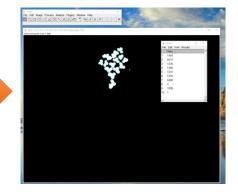
ImageJ

#### <Binarized image>



**ImageJ Threshold** 

<Area measurement>



#### ImageJ Measurement (area)



Image

using

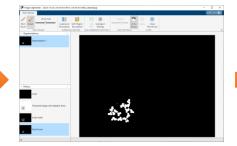
ImageJ



<Original image>

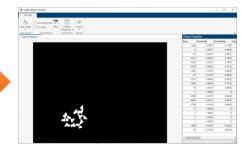
**MATLAB** Image Segmenter

#### <Binarized image>



**MATLAB** Image Segmenter

#### <Area measurement>



**MATLAB Image Region Analyzer** 

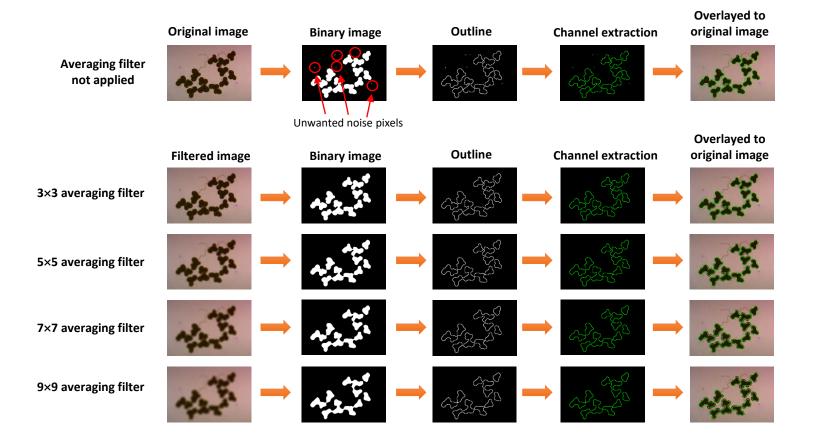
#### Analysis of area calculation accuracy

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  $3 \times 3$  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  $3 \times 3$  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.



#### License info

#### **BSD-3-Clause**

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