We developed the CRISPectra algorithm to measure the pigmentation intensity of the experimental samples which gives us an approximation of gene expression levels.

CRISPectra Rationale

Plate readers are commonly used for measuring gene expression by assessing the absorbance of specific wavelengths associated with pigmentation or fluorescence. However, this specialized instrument, typically only found in professional labs or industry settings, costs upwards of ten thousand dollars, posing a significant financial challenge for many research and educational settings. CRISPectra addresses this gap by enabling accurate approximations of gene expression levels using smartphone cameras. It offers a low-cost, accessible solution: anyone with a smartphone and internet access can analyze and quantify their CRISPRkit data.

Fig. 1: Left - *Plate reader traditionally used for fluorescence/pigmentation expression analysis. Equipment costs >$10,000* ***Right -*** *CRISPectra only requires a smart phone and internet access. Extremely low cost*

Methodology - How the Algorithm Works?

CRISPectra first extracts the mean RGB values for each of the experimental conditions from the smartphone-captured image, using them as a proxy for gene expression levels. To ensure accuracy, the algorithm adjusts these values by comparing them with those from the negative control reaction, effectively filtering out background noise and irrelevant signals.

The algorithm then dimensionally reduces the 3-D RGB model into a singular value that represents the intensity of gene expression for a singular color. This is done by projecting the RGB values onto a vector derived from the positive control - the condition that exhibits maximum gene expression for the color of interest. Once the data is

projected and dimensionally reduced, the algorithm normalizes these values and presents the results as a ratio to the positive control's expression. The outcomes are then graphically illustrated, offering a clear, quantifiable metric of gene expression.

Comparison to Plate Reader

You may be wondering how accurate CRISPectra is at approximating gene expression levels when compared to the established accuracy of fluorescence plate readers. To address this, we put CRISPectra to the test against plate reader data in a series of our dual color CRISPRkit experiments with three biological replicates. The outcomes are plotted in two graphs, with plate reader measurements on the x-axis and CRISPectra measurements on the y-axis. The correlation coefficients for red and yellow chromoproteins stand at 0.939 and 0.943, respectively. Such strong correlation affirms that the estimations of gene expression by CRISPectra are highly consistent with those obtained from plate readers, underscoring the reliability of our algorithm.

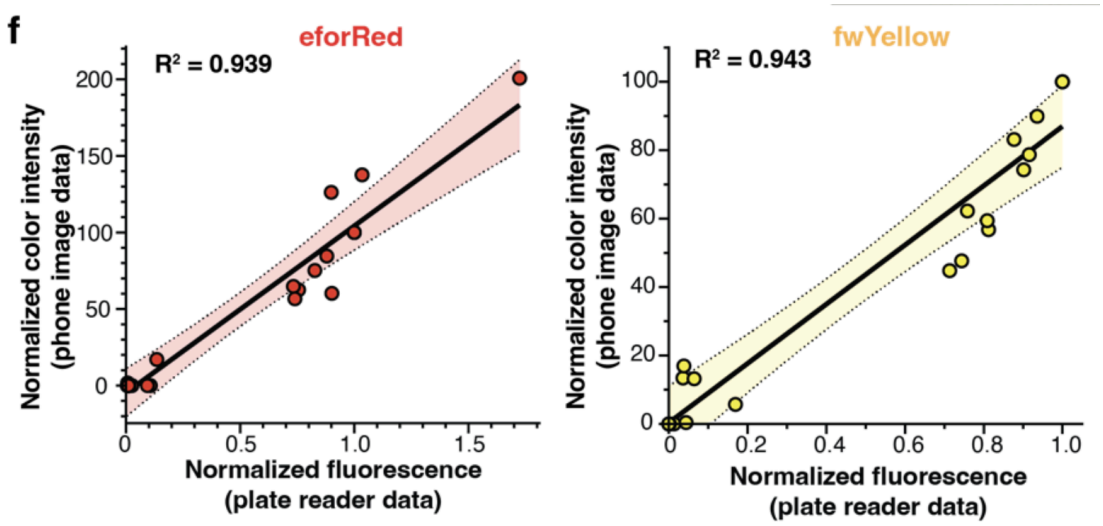

Fig. 2: *Comparison of fluorescence plate reader data (x-axis) and CRISPectra data (y-axis)*

Using CRISPectra

Right now we have only developed and are distributing the Dual Color Kit. You will use CRISPectra to analyze and quantify your CRISPRkit results on Day 2. Detailed instructions on how to use it can be found in the Day 2 Protocol.

Click here to access the CRISPectra GUI.