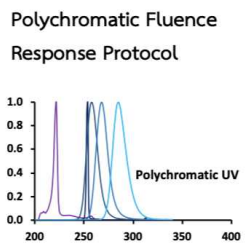
Aug 29, 2023 Version 2

Polychromatic UV Fluence (Dose) Response Determination V.2



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Daniel Ma[1], NATALIE HULL[1]

[1]Ohio State University, Columbus

Water TEAM

Daniel Ma
Ohio State University, Columbus

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We use this protocol and it's working

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Abstract

The purpose of this protocol is to document the steps used for determination of UV doses for polychromatic UV sources such as UV LEDs, excimer lamps, medium pressure mercury lamps. The method is not limited to polychromatic light sources, but can also be used for monochromatic sources, such as low pressure mercury lamps. The method described in this protocol has been modified to account for polychromatic UV radiation sources (**Linden and Darby, 1997**) and is based on method is based on "Standardization of Methods for Fluence (UV Dose) Determination in Bench-Scale UV Experiments" (**Bolton and Linden, 2003**). When using this protocol, we request that you cite the original references. For further information on using UV LEDs and measuring UV intensity, refer to the following publications: **Kheyrandish et al., (2018)**, **Kheyrandish et al., (2017)**, **Kheyrandish et al., (2018)**, **Sholtes et al., (2019)**.

Materials

- UV light source
- Laptop with software for running radiometers and for calculating UV doses
- Spectroradiometer, calibrated with calibration files (e.g., **Ocean Insight HDX**)
- UV-VIS (e.g. **Agilent Cary 4000**)
- Radiometer with detector, calibrated with calibration files (e.g. **ILT 5000 Radiometer**)
- Quartz cuvette (e.g. 1 cm, 5 cm, 10 cm path length)
- Sample containers (e.g. plasticware, glassware, and sterile for microbial samples)
- Magnetic stir plate(s)
- Miniature magnetic stir bars, sterilized
- Lab jacks
- Ruler
- Red light working lamp (not emitting between 300-500 nm, for experiments where photorepair of bacteria is a concern, e.g., **60 W Red LED**)

Troubleshooting

Safety warnings

UV exposure risk! Follow safety precautions for working with UV light sources. Wear PPE such as nitrile gloves, face shield, goggles, and lab coat. Put up signage to warn other lab personnel of potential UV light exposure. Work in biosafety cabinets according to the safety levels required for the microorganisms to be used in experiments.

Before start

For microbial samples, sanitize bench top surfaces with 70% ethanol.

UV Dose Spreadsheet

1 UV Dose Spreadsheet: Blank_UV_Dose.xlsx

Here is an example spreadsheet filled out with radiometer factors, absorbance scan of sample, UV emission spectra of a low pressure mercury lamp, and sample geometry: 254LP_UV_Dose.xlsx

This protocol will guide users through important parts of the UV Dose Spreadsheet.

Preparation of UV Light Source

2 **Obtain UV Source Emission Spectra**. Before your experiment, ensure that you have obtained the UV lamp emission spectra with a UV-VIS spectroradiometer (NIST-traceable is **required**). Warm up the UV source. Typically, 15 minutes is sufficient for light emission to stabilize. UV source stabilization can be confirmed using a radiometer.

Note

Both spectroradiometer and radiometer must have received NIST-traceable calibration within one year of operation.

2.1 Set up the UV-VIS spectroradiometer and the acquisition software. Load calibration data provided with the calibrated UV-VIS spectroradiometer.

2.2 Measure the UV spectra by positioning the spectroradiometer below the UV source. Set scan parameters such as boxcar width, scans to average, and integration time. Obtain background scan (e.g., dark scan with no light exposure) before taking UV measurements.

Note

Remember to use personal protective equipment such as UV-rated goggles, lab coat, face shield, and nitrile gloves. It may be helpful to pull gloves over the sleeves of the lab coat to avoid exposing forearm to UV light during measurements.

Note

Certain spectroradiometers may exhibit noise at low wavelengths. Reducing noise can be achieved by increasing integration time and scans to average. Holding the spectroradiometer still will decrease fluctuations in measurements (e.g. use a clamp to hold it in place, be careful to not damage the instrument).

Note

Avoid saturating the spectroradiometer detector. Saturation will result in "clipped" peaks (e.g., observing a flattened peak near the nominal peak wavelength of the UV device indicates the spectroradiometer is saturated). To correct saturation, move the spectroradiometer detector away from the UV source until a peak is observed.

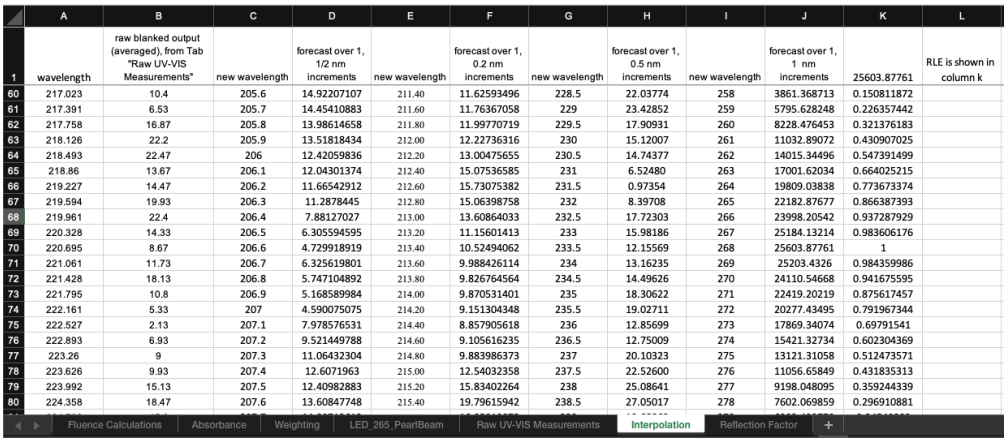
2.3 Export the spectroradiometer measurements. If the spectroradiometer output does not provide irradiance measurements at integer value wavelengths, interpolation of the wavelengths and irradiance measurements is required to use the data in the UV Dose Spreadsheet.

	A	B
152	250.482	2228.44
153	250.842	3361.93
154	251.201	4971.35
155	251.561	8075.35
156	251.921	13253.76
157	252.28	15217.95
158	252.639	15455.67
159	252.999	15519.29
160	253.358	15512.45
161	253.717	15302.24
162	254.076	14595.82
163	254.435	13675.84
164	254.794	12566.11
165	255.153	10972.53
166	255.511	7881.71

Example spectroradiometer data showing non-integer wavelength values. Column A is Wavelength (nm) and Column B is Relative Intensity (a.u.). Data were obtained from Ocean Optics HDX UV-VIS Spectroradiometer.

2.4 Interpolation can be performed on the emission spectra values to obtain integer wavelengths using the tab in the UV Dose Determination spreadsheet titled

"**Interpolation**". Interpolation can be also performed in Microsoft Excel or other computational software (e.g., in R Studio with the 'approx' function).



	A	B	C	D	E	F	G	H	I	J	K	L
1	wavelength	raw blanked output (averaged), from Tab "Raw UV-VIS Measurements"	new wavelength	forecast over 1, 1/2 nm increments	new wavelength	forecast over 1, 0.2 nm increments	new wavelength	forecast over 1, 0.5 nm increments	new wavelength	forecast over 1, 1 nm increments	25603.87761	RLE is shown in column k
60	217.023	10.4	205.6	14.92207107	211.40	11.62593496	228.5	22.03774	258	3861.368713	0.150811872	
61	217.391	6.53	205.7	14.45410883	211.60	11.76367058	229	23.42852	259	5795.628248	0.226357442	
62	217.758	16.87	205.8	13.98614658	211.80	11.99770719	229.5	17.90931	260	8228.476453	0.321376183	
63	218.126	22.2	205.9	13.51818434	212.00	12.22736316	230	15.12007	261	11032.89072	0.430907025	
64	218.493	22.47	206	12.42059836	212.20	13.00475655	230.5	14.74377	262	14015.34496	0.547391499	
65	218.86	13.67	206.1	12.04301374	212.40	15.07536585	231	6.52480	263	17001.62034	0.664025215	
66	219.227	14.47	206.2	11.66542912	212.60	15.73075382	231.5	0.97354	264	19809.03838	0.773673374	
67	219.594	19.93	206.3	11.2878445	212.80	15.06398758	232	8.39708	265	22182.87677	0.866387393	
68	219.961	22.4	206.4	7.88127027	213.00	13.60864033	232.5	17.72303	266	23998.20542	0.937287929	
69	220.328	14.33	206.5	6.305594595	213.20	11.15601413	233	15.98186	267	25184.13214	0.983606176	
70	220.695	8.67	206.6	4.729918919	213.40	10.52494062	233.5	12.15569	268	25603.87761	1	
71	221.061	11.73	206.7	6.325619801	213.60	9.988426114	234	13.16235	269	25203.4326	0.984359986	
72	221.428	18.13	206.8	5.747104892	213.80	9.826764564	234.5	14.49626	270	24110.54668	0.941675595	
73	221.795	10.8	206.9	5.168589984	214.00	9.870531401	235	18.30622	271	22419.20219	0.875617457	
74	222.161	5.33	207	4.590075075	214.20	9.151304348	235.5	19.02711	272	20277.43495	0.791967344	
75	222.527	2.13	207.1	7.978576531	214.40	8.857905618	236	12.85699	273	17869.34074	0.69791541	
76	222.893	6.93	207.2	9.521449788	214.60	9.105616235	236.5	12.75009	274	15421.32734	0.602304369	
77	223.26	9	207.3	11.06432304	214.80	9.883986373	237	20.10323	275	13121.31058	0.512473571	
78	223.626	9.93	207.4	12.6071963	215.00	12.54032358	237.5	22.52600	276	11056.65849	0.431835313	
79	223.992	15.13	207.5	12.40982883	215.20	15.83402264	238	25.08641	277	9198.048095	0.359244339	
80	224.358	18.47	207.6	13.60847748	215.40	19.79615942	238.5	27.05017	278	7602.069859	0.296910881	

Fluence Calculations | Absorbance | Weighting | LED_265_PearlBeam | Raw UV-VIS Measurements | Interpolation | Reflection Factor | +

Interpolation results. Column A = paste raw wavelengths (non-integer). Column B = paste raw irradiance measurements (arbitrary units). Columns C through H = step-wise interpolation calculations. Column I = new integer value wavelengths. Column J = interpolated irradiance values at integer wavelengths. Cell K1 = maximum value from Column J. Column K = relative irradiance calculated by dividing values in each row of Column J by the value in Cell K1.

2.5 **Check UV Spectra Characteristics**. The peak and average wavelength can be obtained from the tab "Spectra Characteristics". The fp radiometer factor should be between 1–1.05. Values higher than this will adversely affect UV exposure times.

	A	B	C	D
1	**Date**	**October 7 2022**		
2	**Spectrometer**	**Ocean Optics HDX UV-VIS (Last Calibrated 9/6/2022)**		
3	**Operator(s)**	**DTM**		
4	**Lamp(s)**	**AquiSense Pearl Beam 265 nm**		
5	**λavg (200-400)**	**272**		
6	**λ peak**	**268**		
7	**Plotted output name**	**AquiSense Pearl Beam 265 nm NEW INTERPOLATION**		
8	Below are calculations, no additional inputs needed on this tab			
9	Update the details of the spectra measurements above			
10				
11	**Calculated**	Sum of NEW INTERPOLATION	SUM OF NEW * Wavelength	
12		15.58720006	4236.421257	
101	264	0.773673374	204.2497708	
102	265	0.866387393	229.5926591	
103	266	0.937287929	249.3185892	
104	267	0.983606176	262.6228489	
105	268	1	268	
106	269	0.984359986	264.7928363	
107	270	0.941675595	254.2524107	
108	271	0.875617457	237.2923308	
109	272	0.791967344	215.4151177	
110	273	0.69791541	190.530907	
111	274	0.602304369	165.0313971	
112	275	0.512473571	140.9302319	
113	276	0.431835313	119.1865463	

Fluence Calculations | Absorbance | Weighting | Spectra Characteristics | Raw UV-VIS Me

Spectra characteristics are calculated in the Spectra Characteristics tab. Cell B12 = sum of all relative irradiance values in Column B starting at row 37. Cell C12 = sum of the product of wavelengths in Column A starting at row 37 and the relative irradiance values in Column B starting at row 37. Cell B5 = average wavelength calculated by the ratio of Cell C12 / Cell B12. Cell B6 = peak wavelength (corresponds with the maximum irradiance value).

	A	B	C	D	E	F
1	IR Radiometer Reading		0.1900	mW/cm2	from fluence calculations sheet	
2	d sample depth		0.4406	cm	from fluence calculations sheet	
3	fp radiometer lamp correction factor		1.0324	unitless	=(sumRLE)/(sumRLE*f_R)	
4	Incident Intensity		0.1860	mW/cm2	=fp*IR*DF*PF*RF	
5	Average Intensity		0.1853	mW/cm2	=sum Iavg	
6	Weighted Average Intensity		0.0000	mW/cm2	=sum Iavg*Rweight	
7	Rλpeak		0.0028	unitless	from calibration data below	

The fp radiometer factor (Cell C3) should be between 1 and 1.05. Obtain another spectra measurement using adjusted acquisition parameters on the spectroradiometer (e.g., integration time, scans to average) and repeat the steps above to interpolate the irradiance values.

Sample Preparation

3 **Select Sample Matrix.** The UV dose responses of different sample matrices may be characterized using this protocol.

Sample types may include, but are not limited to, the following:

- Bio-molecular samples (e.g. bacteria, viruses, fungal spores, etc.)
- Environmental samples (e.g. wastewater, surface water, groundwater, etc.)
- Chemicals

4 **Determine Volume of Working Solution Needed.** To determine the minimum total volume of working solution (V_T) needed:

$$V_T = N \times V_S$$

- N = Number of UV doses you want to include in your experiment (include no dose, i.e. no UV exposure)
- V_S = Sample volume needed per UV dose

Note

When deciding on V_S, consider the following: the amount of sample needed for each downstream analyses, size of the sample container available.

Note

In addition to V_T, prepare additional working solution for determining concentration of target analyte in non-irradiated samples. Sufficient additional volume is also needed for sample absorbance scans. The volume needed for sample absorbance scans will depend on the cuvette size.

Plan to prepare

- Sufficient extra volume to rinse the cuvettes between scans of different sample matrices
- Sufficient volume for at least duplicate absorbance scans per sample

Note

For example, a UV dose response for MS2 bacteriophage might have 7 doses (N): 0, 10, 20, 30, 40, 50, and 60 mJ/cm^2. If 35 mm Petri dishes are used, 4 mL would be an appropriate sample volume (V_S).

$$V_T =$$ (7 doses) x (4 mL/dose) = 28 mL

Since the minimum volume required for the UV exposures is 28 mL, a suitable volume to prepare would be 100 mL, which would provide sufficient volume to rinse the cuvette between absorbance scans, duplicate absorbance scan, and extra solution to repeat UV exposures (if needed).

5 **Choose working stock concentration**. The concentration should be chosen to quantify observable reductions for the highest UV dose. Consider personnel safety when choosing a stock concentration. Avoid risk of exposure to high concentrations of hazardous chemicals.

6 **Prepare working solution for the chosen sample matrix.** After calculating the volume needed and the target concentration, prepare the working solution of your sample by using the appropriate diluent, if necessary. Common diluents of samples include: DI water and phosphate buffered saline (PBS).

Note

- Avoid using DI water for bio-molecular samples such as bacteria and viruses. For bio-molecular samples, use sterilized PBS (e.g., filter sterilized, autoclaved) as the diluent.
- The typical PBS concentration is 0.01 M (e.g., 1X).

6.1 **Preparing bio-molecular samples.** If using bio-molecular samples, prepare microorganisms according to your laboratory protocols. Use best practices and aseptic technique. Biological triplicates are recommended for bio-molecular studies.

Note

Handling BSL2 organisms. Consider setting up your UV experiment in a BSL2 biosafety cabinet for BSL2 microorganisms. If that is not feasible, exercise proper safety and precautions when handling microorganisms outside the BSL2 cabinet. Minimize time BSL2 samples are outside the cabinet.

Exercise extreme caution and only perform UV exposures on BSL3+ organisms in BSL3 certified facilities.

Note

Cell aggregation issues. Previous research suggests that cell aggregation can cause tailing in disinfection kinetics (i.e. leveling off inactivation with increasing UV doses). Tailing can be minimized using several techniques:

- Sonicating aliquots of cells immediately before UV exposure (e.g., 30 seconds) (Sholtes and Linden, 2019)
- Using lower centrifuge speeds to pellet and wash cells to reduce clumping
- Reducing the number of cell washes to reduce clumping (e.g. reduce from 3 washes to 1 wash)
- Add a surfactant to microorganism working stock (e.g. Tween)

Note

Risk of photorepair. Photorepair can influence quantification of microorganisms' UV dose response. When working with microorganisms with the potential to photorepair (e.g. bacteria, fungi), it is recommended to work in a dark environment with minimal exposure to light between 300-500 nm. A red light emitting > 700 nm may be used for visibility when performing UV exposures and sample handling. If a red light is not available, it is recommended to dilute and plate irradiated samples as soon as possible.

To quantify photorepair kinetics, the following protocol can be used:

Protocol

NAME

Photorepair Fluence Response Protocol

CREATED BY

Daniel Ma

Preview

6.2 **Prepare environmental samples**. If using environmental samples, obtain environmental samples and store in appropriate containers and temperatures. Environmental samples may be used to reflect environmental water quality (e.g., wastewater, river water, groundwater). Microorganisms may be spiked into environmental samples. Store chemicals in appropriate storage conditions (e.g., container, temperature, light) before UV exposures.

6.3 **Prepare chemical samples**. If using chemical samples, prepare stock solutions and working stock solutions using best practices for handling and preparing chemicals. Perform dilutions using an appropriate diluent. Store chemicals in appropriate storage conditions (e.g., container, temperature, light) before UV exposures.

7 **Measure sample absorbance.** Use a UV-VIS spectrophotometer to obtain an absorbance spectra of working stock solution. Sample absorbance is required for determining the UV dose by accounting for the UV light absorbance at different wavelengths and for determining the Water Factor, one of the UV irradiance adjustment factors.

Note

Quartz cuvettes are required for obtaining absorbance spectra for UV dose calculations. Follow all best practices for handling and using quartz cuvettes. Cuvettes with various path lengths (e.g., 1 cm, 5 cm, 10 cm) are available.

Important: When using cuvettes that do not have 1 cm path lengths, you must convert absorbance measurements to 1 cm path length (e.g. adjust absorbance values from 10 cm cuvette to 1 cm by dividing by 10). When changing cuvettes, repeat the cuvette zero and baseline measurements for the new cuvette.

7.1 **Prepare sample and dilutions for absorbance scan.** Samples of high concentration may need to be diluted for UV-VIS measurements. Dilute samples using the appropriate diluent. Record the dilution factor, which will be used to correct absorbance scan data for the original solution.

7.2 **Turn on UV-VIS spectrophotometer**. Warm up at least 10 minutes. Longer warm up time for the lamps will increase performance. Adjust the default instrument settings if desired (e.g., scan speed, slit height, wavelength range, etc.).

7.3 **Obtain absorbance measurements**:

1. Load a blank sample (e.g. cuvette with DI water).
2. Zero the instrument. This zeroes the reading at the highest wavelength.
3. Baseline the instrument. This zeroes the readings at all wavelengths. A baseline scan is shown in Figure A below.
4. **Optional**: Obtain absorbance scan of distilled water immediately after the baseline (the absorbance scan values should be near 0 for all wavelengths). If the instrument was properly zeroed and a baseline acquired.
5. Obtain sample absorbance scan(s).
6. Empty cuvette. Dispose of sample in proper containers.
7. Rinse cuvette at least once with working stock solution.
8. Obtain at least duplicate absorbance scan per sample.
9. Export tabulated absorbance scan data. Correct for any dilution factor, if necessary.
10. Calculate the average absorbance from the replicate measurements.
11. Paste the average absorbance at each wavelength in the **Absorbance** tab in the UV Dose Spreadsheet.

Note

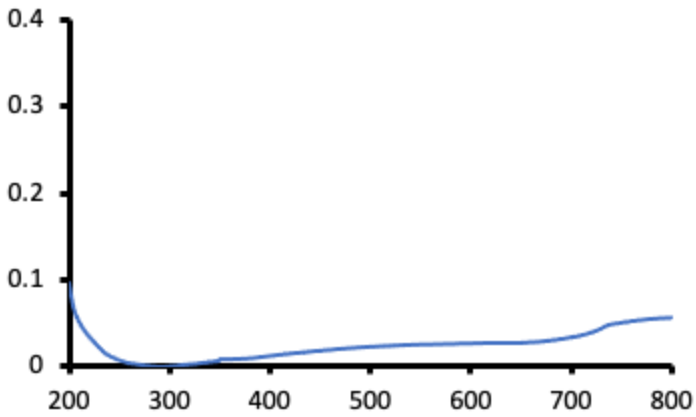


Figure A. Example baseline scan with DI water in 1 cm path length quartz cuvette after zeroing.

Note

When measuring absorbance scan of multiple samples, it is recommended to measure samples from low to high concentrations to avoid contaminating the cuvette.

Note

If the absorbance spectra measurements fall outside the dynamic range of the spectrophotometer, consider diluting the sample and re-measuring or using a larger cuvette (e.g.., 10 cm cuvette). Correct the absorbance by the dilution factor. For example, multiply absorbance values by 10 if the measured sample was diluted 1:10 from the original working solution.

Measuring UV irradiance

16h

8 Set up UV source and radiometer (must be NIST-calibrated). Select the distance between UV source and sample surface. Record this value in the UV Dose Spreadsheet.

To obtain UV irradiance measurements, set up the radiometer so that the radiometer detector plane is at the same level as the sample surface during UV exposures (Figure

A). Use an adjustable lab jack to achieve the same sample surface plane during sample exposure and radiometer measurements. Check the location of the reference plane of the radiometer, as shown in Figure B.

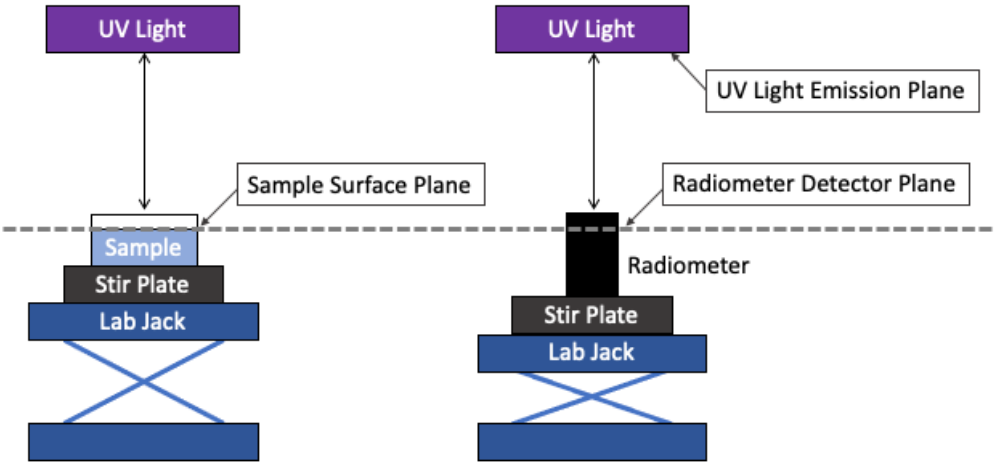


Figure A. Example UV source set up with lab jacks to adjust the elevation of the sample and radiometer. Figure not to scale.

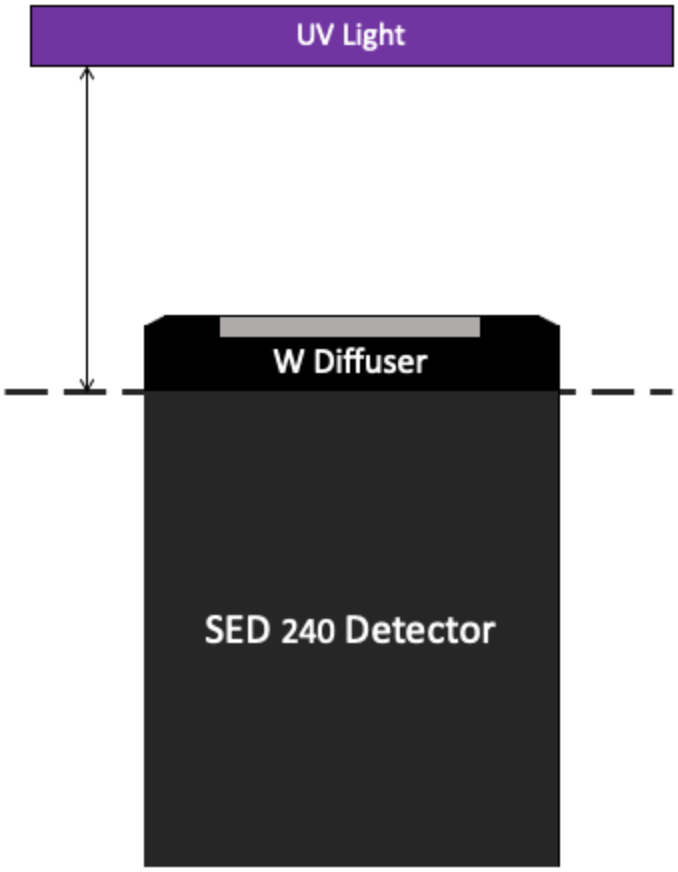


Figure B. Identify the reference plane of the the detector. For the example detector shown, the reference plane is the dashed line and should be at the same plane as the sample surface plane. Contact the manufacturer or consult the instrument manual if needed.

9 **Obtain Petri Factor**. The UV dose calculation requires the Petri Factor to account for the spatial non-uniformity of the UV source emission over the surface of the water sample in the container (e.g., Petri dish). The Petri Factor is obtained by measuring the UV irradiance at the sample surface plane at 0.5 cm intervals in the x- and y-direction starting from the center of the sample dish.

9.1 Ensure the radiometer factor is set to the calibration value for the peak wavelength. The peak wavelength and its corresponding radiometer factor can be found in the **Fluence Calculation** tab.

	λ peak =	268	from Spectra tab
	λ avg =	272	from Spectra tab
Radiometer Factor at λpeak =		2.246E-03	

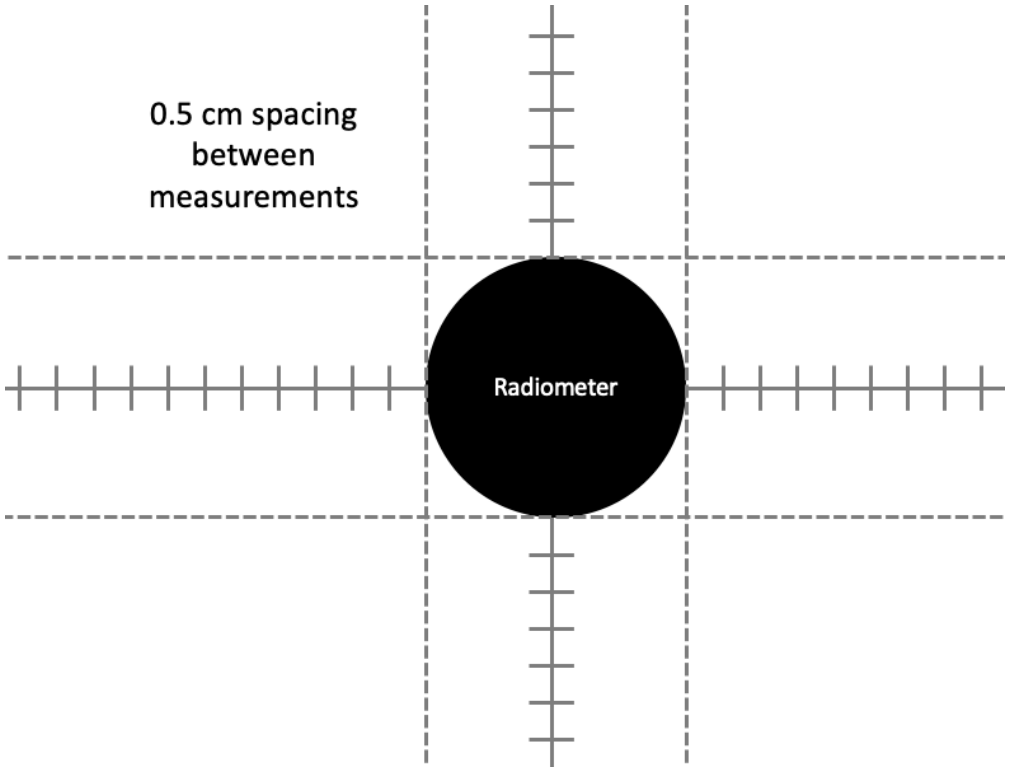
Note

The radiometer factor should be provided by the radiometer manufacturer and must be manually updated in the **Weighting** tab (column with **R** header). The factors should be updated each time the radiometer is re-calibrated.

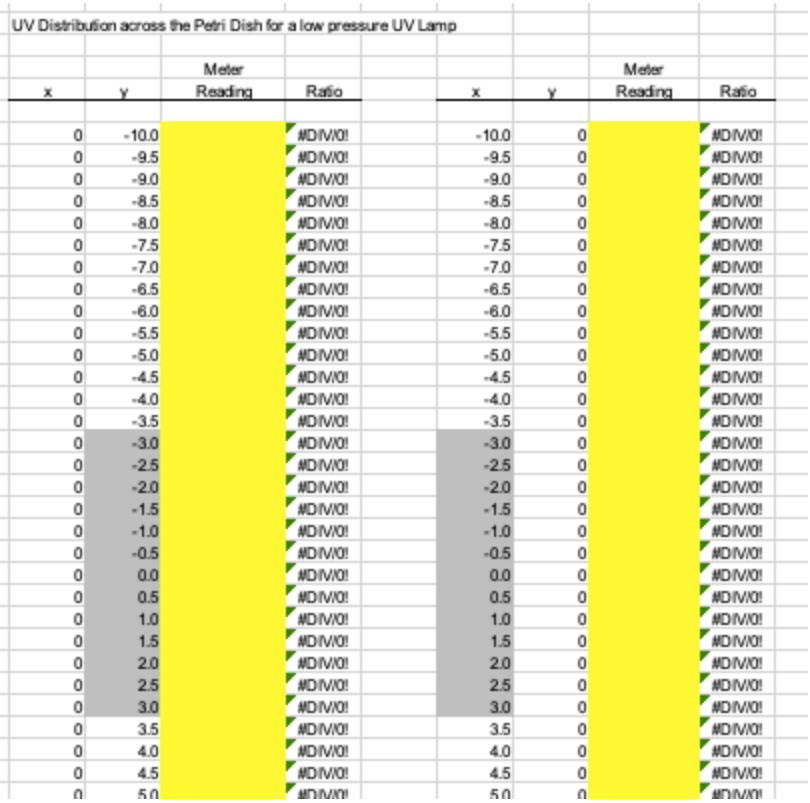
9				
10	COLUMN SUM	#N/A	#N/A	0.0000
11				
12	**λ**	**RLE**	**L(λ)**	**R**
13	Wavelength (nm)	SpectralOutput, relative lamp energy, Spectra tab	RLE/ Total RLE	Calibration Factor at each wavelength, Units are (A)(cm2)(W-1)
14	200	#N/A	#N/A	
15	201	#N/A	#N/A	
16	202	#N/A	#N/A	
17	203	#N/A	#N/A	
18	204	#N/A	#N/A	
19	205	#N/A	#N/A	
20	206	#N/A	#N/A	
21	207	#N/A	#N/A	

If the calibration factors are not provided for each wavelength, it will be necessary to interpolate (e.g., take the average of factors at 250 nm and 252 nm to obtain the factor at 251 nm).

9.2 Create a grid for measuring UV irradiance at 0.5 cm intervals in the x- and y-direction from the center of the sample dish.



9.3 Depending on the size of the dish, the UV Dose Spreadsheet will indicate the minimum number of measurements to take in each direction.



UV Distribution across the Petri Dish for a low pressure UV Lamp

x	y	Meter Reading	Ratio	x	y	Meter Reading	Ratio
0	-10.0		#DIV/0!	-10.0	0		#DIV/0!
0	-9.5		#DIV/0!	-9.5	0		#DIV/0!
0	-9.0		#DIV/0!	-9.0	0		#DIV/0!
0	-8.5		#DIV/0!	-8.5	0		#DIV/0!
0	-8.0		#DIV/0!	-8.0	0		#DIV/0!
0	-7.5		#DIV/0!	-7.5	0		#DIV/0!
0	-7.0		#DIV/0!	-7.0	0		#DIV/0!
0	-6.5		#DIV/0!	-6.5	0		#DIV/0!
0	-6.0		#DIV/0!	-6.0	0		#DIV/0!
0	-5.5		#DIV/0!	-5.5	0		#DIV/0!
0	-5.0		#DIV/0!	-5.0	0		#DIV/0!
0	-4.5		#DIV/0!	-4.5	0		#DIV/0!
0	-4.0		#DIV/0!	-4.0	0		#DIV/0!
0	-3.5		#DIV/0!	-3.5	0		#DIV/0!
0	-3.0		#DIV/0!	-3.0	0		#DIV/0!
0	-2.5		#DIV/0!	-2.5	0		#DIV/0!
0	-2.0		#DIV/0!	-2.0	0		#DIV/0!
0	-1.5		#DIV/0!	-1.5	0		#DIV/0!
0	-1.0		#DIV/0!	-1.0	0		#DIV/0!
0	-0.5		#DIV/0!	-0.5	0		#DIV/0!
0	0.0		#DIV/0!	0.0	0		#DIV/0!
0	0.5		#DIV/0!	0.5	0		#DIV/0!
0	1.0		#DIV/0!	1.0	0		#DIV/0!
0	1.5		#DIV/0!	1.5	0		#DIV/0!
0	2.0		#DIV/0!	2.0	0		#DIV/0!
0	2.5		#DIV/0!	2.5	0		#DIV/0!
0	3.0		#DIV/0!	3.0	0		#DIV/0!
0	3.5		#DIV/0!	3.5	0		#DIV/0!
0	4.0		#DIV/0!	4.0	0		#DIV/0!
0	4.5		#DIV/0!	4.5	0		#DIV/0!
0	5.0		#DIV/0!	5.0	0		#DIV/0!

Gray shaded boxes in **Fluence Calculation** tab indicate the number of measurements required in each direction. The indicated gray boxes are for a dish with diameter of **5 cm** (example). Note that the measurements extend from **-3.0 cm to +3.0 cm** in both x- and y-directions.

UV Distribution across the Petri Dish for a low pressure UV Lamp

x	y	Meter Reading	Ratio	x	y	Meter Reading	Ratio
0	-10.0		#DIV/0!	-10.0	0		#DIV/0!
0	-9.5		#DIV/0!	-9.5	0		#DIV/0!
0	-9.0		#DIV/0!	-9.0	0		#DIV/0!
0	-8.5		#DIV/0!	-8.5	0		#DIV/0!
0	-8.0		#DIV/0!	-8.0	0		#DIV/0!
0	-7.5		#DIV/0!	-7.5	0		#DIV/0!
0	-7.0		#DIV/0!	-7.0	0		#DIV/0!
0	-6.5		#DIV/0!	-6.5	0		#DIV/0!
0	-6.0		#DIV/0!	-6.0	0		#DIV/0!
0	-5.5		#DIV/0!	-5.5	0		#DIV/0!
0	-5.0		#DIV/0!	-5.0	0		#DIV/0!
0	-4.5		#DIV/0!	-4.5	0		#DIV/0!
0	-4.0		#DIV/0!	-4.0	0		#DIV/0!
0	-3.5		#DIV/0!	-3.5	0		#DIV/0!
0	-3.0		#DIV/0!	-3.0	0		#DIV/0!
0	-2.5		#DIV/0!	-2.5	0		#DIV/0!
0	-2.0		#DIV/0!	-2.0	0		#DIV/0!
0	-1.5		#DIV/0!	-1.5	0		#DIV/0!
0	-1.0		#DIV/0!	-1.0	0		#DIV/0!
0	-0.5		#DIV/0!	-0.5	0		#DIV/0!
0	0.0		#DIV/0!	0.0	0		#DIV/0!
0	0.5		#DIV/0!	0.5	0		#DIV/0!
0	1.0		#DIV/0!	1.0	0		#DIV/0!
0	1.5		#DIV/0!	1.5	0		#DIV/0!
0	2.0		#DIV/0!	2.0	0		#DIV/0!
0	2.5		#DIV/0!	2.5	0		#DIV/0!
0	3.0		#DIV/0!	3.0	0		#DIV/0!
0	3.5		#DIV/0!	3.5	0		#DIV/0!
0	4.0		#DIV/0!	4.0	0		#DIV/0!
0	4.5		#DIV/0!	4.5	0		#DIV/0!
0	5.0		#DIV/0!	5.0	0		#DIV/0!
0	5.5		#DIV/0!	5.5	0		#DIV/0!
0	6.0		#DIV/0!	6.0	0		#DIV/0!
0	6.5		#DIV/0!	6.5	0		#DIV/0!
0	7.0		#DIV/0!	7.0	0		#DIV/0!
0	7.5		#DIV/0!	7.5	0		#DIV/0!
0	8.0		#DIV/0!	8.0	0		#DIV/0!

Gray shaded boxes in **Fluence Calculation** tab indicate the number of measurements required in each direction. The indicated gray boxes are for a dish with diameter of **10 cm** (example). Note that the measurements extend from **-5.5 cm to +5.5 cm** in both x- and y-directions.

9.4 Measure the center of dish irradiance. Input the irradiance in the **Fluence Calculations** tab.

Radiometer Reading Center of Dish =		0.0881	mW/cm^2
	Incident Irradiance=		mW/cm^2
	Average Irradiance=	0.0000	mW/cm^2

Note

Use the correct units (e.g., W/cm^2 vs. mW/cm^2) when recording UV irradiance.

9.5 Input values for sample geometry in the **Fluence Calculations** tab: solution volume added to Petri dish, Petri dish internal diameter, distance from UV lamp to top of water surface. The spreadsheet will automatically calculate the water path length.

solution volume added to Petri dish =	4	mL
stirrer volume =	0	mL
Petri dish internal diameter =	3.4	cm
total volume in Petri dish =	4	mL
water path length =	0.44	cm
distance from UV lamp to top of water surface =	10	cm
Absorbance scan =	done	use Absorbance tab
absorption coefficient at 254 nm =	0.0076	cm^{-1}
UVT % at 254 =	98.27	make sure this isn't <90% for disinfection studies

9.6 Check that the UV irradiance adjustment factors in the **Fluence Calculations** tab are within the recommended range of values:

Divergence Factor =	0.9578	=d/(d+l). Target >0.9
Reflection Factor at λpeak =	0.9757	from Reflection Factor tab
Petri Factor =	0.9531	calculated in cell DL90. Target >0.9
Spectral Water Factor =	0.9964	= Incident / Average Intensity. Target >0.9

Divergence Factor = DF; Reflection Factor = RF; Petri Factor = PF; Water Factor = WF

The center of dish irradiance (I_o) is corrected by the adjustment factors to obtain the average irradiance (I_{avg}):

$$I_{avg} = I_0 \times DF \times RF \times PF \times WF$$

Radiometer Reading Center of Dish =	0.0881	mW/cm^2
Incident Irradiance=		mW/cm^2
Average Irradiance=	0.0923	mW/cm^2

9.7 **Weighting factors**. Weighting factors to account for microorganism or biomolecule action spectra can be applied to UV dose calculations. In general, no weighting factor is applied (NA). If weighting is applied, record the weighting type.

Weighting Factor =	NA	choose and apply formula on Weighting tab, record weighting type here
Weighted Average Irradiance=		mW/cm^2

9.8 Calculate exposure times for pre-determined UV doses. The zero (0; unexposed) dose sample is **very important** must be taken. Consider taking the zero dose sample multiple times during the experiment (e.g., beginning, middle, end). Dose responses should have at least 3-5 points in addition to the zero dose. Check the literature for expected dose responses for the specific microorganism.

Seconds for a Fluence (UV Dose) of	1	mJ/cm^2 =	10.833	change formula to use weighted average irradiance, if applicable			
						min	seconds
Time for a Fluence (UV Dose) of	10	mJ/cm^2 =	108.3	s	=	1	48
Time for a Fluence (UV Dose) of	20	mJ/cm^2 =	216.7	s	=	3	37
Time for a Fluence (UV Dose) of	30	mJ/cm^2 =	325.0	s	=	5	25
Time for a Fluence (UV Dose) of	40	mJ/cm^2 =	433.3	s	=	7	13
Time for a Fluence (UV Dose) of	50	mJ/cm^2 =	541.7	s	=	9	2
Time for a Fluence (UV Dose) of	60	mJ/cm^2 =	650.0	s	=	10	50
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0	s	=	0	0
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0	s	=	0	0
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0	s	=	0	0
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0	s	=	0	0
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0	s	=	0	0
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0	s	=	0	0
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0	s	=	0	0

Performing UV exposures

10 Perform UV exposures by transferring working solution of sample to sample container (sterile container for microbial samples), adding a stir bar (sterile for microbial samples), and placing on the magnetic stir plate. Expose the sample to UV irradiation for the calculated exposure times. Expose samples in random order (e.g., not ascending or descending order of UV dose values).

Note

The rotational speed of the magnetic stirrer must be set so the stir bar does not create a vortex in the sample, which would disrupt the sample surface plane.

Note

If available, an automated shutter can be used to control the duration of UV exposure time. If not automated shutter is available, construct the necessary structure(s) to allow a manual shutter (e.g., opaque material like cardboard) to be manually moved to completely block off UV irradiation from the sample.

Note

The shortest exposure time must be at least 30 seconds to minimize effect of timing uncertainty and errors on UV dose delivered when using hand-operated shutters.

11 After the exposure time is complete, replace the shutter to block off UV irradiation. Transfer irradiated samples to appropriate containers. For microbial samples, use sterile technique and equipment (e.g., pipetting) to transfer sample to sterile containers (e.g., test tubes). Save samples for downstream analysis and quantification.

Acknowledgements

12 We acknowledge the past and present Hull Lab members who contributed to the development and review of this protocol. The UV dose spreadsheet provided in this protocol has undergone iterative changes. It has been modified from UV dose spreadsheets from the Linden Lab and changes have been made based on personal communications from Dr. Jim Bolton.