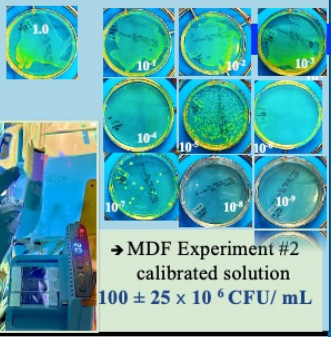
A. Cultured Serial Dilutions Used For Fluorescence

YIELD AFTER 48 HRS INCUBATION VIA CFU COUNTING

# of Bacteria/mL for each bacterial dilution

➔ *Calibrates the bacterial content for each drop fluoresced*

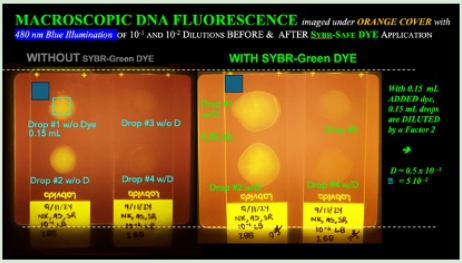
➔ Correlates of measured Ratio of 520 nm Green Fluorescence to 480 nm Blue Excitation $R_{G/B}$ to the actual Bacterial Concentration in the drops



➔ MDF Experiment #2 calibrated solution

$100 \pm 25 \times 10^{6}$ CFU/ mL

B. Macroscopy DNA Fluorescence Detection

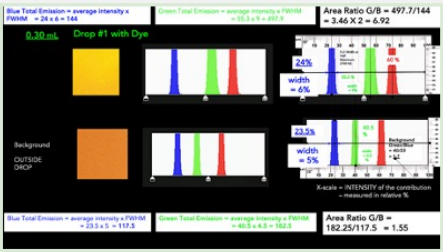


➔ Clear Difference between Different Bacterial Dilution of

➔ Bacterial Load are Detected by MDF

➔ MDF scales with Bacterial Load Magnitude

C. RGB of ratio $R_{G/B}$ of Green Fluorescence Intensity I_G to Blue Illumination Intensity I_B



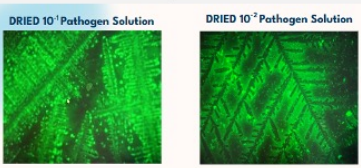
➔ Clear Difference between RGB Spectra can be Measured by $R_{G/B}$ ratio

➔ Bacterial Load are Detected AND MEASURED by MDF

➔ Quantitative Scale between MDF and Bacterial Load Magnitude can be achieved

Correlation of Bacterial DNA/RNA Fluorescence Macroscopic Intensity *with* Microscopic Observation

State of the Art Keyence Fluorescence Microscope used to Image Slides



#1 Bacteria clearly migrate and attach to crystal dendrites formed precipitation of the NaCl (salt) in Luria Broth as it dries,

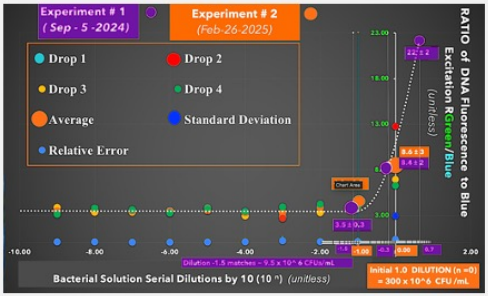
#2 All microscopic fluorescence is caused by bacteria, there is no fluorescence from the NaCl crystallized in dendrites.

#3 Green fluorescence intensity and number of bacteria observed decreases with decreasing bacteria concentration when comparing the 10^{-2} solution to the 10^{-1}

$R_{G/B}$ TABLE A Function of Bacterial Dilution *with Statistical Analysis*

Calibration Solution Dilution in Log Scale	Drop 1	Drop 2	Drop 3	Drop 4	Average	Standard Deviation	Relative Error
1.00							
0.00	8.51	12.74	6.92	6.26	8.61	2.91	33.84%
-1.00	3.32	4.18	4.42	3.98	3.98	0.47	11.88%
-2.00	3.90	4.10	3.30	4.22	3.88	0.41	10.53%
-3.00	3.76	2.66	2.80	3.90	3.28	0.64	19.52%
-4.00	3.62	3.30	2.92	3.42	3.32	0.29	8.89%
-5.00	3.44	3.22	3.78	3.72	3.54	0.26	7.34%
-6.00	3.68	3.46	3.42	3.26	3.46	0.17	5.01%
-7.00	3.50	3.52	3.34	3.60	3.49	0.11	3.12%
-8.00	3.90	4.02	3.34	3.80	3.77	0.30	7.90%
-9.00	3.54	3.28	3.86	3.10	3.45	0.33	9.59%

$R_{G/B}$ Analysis A Function of Bacterial Dilution *Comparing Reproducibility between two experiments*



Conclusions - What did we find?

Conclusion #1 *The Ratio $R_{Green/Blue}$ SCALES with $R_{Green/Blue}$ Bacterial Concentration Consistently in 0.15 mL DROPS*

Conclusion #2 *However, the Fluorescence Ratio $R_{Green/Blue}$ is not SENSITIVE enough to measure low bacterial loads*

➔ **Next Step in DOE**

Use another new, more sensitive dye, Biotium GelGreen™

Conclusion #3 *The Green Fluorescence Ratio $R_{Green/Blue}$ Can Be Considered to be sensitive for the first three orders of magnitude from the 1st three dilutions (10-1, 10-2, 10-3 ..) The relative Error is 10% for the lowest 10^{-2} Dilution.*

Conclusion #4 *Where the $R_{Green/Blue}$ is **not sensitive enough** to measure bacterial loads (10^{-4} to 10^{-9}), the background $R_{Green/Blue}$ Averages 3.50 ± 0.15, about a 4% error.*

- ***The Background Value $R_{Green/Blue}$ in the absence of fluorescence is VERY CONSISTENT,***
- ***The medical gold standard , 10%, error needs a more sensitive dyes such as the new GelGreen for Accuracy***

➔ **Prototyping** *has begun based on this feasibility Study*