

Supplementary Information
AQAFI: A Bioanalytical Method for Automated
KPIs Quantification of Fluorescent Images of
Human Leukocytes and Micro-Nano Particles

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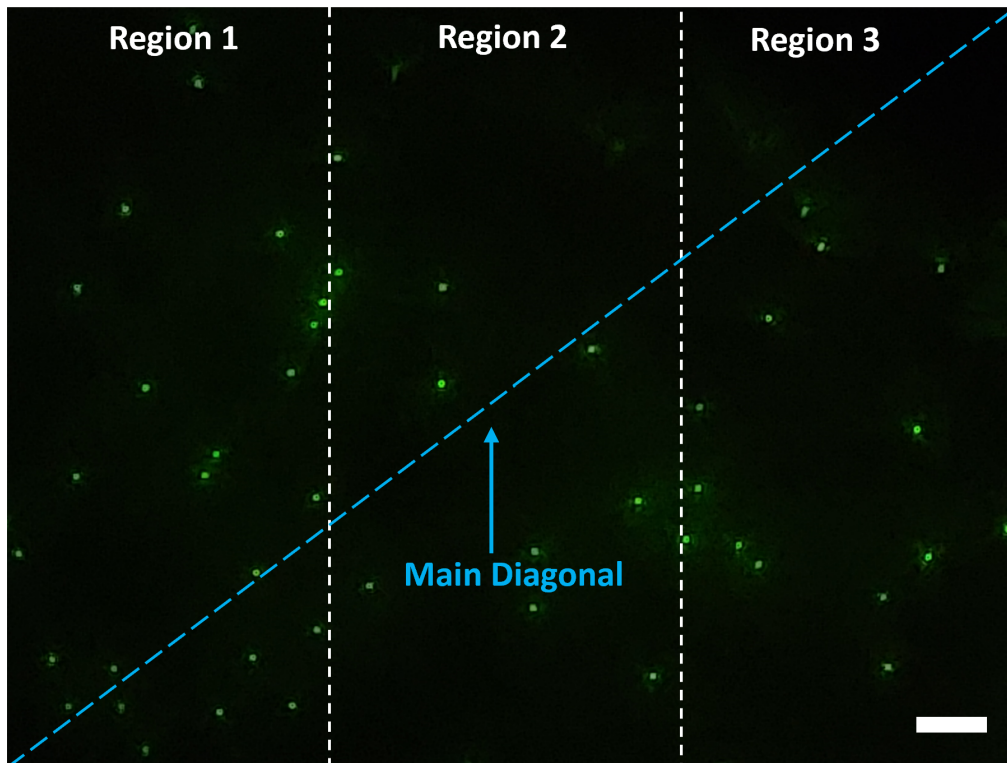


Fig. S 1: Bead selection criteria showcased on $2\ \mu\text{m}$ green fluorescent particles imaged from the smartphone fluorescence microscope at 4.2 V. (Scale bar = $50\ \mu\text{m}$).

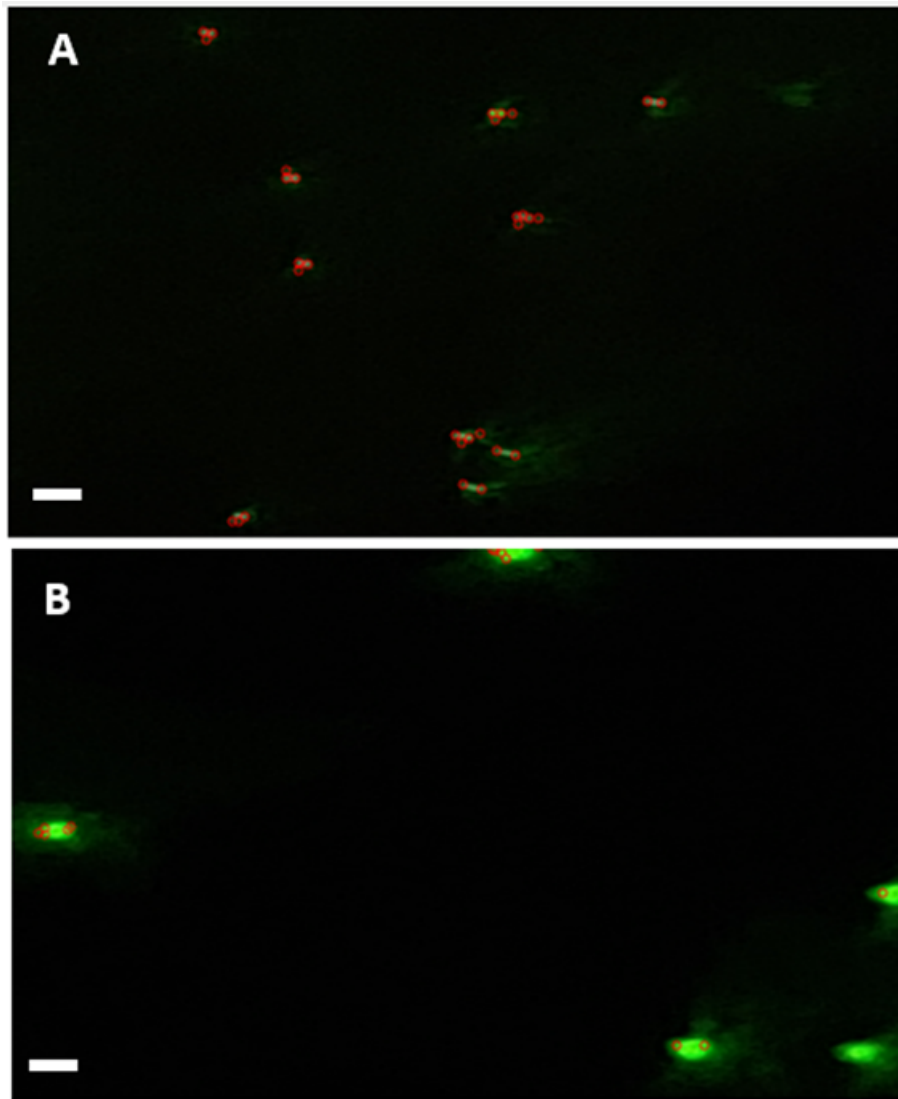


Fig. S 2: (a) Features detected on $2 \mu m$ green fluorescent particles imaged from the smartphone fluorescence microscope at 4.3 V. (b) Features detected on $8 \mu m$ green fluorescent particles imaged from the smartphone fluorescence microscope at 3.8 V. (Scale bar = $10 \mu m$).

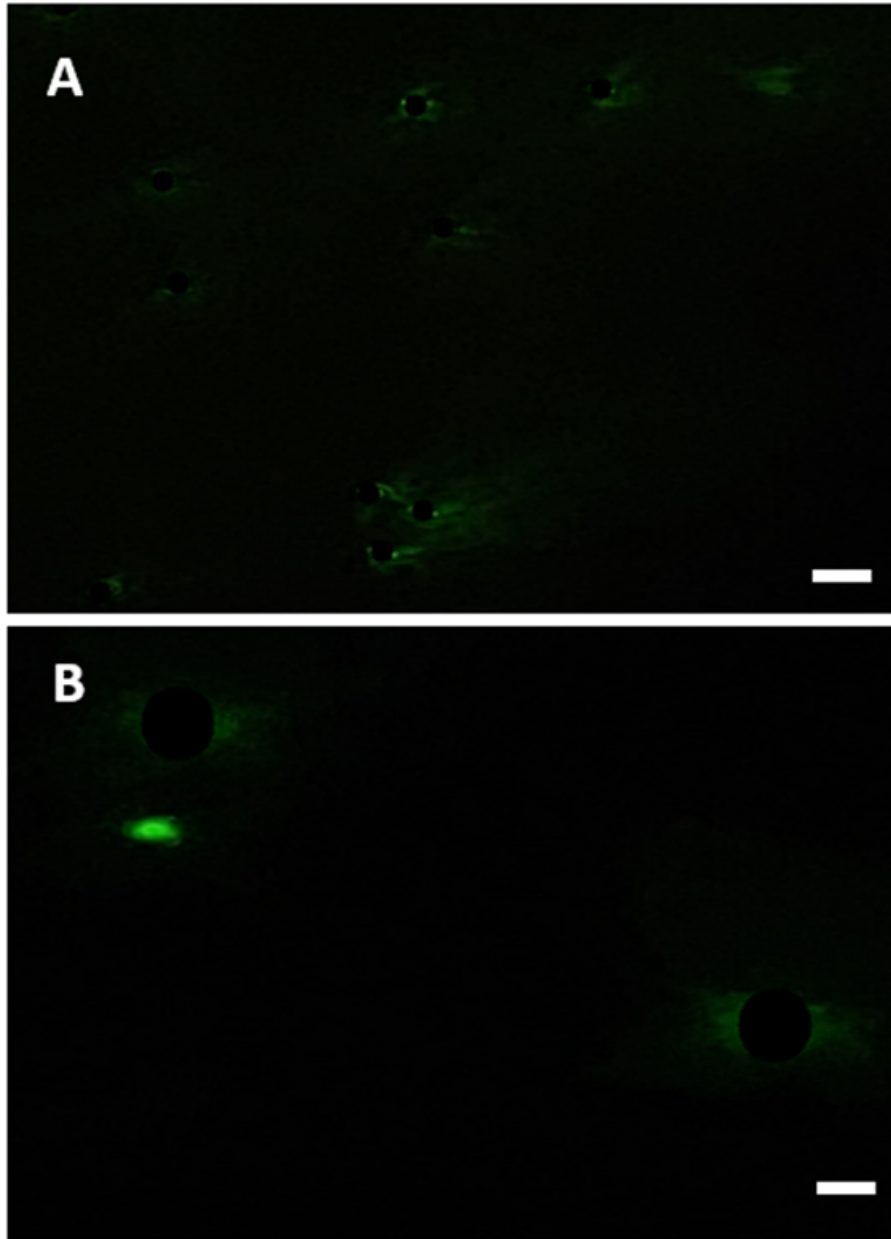


Fig. S 3: (a) Circular bead region removal on $2 \mu m$ green fluorescent particles imaged from the smartphone fluorescence microscope at 4.3 V. (Scale bar = $5 \mu m$) (b) Circular bead region removal on $8 \mu m$ green fluorescent particles imaged from the smartphone fluorescence microscope at 3.8 V. (Scale bar = $10 \mu m$).

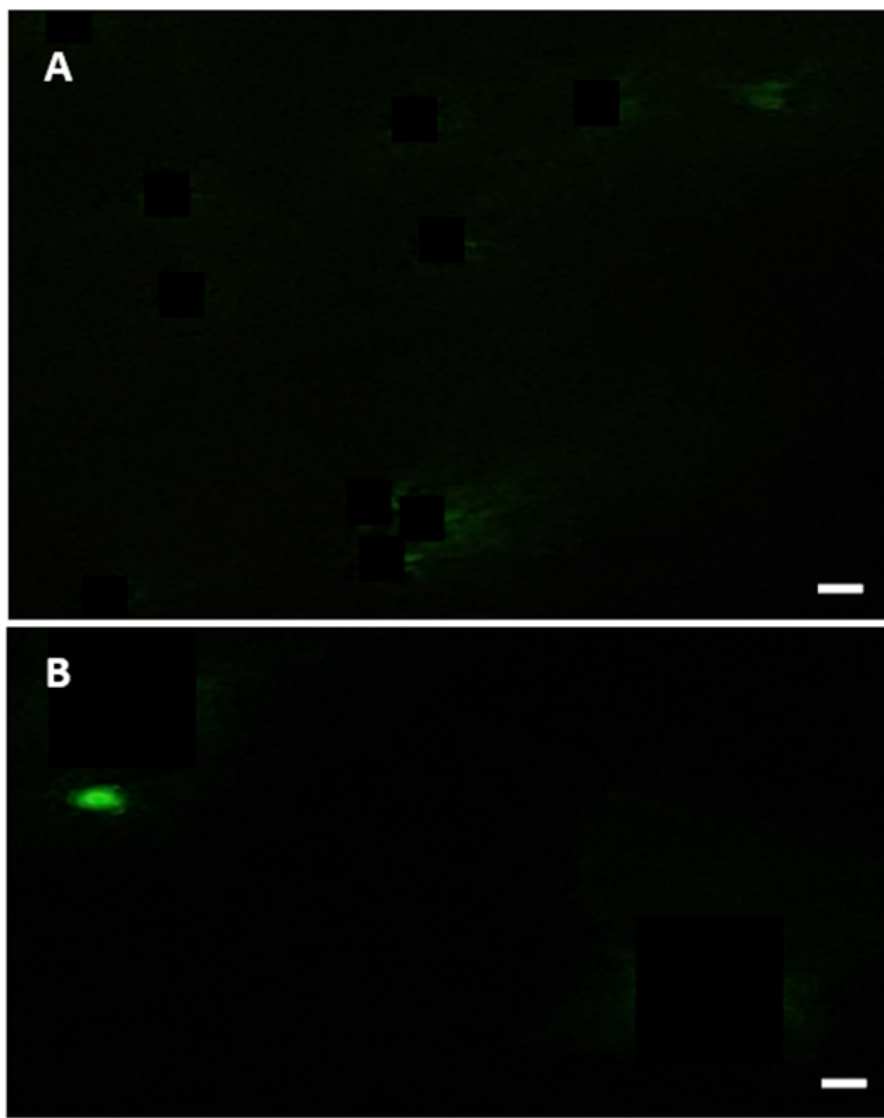


Fig. S 4: (a) Box region removal around detected beads on $2 \mu m$ green fluorescent particles imaged from the smartphone fluorescence microscope at 4.3 V. (Scale bar = $5 \mu m$) (b) Box region removal around detected beads on $8 \mu m$ green fluorescent particles imaged from the smartphone fluorescence microscope at 3.8 V. (Scale bar = $10 \mu m$).

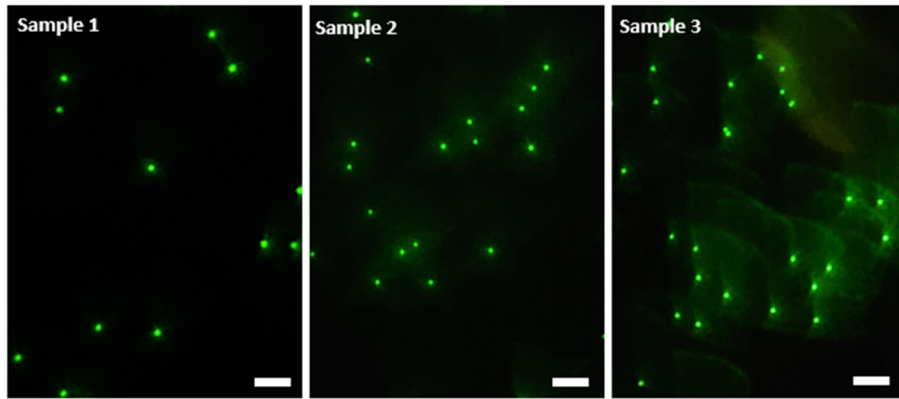


Fig. S 5: Three different samples of $8 \mu m$ green fluorescent particles imaged from the smartphone fluorescence microscope at 3.8 V. (Scale bar = $45 \mu m$).

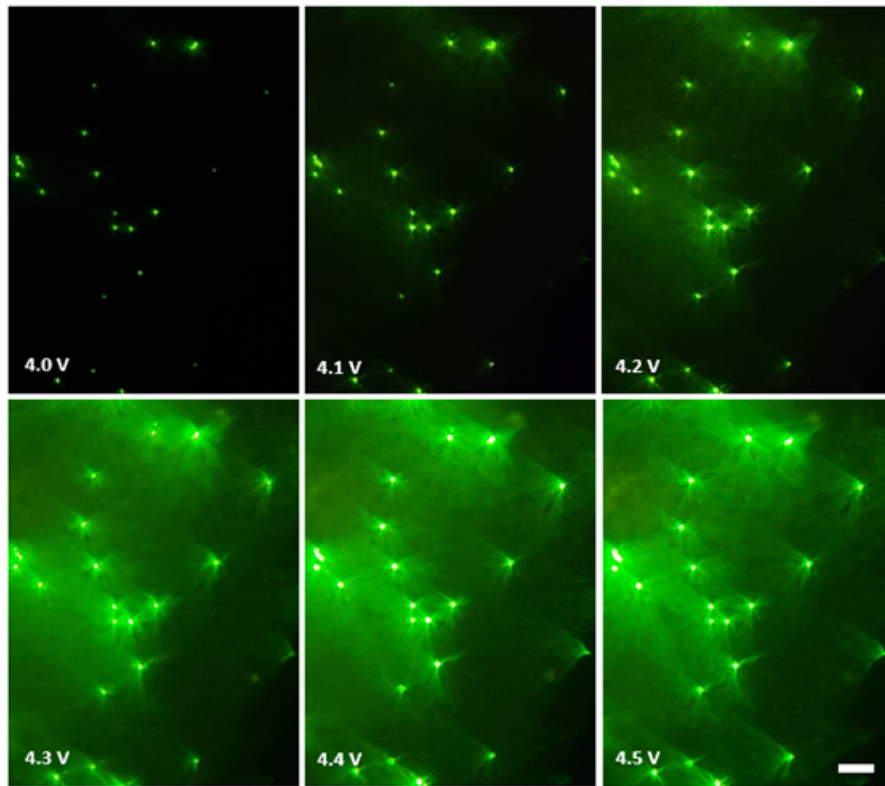


Fig. S 6: Fluorescently tagged human peripheral blood leukocytes imaged using the SFM at excitation voltage ranging from 4.0 to 4.5 V. (Scale bar = $45 \mu m$).

Bead size	Optimal excitation voltage
0.8	4.3 – 4.5 V
1.0	4.3 – 4.5 V
2.0	4.1 – 4.3 V
8.3	3.8V

Table 1: Optimal excitation voltage range for the four different green fluorescent beads imaged using the smartphone fluorescence microscope.

Bead Size	Distance Threshold (pixels)	Fast Feature Intensity Threshold (Intensity)	Bead radius in image space ‘α’ (pixels)	Box size ‘s’ (pixels)
0.8	30	13	4	8
1.0	30	12	4	8
2.0	40	20	6	12
8.3	40	22	13	40

Table 2: Input metrics used by the algorithm for the quantitative assessment of the quantity of fluorescent bead images captured using the smartphone fluorescence microscope.

Sample Number	Bead Intensity		Vicinity Noise		Background Noise		SDNR		CNR	
	ImageJ	Algo	ImageJ	Algo	ImageJ	Algo	ImageJ	Algo	ImageJ	Algo
S1	108.8	117.77	13.6	4.32	3	1.64	31.73	68.89	3.76	16.79
S2	108.7	107.48	15	11.34	3	4.93	31.23	19.49	2.32	1.80
S3	116.5	101.22	18.8	3.44	4	1.02	24.42	95.55	1.35	38.02

Table 3: Image quality metrics for 8.3 μm green fluorescent beads calculated manually using ImageJ and using the designed algorithm.