Optical Tweezers image processing guide

Installing ImageJ

In this work, a free program called ImageJ (available for download at http://rsb.info.nih.gov/ij/) can be used for image processing and analysis. In order to extract the trajectories of the particles, a separate plug-in (MOASIC) has to be installed in ImageJ, which can be found with installation instructions at http://mosaic.mpi-cbg.de/?q=downloads/imageJ. More detailed instructions on the use and the meaning of various parameters of MOASIC can be found on the website.

There is also a pre-packaged version of ImageJ available, called Fiji (http://fiji.sc/Fiji), that I would recommend using instead of the original Image J. Fiji comes with a bunch of plug-ins but the ParticleTracker for extracting particle trajectories is not pre-installed. Here is how to install it:
1. **Help -> Update Fiji**
2. **Manage update sites ->** choose **"MOSAIC ToolSuite"** -> click **Close**
3. **"plugins/Mosaic_ToolSuite/Mosaic_ToolSuite.jar"** should be visible on the list of updates,and after clicking the **"Apply Changes"**-button, Fiji installs the chosen package.
4. Restart Fiji, after which the ParticleTracker-plugin should be found in **Plugins -> Mosaic -> Particle Tracker 2D/3D** –menu.

Determining the motion of the glass spheres

1. Open a series of images you have saved with **File -> Open**
2. In the **Image -> Properties** -menu, change the values of the "slices" and "frames" fields.
3. The contrast of the image can be improved by using the tools found in the Image -> Adjust -> Brightness/Contrast menu. The purpose is to make the particles look approximately the same as in Figure 1. Try also the subtract background function (Process -> Subtract Background)
4. To speed up the extraction of the particle trajectories, it is a good idea to crop the area around the trapped particle to approx. 80x80 pixels from the original video by selecting the desired area and selecting **Image -> Crop**

Finally, the video should look roughly like the one in the lower right corner of Figure 1 and locating the coordinates of the particle can be started.

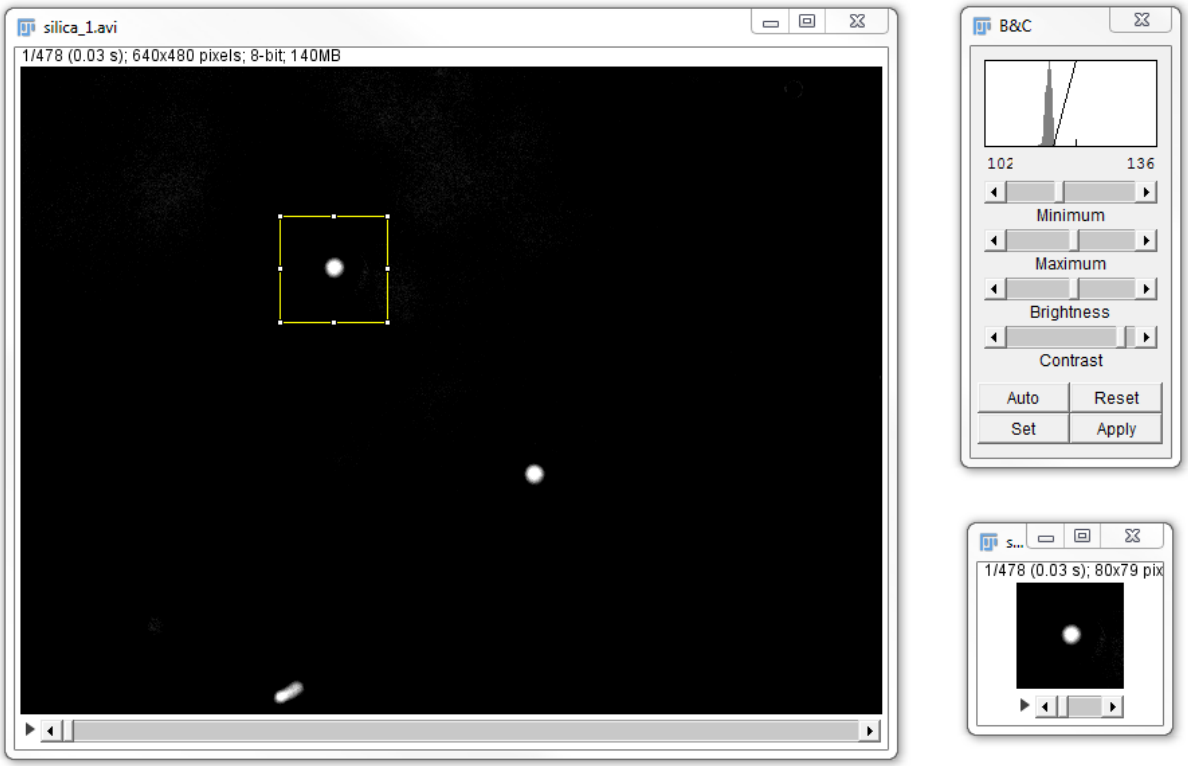


Figure 1

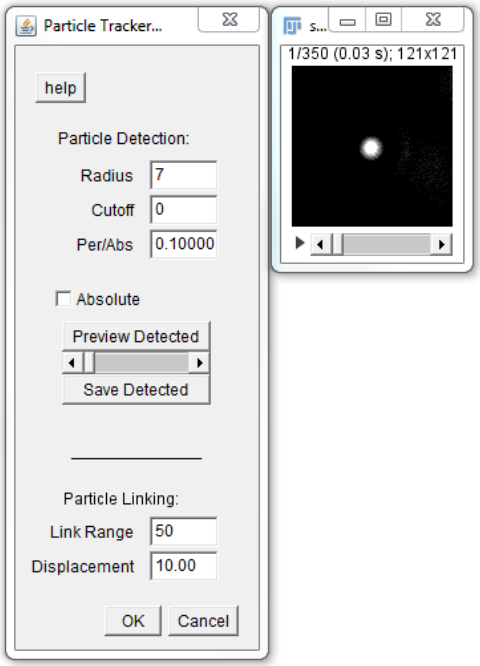
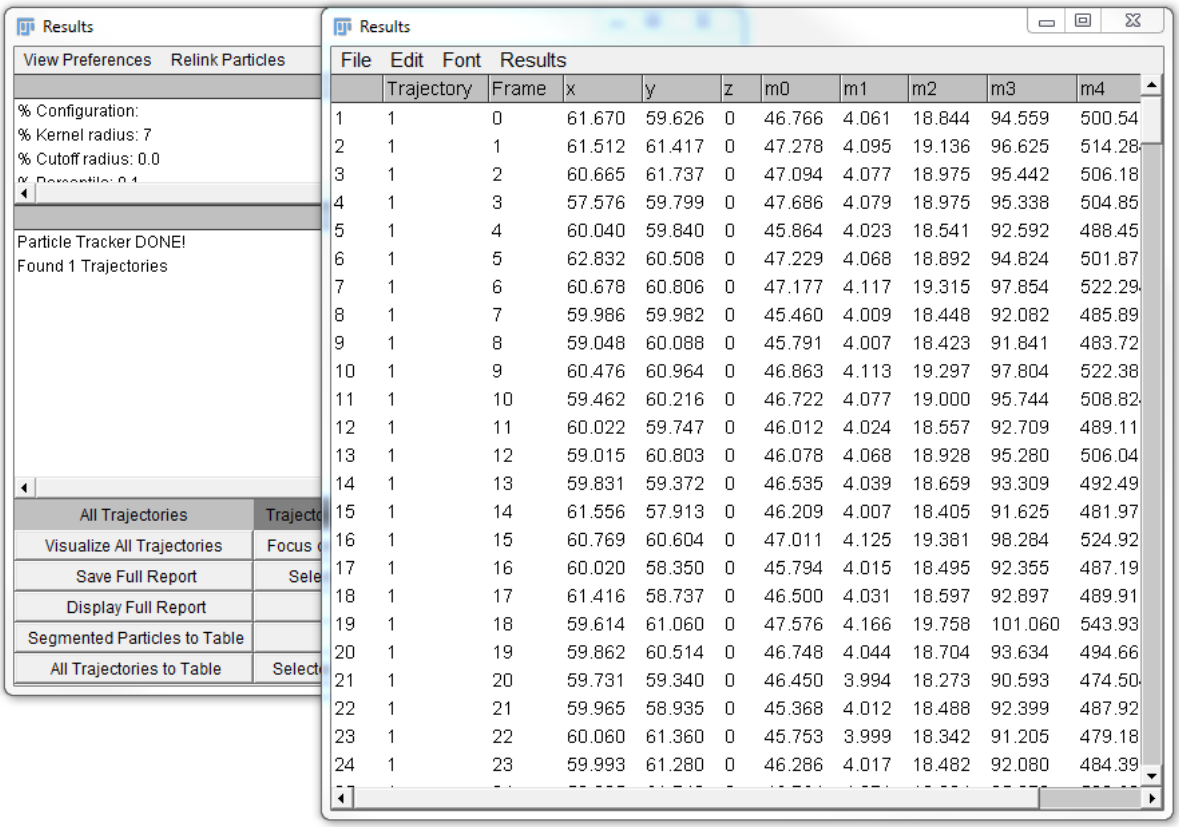


Figure 2

5. Choose **Plugins -> Mosaic -> Particle Tracker 2D/3D**, which opens a window similar to the one shown in Figure 2. The most important parameter to be defined is the radius of the particle, which should be about 7-8 pixels. Set the **"Cutoff"**-value to zero, **"Per/Abs"** between 0.1-0.5 and **"Link Range"** between 50-100. If the contrast in the video is good, then the exact values of these parameters are irrelevant for determining the center of the particle. By clicking the **"Preview Detected"**-button, you can check if there are one or more particles in the image.
6. By pressing the **"OK"**-button, the ParticleTracker will go through the entire series of images and displays a summary of the results shown in Figure 3. If the ParticleTracker finds more than one particle trajectory, then the settings should be changed until only one trajectory remains.
7. By choosing **"All Trajectories to Table"** the coordinates of the particle will be displayed.
8. Choose **File -> Save as** the results can be saved as a text file, which can then be loaded into Matlab (or any other program) for processing the results.



	Trajectory	Frame	x	y	z	m0	m1	m2	m3	m4
1	1	0	61.670	59.626	0	46.766	4.061	18.844	94.559	500.54
2	1	1	61.512	61.417	0	47.278	4.095	19.136	96.625	514.28
3	1	2	60.665	61.737	0	47.094	4.077	18.975	95.442	506.18
4	1	3	57.576	59.799	0	47.686	4.079	18.975	95.338	504.85
5	1	4	60.040	59.840	0	45.864	4.023	18.541	92.592	488.45
6	1	5	62.832	60.508	0	47.229	4.068	18.892	94.824	501.87
7	1	6	60.678	60.806	0	47.177	4.117	19.315	97.854	522.29
8	1	7	59.986	59.982	0	45.460	4.009	18.448	92.082	485.89
9	1	8	59.048	60.088	0	45.791	4.007	18.423	91.841	483.72
10	1	9	60.476	60.964	0	46.863	4.113	19.297	97.804	522.38
11	1	10	59.462	60.216	0	46.722	4.077	19.000	95.744	508.82
12	1	11	60.022	59.747	0	46.012	4.024	18.557	92.709	489.11
13	1	12	59.015	60.803	0	46.078	4.068	18.928	95.280	506.04
14	1	13	59.831	59.372	0	46.535	4.039	18.659	93.309	492.49
15	1	14	61.556	57.913	0	46.209	4.007	18.405	91.625	481.97
16	1	15	60.769	60.604	0	47.011	4.125	19.381	98.284	524.92
17	1	16	60.020	58.350	0	45.794	4.015	18.495	92.355	487.19
18	1	17	61.416	58.737	0	46.500	4.031	18.597	92.897	489.91
19	1	18	59.614	61.060	0	47.576	4.166	19.758	101.060	543.93
20	1	19	59.862	60.514	0	46.748	4.044	18.704	93.634	494.66
21	1	20	59.731	59.340	0	46.450	3.994	18.273	90.593	474.50
22	1	21	59.965	58.935	0	45.368	4.012	18.488	92.399	487.92
23	1	22	60.060	61.360	0	45.753	3.999	18.342	91.205	479.18
24	1	23	59.993	61.280	0	46.286	4.017	18.482	92.080	484.39

Figure 3

Determination of the angular velocity of a calcite particle

1. Select **File -> Open** to open the series of images you have saved.
2. In the **Image -> Properties** -menu change the values of the “slices” and “frames” fields.
3. Crop an area of approx. 160x160 pixels around the trapped particle by choosing the desired area and selecting **Image -> Crop**
4. Choose **Image -> Adjust -> Treshold** and **“Dark Background”**, and change the lower limit of the intensity until you get something as in Figure 4. Then press Apply and OK. If this does not work as desired, try the subtract background function first (Process -> Subtract Background)
5. Chose **Analyze -> Analyze Particles…** and first try the settings shown in Figure 5. In Figure 5 left of the Results window is what the video should look like after step 4. The Results window and the right-hand side image depict what the results could look like after tracking the particle. The idea is that you can find only one particle in every frame of the video, as in this example. The data can then be saved once again as a text file and the rest of the measurement data can be processed in Matlab. The Matft fft function, for example, can be helpful in determining the rotation speed.

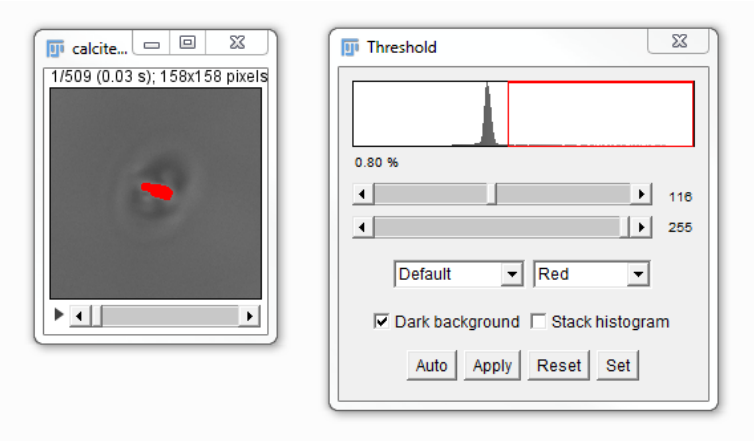


Figure 4

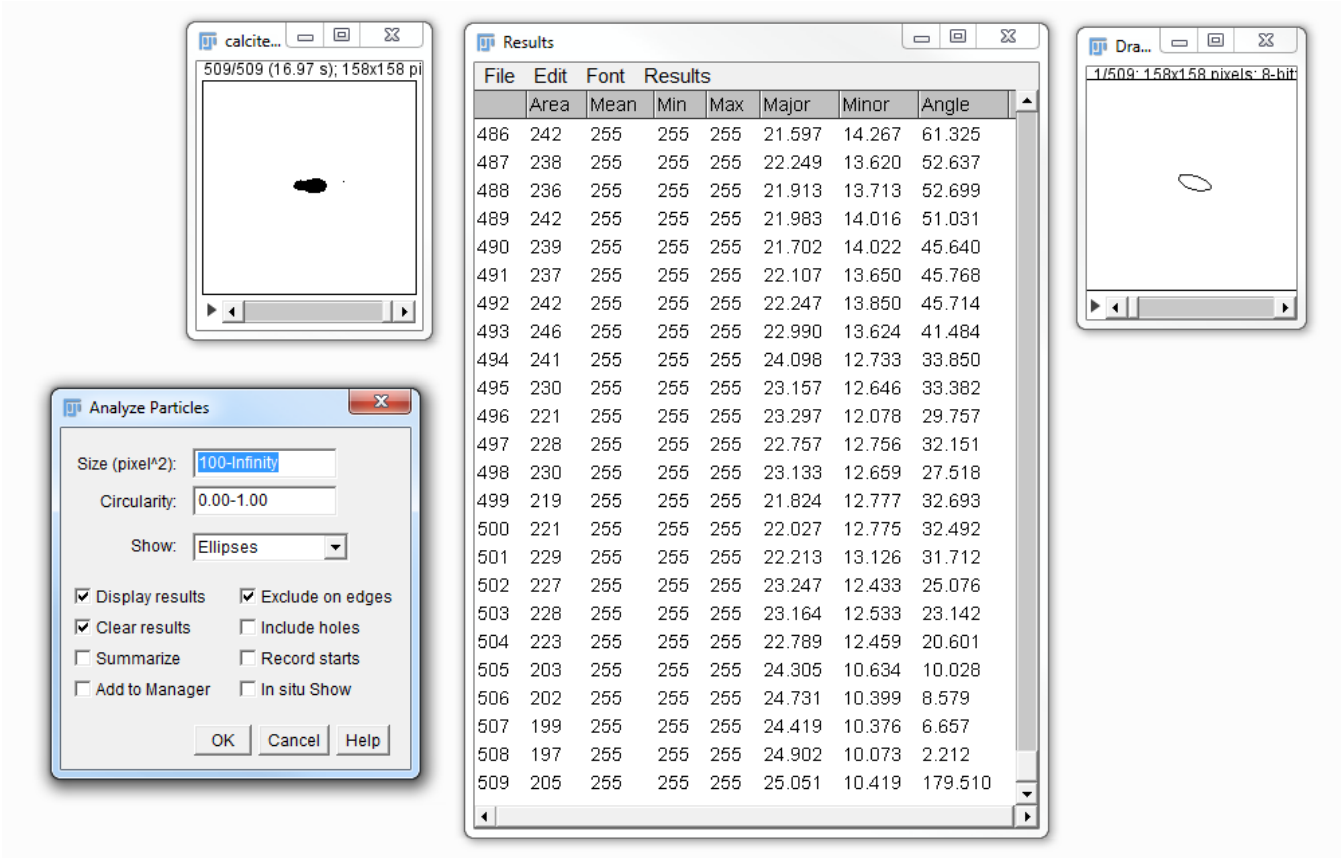


Figure 5