AniWellTracker 1.0

User Manual

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System requirements

- AniWellTracker has been developed with Microsoft Visual Basic. NET (shortly, VB.NET) and therefore runs on a PC with Microsoft Windows operating system (Windows 10 recommended).

- Users may have to install NET Framework 4.5.1 or higher versions.

  https://dotnet.microsoft.com/en-us/download/dotnet-framework

- Recommended hardware specs:

  PC with CPU of 1st generation Intel i3 or higher and RAM of 4GB or more

Preparing for image analysis

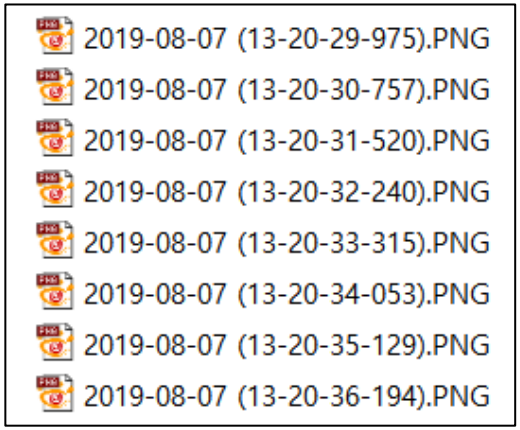
1. Record time-lapse images or videos while performing the experiments.
Users may want to use free PhenoCapture program to record time-lapse images or videos using a web camera. Check out **Appendix I** and **II**.

2. In case of videos, frame images can be extracted from the video file using the provided MATLAB code. check out **Appendix III**.

3. The source image file name should be in the following format.

YYYY-MM-DD (hh-mm-ss-SSS).extension

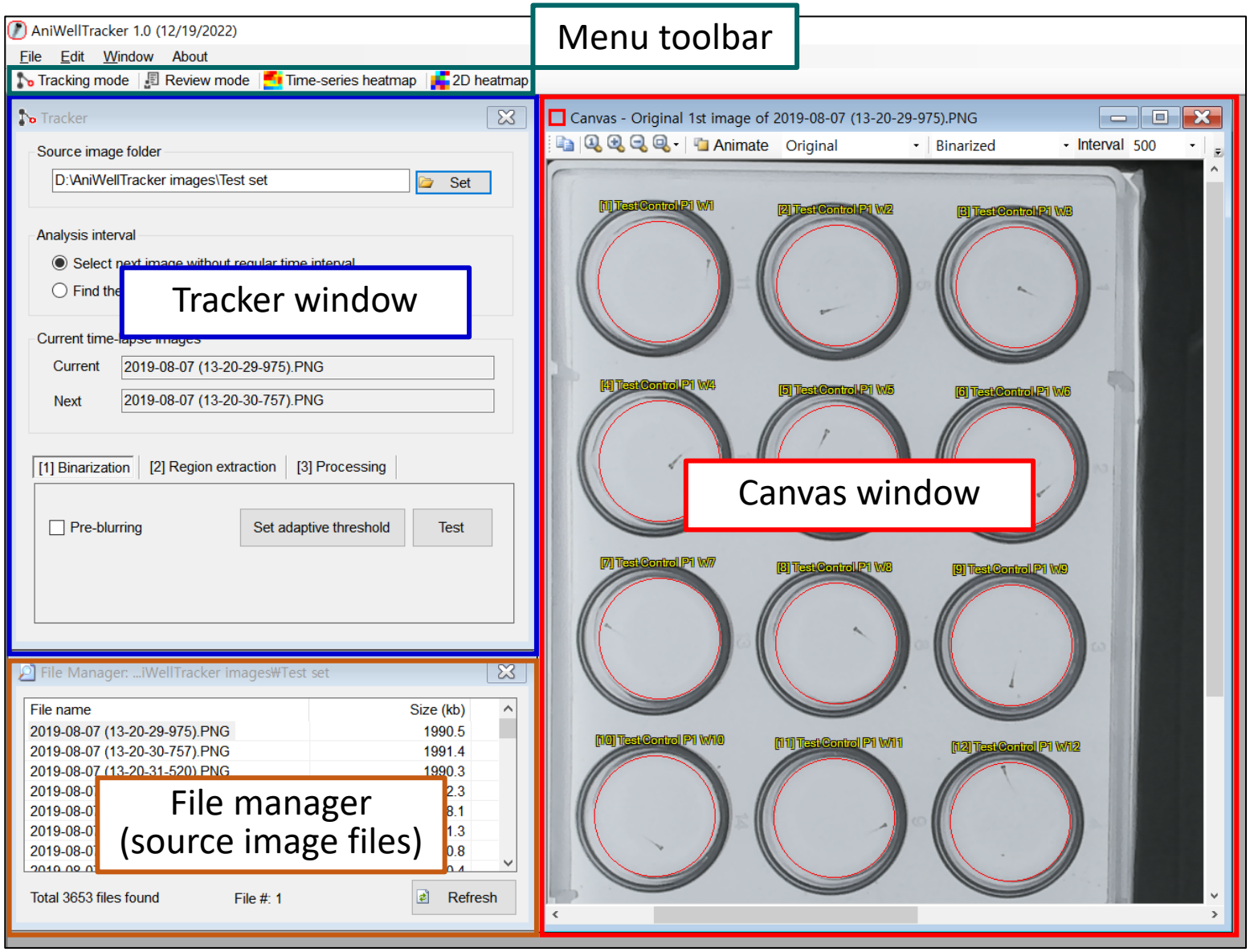
YYYY: four-digit year
MM: two-digit month
DD: two-digit day
hh: two-digit hour
mm: two-digit minute
ss: two-digit second
SSS: three-digit millisecond
One space between 'DD' and '(hh'
Parentheses '(' and ')' required



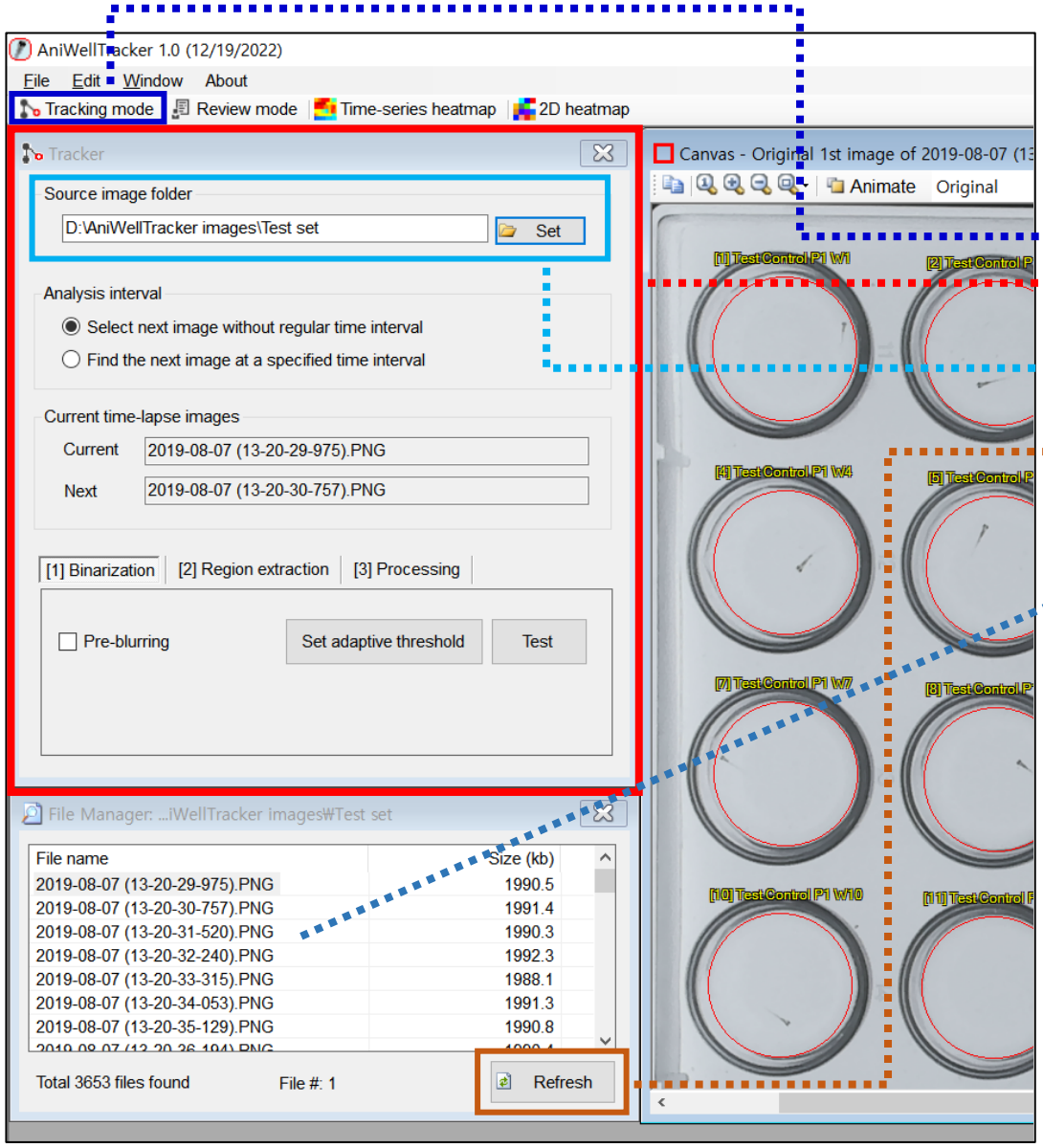
* The PhenoCapture and the MATLAB code generate image files with names compatible with AniWellTracker.

4. The user must create a ROI file (ROI.csv) and locate the file in the folder containing the source images to be analyzed. Check out **Appendix IV**.

User interface



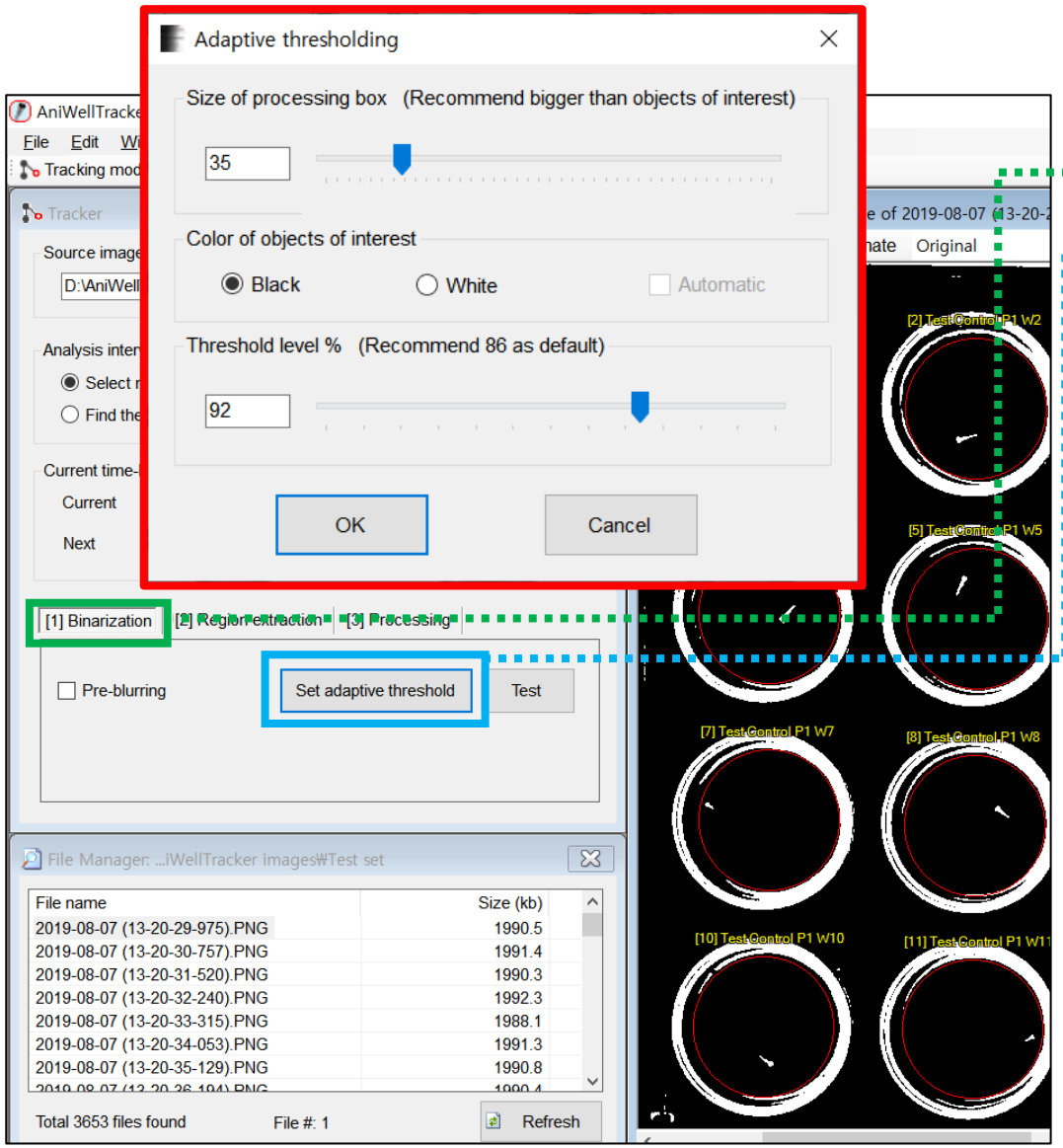
Tracking mode for image analysis



In Tracking mode, users can analyze images to extract animal's centroid coordinate in each well.

1. Click 'Tracking mode' button to show 'Tracker' window.
2. Set the source image folder to be analyzed.
3. Click 'Refresh' button to list image files in the 'File Manager' window.
4. Click any image file in the 'File Manager' to show the image in the 'Canvas' window.
5. If a ROI file (ROI.csv) is found in the folder, red circular or rectangular ROI regions are shown in the 'Canvas' window.

Tracking mode for image analysis



[Step 1: Binarization]

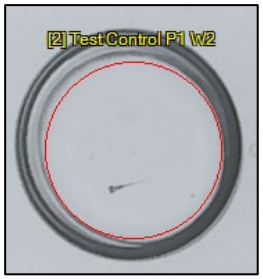
The first step in image analysis is to convert the original image into a black and white image. This is done in the 'Binarization' tab.

AniWellTracker uses an adaptive thresholding algorithm. Click 'Set adaptive threshold'.

1. Set the size of the processing box.
   A slightly bigger number than the size of animals is recommended.
2. Set the color of the objects to be detected.
   If animals are displayed in black, select black.
3. Set the threshold level.
   A number between 80 and 95 is recommended.
4. Click 'OK' button to display the result in the 'Canvas' window.

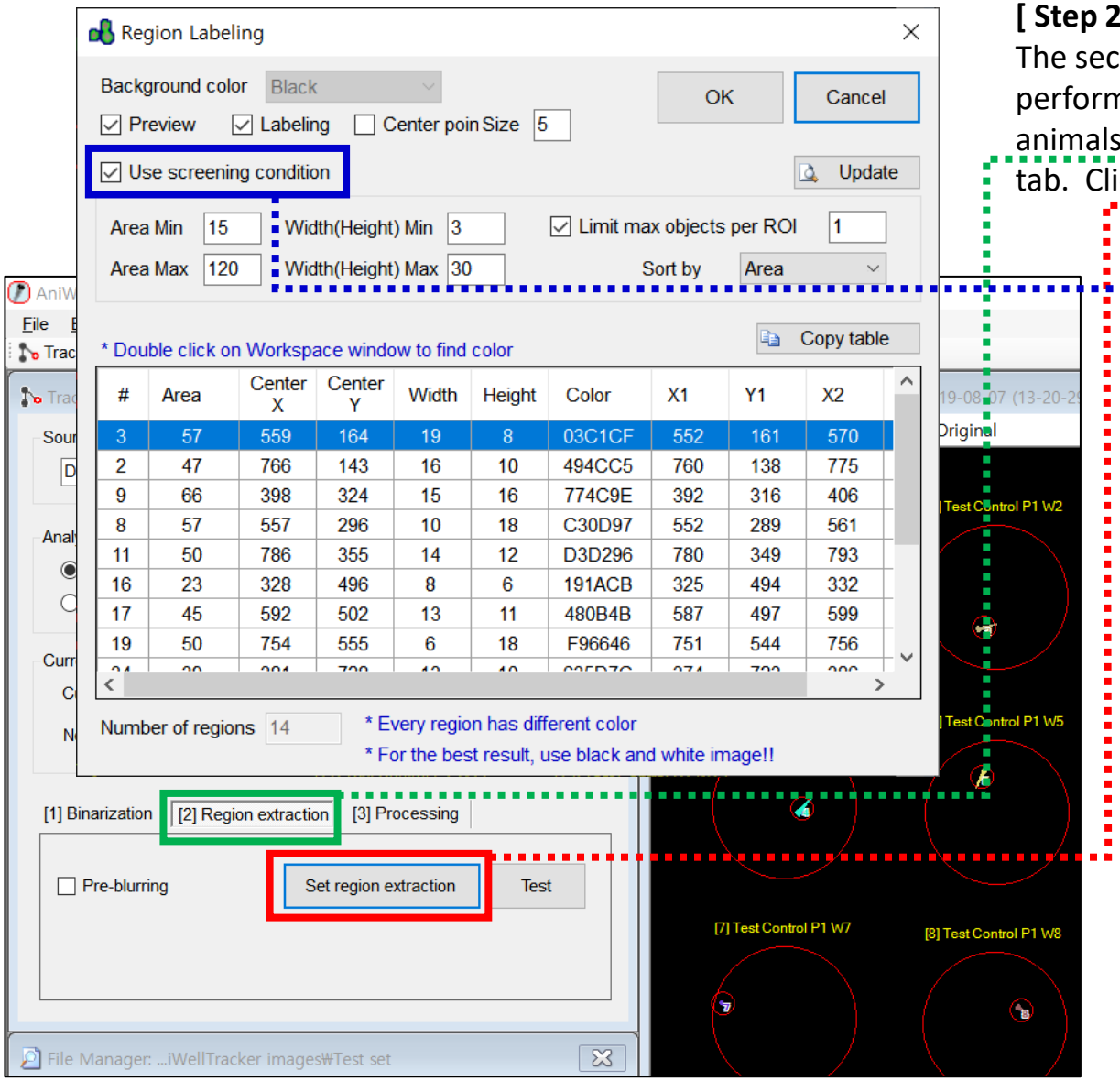
Users can try the default values first and then modify them to improve the binarization.

Original



Binarized

Tracking mode for image analysis



[Step 2: Region extraction]

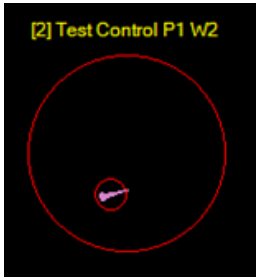
The second step in image analysis is to perform region(blob) extraction to detect animals. This is done in the 'Region extraction' tab. Click 'Set region extraction' to configure.

1. Check 'Use screening condition'. Users must set values for minimum area, maximum area, minimum width(and height), and maximum width values to filter out invalid objects. Only objects within range are detected as animals.

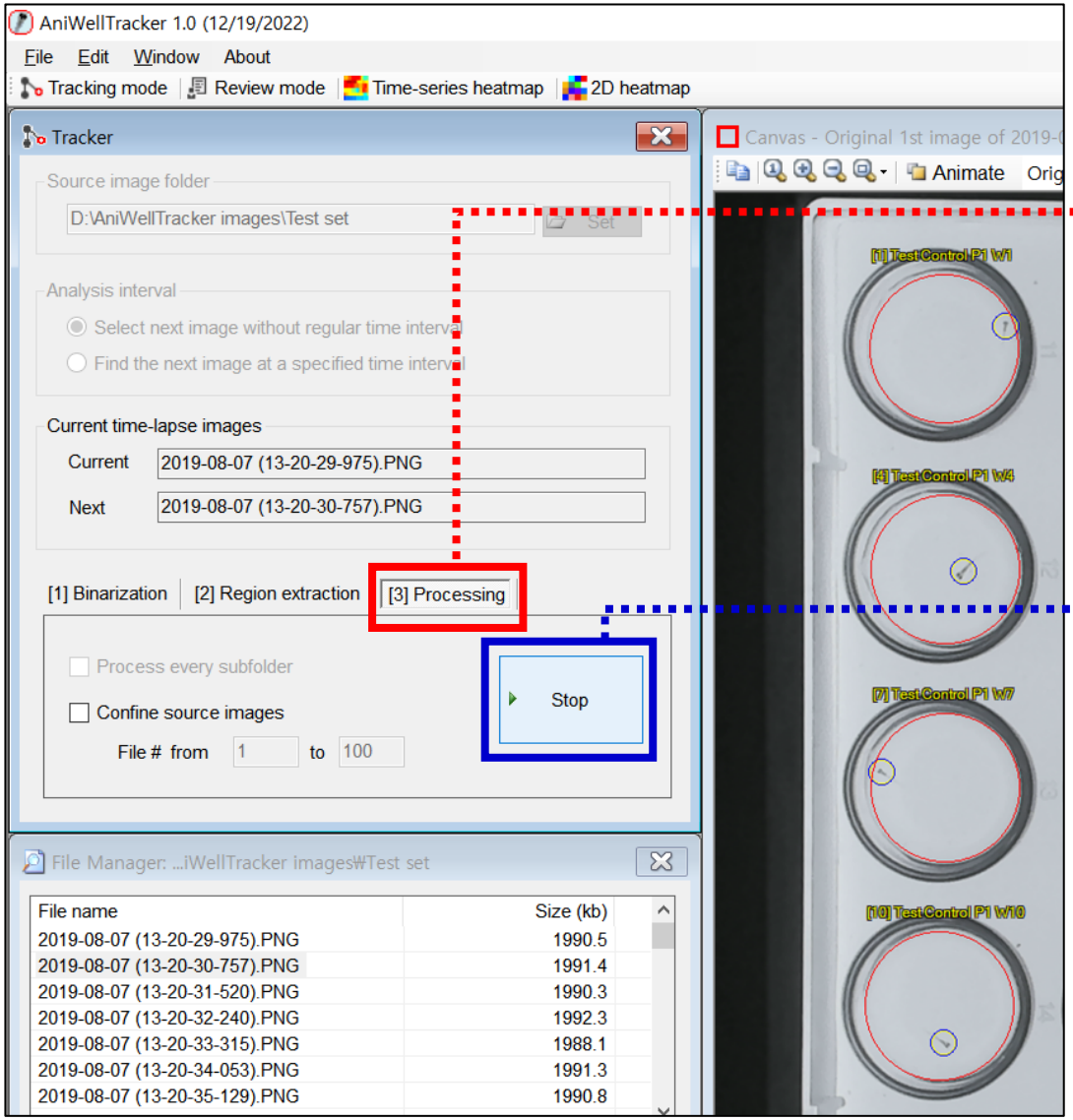
2. The user can find the optimal parameters by checking the information of the detected objects.

Binarized

Region extracted



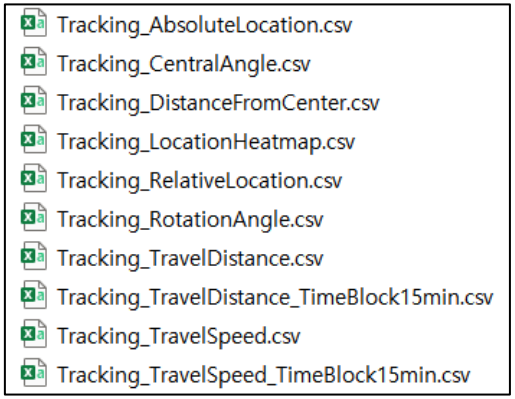
Tracking mode for image analysis



[Step 3: Processing]

After the user finishes optimizing the image analysis parameters in steps 1 and 2, the third step is to analyze all images in the 'Processing' tab.

1. If the user wants to process all subfolders, check 'Process every subfolder'. This option allows you to perform batch processing and to process multiple folders.
2. If the user only wants to process a subset of image files, check 'Confine source images' and set the image numbers in the blanks.
3. Click 'Process' button.
4. When the analysis is completed, multiple output files are generated in the folder analyzed.



Output files

Table. Analysis output files generated by AniWellTracker.

Output file name	Description
Tracking_AbsoluteLocation.csv	Animal's centroid (x and y) in the image
Tracking_RelativeLocation.csv	Animal's centroid (x and y) within the ROI
Tracking_TravelDistance.csv	Animal's travel length between time-lapse images
Tracking_TravelSpeed.csv	Animal's travel speed between time-lapse images
Tracking_CentralAngle.csv	Animal's central angle between the center of the ROI and the l ocation of the animal
Tracking_DistanceFromCenter.csv	Distance between the center of the ROI and the centroid of the animal
Tracking_RotationAngle.csv	Change in the central angle of the animal between time-lapse i mages
Tracking_TravelDistance_TimeBlock00min.csv	Average distance traveled in each time block of 00 min where 0 0 represents a number
Tracking_TravelSpeed_TimeBlock00min.csv	Average speed traveled in each time block of 00 min where 00 represents a number
Tracking_LocationHeatmap.csv	Heatmap for the number of places an animal was located in th e ROI
Tracking_DistanceFromCenterHeatmap.csv	Heatmap of the number of distances the animal was located fr om the center of the ROI.

* AniWellTracker generates 'Tracking_AbsoluteLocation.csv' and then processes the 'Tracking_AbsoluteLocation.csv' to generate all other output files.

Output files

Example of 'Tracking_AbsoluteLocation.csv'

	A	B	C	D	E	F	G	H
1	Filename	Blank	Experiment time (ms)	Time lapse (ms) (n+1 - n)	[X] Test||P1|W1|2021-01-01	[Y] Test||P1|W1|2021-01-01	[X] Test||P1|W2|2021-01-01	[Y] Test||P1|W2|2021-01-01
2	2020-11-18 (15-26-09-452).PNG		0	642	381	129	590	143
3	2020-11-18 (15-26-10-094).PNG		642	966	380	130	590	143
4	2020-11-18 (15-26-11-060).PNG		1608	988	381	130	590	142
5	2020-11-18 (15-26-12-048).PNG		2596	1040	381	130	590	143
6	2020-11-18 (15-26-13-088).PNG		3636	1050	380	129	590	142
7	2020-11-18 (15-26-14-138).PNG		4686	980	380	130	590	141
8	2020-11-18 (15-26-15-118).PNG		5666	1013	380	129	590	142

Image file name analyzed

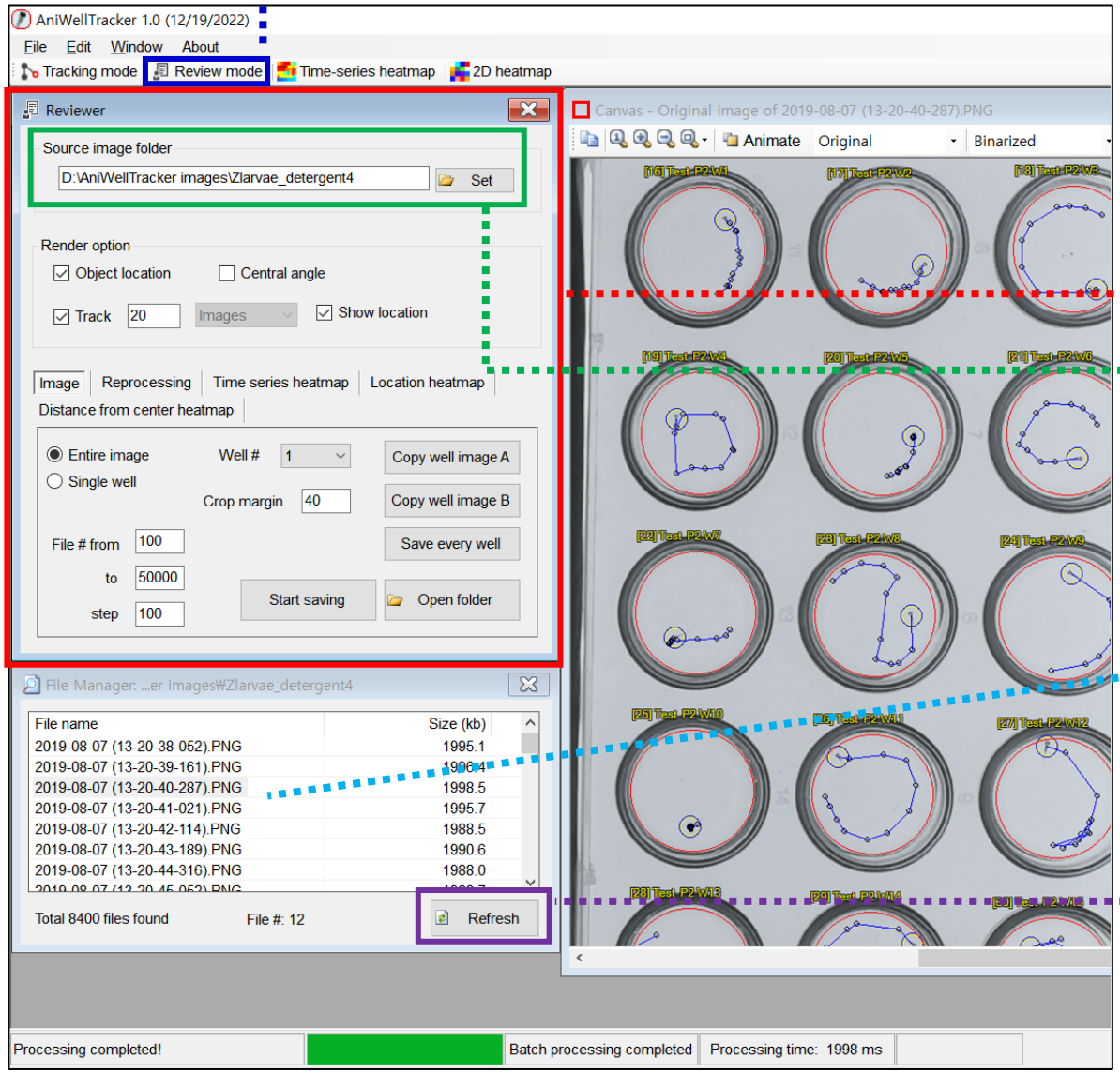
Elapsed time from the beginning

Time interval between two consecutive images

Centroid coordinates of the first well

Centroid coordinates of the second well

Review mode for reprocessing and charting



In Review mode, users can reprocess 'Tracking_AbsoluteLocation.csv'file generated in Tracking mode to create other output files.

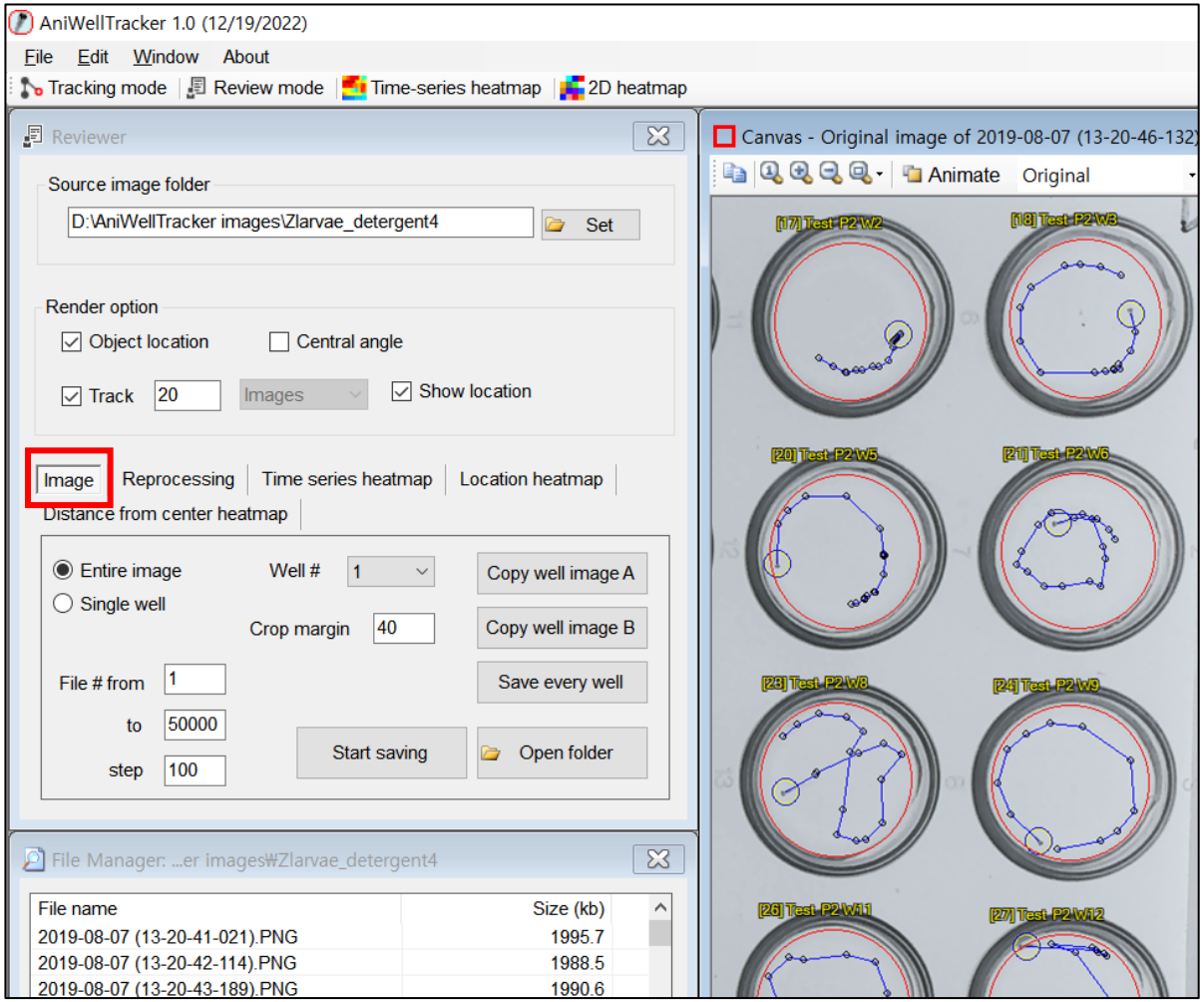
1. Click 'Review mode' button to show Reviewer' window.

2. Set the source image folder where image analysis was completed.

3. Optionally, click 'Refresh' button to list image files in the 'File Manager' window.

4. Click any image file in the 'File Manager' to show the image in the 'Canvas' window.

Review mode for reprocessing and charting



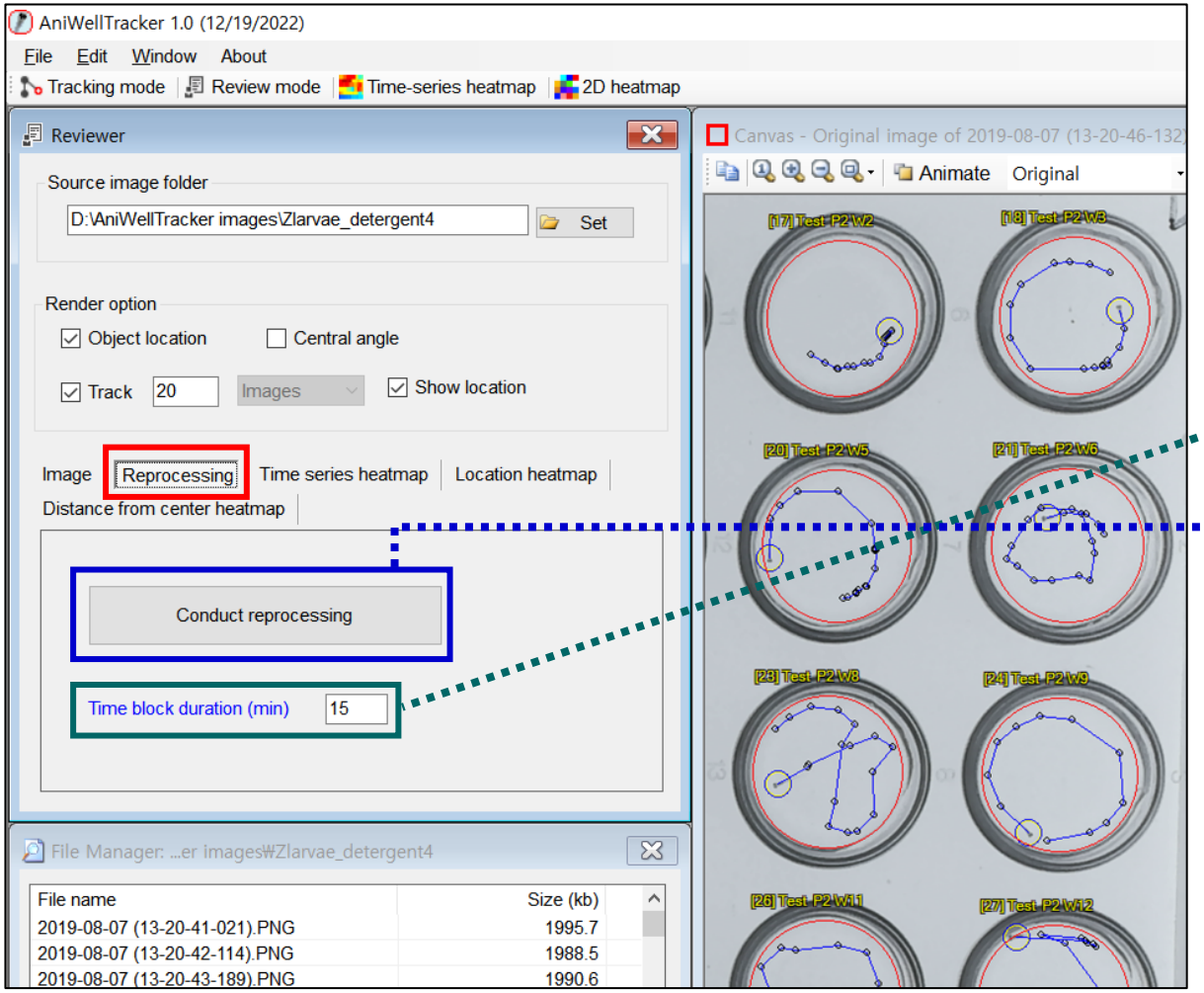
[Image tab]

The first 'Image' tab has a function to automatically save as a file by displaying the tracking path in the original image so that the tracking result can be reviewed.

Not only for one well, but for the entire image, the path of the specified length (number of files) is displayed on one original image, allowing quick review to see if there are any tracking issues. These functions are useful for data quality control.

Check the 'Rendered' subfolder in the source image folder. Users can browse generated images for quick review.

Review mode for reprocessing and charting



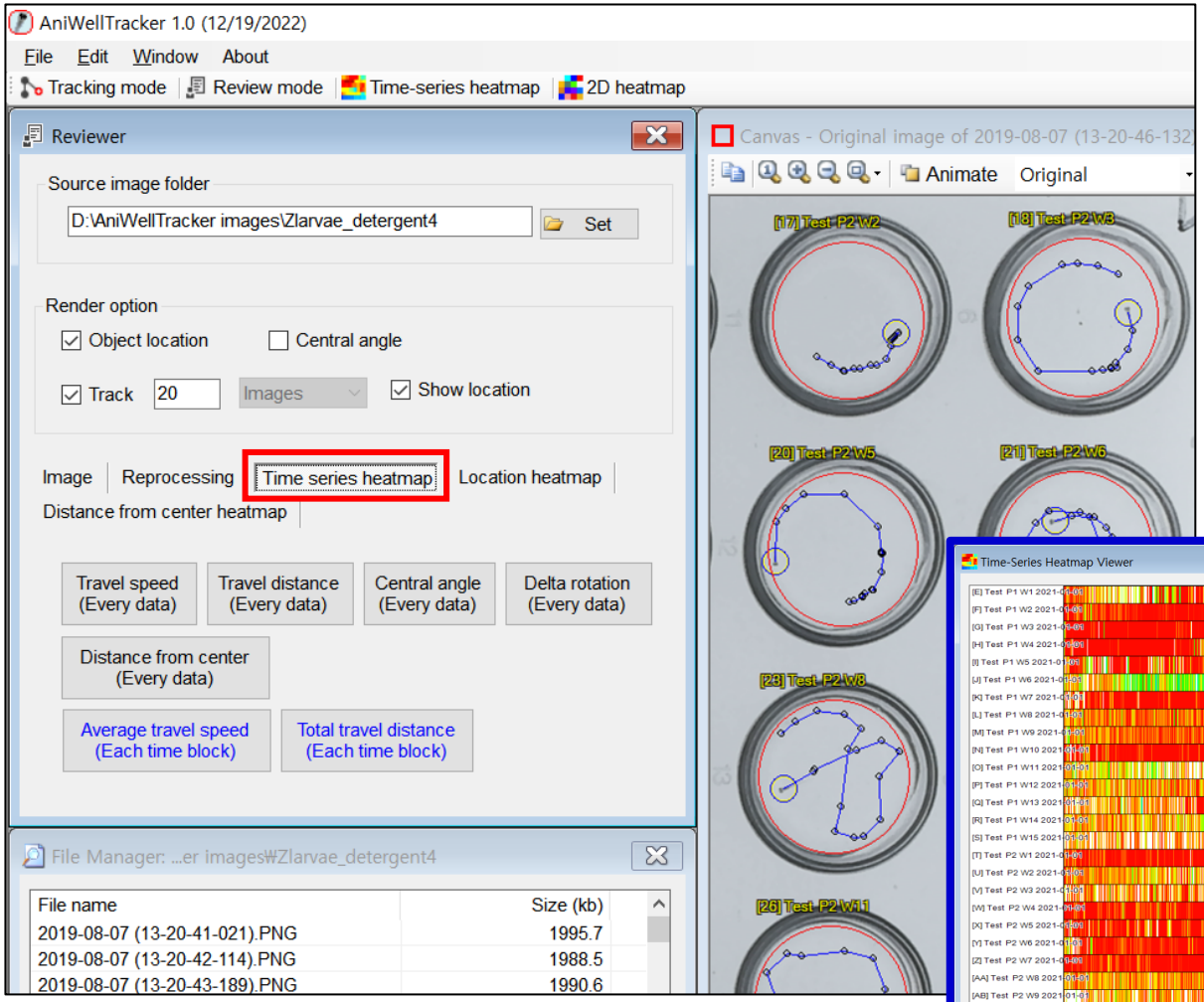
[Reprocessing tab]

In the second 'Reprocessing' tab, the user reprocesses the original files, and especially if the user sets the duration (min), the average speed and distance are recalculated for each time interval and saved in the output file.

1. Time setting

2. Click 'Conduct reprocessing'

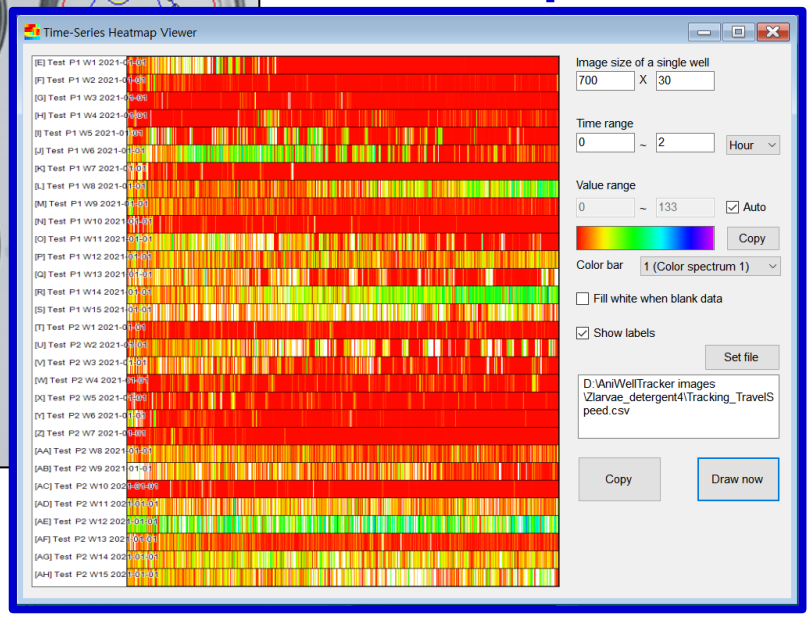
Review mode for reprocessing and charting



[Time-series heatmap tab]

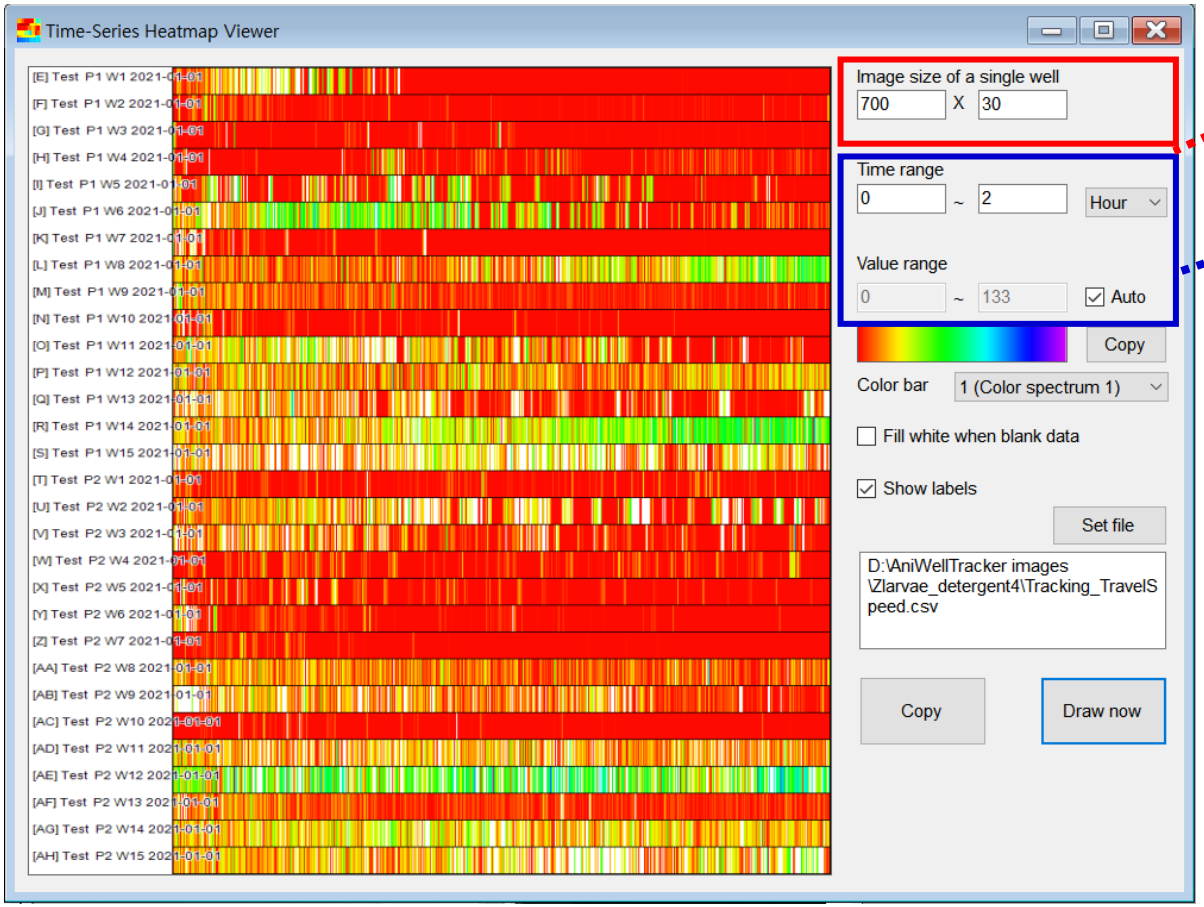
The third "Time Series Heatmap" tab features a heatmap display of raw data for speed, distance traveled, and average speed versus time.

When the user presses buttons such as “Travel speed (every data)” or “Travel distance (every data)”, the analysis result can be immediately displayed in a heatmap chart.



Review mode for reprocessing and charting

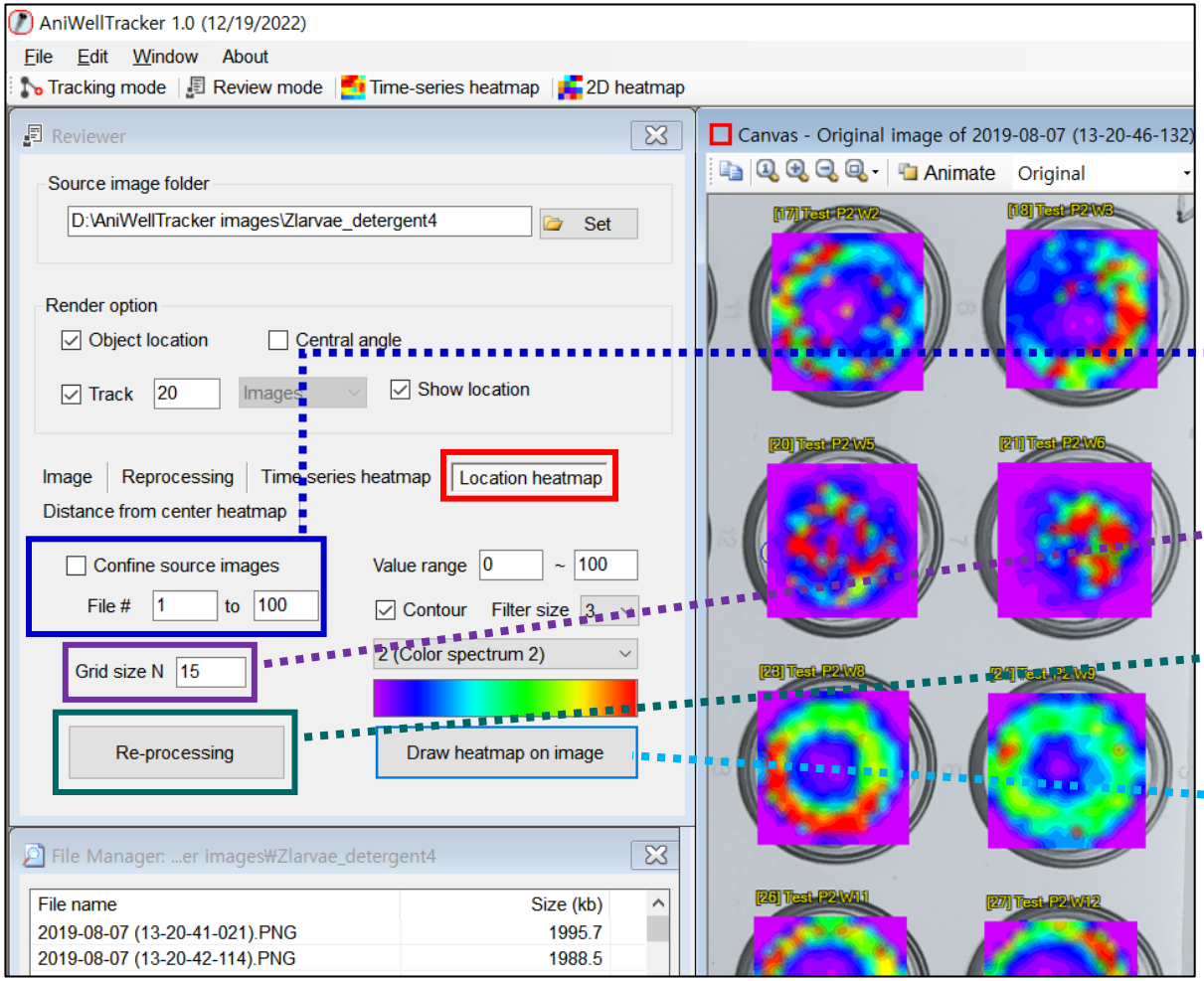
Example of time-series heatmap



[Time-series heatmap tab]

In the time series heatmap, the time interval to be displayed on the heatmap can be input in minutes or hours, and the color corresponding to the numerical value can be set by inputting the range of numerical values.

Review mode for reprocessing and charting



[Location heatmap tab]

In the “Location heatmap” tab, the probability of the location where the animal stays can be displayed as a heatmap.

1. If the user wants to confine a subset of image files, check 'Confine source images' and set the number values for a range.
2. Set the Grid size N to create a N by N location frequency table.
3. Click 'Re-process' button to compute location frequencies.
4. Click 'Draw heatmap on image' to visualize the results.

Review mode for reprocessing and charting

Example of 'Tracking_LocationHeatmap.csv'

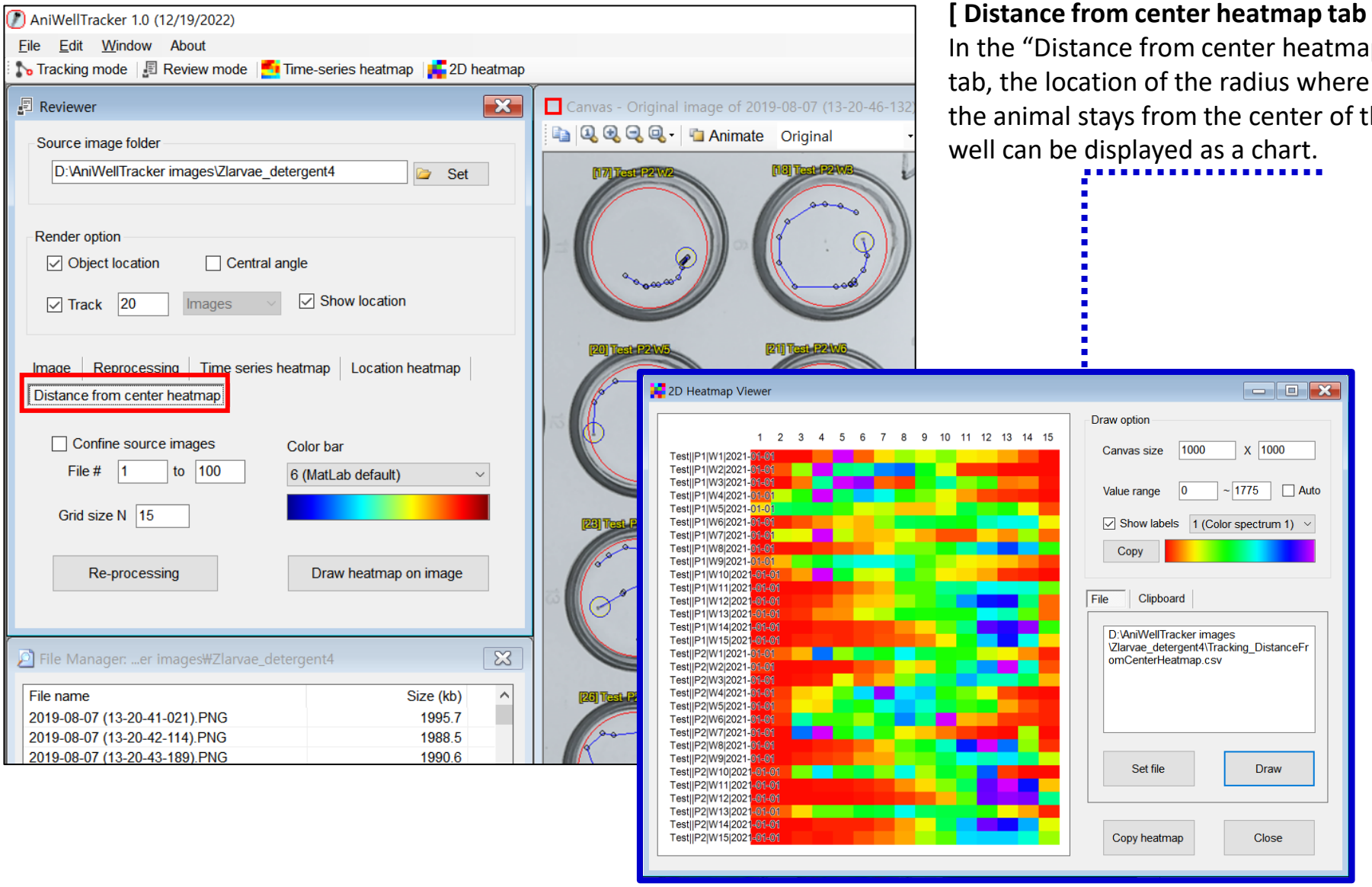
Information of each well

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	ROI ID	1		Total count	8001		Test||P1|W1|2021-01-01								
2	0	0	0	0	0	4	3	7	0	0	0	0	0	0	0
3	0	0	0	12	10	18	15	25	16	17	13	0	0	0	0
4	0	0	3	12	20	17	11	9	15	16	13	3	1	0	0
5	0	1	8	21	16	13	19	21	10	12	17	13	6	3	0
6	0	6	10	12	17	42	3	5	9	7	7	7	14	1	0
7	1	10	28	21	9	9	5	8	7	10	73	62	13	12	2
8	1	14	50	8	11	3	5	4	23	35	250	21	1	9	2
9	4	18	65	17	7	7	1	2	3	2924	1890	121	83	28	2
10	6	9	17	67	2	7	131	5	0	2	15	48	33	15	1
11	2	20	9	21	9	8	9	10	1	4	134	21	10	6	1
12	0	6	20	14	13	8	7	10	2	2	155	63	5	8	1
13	0	2	23	22	18	76	13	31	14	60	118	11	11	1	0
14	0	0	3	20	17	12	5	11	10	19	8	4	9	0	0
15	0	0	0	6	12	17	8	9	15	7	9	4	1	0	0
16	0	0	0	0	1	1	1	2	9	4	1	0	0	0	0
17															
18	ROI ID	2		Total count	8400		Test||P1|W2|2021-01-01								
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	2	0	1	3	5	0	0	0	0	0	0
22	0	0	0	2	1	27	35	92	99	437	37	0	1	0	0
23	0	0	0	4	10	33	93	126	311	955	239	37	0	0	0
24	0	0	1	13	49	139	200	149	301	454	100	93	6	0	0
25	0	0	0	19	76	68	108	254	334	319	84	96	15	1	0

Frequencies in the first well for the heatmap

Frequencies in the second well for the heatmap

Review mode for reprocessing and charting



[Distance from center heatmap tab]
In the “Distance from center heatmap” tab, the location of the radius where the animal stays from the center of the well can be displayed as a chart.

Review mode for reprocessing and charting

Example of 'Tracking_DistanceFromCenterHeatmap.csv'

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	Test||P1|W1|2021-01-01	2	15	25	160	4775	134	329	514	404	550	355	329	253	131	25
3	Test||P1|W2|2021-01-01	36	116	522	1934	904	949	1281	1334	755	421	64	63	19	2	0
4	Test||P1|W3|2021-01-01	0	15	173	966	1994	1683	141	81	700	955	555	657	183	200	44
5	Test||P1|W4|2021-01-01	128	455	684	1796	877	1204	1083	685	561	397	237	115	68	50	17
6	Test||P1|W5|2021-01-01	328	874	836	816	662	428	466	287	288	418	827	645	712	392	105
7	Test||P1|W6|2021-01-01	15	53	124	221	328	259	529	860	838	774	746	991	1086	1058	366
8	Test||P1|W7|2021-01-01	5	431	382	4279	470	259	286	411	241	128	120	318	218	507	161
9	Test||P1|W8|2021-01-01	24	34	42	85	142	203	332	571	565	717	957	1201	1383	1192	673
10	Test||P1|W9|2021-01-01	152	275	618	787	1002	1071	1063	990	638	499	369	334	288	199	97
11	Test||P1|W10|2021-01-01	34	129	238	3757	807	444	412	886	579	361	315	245	76	50	17
12	Test||P1|W11|2021-01-01	1	12	13	27	101	193	271	639	636	942	928	1105	1073	1050	585

Each well

the frequency of the distance from the center of the ROI (the center of the well) to the animal location

License information

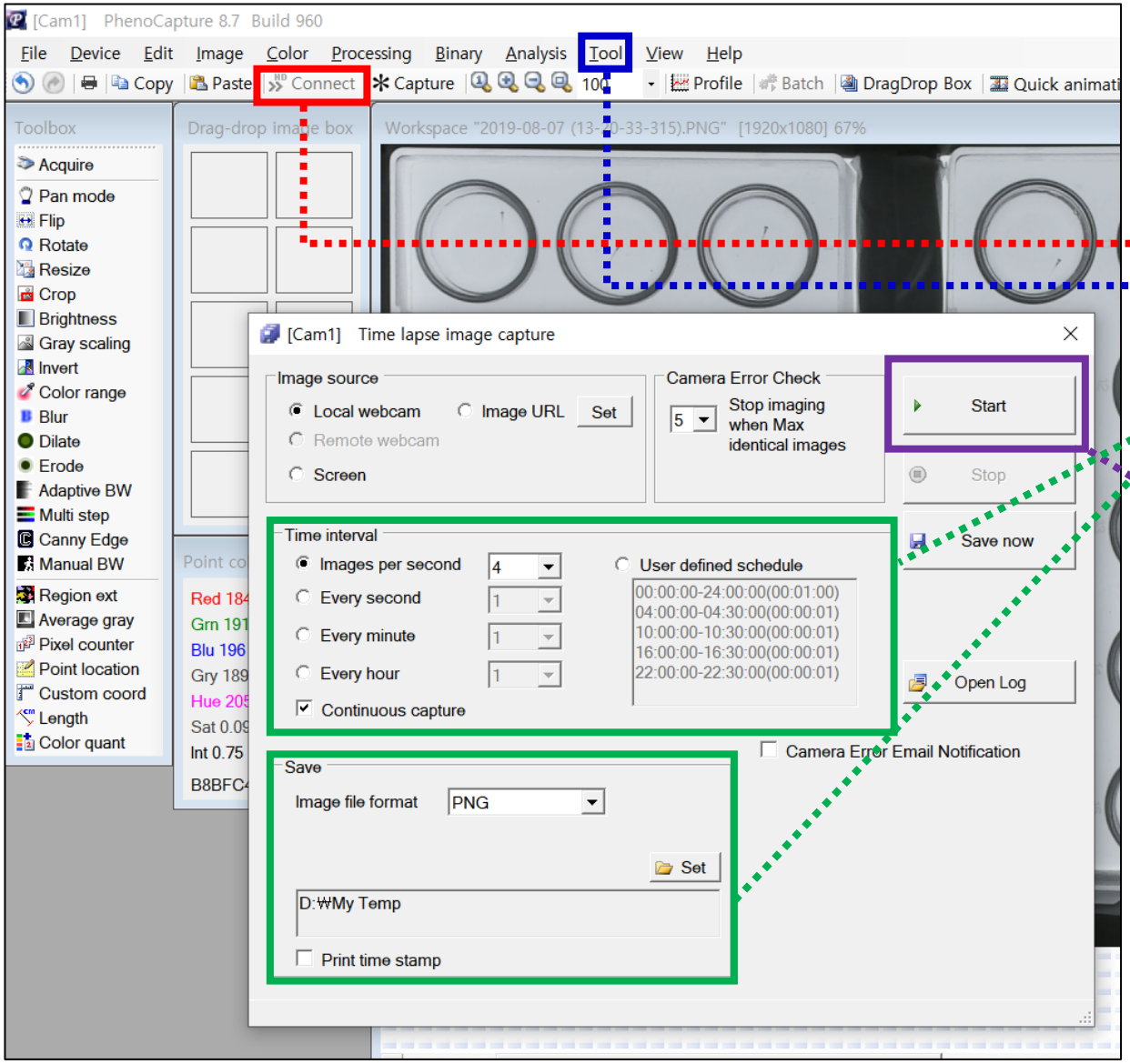
LGPL v3.0

ANIWELLTRACKER IS DISTRIBUTED 'AS IS'. NO WARRANTY OF ANY KIND IS EXPRESSED OR IMPLIED. YOU USE THE PROGRAM AT YOUR OWN RISK.

Alternative license terms are available upon request.

Source codes can be found at *https://github.com/QuantSK/AniWellTracker*. Additional executable files, manual and MATLAB code (frame extraction from a video file) can be found at *https://vgd.hongik.ac.kr/Software/AniWellTracker*.

Appendix I: Capturing time-lapse images

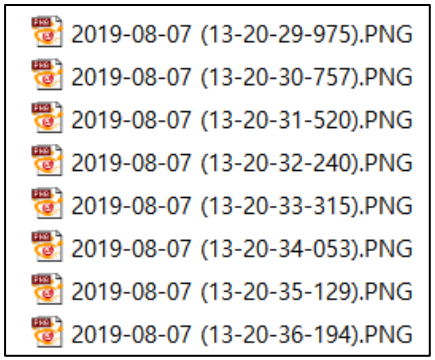


Users may want to use the free PhenoCapture program to capture time-lapse images.

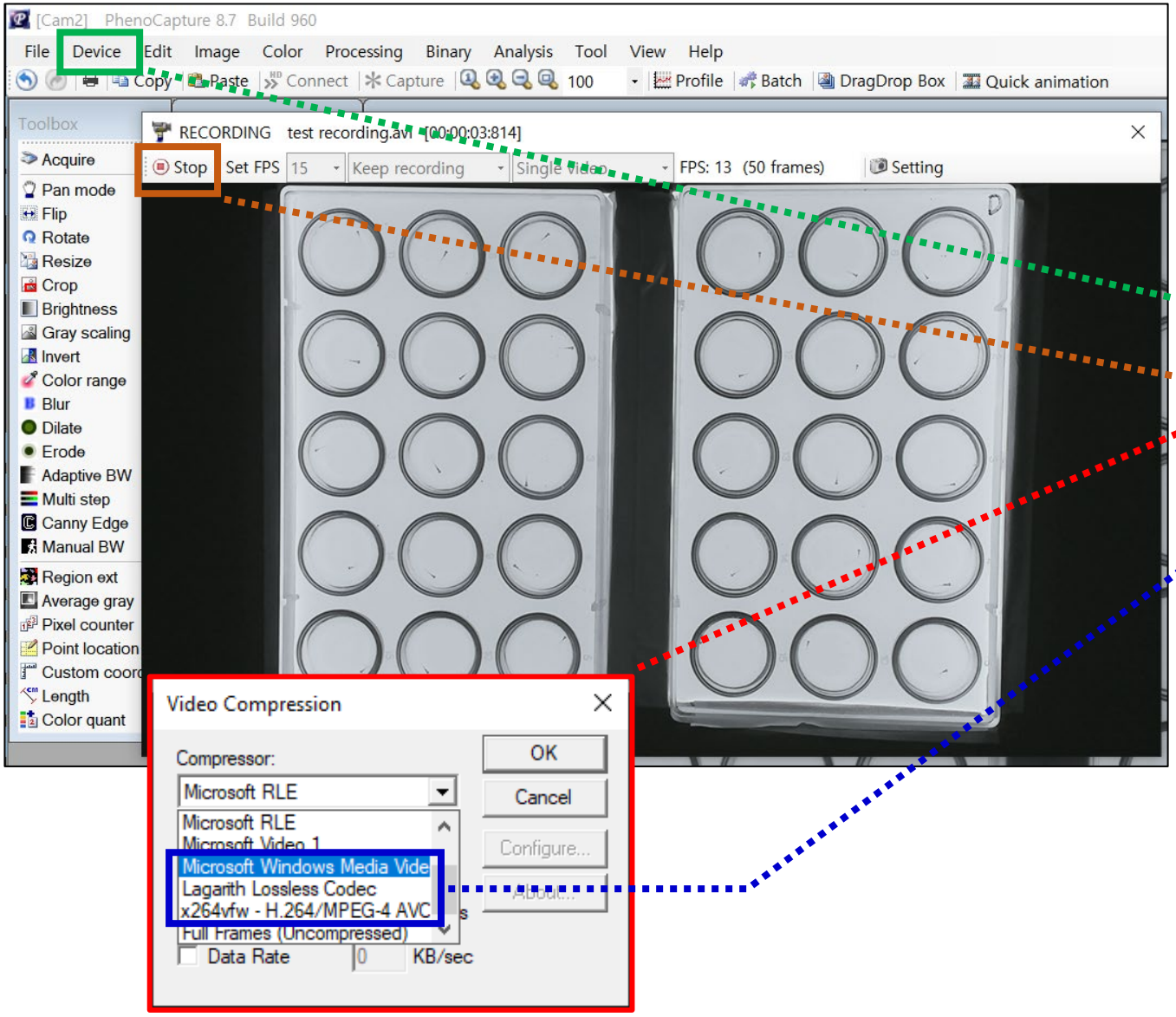
1. Launch PhenoCapture.
2. Connect to the web camera.
3. Select 'Time-lapse image capture' from the 'Tool' menu.
4. Set time interval and target folder.
5. Click 'Start' button to start recording.

For more details, check out the PhenoCapture manual.

[Captured images in the folder]



Appendix II: Recording videos

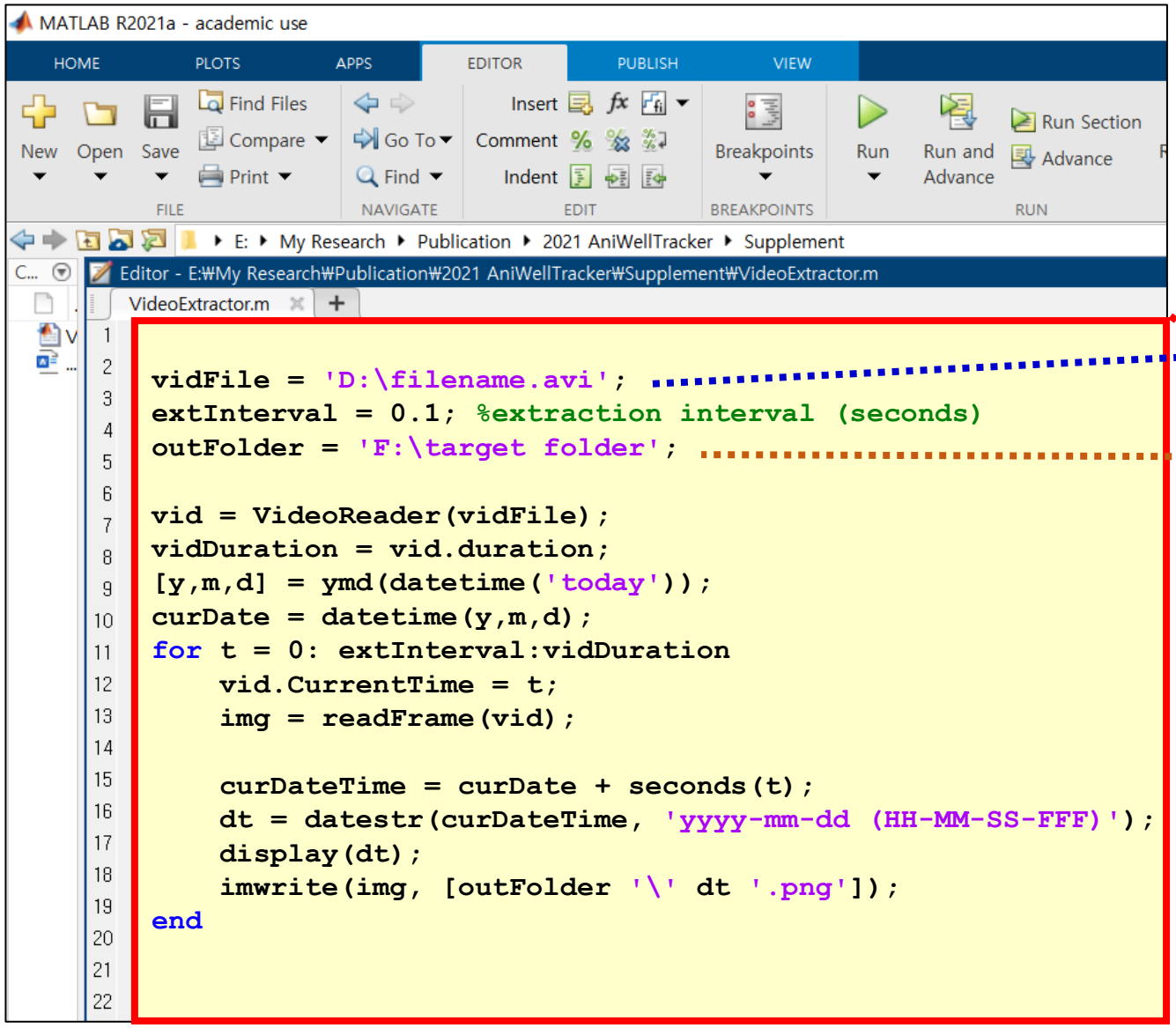


Users may want to use the free PhenoCapture program to record videos. The PhenoCapture has been developed by this author.

1. Launch PhenoCapture.
2. Select 'Record HD video' from the 'Device' menu.
3. Click 'Record' button.
4. Select a video compressor.
5. One of the following video codecs is recommended: Microsoft Windows Media Video (WMV), x264vfw or Lagarith Lossless Codec (users must install these codecs first).

For more details, check out the PhenoCapture manual.

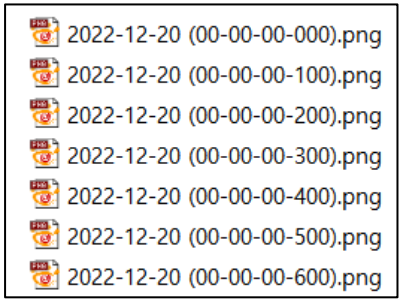
Appendix III: Extracting images from videos



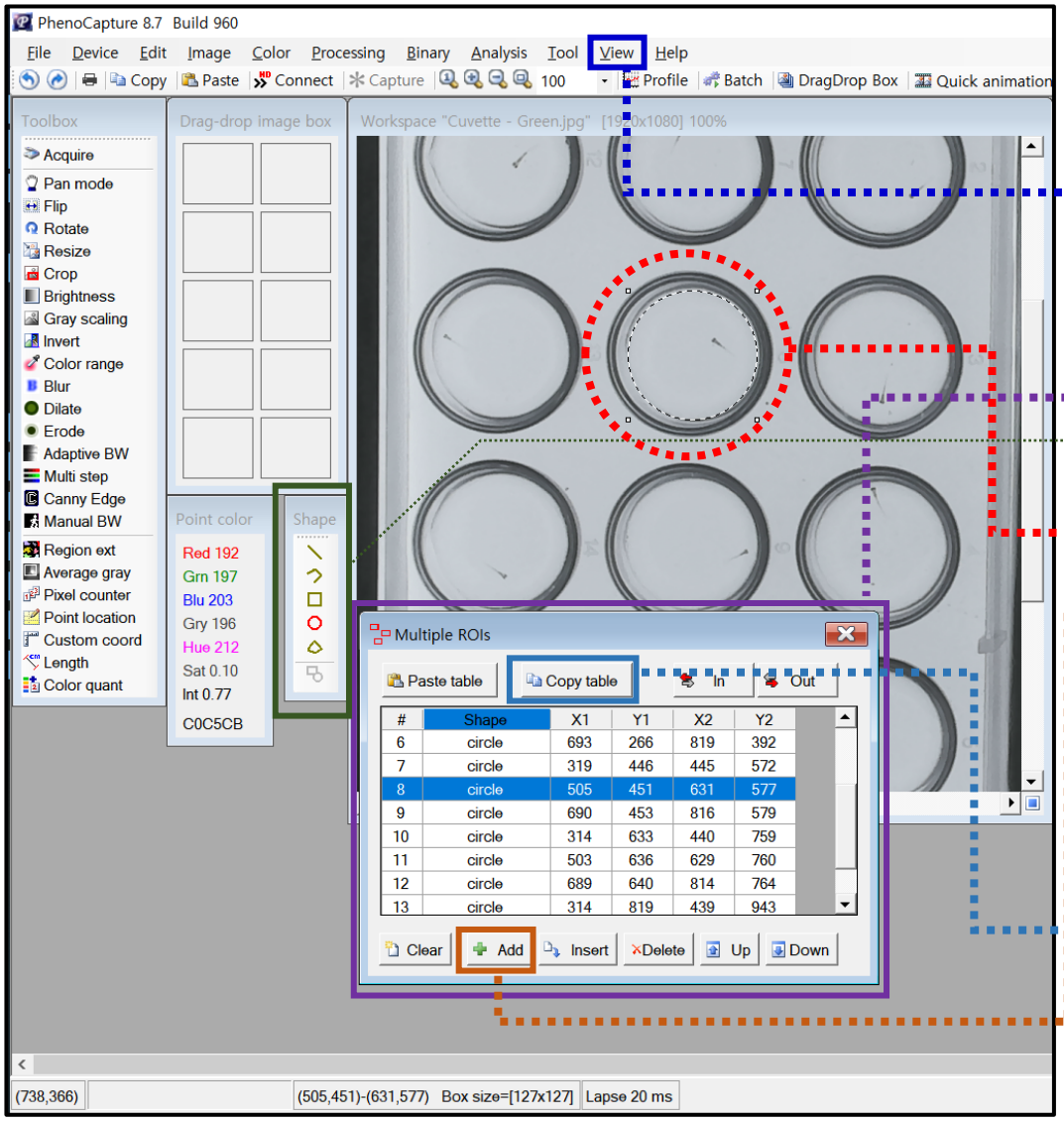
Users can use MATLAB to extract frame images from video files.

1. Start MATLAB.
2. Copy and paste the code shown on the left into MATLAB.
3. Set the video file name, destination folder and extraction interval, and save the code as a file in MATLAB.
4. Run the MATLAB code.
5. Users can view the frame images created in the target folder.

[Generated frame images]



Appendix IV: ROI file generation



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

Appendix IV: ROI 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

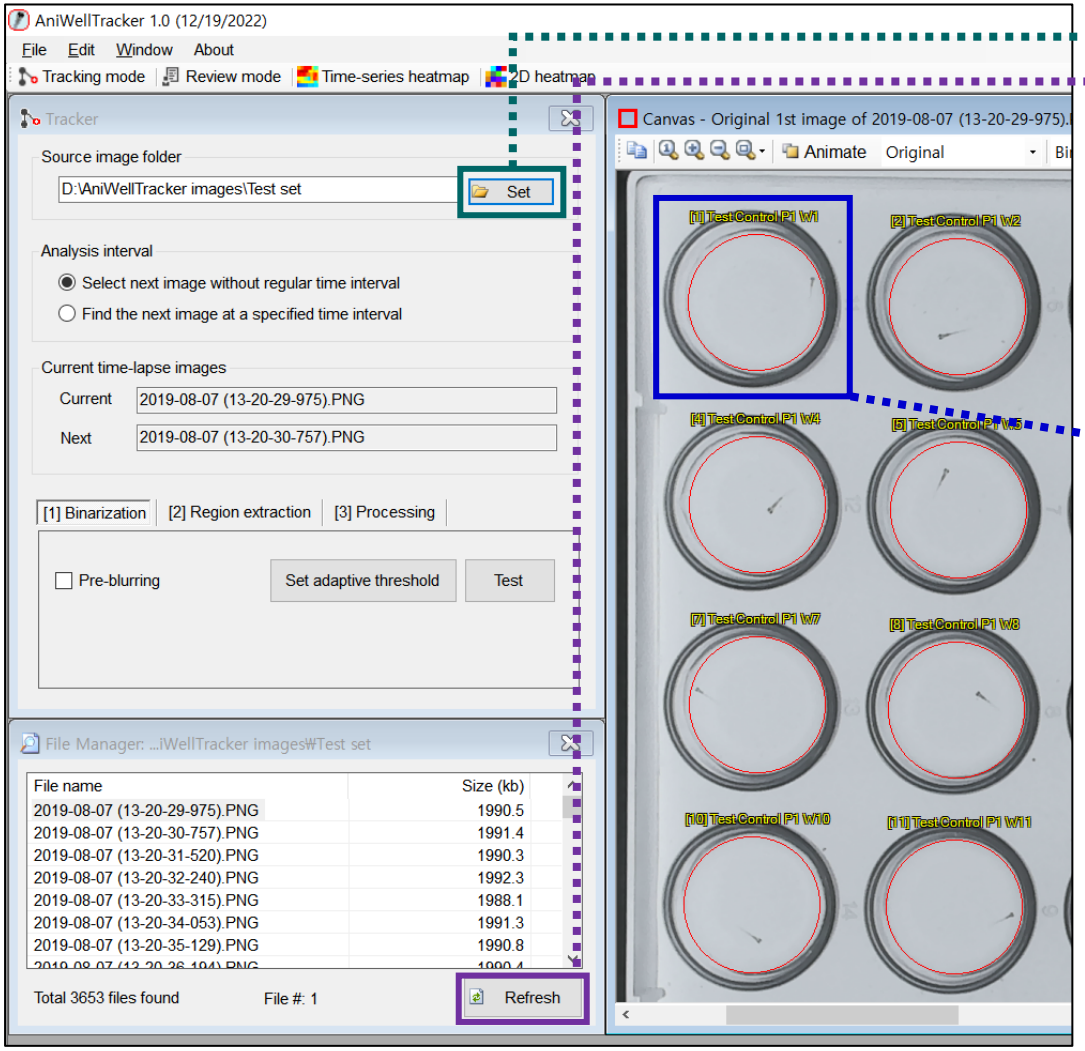
Additional information that users may enter

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!

Appendix IV: ROI file generation



Set the source image folder in AniWellTracker or click the 'Refresh' button in the File Manager window. Users see a red ROI region.

[Well label description]