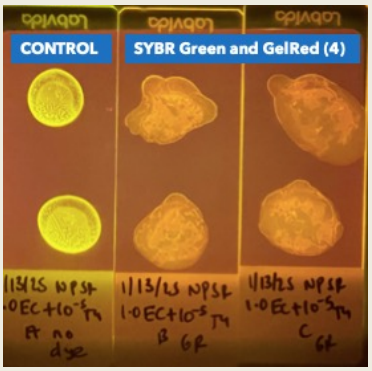
# Differential Detection Of Viral and Bacterial Infections In Small Volume Diagnostic Via Macroscopic Epi-Fluorescence From DNA/RNA Specific Stains

N. Prakash[1,2] , S. Ram [1,2] , Nicole Herbots [1,2, a)], Robert J. Culbertson [1], E.J. Culbertson, MD [2]

[1]**Arizona State University**, *[1]Department of Physics, Tempe, AZ, United States,*

[2]**SiO2 Innovates LLC** (*Est 2016),* 211 E Balboa Dr, Tempe, AZ 85282

***Fig. 1*** *shows the fluorescence of 0.1mL flattened drops from viral and bacterial solutions on super-hydrophilic testing strips. Testing strip B & C contain green DNA fluorescent dye and red RNA fluorescent dye exhibit a color change that can be observed by comparing to the un-dyed control strip. These drops are photographed and analyzed to correlate viral load values with the green fluorescence ratios via RGB analysis shown in Figures 4 and 6.*



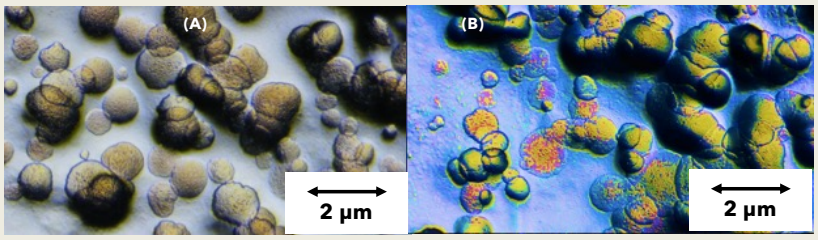




***Fig. 2*** *shows that the observed macroscopic epi-fluorescence originates from the red and green dyes fluorescing when bound to DNA and RNA respectively and when observing bacteria and viruses directly by microscopy. (A) shows bacteria and viruses interacting under white light illumination without fluorescence to identify them. (B) shows bacteria and viruses under blue light illumination when epi-fluorescence clearly separates viruses from bacteria.*

***Fig. 3*** *depicts proof by microscopy of bacterial lysis, due to viral presence. When the DNA is damaged, SYBR-safe green dye does not bind to the DNA and fluoresce. As viral concentration is increased, there is less intact DNA, creating a decrease in green fluorescence.*

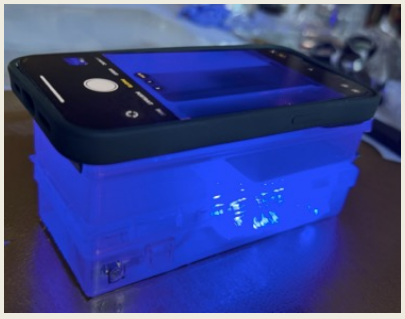


***Fig. 4*** *is the prototype for viral diagnostics. A testing strip is inserted into the side slit of the device. The blue light is diffused onto the testing strip in order to fluoresce the drops (which contain patient biofluids and dye). After taking a picture of the drops and uploading it to the self-built app, color emissions from fluorescence can be analyzed.*

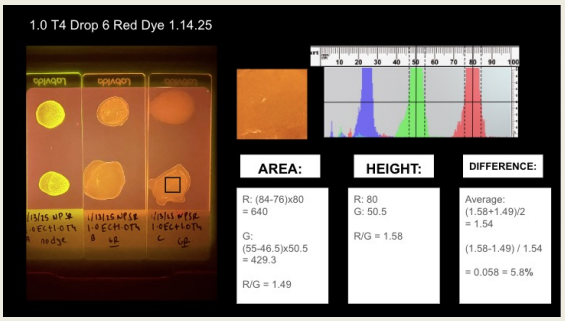



***Fig. 5*** *shows how manual ratio calculations are conducted. Using 250 x 250 pixel sections of each drop, RGB color graphs are generated and fluorescent color emission ratios are found. Here, manual calculations, a time-consuming and inconsistent method, proves inefficient for rapid analysis, which is why an app was developed to accurately find color emission ratios.*

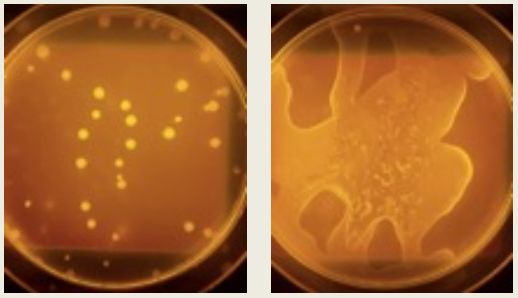
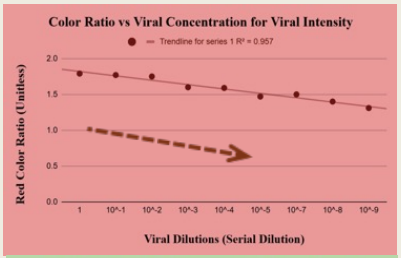


***Fig. 6*** *show two bacterial cultures plate with different bacterial loads: the culture plate on the left has an E. Coli concentration of 70 CFUs/mL while the right has an E. Coli load of 70 Billions/mL .To find the CFUs (Colony Forming Units) count of the E. Coli solution used, a serial dilution of the 1.0 concentrated solution was used. 42 CFU were counted on the $10^{-9}$ plate, where 0.5 mL of the $10^{-9}$ plate, a total of 8.2 x $10^{10}$ CFUs.*



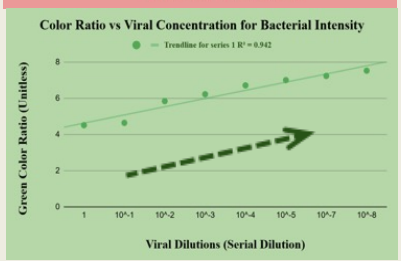




***Fig. 7*** *shows the app analysis using the three RGB color emissions measured via photographs of fluorescing drops. The ratio of red to green epi-fluorescence is calculated for a specific drop is calculayed as follows*

$R_{R/G} = I_{Red} / I_{Green}$

*= (850 Pixels/ 424 Pixels)*

*= 2.00*

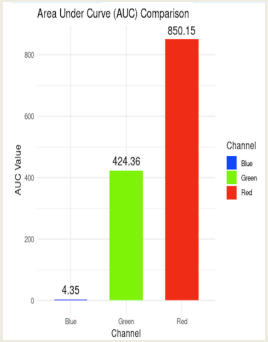



***Fig. 8*** *depicts linear trends in epi-fluorescence as viral concentration is decreased. As the virus infects and damages the DNA of host cells, which the safe green dye binds to, less green epi-fluorescence is detected.*

*As more viruses are present, which the red RNA stain binds to, more red epi-fluorescence is measured.*

*As the virus is diluted,* $R_{Green} / R_{Blue}$ *increases, while* $R_{Red} / R_{Green}$ *decreases, as shown in the graphs.*