



Lab on a Chip

Early detection of E. coli and total coliform using an automated, colorimetric and fluorometric fiber optics-based device

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Early detection of *E. coli* and total coliform using an automated, colorimetric and fluorometric fiber optics-based device

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Abstract

Lack of access to clean water is a major global issue that affects millions of people worldwide. Drinking contaminated water can be extremely hazardous, so it is imperative that it is tested sufficiently. One method commonly used to determine the quality of water is testing for both *E. coli* and total coliform. Here, we present a cost-effective and automated device which can concurrently test drinking water samples for both *E. coli* and total coliform using an EPA-approved reagent. Equipped with a Raspberry Pi microcontroller and camera, we perform automated periodic measurements of both the absorption and fluorescence of the water under test over 24 hours. In each test, 100 mL of the water sample is split into a custom designed 40-well plate, where the transmitted blue light and the fluorescent light (under UV excitation) are collected by 520 individual optical fibers. Images of these fiber outputs are then acquired periodically, and digitally processed to determine the presence of the bacteria in each well of the 40-well plate. We demonstrate that this cost-effective device, weighing 1.66 kg, can automatically detect the presence of both *E. coli* and total coliform in drinking water within ~16 hours, down to a level of one colony-forming unit (CFU) per 100 mL. Furthermore, due to its automated analysis, this approach is also more sensitive than a manual count performed by an expert, reducing the time needed to determine whether the water under test is safe to drink or not.

Introduction

The World Economic Forum's "The Global Risks Report 2019" states that water crises have been one of the top 5 global risks in terms of impact, and the top societal risk for the past 5 years¹. According to the World Health Organization (WHO), 785 million people lack safe drinking water, and at least 2 billion people use water sources contaminated with feces². Lack of access to safe, contaminant-free water, severely threaten public health due to waterborne illnesses³⁻⁵. It's estimated that 1 million people die every year due to water, sanitation or hygiene related problems, and every 2 minutes a child dies due to poor quality water^{6,7}. Therefore, effective monitoring of water quality is urgently needed to prevent waterborne diseases, improve public health, and save lives.

Solving these problems is no simple task. Water can contain hundreds of different microorganisms, making the analysis of all possible pathogenic microorganisms very challenging. However, the presence of *E. coli* and total coliform in a water sample is widely accepted as evidence for contamination of a water supply^{8,9}. Total coliform bacteria are commonly found in the environment. Therefore, the presence of total coliform in water samples is an indicator of contamination from the surrounding environment¹⁰. While the coliform bacteria do not necessarily cause disease, their presence can indicate that other pathogens may potentially exist within the water sample¹⁰. On the other hand, *E. coli* is a member of the fecal coliform group, which exists in the intestines and feces of human and other warm-blooded animals¹¹. Therefore, the presence of fecal coliform, more specifically *E. coli*, indicates the presence of disease-causing pathogens¹²⁻¹⁴. In practice, monitoring only *E. coli* and total coliform contamination is sufficient to analyze water quality for health-related risks. According to the United States Environmental Protection Agency (EPA)^{8,15,16} in order to determine whether or not a water source is safe for drinking, the sensitivity of the measurement technique must be at least 1 CFU/100 mL.

There are several EPA-approved methods used to monitor water quality which employ conventional microbiological techniques such as multiple tube fermentation and membrane filtration¹⁷⁻²⁰. However, these microbiological methods have some limitations, such as a long total analysis time, interference from non-coliform bacteria, limited detection of slow-growing or stressed coliform, viable but non-culturable (VBNC) bacteria, and requiring transportation to central lab facilities with trained professionals^{12,17,21-25}. There are other emerging methods such as immunological assays²⁶⁻²⁹ and polymerase chain reaction (PCR) based methods³⁰⁻³² which in general provide faster detection. However, these methods require relatively complex procedures and trained specialists. Additionally, these methods do not allow on-site water quality monitoring. Instead, water samples need to be transported to central lab facilities, resulting in an additional delay. Water quality can also be monitored by optical, electrochemical, piezoelectric or plasmonic biosensors³³⁻³⁶. However, such biosensor technologies typically lack sensitivity and/or are constrained to very small sample volumes, in addition to requiring complex and expensive benchtop equipment to operate.

One of the EPA-approved methods for *E. coli* and total coliform detection is Colilert^{37,38}. This is one of the most widely used technique, and is an enzymatic method which uses Defined Substrate Technology (DST)³⁹ to simultaneously detect *E. coli* and total coliform in drinking

water. Within the Colilert reagent there are two substrates: *o*-nitrophenyl- β -D-galactopyranoside (ONPG) and 4-methylumbelliferyl- β -D-glucuronide (MUG)⁴⁰, which are metabolized by the coliform enzyme β -galactosidase and *E. coli* enzyme β -glucuronidase respectively. When total coliform bacteria are present in the water sample, they use the β -galactosidase to metabolize ONPG, which releases *o*-nitrophenol and changes the sample from being colorless to yellow. *E. coli* use β -glucuronidase to metabolize MUG and release 4-methylumbelliferone (4-MU), which is a fluorescent molecule and emits blue light when excited by ultraviolet (UV) light^{38,41,42}. This method is sensitive and can be used to detect concentrations as low as 1 CFU/100 mL, and quantification is available using a most probable number (MPN) table or software. However, when used to quantify coliform bacteria concentration in water samples, the Colilert method has drawbacks as well. Notably, the total process takes 24 to 28 hours, and similar to those listed above, it is not an on-site method (i.e., the samples must be transported to a lab with trained personnel and special equipment).

In case of fecal contaminated water sources, it is crucial to detect the presence of the bacteria as early as possible to prevent illness. To achieve this, sensitive, portable and cost-effective water quality sensors which can be operated by non-specialists are urgently needed. In this paper we demonstrate a cost-effective and highly sensitive water quality monitoring device, which can perform automatic early detection of both *E. coli* and total coliform using the Colilert reagent, mixed with the sample water under test, which is then placed inside a custom-designed 40-well plate to be automatically imaged all in parallel using fiber optic cables. Our device weighs 1.66 kg and can automatically detect 1 CFU/100 mL in less than 16 h, which allows the sample to be processed using limited laboratory equipment and without requiring specialized personnel. At higher concentrations of *E. coli* and/or total coliform the automated detection time can be further decreased.

Methods

Materials

The three different types of bacteria, *Escherichia coli* (*E. coli*) (ATCC® 25922™), *Klebsiella aerogenes* (*K. aerogenes* or *Enterobacter aerogenes* (*E. aerogenes*)) (ATCC® 49701™), and *Citrobacter freundii* (*C. freundii*) (ATCC® 43864™) were obtained from American Type Culture Collection, Manassas, VA, USA. Tryptic Soy Agar (TSA) (BD Difco) and Nutrient Agar (NA) (BD Difco) were obtained from Fisher Scientific, CA, USA. Colilert reagent for 100 mL water sample (Colilert snap packs) and Colilert sterile vessels were obtained from IDEXX Laboratories, Inc., Westbrook, USA. Multimode optical fibers (FT400UMT), the ground glass diffuser (DG100X100-220 N-BK7) and a plano-convex lens (LA1027-A) were purchased from Thorlabs, Newton, NJ, USA. The ultraviolet light emitting diodes (UV LEDs) (VLMU1610-365-135CT-ND) and drivers (296-31235-ND) were obtained from Digi-Key Electronics, Thief River Falls, MN, USA. The blue LEDs (749-SM1206UV-400-IL) were obtained from Mouser Electronics, Mansfield, TX, USA. The Raspberry Pi Board (Raspberry Pi 3 Model B) was purchased from Newark element 14, Chicago, IL, USA, and the camera (Raspberry Pi Camera Module V2-8 Megapixel, 1080p) was purchased from Raspberry Pi Foundation, UK. Rechargeable batteries were obtained from EBL, LA, USA. The Plexiglas

MC acrylic sheet UF-5 was purchased from Altuglas International Arkema Inc., Philadelphia, PA, USA. The glass beads (ColiRollers Sterile Plating Beads (Novagen)) were purchased from MilliporeSigma, Burlington, MA, USA. The sealing film (ThermalSeal RTS™, Sterile) was obtained from Sigma-Aldrich, Burlington, MA, USA. Reagent Grade Water (Nerl, Reagent Water (CLRW) and USP/NF Purified Water) and Petri dishes (100 mm x15mm, Sterile) were obtained from Fisher Scientific, CA, USA. Microcentrifuge tubes (Microwtube, 2mL, o-ring seal screw cap, sterile) were obtained from Thomas Scientific, Swedesboro, NJ, USA. Glass shell vials (Kimble) were obtained from Carolina Biological Supply Company, Burlington, NC, USA. The microbiological incubator (Isotemp) and autoclave (SterilElite) were obtained from Fisher Scientific, CA, USA. The incubator (Heracell VIOS CO₂) was obtained from Thermo Scientific, Waltham, MA, USA.

Culture Based Assays and Sample Preparation

Pure cultures of *E. coli* (ATCC 25922) and *E. aerogenes* (ATCC 49701) were grown on Tryptic Soy Agar (TSA) for 24 h in a microbiological incubator at 37 °C and 35 °C, respectively. *C. freundii* (ATCC 43864) was cultured on Nutrient Agar (NA) at 37 °C in an incubator. TSA and NA plates were prepared according to the manufacturers specifications⁴³. Following this, 20 mL was poured into each 100 mm diameter plate and used or stored at 4 °C until use. The agar plates are used to culture the bacteria and to perform quantitative measurements of the bacteria concentration for *comparison* to our presented method. This plate count, in which bacteria were grown on agar plates and counted, was used as the gold standard for determining the concentration added to the device during testing. Bacteria from an overnight culture were resuspended in 1 mL sterile reagent grade water and serially diluted (10-fold) as required and 100 µL of bacteria contaminated water (BCW) sample was added to each agar plate (n=5). Samples were spread onto the agar surface with sterile glass beads using the Copacabana method^{44,45} and the plates were incubated for 24 h. After overnight incubation, individual colonies of bacteria on the agar plates were counted and averaged (see Supplementary Figure 1). In addition to the bacteria samples, negative control experiments were performed using the same procedures without any addition of bacteria (see Supplementary Figure 2).

To prepare the samples used to validate the performance of the device, 100 µL of the same BCW sample described above was added to 100 mL of sterile reagent grade water and mixed with the Colilert reagent until dissolved. 2.5 mL of the contaminated water sample was then added to each sterile glass vial within the 40-well plate. The filled vials were then sealed with sterile, non-fluorescent, UV-transmitting sealing film, put into the device and incubated for 24 h at 35 °C inside the incubator. Following the incubation, the concentration of bacteria which was determined with the plate count (n=5) was compared with the automated counting results of our device (see Supplementary Figure 1). Negative control experiments were performed using the same procedure without bacteria added to the initial sample.

To test the performance of the device at higher concentrations of bacteria, a modified version of the above test was used. The sample preparation steps for these tests can be visualized in Supplementary Figure 3. For these tests, eight different *E. coli* concentrations were prepared with 10-fold serial dilution between each. The Colilert reagent was added to 100 mL sterile reagent grade water and 900 µL of this sample was in turn added to each vial of the 40-well plate. 100 µL of each *E. coli* concentration was added to 5 vials, and similar to the procedures outlined above, the 40-well plates were sealed and incubated for 24 h at 35 °C. To

quantitatively determine the precise bacteria concentrations used for these tests, the plate count method ($n=3$) was applied following the procedure described in the earlier section. Since higher concentrations are too numerous to count (TNTC), the plate count method is applied to only the four lowest concentrations.

Device design

For use in the presented device a water sample of interest is split evenly into 40 disposable glass vials which are held by a custom 3D-printed 40-well plate. There are two LEDs (one UV, one blue) above each one of these wells which illuminate the sample, and 13 fiber optic cables below each well collect the sample's signal. The blue LEDs are used to detect the presence of total coliform, with the sensor indirectly measuring the absorption of the transmitted light. The UV LEDs are used to detect the presence of *E. coli* by exciting fluorophores in the sample. Therefore, when fluorescence is detected in a vial, it is classified as containing *E. coli*. The fiber optic cables are used to map the light passing through the 40 wells onto the camera, without the use of any mechanical scanning.

A diagram of our device design is shown in Figure 1. The device uses a 3D-printed structure to hold the components together, and the entire device is placed within an incubator to ensure a constant temperature of 35 °C. A Raspberry Pi microcontroller controls the illumination and a Raspberry Pi camera is used to periodically detect the light from all the 40 wells. A total of 520 fibers are used, with 13 collecting light from a given well. A plano-convex lens is used below the fiber bundles to help focus the light on the camera sensor.

The blue LEDs are used to detect the colorimetric/absorption signal indicating the presence of total coliform and they operate at a peak wavelength of 400 nm. This gives a strong overlap with *o*-nitrophenol's absorption spectrum, which is centered at 420 nm. The UV LEDs used to detect the fluorometric channel operate at a peak wavelength of 365 nm, and are used to excite the 4-MU fluorophores. To eliminate the need for expensive and bulky UV excitation filters, a UV LED with minimal emission above 400 nm was chosen, which allows the light to be blocked solely by an emission filter. Between the LEDs and the glass vials there is a UV-transmitting glass diffuser, which is used to make the illumination more uniform and reduce the effects of any small movement of the device. The LEDs are powered by constant current drivers, which output a current of 20 mA. All of the LEDs are surface mounted to a custom printed circuit board (PCB). To allow for flexibility, the device can either be powered by a rechargeable battery or plugged into a standard outlet.

A 3 mm thick UF-5 Plexiglas sheet is used as a longpass filter, which blocks light below 400 nm, and filters out the light produced by the UV LEDs. This Plexiglas sheet is an ideal UV filter for our application as it completely blocks the wavelengths desired, does not produce auto-fluorescence, and unlike custom-designed filters, is very cost-effective. This cost-effectiveness is particularly useful as the filter needs to be large (165 × 110 mm) to cover all the fibers.

Once the device has been loaded with the 40 vials, the Raspberry Pi begins to activate the LEDs and takes an image of the fibers using one wavelength at a time. When the images have been taken, the device waits for 15 minutes with the LEDs off before taking another image, which are all saved as raw '.mat' files for processing. The images using UV excitation have an exposure time of 30 ms while the blue excitation images use an exposure time of 2 ms.

Image processing

The raw images were processed using MATLAB (The MathWorks, Inc., release R2018a). First, the exact location of the center of each fiber is determined using the first image illuminated by the blue LEDs. These fiber locations are then associated with the different wells using pre-set manually labeled groupings. To account for minor shifts of the setup, subsequent images are then registered to the initial image using cross correlation.

Following these pre-processing steps, the fiber intensities in each image are measured by summing up the intensities of all the pixels within a 14-pixel radius of the fiber's center. As there is some crosstalk between the different wells in the fluorescence channel, the intensity values for the fibers in each well are reduced according to the intensity of those around them. This normalization is done by multiplying the average intensity of the 13 fibers under each vial by an empirically determined constant and then dividing it by the square of the distance between the wells.

The normalized intensities of the fibers in each well are averaged for the final classification. The absorption channel, which is used for the detection of total coliform, is classified as positive when the intensity drops by 5% over 10 successive images. The fluorescence channel is classified using a manually-chosen threshold, where the well is marked as positive if the intensity increases more than 20% of the value after the first 75 min, indicating that *E. coli* is present in the sample. In both cases, the first 5 time points (i.e., the first hour after loading the sample inside the incubator) are ignored as there are significant fluctuations in the signal intensities due to the liquid in the vials slowly warming to the temperature of the incubator. This causes condensation to form on the sealing film covering the wells over the course of the first hour. The classification threshold for the fluorescence channel was set to be higher than the colorimetric channel as the crosstalk between wells cannot be completely eliminated in the fluorescence detection channel. A visualization of the intensity for the fibers in each well can be seen in Supplementary Figure 1.

Results and Discussion

Our detection device was validated using *E. coli* and two different types of total coliform bacteria. Using the procedures described in the Methods section, 51 tests were performed using *E. coli* samples, 27 using *E. aerogenes* samples, and 19 using *C. freundii* samples as well as 3 negative samples, in which no bacteria was added, to determine the device performance, sensitivity and limit of detection. Figure 2 provides a comparison of our device's counting efficiency against the gold standard plate count method (see the Methods section). In this plot, the number of wells that turned out to be positive is compared with the average of 5 plate counts. Plate count measurements are required to quantify the concentration of bacteria in the sample tested by our device and constitute our ground truth measurements. The figure also shows the statistically expected number of wells that should be positive for a given plate count measurement. The horizontal error bars for these values are calculated by finding the standard deviation of the plate count ($n=5$), while the vertical error bars are calculated using the 95% confidence interval of a Monte Carlo simulation, based on the experimental measurement. This simulation was performed by taking the plate count, adding or subtracting a random number of

bacteria corresponding to a normal distribution using the measured standard deviation, and finally randomly placing the bacteria into the wells of the 40-well plate.

In this Figure 2, the line of best fit is calculated as:

$$\text{Expected number of positive wells} = N - N * \left(\frac{N-1}{N}\right)^{\alpha * P} \quad [1]$$

where $N=40$ is the number of wells per plate, P is the ground truth bacteria number in the sample (which is the plate count in our case) and α is the constant being fitted for, which represents the efficiency of our device at measuring bacteria concentration compared to the ground truth. When α is equal to one, Eq. (1) gives the theoretical number of positive wells containing bacteria for a given P . For *E. coli* detection experiments, α was found to be 1.154 (95% confidence between 1.064 and 1.244), which is close to the theoretical detection efficiency. The two total coliform tests had larger deviations from the plate count method, with *E. aerogenes* having an α of 0.602 (95% confidence between 0.46 and 0.745), and *C. freundii* having an α of 1.707 (95% confidence between 1.126 and 2.288). These measurements show that there is no consistent trend for either over counting or under counting the bacteria.

The lower α value of *E. aerogenes* experiments is likely due to a portion of the bacteria being stressed or injured, and not multiplying efficiently for the test to report positive within the 24 hour window. It is important to note that for the undercounted *E. aerogenes* measurements, our detection platform was able to obtain better results than a standard visual count of the positive wells since our automated detection is more sensitive than the human eye. One example of this can be seen in Figure 4. For all other bacteria measurements, the visual positive well counts and the device counts were the same.

The overcounting of the *C. freundii* samples compared to the plate counts, with an α of 1.707, can potentially be due to the bacteria being in the viable but non-culturable (VBNC) state. Bacteria can enter the VBNC state for reasons such as environmental stress. When this happens, they can preserve some metabolic activity (detected by our platform) but lose their ability to grow on an agar plate. Therefore, the concentration of bacteria detected by enzyme-based methods, such as the presented device, can be higher than the concentration detected using culture-based methods.

If the number of bacteria detected by the device needs to be quantified, Eq. 1 can be rearranged to provide a probabilistic estimation:

$$\text{Estimated number of bacteria} = \frac{\log(N - W)}{\log\left(\frac{N-1}{N}\right)} \quad [2]$$

where W is the number of positive wells counted by the device. At low concentrations the number of bacteria tested by the sample can be accurately estimated using Eq. (2). However, at higher concentrations as the probability of multiple bacteria to end up at the same well increases, Eq. (2) will undercount bacteria.

Figure 3 demonstrates one of the important benefits of the presented device: it can automatically detect bacteria several hours faster than the standard Colilert method. While the exact detection time depends on several factors, Figure 3 shows that our device is capable of bacteria detection in < 16 hours, i.e., 8 hours faster than manual inspection. This is even the

case at lower bacteria concentrations: for water samples with a concentration of ≤ 5 CFU/100 mL, our device was able to detect the presence of bacteria on average within 15.0 and 15.1 hours of the start of the incubation period for *E. coli* and *C. freundii*, respectively. The exception to this trend is a portion of the *E. aerogenes* samples, which, as discussed earlier, take significantly longer to be detected, e.g., 21.4 hours after the start of the incubation for the same concentration range. We should note that for these *E. aerogenes* samples it also took significantly longer than 24 hours to exhibit color change or fluorescence signal using the standard Colilert_method, which indicates potentially damaged or stressed bacteria. See for example Figure 4, which reports that the visual signal change, indicative of the presence of the bacteria, occurred only after 28 hours for some of the vials in this *E. aerogenes* sample, whereas for the same vials our device detected the presence of the bacteria several hours earlier compared to the visual inspection.

This significant decrease in the detection time when compared to the traditional Colilert method is a result of two factors. First, since our device is completely automated, it performs measurements at regular intervals rather than waiting for the full 24 hours. Additionally, since it performs *comparative quantitative* analysis, our device can make more sensitive measurements than is possible with a manual end-point qualitative measurement using the Colilert method. Figure 4 gives a demonstration of this increased sensitivity, showing that the device can detect all of the 5 positive wells under 24 hours while a visual inspection at 24 hours is unable to detect any color changes that indicate the growth of the *E. aerogenes*. Only after the same samples have been incubated for an additional four hours, visual inspection was then able to determine that all of these wells are indeed positive.

Figure 5 also shows that as the bacteria concentration increases, the detection time further decreases as a larger number of bacteria can interact with the enzymes at an earlier time point. This point is illustrated in Figure 5 (a), which reports the reduction in the detection time as the concentration of bacteria is raised to higher levels. Using the average of 5 measured wells at each concentration, the detection time is found to get ~ 0.66 hours less for each order of magnitude increase in the concentration of bacteria over the range of 1 to 1.2×10^7 CFU/mL. For example, at a concentration of 1 CFU/mL, our device takes an average of 13.7 hours to automatically detect the presence of the bacteria, while at a concentration of 1.2×10^7 CFU/mL it only takes 2.8 hours. Figure 5 (b) and (c) demonstrate how the intensity measured by the device changes over time for the fluorescence and absorption channels of our device, respectively.

As our device is designed to test drinking water, all the testing has been performed using non-turbid water. EPA regulations¹⁶ require the turbidity in drinking water to be below 1 nephelometric turbidity unit (NTU) when direct or conventional filtration is used, and below 5 NTU otherwise. At a turbidity level of 5 NTU, less than 7% of the light passing through the sample will be absorbed⁴⁶. Since our device makes a determination of bacterial growth according to the relative changes in the signal intensity (as a function of time), any absorption from these low levels of turbidity that drinking water might exhibit will therefore not impact the operation of our device.

Conclusions

We presented a custom designed, 3D-printed opto-mechanical platform controlled by a Raspberry Pi microcontroller which can perform automated detection of both *E. coli* and total coliform in a 100 mL water sample using the Colilert reagent. We demonstrated that the presented device is more sensitive than a manual count, and that, because of this increased sensitivity, it can automatically detect the presence of bacteria faster than it is possible through a manual measurement. The device is able to perform these measurements according to the EPA standard, which requires testing of 100 mL of water sample, and it can identify a single organism in this 100 mL sample (i.e., 1 CFU/100 mL). Additionally, it is capable of performing this automated detection within less than 16 hours. This time can be further reduced as the bacteria concentration is increased.

The automatic classification of the wells eliminates the need for a trained operator as well as the risk of a counting error. Additionally, since no specialized skills are required for its operation, it can be used with minimal training. This simplicity also allows the sample preparation to be performed in only a few minutes, while still being effective, giving a definitive result in less than 24 hours.

By using a Raspberry Pi microcontroller and CMOS camera to perform the detection, optical fibers to collect the light, Plexiglas as an inexpensive fluorescence emission filter, and UV LEDs for illumination (without the need for an excitation filter), our device is rather cost-effective, with its parts costing ~\$600 under low volume manufacturing, which can be significantly reduced with economies of scale. Therefore, it is applicable in a variety of settings, particularly in areas where access to a central lab or transportation of the sample are not feasible. In the future, the device can be modified to use a custom incubator, which would allow the device to be field-portable and even more cost-effective to use.

Acknowledgements

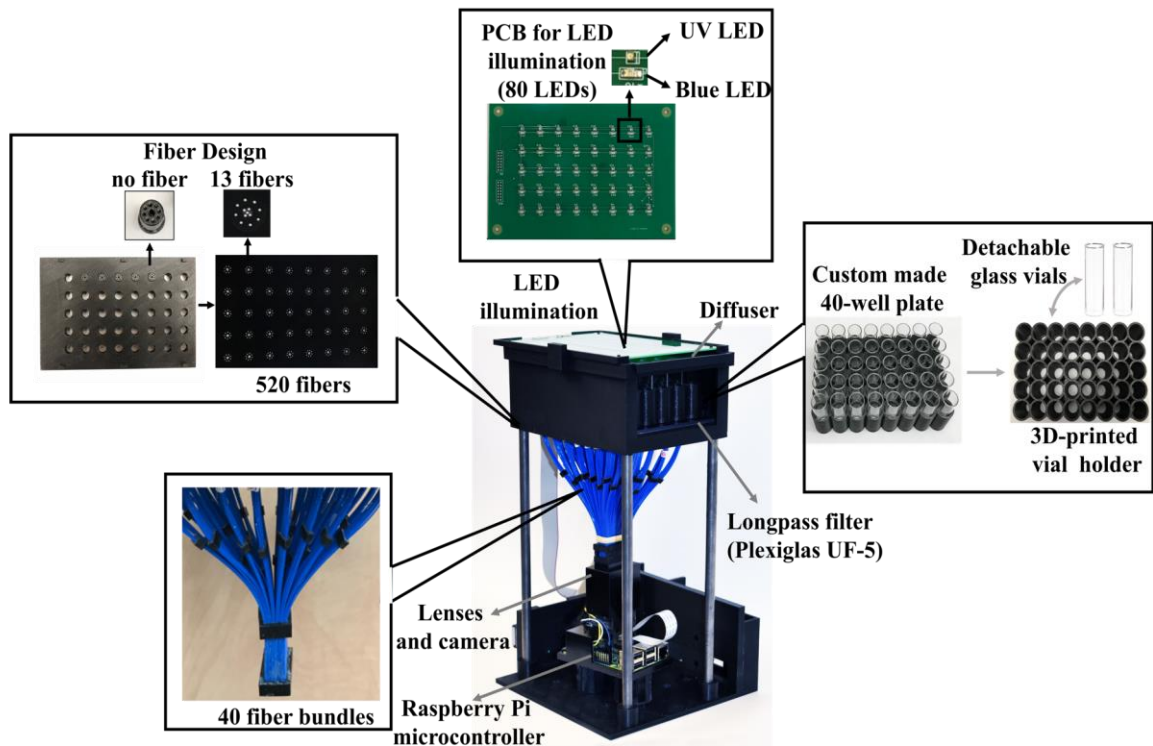
The Ozcan Research Group at UCLA acknowledges the support of the Koç Group, ARO Life Sciences Division (W911NF-17-1-0161) and the Howard Hughes Medical Institute (HHMI).

Conflicts of interest

There are no conflicts of interest to declare.

Figures

a.



b.

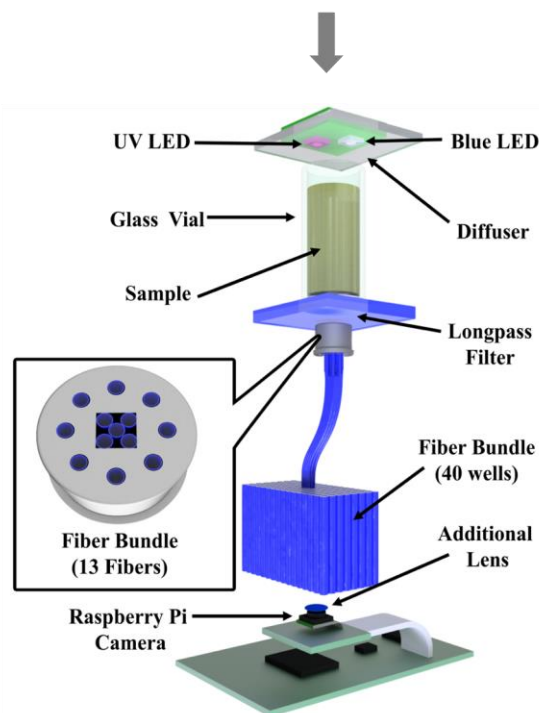


Figure 1: Schematics and components of the *E. coli* and total coliform detection device. a) Detailed illustration of the components. b) Illustration of the working principle for a single well. A pair of blue and UV LEDs, controlled by the Raspberry Pi, are used to illuminate each well of the 40-well plate. The blue light passing through the well and the fluorescence emitted by the sample in response to UV excitation are long-pass filtered. Then the light passing through each well is collected by fibers and mapped onto the camera.

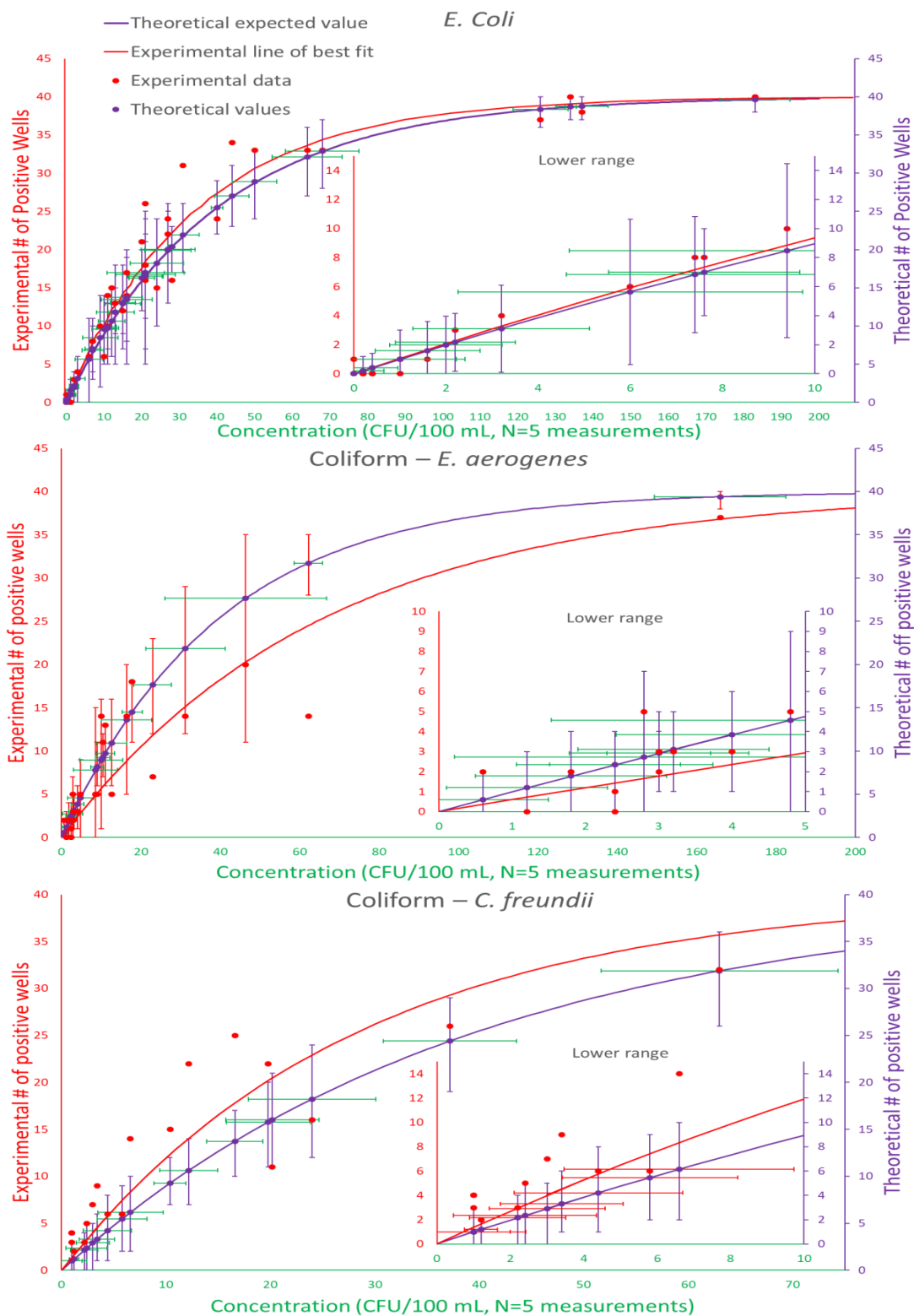


Figure 2: *E. coli* and coliform concentrations detected by our device up to 200 CFU/100 mL, compared to the plate count method used as the gold standard, ground truth. The x-axis represents the number of bacteria measured by the plate count method and the y-axis represents the number of positive wells detected by our device. a) *E. coli* measurements, b) *E. aerogenes* measurements, c) *C. freundii* measurements.

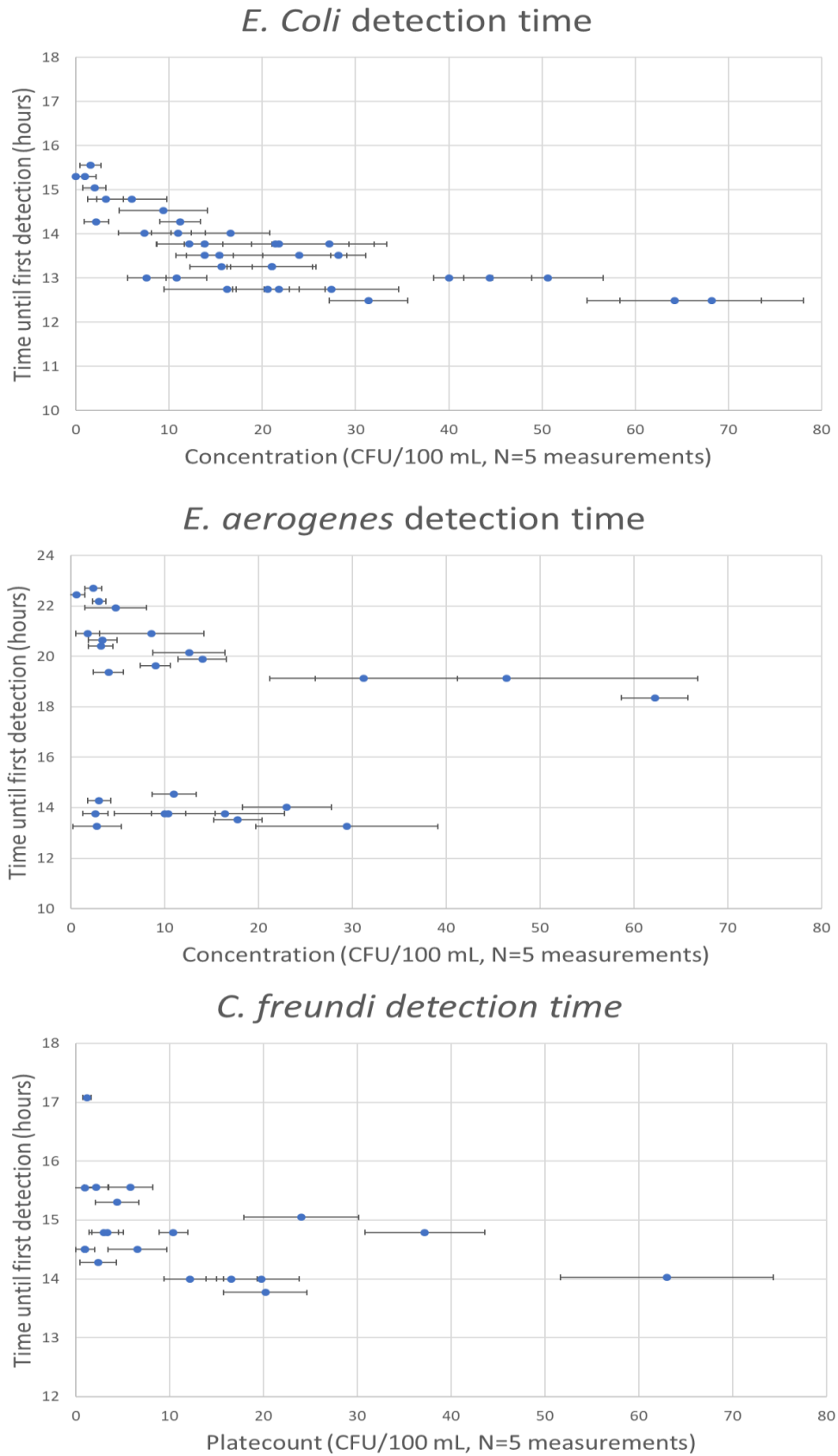


Figure 3: Time that our device takes until the first detection of a positive signal in the sample being measured. a) *E. coli* measurements, b) *E. aerogenes* measurements, c) *C. freundii* measurements.

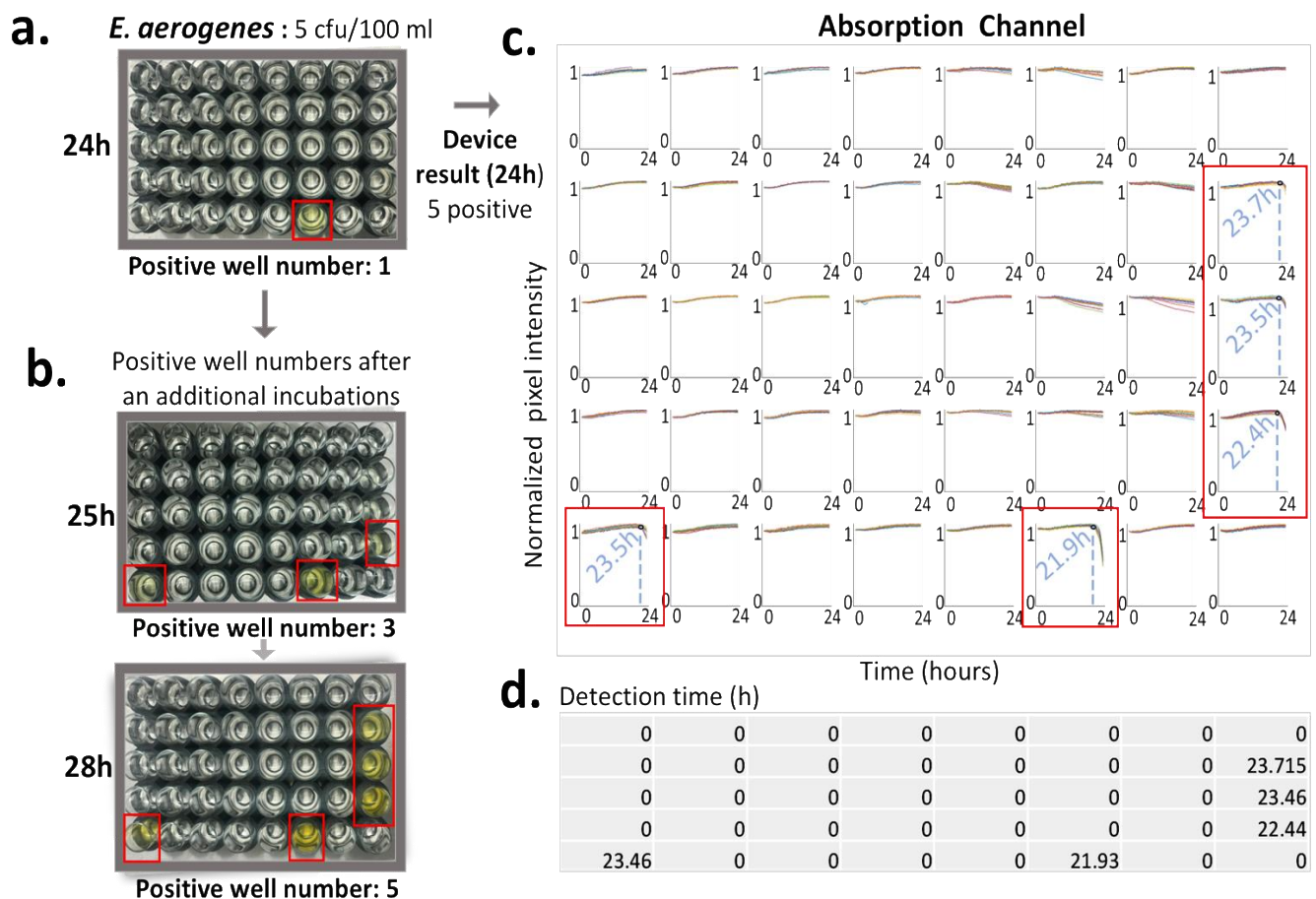


Figure 4: Demonstration of how our detection device is capable of performing measurements faster than a manual count. The device detects the slow-growing coliforms in less than 24 hours, while the visual inspection cannot. a) Picture of the wells after 24 hours of incubation. b) Picture of the wells after 28 hours of incubation, where four additional wells have become positive. c) Plot of the absorption channel intensity over time measured by our device. d) Time at which our device was able to detect each positive well.

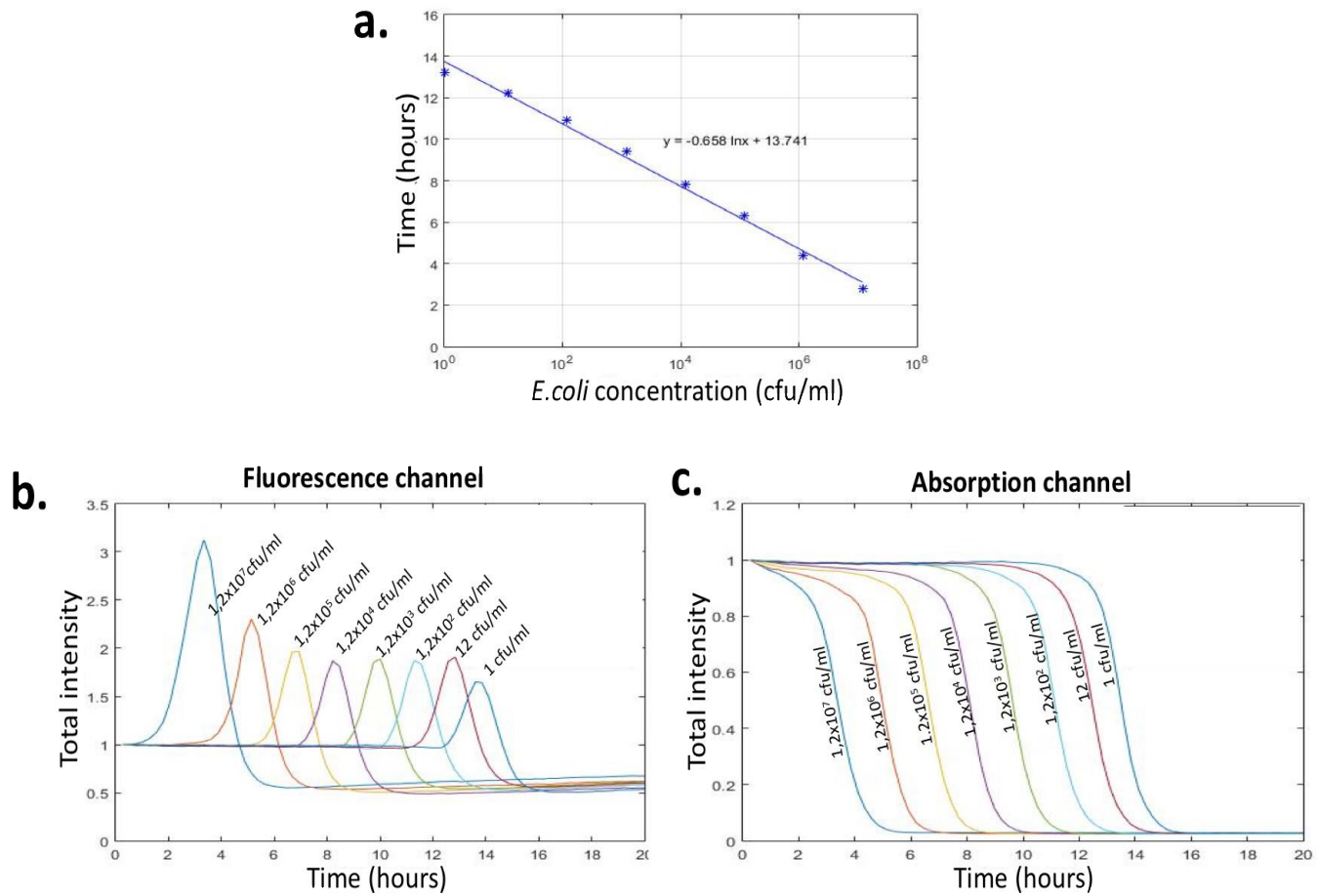
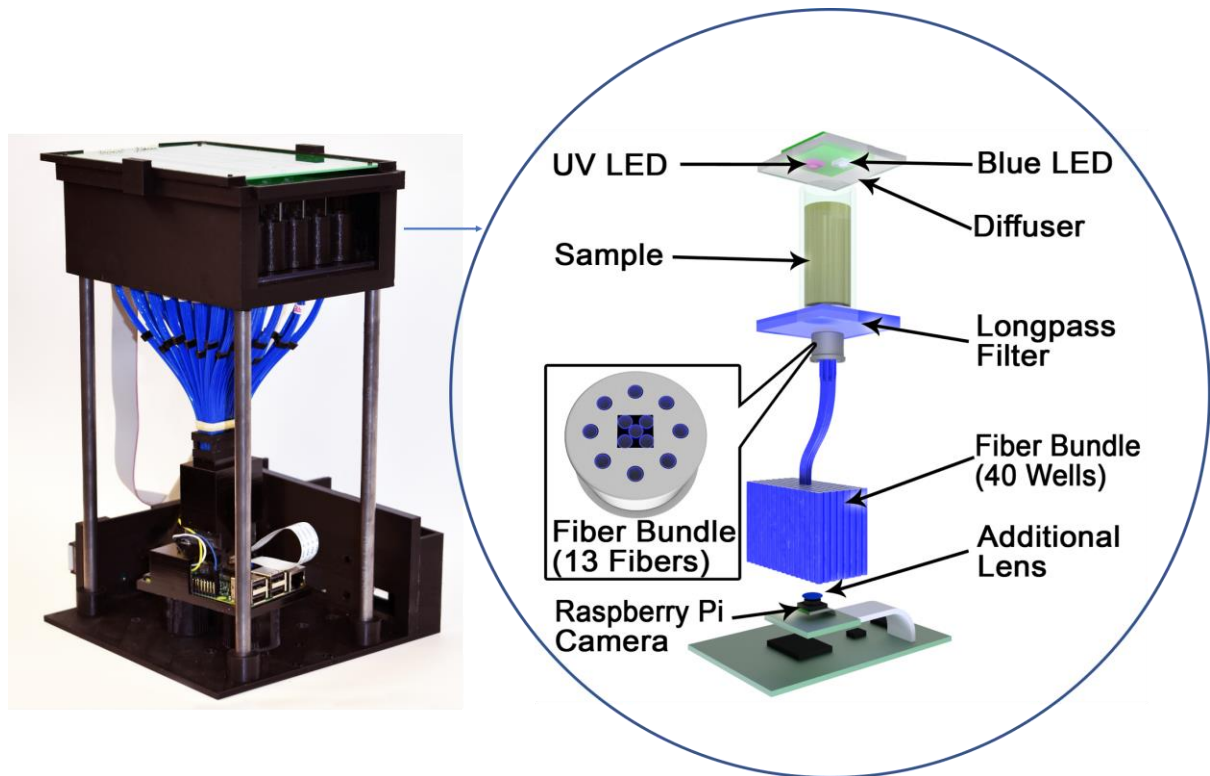


Figure 5: Detection times for different *E. coli* concentrations. a) Time until the first detection of *E. coli* as a function of the bacteria concentration. b) The fluorescence channel, and c) the absorption channel intensity measured by our device over time, for increasing concentrations of *E. coli*.

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We report early detection of *E. coli* and total coliform bacteria using an automated, colorimetric and fluorometric fiber optics-based device.

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