

# Protocol of 3D registration

Title:

An ImageJ-based tool for three-dimensional registration between different types of microscopic images

Koyama et al.

Our method is composed of the following three macros. For each macro opened, users can find the essential information; descriptions, requirements, setting of parameter values to be done by users, tips, etc. Only the most fundamental processes and parameter settings are explained in this presentation.

Macro\_3D\_particle\_registration\_06\_v2.ijm

Macro\_particle\_drawing\_02.ijm

Macro\_3D\_image\_rotation\_02.ijm

# Macro name: Macro\_3D\_particle\_registration\_06\_v2.ijm

## Format of input file

File name should be "input\_xyz\_registration.csv"

(Csv files are generated by ImageJ>"Multi-point tool" followed by "Analyze>Measure", and then edited on the Excel software.)

From 1<sup>st</sup> or 2<sup>nd</sup> image

Paired landmarks should have the same ID.

Objects of interest should have the different IDs.

	x	y	z		ID
	A	B	C	D	E
1	42.813	12.706	39.1	1	1
2	34.665	18.921	26.45	1	2
3	72.92	74.025	10.925	1	3
4	50.547	86.869	12.65	1	4
5	33.836	89.355	29.325	1	5
6	15.744	70.573	31.05	1	6
7	21.821	31.074	35.65	1	7
8	21.821	58.833	14.375	1	8
9	18.23	45.023	20.7	1	9
10	28.588	37.703	11.5	1	10
11	65.325	26.24	13.8	1	11
12	81.207	47.923	13.225	1	12
13	85.902	67.81	14.95	1	13
	'''	'''	'''	'''	'''
	'''	'''	'''	'''	'''

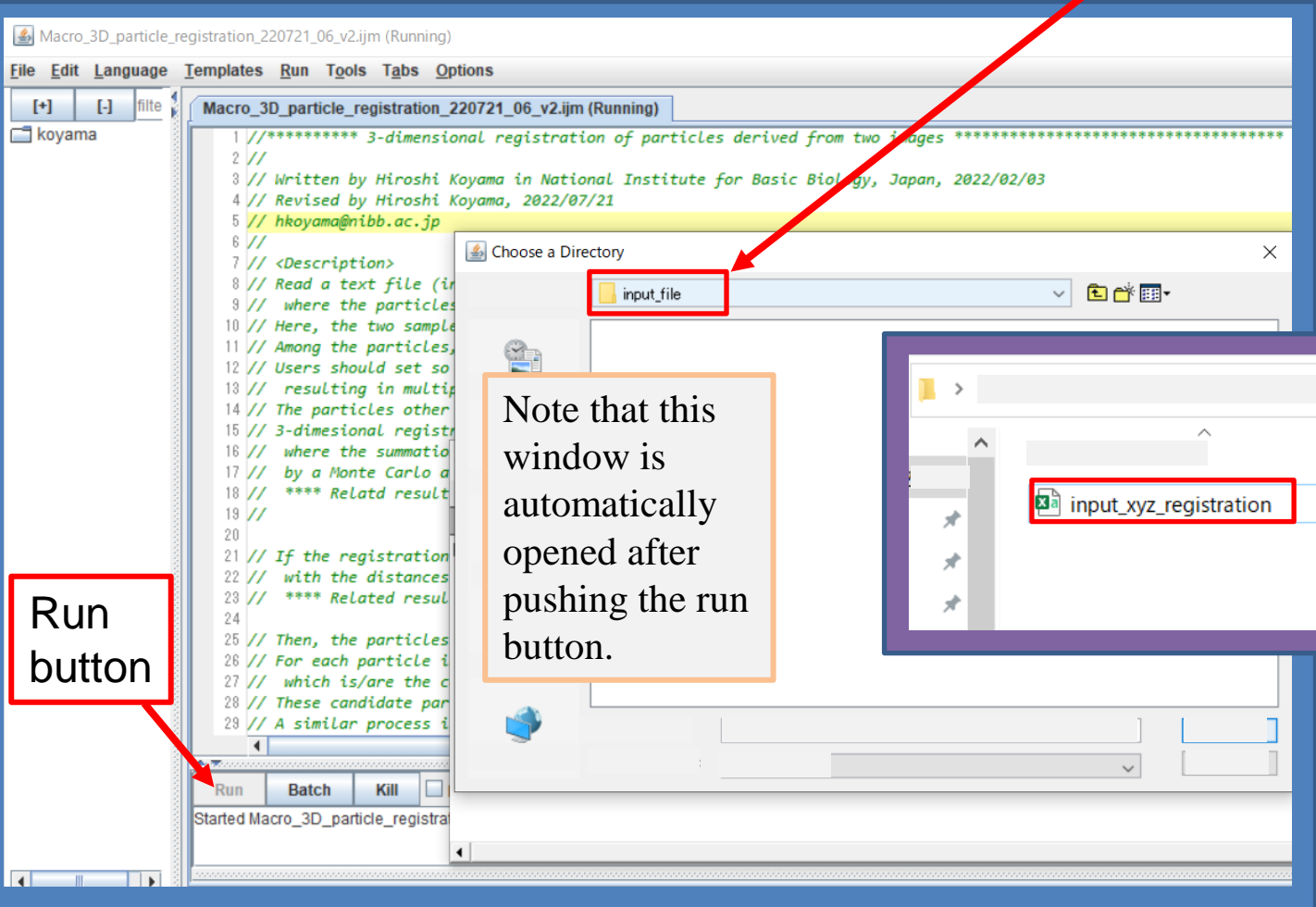
	A	B	C	D	E
43	25.135	62.286	31.05	1	43
44	33.146	64.496	27.025	1	44
45	39.913	73.887	27.025	1	45
46	31.35	68.501	9.2	1	46
47	52.619	12.015	45.625	2	1
48	43.227	13.258	33.125	2	2
49	59.8	66.844	3.75	2	3
50	35.632	73.058	7.5	2	4
51	20.578	72.506	21.25	2	5
52	11.049	52.757	29.375	2	6
53	28.45	21.821	43.75	2	7
54	20.025	41.57	16.875	2	8
55	20.302	29.831	25.625	2	9
56	31.903	26.102	16.875	2	47
57	67.396	25.964	17.5	2	48
58	74.025	46.956	9.375	2	49
	'''	'''	'''	'''	'''
	'''	'''	'''	'''	'''

Macro name: Macro\_3D\_particle\_registration\_06\_v2.ijm

Run of the macro

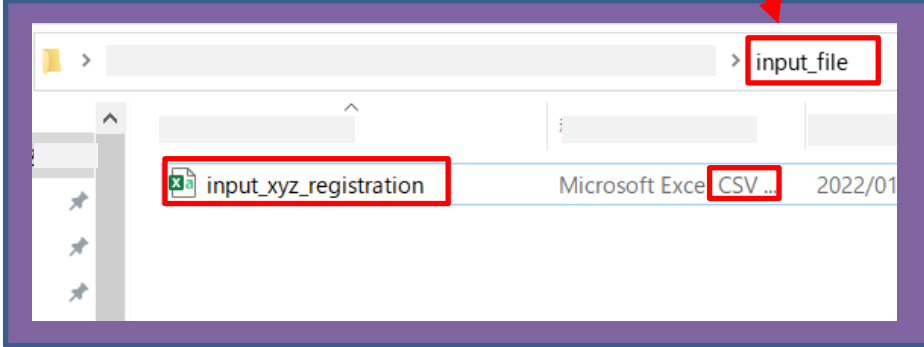
Window of ImageJ/Fiji (in the case of Windows OS)

Manually choose the folder where input\_xyz\_registration.csv is put.



Note that this window is automatically opened after pushing the run button.

Run button



Window of OS

# Macro name: Macro\_3D\_particle\_registration\_06\_v2.ijm

## Output files of the macro

input\_xyz\_registration

Log\_registration

output\_bad\_results\_rotation

output\_condition\_for\_registration

output\_registered\_particles\_1neighbors

output\_registered\_particles\_3neighbors

output\_registered\_particles\_all

output\_registered\_particles\_landmarks

output\_rotation\_results

Log file

Unsuccessful minimization

Parameter values/conditions

List of candidates: 1 nearest neighbor

List of candidates: 1-3 nearest neighbors (Fig. 4C)

IDs and xyz-coordinates of paired landmarks/objects between the 1<sup>st</sup> and the rotated 2<sup>nd</sup> images (Fig. 4B,D)

IDs and xyz-coordinates of paired landmarks between the 1<sup>st</sup> and the rotated 2<sup>nd</sup> images (Fig. 4A)

List of successful minimization with the values of the three angles, and the best values (Fig. 5)

List of successful minimization

```
1: 9636 xyz-error(2357.9223 -> 112.7069) mean-distance(2.6259, 3.4258) angle(5.9524, 6.229, 0.2563)
```

```
'''
```

```
best rotational angles: 5.9524, 6.229, 0.2563
```

The best values

Macro name: Macro\_3D\_particle\_registration\_06\_v2.ijm

A format of an output file (output\_registered\_particles\_3neighbor.txt, related to Fig. 4C)

At the end of the text file in Fig. 4C, multiply assigned objects are listed as follows.

```
''' ''' '''
''' ''' '''
''' 61 '''
''' ''' '''
''' ''' '''
```

	A	B	C	D	E	F	G
38	45	82	72	61	5.46	12.108	12.215
39	46	61	56	62	17.083	17.87	19.572
40	multiply counted particles = 2						
41	55	61					

See Fig. 4C

This means that ID = #55 and #61 in the 2<sup>nd</sup> image are multiply assigned as the nearest neighbor.

Macro name: Macro\_3D\_particle\_registration\_06\_v2.ijm

Optional for correction of distortion of xyz-coordinates: step-3 in Fig. 3A

Window of the macro on ImageJ/Fiji

```
Macro_3D_particle_registration_220721_06_v2.ijm
66 //
67 //*****
68
69 //***** Parameters to be defined by users **
70
71 print("Please set several values in 'Parameters to
72
73 /** Scaling of xyz-coordinates: if the values =
74 //In spite of these parameters, I recommend that
75 //This is because the scaling toward the text fil
76 // "Macro_particle_drawing_220721_02.ijm and "Mac
77 //for before-image
78 x_scale_before = 1.0;
79 y_scale_before = 1.0;
80 z_scale_before = 1.0;
81 //for after-image
82 x_scale_after = 1.0;
83 y_scale_after = 1.0;
84 z_scale_after = 1.0;
```

Enter manually the magnification values for xyz-coordinates in the 1<sup>st</sup> image

Enter manually the magnification values for xyz-coordinates in the 2<sup>nd</sup> image

Before running the macro, users rewrite these values.

Important!

Note that these values will be used in the following two macros,

“Macro\_particle\_drawing\_02.ijm” and “Macro\_3D\_image\_rotation\_02.ijm”, where **users should manually rewrite the corresponding lines in the two macros.** Otherwise, images with different xyz-scales are generated.

Macro name: Macro\_particle\_drawing\_02.ijm

## Parameter setting

Window of the macro on ImageJ/Fiji

```
Macro_particle_drawing_220721_02.ijm
31
32
33 //***** Basic parameters to be defined by users *****
34
35 print("Please set several values in 'Parameters to be defined by us
36
37 //Choose input text file
38 input_name = "output_registered_particles_all.txt";
39 //input_name = "output_registered_particles_Landmarks.txt";
40
41 image_size_x = 262; //The pixel number of output images along x
42 image_size_y = 262; //The pixel number of output images along y
43
44 //Unit of input text file
45 //If the unit is pixel, um_pixel should be set as 1.0.
46 //If the unit is um or something, um_pixel should be set so that th
47 ///The relationship between um and pixel in the original before-imag
48 um_per_pixel = 0.4143204;
49
50 //These values should be the same as those used in Macro_3D_particl
51 x_scale_before = 1.0;
52 y_scale_before = 1.0;
53
54 radius_of_particle=5.0; //Particle radius to be drawn
55
```

Users should manually write the following parameters.

Choose one of two possible input files obtained from the previous macro.

Image sizes

Unit of length:  $\mu\text{m}/\text{pixel}$

See the previous slide.

Size of particles to be drawn



# Macro name: Macro\_3D\_image\_rotation\_02.ijm

## Parameter setting

Window of the macro on ImageJ/Fiji

```
Macro_3D_image_rotation_220722_02.ijm
91 /** Rotation angles for after-image, which should be
92  //output_rotation_results.txt after running the Macro_
93  //The values are written in the Last line of the text
94 angle1=5.9529;
95 angle2=6.2313;
96 angle3=0.2541;
97
98 /** Normalization of intensity gradient along z-slices
99  //If your images exhibit severe decay of intensity in
100 z_normalize = 1;  //1 for Yes, 0 for No.
101
102 /** Averaging of 3D image drawing
103  //If range_ave = 0, no averaging. If range_ave = 1, 3x
104  // This averaging improves image quality, but time con
105 range_ave = 1;
106
107 /*******
108
109
110 /******* input text file name and input after-image nam
111 dir_input = getDirectory("Choose a Directory ");
112 input_file = dir_input+"input_xyz_registration.csv";
113 input_image = dir_input+"input_after_image.tif";
114
```

Users should manually write the following parameters.

The values of the three angles obtained from the 1<sup>st</sup> macro.

Before rotation, the intensities of the 2<sup>nd</sup> image is normalized or not.

Input file also used in the 1<sup>st</sup> macro  
The name of the original 2<sup>nd</sup> image to be rotated.

Note that the parameters of xyz-scaling are also to be set as previously explained.