Fig. S1 How to use the "Load" macro

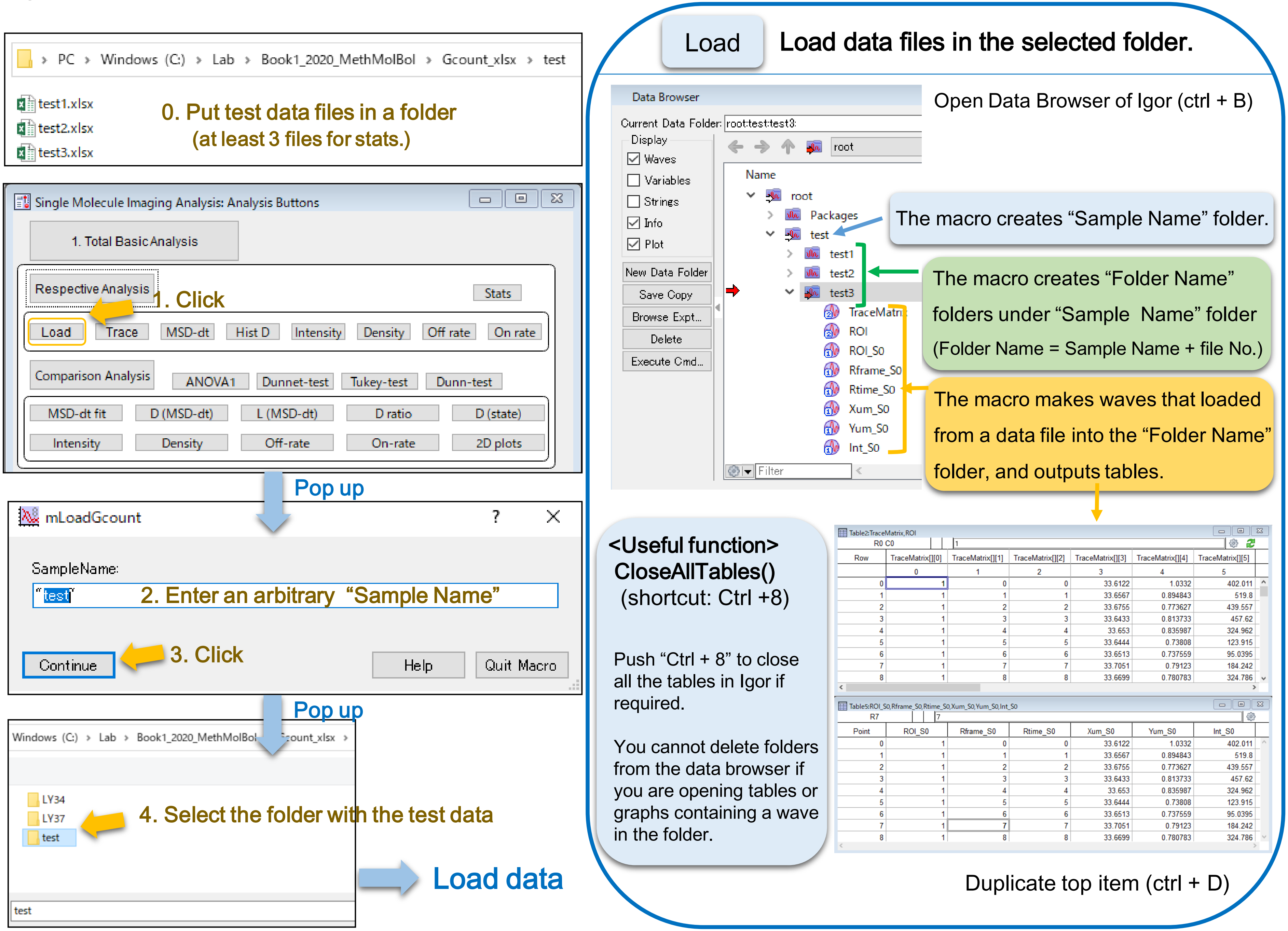


Fig. S2 How to use the “Trace” macro

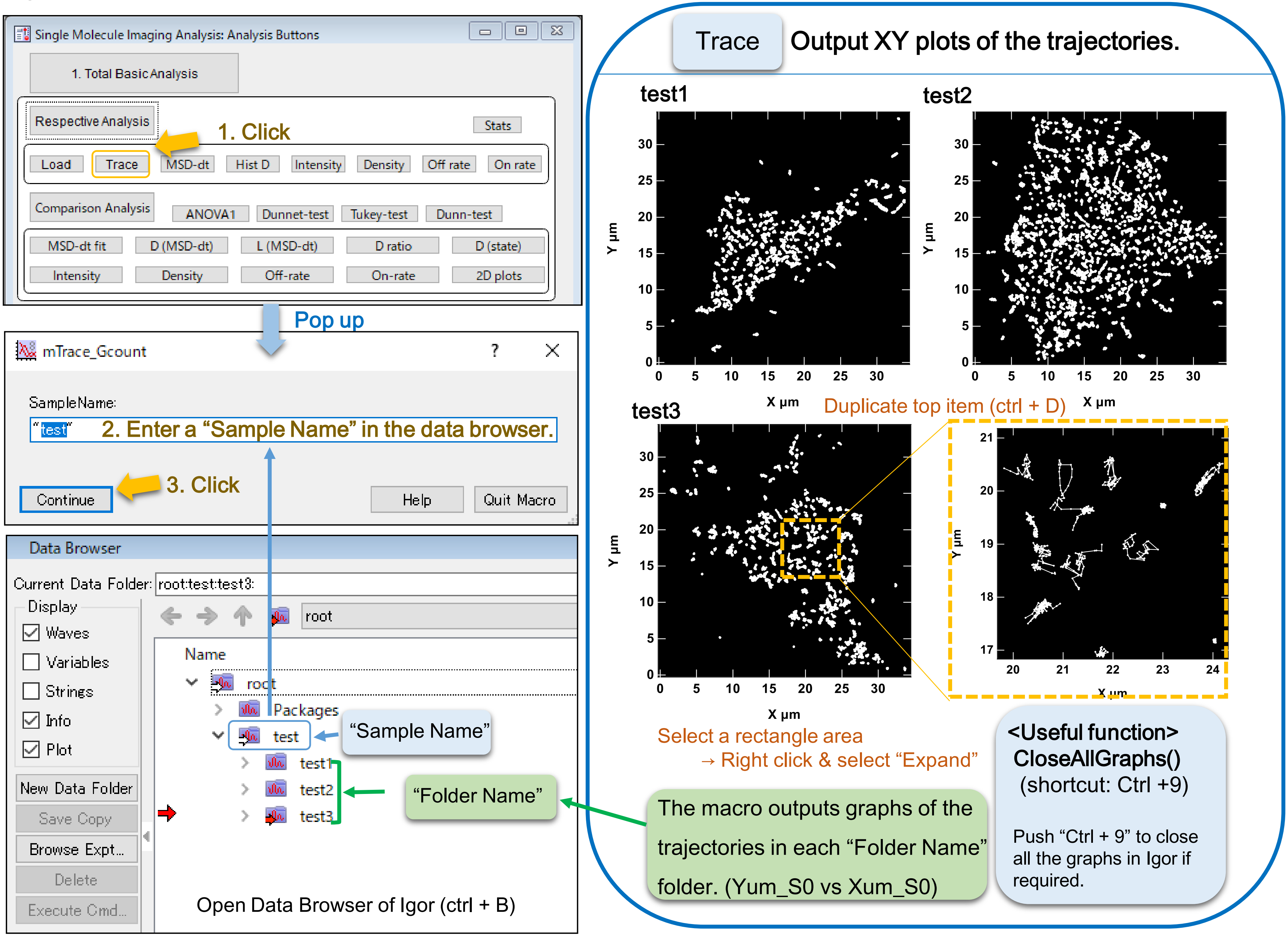


Fig. S3 How to use the “Trace” macro (HMM format)

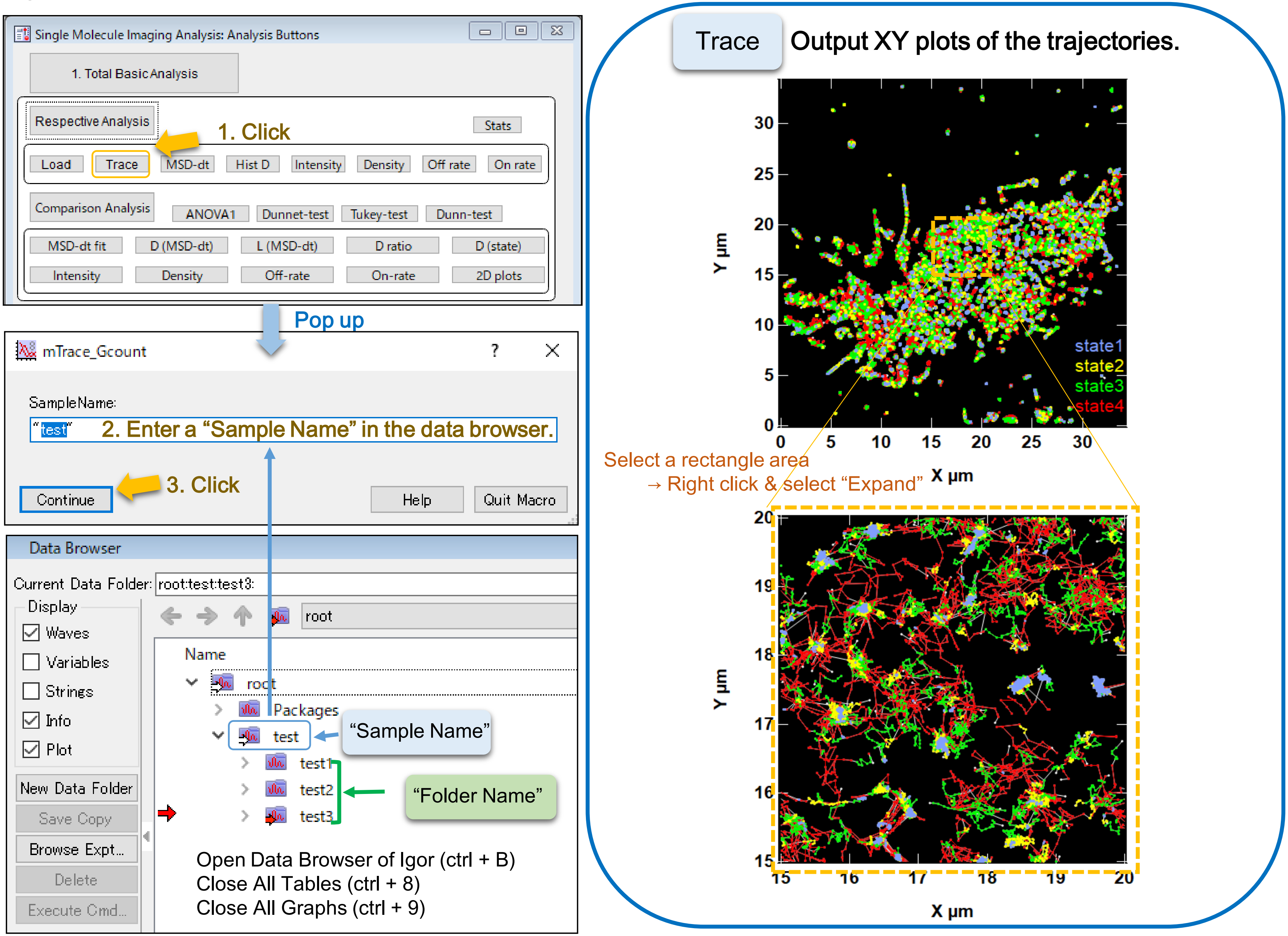


Fig. S4 How to use the “MSD-dt” macro

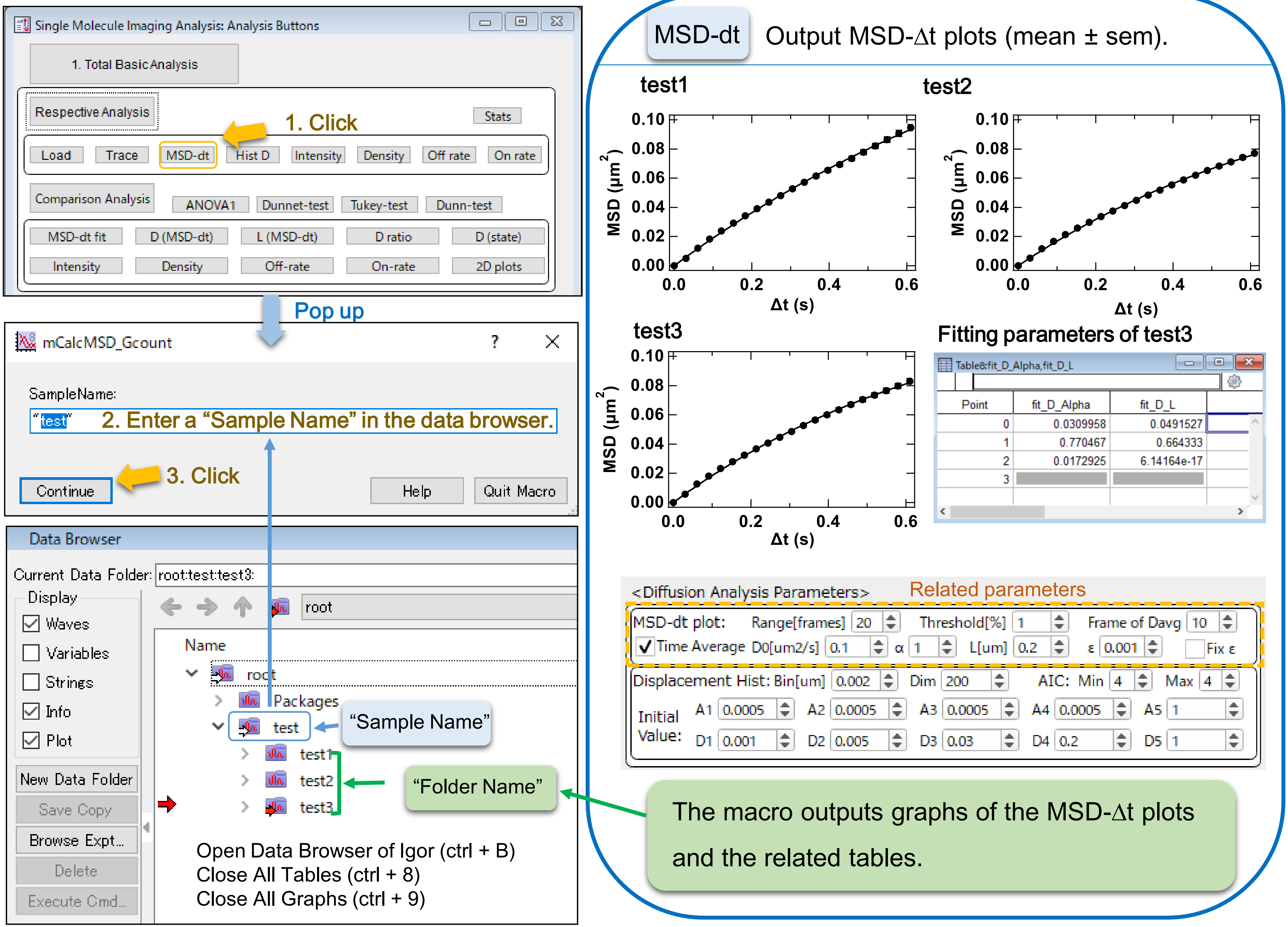


Fig. S5 Parameter settings of the “MSD-dt” macro

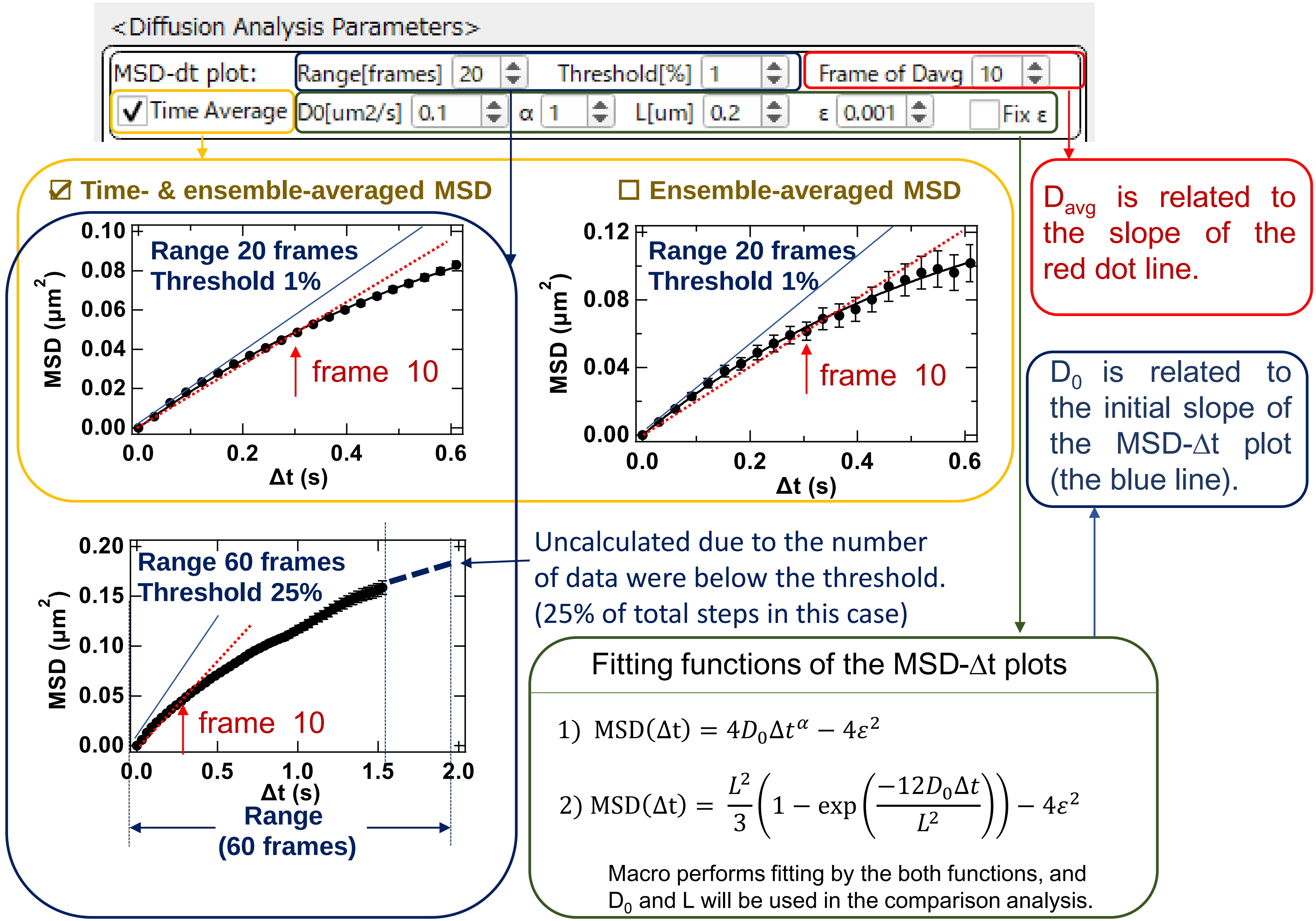


Fig. S6 How to use the “MSD-dt” macro (HMM format)

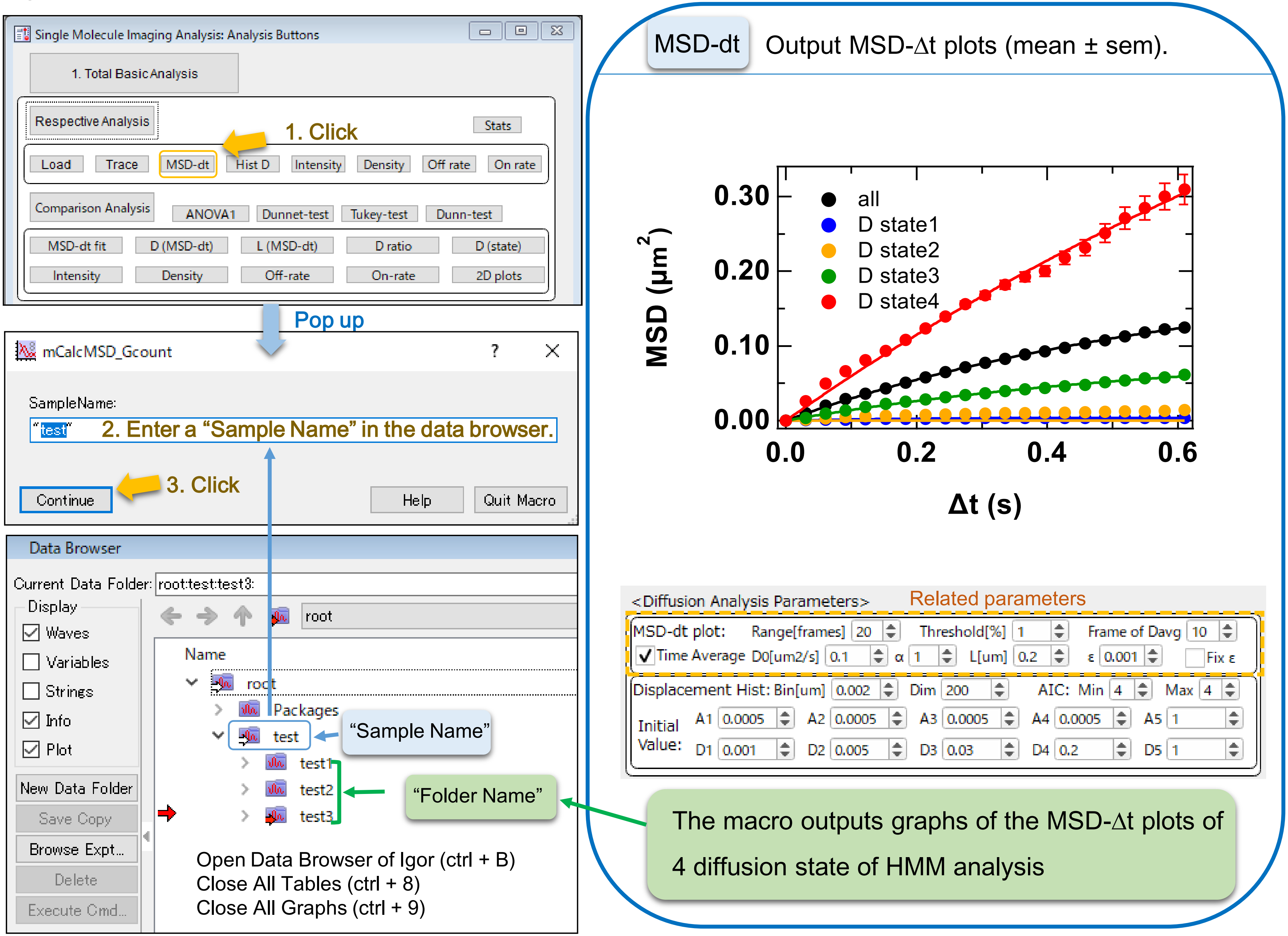


Fig. S7 How to use the “Hist D” macro

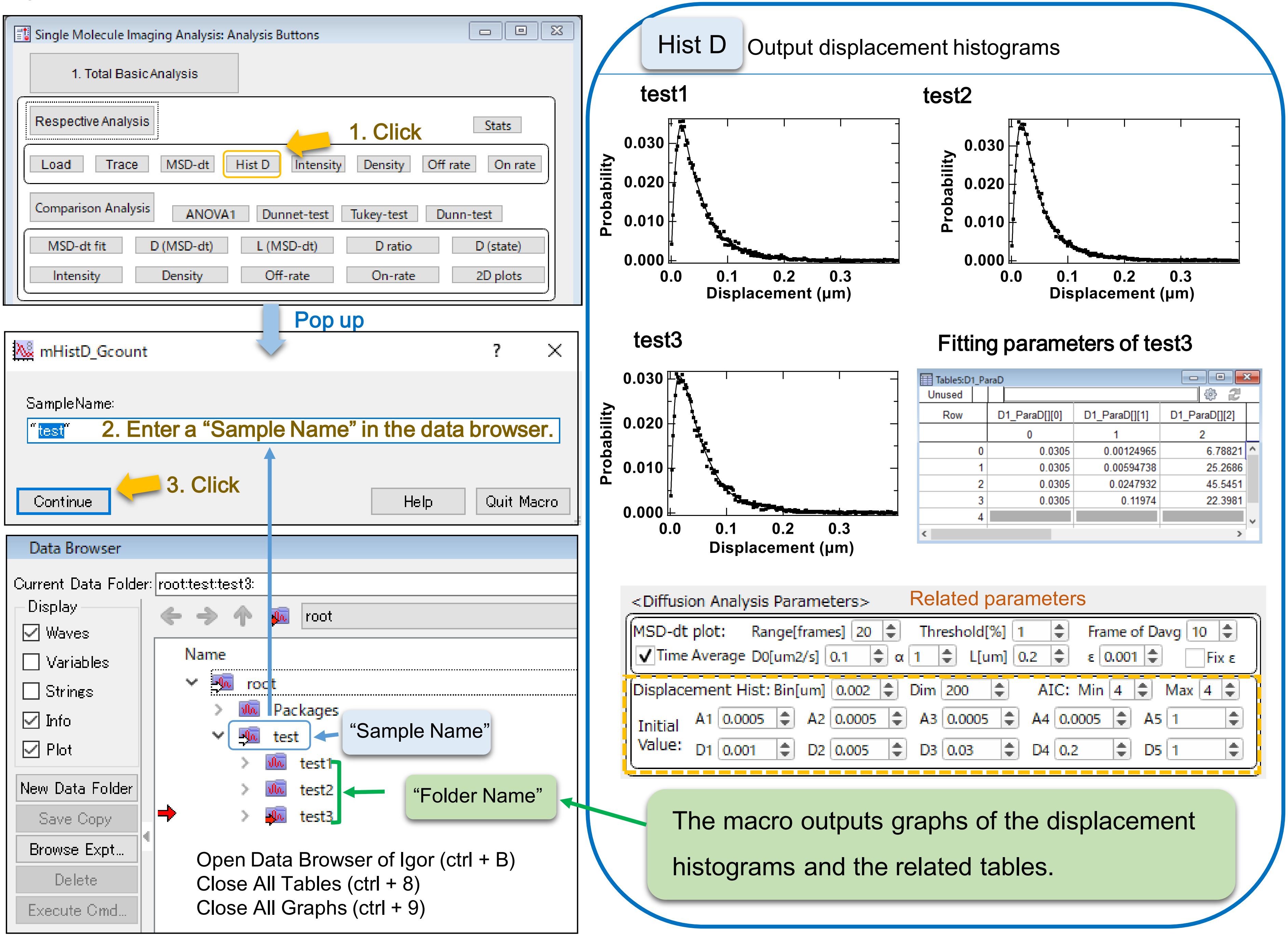
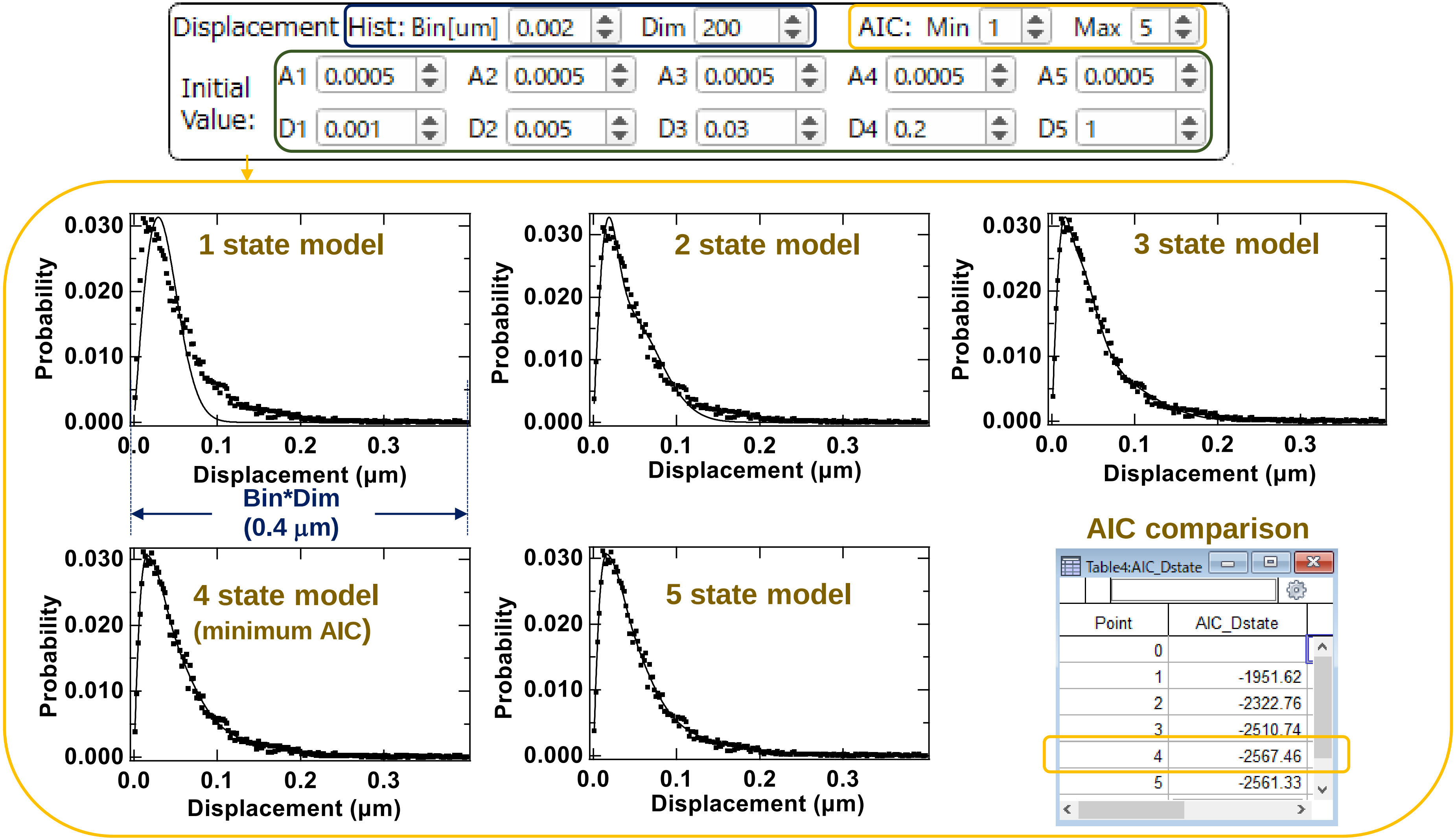


Fig. S8 Parameter settings of the “Hist D” macro



Fitting function of the displacement histogram

$$P(r) = \sum_{i=1}^{n} A_i \frac{r}{2D_i\Delta t} \exp\left(\frac{-r^2}{4D_i\Delta t}\right)$$

You can compare Min (1) to Max (5) state fitting models by AIC, but the fitting result is sensitive to the initial values. Check the fitting result of each model if the error message outputs as below .

```
error: singular matrix or other numeric error
```

Fig. S9 How to use the “Hist D” macro (HMM format)

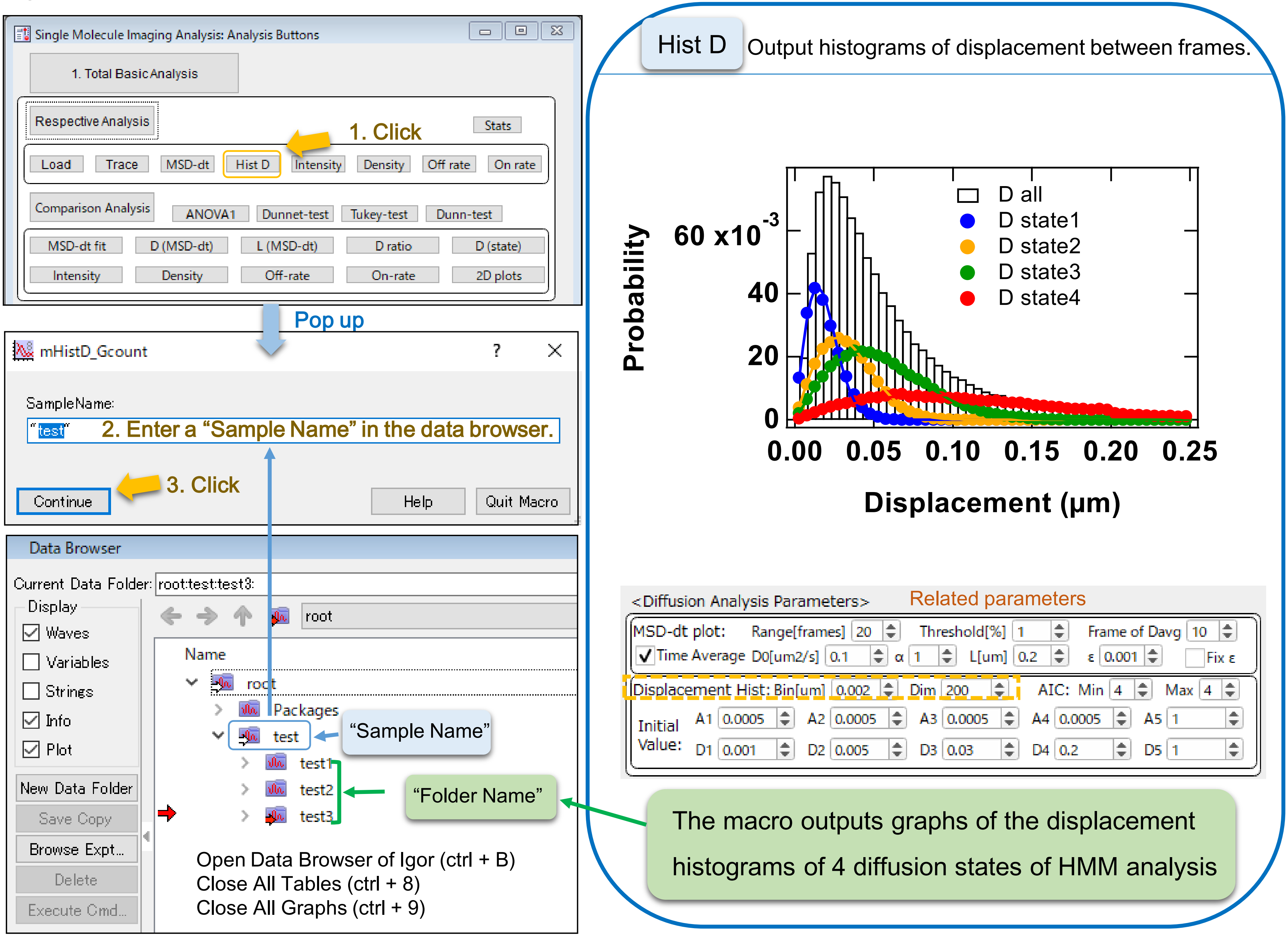


Fig. S10 How to use the “Intensity” macro

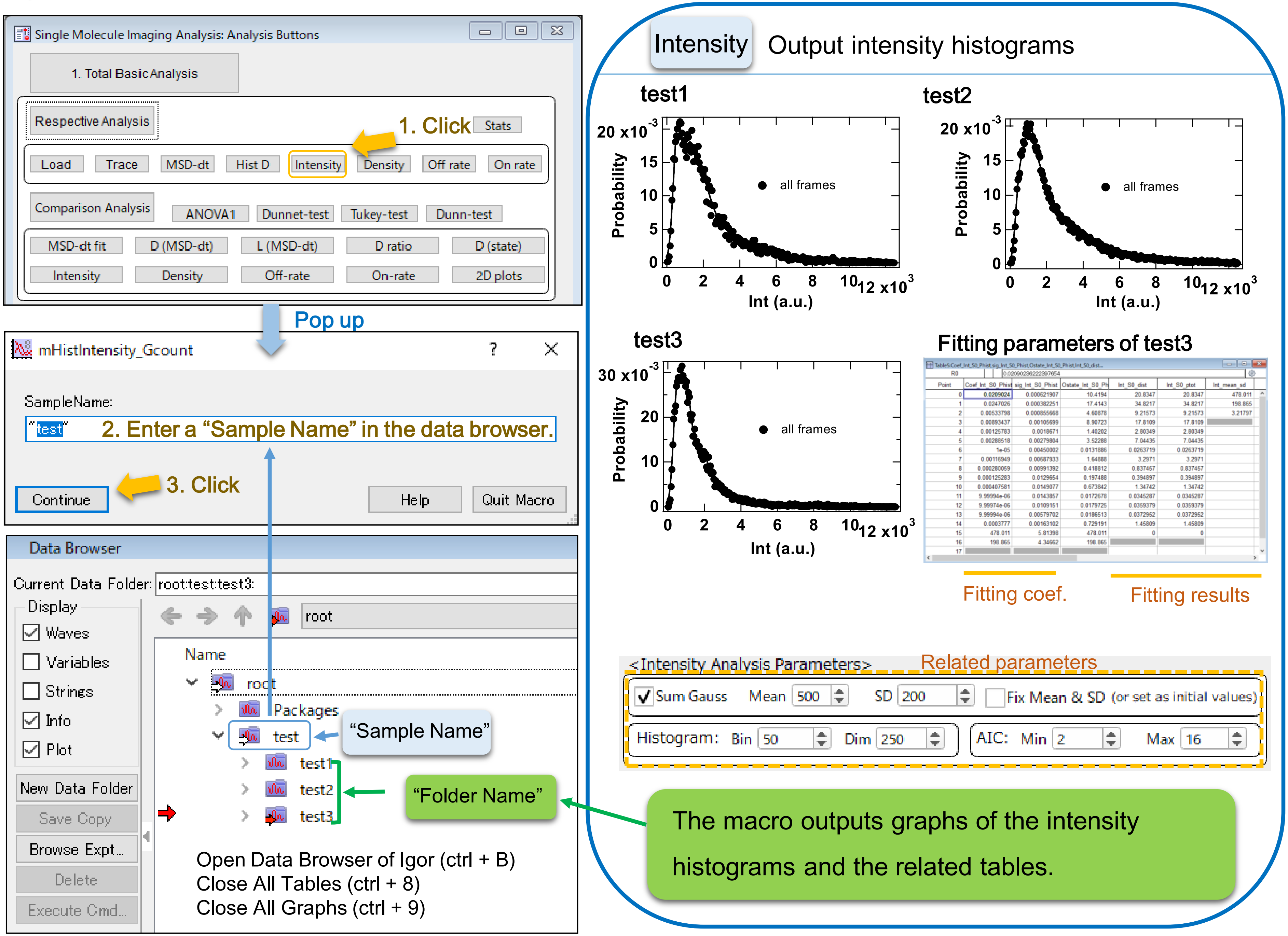
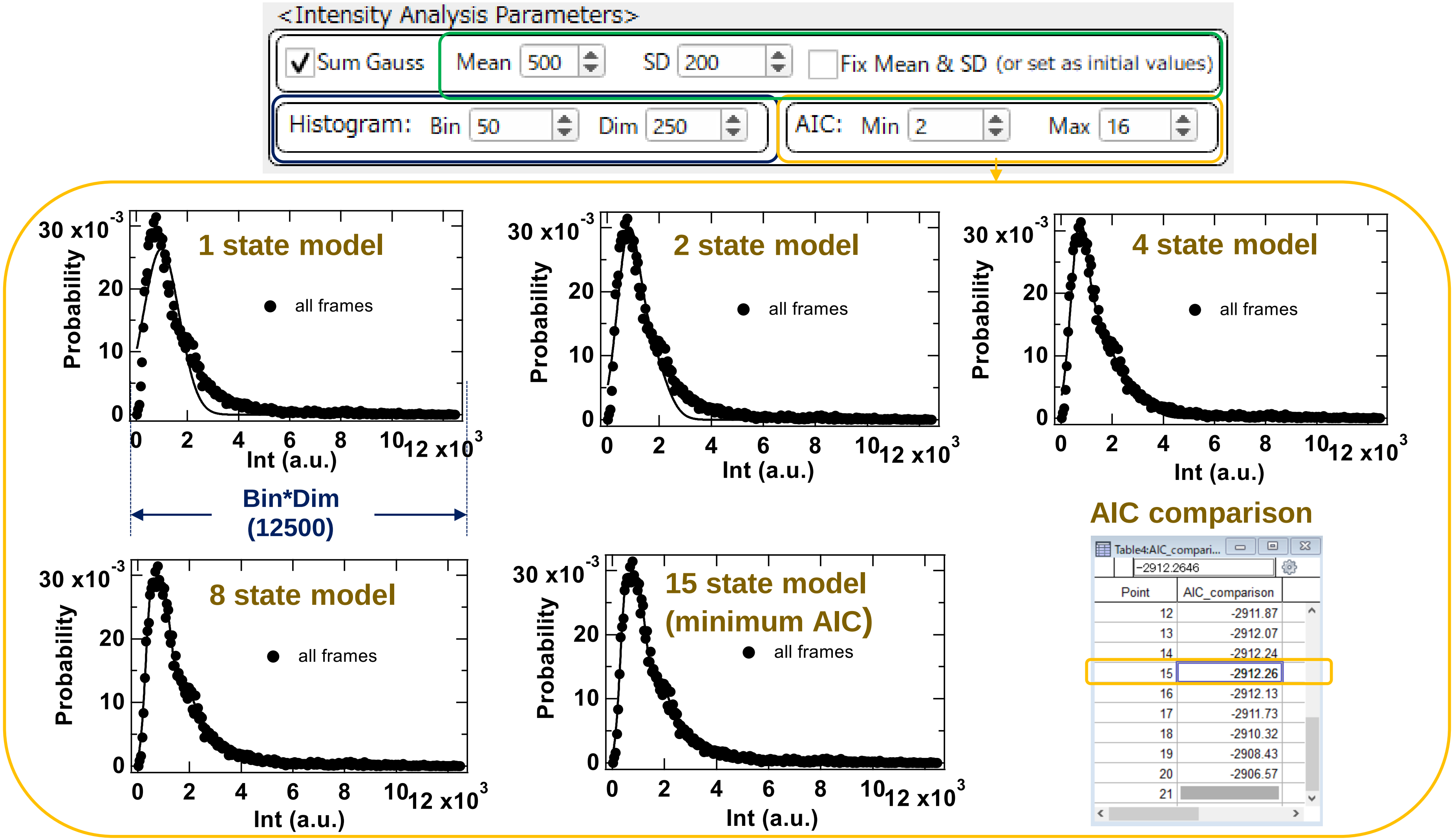


Fig. S11 Parameter settings of the “Intensity” macro



Fitting function of the intensity histogram

$$P(x) = \sum_{n=1}^{N} A_n \exp\left(-\frac{(x-nI)^2}{2n\sigma^2}\right)$$

You can compare Min (1) to Max (20) state fitting models by AIC. Check the fitting results to confirm the Mean and SD values are within the appropriate range as a single-molecule intensity.

The Mean and SD values are fixed to the input values when the checkbox is checked.

Fig. S12 How to use the “Intensity” macro (HMM format)

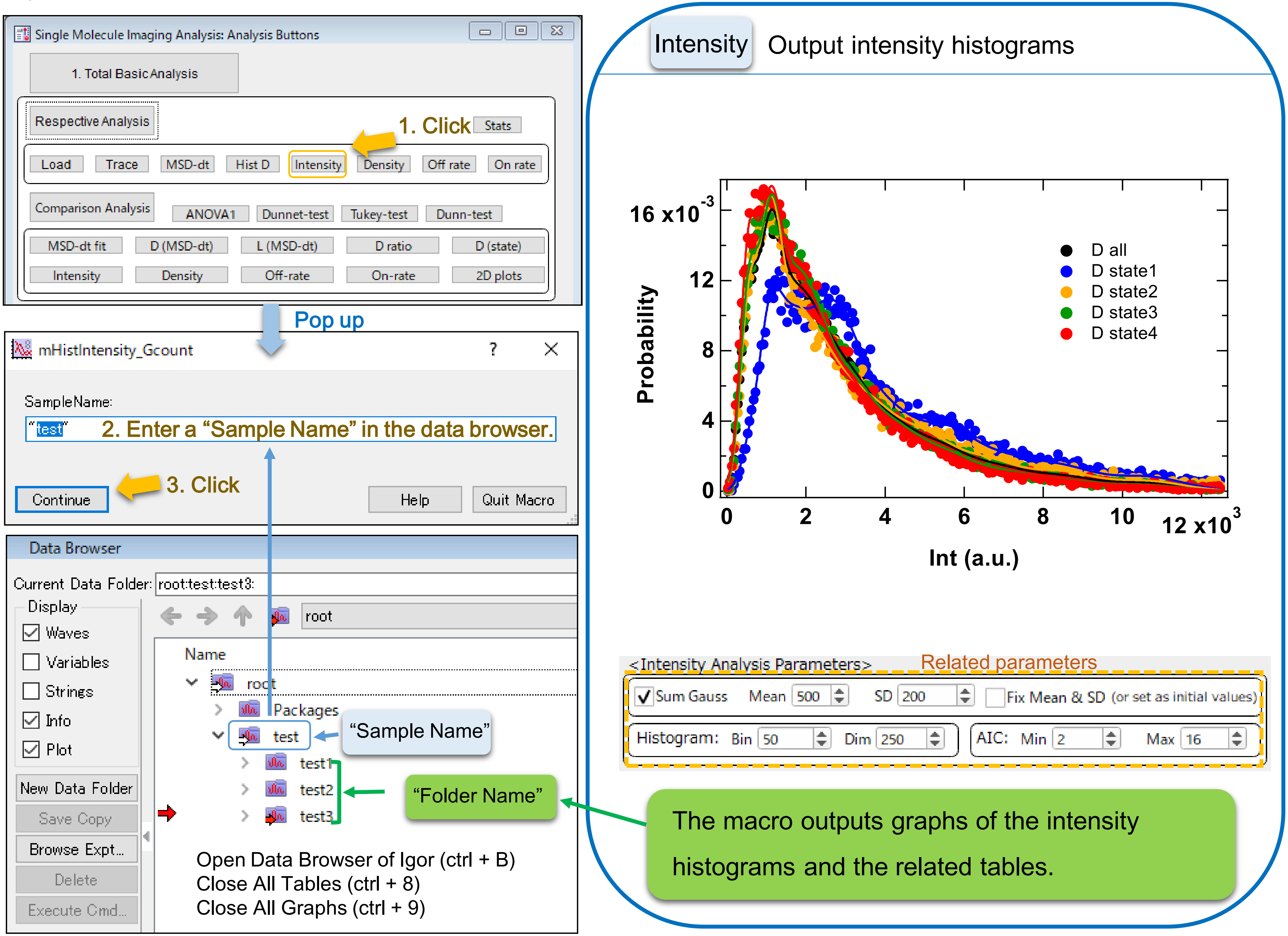


Fig. S13 How to use the "Density" macro

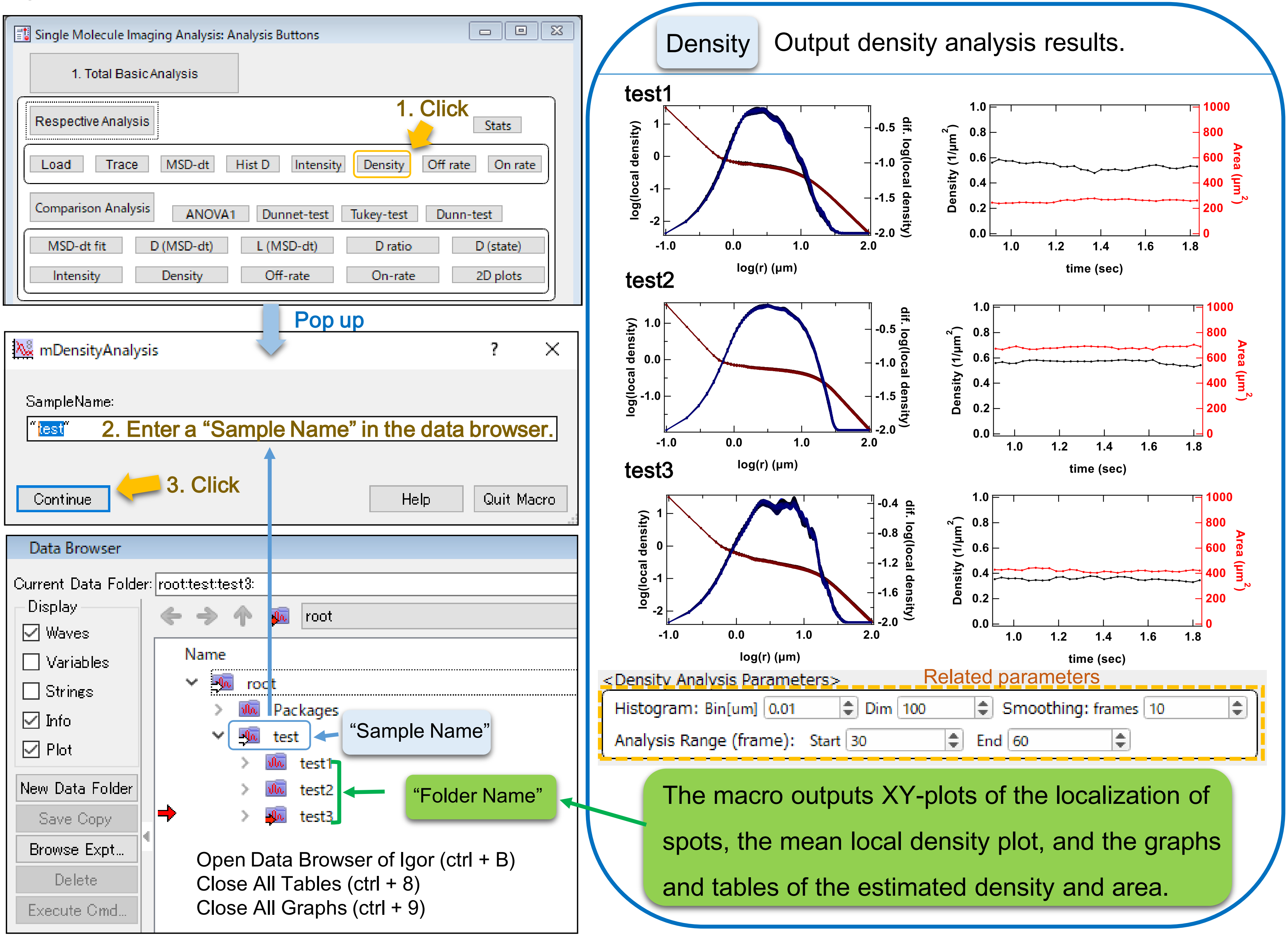
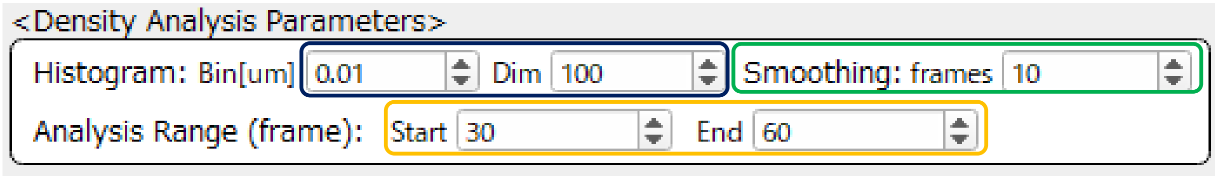
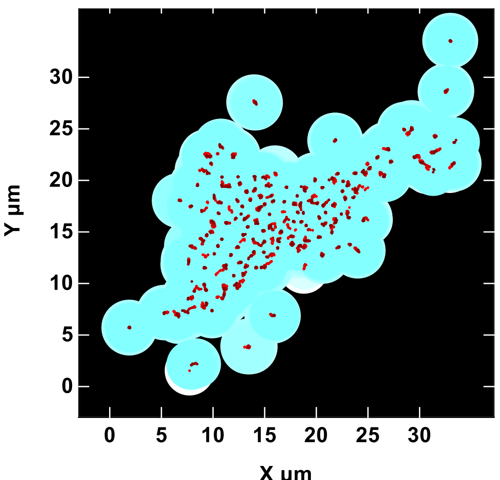


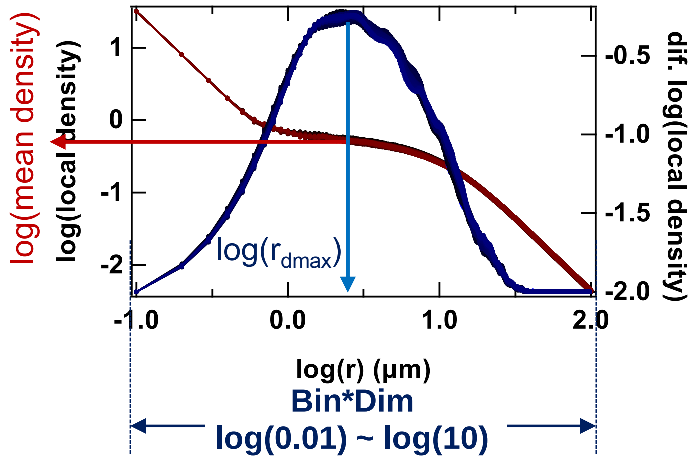
Fig. S14 Parameter settings of the “Density” macro



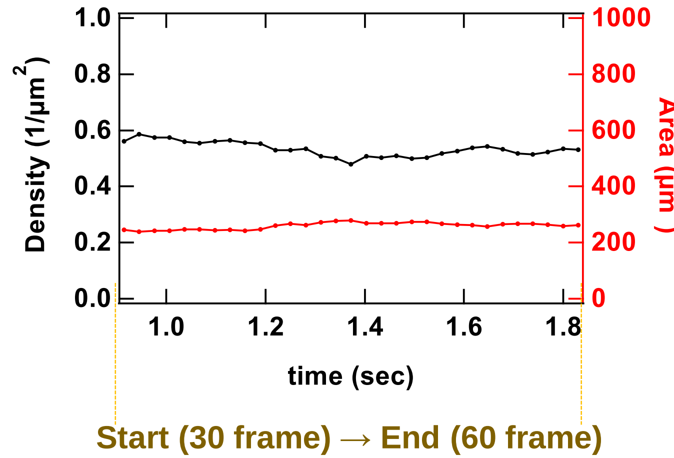
Single particle localization



Mean local density plots



Estimated density and area



<Red dots>
The particle localization within the selected frames (Start: 30 ~ End: 60) are plotted.

<Blue circles around the red dots>
Circles with radius (r_{dmax}) that gives the plateau of the mean local density curve (middle panel) are plotted around each localization.

<Red curves>
The mean local density around in the vicinity of r from each localization within a frame are plotted against r.

<Blue curves>
First-order derivative of the mean local density curves. The local density in the vicinity of r_{dmax}, which gives the peak, is adopted as the mean density of particles at each frame.

<Black plots>
The mean density at each frame are plotted against time.

<Red plots>
The area of cell region that was estimated from the mean density and particle number are plotted against time.

The mean local density function

$$d_{avg}(r) = \frac{1}{n}\sum_{i=1}^{n}\frac{N_{Pi}(r)}{\pi r^2}$$

($N_{Pi}(r)$: number of points N within a distance r of point i)

A parameter to find peak (r_{dmax}) in the middle panel.

Increase it if the peak detection was improper due to the noise.

Fig. S15 How to use the “Off rate” macro

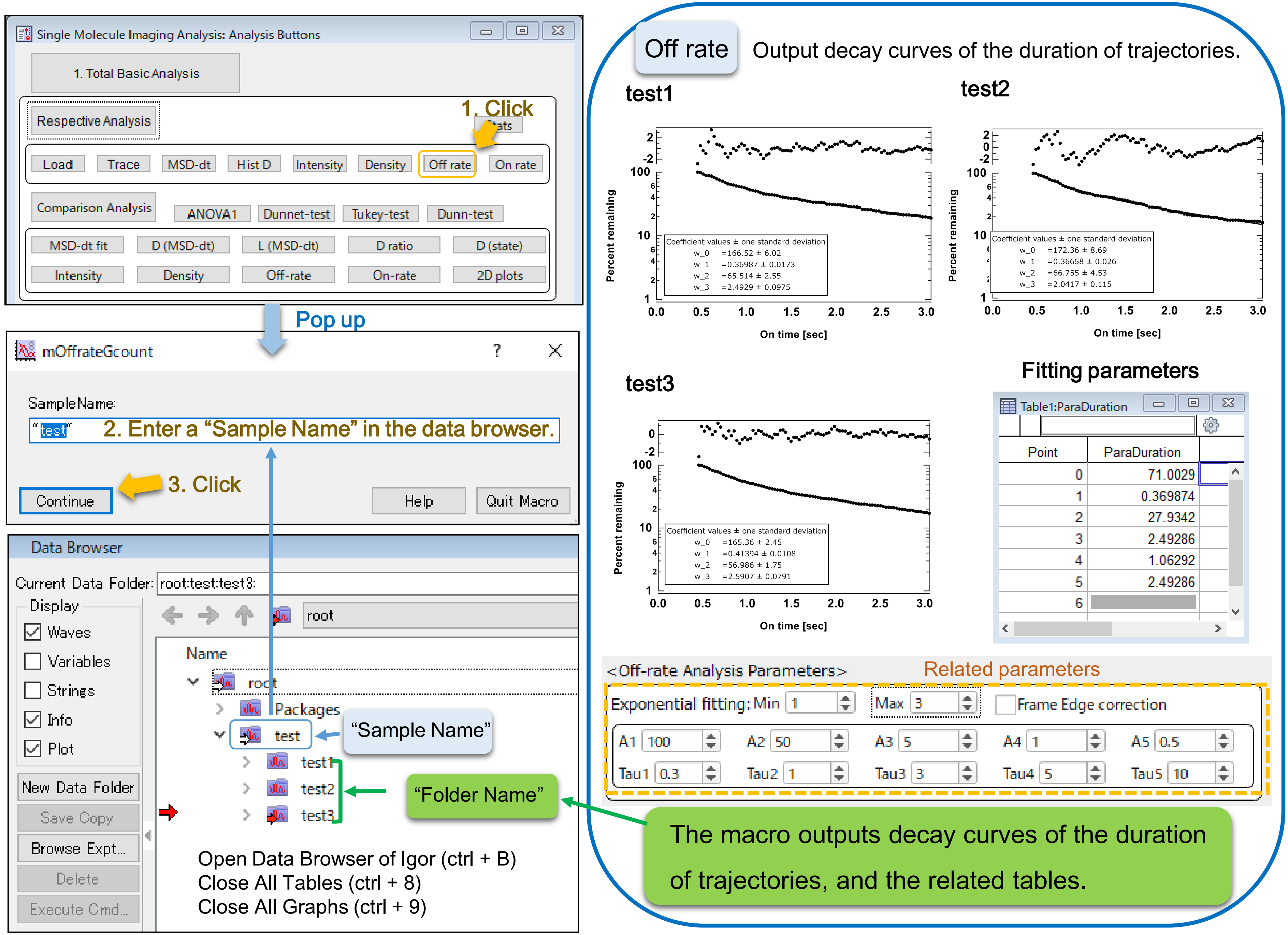
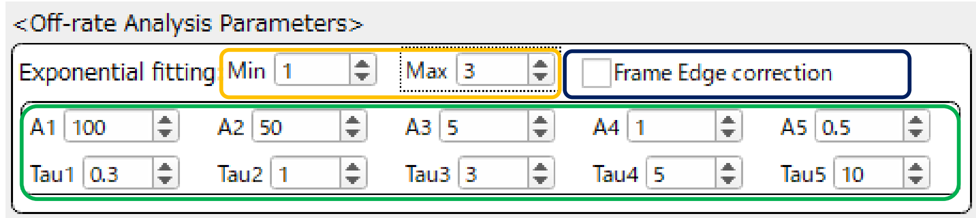
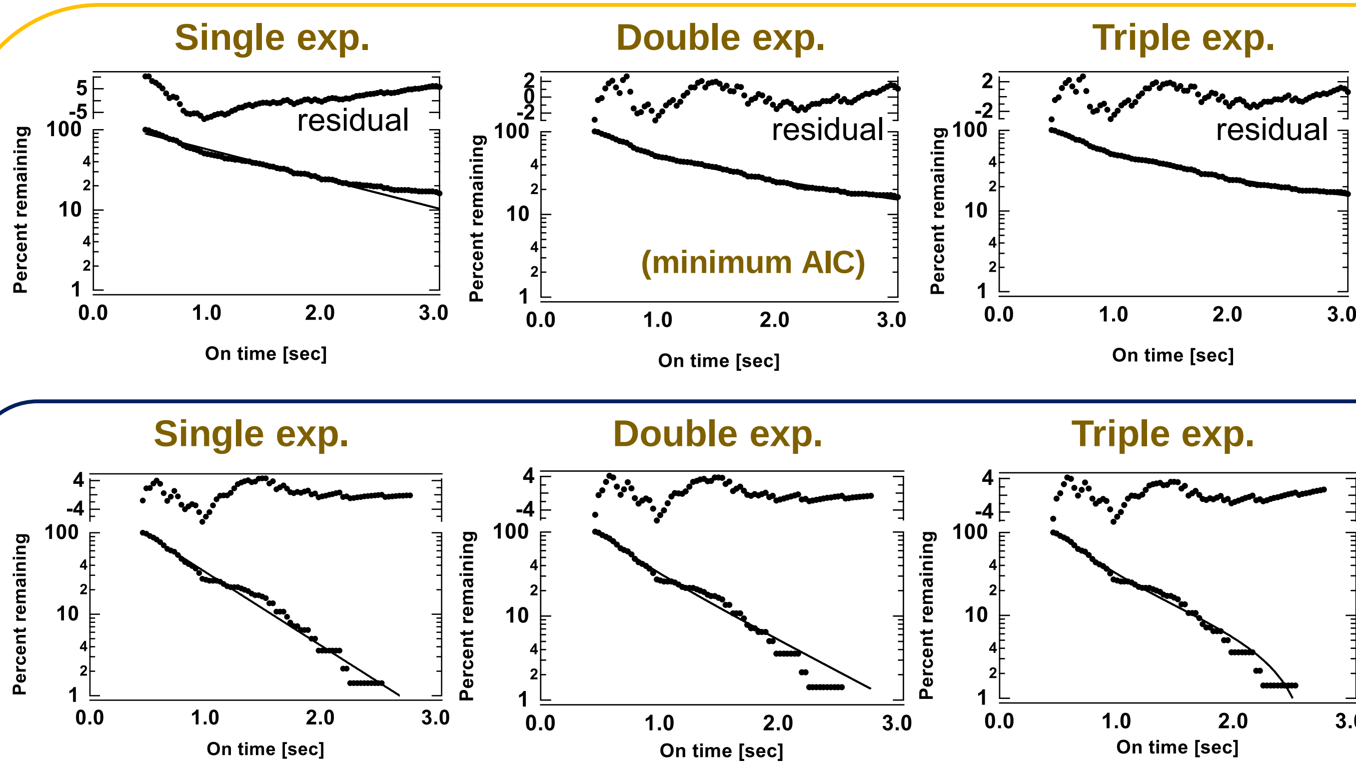
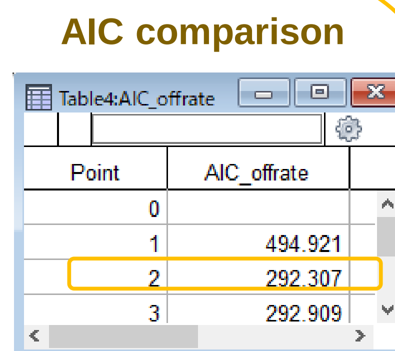


Fig. S16 Parameter settings of the “Off rate” macro







If the check box is checked, the plot is created except for the trajectories that start in the first frame or ends in the last frame.

Fitting function of the decay curve

$$P(t) = \sum_{\mathrm{i}=1}^{\mathrm{n}} A_i \exp\left(\frac{-t}{\tau_{\mathrm{i}}}\right)$$

You can compare Min (1) to Max (5) state fitting models by AIC, but the fitting result is sensitive to the initial values. Check the fitting result of each model if the error message outputs as below .

```
error: singular matrix or other numeric error
```

Fig. S17 How to use the “On rate” macro

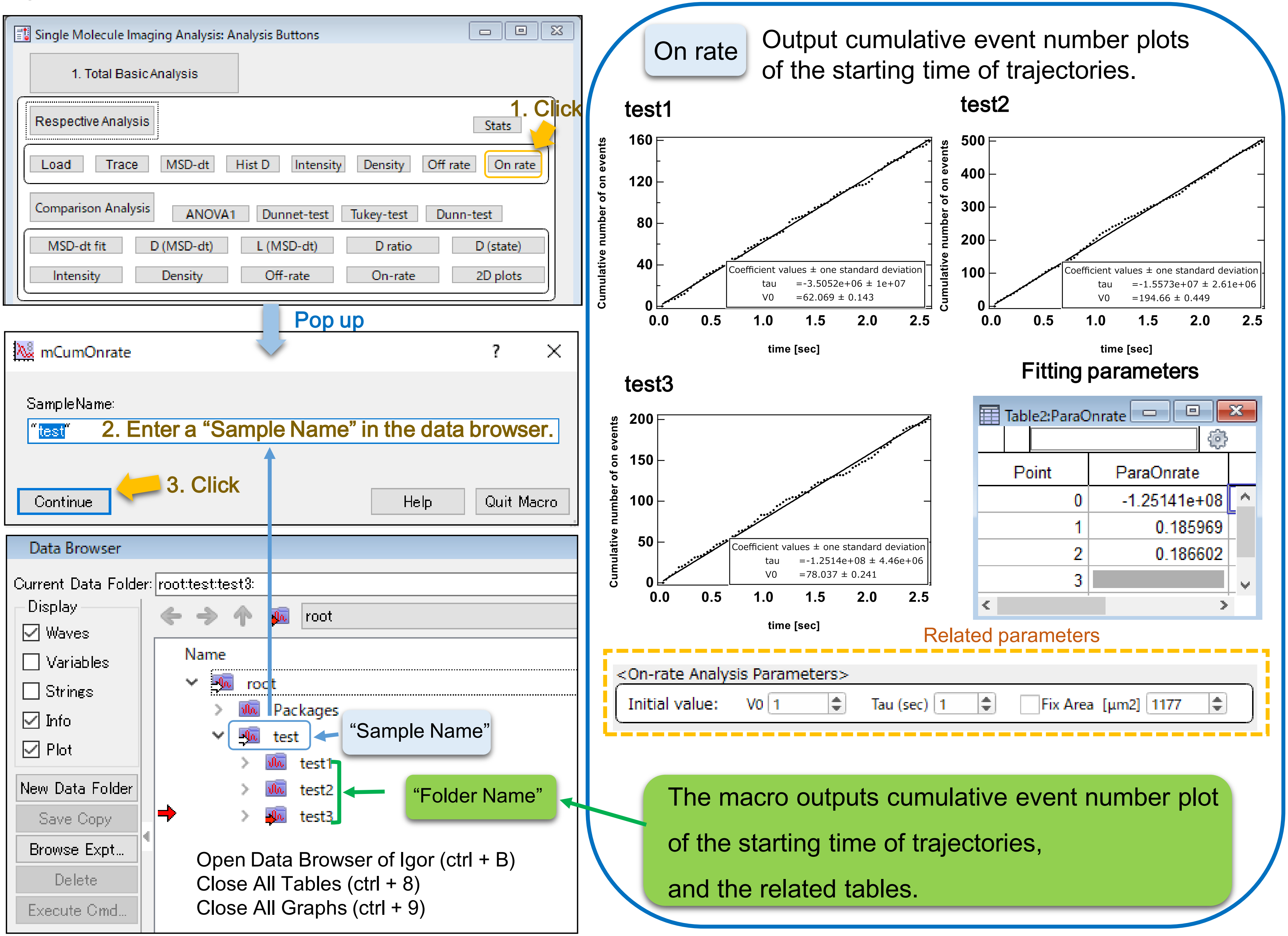
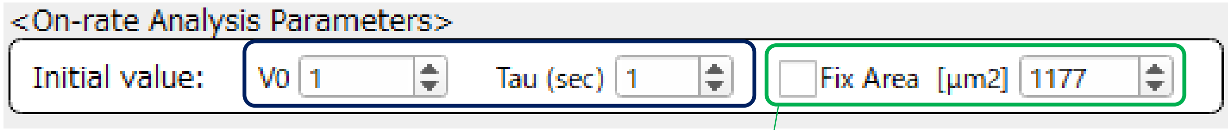
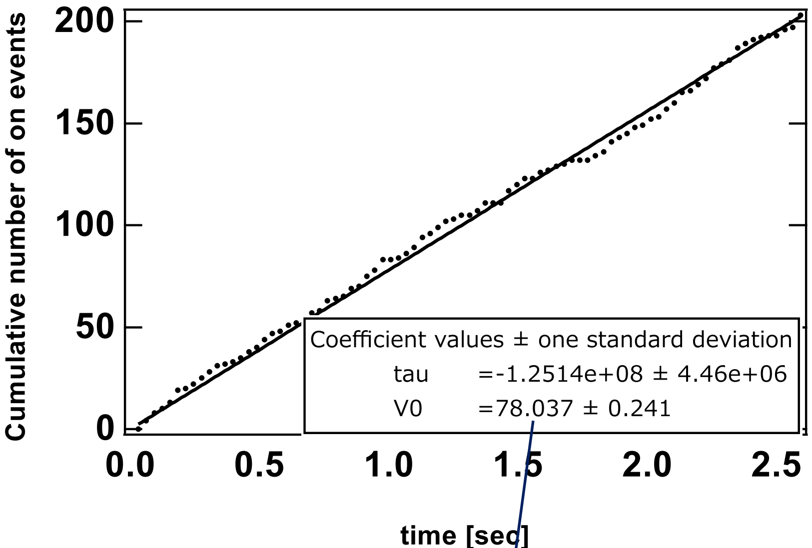


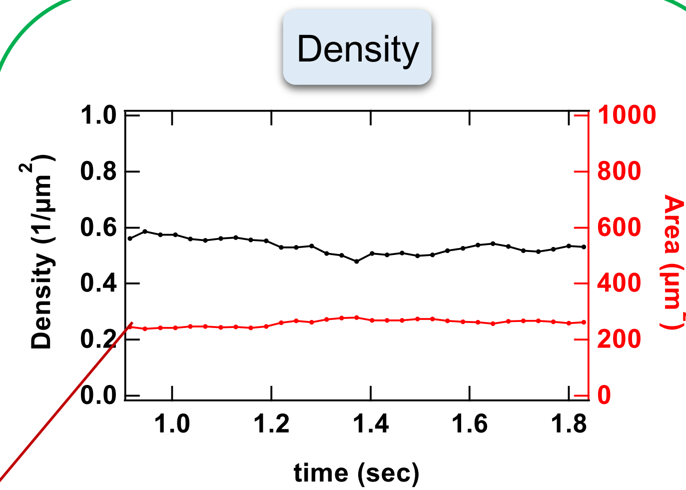
Fig. S18 Parameter settings of the "On rate" macro



On-rate analysis should be done after the density analysis



$$\text{on-rate} = V_0 \,/\, \text{Area}_{\text{cell}}$$



If the check box is unchecked, the $\text{Area}_{\text{cell}}$ estimated from the density analysis is used for the calculation of the on-rate.

If checked, the input value is used for the calculation of the on-rate.

Fitting function of the plot

$$f(t) = V_0\left(1 - \exp\left(\frac{-t}{tau}\right)\right)$$

V0 is the initial slope of the cumulative event number plot.

Tau is required when the cumulative event number plot is not linear due to the photo bleaching.

Fig. S19 How to use the "Stats" macro

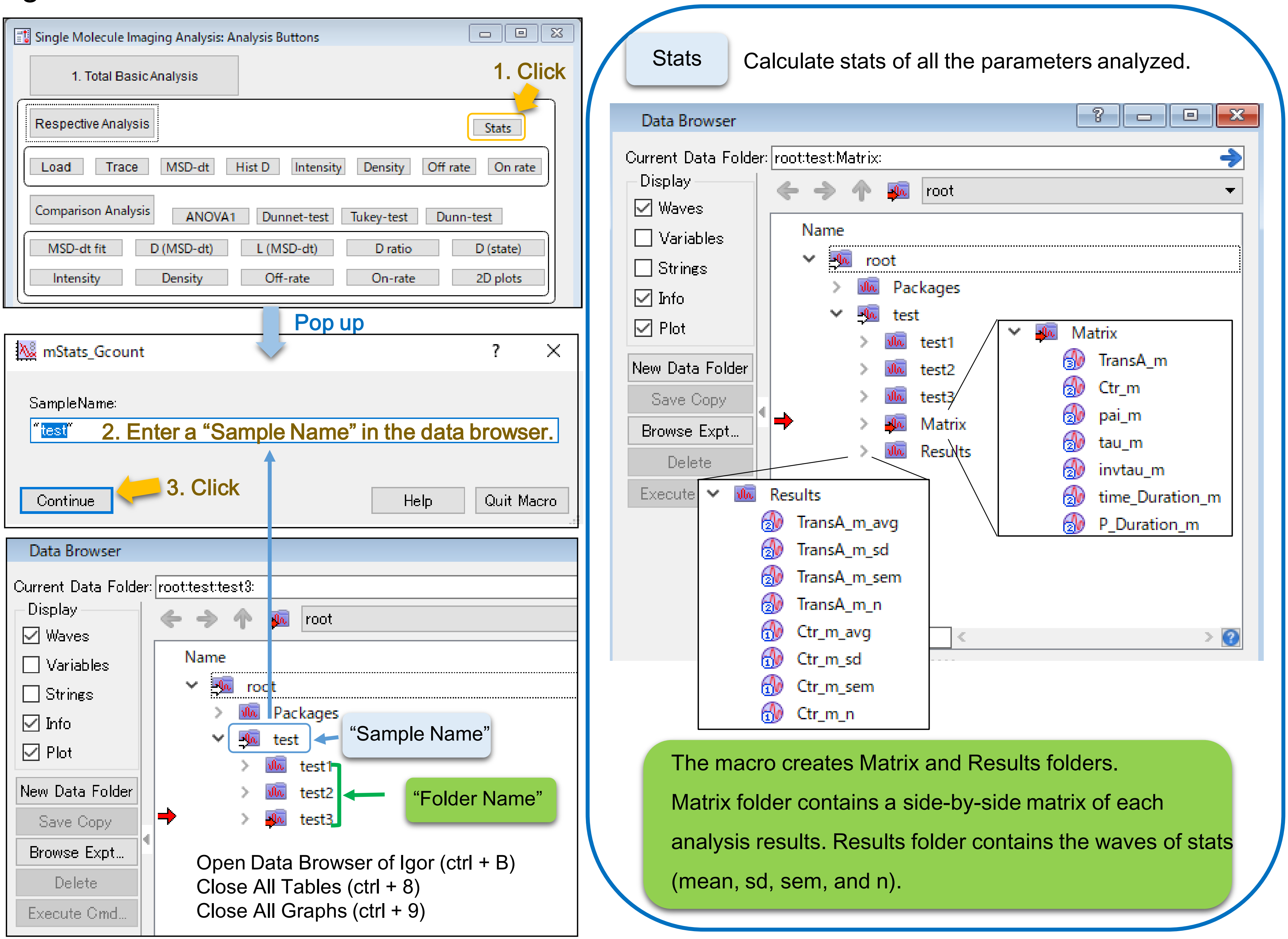
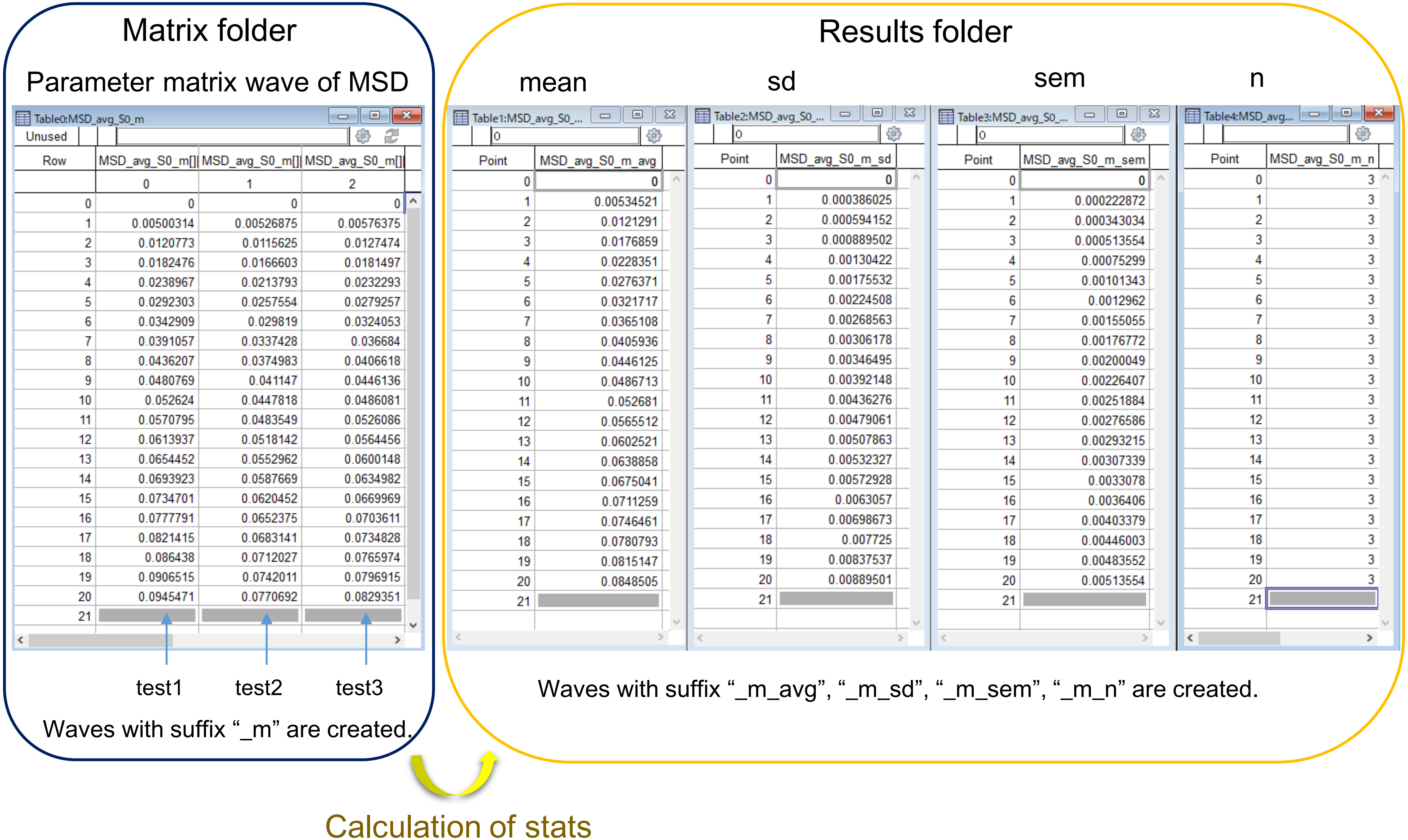


Fig. S20 Example waves in the Matrix and Results folders



Row	MSD_avg_S0_m[][0]	MSD_avg_S0_m[][1]	MSD_avg_S0_m[][2]
	0	1	2
0	0	0	0
1	0.00500314	0.00526875	0.00576375
2	0.0120773	0.0115625	0.0127474
3	0.0182476	0.0166603	0.0181497
4	0.0238967	0.0213793	0.0232293
5	0.0292303	0.0257554	0.0279257
6	0.0342909	0.029819	0.0324053
7	0.0391057	0.0337428	0.036684
8	0.0436207	0.0374983	0.0406618
9	0.0480769	0.041147	0.0446136
10	0.052624	0.0447818	0.0486081
11	0.0570795	0.0483549	0.0526086
12	0.0613937	0.0518142	0.0564456
13	0.0654452	0.0552962	0.0600148
14	0.0693923	0.0587669	0.0634982
15	0.0734701	0.0620452	0.0669969
16	0.0777791	0.0652375	0.0703611
17	0.0821415	0.0683141	0.0734828
18	0.086438	0.0712027	0.0765974
19	0.0906515	0.0742011	0.0796915
20	0.0945471	0.0770692	0.0829351
21			

Point	MSD_avg_S0_m_avg	MSD_avg_S0_m_sd	MSD_avg_S0_m_sem	MSD_avg_S0_m_n
0	0	0	0	3
1	0.00534521	0.000386025	0.000222872	3
2	0.0121291	0.000594152	0.000343034	3
3	0.0176859	0.000889502	0.000513554	3
4	0.0228351	0.00130422	0.00075299	3
5	0.0276371	0.00175532	0.00101343	3
6	0.0321717	0.00224508	0.0012962	3
7	0.0365108	0.00268563	0.00155055	3
8	0.0405936	0.00306178	0.00176772	3
9	0.0446125	0.00346495	0.00200049	3
10	0.0486713	0.00392148	0.00226407	3
11	0.052681	0.00436276	0.00251884	3
12	0.0565512	0.00479061	0.00276586	3
13	0.0602521	0.00507863	0.00293215	3
14	0.0638858	0.00532327	0.00307339	3
15	0.0675041	0.00572928	0.0033078	3
16	0.0711259	0.0063057	0.0036406	3
17	0.0746461	0.00698673	0.00403379	3
18	0.0780793	0.007725	0.00446003	3
19	0.0815147	0.00837537	0.00483552	3
20	0.0848505	0.00889501	0.00513554	3
21				

Note: At least 3 data are required to use the stats macro.

Fig. S21 Work flow of the “Comparison Analysis” and “Multiple comparison test” macros

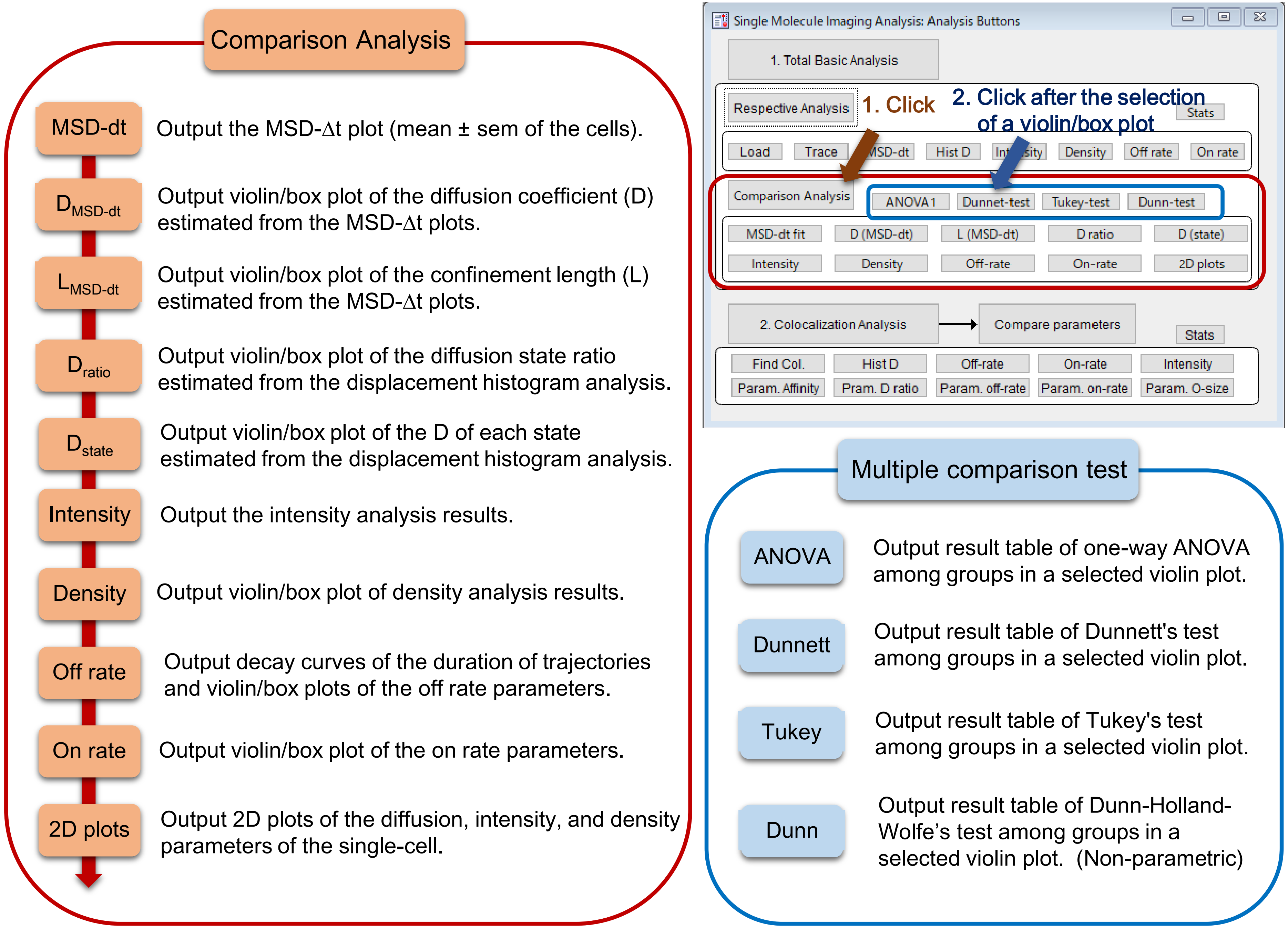
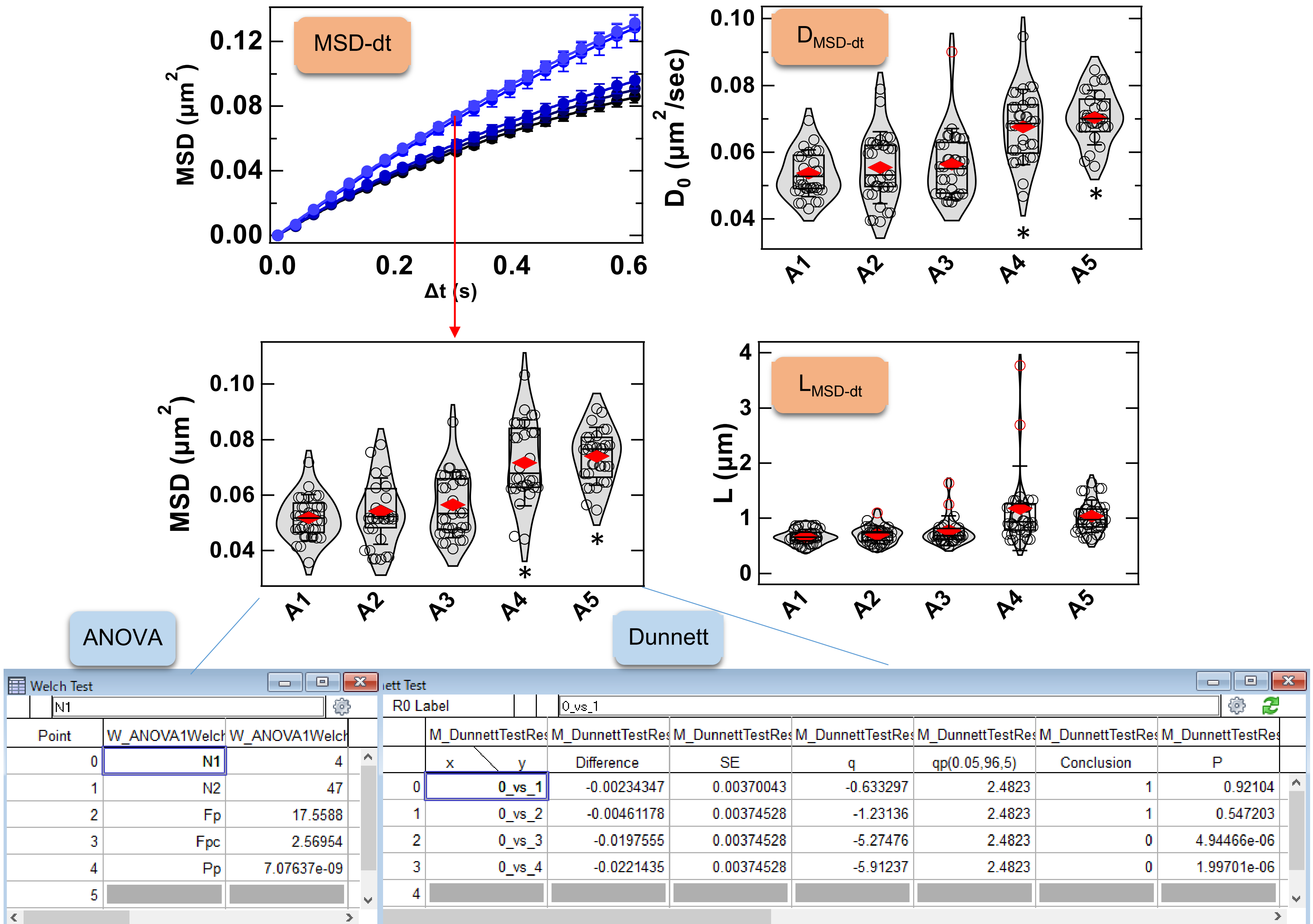


Fig. S22 Example of the violin/box plots (MSD-Δt plot analysis) and the multiple comparison test



Point	W_ANOVA1Welch	W_ANOVA1Welch
0	N1	4
1	N2	47
2	Fp	17.5588
3	Fpc	2.56954
4	Pp	7.07637e-09
5		

R0 Label	M_DunnettTestRes x y	M_DunnettTestRes Difference	M_DunnettTestRes SE	M_DunnettTestRes q	M_DunnettTestRes qp(0.05,96,5)	M_DunnettTestRes Conclusion	M_DunnettTestRes P
0	0_vs_1	-0.00234347	0.00370043	-0.633297	2.4823	1	0.92104
1	0_vs_2	-0.00461178	0.00374528	-1.23136	2.4823	1	0.547203
2	0_vs_3	-0.0197555	0.00374528	-5.27476	2.4823	0	4.94466e-06
3	0_vs_4	-0.0221435	0.00374528	-5.91237	2.4823	0	1.99701e-06
4							