Deep Learning Enabled Universal Multiplexed Fluorescence Detection for Point-of-Care Applications

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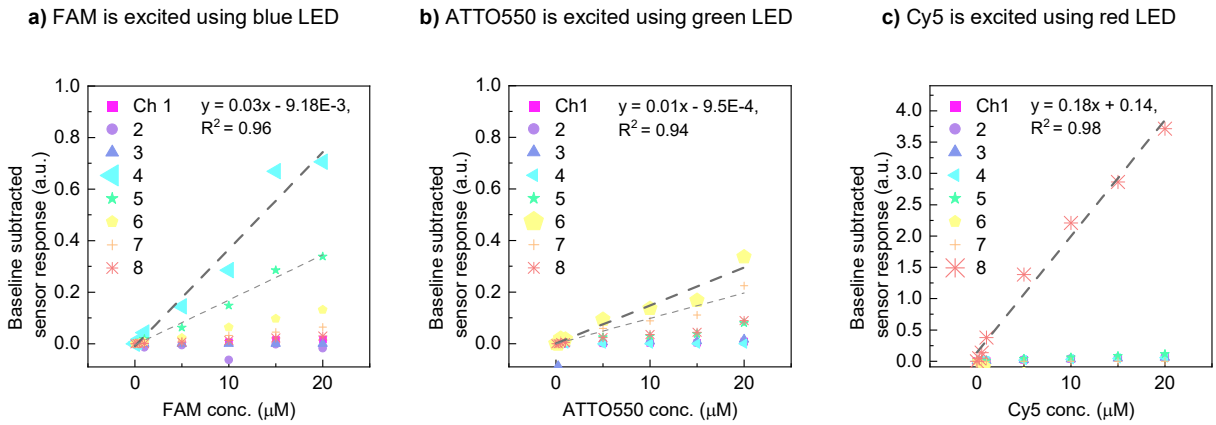


Figure S1. Choosing optimal excitation sources and detection channels for FAM, ATTO550, and Cy5 individually. a) The responses of all channels of the spectral sensor for increasing concentration when only FAM is excited with the blue light of the RGB LED. Channel #3 (470 nm) captures the 466 nm blue LED excitation, while channel #4 (510 nm) measures the fluorescence emission. Adjusting channel 4's reading by dividing its value by channel 3's negates the leaked excitation light into the sensor and mitigates excitation brightness variations. After subtracting the baseline from this adjusted value, channel #4 is the most responsive for FAM, evidenced by the steepest linear fit slope for each channel's adjusted RFUs. b) The spectral sensor's channel responses to increasing concentration when only ATTO550 is excited by green light. Similar to part a, adjusting channel #6's (583 nm) reading with channel #5's (510 nm) reading shows that channel #6 is the most responsive. **c)** The spectral sensor's channel responses to increasing concentration when only Cy5 is excited by red light. Adjusting channel #8's (670 nm) reading with channel #7's (620 nm) reading shows that channel #8 is the most responsive. These analyses match the simple theoretical alignment of spectra presented in Figure 2a.

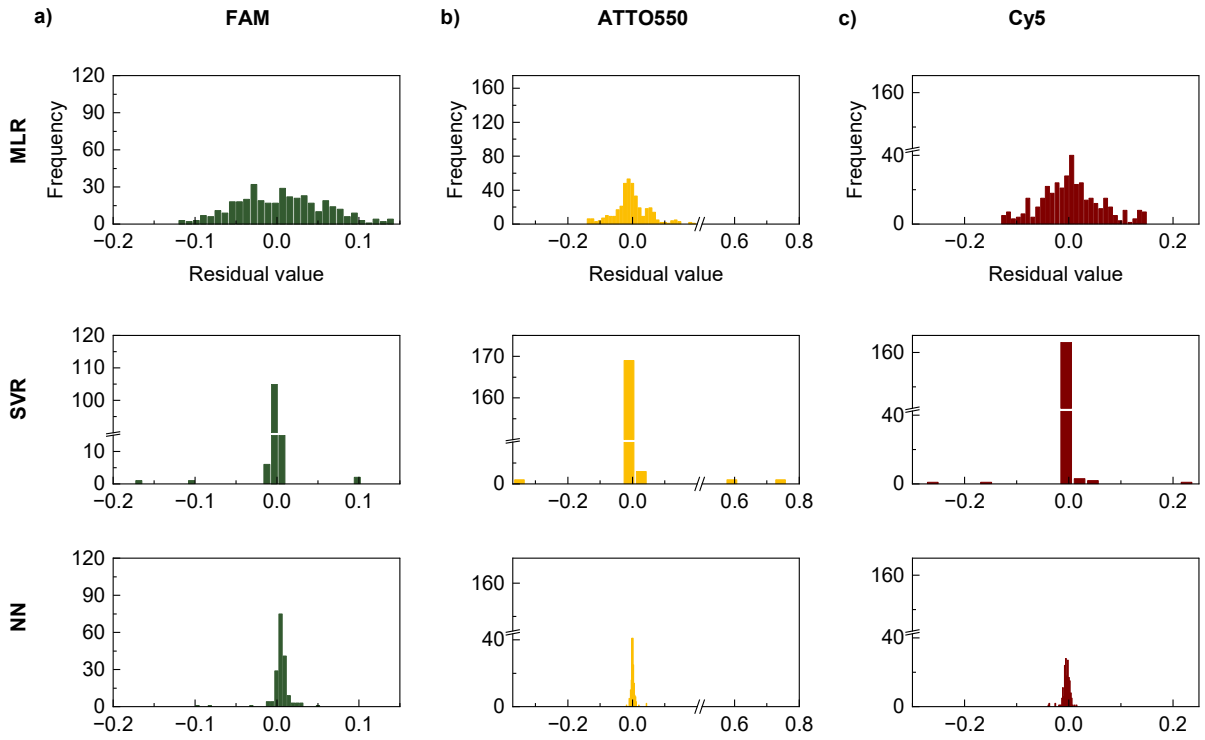


Figure S2. Residual analyses of the machine learning models used for fluorophore concentration prediction in Section 3.2. a) Histograms of residuals for FAM: MLR (top), SVR (middle), and NN (bottom). The MLR model shows a broad distribution, indicating variability in its predictions for FAM. SVR and NN models display tighter distributions, suggesting more consistent predictions, but SVR exhibits outliers. **b)** Histograms of residuals for ATTO550: MLR (top), SVR (middle), and NN (bottom). The MLR model again shows a broader distribution. The SVR model is prone to outliers, indicating extreme residual values far from the central cluster of residuals. The NN model presents a very tight distribution, indicating high prediction consistency. **c)** Histograms of residuals for Cy5: MLR (top), SVR (middle), and NN (bottom). All models show residuals clustered around zero, with the NN model exhibiting the tightest distribution, suggesting it may provide the most accurate and consistent predictions for Cy5. Overall, while the SVR model demonstrates outliers potentially caused by non-linear interactions, heteroscedasticity, or measurement errors, the NN model shows a higher frequency of small residuals, suggesting it offers the most precise predictions across fluorophores.

Supplementary Tables

Table S1. MAE, LoD, and LoQ: scenario-based comparison

	Best case scenario (conc. of other two fluorophores is 0 µM)			Worst case scenario (conc. of other two fluorophores is 1 µM)		
	% MAE	**LoD (µM)**	**LoQ (µM)**	**% MAE**	**LoD (µM)**	**LoQ (µM)**
			Multiple Linear Regression			
FAM	5.79	0.15	0.48	13.62	0.17	0.55
ATTO550	6.19	0.15	0.50	2.70	0.05	0.16
Cy5	11.31	0.27	0.88	7.65	0.13	0.42
			Support Vector Regression			
FAM	0.36	0.02	0.05	0.30	0.01	0.05
ATTO550	0.06	0.01	0.01	0.03	0.01	0.01
Cy5	0.12	0.01	0.02	0.07	0.01	0.01
			Multilayer Perceptron (NN) Regression			
FAM	0.04	0.01	0.02	0.27	0.02	0.06
ATTO550	0.32	0.01	0.02	1.37	0.03	0.10
Cy5	0.25	0.01	0.05	0.4	0.02	0.06

Table S2. Cost estimate

Vendor	**Description**	**Function**	**Unit cost ($)**	**Quantity**	**Extended cost ($)**
Adafruit	DC/DC step-down	Voltage converter	8.96	1	8.96
Adafruit	RGB led	Excitation source	0.5	1	0.5
Adafruit	0.1” spaced pin strip (male and female)	Connect the LED and spectral sensor PCBs to Raspberry Pi	0.9	1	0.9
Digikey	Thermistor	Temperature feedback	7.22	1	7.22
Digikey	4 channel 16-bit ADC	Convert voltage to digital values	6.35	1	6.35
Digikey	Spectral sensor	Fluorescence detection	7.5	1	7.5
Digikey	DC barrel jack	Power connector	0.76	1	0.76
Digikey	0.1 and 10 µF SMD capacitors and 10k ohm SMD resistor	Passive components for power regulation of ICs	0.02	5	0.1
Digikey	2 ohm 1% 25W power resistor	Heat generation	7.63	1	7.63
Digikey	Microprocessor board	Electronic coordination and data logging	10	1	10
Digikey	SPDT switch	Power delivery	0.07	1	0.07
OSH park	Two PCBs for LED and spectral sensor	Interface LED and spectral sensor with Raspberry Pi	4.34	1	4.34
				Total cost ($)	**54.33**

Table S3. RT-LAMP assay primer sequences

Primer	Sequence 5' → 3'
RSV F3	GAGTTGAAGGGATTTTTGCA
RSV B3	TGGGTTGTTCAATATATGGTAGA
RSV FIP	TAACTGATTTTGCTAAGACCCCCGGATTGTTTATGAATGCCTATGG
RSV QFIP	/5IABKFQ/TAACTGATTTTGCTAAGACCCCCGGATTGTTTATGAATGCCTATGG
RSV Fd	GGGTCTTAGCAAAATCAGTTA/3FLOURT/
RSV BIP	CAAGCAGAAATGGAACAAGTTGTGCTGCTTCTCCACCCAATT
RSV LF	CACCGTAACATCACTTG
RSV LB	GAGGTGTATGAGTATGCTCAGA
IAV F3	GACTTGAAGATGTCTTTGC
IAV B3	TRTTATTTGGGTCTCCATT
IAV FIP	TTAGTCAGAGGTGACARRATTGCAGATCTTGAGGCTCTC
IAV QFIP	TTGTKTTCACGCTCACCGTGTTTGGACAAAGCGTCTACG
IAV Fd	GTCTTGTCTTTAGCCA
IAV BIP	CMAGTGAGCGAGGACTG
IAV LF	/5IABkFQ/TTAGTCAGAGGTGACARRATTGCAGATCTTGAGGCTCTC
IAV LB	CAATYYTGTCACCTCTGACTAA/3ATTO550N/
SARS-CoV-2 F3	AACACAAGCTTTCGGCAG
SARS-CoV-2 B3	GAAATTTGGATCTTTGTCATCC
SARS-CoV-2 FIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG
SARS-CoV-2 QFIP	TGCGGCCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC
SARS-CoV-2 Fd	TTCCTTGTCTGATTAGTTC
SARS-CoV-2 BIP	ACCTTCGGGAACGTGGTT
SARS-CoV-2 LF	/5IAbRQ/CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG
SARS-CoV-2 LB	AAGTGACTTCCATGCCAATGCG/3Cy5Sp/