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

Supplementary Information

Excitation Schemes for Enhanced Micro and Nanoparticle Imaging In 3D Printed Smartphone Based Fluorescent Microscope

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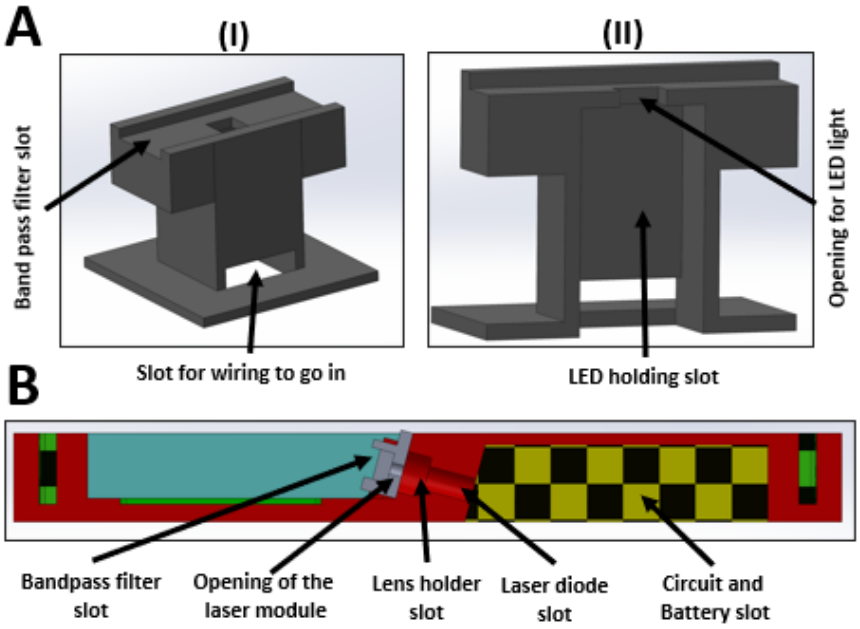

Fig S1. (a) (i) The CAD design of the LED holder used in SFM with a perpendicular excitation scheme. (ii) Sliced view of the LED holder used in SFM with a perpendicular excitation scheme. (b) Sliced view of the bottom portion used in a SFM with an oblique excitation scheme.

Fig S2. The 3D printed prototype of the SFM working in conjunction with a Samsung Galaxy S21 Ultra to image green fluorescent microparticles.

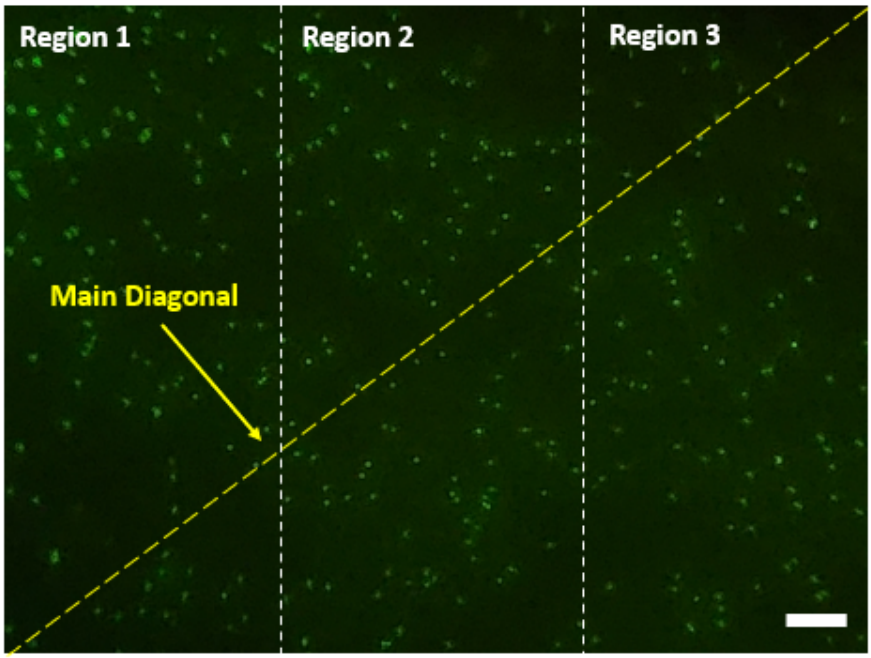

Fig S3. Bead selection process for SDNR/CNR calculation on an image of 1 µm green fluorescent particles obtained from the smartphone attachment using oblique excitation scheme at 4.4 V.(Scale bar = 50µm).

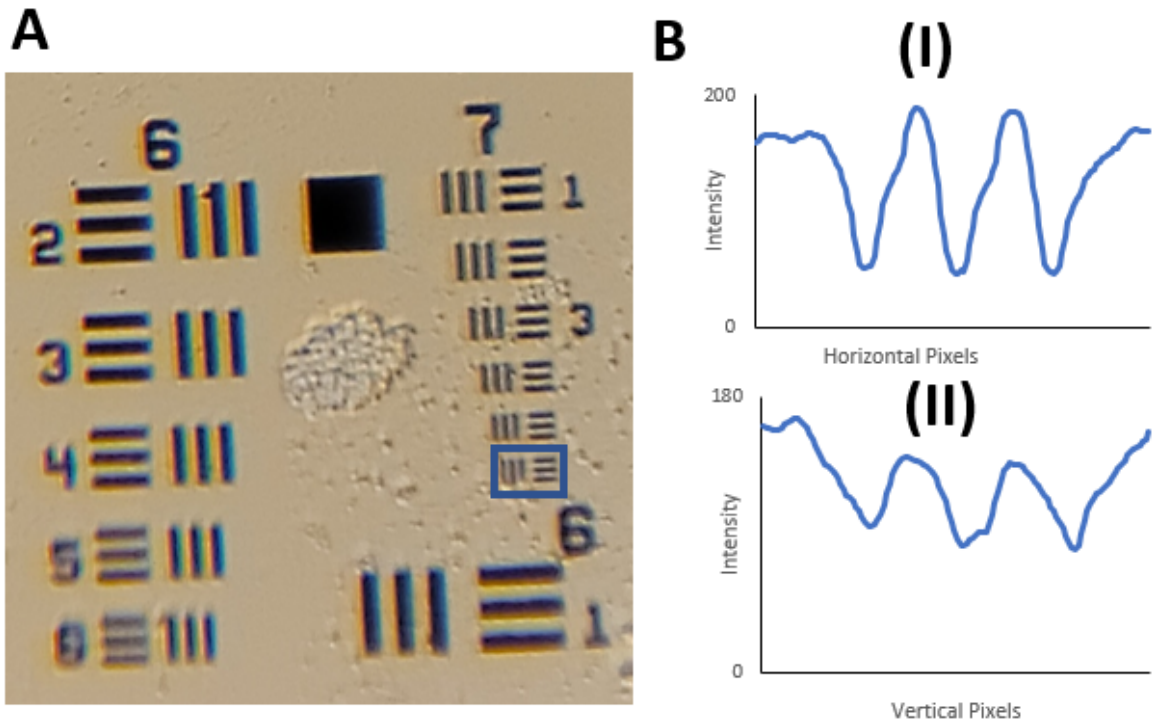

Fig S4. (a) 1951 USAF resolution test chart imaged using the magnification module of the designed SFM. (b) (i) Pixel intesnities of horizontal lines of Group 7 Element 6. (Ii) Pixel intesnities of vertical lines of Group 7 Element 6.

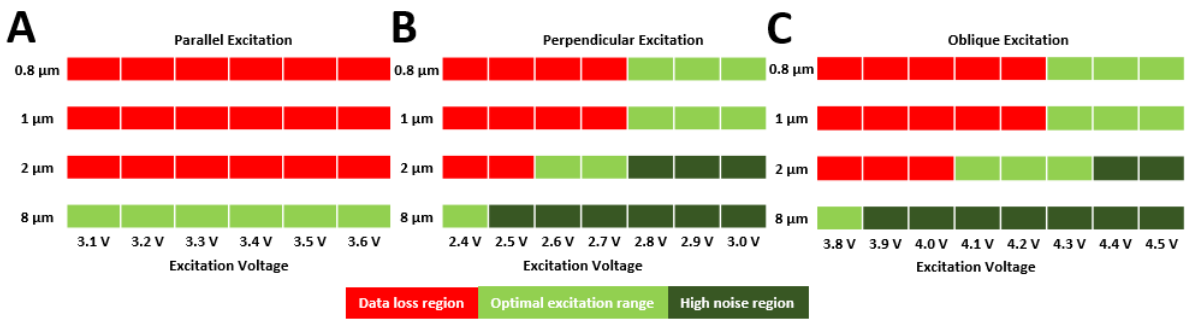

Fig S5. (a) The position of data loss, optimal, and high noise regions for the SFM design variant with a parallel excitation scheme. (b) The position of data loss, optimal, and high noise regions for the SFM design variant with a perpendicular excitation scheme. (b) The position of data loss, optimal, and high noise regions for the SFM design variant with an oblique excitation scheme.

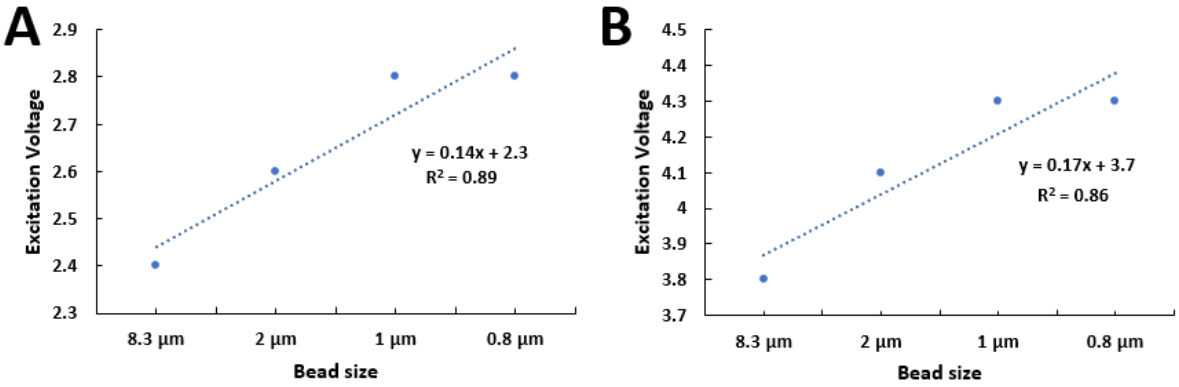

Fig S6. (a) Lower end of the optimal excitation range for the four bead sizes (8.3,2,1,0.8 µm) imaged using the SFM using perpendicular excitation modality. (b) Lower end of the optimal excitation range for the four bead sizes (8.3,2,1,0.8 µm) imaged using the SFM using oblique excitation modality.

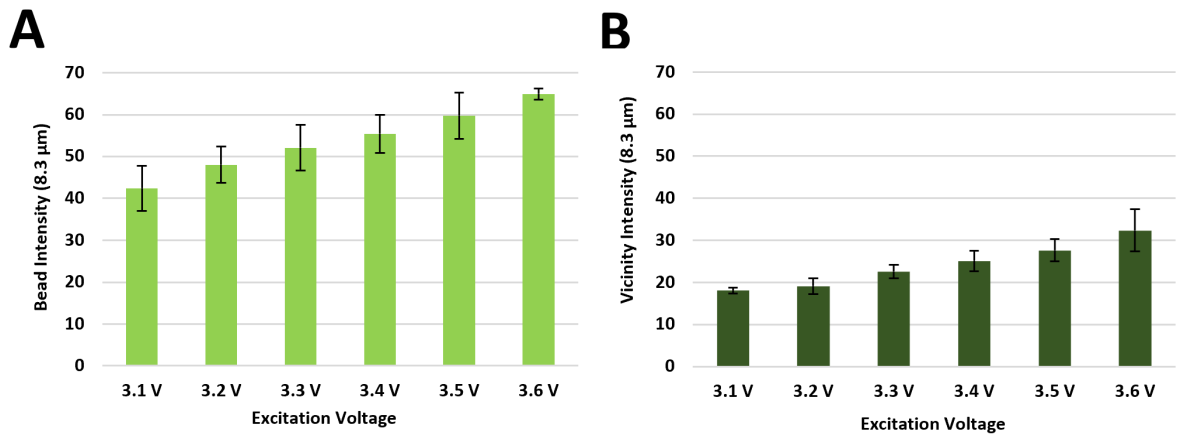

Fig S7. (a) Bead intensities in optimal excitation voltage range for 8.3 µm fluorescent beads imaged using the SFM employing a parallel excitation scheme. (b) Corresponding intensity of vicinity noise.

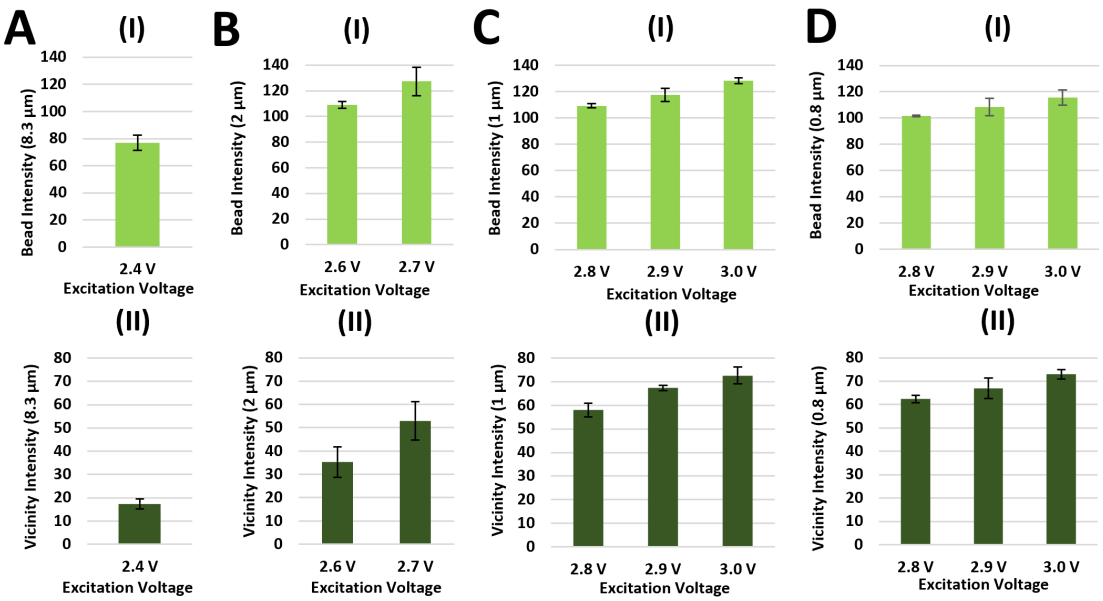

Fig S8. (a) (i) Bead intensities in optimal excitation voltage range for 8.3 µm fluorescent beads imaged using the SFM employing a perpendicular excitation scheme. (ii) Corresponding intensity of vicinity noise. (b) (i) Bead intensities in optimal excitation voltage range for 2 µm fluorescent beads imaged using the SFM employing a perpendicular excitation scheme. (ii) Corresponding intensity of vicinity noise. (c) (i) Bead intensities in optimal excitation voltage range for 1 µm fluorescent beads imaged using the SFM employing a perpendicular excitation scheme. (ii) Corresponding intensity of vicinity noise. (d) (i) Bead intensities in optimal excitation voltage range for 0.8 µm fluorescent beads imaged using the SFM employing a perpendicular excitation scheme. (ii) Corresponding intensity of vicinity noise.

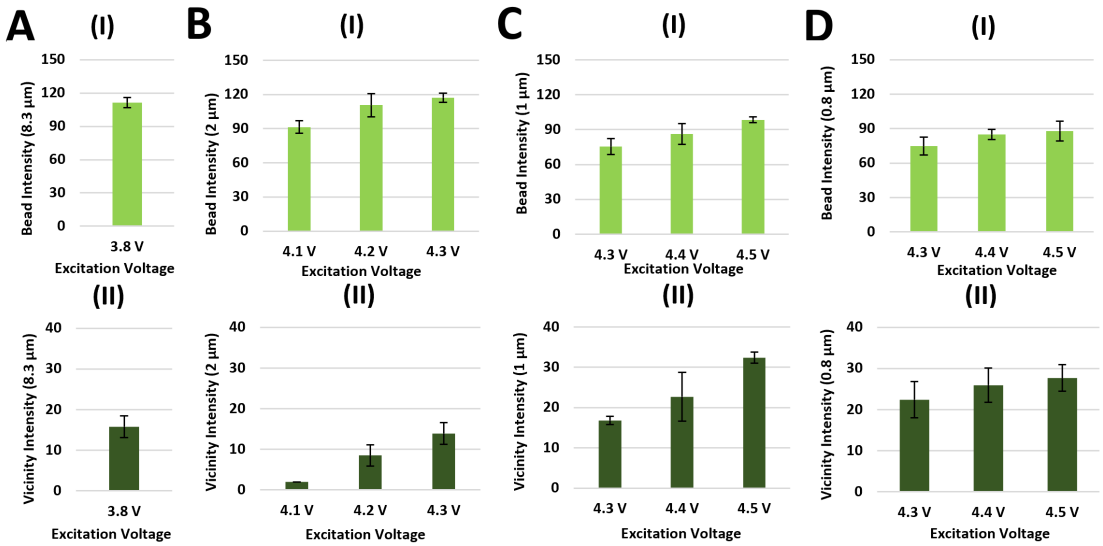

Fig S9. (a) (i) Bead intensities in optimal excitation voltage range for 8.3 µm fluorescent beads imaged using the SFM employing an oblique excitation scheme. (ii) Corresponding intensity of vicinity noise. (b) (i) Bead intensities in optimal excitation voltage range for 2 µm fluorescent beads imaged using the SFM employing an oblique excitation scheme. (ii) Corresponding intensity of vicinity noise. (c) (i) Bead intensities in optimal excitation voltage range for 1 µm fluorescent beads imaged using the SFM

employing an oblique excitation scheme. (ii) Corresponding intensity of vicinity noise. (d) (i) Bead intensities in optimal excitation voltage range for 0.8 µm fluorescent beads imaged using the SFM employing an oblique excitation scheme. (ii) Corresponding intensity of vicinity noise.

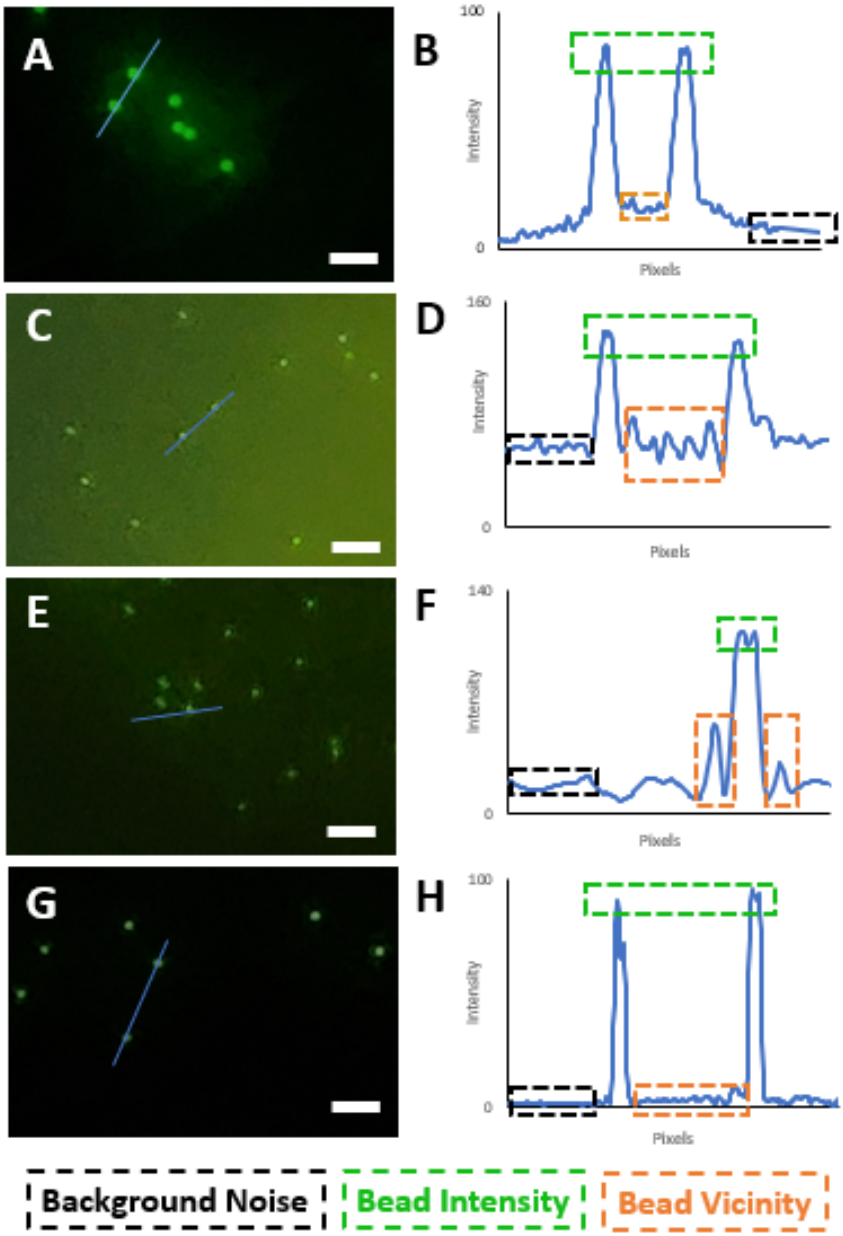

Fig S10. Fluorescent images (A, C, E, G) and corresponding bead intensity profiles for the captured images in the study (B, D, F, H). Individual profile types with detailed description of unique features are mentioend in the Discussion section of the manuscript.

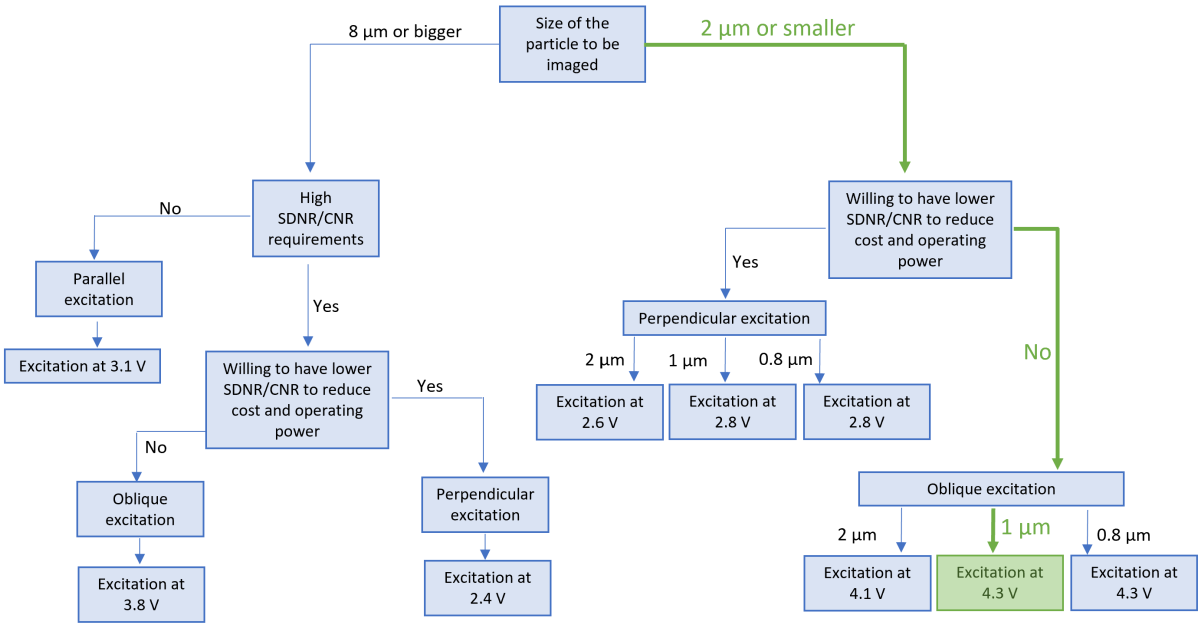

Fig S11. A use case for the general process flow model depicting the choices made when 1 µm beads have to be image at high SDNR/CNR values.

		Current Drawn (A)					
	Sample #	3.1 V	3.2 V	3.3 V	3.4 V	3.5 V	3.6 V
8.3 µm	1	0.03	0.04	0.06	0.08	0.11	0.12
	2	0.03	0.04	0.06	0.07	0.1	0.12
	3	0.02	0.04	0.06	0.08	0.09	0.12

Table S1: Current drawn by the SFM with a parallel excitation scheme during the triplicate imaging experiments.

		Current Drawn (A)						
	Sample #	2.4 V	2.5 V	2.6 V	2.7 V	2.8 V	2.9 V	3.0 V
8.3 µm	1	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
2 µm	Sample #	2.4 V	2.5 V	2.6 V	2.7 V	2.8 V	2.9 V	3.0 V
	1	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
1 µm	Sample #	2.4 V	2.5 V	2.6 V	2.7 V	2.8 V	2.9 V	3.0 V
	1	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
0.8 µm	Sample #	2.4 V	2.5 V	2.6 V	2.7 V	2.8 V	2.9 V	3.0 V
	1	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01

Table S2: Current drawn by the SFM with a perpendicular excitation scheme during the triplicate imaging experiments for all four beads (8.3, 2, 1, 0.8 µm).

		Current Drawn (A)							
	Sample #	3.8 V	3.9 V	4.0 V	4.1 V	4.2 V	4.3 V	4.4 V	4.5V
8.3 µm	1	0.01	0.02	0.03	0.04	0.06	0.07	0.09	0.12
	2	0.02	0.02	0.03	0.04	0.06	0.08	0.10	0.13
	3	0.02	0.02	0.03	0.04	0.05	0.07	0.11	0.15
2 µm	Sample #	3.8 V	3.9 V	4.0 V	4.1 V	4.2 V	4.3 V	4.4 V	4.5V
	1	0.02	0.03	0.04	0.05	0.06	0.07	0.09	0.14
	2	0.02	0.03	0.03	0.04	0.05	0.07	0.09	0.13
	3	0.03	0.04	0.05	0.06	0.07	0.07	0.10	0.13
1 µm	Sample #	3.8 V	3.9 V	4.0 V	4.1 V	4.2 V	4.3 V	4.4 V	4.5V
	1	0.02	0.03	0.04	0.05	0.07	0.08	0.09	0.15
	2	0.02	0.03	0.03	0.04	0.07	0.07	0.08	0.14
	3	0.02	0.02	0.04	0.05	0.07	0.09	0.10	0.15
0.8 µm	Sample #	3.8 V	3.9 V	4.0 V	4.1 V	4.2 V	4.3 V	4.4 V	4.5V
	1	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.11
	2	0.02	0.03	0.05	0.06	0.07	0.09	0.13	0.14
	3	0.03	0.04	0.05	0.07	0.08	0.11	0.10	0.13

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Table S3: Current drawn by the SFM with an oblique excitation scheme during the triplicate imaging experiments for all four beads (8.3, 2, 1, 0.8 µm).

	Voltage (V)					
	3.1 V	3.2 V	3.3 V	3.4 V	3.5 V	3.6 V
Excitation source average current (mA)	27	40	60	77	100	120
Relative Luminous Intensity	1.5	2.4	3.0	3.7	4.2	5.1

Table S4: Approximate relative luminous intensity of the excitation source in the SFM with parallel excitation scheme. Normalized to 20 mA forward current for a single blue LED (Digi Key, Product no: 516-2800-1-ND).

	Voltage (V)							
	3.8 V	3.9 V	4.0 V	4.1 V	4.2 V	4.3 V	4.4 V	4.5 V
Average current (mA)	21.67	29.17	39.17	50	65	79.17	97.50	135
Optical power (mW)	5	15	30	43	65	88	112	120

Table S5: Approximate optical power output of the excitation source used in SFM with an oblique excitation corresponding to the excitation volatge and current drawn.

	Bead Size	Excitation Voltage (V)	Current (A)	Power (W)	Bead Intensity	Vicinity Intensity	SDNR	CNR
Parallel Excitation	0.8 µm	x	x	x	x	x	x	x
	1 µm	x	x	x	x	x	x	x
	2 µm	x	x	x	x	x	x	x
	8.3 µm	3.1 V	0.03	0.093	42.3 ± 5.4	18.1 ±0.7	2 ± 0.7	0.1 ± 0.04
Perpendicular Excitation	0.8 µm	2.8 V	0.01	0.028	101.6 ± 0.6	62.3 ± 1.5	0.7 ± 0.1	0.01 ± 0.0
	1 µm	2.8 V	0.01	0.028	109.3 ± 1.5	58 ± 3.0	1.1 ± 0.2	0.01 ± 0.0
	2 µm	2.6 V	0.01	0.026	109 ± 2.6	35.33 ± 6.50	2.6 ± 0.4	0.08 ± 0.02
	8.3 µm	2.4 V	0.01	0.024	76.9 ± 5.7	17.40 ± 2.10	8.7 ± 2.5	0.55 ± 0.24
Oblique Excitation	0.8 µm	4.3 V	0.09	0.39	74.7 ± 7.8	22.4 ± 4.4	3.1 ± 0.4	0.2 ± 0.04
	1 µm	4.3 V	0.08	0.34	75.3 ± 6.8	16.8 ± 1.05	4.8 ± 0.4	0.3 ± 0.03
	2 µm	4.1 V	0.04	0.16	91.3 ± 5.4	2 ± 0	29.4 ± 1.8	9.7 ± 0.6
	8.3 µm	3.8 V	0.03	0.11	111.3 ± 4.4	15.8 ± 2.7	29.1 ± 4.0	2.5 ± 1.2

Table S6: Optimal imaging parameters for the four beads (8.3, 2, 1, 0.8 µm) in each SFM design variant.

	Performance parameters of the SFM variants		
	Imaging performance	Field of View (FOV)	Power Requirements
Parallel Excitation	Third	First	Second
Perpendicular Excitation	Second	Second	First
Oblique Excitation	First	Third	Third

Table S7: A relative comparison of the major performance parameters of the three SFM variants.