Supplementary protocol for article :

A Simple Setup to Perform 3D Locomotion Tracking in Zebrafish by Using a Single Camera

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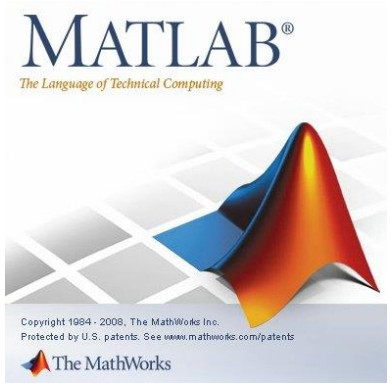
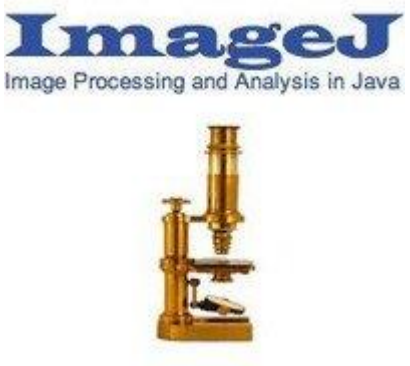
Required Programs summarized

- **Matlab Compiler Runtime**



- **EOS Utility**

- **ImageJ**



- **Origin 9.1**

- **idTracker**

- **Microsoft Excel**

- **GraphPad Prism**

Summary of analysis pipeline

Video recording for 5 min@50fps by a single camera (Canon 600D)

idTracker

MATLAB®
The Language of Technical Computing

Use idTracker to track fish movement

Excel

Output fish xyz position pixel data in excel format

ImageJ
Image Processing and Analysis in Java

Convert xyz pixel data into cm by using ImageJ software

ORIGIN 9.1
Data Analysis and Graphing Software

Establish 3D swimming trajectory by using Oringin9.1 software

GraphPad PRISM VERSIONE 6

Perform statistic analysis by using GraphPad Prism software

Tips for Video Recording

- Frame rate per second (fps)
  - **↑ : more accurate tracking, slower process**
  - ↓ : less accurate tracking, faster process
- Use a good contrast vessel for the fishes
- Using Black and White image setting is recommended because it can enhance the image contrast
- There must be free space in the disk of your computer because high resolution and high frame rate recording will generate a large amount of data
- Internal hard drive’s video usage is strongly recommended

Example video recorded by Canon 600D camera

1280x720; 50fps

***original video is available upon request**

idTracker

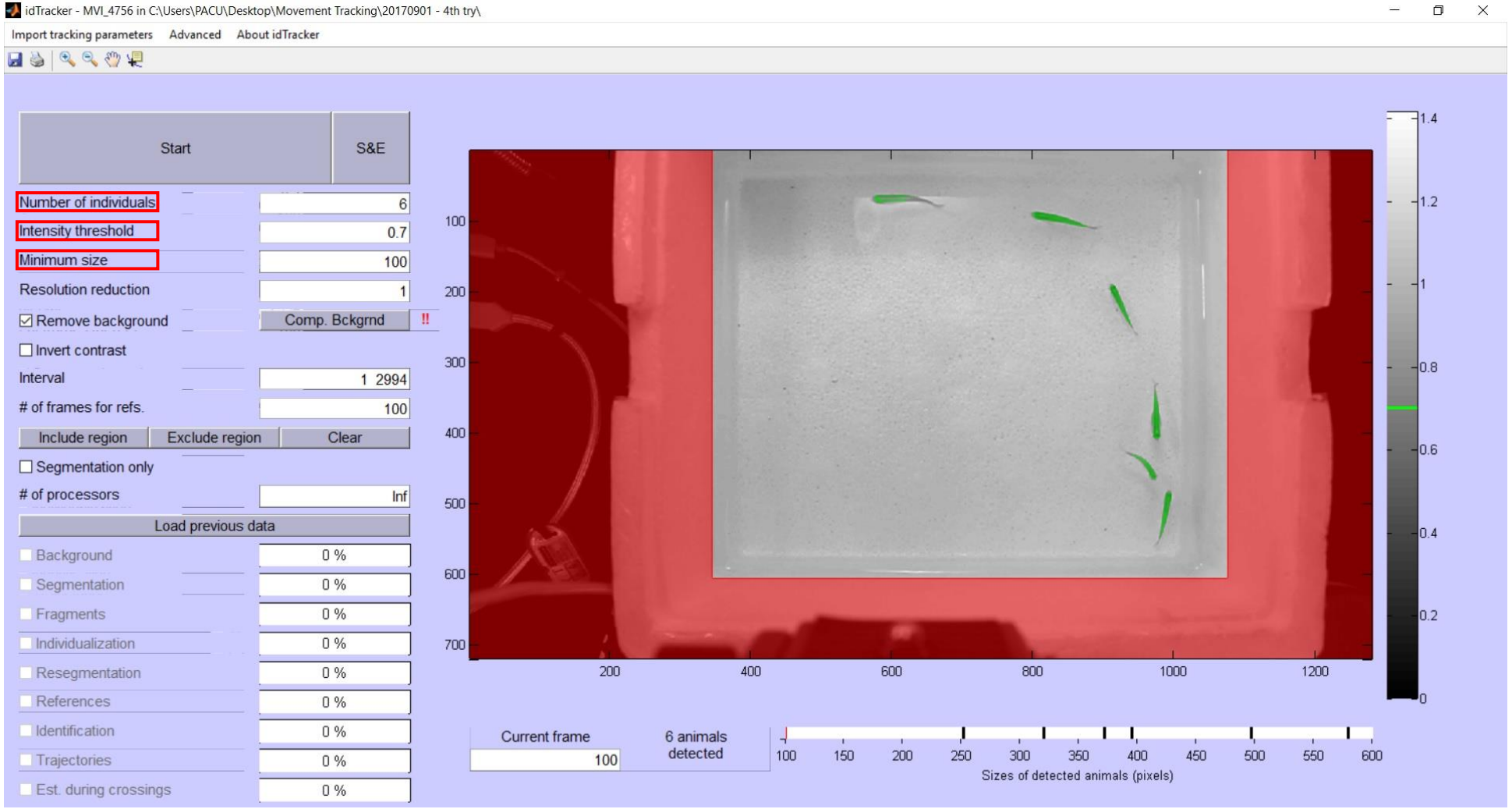
Parameters adjustments for idTracker

Execute idTracker.exe

Select the file with the video

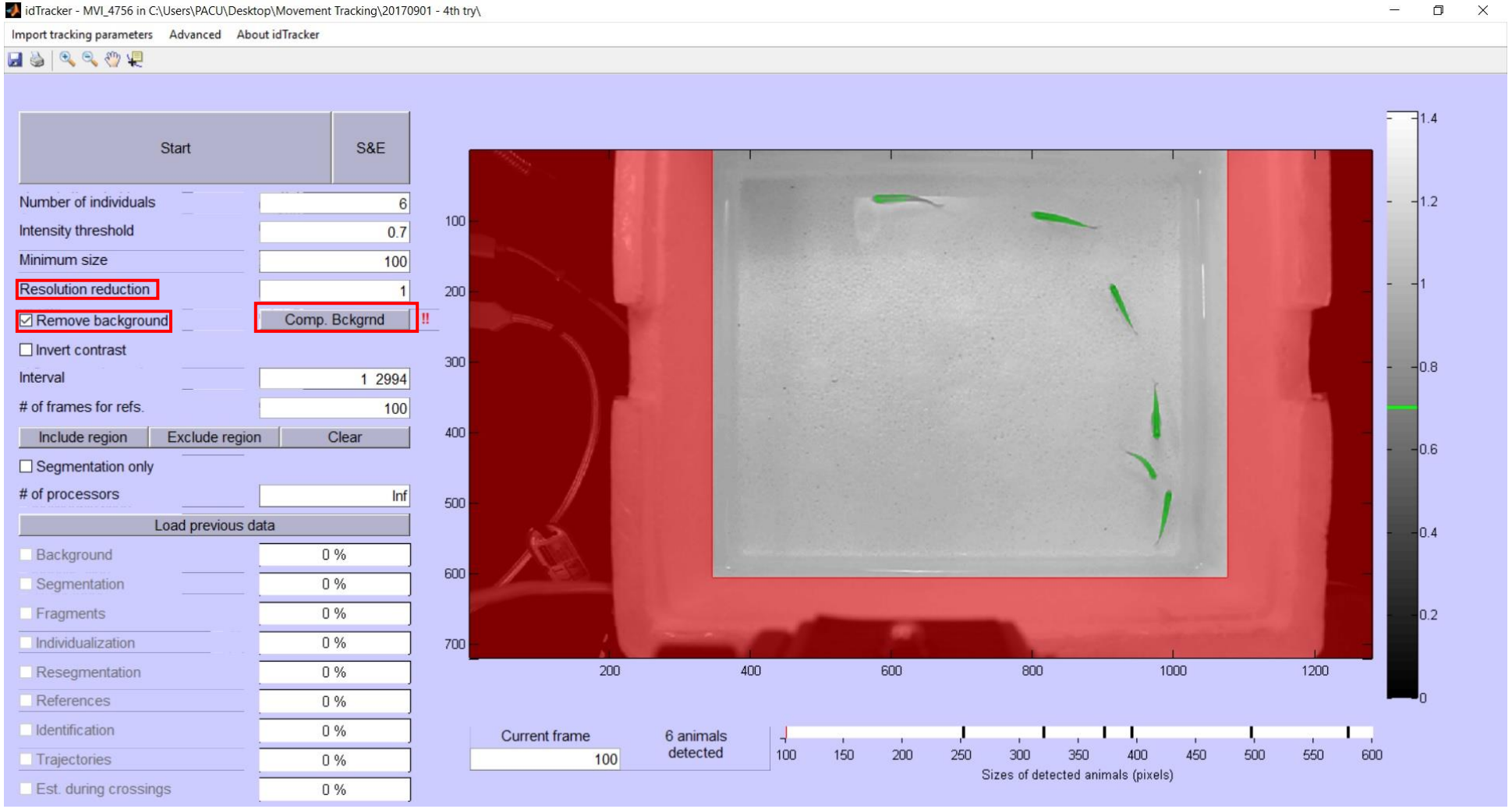
Load the video

idTracker



1. Enter the number of individuals
2. Intensity threshold
   - The system is going to considers that pixels with lower intensity than this threshold belong to the animals (*vice versa* if “invert contrast” is checked)
3. Minimum size
   - The programs will reject blobs smaller than the minimum size entered

idTracker



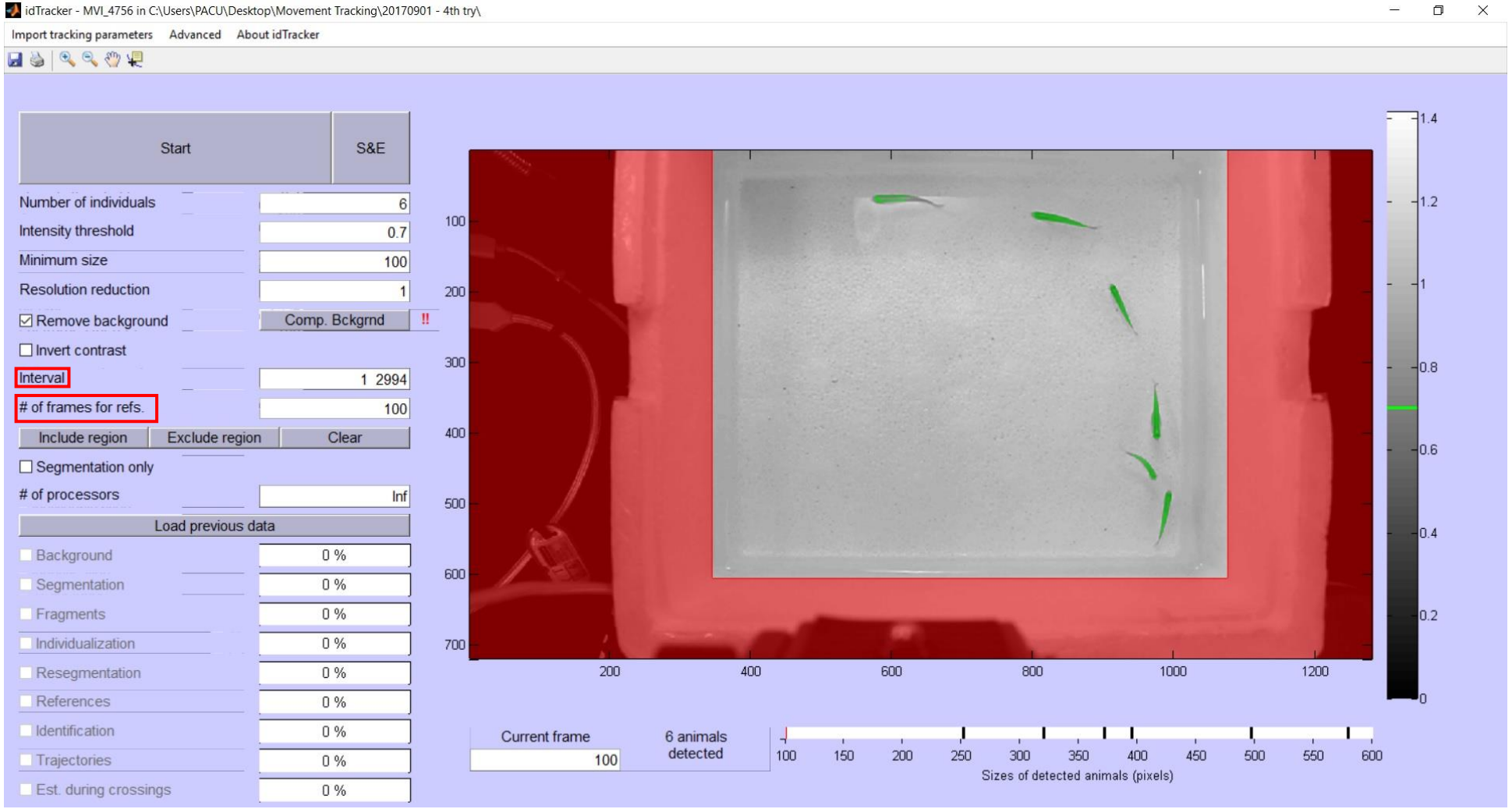
4. Choose resolution reduction

- If the sizes of the animals are bigger than 2000 pixels, input a number higher than 1 (the number of pixels will be divided by n^2, where n is the number in the box)

5. Background removal option

- Check if you want to activate the background removal option
- To compute it before, click on the 'Compute Bckgrnd' button

idTracker



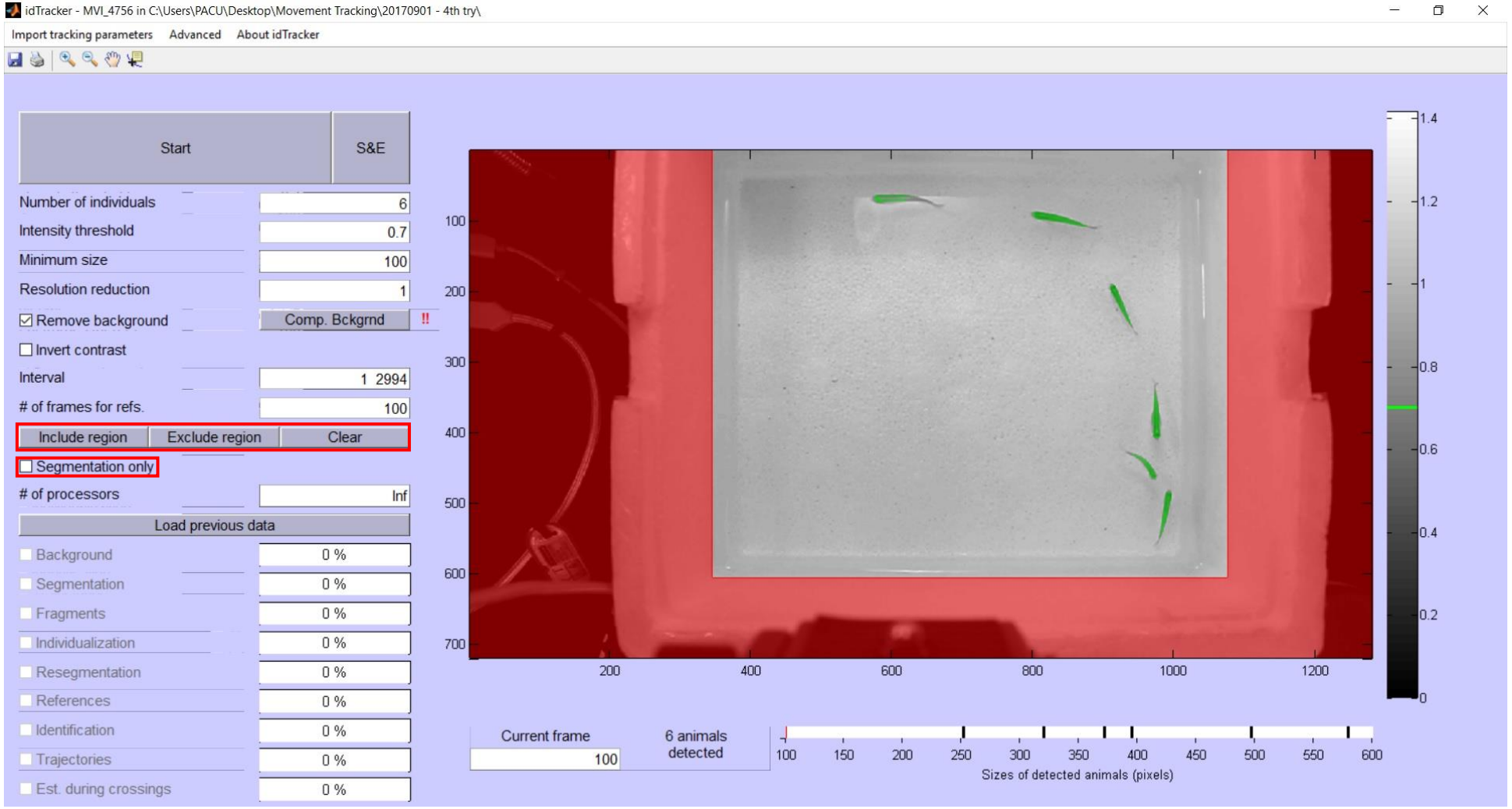
6. Choose an interval

- If you want to track only part of video, enter the interval that you want to track

7. Number of references frames

- Choose a lower number for increased speed, a higher number for increased accuracy

idTracker



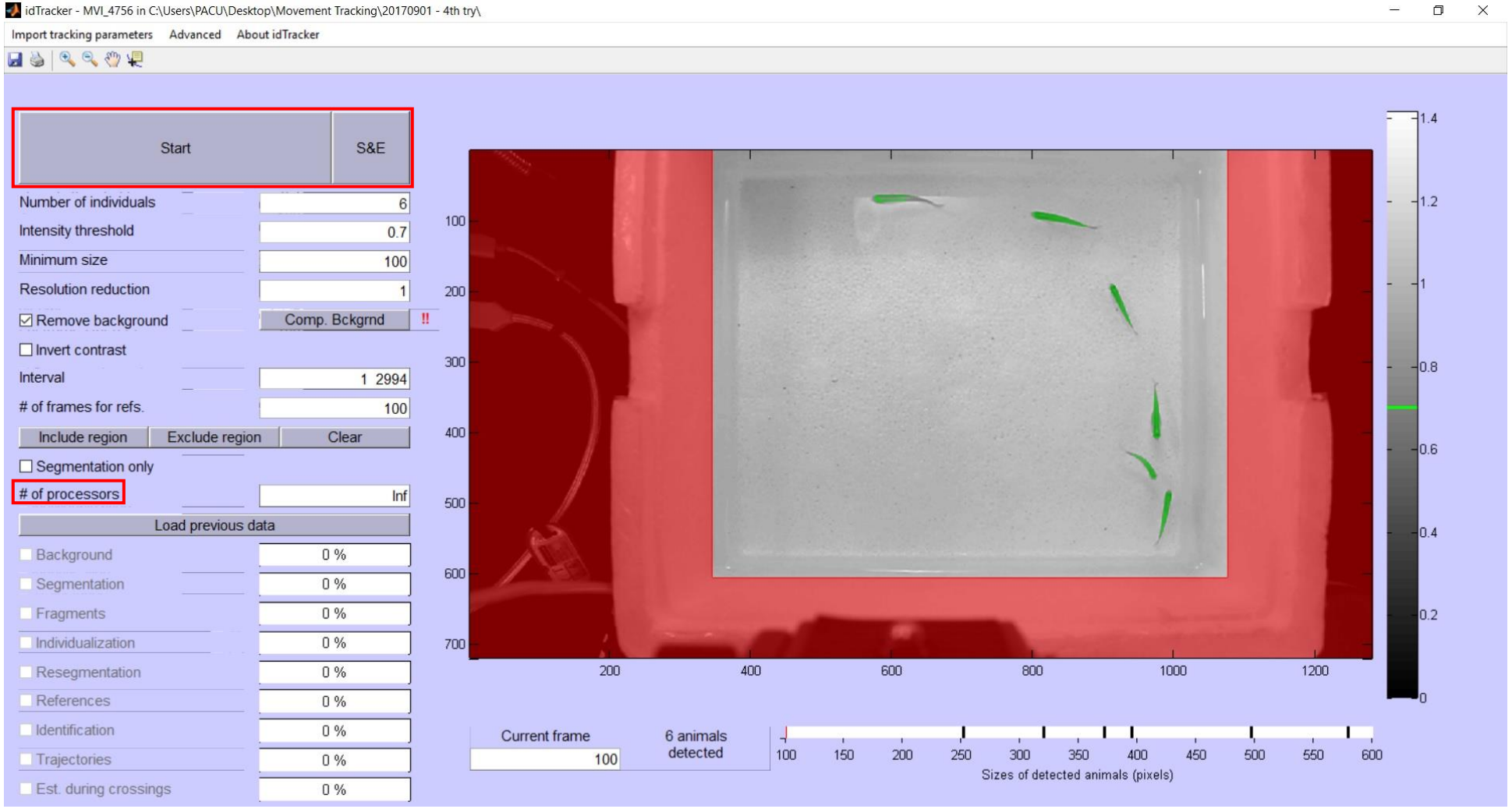
8. Select Region Of Interest (ROI) and/or exclude regions

- Click on button ‘Clear’ to clear all previously defined ROS’s or excluded regions

9. Segmentation only

- If this box is checked, idTracker will exit after the segmentation step, leaving the tracking unfinished.
- The data can be recovered later using button ‘Load Previous Data’

idTracker



10. Number of processors

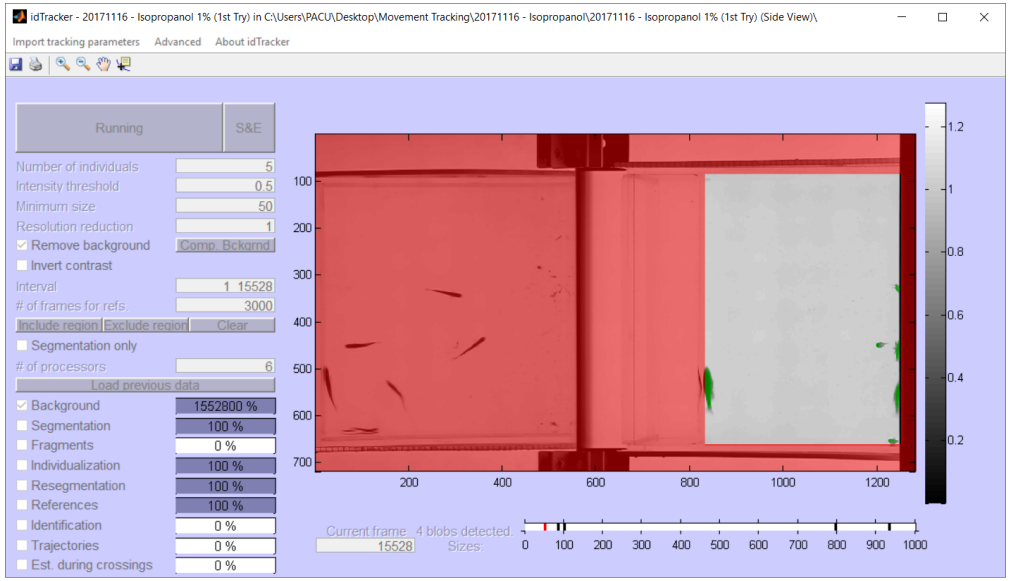
- The number of this box indicates how many processors idTracker will use (‘Inf’ means that idTracker will use all available processors)

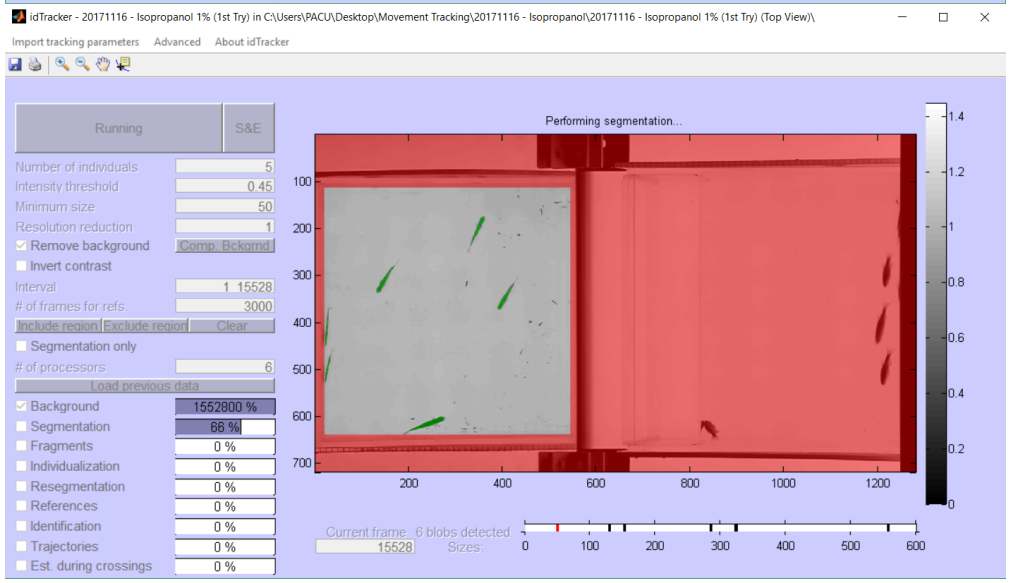
11. Start tracking

- Click the ‘Start’ button
- If ‘S&E’ (Save & Exit) button pressed, the programs ends, but the tracking does not start. All tracking parameters can be used later using ‘Load Previous Data’ button

idTracker

Movement Tracking



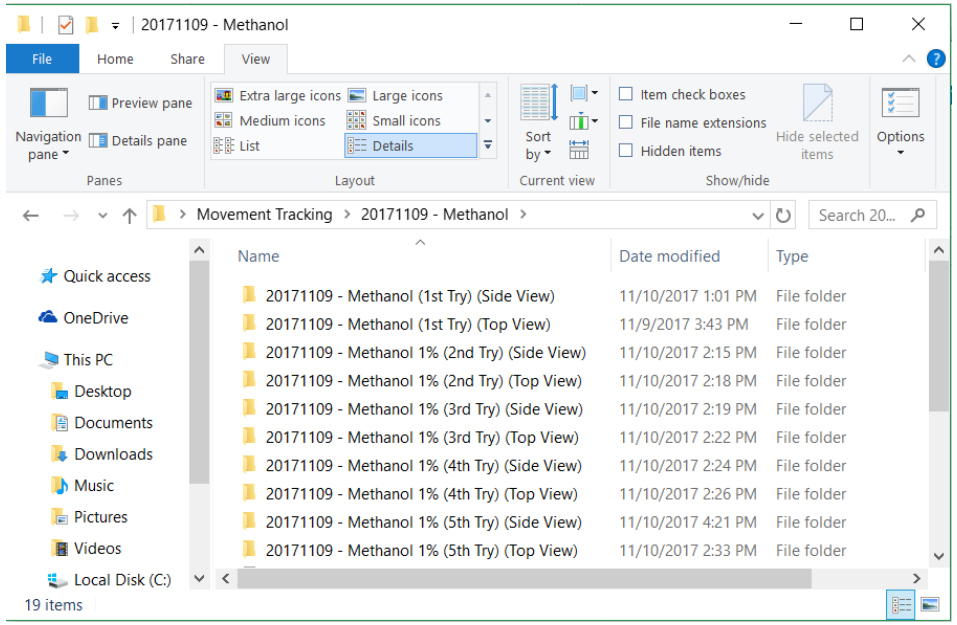


- **Separated** tracking is **better**
- Advantages
  - **Reduce** error occurrence
  - Easy to **match** the fish with their number
- Disadvantages
  - Tracking **duration** will take **more** time

idTracker

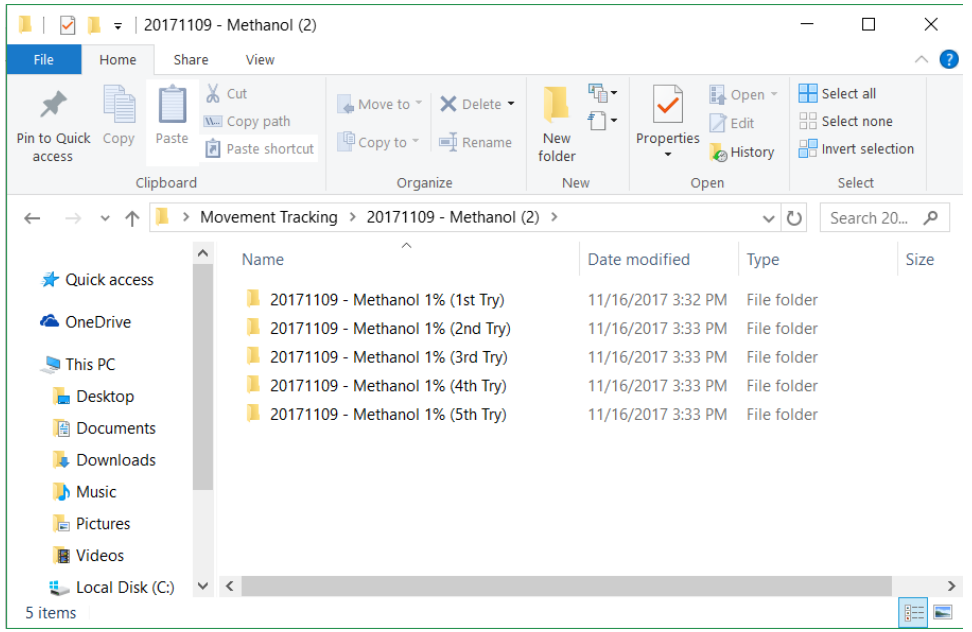
Save side view and top view movement tracking separated

√



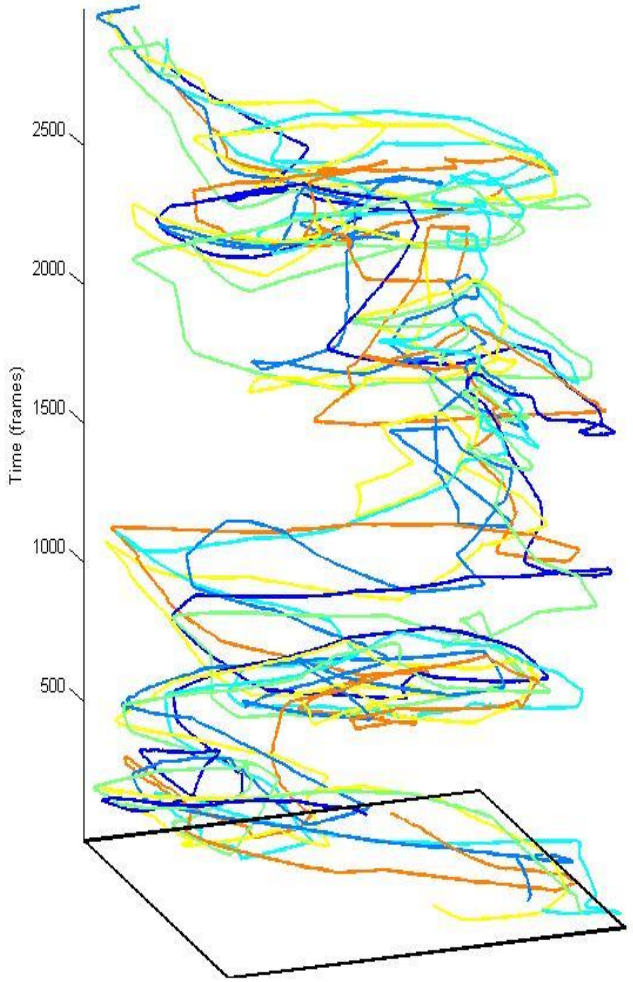
- Advantages
  - **Reduce** error occurrence
  - Easier to **maintain** the trajectories files
- Disadvantages
  - Need **more space** to store the video

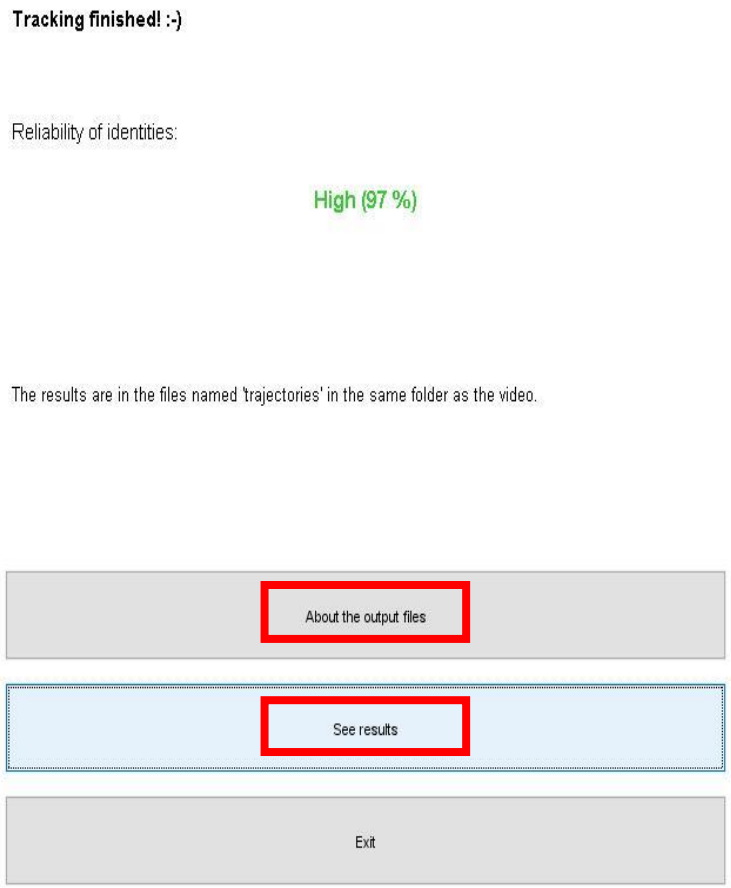
X



- Advantages
  - Just **one** video needs to be saved
- Disadvantages
  - Must **backup** the trajectories and video result file **before** continue to the 2nd tracking
  - Error **often** occurred

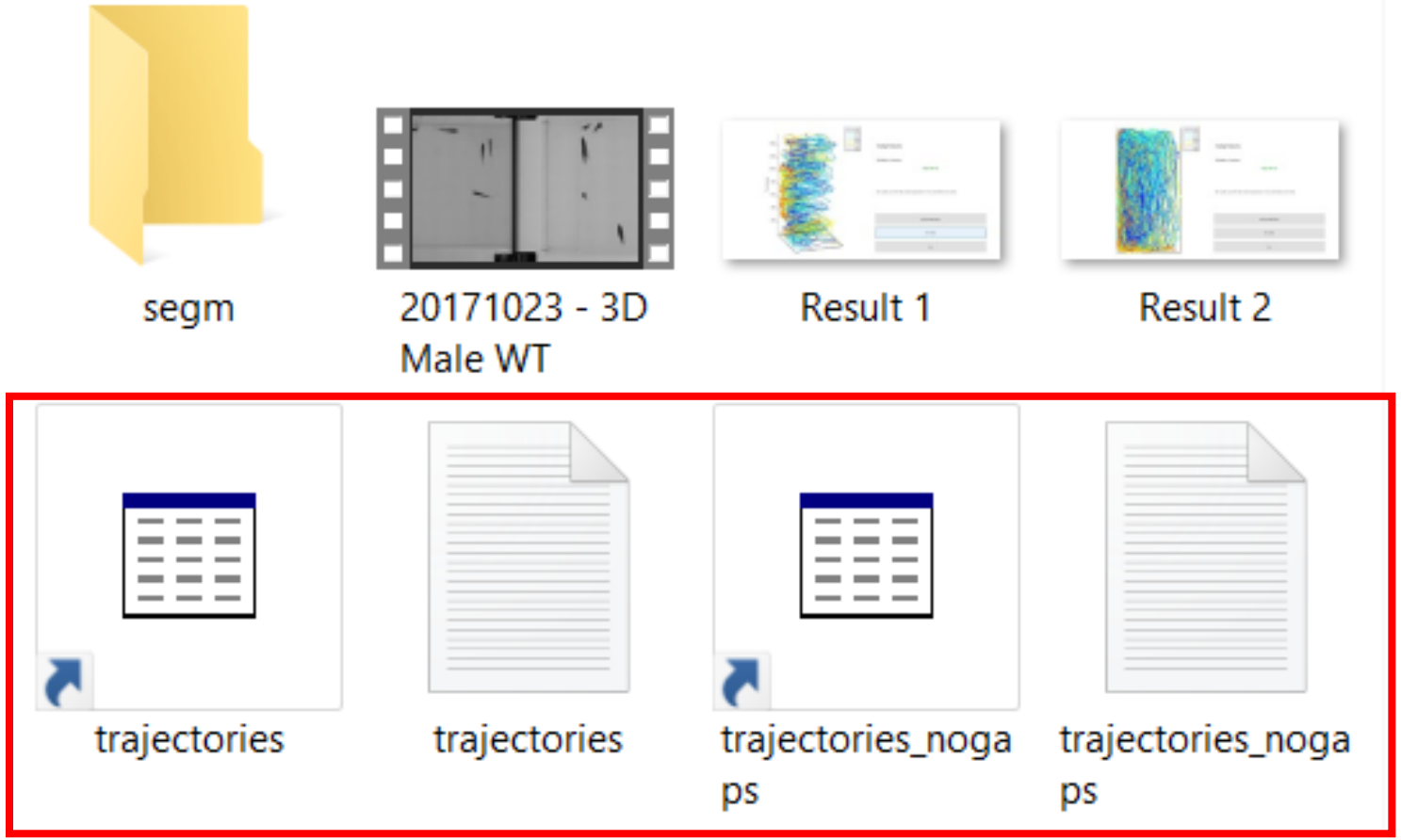
Analysis results of idTracker





- The results are in a folder called '**segm**' located in the same folder as the video
- Press 'About the output files' for more information about the results
- 'See results' button will play the result's video

Analysis result of idTracker



- There will be four output files
  - Two of them are .mat files, to be loaded into Matlab
  - The others two are .txt files to import the data into any data analysis software
- Both of the files contain identical information, which is the following
  - X and Y coordinates of each individual in each frame
  - Probability of correct assignment

Copy XY position information and paste to Excel

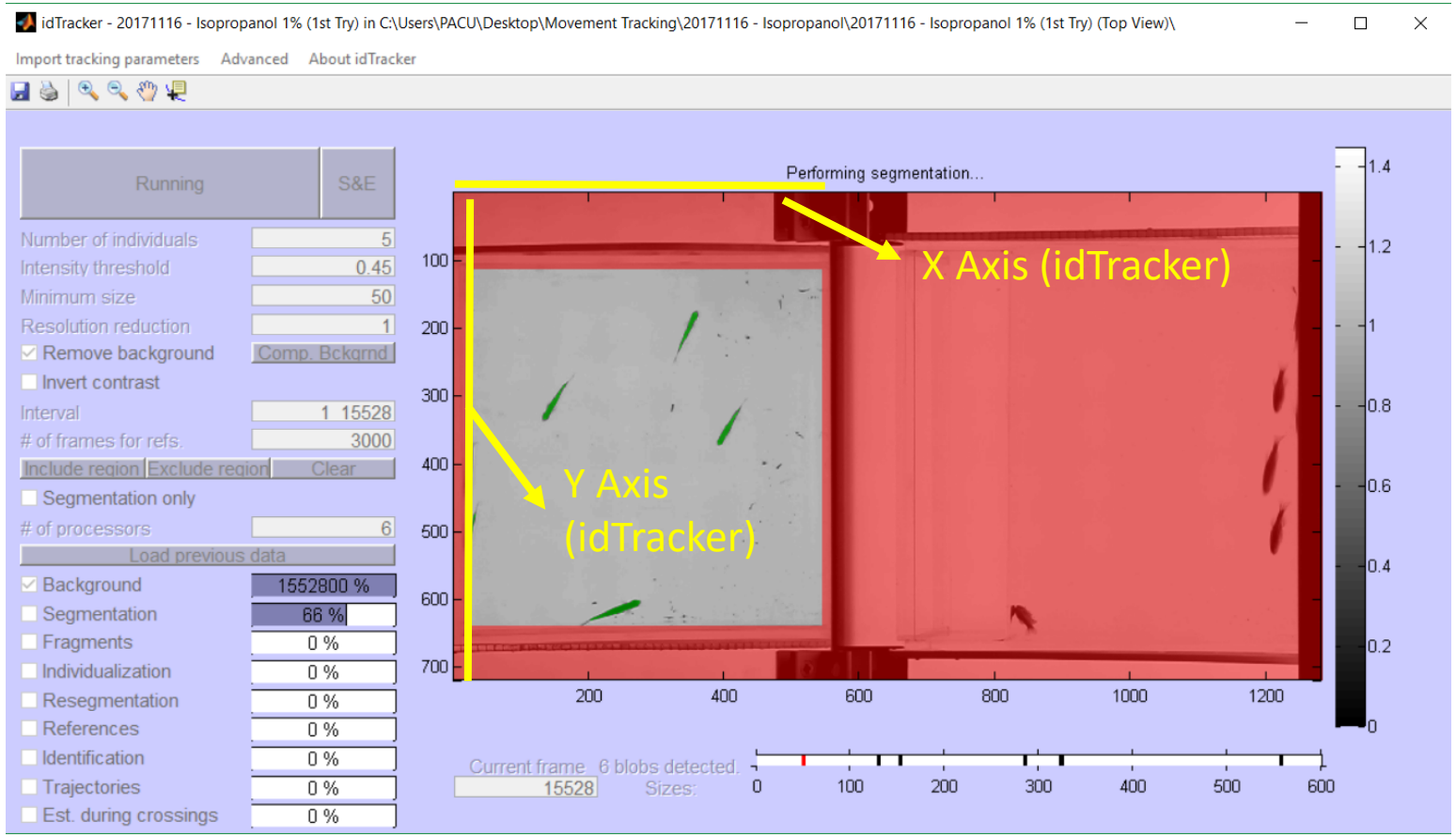
	A	B	C	D	E	F	G	H	I	J	K
1	X1	Y1	ProbId1	X2	Y2	ProbId2	X3	Y3	ProbId3	X4	Y4
2	815.46	77.413	0.92244	912.39	436.59	0.82216	921.27	469.88	0.97348	971.75	500.04
3	815.92	77.648	0.92244	913.06	435.82	0.82216	922.19	473.83	0.97348	972.1	496.19
4	815.46	77.753	0.92244	913.97	434.78	0.82216	924.38	478.29	0.97348	972.6	492.04
5	814.52	77.902	0.92244	914.61	434.15	0.82216	926.51	481.48	0.97348	973.08	488.54
6	814.22	78.115	0.92244	915.41	432.96	0.82216	928.77	484.44	0.97348	973.79	484.39
7	813.9	78.295	0.92244	917.08	430.22	0.82216	930.6	486.73	0.97348	974.51	481.25
8	813.23	78.525	0.92244	921.9	424.18	0.82216	932.43	489.16	0.97348	975.26	478.36
9	813.51	78.757	0.92244	920.3	427.35	0.82216	934.16	491.56	0.97348	976.17	475.26
10	811.58	78.627	0.92244	919.75	428.61	0.82216	935.9	494.05	0.97348	977.12	472.06
11	811.53	78.856	0.92244	920.27	428.56	0.82216	937.51	496.27	0.97348	977.98	469.51
12	811.29	79.16	0.92244	920.87	428.05	0.82216	939.07	498.75	0.97348	978.63	466.99
13	811.3	79.39	0.92244	921.6	427.73	0.82216	940.21	500.37	0.97348	979.42	464.4
14	810.77	79.569	0.92244	923.72	425.48	0.82216	941.11	501.61	0.97348	980.22	461.87
15	809.54	79.505	0.92244	924.99	422.94	0.82216	942.16	503.18	0.97348	980.85	459.7
16	809.16	79.682	0.92244	924.45	423.69	0.82216	943.96	506.17	0.97348	981.38	457.67
17	807.58	79.756	0.92244	924.21	420.8	0.82216	945.6	508.21	0.97348	982.12	455.55
18	806.4	79.931	0.92244	924.84	416.07	0.82216	949.49	510.02	0.97348	983.25	451.69
19	804.85	80.023	0.92244	926.94	409.29	0.82216	953.36	511.29	0.97348	982.87	448.93
20	805.59	80.224	0.92244	928.3	406.7	0.82216	958.26	513.12	0.97348	981.49	445.08
21	805.6	80.473	0.92244	930.27	401.99	0.82216	962.85	514.91	0.97348	979.51	438.45
22	804.76	80.668	0.92244	932.17	397.04	0.82216	966.78	516.47	0.97348	978.31	431.31
23	803.7	80.823	0.92244	933.49	393.24	0.82216	970.25	517.97	0.97348	977.72	424.29
24	802.38	80.939	0.92244	934.51	389.82	0.82216	972.77	519.12	0.97348	977.31	417.3
25	802.44	81.142	0.92244	935.63	385.52	0.82216	974.81	520.13	0.97348	977.13	411.91
26	803.08	81.373	0.92244	936.67	381.78	0.82216	976.99	521.29	0.97348	976.79	406.14
27	803.46	81.726	0.92244	937.5	378.46	0.82216	979.06	522.53	0.97348	976.28	400.99
28	801.26	81.752	0.92244	938.17	374.61	0.82216	980.81	523.39	0.97348	975.65	395.89

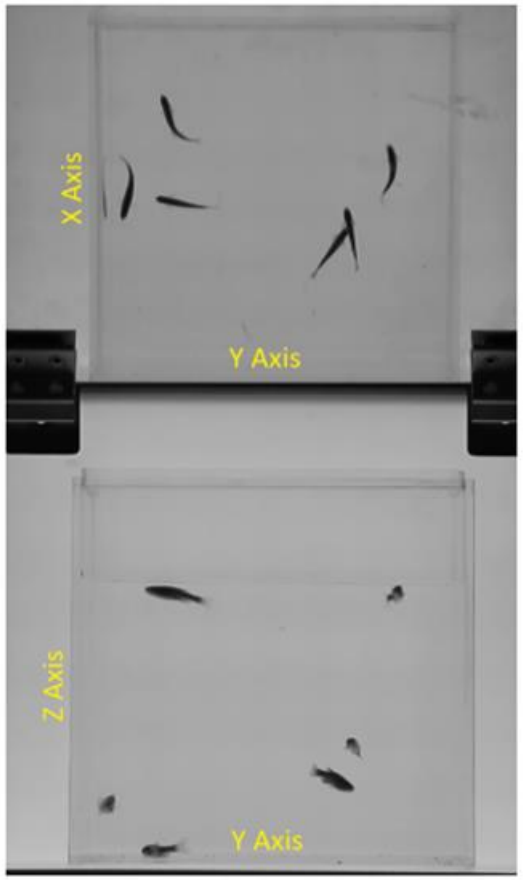
- Each row corresponds to one frame of the video
  - Columns 1 and 2 are the x and y coordinates of individual 1, respectively, and so forth
- There is an estimation of the probability of correct assignment for each frame and it is usually conservative
  - The probabilities for individual 1 are in column 3, for individual 2 in column 6, and so on

idTracker

3D tracking by running idTracker twice

- XYZ Axis

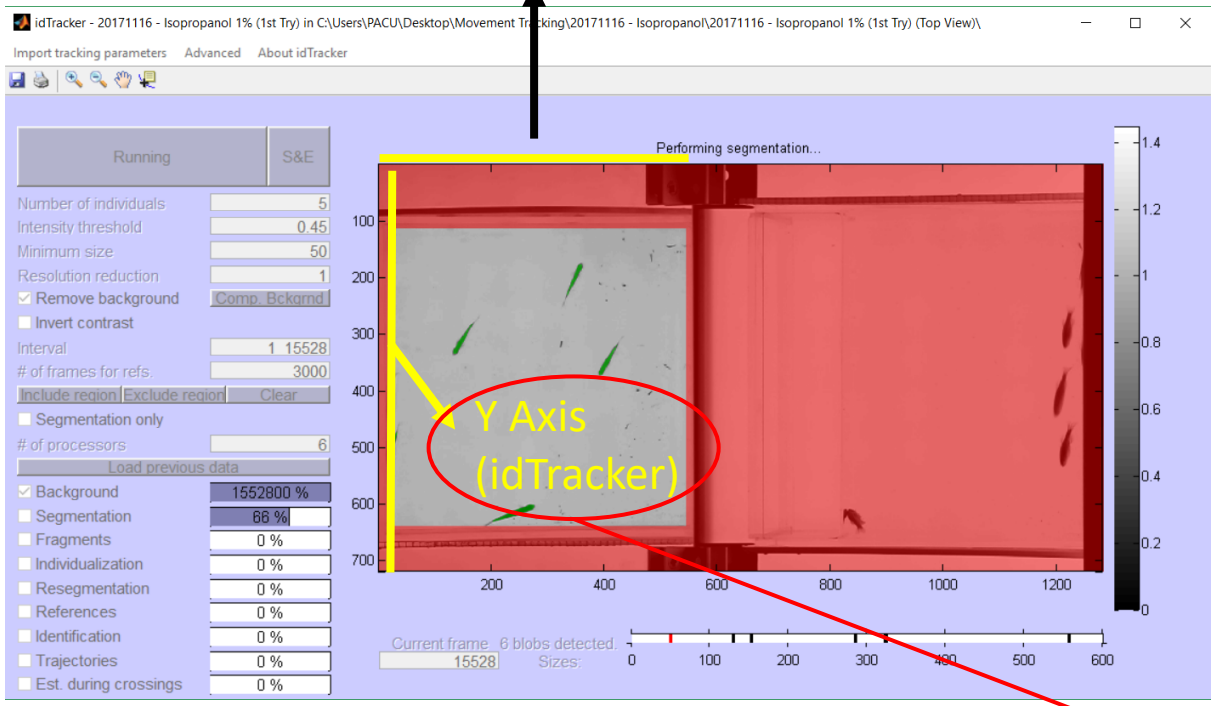




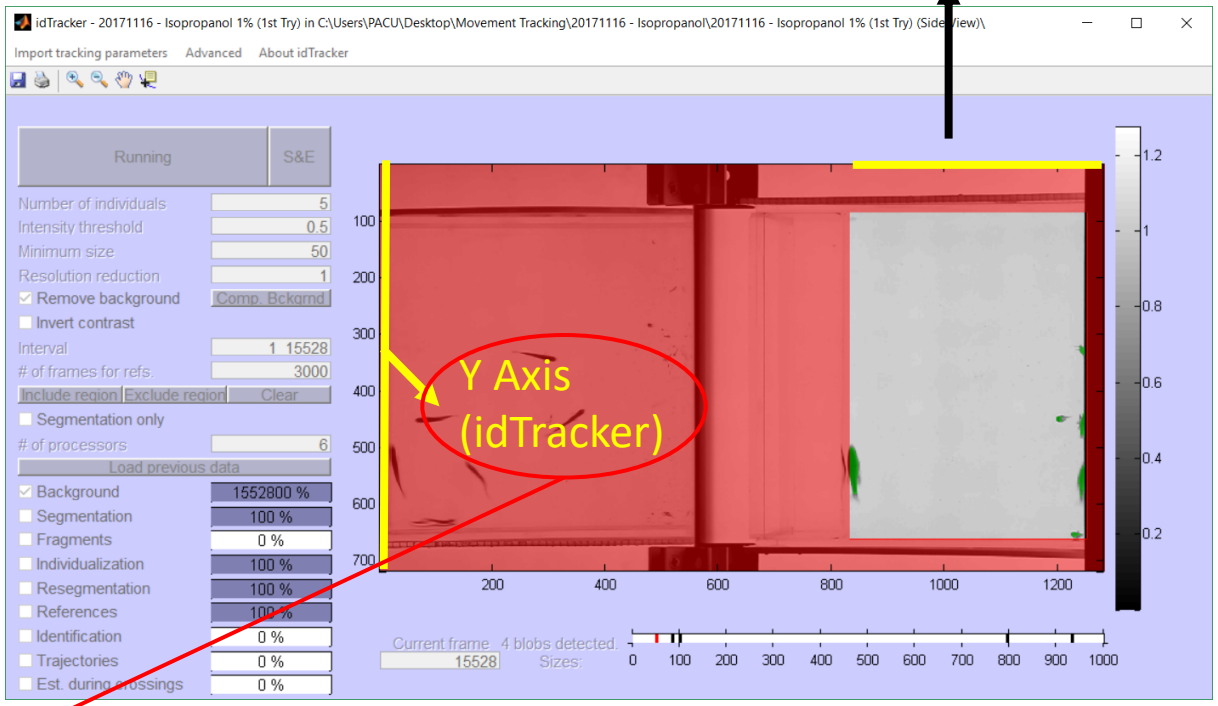
idTracker

3D tracking by running idTracker twice

X Axis in idTracker = X axis in real image



X Axis in idTracker = Z axis in real image



Both two images share the <u>similar</u> Y axis position <u>pattern</u>

Merge two XY and YZ dataset by searching object in specific frame with the same Y position

data matrix before merging

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1	Fish 2		Fish 4		Fish 3		Fish 5		Fish 1		Fish 6			Fish 1		Fish 2		Fish 3		Fish 4		Fish 5		Fish 6	
2	X1 (SV)	Y1 (SV)	X2 (SV)	Y2 (SV)	X3 (SV)	Y3 (SV)	X4 (SV)	Y4 (SV)	X5 (SV)	Y5 (SV)	X6 (SV)	Y6 (SV)		X1 (TV)	Y1 (TV)	X2 (TV)	Y2 (TV)	X3 (TV)	Y3 (TV)	X4 (TV)	Y4 (TV)	X5 (TV)	Y5 (TV)	X6 (TV)	Y6 (TV)
3	887.02	474.96	1156.3	116.32	994.96	241.92	900.06	417.6	1003.9	337.76	1040.1	108.94		166.46	338.41	268.05	457.12	106.25	254.12	400.17	124.6	460.84	420.91	279.88	132.92
4	887.31	473.95	1156	117.49	995.35	242.58	900.04	420.52	1004.3	340.23	1041.8	109.99		168.7	340.56	266.85	456.3	106.35	254.69	398.24	124.82	461.45	423.28	279.68	131.6
5	887.75	472.69	1156.4	117.64	996.07	243.31	900.35	423.05	1005.1	342.62	1043.5	111.26		171.79	342.82	265.41	454.97	106.84	255.56	396.69	124.84	462.6	425.69	279.78	130.83
6	888.69	472.51	1157	117.15	997.18	245.32	901.04	427.07	1006.2	346.22	1045.6	113.21		174.8	345.8	264.59	454.82	107.53	258.2	394.96	125.11	464.04	429.08	279.99	131.26
7	889.39	472.01	1157.6	115.32	998.04	247.71	901.65	430.19	1007.1	349.32	1048	114.37		177.62	348.55	263.28	454.11	108.32	258.94	393.8	125.53	465.31	432.15	280.87	130.73
8	889.85	470.21	1157.9	113.3	998.69	248.09	901.9	432.24	1007.5	350.83	1050.3	114.06		180.31	350.49	261.72	452.53	108.51	259.5	392.57	125.21	466.4	434.62	281.73	129.43
9	890.16	469.52	1158.5	110.83	999.27	249.1	902.13	433.96	1008.1	353.25	1052.4	113.94		182.41	352.4	260.39	451.75	108.7	261.1	391.84	124.7	467.02	436.85	282.44	128.92
10	890.46	469.16	1158.4	110.3	999.61	250.71	902.34	433.81	1008.4	355.75	1054.3	113.32		184.47	354.48	259.29	451.35	108.66	261.87	390.09	123.3	467.66	439.59	283.39	128.78
11	891	468.78	1158.2	110.33	1000.3	251.78	902.71	435.51	1009.1	358.32	1056.6	111.33		186.93	356.96	257.91	450.64	108.89	263.41	388.96	122.96	468.64	442.72	284.79	127.79
12	891.51	467.66	1157.6	110.25	1001.1	253.06	903.45	436.54	1009.7	360.34	1058.7	110.1		189.33	359.06	256.65	449.67	109.34	264.42	387.8	122.41	469.57	445.34	286.95	126.2
13	892.34	466.77	1157.8	109.43	1002.1	253.81	904.07	437.46	1010.4	362.37	1059.4	110.36		192	361.05	255.6	448.55	109.69	265.17	385.86	121.51	470.65	447.65	290.29	124.7
14	892.78	466.16	1158.4	108.63	1002.7	255.21	904.5	438.02	1010.9	364.22	1060.5	109.96		193.77	362.84	254.37	447.92	109.75	266.4	383.12	121.73	471.31	450.32	285.94	125.72
15	893.53	466.16	1158.6	108.04	1003.2	256.68	904.82	438.9	1011.3	366.73	1061.8	109.92		195.84	365.19	252.83	447.53	109.8	267.47	380.4	122.59	471.87	453.17	296.72	124.26
16	893.68	465.21	1158.4	107.1	1003.7	257.27	905.13	440.47	1011.5	368.34	1062.9	108.57		197.3	366.6	251.95	446.93	109.71	268.19	378.34	123.26	472.34	455.31	299.76	124.03
17	894.32	464.93	1157.8	105.62	1004.3	258.59	905.47	440.66	1011.9	370.33	1064.3	108.15		199.15	368.08	250.07	445.68	109.75	268.55	376.34	123.56	472.8	456.96	303.91	123.28
18	895.29	465.07	1157.9	104.57	1005	259.5	905.96	441.37	1012.5	372.43	1065.7	107.55		201.13	369.71	249.06	445.18	109.93	270.03	373.68	123.81	473.55	459.42	309.1	122.49
19	895.92	465.41	1157.3	104.63	1005.6	261.3	906.46	441.92	1012.8	375.14	1066.8	107.86		203.21	372.39	247.91	445.14	110.04	271.14	370.52	124.46	474.06	461.87	315.94	122.13
20	896.59	464.68	1156.9	103.84	1006	263.07	907.01	442.78	1013.4	376.61	1067.4	107.91		204.84	373.52	246.82	444.21	110.49	273.18	370.33	124.06	474.9	463.79	314.55	121.77
21	899.09	465.89	1156.5	103.34	1006.6	264.9	906.78	445.59	1013.8	377.73	1068.1	107.75		206.29	374.83	245.84	443.55	111.15	274.63	370.13	123.66	475.52	465.59	313.16	121.4
22	898.91	464.14	1155.6	102.91	1006.8	266.81	907.01	448.94	1014.2	380.01	1068.4	107.48		208.05	376.65	244.89	443.25	111.74	275.79	370	124	476.12	467.52	313	121
23	899.83	466.04	1154.5	102.34	1007.5	268.88	909.5	455.5	1014.7	382.14	1069	107.42		210	378.27	244.34	442.59	112.66	277.79	369	124	476.86	469.59	313	120
24	899.54	463.95	1152.6	101.45	1007.8	271.7	911	465	1015.2	384.28	1069.3	108.03		212.06	380.44	242.69	440.3	113.6	280.77	369	124	477.62	471.22	313	121
25	899.26	461.87	1151	100.26	1008.5	273.93	910.88	467.41	1015.4	387.03	1069.1	108.43		213.77	383.16	242.54	438	114.52	283.33	368.71	123	478.44	472.78	316	123
26	898.97	459.79	1148.2	99.831	1008.8	276.84	910	470	1015.2	390.03	1069.1	106.39		214.38	386.04	242.72	435.69	114.99	286.14	368	123	479.06	474.66	317	124
27	898.69	457.71	1146.4	100.16	1009.1	280.17	910	472.38	1014.7	393.59	1069.5	103.43		214.82	389.85	243.18	434.4	115.22	288.62	368	125	479.56	476.5	319	124
28	898.41	455.63	1144.9	100.54	1009.3	282.66	910	474.75	1014	397.28	1068	103.35		215.08	393.04	243.55	433.26	115.4	291.62	365	115	480.08	478.35	320	124
29	898.12	453.55	1142.4	99.617	1009.5	285.52	910.01	477.13	1013.5	400.73	1067	104.15		215.24	395.78	244.46	430.33	115.61	293.74	367	115	480.67	480.01	318	112
30	897.84	451.47	1139.3	98.83	1009.9	287.8	910.01	479.51	1012.7	404.72	1066.2	104.34		215.14	400.32	244.78	428.93	115.75	295.2	367	116	481.32	481.49	311	113
31	897.56	449.39	1136.6	98.217	1010.4	290.48	910.01	481.88	1012	409.14	1064.9	102.22		215.16	404	245.58	426.99	116.03	296.68	367	117	481.87	483.04	306	114
32	897.27	447.31	1134	98.364	1010.7	293.01	910.01	484.26	1011.5	412.28	1063.9	101.39		215.24	407.38	245.97	426.4	116.45	299.65	367	118	482.6	485.01	298	114
33	896.99	445.23	1131.8	98.353	1010.9	295.9	909.89	486.67	1010.6	416.01	1063.5	101.05		214.96	410.77	247.23	425.01	116.91	302.41	367	119	482.85	486.49	296	115
34	896.7	443.15	1129.2	97.914	1010.9	298.63	909.77	489.07	1009.8	418.77	1062.5	100.45		214.73	413.09	249.33	423.76	117.5	304.66	367.62	118.48	483.26	487.95	295	116
35	896.52	441.4	1127.7	98.235	1011.6	301.49	910	492.42	1009.2	422.18	1062.6	100.56		214.61	416.1	251.31	422.71	118.7	306.9	367.42	118.08	483.71	489.09	293.72	116.3
36	896.23	438.18	1127	98.323	1011.9	304.52	910.36	492.67	1008.6	424.73	1062.7	100.33		214.4	418.34	253.95	420.34	119.77	309.67	367.23	117.68	484.29	490.85	292.33	115.93
37	896.17	434.81	1122.2	98.915	1012.5	306.95	910.52	492.95	1008.2	427.67	1062.9	101.22		214.31	421.02	257.69	416.89	120.85	312.33	375.39	118.53	485.03	492.23	289.28	116.69
38	896.11	430.86	1116.1	101.58	1012.9	309.58	910.72	495.76	1007.6	430.47	1062.7	102.96		214.05	423.59	260.32	413.71	121.78	315.52	383.66	120.43	485.38	493.23	289.92	119.74
39	896.11	427.3	1113.2	104.3	1013.2	313.65	911	495.92	1007	433.05	1062.9	106.11		213.88	426.04	263.77	407.5	122.47	319.23	388.78	122.73	485.78	494.88	290.31	123.09
40	896	424.17	1109.8	107.27	1013.4	316.81	911.1	497.26	1006.2	436.17	1063	110.16		213.51	428.97	263.67	406.05	122.93	322.28	392.12	124.87	486.09	496.61	290.66	126.75

- Use the video for confirmation
- SV : Side view
- TV : Top view

Merge two XY and YZ dataset by searching object in specific frame with the same Y position

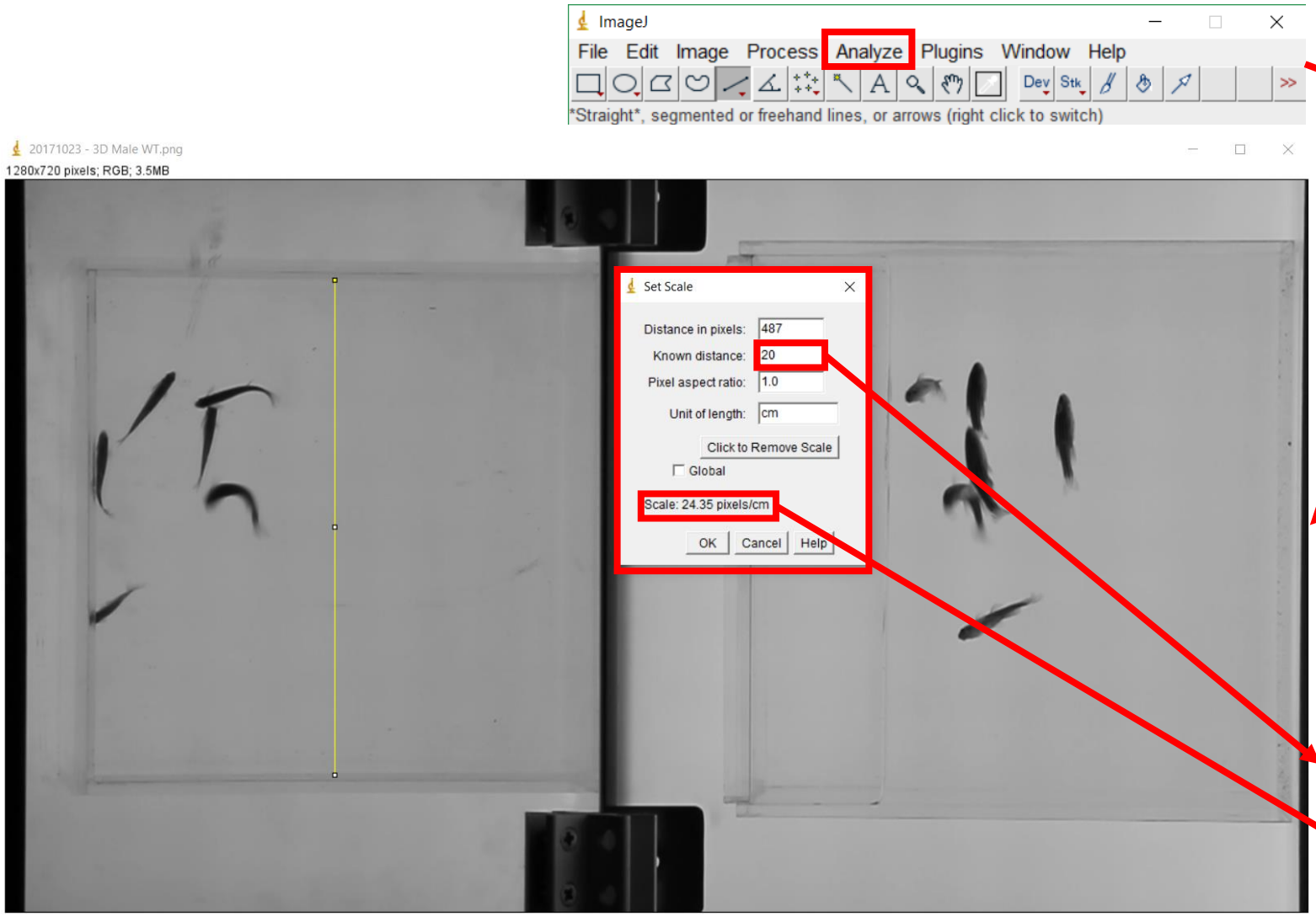
data matrix after merging

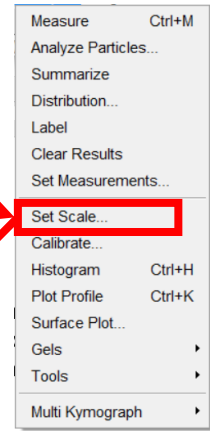
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
1	Fish 1					Fish 2					Fish 3					Fish 4					Fish 5					Fish 6			
2	X1 (TV)	Y1 (TV)	X5 (SV)	Y5 (SV)		X2 (TV)	Y2 (TV)	X1 (SV)	Y1 (SV)		X3 (TV)	Y3 (TV)	X3 (SV)	Y3 (SV)		X4 (TV)	Y4 (TV)	X2 (SV)	Y2 (SV)		X5 (TV)	Y5 (TV)	X4 (SV)	Y4 (SV)		X6 (TV)	Y6 (TV)	X6 (SV)	Y6 (SV)
3	166.46	338.41	1003.9	337.76		268.05	457.12	887.02	474.96		106.25	254.12	994.96	241.92		400.17	124.6	1156.3	116.32		460.84	420.91	900.06	417.6		279.88	132.92	1040.1	108.94
4	168.7	340.56	1004.3	340.23		266.85	456.3	887.31	473.95		106.35	254.69	995.35	242.58		398.24	124.82	1156	117.49		461.45	423.28	900.04	420.52		279.68	131.6	1041.8	109.99
5	171.79	342.82	1005.1	342.62		265.41	454.97	887.75	472.69		106.84	255.56	996.07	243.31		396.69	124.84	1156.4	117.64		462.6	425.69	900.35	423.05		279.78	130.83	1043.5	111.26
6	174.8	345.8	1006.2	346.22		264.59	454.82	888.69	472.51		107.53	258.2	997.18	245.32		394.96	125.11	1157	117.15		464.04	429.08	901.04	427.07		279.99	131.26	1045.6	113.21
7	177.62	348.55	1007.1	349.32		263.28	454.11	889.39	472.01		108.32	258.94	998.04	247.71		393.8	125.53	1157.6	115.32		465.31	432.15	901.65	430.19		280.87	130.73	1048	114.37
8	180.31	350.49	1007.5	350.83		261.72	452.53	889.85	470.21		108.51	259.5	998.69	248.09		392.57	125.21	1157.9	113.3		466.4	434.62	901.9	432.24		281.73	129.43	1050.3	114.06
9	182.41	352.4	1008.1	353.25		260.39	451.75	890.16	469.52		108.7	261.1	999.27	249.1		391.84	124.7	1158.5	110.83		467.02	436.85	902.13	433.96		282.44	128.92	1052.4	113.94
10	184.47	354.48	1008.4	355.75		259.29	451.35	890.46	469.16		108.66	261.87	999.61	250.71		390.09	123.3	1158.4	110.3		467.66	439.59	902.34	433.81		283.39	128.78	1054.3	113.32
11	186.93	356.96	1009.1	358.32		257.91	450.64	891	468.78		108.89	263.41	1000.3	251.78		388.96	122.96	1158.2	110.33		468.64	442.72	902.71	435.51		284.79	127.79	1056.6	111.33
12	189.33	359.06	1009.7	360.34		256.65	449.67	891.51	467.66		109.34	264.42	1001.1	253.06		387.8	122.41	1157.6	110.25		469.57	445.34	903.45	436.54		286.95	126.2	1058.7	110.1
13	192	361.05	1010.4	362.37		255.6	448.55	892.34	466.77		109.69	265.17	1002.1	253.81		385.86	121.51	1157.8	109.43		470.65	447.65	904.07	437.46		290.29	124.7	1059.4	110.36
14	193.77	362.84	1010.9	364.22		254.37	447.92	892.78	466.16		109.75	266.4	1002.7	255.21		383.12	121.73	1158.4	108.63		471.31	450.32	904.5	438.02		285.94	125.72	1060.5	109.96
15	195.84	365.19	1011.3	366.73		252.83	447.53	893.53	466.16		109.8	267.47	1003.2	256.68		380.4	122.59	1158.6	108.04		471.87	453.17	904.82	438.9		296.72	124.26	1061.8	109.92
16	197.3	366.6	1011.5	368.34		251.95	446.93	893.68	465.21		109.71	268.19	1003.7	257.27		378.34	123.26	1158.4	107.1		472.34	455.31	905.13	440.47		299.76	124.03	1062.9	108.57
17	199.15	368.08	1011.9	370.33		250.07	445.68	894.32	464.93		109.75	268.55	1004.3	258.59		376.34	123.56	1157.8	105.62		472.8	456.96	905.47	440.66		303.91	123.28	1064.3	108.15
18	201.13	369.71	1012.5	372.43		249.06	445.18	895.29	465.07		109.93	270.03	1005	259.5		373.68	123.81	1157.9	104.57		473.55	459.42	905.96	441.37		309.1	122.49	1065.7	107.55
19	203.21	372.39	1012.8	375.14		247.91	445.14	895.92	465.41		110.04	271.14	1005.6	261.3		370.52	124.46	1157.3	104.63		474.06	461.87	906.46	441.92		315.94	122.13	1066.8	107.86
20	204.84	373.52	1013.4	376.61		246.82	444.21	896.59	464.68		110.49	273.18	1006	263.07		370.33	124.06	1156.9	103.84		474.9	463.79	907.01	442.78		314.55	121.77	1067.4	107.91
21	206.29	374.83	1013.8	377.73		245.84	443.55	899.09	465.89		111.15	274.63	1006.6	264.9		370.13	123.66	1156.5	103.34		475.52	465.59	906.78	445.59		313.16	121.4	1068.1	107.75
22	208.05	376.65	1014.2	380.01		244.89	443.25	898.91	464.14		111.74	275.79	1006.8	266.81		370	124	1155.6	102.91		476.12	467.52	907.01	448.94		313	121	1068.4	107.48
23	210	378.27	1014.7	382.14		244.34	442.59	899.83	466.04		112.66	277.79	1007.5	268.88		369	124	1154.5	102.34		476.86	469.59	909.5	455.5		313	120	1069	107.42
24	212.06	380.44	1015.2	384.28		242.69	440.3	899.54	463.95		113.6	280.77	1007.8	271.7		369	124	1152.6	101.45		477.62	471.22	911	465		313	121	1069.3	108.03
25	213.77	383.16	1015.4	387.03		242.54	438	899.26	461.87		114.52	283.33	1008.5	273.93		368.71	123	1151	100.26		478.44	472.78	910.88	467.41		316	123	1069.1	108.43
26	214.38	386.04	1015.2	390.03		242.72	435.69	898.97	459.79		114.99	286.14	1008.8	276.84		368	123	1148.2	99.831		479.06	474.66	910	470		317	124	1069.1	106.39
27	214.82	389.85	1014.7	393.59		243.18	434.4	898.69	457.71		115.22	288.62	1009.1	280.17		368	125	1146.4	100.16		479.56	476.5	910	472.38		319	124	1069.5	103.43
28	215.08	393.04	1014	397.28		243.55	433.26	898.41	455.63		115.4	291.62	1009.3	282.66		365	115	1144.9	100.54		480.08	478.35	910	474.75		320	124	1068	103.35
29	215.24	395.78	1013.5	400.73		244.46	430.33	898.12	453.55		115.61	293.74	1009.5	285.52		367	115	1142.4	99.617		480.67	480.01	910.01	477.13		318	112	1067	104.15
30	215.14	400.32	1012.7	404.72		244.78	428.93	897.84	451.47		115.75	295.2	1009.9	287.8		367	116	1139.3	98.83		481.32	481.49	910.01	479.51		311	113	1066.2	104.34
31	215.16	404	1012	409.14		245.58	426.99	897.56	449.39		116.03	296.68	1010.4	290.48		367	117	1136.6	98.217		481.87	483.04	910.01	481.88		306	114	1064.9	102.22
32	215.24	407.38	1011.5	412.28		245.97	426.4	897.27	447.31		116.45	299.65	1010.7	293.01		367	118	1134	98.364		482.6	485.01	910.01	484.26		298	114	1063.9	101.39
33	214.96	410.77	1010.6	416.01		247.23	425.01	896.99	445.23		116.91	302.41	1010.9	295.9		367	119	1131.8	98.353		482.85	486.49	909.89	486.67		296	115	1063.5	101.05
34	214.73	413.09	1009.8	418.77		249.33	423.76	896.7	443.15		117.5	304.66	1010.9	298.63		367.62	118.48	1129.2	97.914		483.26	487.95	909.77	489.07		295	116	1062.5	100.45
35	214.61	416.1	1009.2	422.18		251.31	422.71	896.52	441.4		118.7	306.9	1011.6	301.49		367.42	118.08	1127.7	98.235		483.71	489.09	910	492.42		293.72	116.3	1062.6	100.56
36	214.4	418.34	1008.6	424.73		253.95	420.34	896.23	438.18		119.77	309.67	1011.9	304.52		367.23	117.68	1127	98.323		484.29	490.85	910.36	492.67		292.33	115.93	1062.7	100.33
37	214.31	421.02	1008.2	427.67		257.69	416.89	896.17	434.81		120.85	312.33	1012.5	306.95		375.39	118.53	1122.2	98.915		485.03	492.23	910.52	492.95		289.28	116.69	1062.9	101.22
38	214.05	423.59	1007.6	430.47		260.32	413.71	896.11	430.86		121.78	315.52	1012.9	309.58		383.66	120.43	1116.1	101.58		485.38	493.23	910.72	495.76		289.92	119.74	1062.7	102.96
39	213.88	426.04	1007	433.05		263.77	407.5	896.11	427.3		122.47	319.23	1013.2	313.65		388.78	122.73	1113.2	104.3		485.78	494.88	911	495.92		290.31	123.09	1062.9	106.11
40	213.51	428.97	1006.2	436.17		263.67	406.05	896	424.17		122.93	322.28	1013.4	316.81		392.12	124.87	1109.8	107.27		486.09	496.61	911.1	497.26		290.66	126.75	1063	110.16

X Axis Y Axis Z Axis | X Axis Y Axis Z Axis | X Axis Y Axis Z Axis | X Axis Y Axis Z Axis | X Axis Y Axis Z Axis | X Axis Y Axis Z Axis

- Use the Y Axis from the Top View (TV) if the conversion that will be used is also TV conversion

Convert pixel to real distance by using ImageJ (Top View Conversion)





- Convert the pixel dimension into the standard length dimension using **ImageJ**

Real Size measured by ruler

Top View Conversion from pixel to cm

Calculate fish movement by using Excel

		Distance and Velocity						
X1	Y1	Distance (pixel)	Distance (cm)	Speed (cm/s)	Maximum speed (cm/s)	Minimum speed (cm/s)	Total distance (cm)	Average speed (cm/s)
866.58	409.89	0.272946881	0.009821943	0.491097143	124.6146758	0	433.1221536	7.201898132
866.54	410.16	2.646091457	0.09521911	4.7609555				
865.91	412.73	2.500599928	0.089983624	4.499181216				
865.32	415.16	2.822357171	0.101561999	5.078099949				
864.73	417.92	6.246350935	0.224773779	11.23868896				
863.53	424.05	2.156965461	0.077618002	3.88090009				
863.19	426.18	0.471699057	0.016974003	0.84870015				
863.36	425.74	2.025438224	0.072885019	3.64425093				
863.04	427.74	1.808535319	0.065079808	3.25399039				
862.72	429.52	2.526855754	0.090928435	4.546421767				
862.29	432.01	1.969009903	0.070854456	3.542722797				
861.9	433.94	2.546566316	0.091637716	4.581885812				
861.47	436.45	1.62431524	0.058450682	2.922534123				
861.19	438.05	5.246722787	0.188802346	9.440117286				
860.24	443.21	1.019803903	0.036697454	1.834872709				
860.44	442.21	5.089214085	0.183134424	9.156721216				
859.44	447.2	1.223315168	0.044020769	2.201038464				
859.26	448.41	1.170042734	0.042103771	2.105188532				
859.63	447.3	2.211990054	0.079598052	3.979902579				
859.4	449.5	1.568470593	0.056441123	2.822056159				
859.16	451.05	0.850529247	0.030606137	1.530306855				
859.13	451.9	1.77552809	0.063892049	3.194602441				
858.99	453.67	1.520822146	0.054726503	2.736325134				
858.94	455.19	2.377603836	0.085557633	4.277881638				
858.75	457.56	2.293686988	0.082537901	4.126895029				
858.62	459.85	0.830240929	0.029876066	1.493803287				
858.64	460.68	1.540811475	0.055445815	2.772290747				
858.59	462.22	1.210041322	0.043543112	2.177155619				
858.6	463.43	1.350037037	0.048580832	2.42904161				
858.59	464.78	1.694992625	0.060993995	3.049699752				
858.46	466.47	0.820060973	0.029509742	1.475487096				
858.45	467.29	0.672681202	0.024206308	1.210315411				
858.51	467.96	1.562049935	0.056210077	2.810503851				

- Use the Microsoft Excel's formula to calculate the fish's movement frame by frame
- Example : measure the fish's rate of speed by dividing the calculation results with the time

Some results we can get from this analysis

- **Total distance traveled (cm)**

$$\Sigma\sqrt{(X_2-X_1)^2+(Y_2-Y_1)^2+(Z_2-Z_1)^2}$$

- **Avg. swimming speed (cm/s)**

$$\frac{\text{Total distance (cm)}}{\text{Total Time (s)}}$$

- **Maximum speed (cm/s)**
- **Minimum speed (cm/s)**
- **Freezing Time (s)**
  (Total Time when speed less than 1cm/s)
- **Swimming Time (s)**
  (Total Time when speed 1-10 cm/s)
- **Rapid Movement Time (s)**
  (Total Time when speed more than 10 cm/s)

Some results we can get from this analysis

- **Turning Angle (^{0})**

$$\frac{1}{\frac{tan(\frac{\Delta Y}{\Delta X}) \times 180}{\pi}}$$

- **Fast Type Latent Time (s)**
  (Total time when Angular velocity above 0.5^0/ms)

- **Slow Type Latent Time (s)**
  (Total time when Angular velocity below 0.5^0/ms)

- **Angular Velocity (^{0}/ms)**

$$\text{Turning Angle} \times (\frac{\text{Video duration (ms)}}{\text{Frame per second}})$$

- **Absolute Turn Angle**
  (Sum of all the Turning Angles without considering the sign of angular direction)

Some results we can get from this analysis

- **Time spent in the top (%)**

  Total time spent in the top part of the novel tank

- **Time spent in the bottom (%)**

  Total time spent in the bottom part of the novel tank

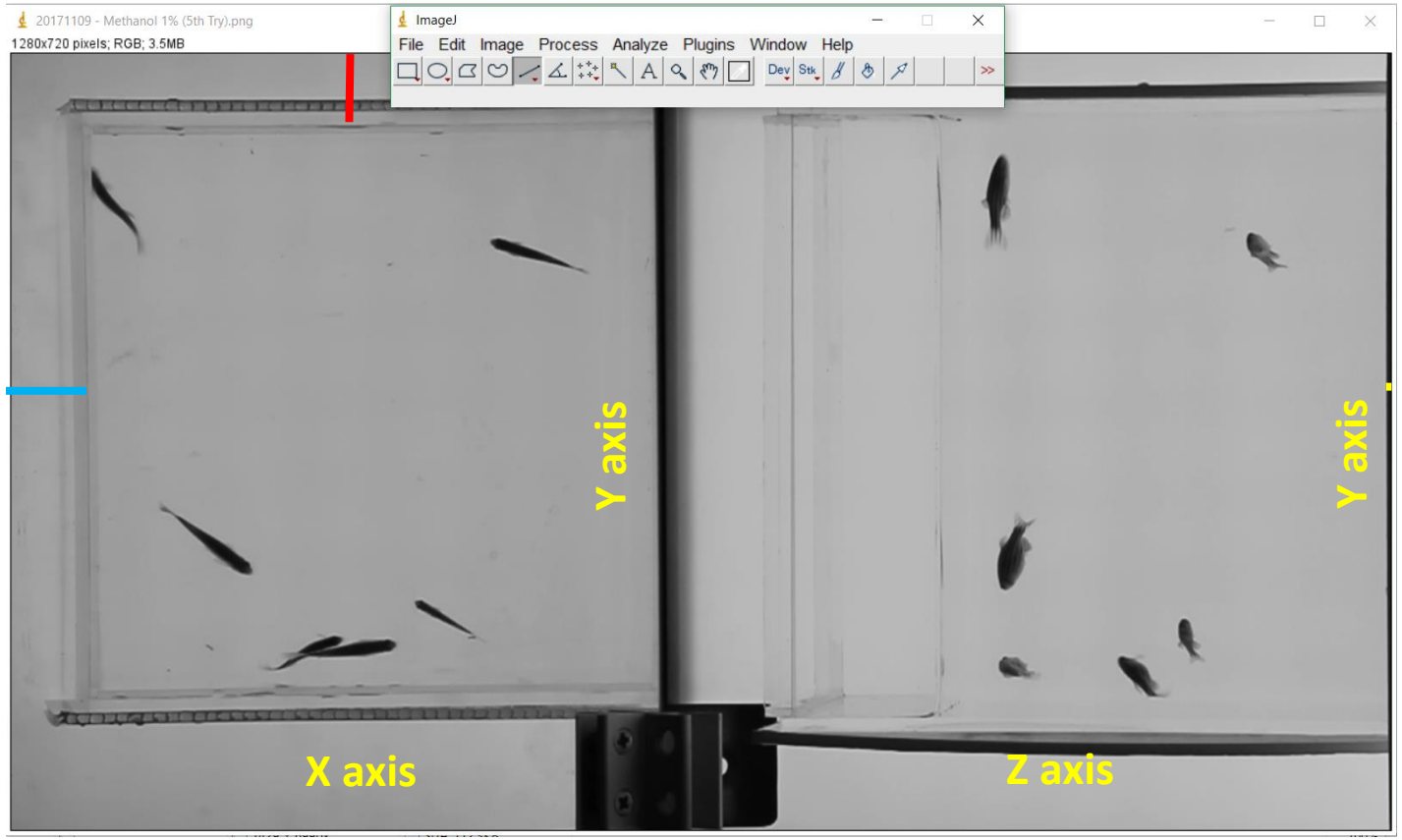
- **Meandering (°/m)**

  (The degree of turning vs. travel distance)

$$\left(\frac{\text{Absolute turn angle (°)}}{\text{Total distance (cm)}} \times 100\right)$$

Calculation of fish trajectory by ImageJ

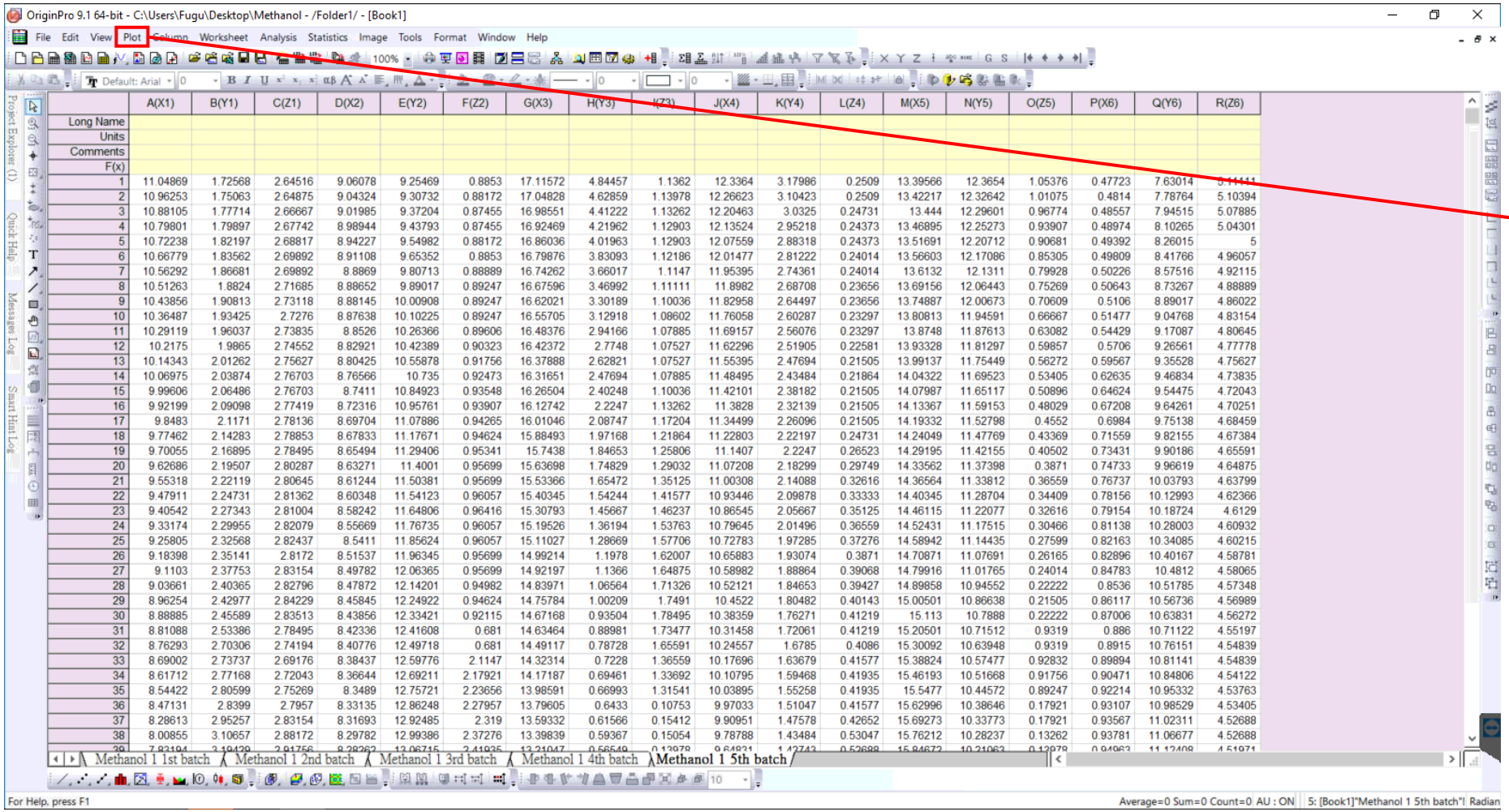
- Trajectories → Fish position (cm) on every frames

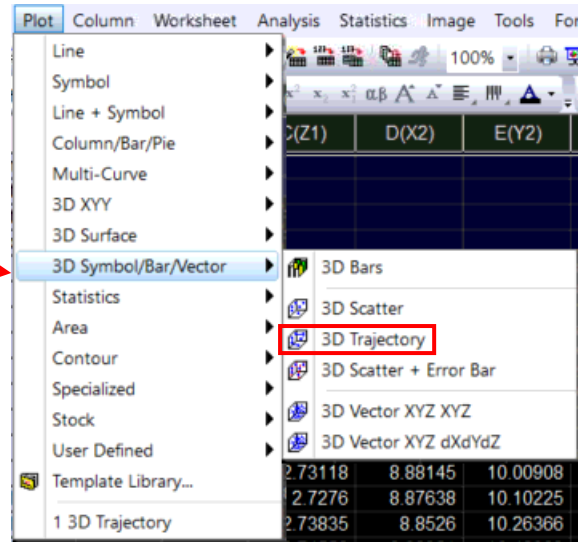


1280X720 pixel

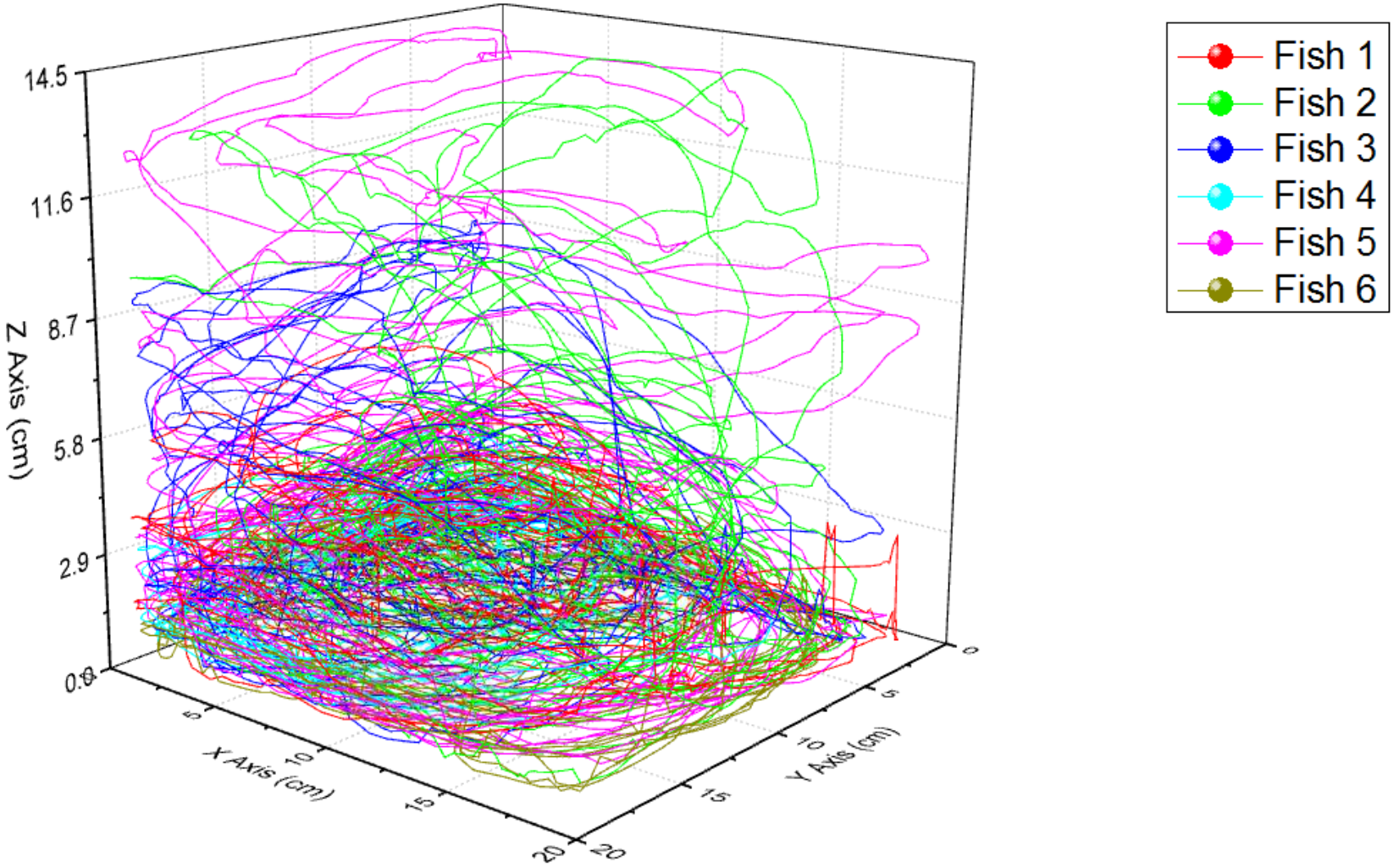
- X position
  - (X(TV) – Blue)/TV conversion
- Y Position
  - (Y(TV) – Red)/TV conversion
- Z Position
  - (1280 – X(SV) – Yellow)/SV conversion

Plot fish 3D spatial trajectory by Origin 9.1





Plot fish 3D spatial trajectory by Origin 9.1



Plot fish 3D temporal trajectory by Origin 9.1

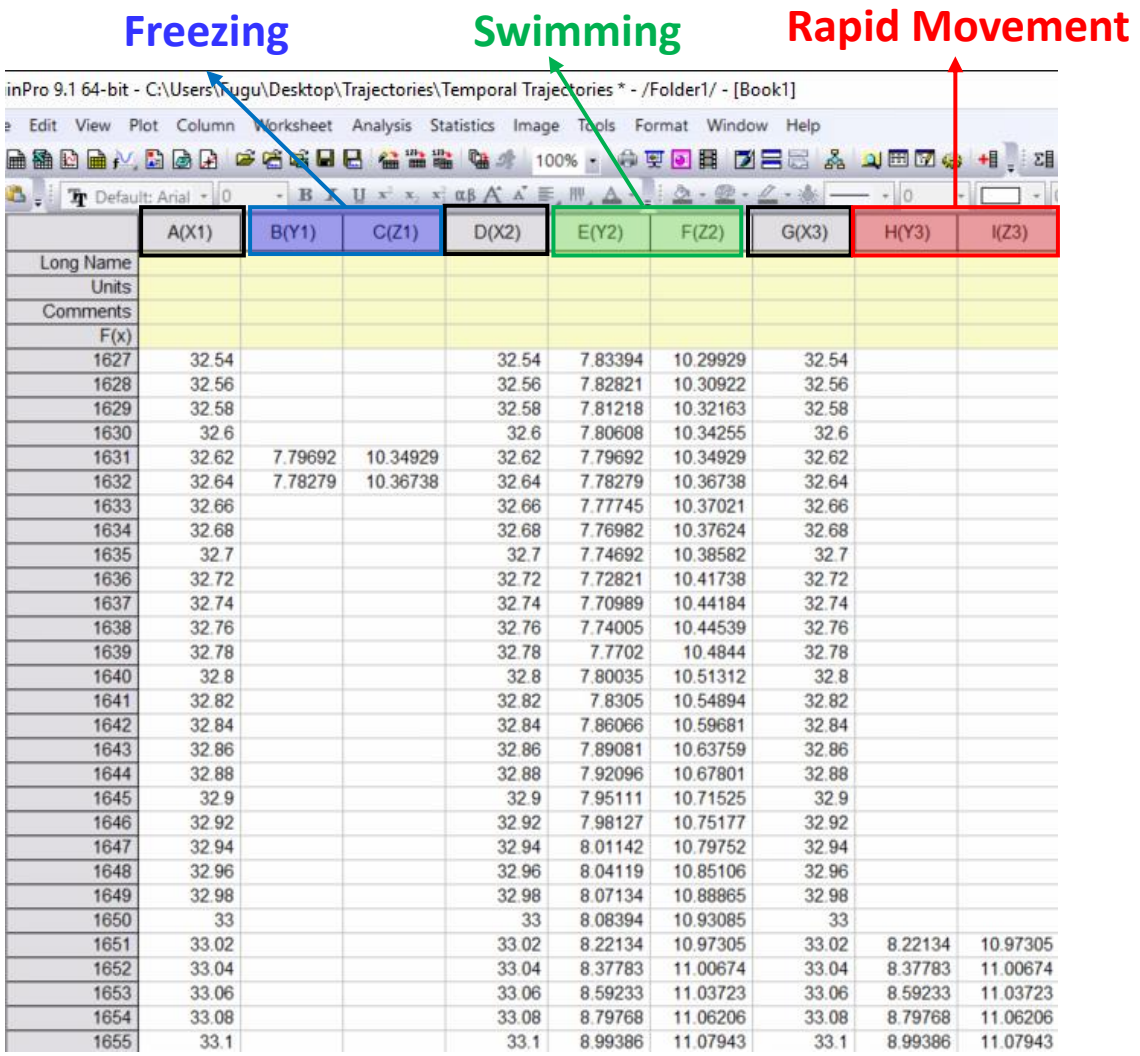
	A	B	C	D	E	F	G	H	I
1				Freezing		Swimming		Rapid	
2	Y1 (cm)	Z5 (cm)	Speed 3D (cm/s)	Y1(cm)	Z1(cm)	Y1(cm)	Z1(cm)	Y1(cm)	Z1(cm)
3	12.4255	12.9791	2.916404055			12.4255	12.9791		
4	12.3793	12.9762	3.524997699			12.3793	12.9762		
5	12.324	12.9699	3.416443925			12.324	12.9699		
6	12.274	12.9691	2.552823399			12.274	12.9691		
7	12.237	12.9574	2.806436078			12.237	12.9574		
8	12.1916	12.9489	3.282763062			12.1916	12.9489		
9	12.1389	12.9351	2.716155436			12.1389	12.9351		
10	12.0992	12.9298	2.969309588			12.0992	12.9298		
11	12.0564	12.9294	2.322362028			12.0564	12.9294		
12	12.0229	12.9284	2.716423592			12.0229	12.9284		
13	11.9832	12.9262	2.436394078			11.9832	12.9262		
14	11.9438	12.9195	1.808948055			11.9438	12.9195		
15	11.9167	12.916	2.977271367			11.9167	12.916		
16	11.8683	12.9099	2.699680083			11.8683	12.9099		
17	11.8309	12.9106	2.844204949			11.8309	12.9106		
18	11.7874	12.9106	1.322584256			11.7874	12.9106		
19	11.7687	12.9103	1.640993189			11.7687	12.9103		
20	11.7416	12.906	2.858128116			11.7416	12.906		
21	11.6938	12.9028	1.963787673			11.6938	12.9028		
22	11.6641	12.9043	2.966916888			11.6641	12.9043		
23	11.6171	12.9028	2.105637973			11.6171	12.9028		
24	11.5969	12.911	1.489766761			11.5969	12.911		
25	11.5729	12.9099	1.624374826			11.5729	12.9099		
26	11.5473	12.8979	2.168018011			11.5473	12.8979		
27	11.511	12.8908	2.010250966			11.511	12.8908		
28	11.4797	12.8894	2.036172178			11.4797	12.8894		
29	11.4484	12.8915	1.78666453			11.4484	12.8915		
30	11.4198	12.8908	3.392642373			11.4198	12.8908		
31	11.3671	12.8922	3.181578052			11.3671	12.8922		
32	11.3286	12.8968	5.240571446			11.3286	12.8968		

- Temporal Trajectories

- Grouped the average speed (1 fish) for each frame into 3 different types of movement
  - **Freezing Time (Speed < 1cm/s)**
  - **Swimming Time (1cm/s ≤ Speed ≤ 10cm/s)**
  - **Rapid Movement Time (Speed > 10cm/s)**

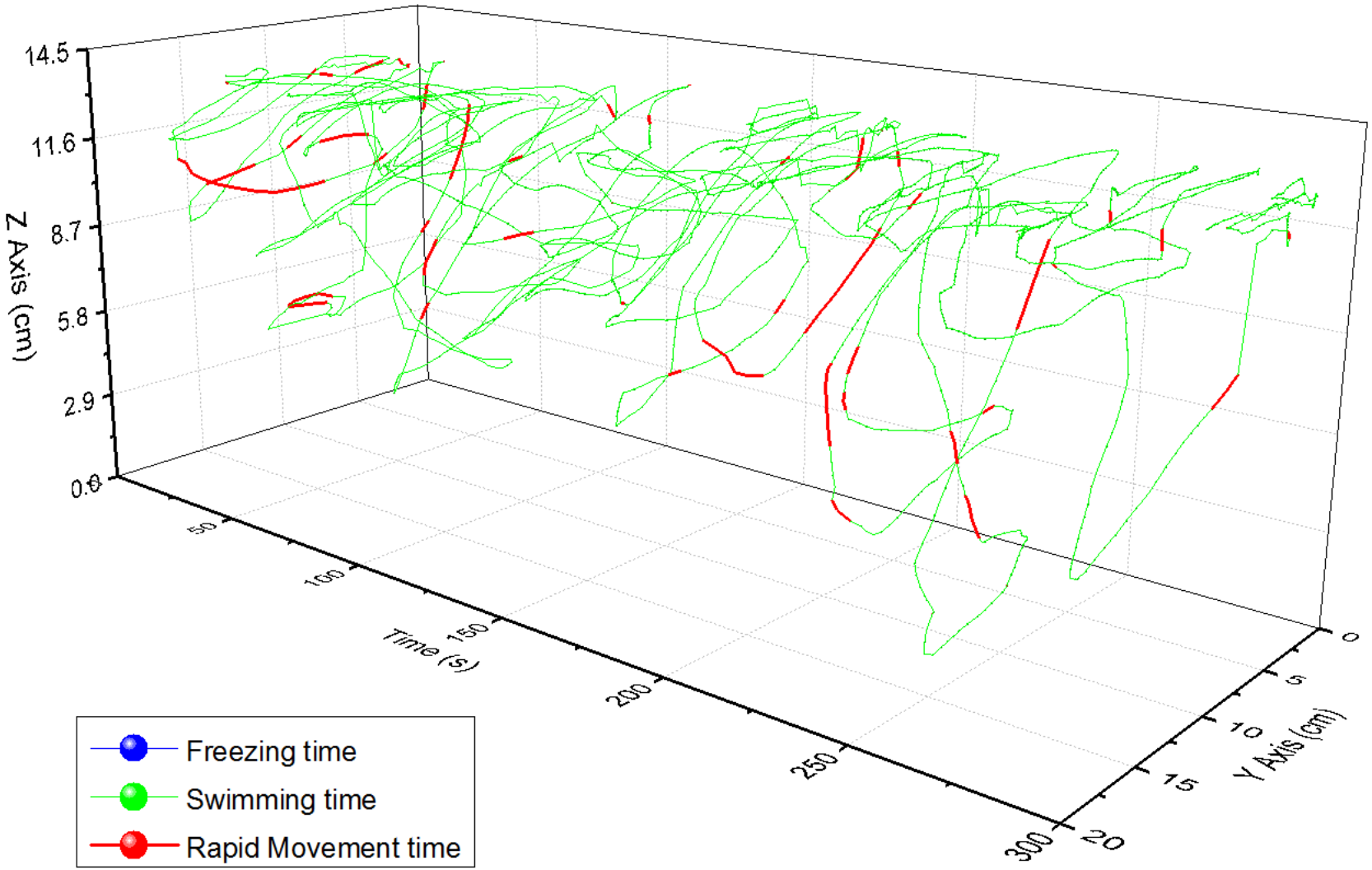
Plot fish 3D temporal trajectory by Origin 9.1

- Temporal Trajectories
- Copy the grouped speeds into Origin 9.1 (Y&Z column)
- Add the time in the X column
- Make the 3D temporal trajectories



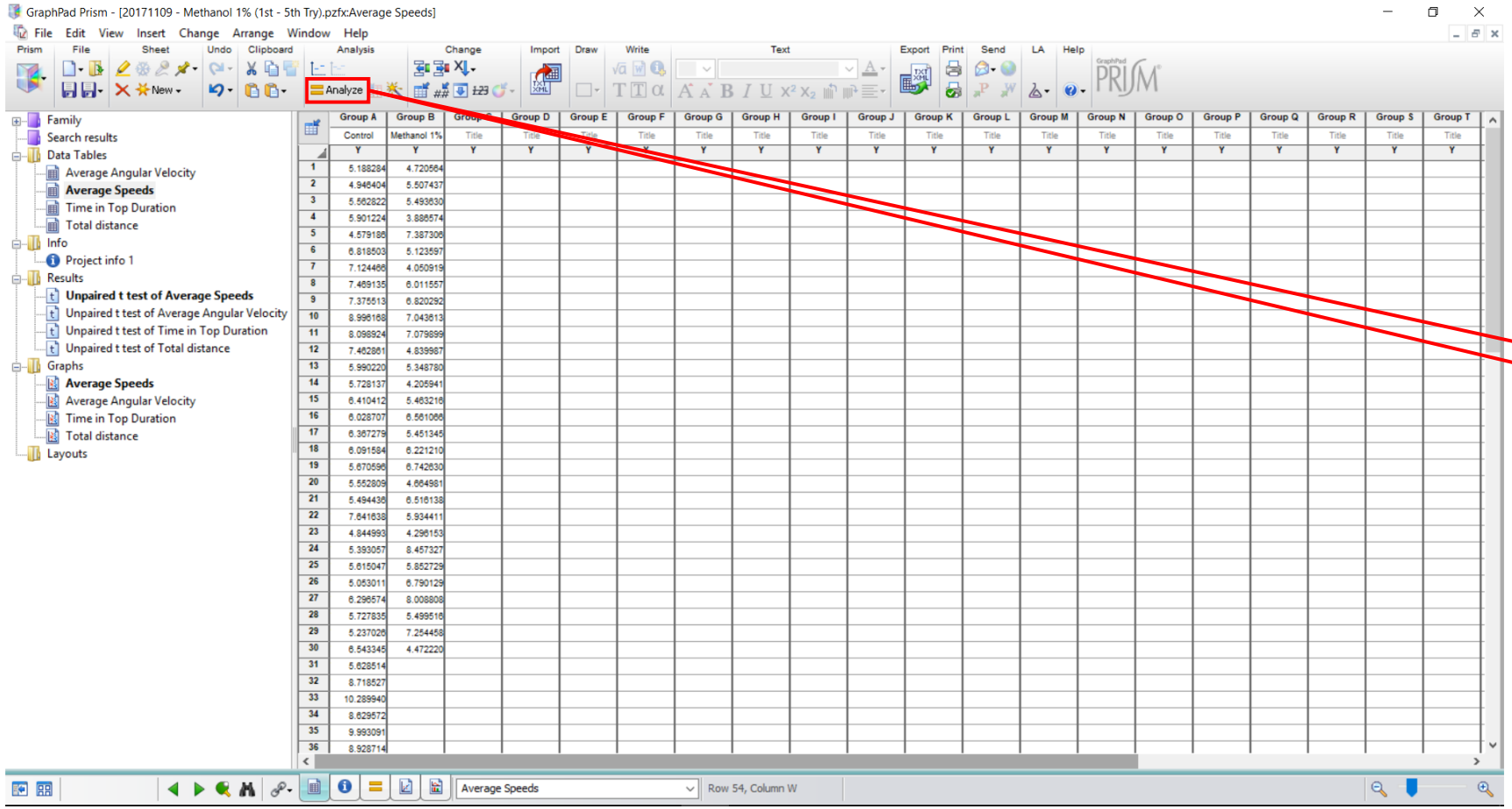
	A(X1)	B(Y1)	C(Z1)	D(X2)	E(Y2)	F(Z2)	G(X3)	H(Y3)	I(Z3)
Long Name									
Units									
Comments									
F(x)									
1627	32.54			32.54	7.83394	10.29929	32.54		
1628	32.56			32.56	7.82821	10.30922	32.56		
1629	32.58			32.58	7.81218	10.32163	32.58		
1630	32.6			32.6	7.80608	10.34255	32.6		
1631	32.62	7.79692	10.34929	32.62	7.79692	10.34929	32.62		
1632	32.64	7.78279	10.36738	32.64	7.78279	10.36738	32.64		
1633	32.66			32.66	7.77745	10.37021	32.66		
1634	32.68			32.68	7.76982	10.37624	32.68		
1635	32.7			32.7	7.74692	10.38582	32.7		
1636	32.72			32.72	7.72821	10.41738	32.72		
1637	32.74			32.74	7.70989	10.44184	32.74		
1638	32.76			32.76	7.74005	10.44539	32.76		
1639	32.78			32.78	7.7702	10.4844	32.78		
1640	32.8			32.8	7.80035	10.51312	32.8		
1641	32.82			32.82	7.8305	10.54894	32.82		
1642	32.84			32.84	7.86066	10.59681	32.84		
1643	32.86			32.86	7.89081	10.63759	32.86		
1644	32.88			32.88	7.92096	10.67801	32.88		
1645	32.9			32.9	7.95111	10.71525	32.9		
1646	32.92			32.92	7.98127	10.75177	32.92		
1647	32.94			32.94	8.01142	10.79752	32.94		
1648	32.96			32.96	8.04119	10.85106	32.96		
1649	32.98			32.98	8.07134	10.88865	32.98		
1650	33			33	8.08394	10.93085	33		
1651	33.02			33.02	8.22134	10.97305	33.02	8.22134	10.97305
1652	33.04			33.04	8.37783	11.00674	33.04	8.37783	11.00674
1653	33.06			33.06	8.59233	11.03723	33.06	8.59233	11.03723
1654	33.08			33.08	8.79768	11.06206	33.08	8.79768	11.06206
1655	33.1			33.1	8.99386	11.07943	33.1	8.99386	11.07943

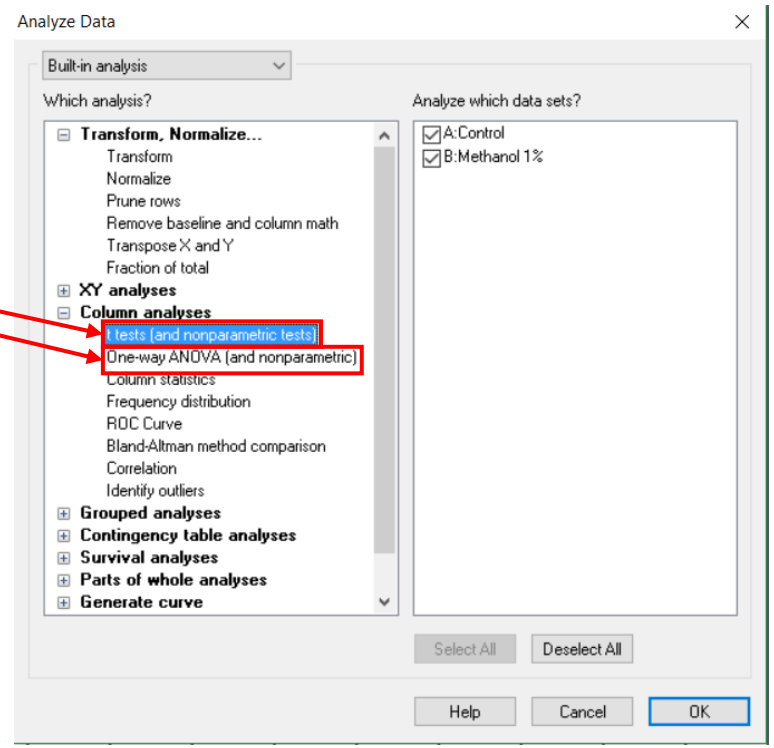
Plot fish 3D temporal trajectory by Origin 9.1



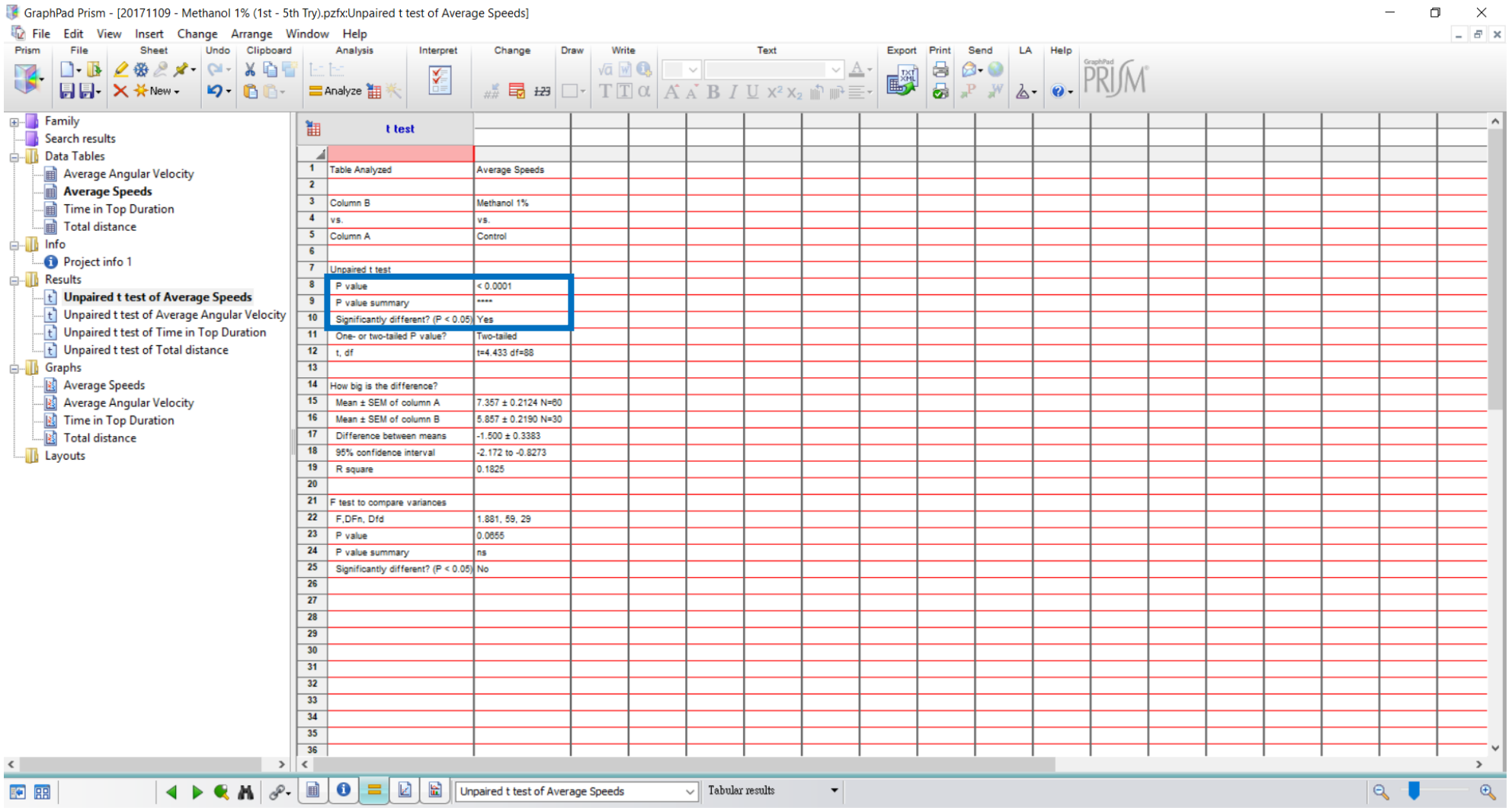
Perform statistic analysis by GraphPad Prism

- ANOVA & t-test using GraphPad Prism

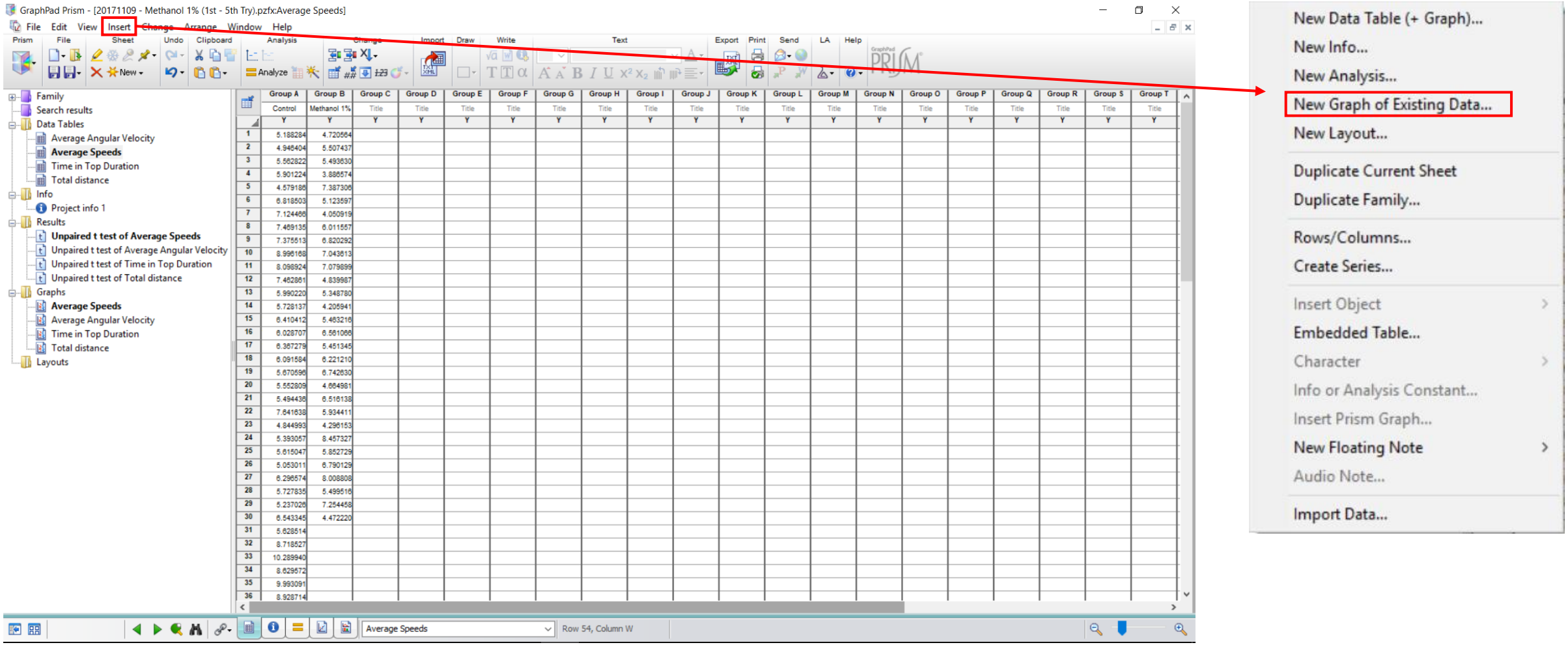




Perform statistic analysis by GraphPad Prism



Perform statistic analysis by GraphPad Prism



Perform statistic analysis by GraphPad Prism