Supplementary Information

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A. Additional figures for background information and CAD diagrams

A.1 Relative emission intensity (%) of a UV LED and a typical xenon arc lamp

Figure S1 is an unused figure demonstrating the relative emission intensity (%) of a UV LED and a typical xenon arc lamp.[1,2] An additional advantage of the LED is the power output at the UV LED emission wavelength. While xenon arc lamps operate at a higher overall power output, this power is distributed over a much larger spectral range (180 – 1100 nm) whereas the LED may be purchased for a specific peak wavelength.

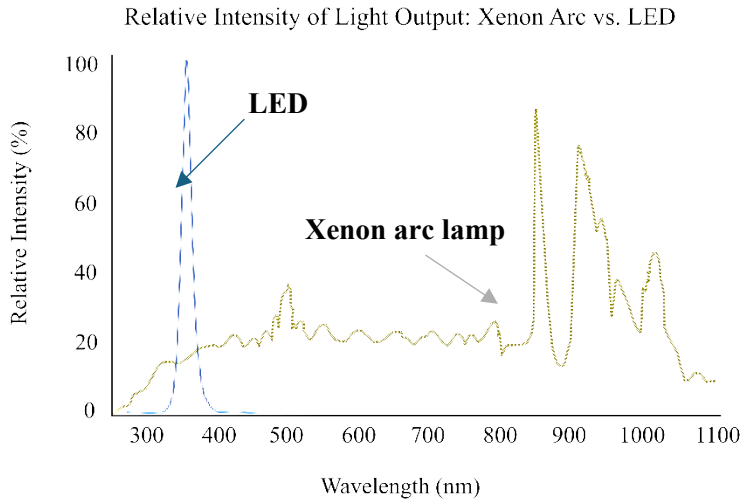

Fig. S1 Overlay of the relative emission intensity (%) of a UV LED and a typical xenon arc lamp. In blue dashed line, the LED spectral power (adapted from [1]) distribution for a 365 nm peak wavelength LED overlaid on the xenon arc lamp (solid yellow) relative light output (adapted from [2]).

A.2 Additional CAD models for detector body (side view and top-down view)

IGES and STEP files for the 3D printed components are provided as a zip file.

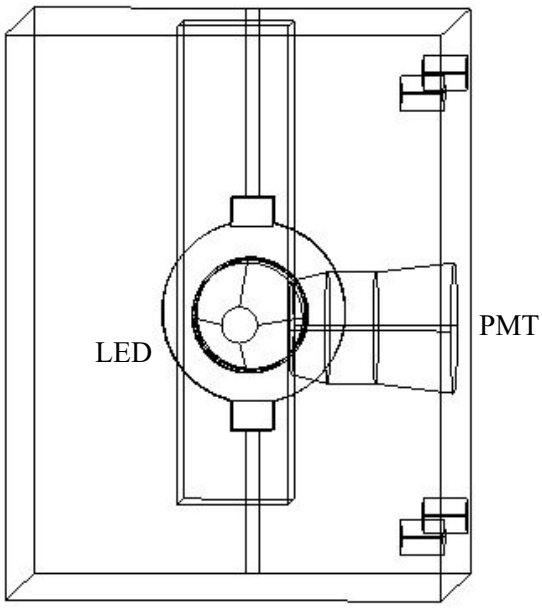

Fig. S2 CAD model (side view) of detector body.

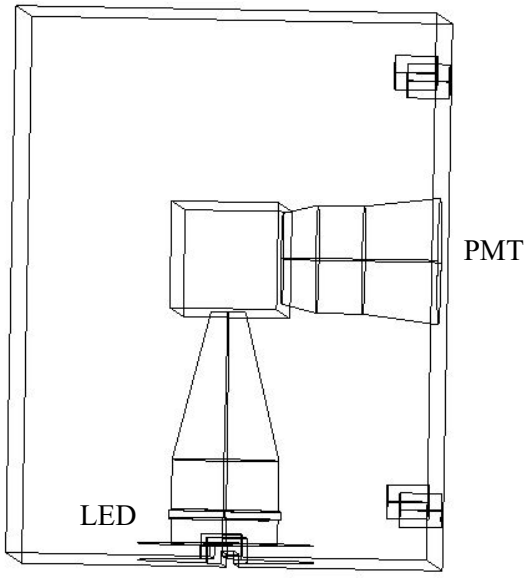

Fig. S3 CAD model (top-down view) of detector body.

B. Additional figures with data for emission filter testing and SNR estimation

B.1 Signal output from the 3DFL detector using a 400 nm CWL emission filter

The 365 nm excitation wavelength filter used for the HAA system overlapped with the 400 nm emission causing a high and erratic signal output from the PMT, as shown in Figure S4.

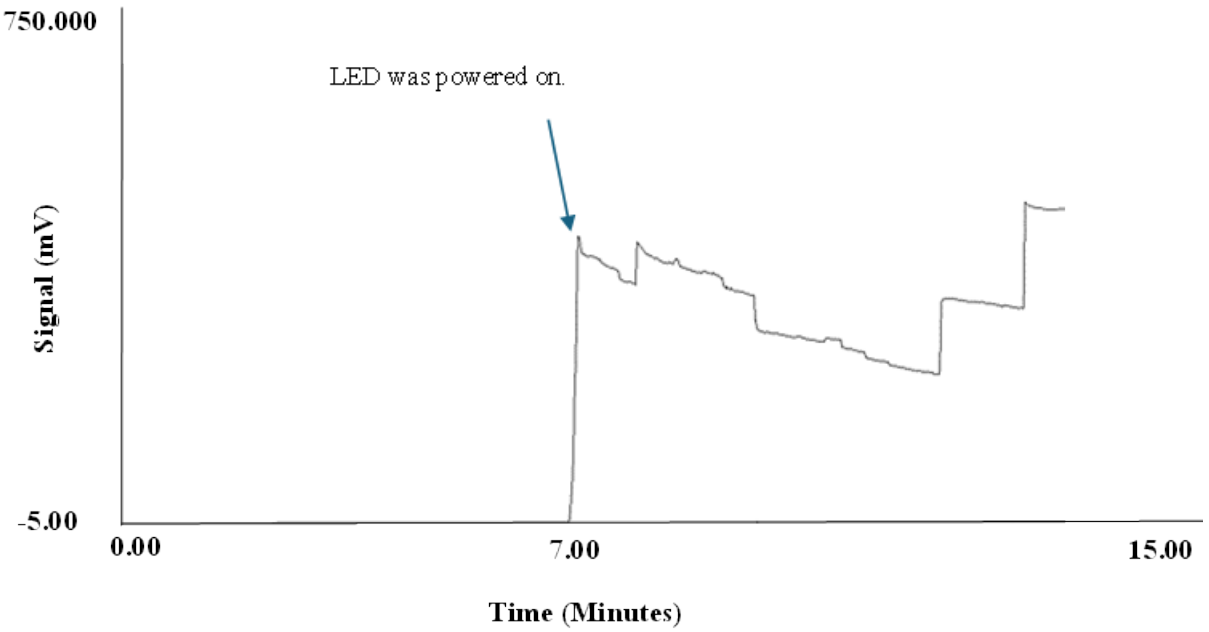

Fig. S4 The signal output from the 3DFL detector using the 400 nm CWL emission filter. From time 0 to 7 minutes the excitation light was not powered on. At 7 minutes, the LED was powered on. As can be seen in the signal, upon turning the LED on, the PMT signal was unable to stabilize and reach a flat baseline signal.

B.2 Raw data from an example blank run on the 3DFL detector to estimate noise level

A blank run was performed by running the reagent water carrier stream, injecting a plug of reagent water and recording the signal over two minutes. Data acquisition was performed using PeakSimple hardware (analog to digital converter) and integration software (SRI Instruments, California, USA). To obtain the peak area of the noise a baseline was placed by the PeakSimple software shown as a blue line in Figure S5, and the peaks of the signal (signal line shown in black, Figure S5) noise above this baseline were integrated to obtain a peak area. Each vertical blue line in Figure S5 is automatically dropped by the software and corresponds to a peak. The area between the blue baseline and the black signal line is integrated (mathematically) in the software to obtain a peak area of the noise. The higher these peaks are above the baseline, the greater the noise in the instrument.

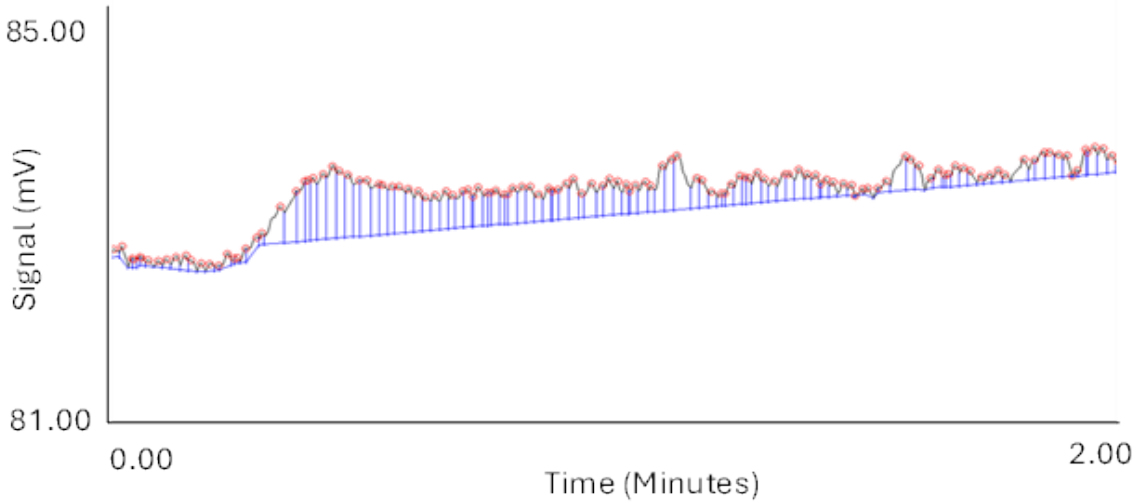

Fig. S5 A blank run on the 3DFL detector used to calculate the noise in the instrument. The blue line represents the baseline signal. Deviations of the signal (black line) above this baseline are recorded as peak areas to represent an estimation of the noise. Each red circle indicates a peak.

C. Information for nicotinamide standard preparation for SNR estimation and MDL, accuracy, and precision studies

SNR Estimation Study: To obtain an estimation of the SNR for the 3DFL and commercial detectors, a **19.8 µM** nicotinamide check standard (Table S1, Alt1) was analyzed to estimate the signal whereas the noise was estimated by averaging 100 data points from peak area data collected from a two-minute analysis of a blank. The average peak area from the *n* replicate check standards at 19.8 µM were divided by the average noise from the blank runs to obtain the representative SNR.

Calibration Studies: As summarized in Table S1, the calibration standards ranged from **1.4 to 141.7 µM** nicotinamide (Figure S1, Level 1 – 8), with a check standard (Table S1, Alt1) of 19.8 µM. The check standard (Alt1) was analyzed eight consecutive times. Examples of raw and treated data are featured in Section D.

Table S1 Typical nicotinamide (NCA) concentrations for signal-to-noise (SNR) and MDL, accuracy, and precision studies.

NCA Preparation Calculations				
Mass NCA (g)	**NCA MW (g/mol)**	**Final Volume (L)**	**[NCA] (M)**	**[NCA] (mM)**
0.0173	122.12	0.100	0.001417	1.417
Standards	**Volume (mL)**	**Total Volume (mL)**	**Actual [NCA] (µM)**	**[NCA] (µM)**
Level 1	0.10	100.00	1.42	1417
Level 2	0.50	50.00	14.17	
Level 3	1.20	50.00	34.00	
Level 4	1.70	50.00	48.17	
Level 5	2.50	50.00	70.83	
Level 6	3.20	50.00	90.66	
Level 7	3.70	50.00	104.83	
Level 8	5.00	50.00	141.66	
Alt1	0.70	50.00	19.83	
Alt2	2.80	50.00	79.33	
Alt3	0.20	50.00	5.67	

D. Treated data for MDL, accuracy, and precision studies for the 3DFL and commercial fluorescence detectors

D.1 MDL, accuracy, and precision results for the 3DFL detector

Table S2 Linear regression and MDL, accuracy, and precision results for nicotinamide (NCA) for the 3DFL detector.

Calibration Std.#	NCA Concentration (µM)	Peak Area (mV*s)		
Level 1	1.4	11.48		
Level 2	14.2	55.45		
Level 3	34.0	103.48		
Level 4	48.2	123.94		
Level 5	70.8	200.40		
Level 6	90.7	252.12		
Level 7	104.8	296.05		
Level 8	141.7	369.34		
Count (N) =	**Σx_i=**			
8	505.74			
Average	63.22			
S_{xx} =	16002.51			
S_{yy} =	1.08E+05			
S_{xy}=	4.14E+04			
$SS_{residual}$ =	5.35E+02	**Inst. LOD**	10.95	**µM**
s_y =	9.44	**Inst. LOQ**	36.48	**µM**
m =	2.5884	**s_m =**	0.0747	
b =	12.9026	**s_b =**	5.7809	
R^2=	0.9950	**sR^2 (s_y) =**	9.4434	

Check Standard Replicate # (or Detection Limit Study)	Peak Area (mV*s)	NCA Concentration (µM)	
1	70.01	22.06	
2	68.67	21.55	
3	67.53	21.10	
4	68.72	21.56	
5	74.71	23.88	
6	76.32	24.50	
7	72.80	23.14	
8	66.84	20.84	
Mean	70.70	22.33	
Stdev	3.50	1.35	
Count (N) =	**Prop Unc.**	2.17	
8	**CI95%**	1.13	
	High	23.46	
Check Standard "True" Value	**Low**	21.20	
19.8			
Precision	**%RSD**	6.05	**%RSD Prop.**
Accuracy	**Mean % Recovery**	112.59	9.73
	Mean Absolute Error	2.50	**µM**
	% Relative Error	12.59	**µM**
Detection Limits	**Harris LOD**	4.05	**µM**
	Harris LOQ	13.51	**µM**
	Error Prop DL (Unc)	6.52	**µM**
	USEPA MDL	4.05	**µM**
	ISO DL	10.02	**µM**
	Instrument LOD	10.95	**µM**
	Instrument LOQ	36.48	**µM**
	Average Blank Area	0.18	**µM**
	Signal DL	10.67	**mV*s**
	Traditional DL	10.95	**µM**

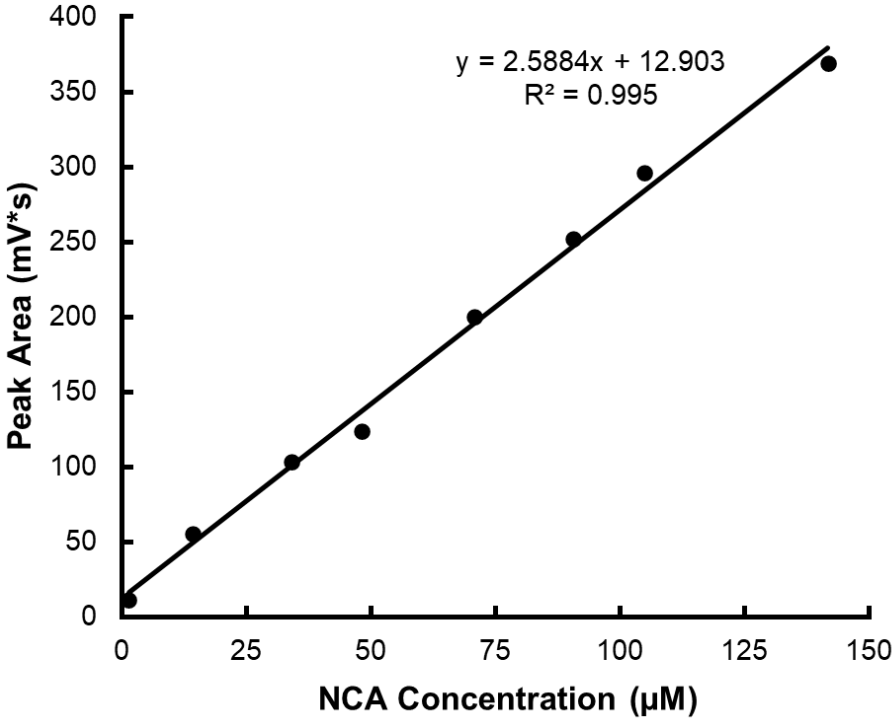

Fig. S6 Calibration curve for nicotinamide (NCA) using the 3DFL detector.

D.2 MDL, accuracy, and precision results for the commercial detector (Waters)

Table S3 Linear regression and MDL, accuracy, and precision results for nicotinamide (NCA) for the commercial detector.

Calibration Std.#	NCA Concentration (µM)	Peak Area (mV*s)		
Level 1	1.4	0.73		
Level 2	14.2	2.06		
Level 3	34.0	3.82		
Level 4	48.2	4.64		
Level 5	70.8	8.10		
Level 6	90.7	8.85		
Level 7	104.8	10.49		
Level 8	141.7	13.44		
Count (N) =	Σx_i=			
8	505.74			
Average	63.22			
S_{xx} =	16002.51			
S_{yy} =	1.36E+02			
S_{xy}=	1.47E+03			
$SS_{residual}$ =	1.18E+00	Inst. LOD	14.50	µM
s_y =	0.44	Inst. LOQ	48.34	µM
m =	0.0917	s_m =	0.0035	
b =	0.7186	s_b =	0.2715	
R^2=	0.9913	sR^2 (s_y) =	0.4435	

Check Standard Replicate # (or Detection Limit Study)	Peak Area (mV*s)	NCA Concentration (µM)	
1	2.40	18.28	
2	2.38	18.15	
3	2.38	18.15	
4	2.13	15.39	
5	2.43	18.65	
6	2.76	22.26	
7	2.61	20.57	
8	2.65	21.08	
Mean	2.47	19.07	
Stdev	0.20	2.15	
Count (N) =	Prop Unc.	2.95	
8	CI95%	1.80	
	High	20.87	
Check Standard "True" Value	Low	17.26	
19.8			
Precision	%RSD	11.30	%RSD Prop.
Accuracy	Mean % Recovery	96.13	15.46
	Mean Absolute Error	-0.77	µM
	% Relative Error	-3.87	µM
Detection Limits	Harris LOD	6.46	µM
	Harris LOQ	21.55	µM
	Error Prop DL (Unc)	8.84	µM
	USEPA MDL	6.46	µM
	ISO DL	13.28	µM
	Instrument LOD	14.50	µM
	Instrument LOQ	48.34	µM
	Average Blank Area	0.020	µM
	Signal DL	0.61	mV*s
	Traditional DL	14.50	µM

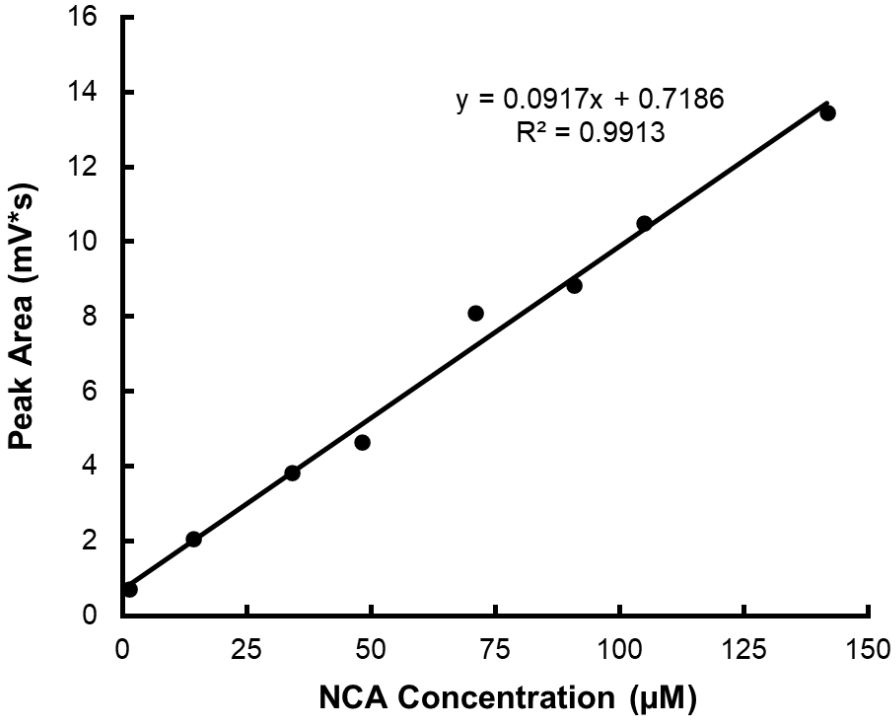

Fig. S7 Calibration curve for nicotinamide (NCA) using the commercial detector.

E. References

1 USHIO, USHIO Xenon Short arc UXL Specifications, https://www.ushio.com/product/xenon-short-arc-uxl/, (accessed July 23, 2019).

2 DOE, Lifetime of White LEDs, https://betterbuildingssolutioncenter.energy.gov/sites/default/files/attachments/lifetime_white_leds.pdf, (accessed July 1, 2024).