Electronic Supplementary Material (ESI) for Lab on a Chip.

1 Characteristic of the focusing condition by observation of the particle flow velocity standard deviation of 2.2 µm polystyrene beats

Table 1 Characterization of the focusing and throughput conditions of 2.2 µm polystyrene beads for a defined observation window in dependency of alpha

Alpha	Center position of the observed area in µm	Width of the observed area in µm	U mean in mm / s	standard deviation in %	Observations per particle	Observed particles
0.02	58.88	17.4	2.37	2.72	7	187
0.05	58.88	17.4	2.35	3.32	7	301
0.11	58.88	17.4	2.35	3.80	7	588
0.17	58.88	17.4	2.34	5.52	7	877
0.24	58.88	17.4	2.35	7.70	7	1266
0.40	58.88	17.4	2.31	11.06	6	1954
0.64	58.88	17.4	2.33	12.31	6	2312

Table 2 Particle throughput at full frame for increasing alpha with constant flow velocity

Alpha	N Frames	Mean Observations per particle	Observed particles	Particles per second
0.02	650	7.2	1100	102
0.05	480	7.3	1572	197
0.11	480	7.0	3314	414
0.17	480	7.0	4714	589
0.24	480	6.8	5784	723
0.40	480	6	9182	1 148
0.64	480	5.8	12015	1 502

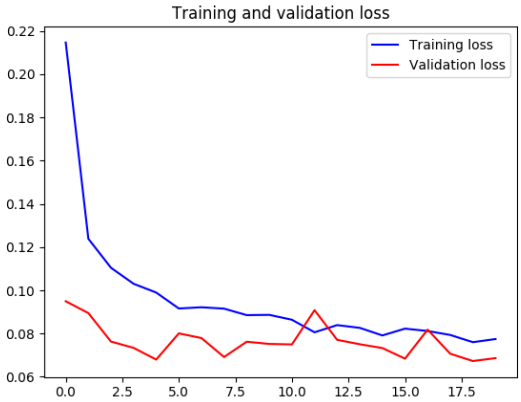
Table 3 Characterization of the focusing and throughput conditions of 2.2 µm polystyrene beads for a defined observation window in dependency of the particle flow velocity at constant alpha

Alpha	Center position of the observed area in µm	Width of the observed area in µm	U mean in mm / s	standard deviation in %	Observations per particle	Observed particles
0.05	58.88	17.4	0.20	8.59	42	60
0.05	58.88	17.4	0.40	5.99	45	57
0.05	58.88	17.4	0.78	4.12	29	58
0.05	58.88	17.4	1.60	2.39	15	118
0.05	58.88	17.4	2.36	3.26	10	151
0.05	58.88	17.4	3.18	2.61	6	208
0.05	58.88	17.4	3.96	2.31	5	274
0.05	58.88	17.4	4.71	3.05	4	366
0.05	58.88	17.4	6.47	3.17	3	556
0.05	58.88	17.4	9.67	2.11	2	885

Table 4: Particle throughput at full frame with constant alpha and increasing flow velocity

Alpha	N Frames	Mean Observations per particle	Observed particles	Particles per second
0.20	580	41.65	327	44
0.40	580	45	277	37
0.78	580	28.8	370	50
1.60	580	14.57	680	91
2.36	482	10	515	83
3.18	580	6.4	1066	143
3.96	580	5.5	1222	164
4.71	580	4.46	1847	248
6.47	580	2.97	2733	367
9.67	580	1.6	4815	647

2. Training performance of the DCNN



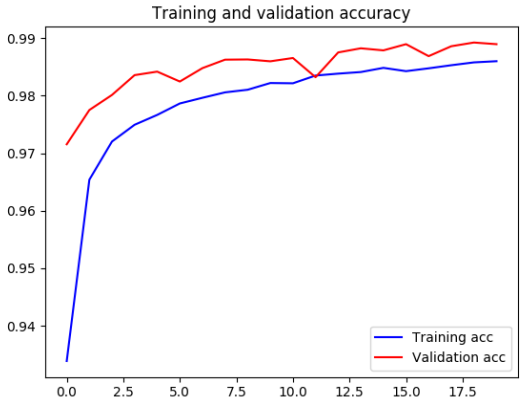


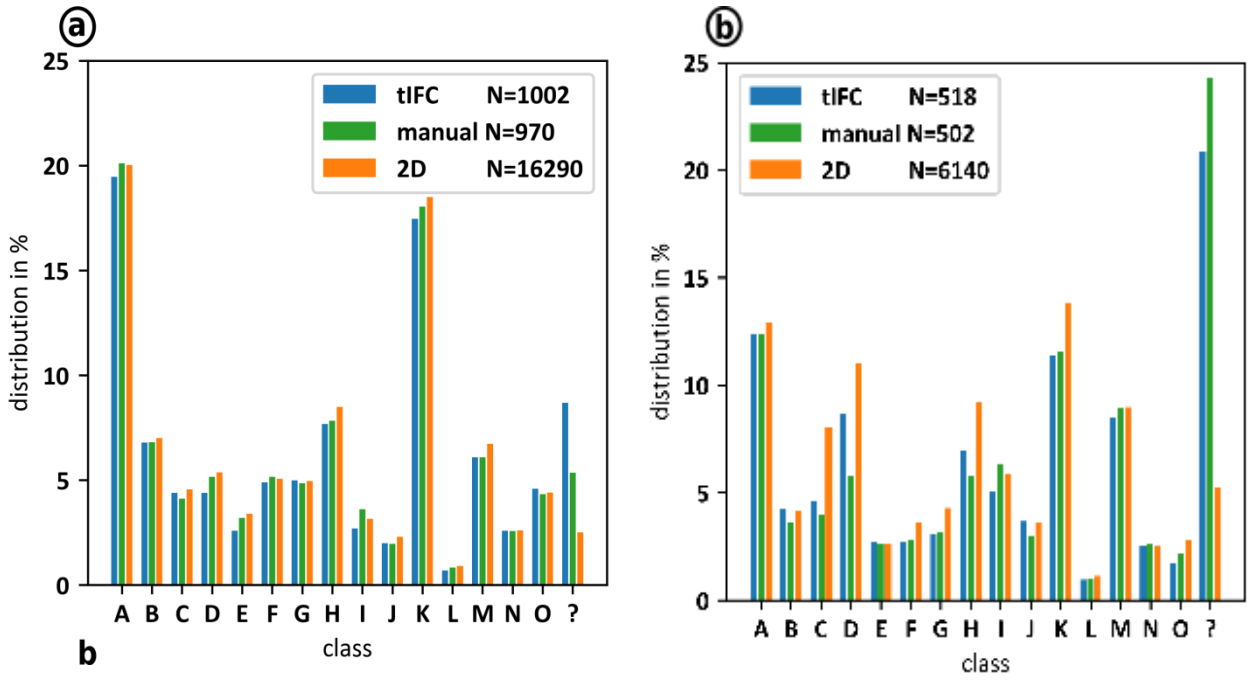
Table 2 gives a more detailed overview about the validation accuracy for each single species.

Table 4 Validation accuracy for each Species

Species	Validation images	Validation accuracy
Cyclamen	2000	99.7
Rumex	2000	98.65
Alnus	2000	97.5
Fraxinus excelsior	2000	99.1
Carpinus betulus	2000	99.1
Corylus	2000	98
Rosa corymbifera	2000	99.4
Sambucus	2000	98.1
Populous	2000	98.7
Molinia	2000	98.25
Ambrosia	2000	99.85
Fagus sylvatica	815	99.75
Scripus sylvaticus	1492	99.53
Helianthus annuus	1546	100
Ulmus	2000	98.75

3. Pollen population analysis

To outline the impact of the foreign species to the classification results we compared the population analysis shown in the manuscript (b) with a population only containing the 15 trained pollen species (a) Automated classification was run applying a P_{thresh} = 0.735. The population results for sample A (a) show a high conformity between all three methods. Due to the reliable classification accuracy of the DCNN network (98.90%) a similar pollen distribution between 2D and mIFC classification was expected.



Pollen population analysis: Comparison of the pollen distribution for 2D-. mIFC- and manual classification. (a) Population analysis results for a sample containing 15 different pollen species with low foreign particle concentration. (b) Population analysis results for a sample containing 15 different pollen species with high foreign particle concentration. The numbers in the inset of the diagrams denote number of analyzed cells/images per method.

4 Calculation of the angular velocity of red blood cells (RBC's)

Assumption:

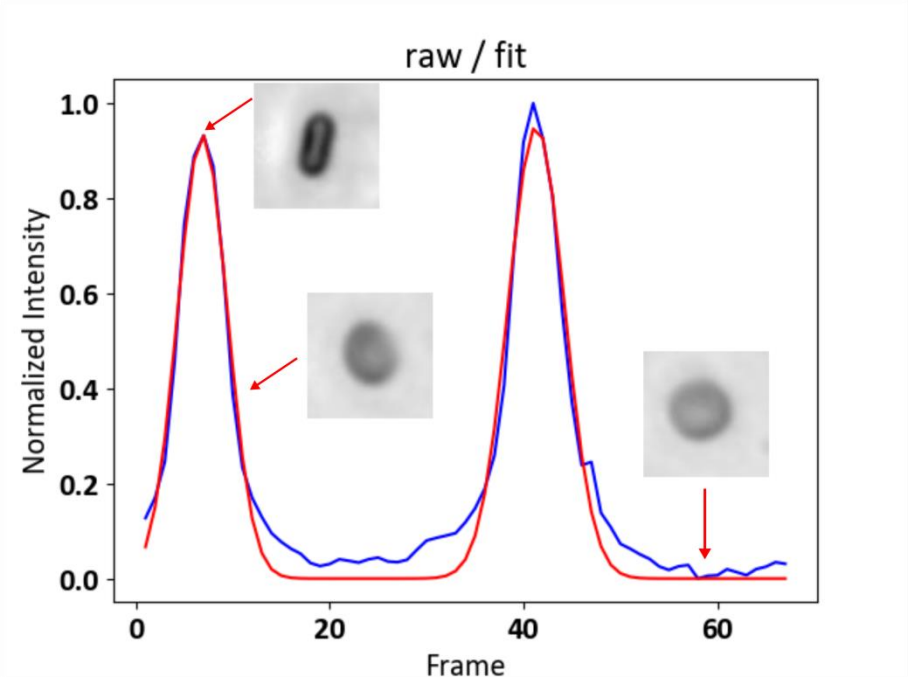
- The absorbance of the bright field illumination changes with the orientation of the major and minor axis of the RBC`S
- Highest absorbance when light passes through major axis

Exception:

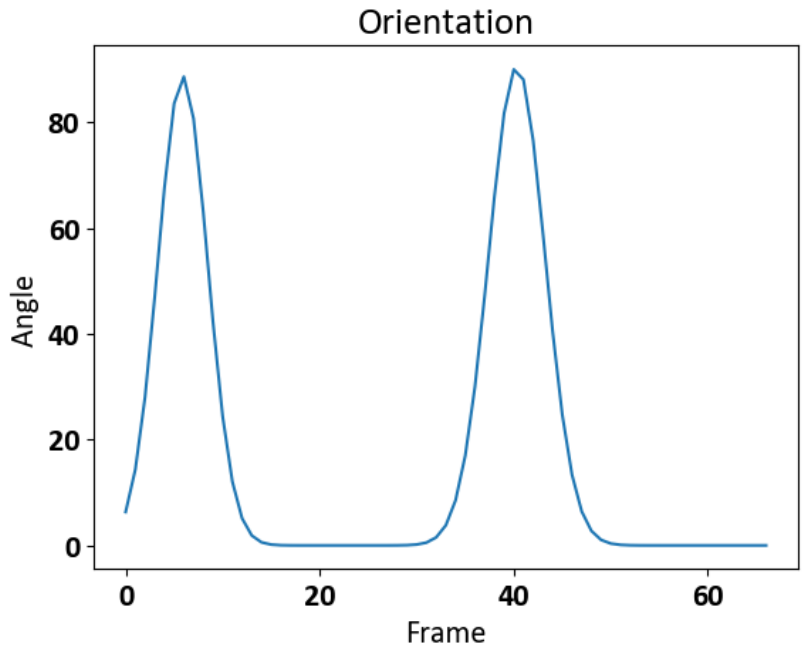
- No significant intensity changes when the RBC only rotates around its minor axis

Procedure:

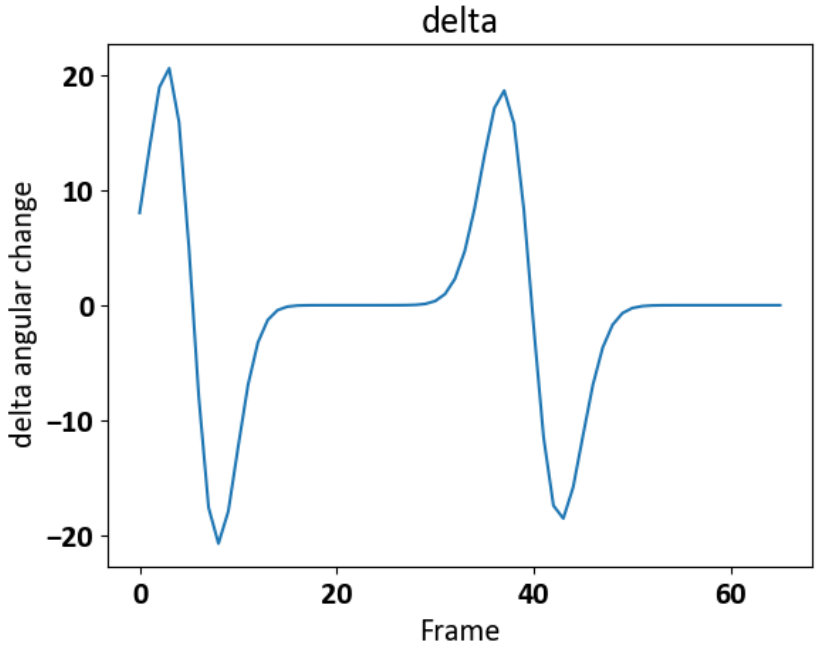
1. **Plot of the normalized Gray Value Intensity at 25 percentile (blue) and Gaussian fit (red) for each image of the tilt series.**



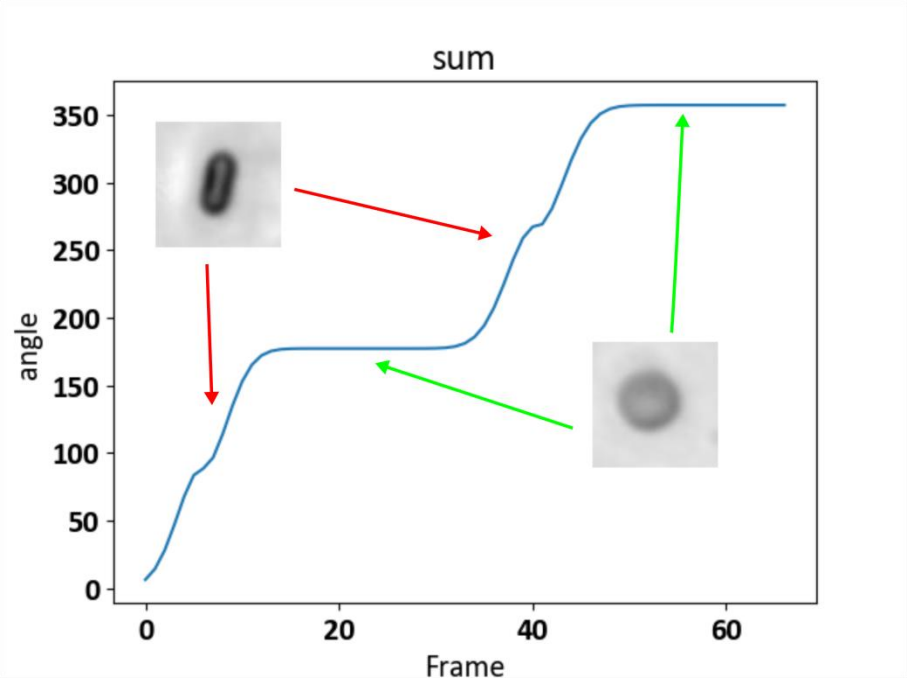
2. **Normalization between 0 and 90 where the maximum intensity corresponds to 90 degree and the minimum intensity to 0 degree.**



3. Angular change between neighboring frames



4. summation of the absolute value of delta angle



The plot outlines the flipping effect where we have two plateau area (green) without angular rotation and two areas with a step angular velocity (red) covering a 360° rotation. The summit angular shift between neighboring frames is saved in csv file and can be directly imported to tomviz.