Supporting Information

Materials and Methods

Generation 1 Raspberry Pi Holder Design. The first Raspberry Pi holder was designed using Autodesk Inventor software. The holder (Figure SI3B and SI3C) measures 5 cm in diameter at the top of the holder and 10 cm in diameter at the bottom of the holder, and is overall 10 cm tall, not including the pegs on top of the holder. Pegs on top of the device were used to hold the Raspberry Pi camera in the same position between experiments. Pegs were also added on the side of the device for the option of using a rubber band to hold the camera down for more consistent placement between experiments (seen in use in Figure SI3C). Pegs were used on another side of the holder and measured 4 mm at the base and 6 mm at the top. The pegs were used to hold the Raspberry Pi board which is housed in a plastic case. This holder contains notches that the pegs were specifically designed to hold for the Raspberry Pi board case to snap into place. The interior of the Raspberry Pi holder was spray painted with matte-white spray paint to help reflect light for optimal images. The lighting system to illuminate the inside of the holder was a standard light circuit where the bulbs were designed to be in parallel. A 9V battery was wired first to a toggle switch, then to the light bulbs in parallel, then to the resistor, back to the negative input of the battery. 3 white LEDs and an 820 Ω resistor were used. The wires, bulbs, and resistor were soldered together on a breadboard using a soldering iron for optimal electrical connection. Holes were drilled into the side of the holder using a 15/64 inch drill bit for the LEDs to insert into the device. The breadboard was taped to the side of the holder using electrical tape to hold the lighting system in place.

Results and Discussion

The initial Raspberry Pi holder was like the updated holder in that it was composed of two different parts (Figure SI4). The bottom component of the holder was designed to hold the PADs in the same location between experiments. 3 mm pegs were used in the bottom component, and 3 mm holes were designed into the PADs via laser cutting for the 3 mm holes to be in the same location as the holder's pegs for consistent device placement (Figure SI4A). The top component of the holder secures the Raspberry Pi computer board and places the attached camera in the same location between experiments using pegs, like the PAD. The Raspberry Pi board was housed in a plastic case with notches that were designed for wall-mounting, so pegs were designed into the side of the holder for the Raspberry Pi to have a secure fitting. The camera, which is attached to the board through a ribbon, is placed at the top of the holder where pegs can hold the camera over a window for the camera to image the paper-based device (Figure SI4B and SI4C). Additional pegs were placed on either side of the camera for the option of securing the camera using a rubber band. The holder is 10 x 10 cm on the bottom of the holder, but decreases to 5 x 5 cm at the top of the holder where the camera is held, similar to a pyramid. This design was initially chosen instead of a square device to enhance the system's portability, along with generating an overall cheaper holder by using less materials. To illuminate the inside of the holder, a light circuit was fabricated using a toggle switch, three white LEDs, an 820 Ω resistor, and a 9V battery. Although it was calculated that only a 75 Ω resistor was necessary, using a larger resistor dimmed the LEDs to an appropriate brightness as to not “wash out” the devices. The inside of the holder was also spray-painted with matte-white paint to defuse light more evenly across the device. Finally, a dark cloth covered the entire holder during experiments due to natural light affecting the photos through the camera’s viewing window on top of the holder.

Kinetic Experiments and Analysis. For the first Raspberry Pi system, we compared Raspberry Pi photo analysis to manual photo analysis using β-lactamase and nitrocefin on Whatman grade 1 and Whatman grade 4 chromatography papers. The Raspberry Pi camera obtained images at minutes 1, 2, and 3 throughout the reaction once the program was initialized (Figure SI3). The same images were analyzed whether it was the Raspberry Pi program or manual analysis (detailed photo analysis can be found in the materials and methods of the main manuscript). For manual analysis, NIH ImageJ was used to measure the mean color intensity of each sample spot using the circle tool to encompass the entire spot, around 1000 pixels. When the Raspberry Pi analyzed the images, one specific pixel from each sample spot was chosen to measure the light intensity instead of averaging the color intensity of all pixels within the sample spot. The obtained color intensity pixel values, whether manual or Raspberry Pi analysis, both used the same data analysis process to calculate V_{max} and K_M as explained in detail in the materials and methods section.

Manual vs. Raspberry Pi Analysis. The goal of this project was to design a program that obtains similar values of color intensity and Michaelis-Menten values whether it is a person or a Raspberry Pi program analyzing the images. Michaelis-

Menten constants, V_{max} and K_M, were calculated through Raspberry Pi and manual analysis, then compared for overall average. As seen in Figure SI5, the average V_{max} and K_M calculated using the Raspberry Pi program were similar to values obtained through manual analysis, especially for Whatman 1 paper. In Whatman 4 paper, the averages were similar value, but the error was much larger at 30-50% relative standard deviation compared to 2% in manual analysis. This only occurred for Whatman 4, therefore this error could be indicative of inconsistent color formation across each sample spot. In manual analysis, the color intensity values are based off an average of 1000 pixels across the sample, *vs*. Raspberry Pi analysis, the color intensity is based off one pixel. If color formation across the sample spot is consistent, obtaining color intensity based on one pixel is satisfactory. However, if color intensity is inconsistent, using one pixel could lead to significant error. When comparing Whatman 1 and Whatman 4, whether manual or Raspberry Pi analysis, there is no statistically significant difference in either V_{max} or K_M. Whatman 4 appears to be more consistent across experiments, but only when averaging all 1000 pixels across the sample spot. This implies that although color formation rate is consistent between experiments, it is not consistent across the sample spot. When comparing the calculated V_{max} and K_M between manual and Raspberry Pi analysis in the same experiment, values could be anywhere from 2% off to 44% off.

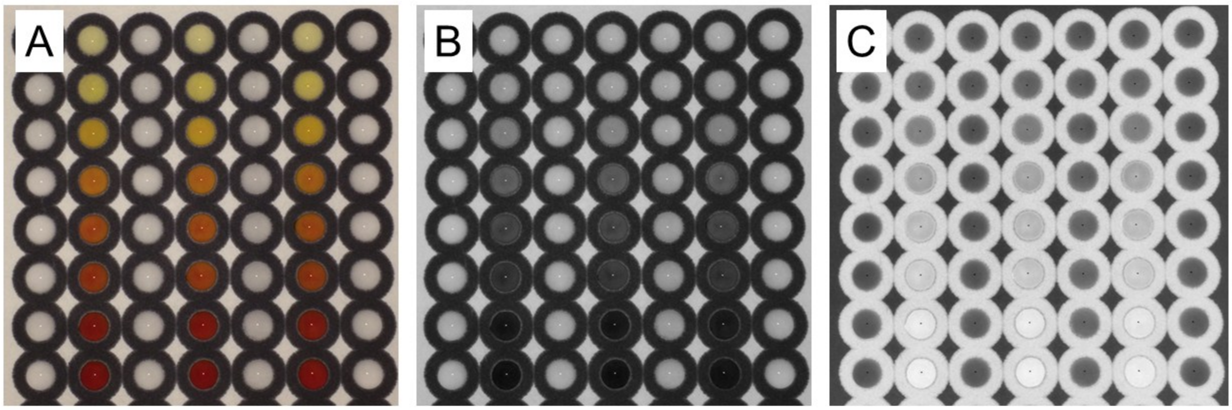

Figure SI1 | Analyzing images in NIH ImageJ (beta-lactamase and nitrocefin). **(A)** Original image. **(B)** The green channel was selected after splitting the original image into red, blue, and green color channels. **(C)** Inverting the image for light intensity to be a positive trend as chemical concentration increases.

Image Analysis Program

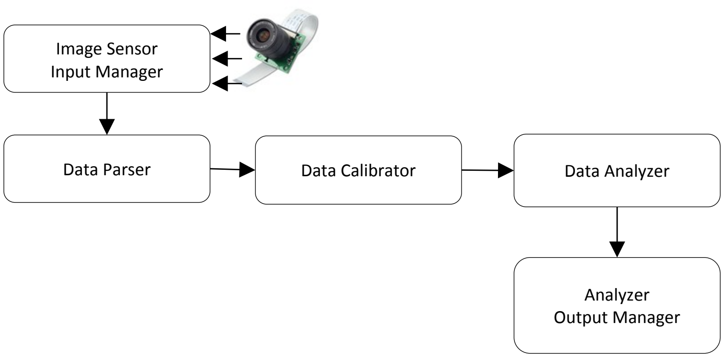

Figure SI2. Software Architecture of KineticsAnalyzer deployed on a Raspberry Pi

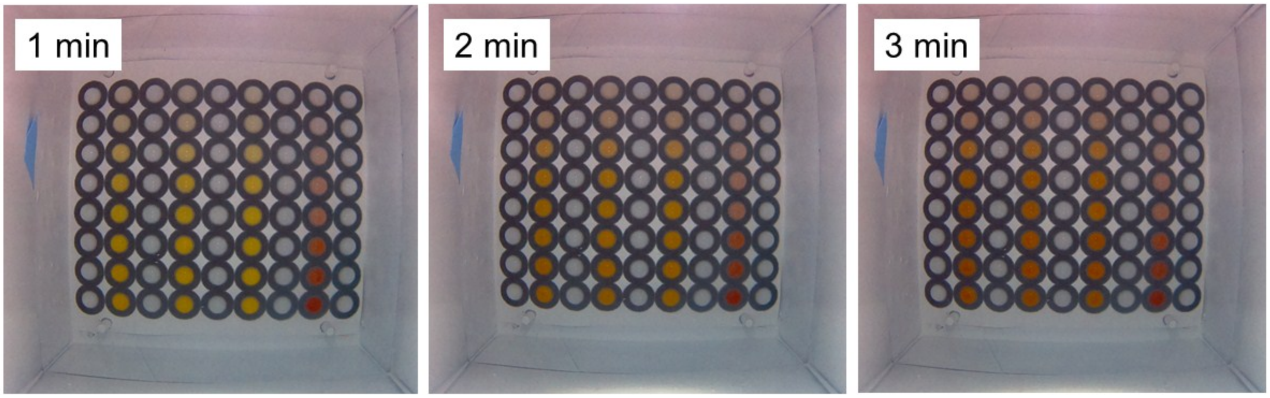

Figure SI3 | Images obtained from the Raspberry Pi during a kinetic experiment.

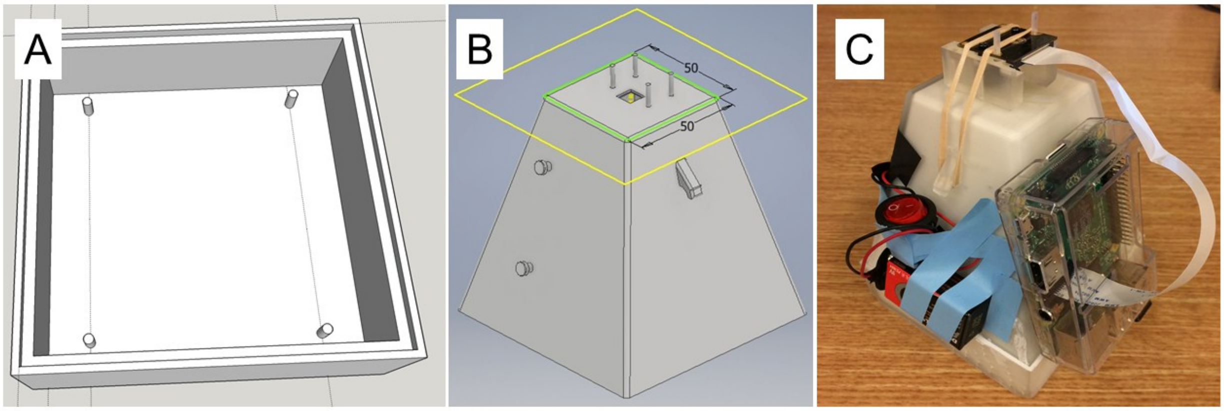

Figure SI4 | The holder for the Generation 1 Raspberry Pi. **(A)** CAD image of the bottom component that houses the paper-based devices. **(B)** CAD image of the top component that holds the Raspberry Pi and attached camera. **(C)** How the Raspberry Pi and attached camera fit onto the entire holder put together.

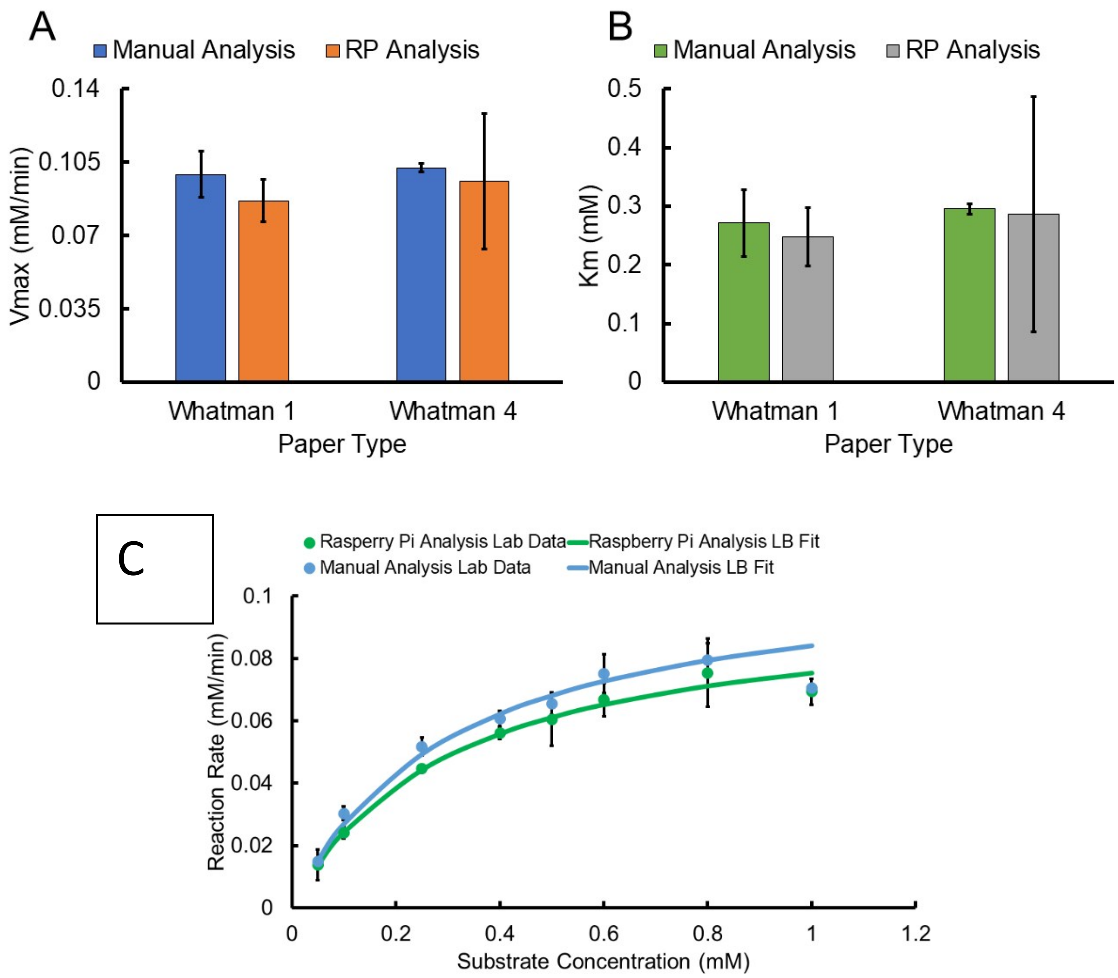

Figure SI5 | Comparing **(A)** V_{max} and **(B)** K_M of β-lactamase and nitrocefin on Whatman 1 and Whatman 4 papers as calculated via manual analysis or Raspberry Pi (RP) analysis. **(C)** A sample Michaelis-Menten kinetic curve comparing manual analysis and Raspberry Pi computer analysis.