Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

A Modular Microscopic Smartphone Attachment for Imaging and Quantification of Multiple Fluorescent Probes using Machine Learning

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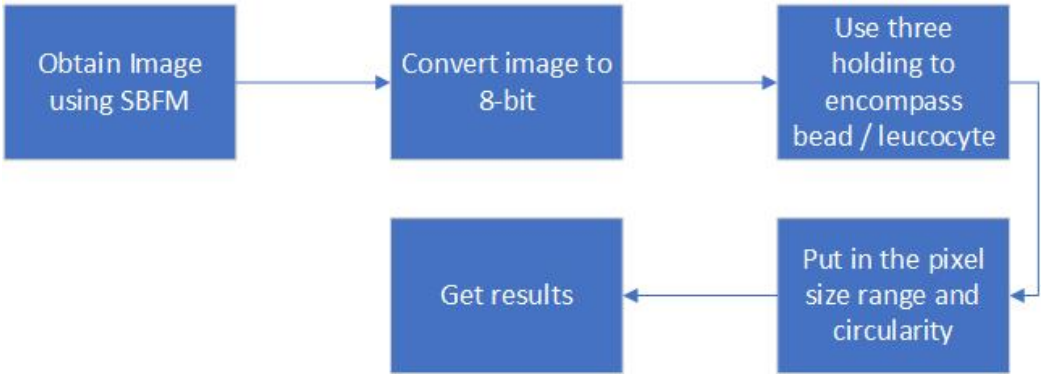

Figure S1. Flowchart outlining the steps involved in obtaining leukocyte/bead count from a smartphone image using ImageJ.

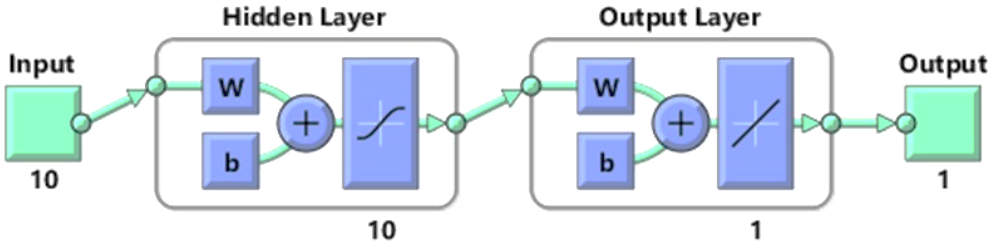

Figure S2. The network diagram of the feed forward artificial neural network with sigmoid hidden and softmax output neurons. The neural network consists of 10 in hidden and 1 neuron in output layers. Input to the network consists of the 10 counts generated from using different sensitivity values in MATLAB. The network was trained using multiple training algorithms.

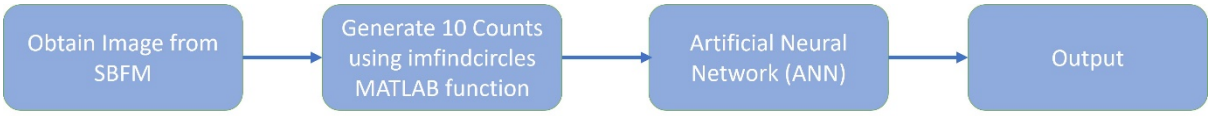

Figure S3. Flowchart outlining the steps involved in obtaining leukocyte/bead count from any image using the developed ANNs.

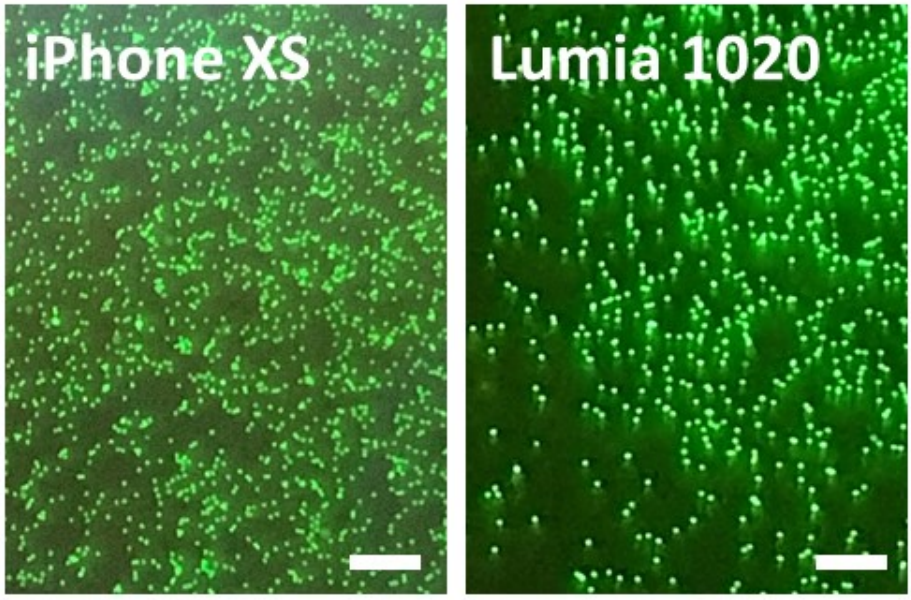

Figure S4. Green fluorescent beads imaged using the SBFM along with and iPhone XS and Nokia Lumia 1020 (Scale bar = 100µm).

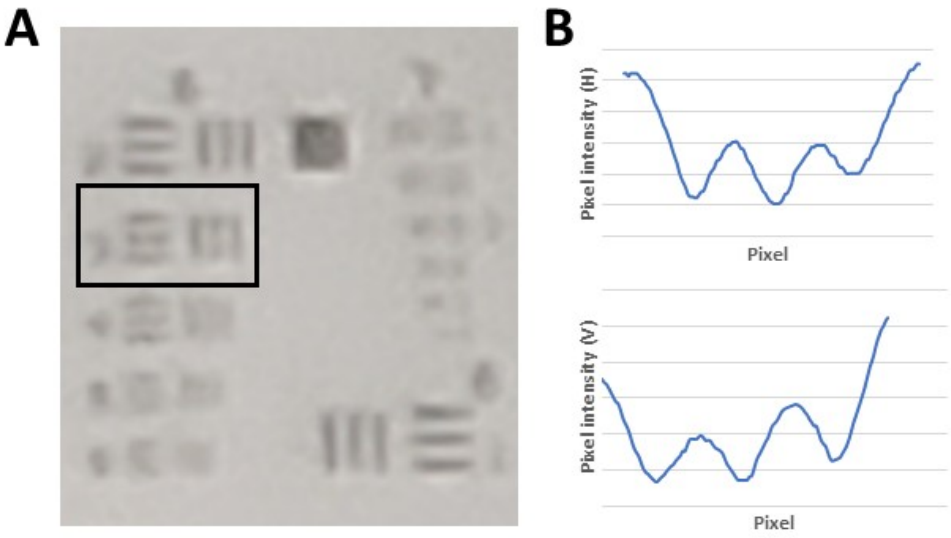

Figure S5: 1951 USAF resolution test chart imaged using S9+ and lens B (10 mm focal length).

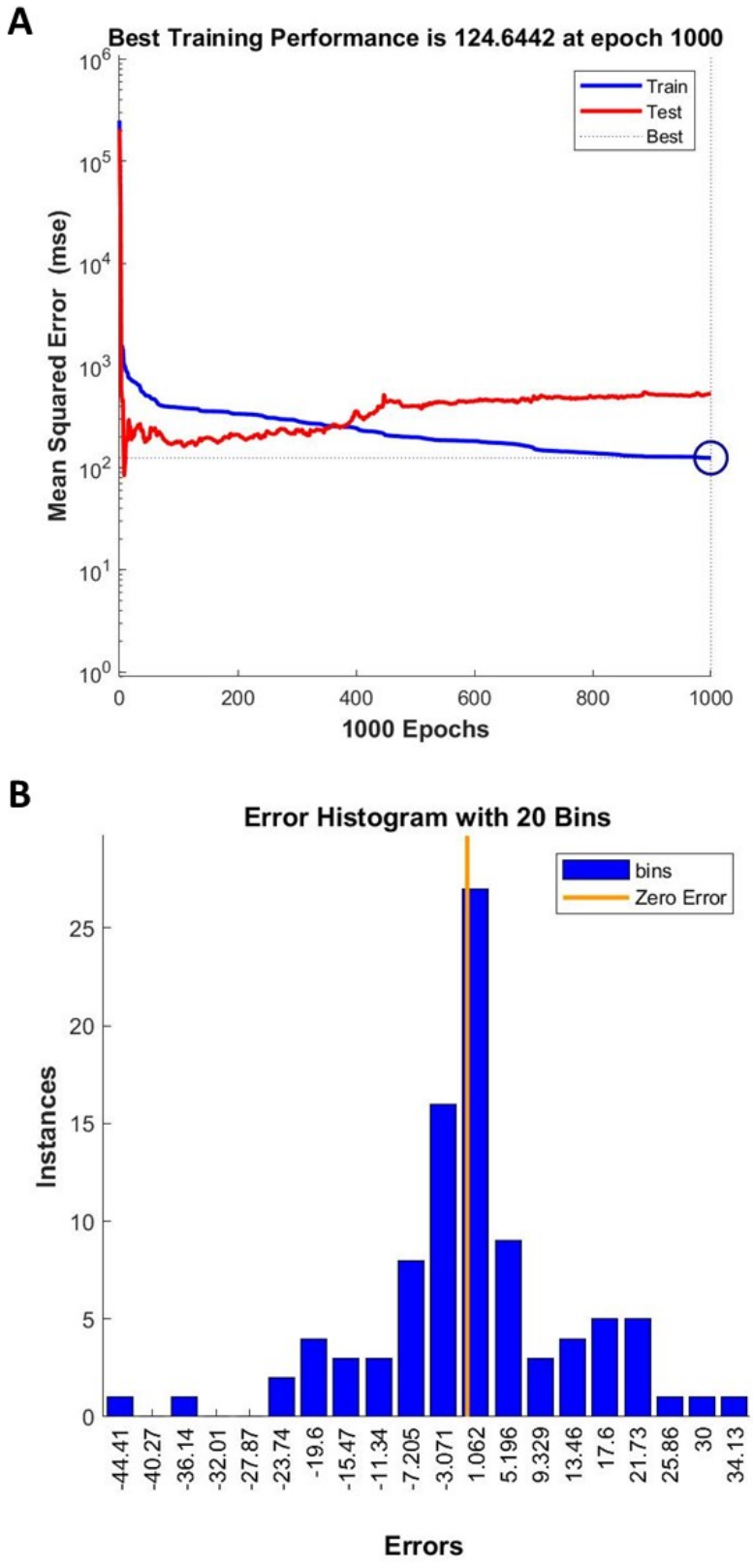

Figure S6: First artificial neural network performance using scaled conjugate gradient method for training. (a) mean squared error at different epochs. (b) the error histogram of the model.

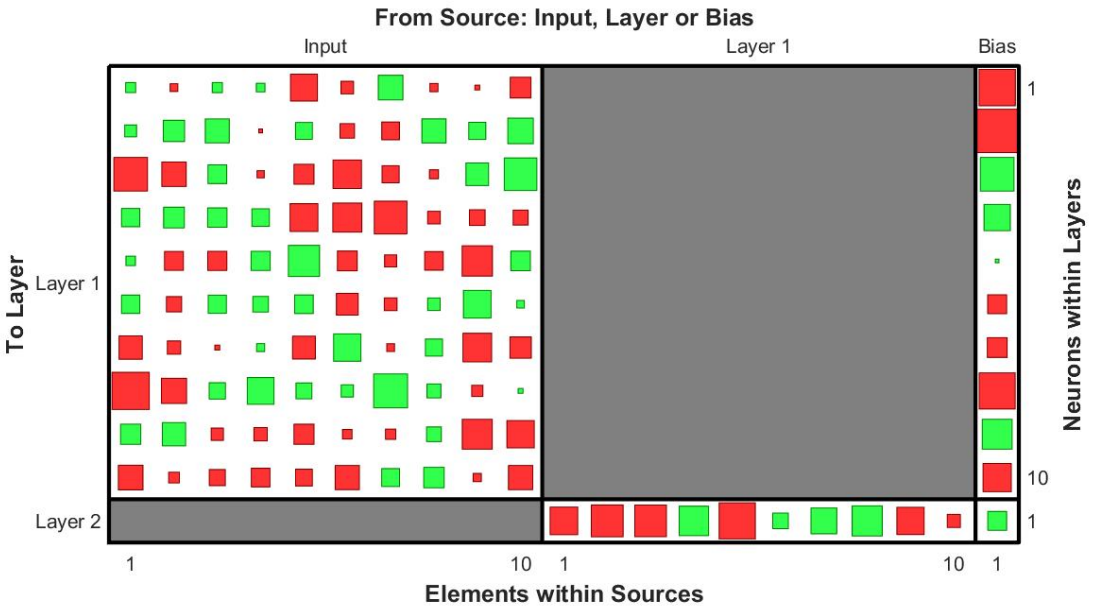

Figure S7: Hinton diagram representing the weight and bias values of the input (hidden) layers and output layers of neurons in our neural network that was trained using scaled conjugate gradient method.

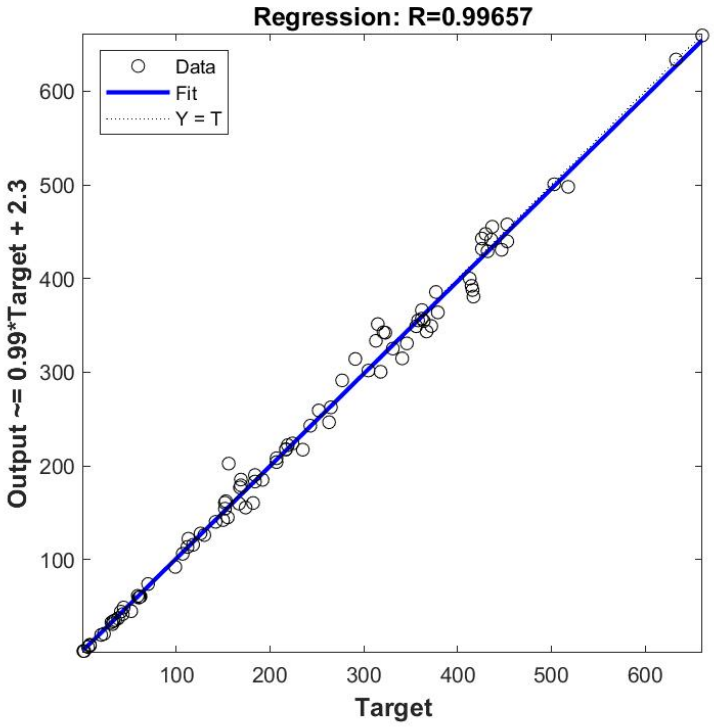

Figure S8: The 10 counts generated on the basis of different sensitivity values are fed to the neural network shown in Figure S2. The network was then trained using scaled conjugate gradient algorithm. The resulting regression plot consisting of training, validation, and testing data sets is shown above.

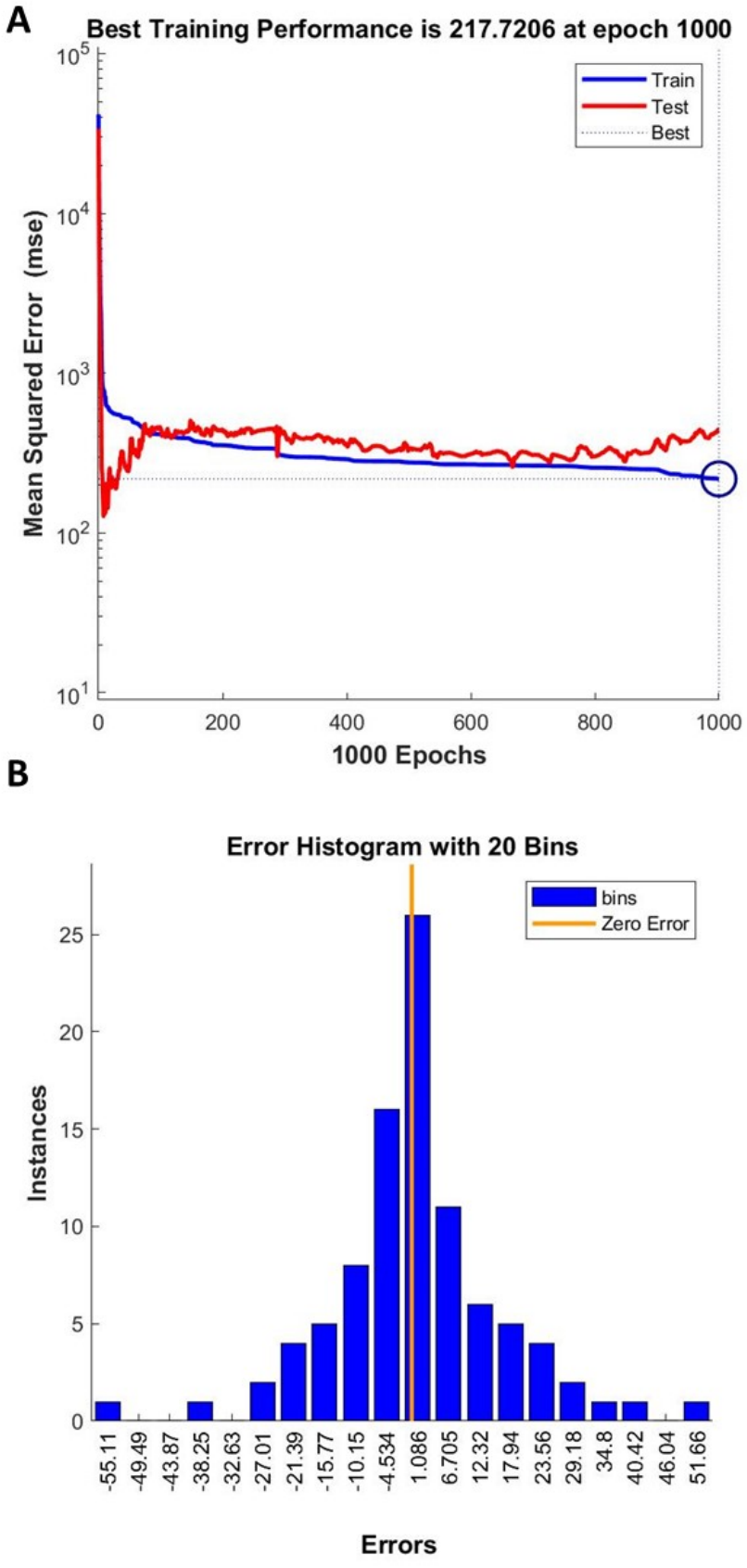

Figure S9: Second artificial neural network performance using scaled conjugate gradient method for training. (a) mean squared error at different epochs. (b) the error histogram of the model.

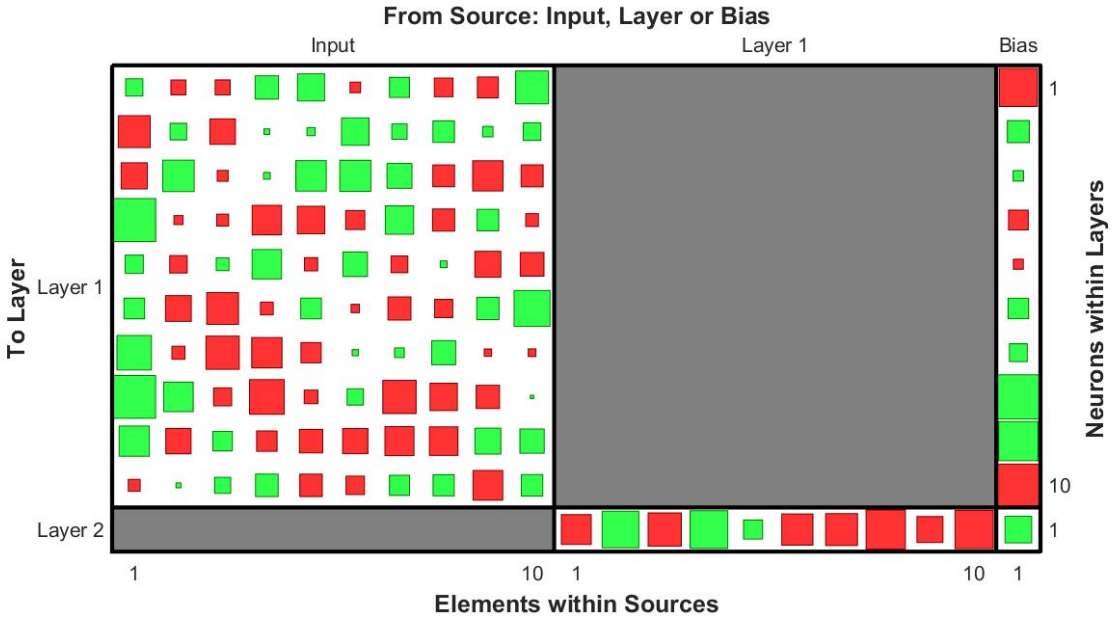

Figure S10: Hinton diagram representing the weight and bias values of the input (hidden) layers and output layers of neurons in our neural network that was trained using scaled conjugate gradient method.

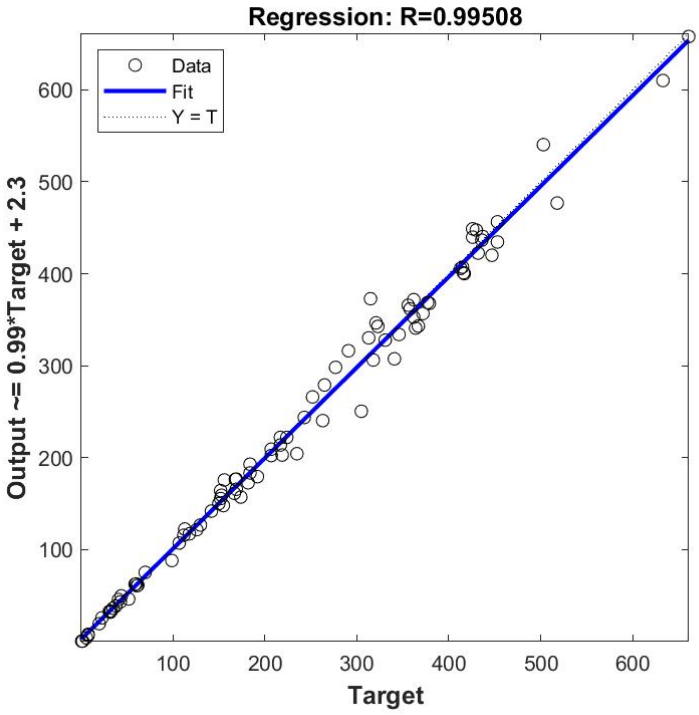

Figure S11: The 10 counts generated on the basis of different sensitivity values are fed to the neural network shown in Figure S2. The network was then trained using scaled conjugate gradient algorithm. The resulting regression plot consisting of training, validation, and testing is shown above.

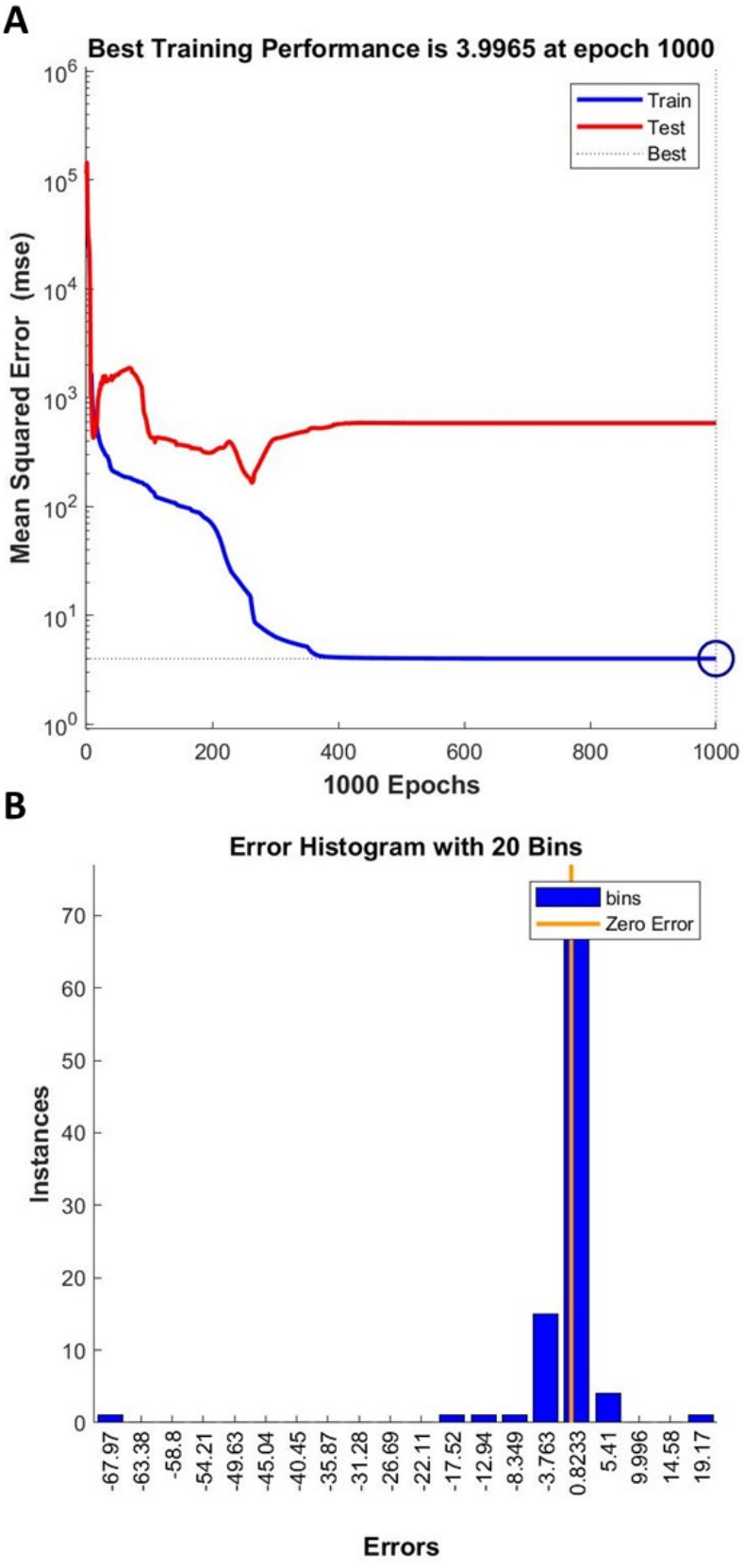

Figure S12: Third artificial neural network performance using Bayesian regularization method for training. (a) mean squared error at different epochs. (b) the error histogram of the model.

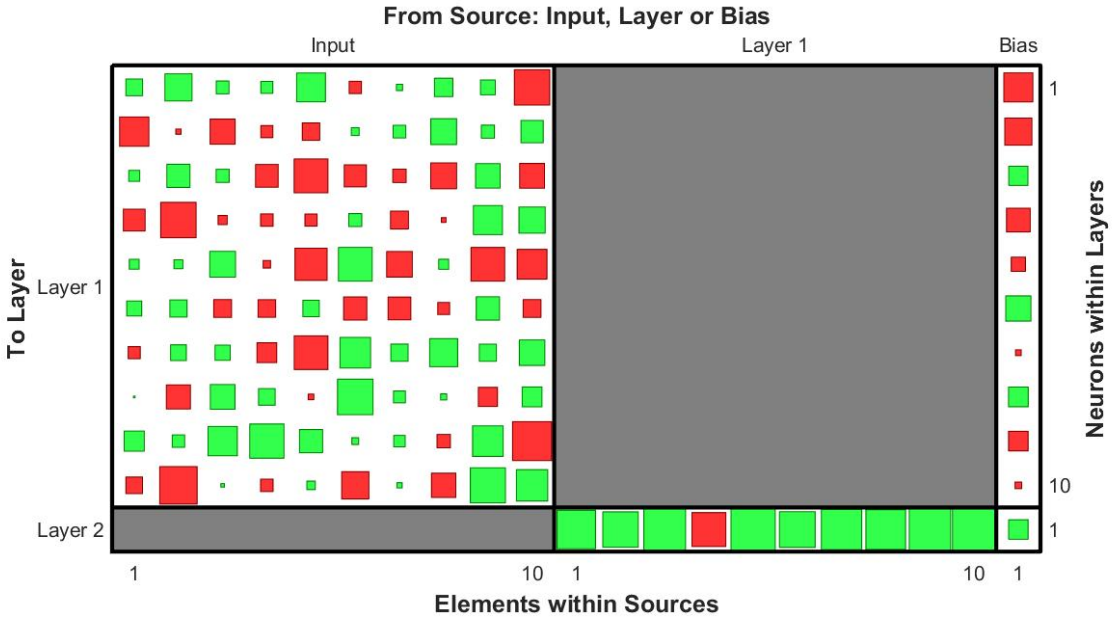

Figure S13: Hinton diagram representing the weight and bias values of the input (hidden) layers and output layers of neurons in our neural network that was trained using Bayesian regularization method.

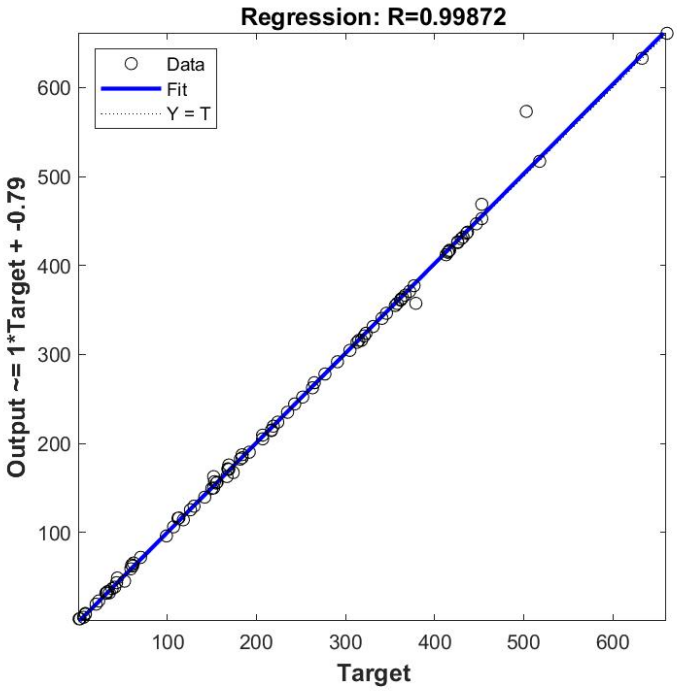

Figure S14: The 10 counts generated on the basis of different sensitivity values are fed to the neural network shown in Figure S2. The network was then trained using Bayesian regularization algorithm. The resulting regression plot consisting of training, validation, and testing is shown above.

Image 1	Image 2	Image 3	Image 4	Image 5	Image 6	Image 7	Image 8	Image 9	Image 10
219	518	633	367	182	150	118	35	33	142
Image 11	**Image 12**	**Image 13**	**Image 14**	**Image 15**	**Image 16**	**Image 17**	**Image 18**	**Image 19**	**Image 20**
61	126	341	192	107	174	167	155	38	32
Image 21	**Image 22**	**Image 23**	**Image 24**	**Image 25**	**Image 26**	**Image 27**	**Image 28**	**Image 29**	**Image 30**
43	235	364	62	8	1	20	41	2	23
Image 31	**Image 32**	**Image 33**	**Image 34**	**Image 35**	**Image 36**	**Image 37**	**Image 38**	**Image 39**	**Image 40**
6	377	318	346	265	8	169	169	52	152
Image 41	**Image 42**	**Image 43**	**Image 44**	**Image 45**	**Image 46**	**Image 47**	**Image 48**	**Image 49**	**Image 50**
436	168	217	453	426	207	59	153	112	112
Image 51	**Image 52**	**Image 53**	**Image 54**	**Image 55**	**Image 56**	**Image 57**	**Image 58**	**Image 59**	**Image 60**
313	453	437	503	60	70	331	432	152	184
Image 61	**Image 62**	**Image 63**	**Image 64**	**Image 65**	**Image 66**	**Image 67**	**Image 68**	**Image 69**	**Image 70**
379	417	243	31	113	130	44	315	277	413
Image 71	**Image 72**	**Image 73**	**Image 74**	**Image 75**	**Image 76**	**Image 77**	**Image 78**	**Image 79**	**Image 80**
430	321	372	362	358	252	447	415	356	323
Image 81	**Image 82**	**Image 83**	**Image 84**	**Image 85**	**Image 86**	**Image 87**	**Image 88**	**Image 89**	**Image 90**
416	263	291	426	99	224	362	184	305	156
Image 91	**Image 92**	**Image 93**							
169	207	217							

Table S1: Table showing the particle counts in the SBFM images that were used to train the artificial neural networks for multivariate regression.

		Percent Error in Each Image											
		Image 1	Image 2	Image 3	Image 4	Image 5	Image 6	Image 7	Image 8	Image 9	Image 10	Mean	Std
Scale Conjugate Gradient	Model 1	-38.5681	-8.2067	-9.13855	-18.5362	-0.72182	-9.86399	-6.04837	-3.67589	-10.5798	0.521448	-10.4818	10.69294
	Model 2	-31.5459	-17.6315	-9.94139	-15.3311	-6.96423	-13.9385	-4.31045	-4.22289	-14.0415	-0.03492	-11.7962	8.513127
	Model 3	-20.5842	-24.8252	-15.8416	-23.4746	-9.01918	-13.4395	-9.62523	-1.9957	-24.8414	-3.3038	-14.6951	8.180322
	Model 4	-31.2361	-9.77106	-15.5563	-18.0768	-3.6118	-10.9944	-5.31419	-4.62732	-22.9069	0.989919	-12.1105	9.394545
	Model 5	-15.084	-21.607	-6.24171	-18.9742	-3.19904	-11.095	-11.7511	-1.55591	0.925646	-1.84418	-9.04264	7.44159
	Model 6	-28.7524	-17.775	-15.2954	-19.6789	-8.52275	-10.7483	-5.25968	-4.8687	-19.1008	2.022869	-12.7979	8.570069
	Model 7	28.38254	-14.7083	-19.7033	-21.8698	-5.89934	-11.635	-10.9942	-2.91826	21.73549	2.514835	-3.50953	15.94855
	Model 8	-128.214	-13.9348	-15.186	-22.1569	-7.63767	-11.4345	-14.8159	-2.14723	-76.3364	0.805258	-29.1058	38.88973
	Model 9	-62.4452	-14.9175	-8.61804	-17.5327	-5.70997	-10.0313	-6.29333	-1.46187	-17.3063	2.183692	-14.2133	17.21247
	Model 10	0.039059	-13.6191	-8.71229	-20.107	-5.43982	-10.898	-5.19009	-3.00427	5.172576	-1.38724	-6.31462	6.927719
Lavenberg Marquardt	Model 1	57.24579	-37.8207	-12.8837	-14.1707	-8.09244	41.34562	49.91755	8.003829	-118.618	1.413251	-3.36598	48.33636
	Model 2	-308.268	-91.8685	6.844112	-30.4312	-5.92648	25.04175	-39.2008	-70.3969	-353.508	6.384941	-86.1329	127.4427
	Model 3	-78.306	-49.6333	10.56098	-3.70241	-6.37033	-8.93238	19.55318	-6.45044	70.07158	-9.15869	-6.23679	37.24215
	Model 4	68.05582	-176.037	12.80925	-18.8178	5.045897	69.65707	-40.1528	2.525997	-178.98	30.8954	-22.4998	84.00061
	Model 5	0.611291	-45.0454	-13.6728	-28.8221	-35.588	2.913837	40.52337	-12.0669	34.22507	7.604024	-4.93177	26.63799
	Model 6	-380.57	57.91063	5.595237	-7.40797	-19.8829	12.12262	-2.75707	-4.70983	14.79101	-25.2167	-35.0125	117.2354
	Model 7	27.88048	12.63154	-9.42278	-17.9903	-14.1048	5.157417	-3.03357	-9.99972	116.6201	-0.75348	10.6985	37.60359
	Model 8	144.1108	111.5149	12.29234	-12.3061	3.138749	-17.7259	-10.0364	-48.0869	-27.1577	3.57903	15.93229	58.69852
	Model 9	-129.429	72.07143	8.435833	-27.4393	11.14103	68.65003	-3.53102	-17.9712	-45.6948	253.8515	19.00845	95.31949
	Model 10	-43.4569	-21.6464	-12.7252	-3.8927	2.530305	-7.91705	16.50071	16.24854	-68.5614	5.609481	-11.7311	25.48622
Bayesian Regularization	Model 1	-139.329	-43.7288	4.994971	-16.1199	3.060797	-8.51007	-1.84716	3.969962	-187.319	-5.15075	-38.9979	64.51128
	Model 2	-7.60007	-23.243	-9.88813	-15.8701	-0.52444	-13.4442	6.264494	3.667444	27.39919	4.223016	-2.90159	13.6591
	Model 3	-216.199	-13.0643	-9.90838	-14.3042	0.435596	-20.0737	2.73838	0.295824	-163.939	1.028809	-43.299	74.66942
	Model 4	2.814407	-11.4153	-6.63475	-20.4427	1.040819	-20.0762	-5.17349	7.689511	17.66144	-13.5775	-4.81137	11.6403
	Model 5	-117.414	-29.6527	-4.70712	-8.94218	-6.74286	-13.6359	4.468904	3.694926	-52.18	0.449193	-22.4661	35.64842
	Model 6	-155.738	-20.7241	-10.2336	-12.1808	2.005576	-8.47152	5.533297	2.607704	-137.557	-2.52987	-33.7289	57.09831
	Model 7	-72.7531	-16.1429	-17.5896	-21.7669	-7.92389	-9.35012	-9.01389	-3.91084	-39.0715	2.482177	-19.5041	20.76586
	Model 8	30.45721	-29.6066	-21.4643	-5.95284	0.455467	-13.5544	7.82871	-1.47792	37.06464	0.532394	0.42823	19.79896
	Model 9	-100.371	-33.0707	-19.7827	-14.8536	-4.88624	-23.778	9.050812	9.908433	-61.3915	-3.32559	-24.25	32.32436
	Model 10	-94.6029	-17.823	-12.6192	-22.0117	-0.79678	-9.00232	-7.90073	-3.83209	-80.0222	1.719769	-24.6891	32.21939

Table S2: Table showing the corresponding percentage errors and standard deviation for each trained network for the ten blinded test images. The best three performing networks have been highlighted.

	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	Bias	O
w1	0.156927	-0.09443	0.16078	0.116254	-1.13527	-0.24872	0.951112	-0.10562	-0.03682	-0.67684	-2.07819	-1.20126
w2	0.221045	0.711481	0.903881	-0.02272	0.446455	-0.3313	-0.49521	0.91435	0.458135	1.024905	-2.95416	-1.59166
w3	-1.78261	-0.9369	0.550892	-0.08215	-0.62871	-1.27334	-0.46311	-0.12669	0.80314	1.66802	1.782773	-1.57706
w4	0.522355	0.677722	0.587892	0.484589	-1.21993	-1.32384	-1.68996	-0.25118	-0.38597	-0.3514	1.081087	1.363382
w5	0.139977	-0.56268	-0.57326	0.575295	1.519453	-0.61607	-0.2276	-0.54144	-1.44526	0.600633	0.020855	-2.05648
w6	0.526816	-0.36431	0.522692	0.379386	0.54162	-0.7465	-0.25394	0.24466	1.196136	0.089684	-0.56447	0.382139
w7	-0.85542	-0.27931	-0.03779	0.099641	-0.8211	1.171898	-0.09476	0.460604	-1.29537	-0.71718	-0.61693	1.049347
w8	-2.17283	-0.9969	0.418928	1.12595	0.39637	0.236232	1.807338	0.305608	-0.19788	0.037378	-2.04882	1.429442
w9	0.638884	0.850866	-0.23787	-0.27852	-0.63536	-0.14149	-0.16481	0.338834	-1.41036	-1.17344	1.406274	-1.16555
w10	-0.95266	-0.18214	-0.40624	-0.54896	-0.44197	-0.91688	0.504135	0.660171	-0.10691	-0.92448	-1.25608	-0.27041

Table S3: Table showing the input and output weights along with the bias value of the Neural Network 1, trained using scaled conjugate gradient algorithm. Bias value of output neuron is 0.559311.

	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	Bias	O
w1	0.340804	-0.25451	-0.25741	0.623186	0.83953	-0.12726	0.458142	-0.39938	-0.50163	1.237552	-1.01998	-1.65325
w2	-1.16708	0.316284	-0.74328	0.03692	0.07556	0.859049	0.267068	0.527801	0.122991	0.354812	1.535574	0.550787
w3	-0.77902	1.132483	-0.13711	0.051578	1.059387	1.106365	0.713038	-0.54343	-1.05067	-0.55378	-1.26264	0.122835
w4	2.11044	-0.0936	-0.16109	-0.99315	-0.86287	-0.41514	0.919919	-0.56108	0.529852	-0.18738	1.620775	-0.44483
w5	0.379927	-0.34662	0.187267	0.987913	-0.19855	0.684744	-0.31945	0.052116	-0.77319	-0.64216	0.412302	-0.11448
w6	0.487237	-0.76155	-1.15441	-0.18835	0.493057	-0.07936	-0.6084	-0.38008	0.567484	1.474693	-1.10932	0.480254
w7	1.356049	-0.19399	-1.26437	-1.04737	-0.4674	0.042151	0.106636	0.659792	-0.06153	-0.06683	-1.16398	0.368343
w8	2.090725	0.994275	-0.36977	-1.37661	-0.21228	0.305071	-1.26484	-0.85253	-0.62038	0.012549	-1.76161	2.235846
w9	1.048044	-0.73163	0.434118	-0.4728	-0.62732	-0.72851	-0.96662	-0.92894	0.764957	0.673344	-0.7726	1.750524
w10	-0.16352	0.027723	0.295012	0.583653	-0.57697	-0.38982	0.459392	0.511915	-1.01511	0.528175	-1.67907	-2.07001

Table S4: Table showing the input and output weights along with the bias value of the Neural Network 2, trained using scaled conjugate gradient algorithm. Bias value of output neuron is 0.810424.

	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	Bias	O
w1	0.577352	1.513568	0.337433	0.292214	1.705936	-0.30633	0.079619	0.691268	0.451605	-2.56264	3.043035	-1.7332
w2	-1.76735	-0.05686	-1.29623	-0.29862	-0.62226	0.125867	0.328523	1.368704	0.349812	1.019168	2.575995	-1.51357
w3	0.247057	1.099893	0.351843	-1.06112	-2.35103	-1.0039	-0.3688	-1.37898	1.233382	-1.26418	3.584894	0.752884
w4	-0.9797	-2.57384	-0.16993	-0.31543	-0.30123	0.344065	-0.65887	-0.04644	1.73377	1.407588	-2.36269	-1.15036
w5	0.19875	0.161941	1.366252	-0.1167	-2.12102	2.340452	-1.3529	0.207834	-2.33352	-1.82211	4.017845	-0.41558
w6	0.458301	0.605998	-0.64907	-0.63377	0.554874	-1.10631	-1.06276	-0.29514	1.116189	-0.65059	2.625013	1.301798
w7	-0.30077	0.509725	0.473738	-0.80813	-2.32798	1.926679	0.607765	1.61303	0.602343	1.335759	3.345329	-0.06747
w8	0.005282	-1.19436	1.252517	0.564789	-0.06532	2.590052	0.290951	0.076409	-0.75169	0.804598	3.229693	0.799733
w9	0.828429	0.31573	1.761224	2.40865	1.104454	0.093962	0.268645	-0.36816	1.920531	-3.12425	3.532193	-0.78412
w10	-0.56593	-2.87765	0.025169	-0.31624	0.145819	-1.5246	0.0567	-1.2134	2.533574	2.019321	3.850481	-0.08935

Table S5: Table showing the input and output weights along with the bias value of the Neural network 3, trained using Bayesian regularization algorithm. Bias value of output neuron is 0.779855599.

Reference Cited	Multiple magnification	Multiple fluorophores	Multiple smartphones	Microfluidic chip	Custom image-processing algorithm	complexity/time Analysis
7	✗	✗	✗	✓	✓	✗
8	✗	✗	✗	✓	-	✗
9	✗	✗	✗	✗	✓	✗
10	✗	✗	✗	✗	✓	✗
11	✗	✓	✗	✗	✓	✗
12	✗	✗	✗	✓	✓	✗
13	✗	✗	✗	✓	✓	✗
14	✗	✓	✗	✗	✗	✗
15	✓	✓	✗	✗	✓	✗
16	✗	✓	✓	✗	✓	✗
17	✗	✗	✗	✓	✗	✗
18	✗	✗	✗	✗	✓	✗
19	✓	✓	✗	✗	✓	✗
20	✗	✓	✓	✗	✓	✗
21	✓	✗	✗	✗	✓	✗
22	✗	✓	✗	✓	✓	✗
23	✗	✗	✗	✗	✓	✗
24	✗	✗	✗	✓	✓	✗
26	✗	✓	✗	✗	✗	✗
27	✗	✗	✗	✓	✓	✗
30	✗	✗	✗	✓	✓	✗
Presented design	✓	✓	✓	✓	✓	✓

Table S6: Comparison between the different functionalities and capabilities offered by previously discussed SBFMs and the presented SBFM.

Smartphone	Aspect ratio	Zoom level	Image dimensions	Image size	Processing time (s)
Galaxy S9+	3:4	Min	3024 x 4032	3.2 MB	27
		Max	3024 x 4032	1.19 MB	35
	9:16	Min	2268 x 4032	2.43 MB	19
		Max	2268 x 4032	978 KB	26
	1:1	Min	3024 x 3024	2.41 MB	20
		Max	3024 x 3024	0.99 MB	21
	Full	Min	1960 x 4032	2.16 MB	17
		Max	1960 x 4032	860 KB	25
Lumia 1020	3:4	Min	2592 x 1936	982 KB	9
		Max	2592 x 1936	1.46 MB	8
	16:9	Min	3072 x 1728	911 KB	8
		Max	3072 x 1728	1.64 MB	9
iPhone XS	3:4	Min	3024 x 4032	1.55 MB	19
		Max	3024 x 4032	746 KB	78
	9:16	Min	2268 x 4032	1.66 MB	14
		Max	2268 x 4032	800 KB	50
	1:1	Min	3024 x 3024	1.54 MB	15
		Max	3024 x 3024	749 KB	50

Table S7: Comparison of processing times for images obtained under different aspect ratios and zoom setting using the developed ANN based image processing algorithm.