

Advice:

Download “manual tracking plug-in” to plug-in folder of ImageJ(I saved it to the “stacks” sub-folder in the plug-ins folder. Restart ImageJ. Manual Tracking should show up where you saved it i.e. under stacks under plug ins.

As we are interested in the average velocity of the beads, not their instantaneous velocity from frame to frame, you do not have to click on every frame (1-30). Instead, you need only look at the first and last frames in which the bead appears, find the displacement of the bead from first frame to last frame and divide by the time elapsed between those two frames to find the average velocity of your bead.

However, if you had many beads in your images, you may find it difficult to identify the same bead from frame 1 to frame 30. If you can confidently identify the beads, then please take this shortcut. Note also, that if the beads you are tracking were not visible over the entire duration, you must account for this in your calculation of avg velocity! Say a bead enters on frame 3, then your time interval corresponds to 27 frames. Or say your bead leaves the field of view at frame 25, then your time interval corresponds to 25 frames. If you do not account for this and unilaterally impose the time interval as corresponding to 30 frames, you will be artificially lowering the average velocity of those beads that were not tracked for a full 30 frames!

If you recorded motion of more than 10 beads, include them in your analysis. Do not simply analyze 10 beads if you collected images of more than 10. 10 was the MINIMUM. You will be better able to see trends in your data if you analyze the motion of more than 10 beads.

Results:

Average velocity of beads in each channel before blockage:

Both histograms showing frequency (number of beads) per velocity interval (bin) And a final value (weighted average)

You can define the number of intervals and their range (extent)

Weighted average calculation:

Average velocity for interval x = sum of velocities of beads in interval x / number of beads in interval x

Weighted velocity for channel = sum of (average interval velocity X number of beads in interval / total number of beads)

In this case, the weighted average works out to equivalent to the arithmetic mean: sum of average bead velocities / total number of beads

Average velocity of beads in each channel after blockage:

Both histograms showing frequency (number of beads) per velocity interval (bin) And a final value (weighted average)

Prediction of how flow would be altered by blockage

Salient images – of blockage, of example analysis (ImageJ allows you to overlay a line corresponding to the path / trajectory taken by your particle if you click through the stack frame by frame)