Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Cell Phone based Colorimetric Analysis for Point-of-Care Settings

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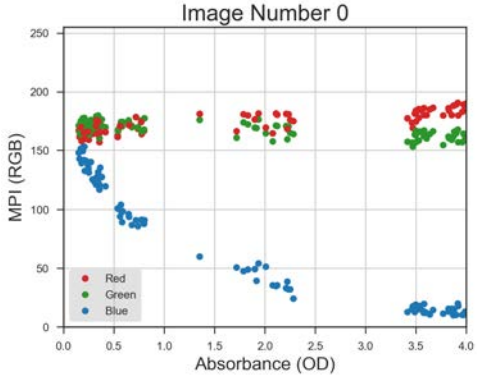
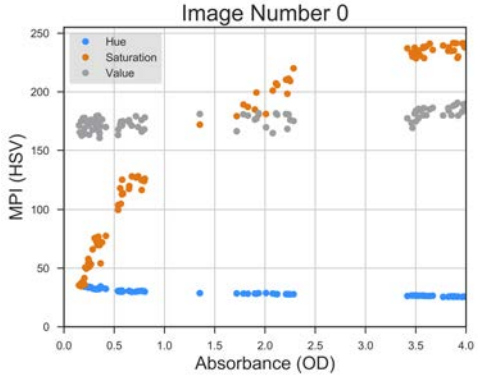
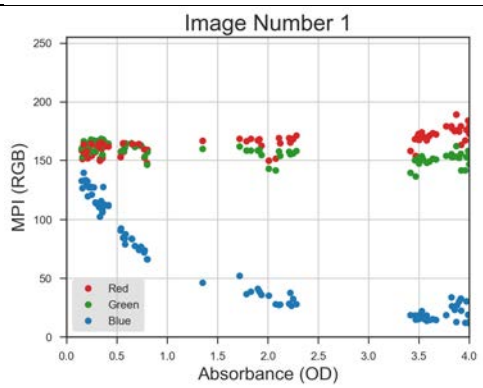
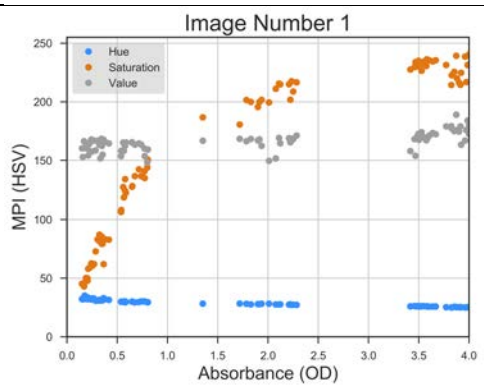
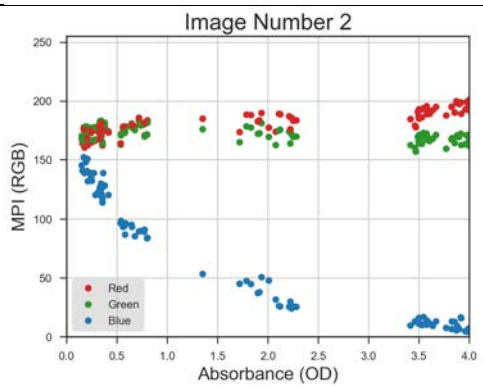
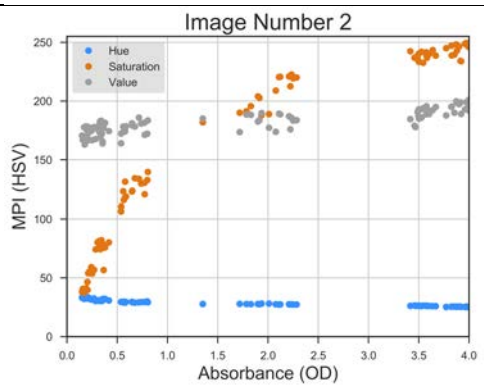
Dataset 1 for Supplementary Table 1: HRP conjugate concentrations

This dataset consists of 13 images of a serial dilution of HRP conjugate concentrations. All 96 wells were used, with each horizontal (8 well) series used for the same concentration. With the exception of image 11, all images were taken under fluorescent white light, without an image capture enclosure, against a white background at a constant angle. Image 11 was taken against a dark background. The distance to the plate was also kept approximately constant. Images were taken with the 12 MP camera of the iPhone 6s. For this dataset, absorbance values range from OD = 0 to OD = 4. There are a total of 1,248 sample region of interest (ROI) images of individual samples and 1,248 sets of six MPI values. The average ROI size is 30 pixels by 30 pixels, or 900 individual RGB values.

Table 1: Response of RGB, HSV MPIs to Optical Density in Range [0, 4]

Image	RGB MPIs vs. Absorbance	HSV MPIs vs. Absorbance
		
		
		

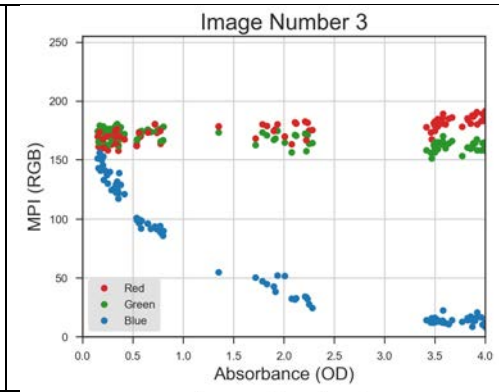
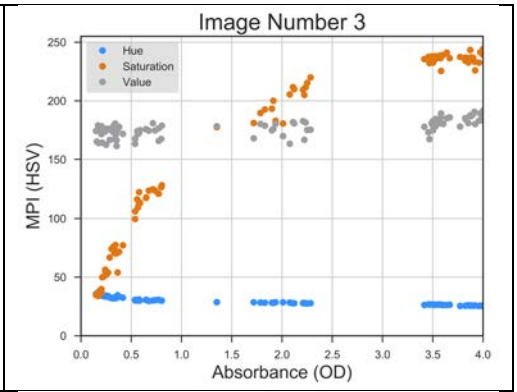


Image Number 3
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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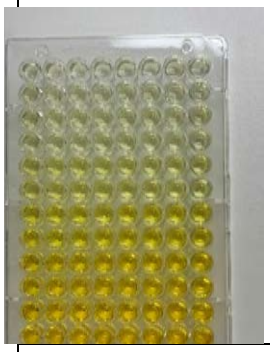
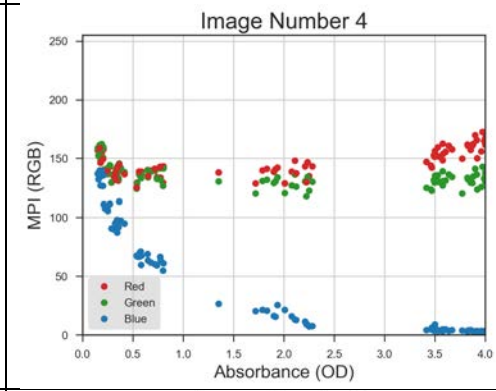
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

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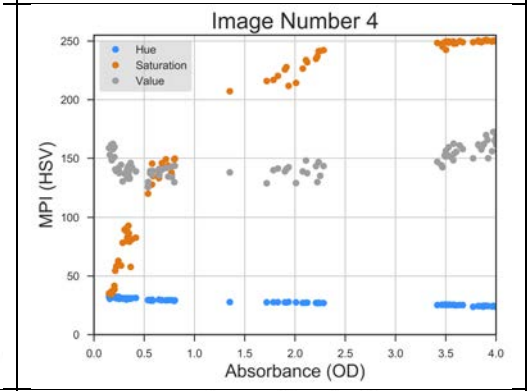
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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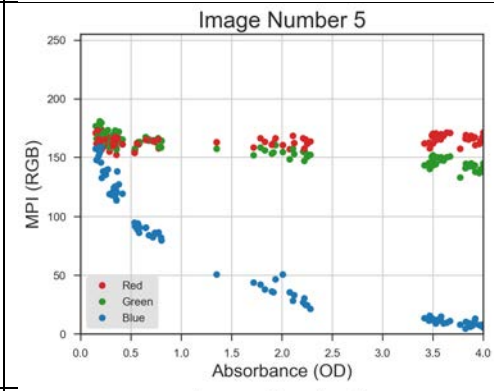
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value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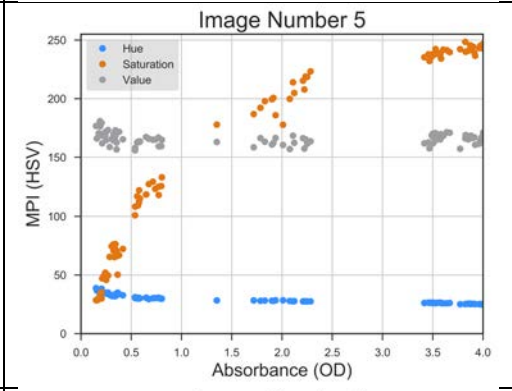
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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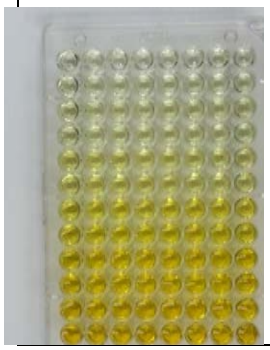
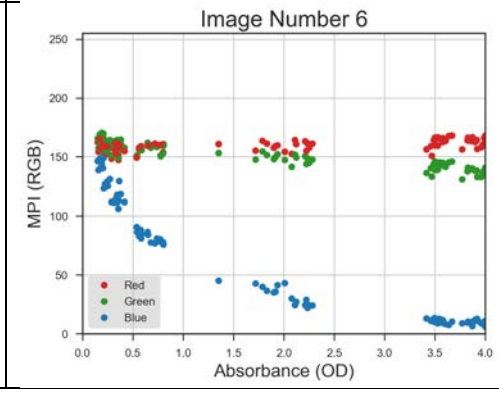
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

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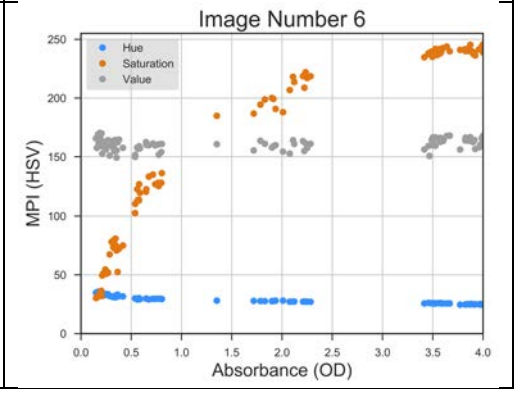
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

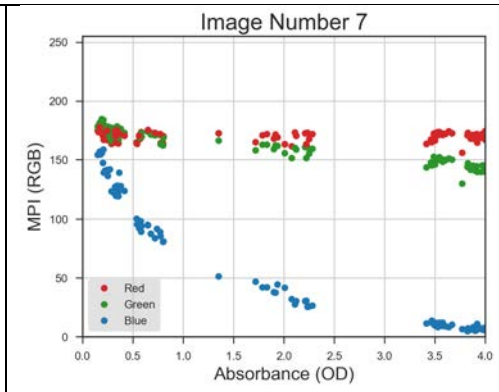
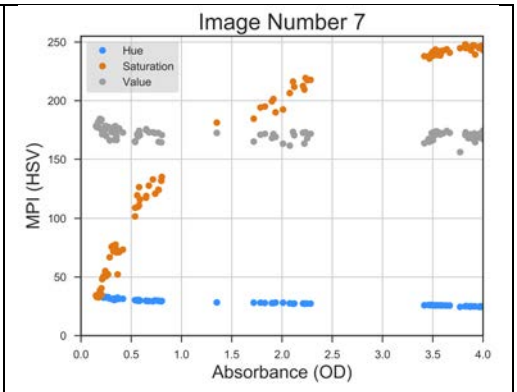


Image Number 7
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

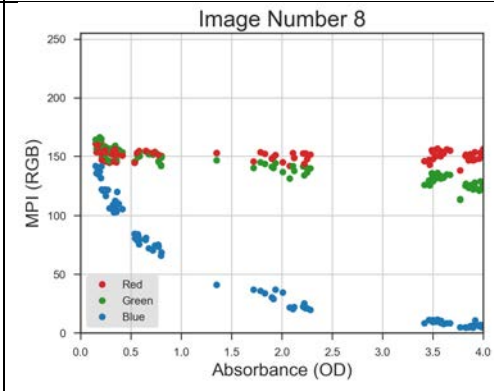
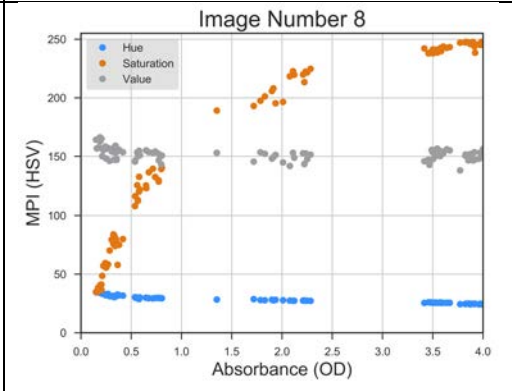


Image Number 8
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

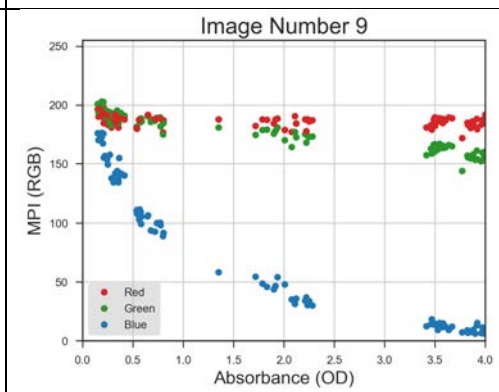
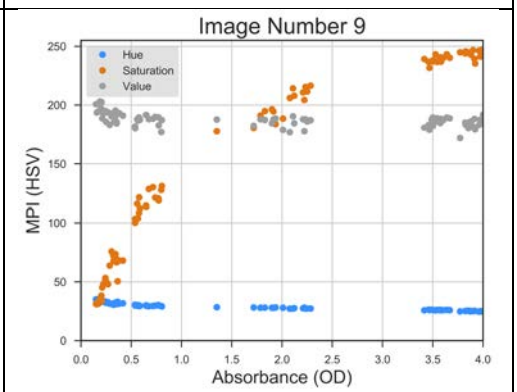


Image Number 9
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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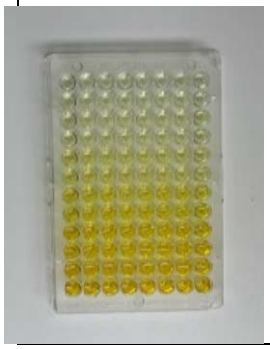
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



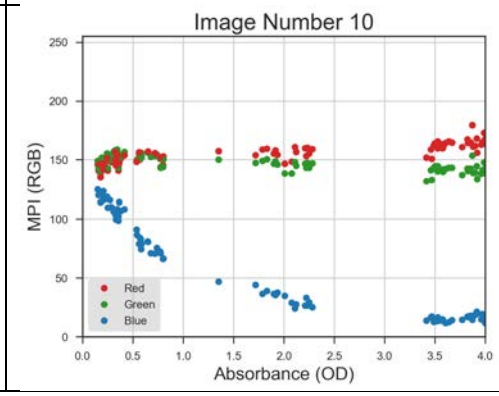
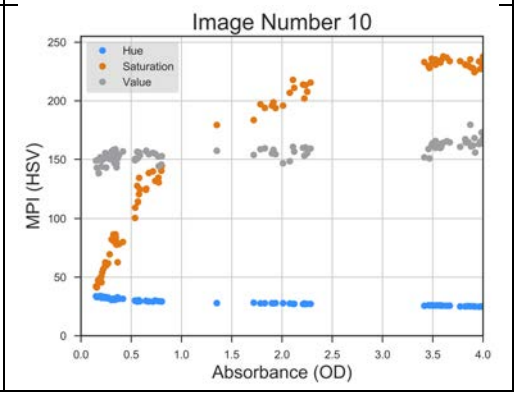


Image Number 10
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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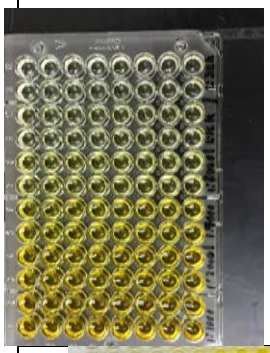
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



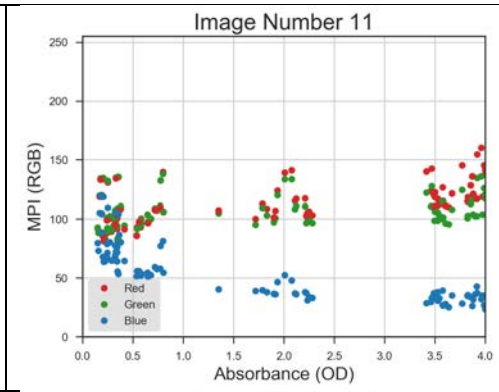


Image Number 11
MPI (RGB)
Absorbance (OD)
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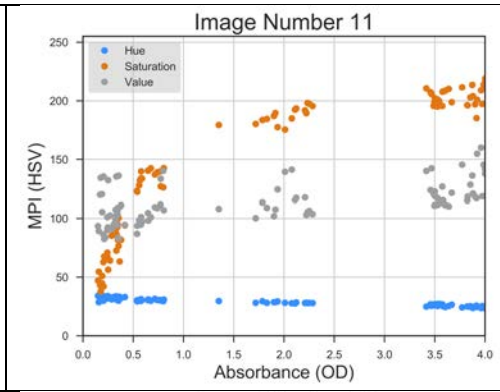
Green
Blue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MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

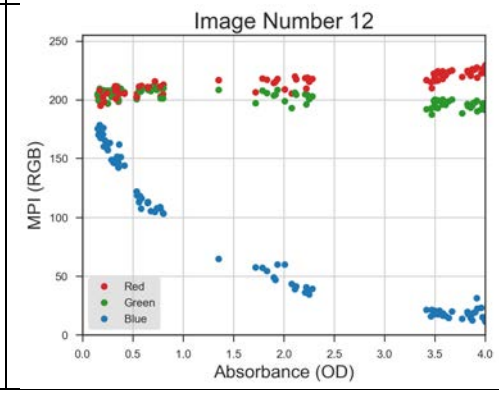


Image Number 12
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

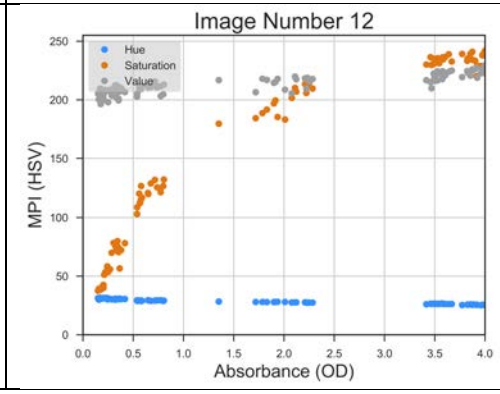
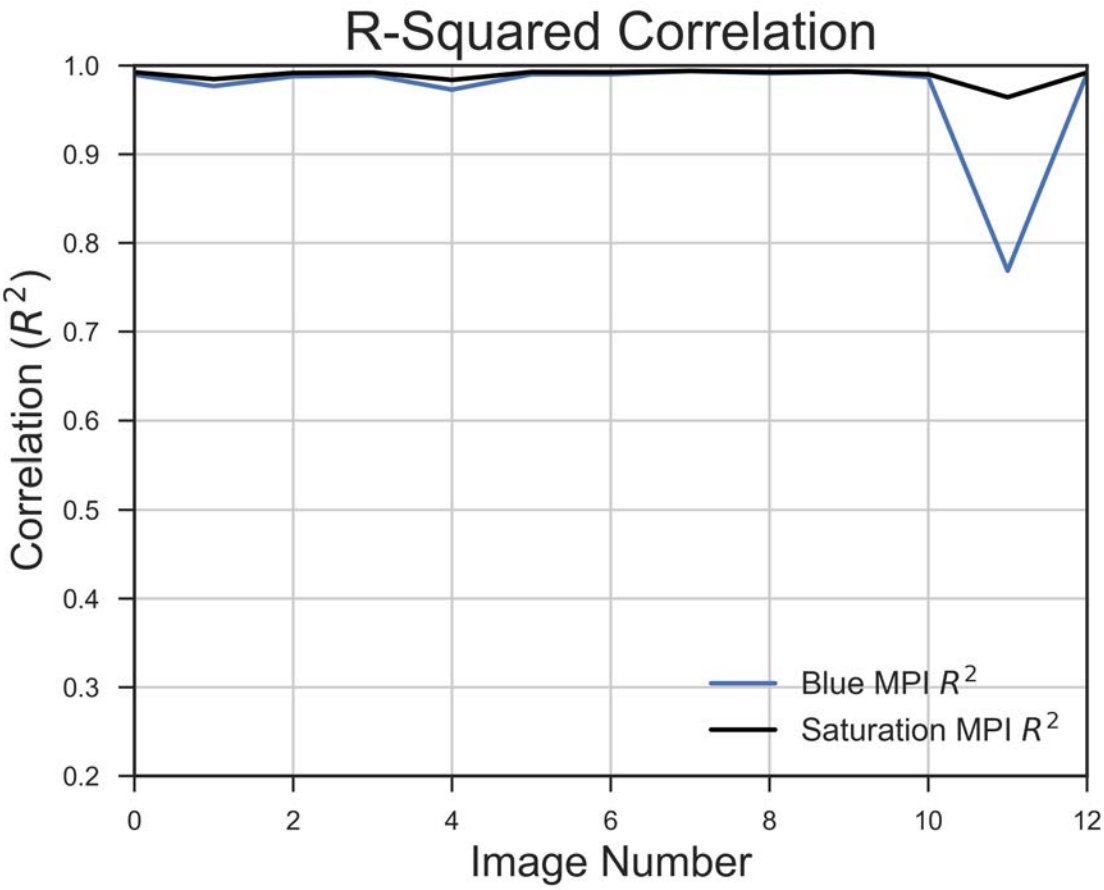


Image Number 12
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



Supplementary Figure 1: Logarithmic regression model correlation coefficient (R^2) for each image in Table 1. The values were obtained by fitting a logarithmic model for the MPI-absorbance relationship. The entire set of MPI data was used.

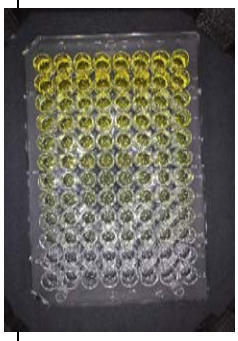
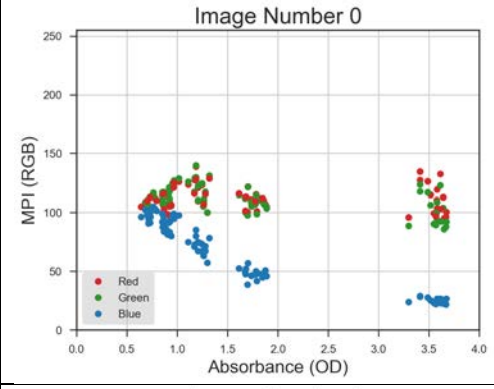
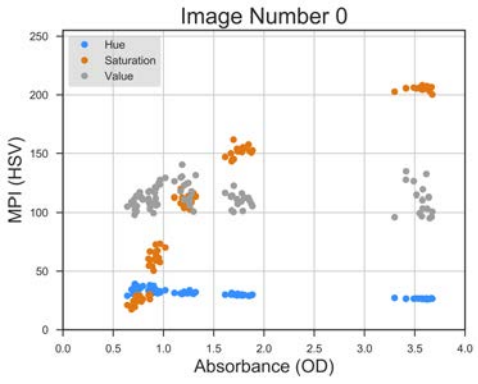
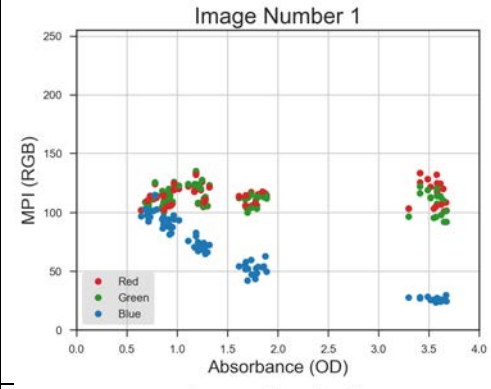
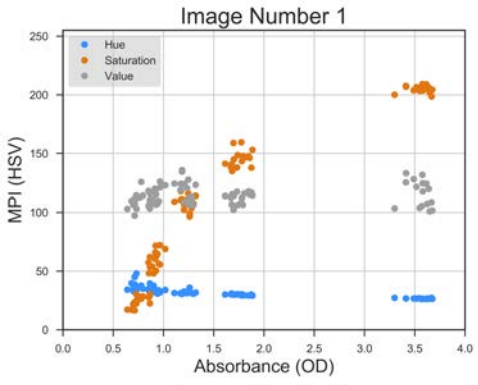
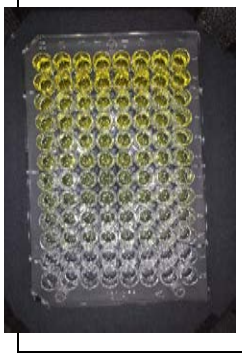
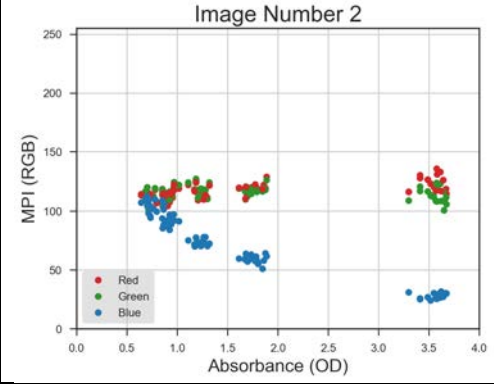
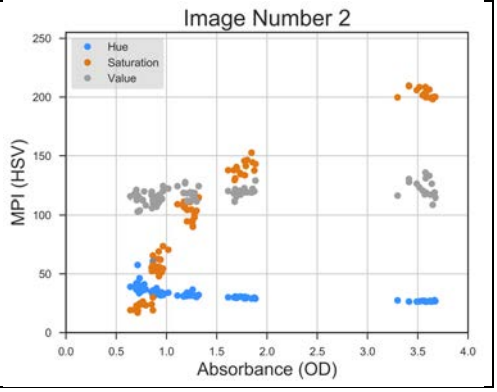
From the scatterplots for each image, we calculated the correlation coefficient of a logarithmic line of best fit. These values are plotted for each of the images in Table 1, in Supplementary Figure 1.

Dataset 2: Dilution Curve

Dataset 2-A for Table 2: iPhone Images

This dataset consists of 42 images taken with the 12 MP camera of the iPhone 6s. All 96 wells were used, for a total of 4,032 ROI images and MPI sets. In this dataset, images were taken under a wide range of image capture conditions, with variable light level, background, and distance to the sample. Absorbance values ranged from OD = 0 to OD = 3.5, but the highest cluster of values near OD = 3.5 was outside the linear response range for MPI values and was discarded for the regression analysis.

Table 2: iPhone HSV and RGB Response Within Linear Range

Image	RGB MPIs vs. Absorbance	HSV MPIs vs. Absorbance
		
		
		

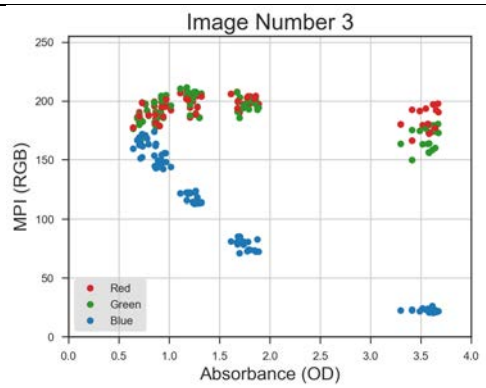


Image Number 3
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

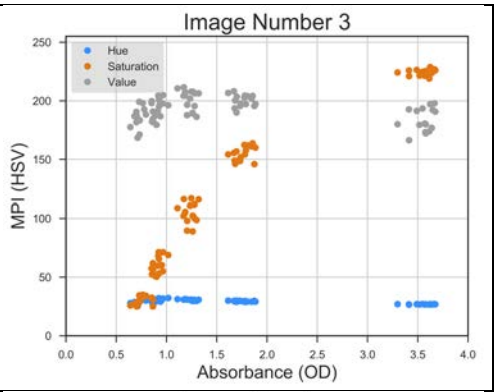


Image Number 3
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

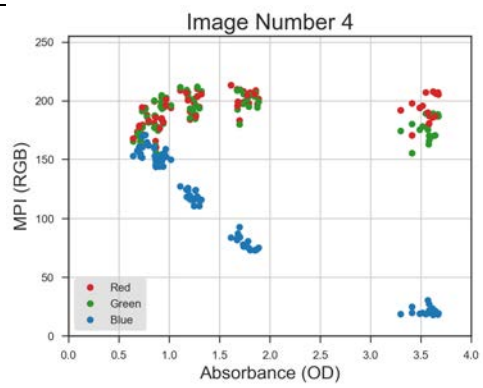


Image Number 4
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

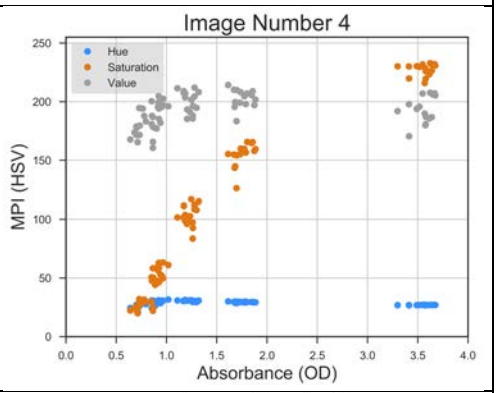


Image Number 4
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

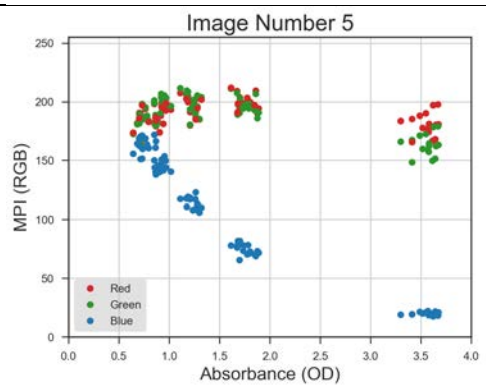


Image Number 5
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

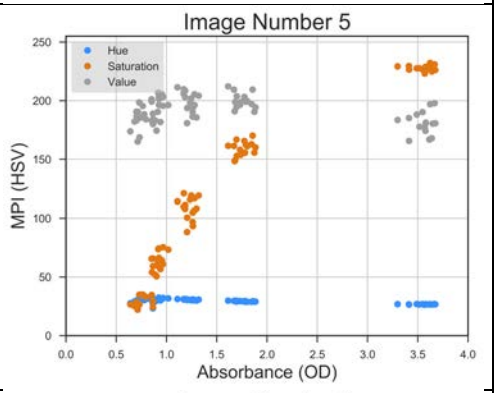


Image Number 5
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

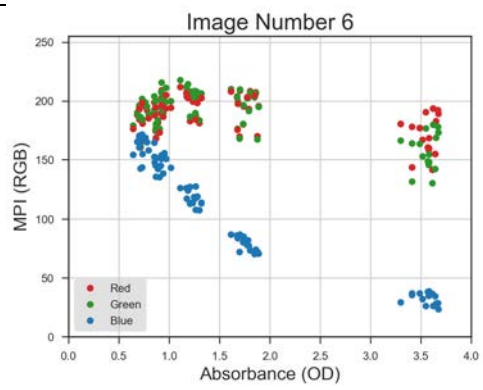


Image Number 6
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

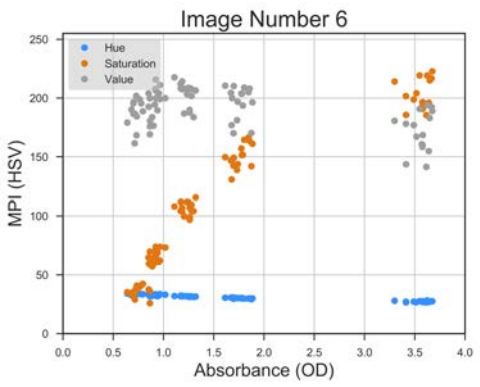


Image Number 6
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

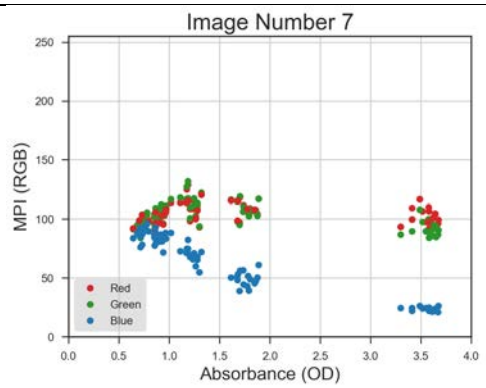


Image Number 7
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

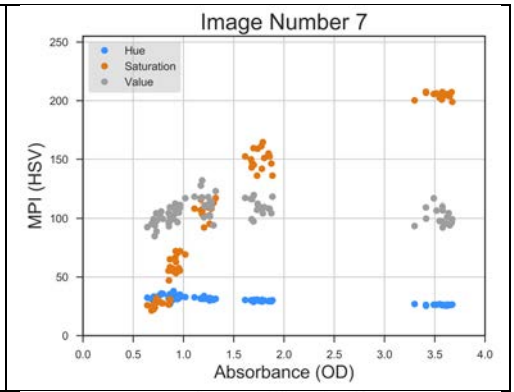


Image Number 7
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

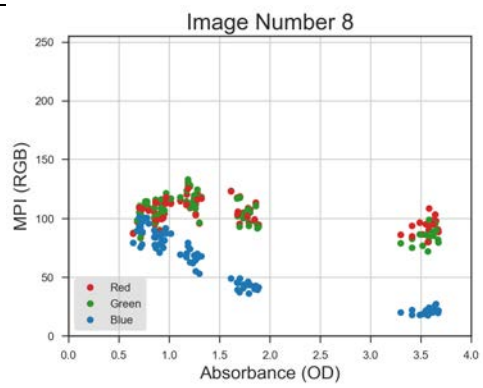


Image Number 8
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

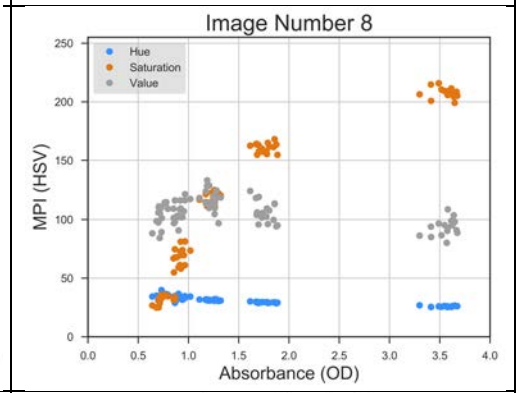
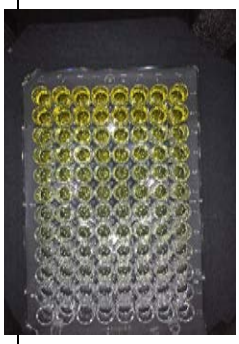


Image Number 8
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



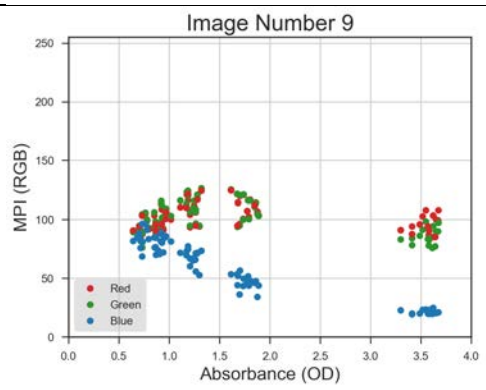


Image Number 9
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

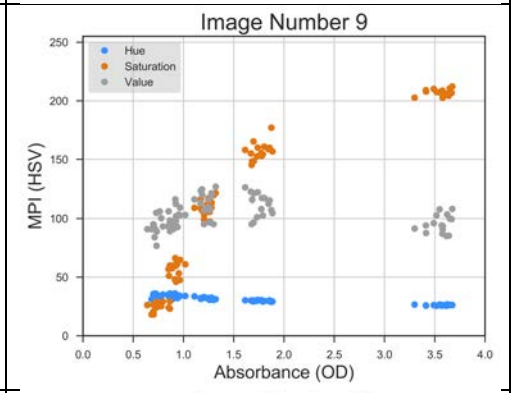


Image Number 9
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

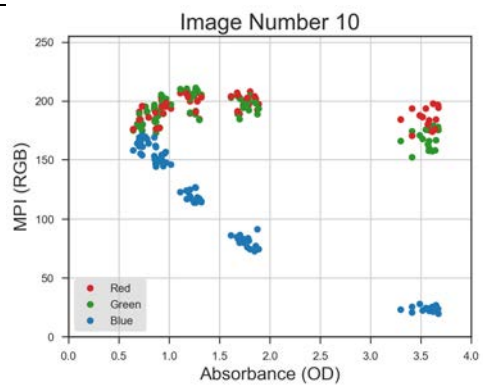


Image Number 10
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

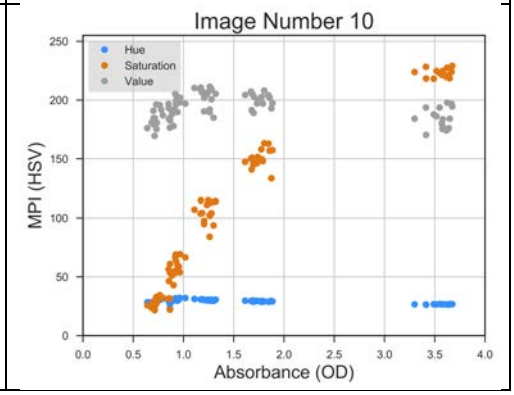


Image Number 10
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

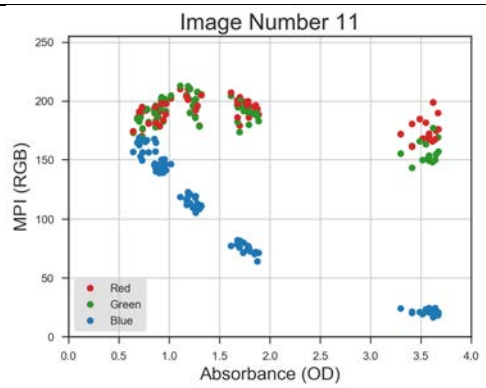


Image Number 11
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

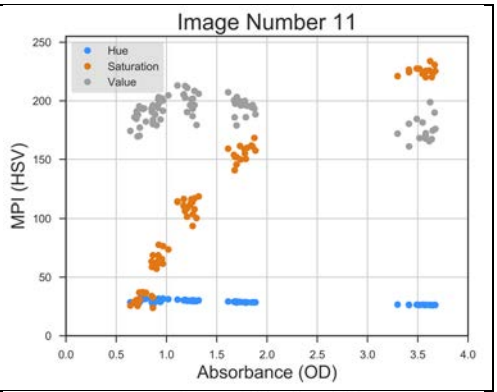


Image Number 11
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

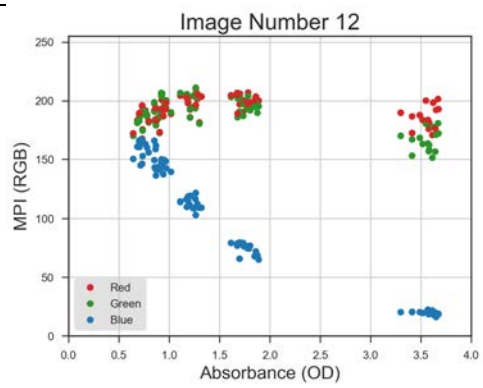
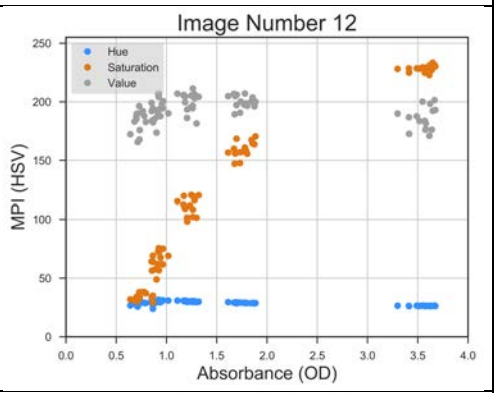


Image Number 12
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

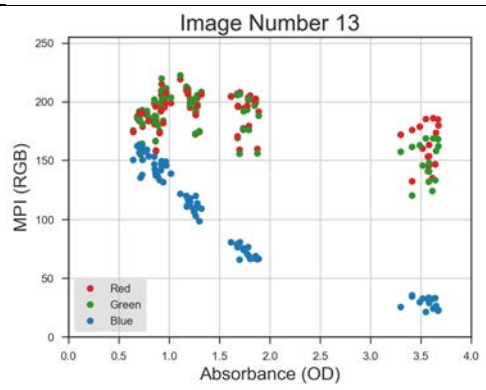
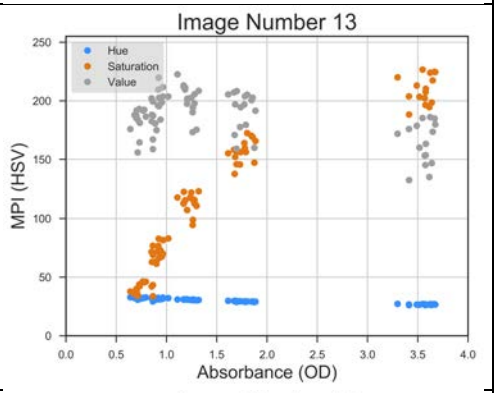


Image Number 13
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

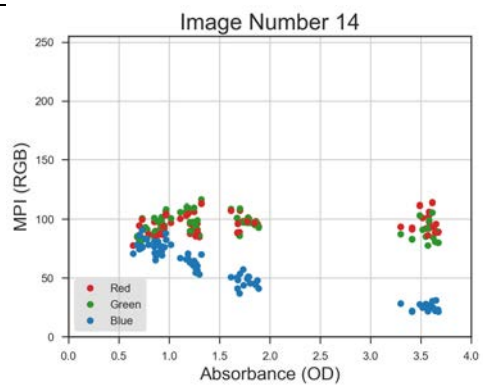


Image Number 14
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

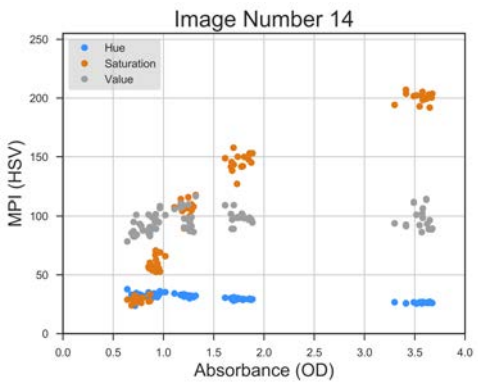
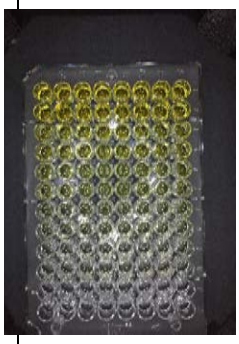


Image Number 14
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



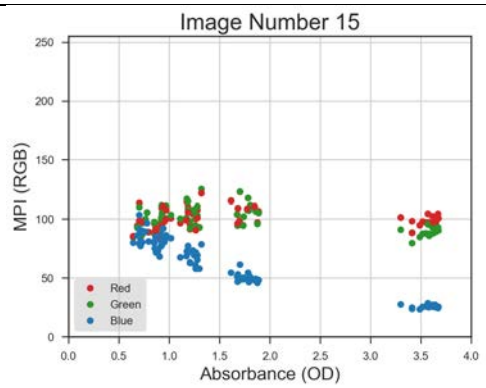
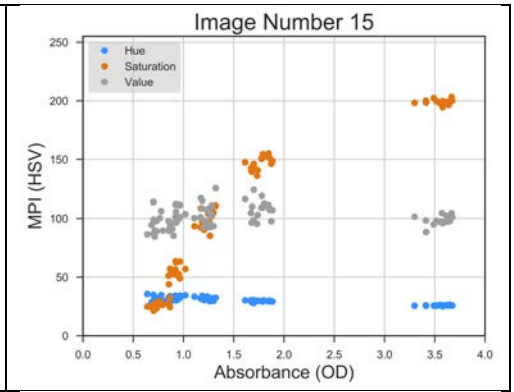


Image Number 15
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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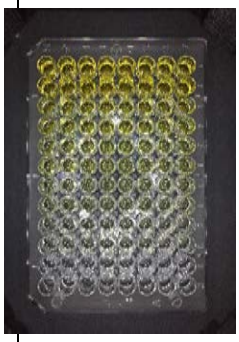
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



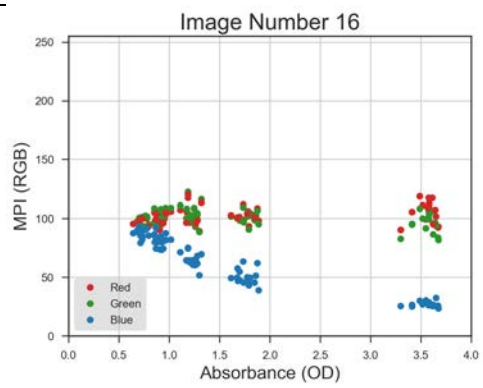


Image Number 16
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

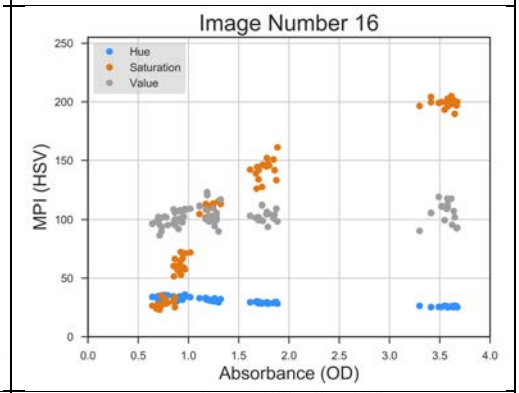


Image Number 16
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

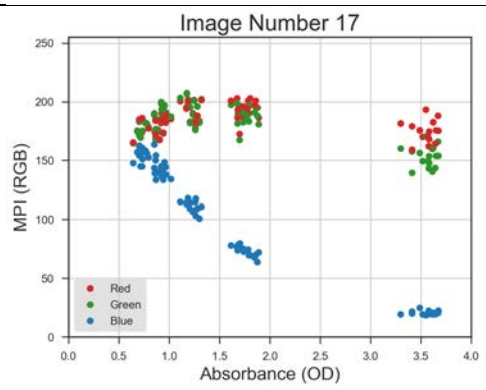


Image Number 17
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

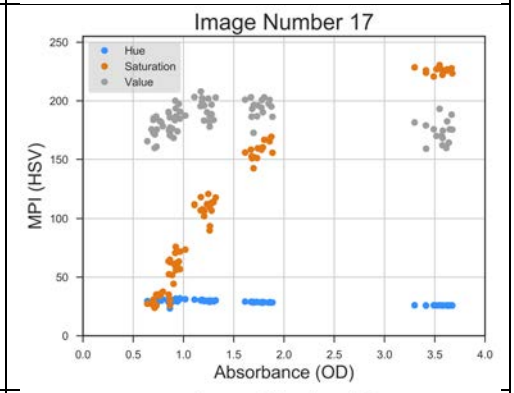


Image Number 17
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

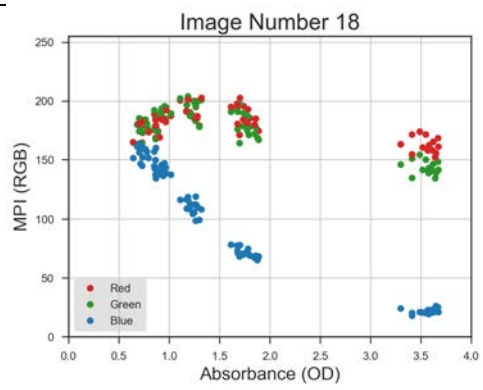


Image Number 18
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

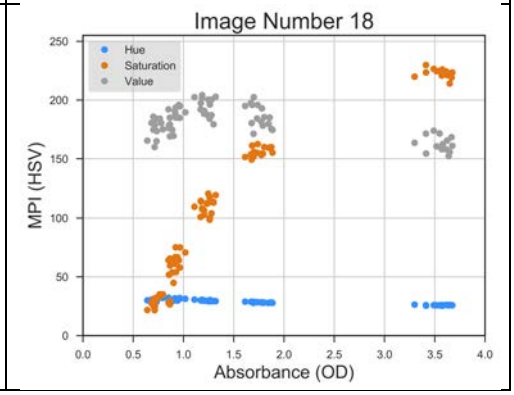


Image Number 18
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

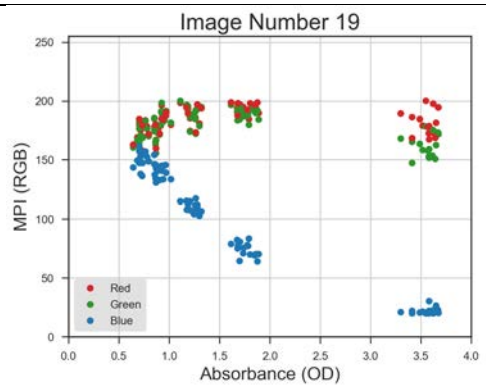


Image Number 19
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

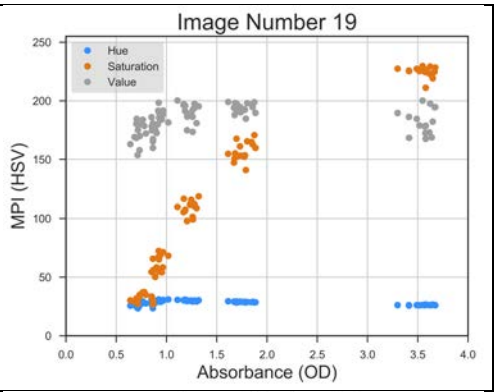


Image Number 19
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

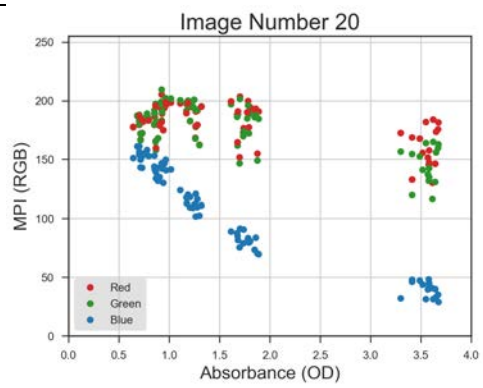


Image Number 20
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

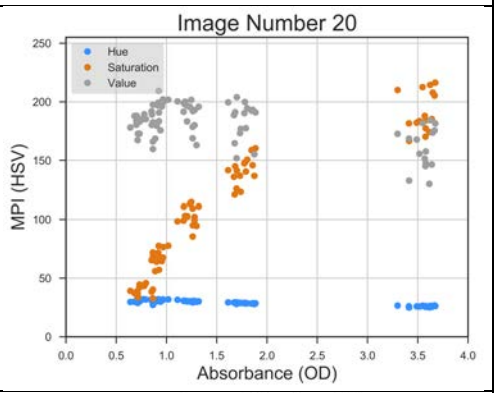


Image Number 20
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

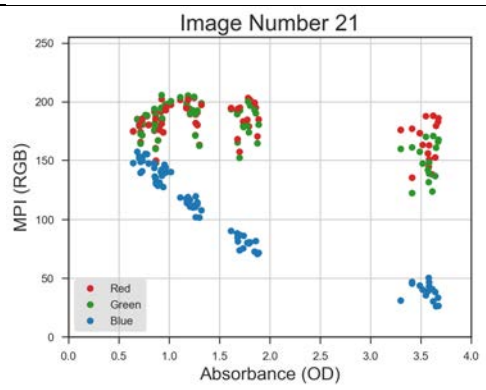


Image Number 21
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

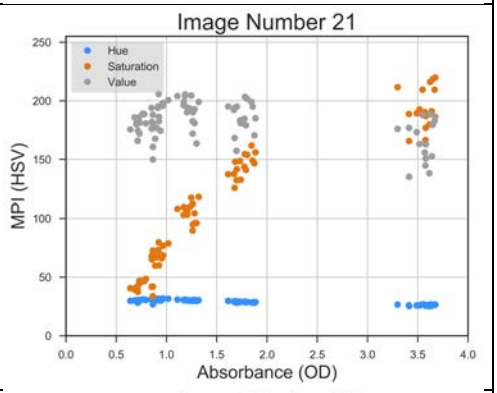
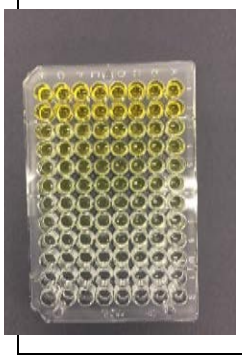


Image Number 21
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



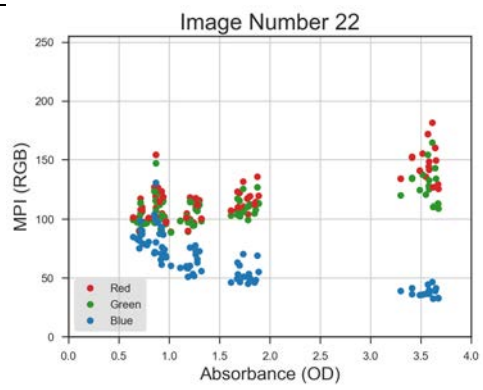


Image Number 22
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

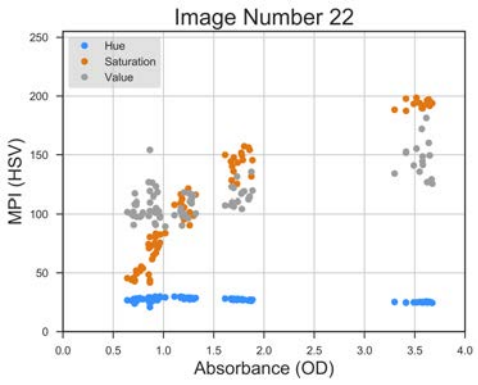
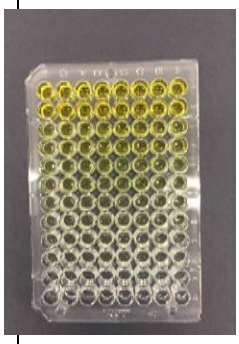


Image Number 22
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



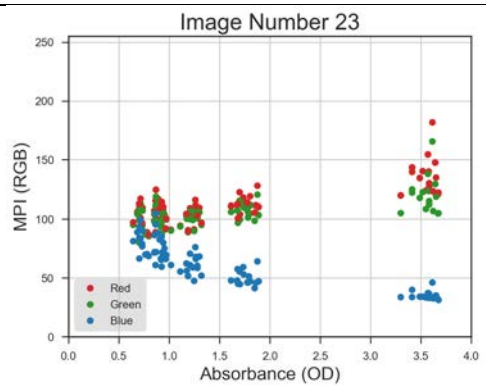


Image Number 23
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

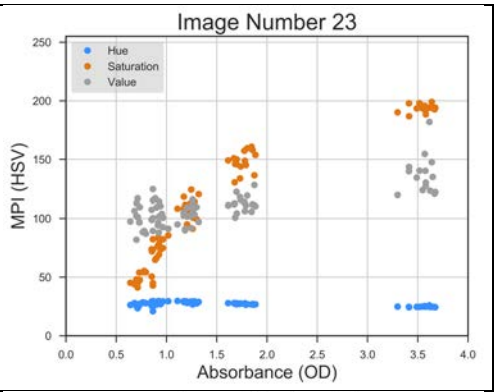
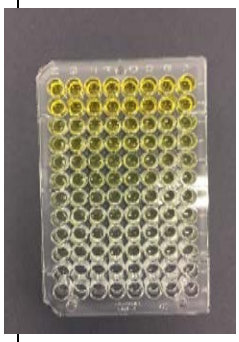


Image Number 23
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



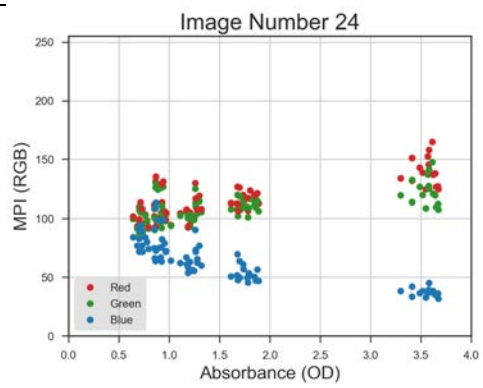


Image Number 24
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

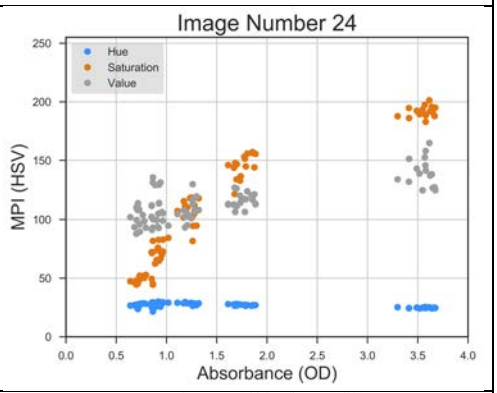
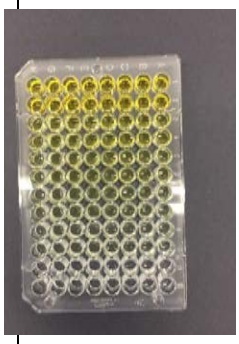


Image Number 24
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



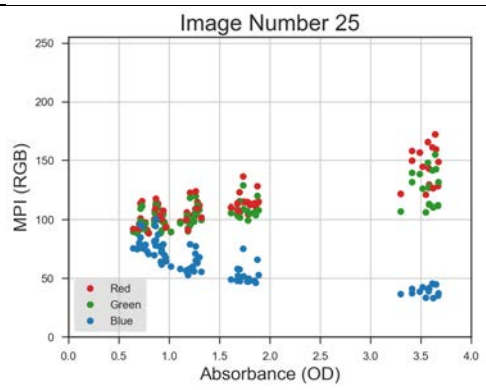


Image Number 25
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

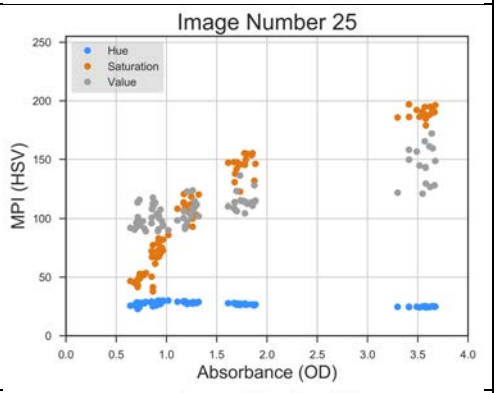
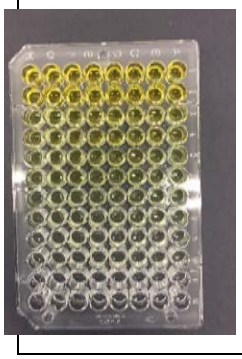


Image Number 25
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



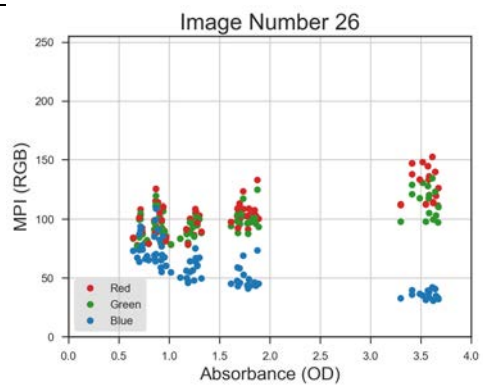


Image Number 26
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

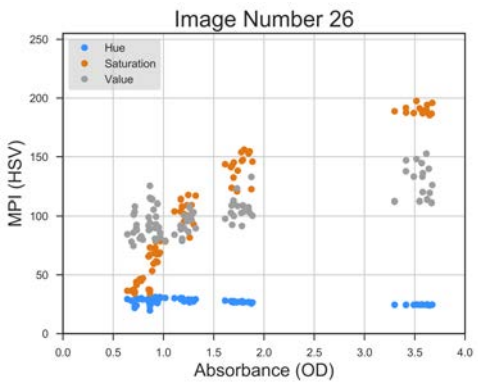
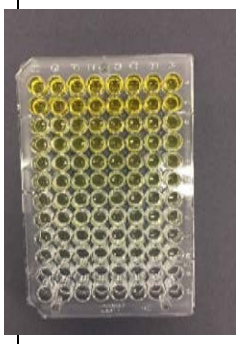


Image Number 26
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



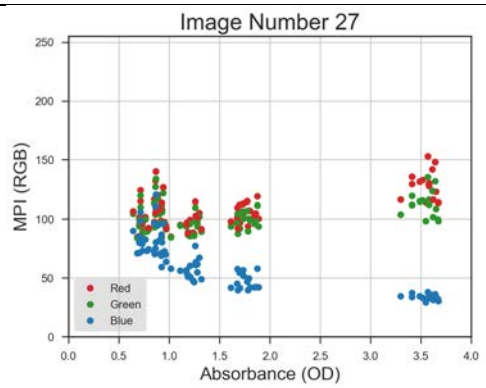


Image Number 27
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

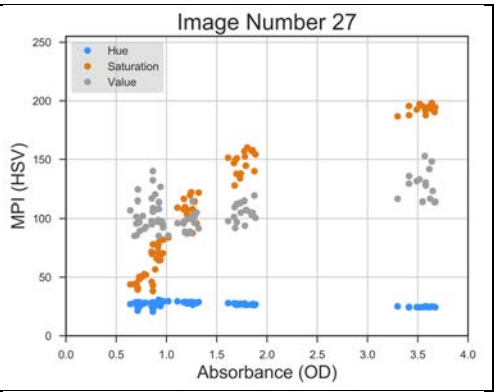
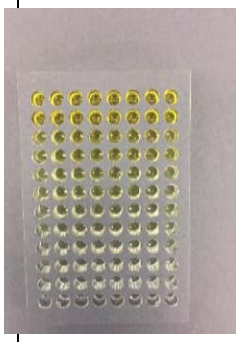


Image Number 27
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



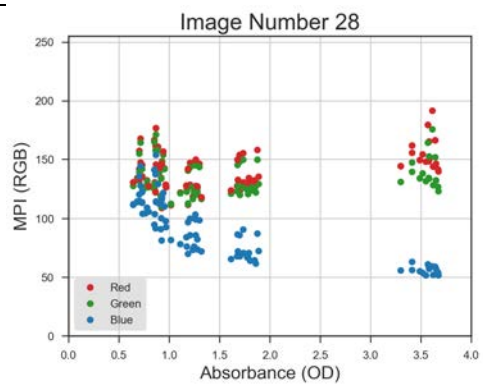


Image Number 28
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

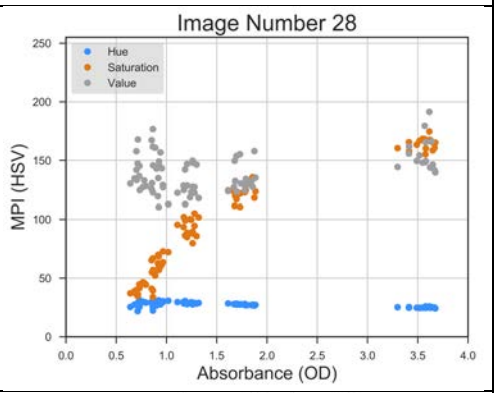
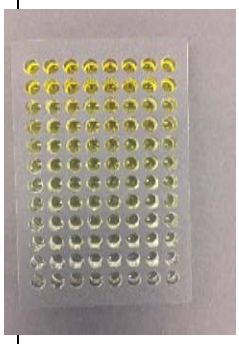


Image Number 28
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



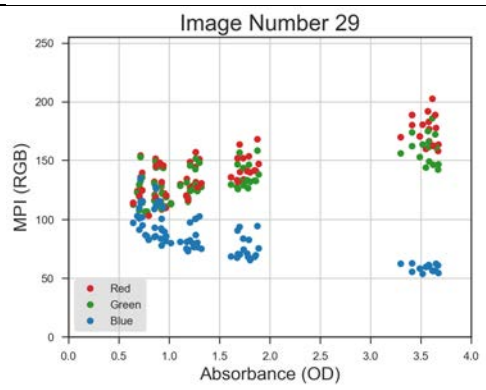


Image Number 29
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

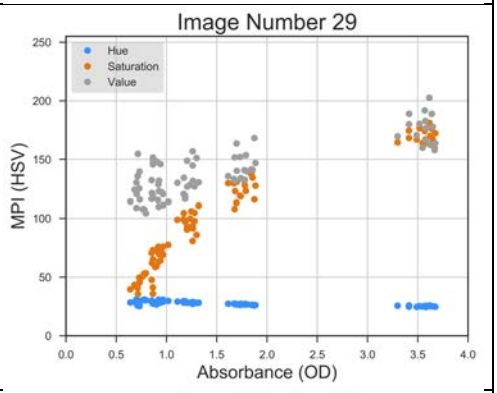
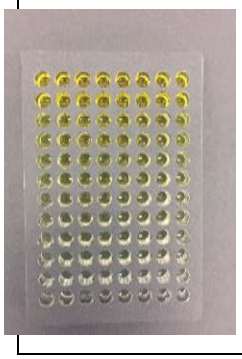


Image Number 29
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



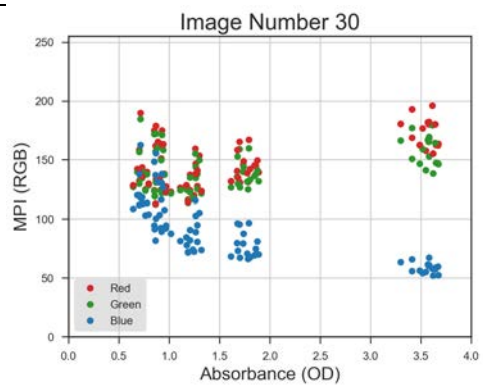


Image Number 30
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

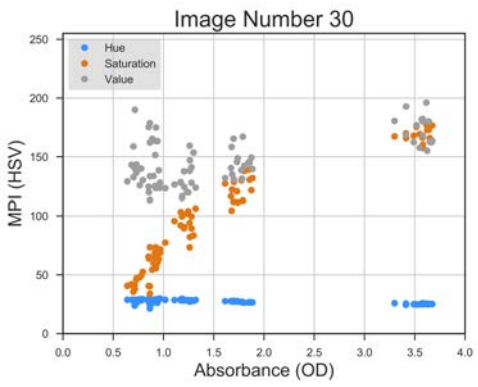


Image Number 30
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

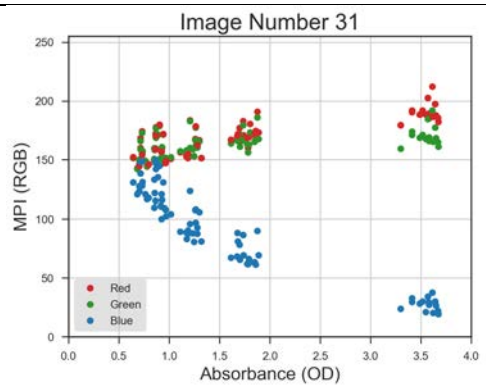


Image Number 31
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

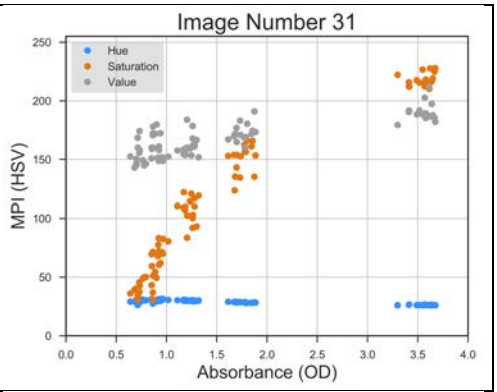
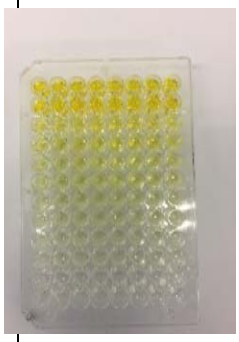


Image Number 31
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



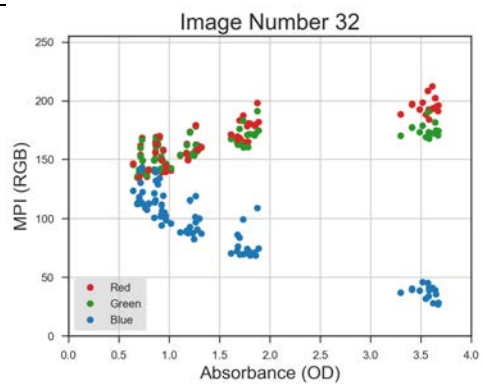


Image Number 32
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

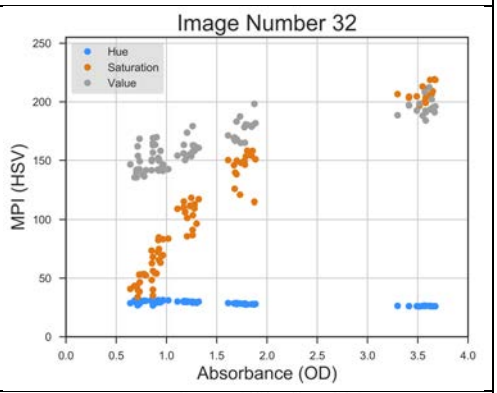
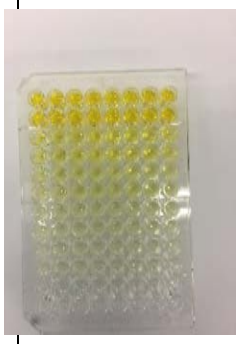


Image Number 32
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



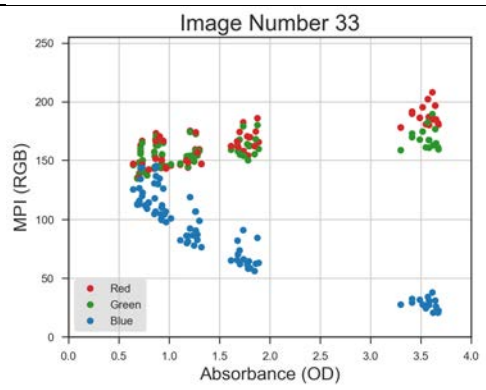


Image Number 33
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

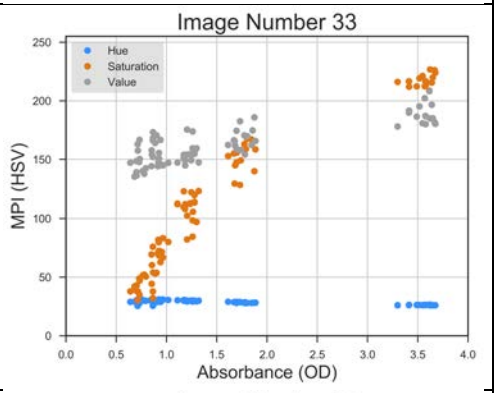


Image Number 33
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

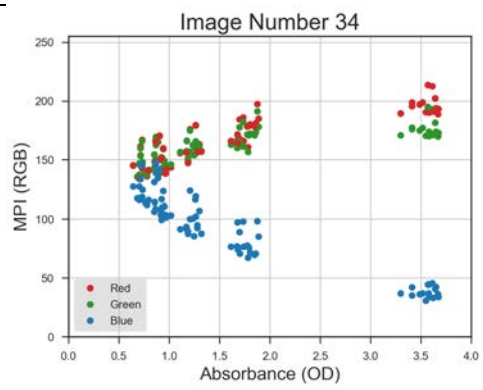


Image Number 34
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

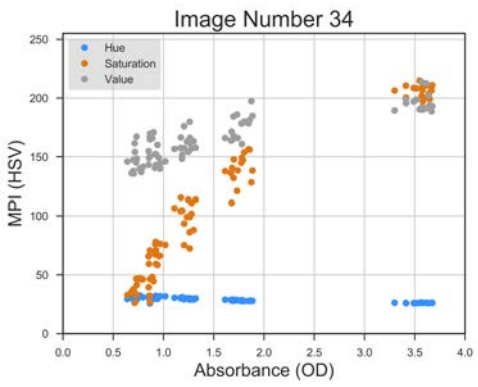
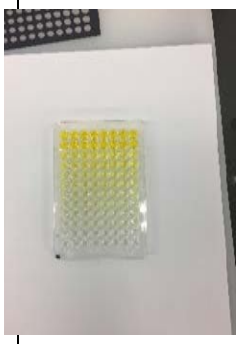


Image Number 34
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



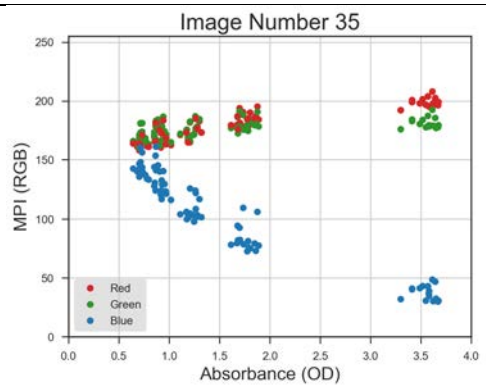


Image Number 35
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

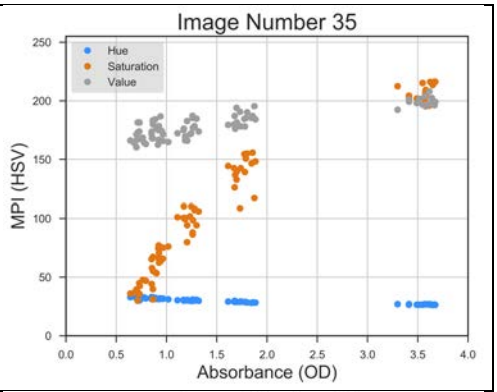


Image Number 35
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

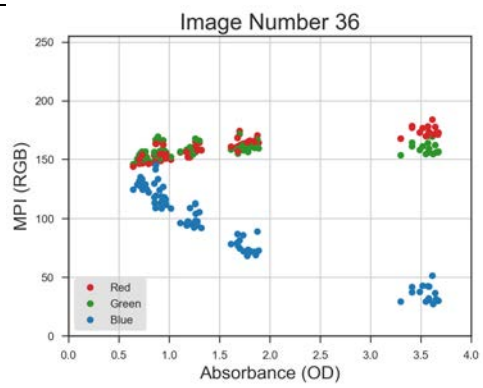


Image Number 36
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

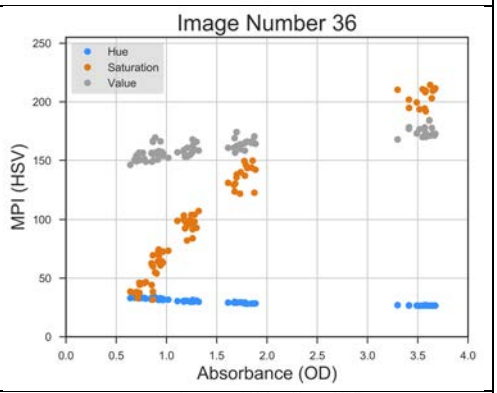


Image Number 36
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

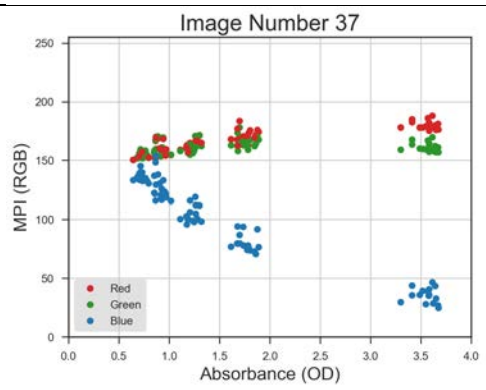


Image Number 37
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

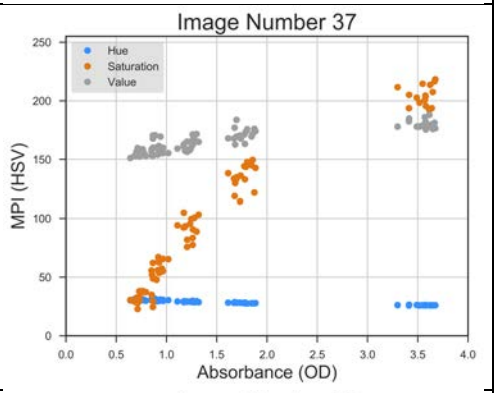
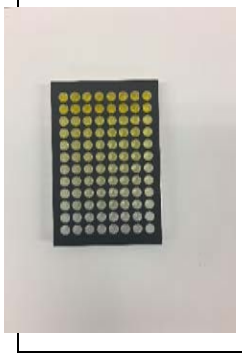


Image Number 37
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



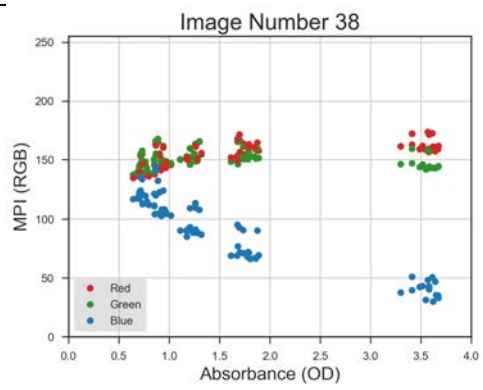


Image Number 38
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

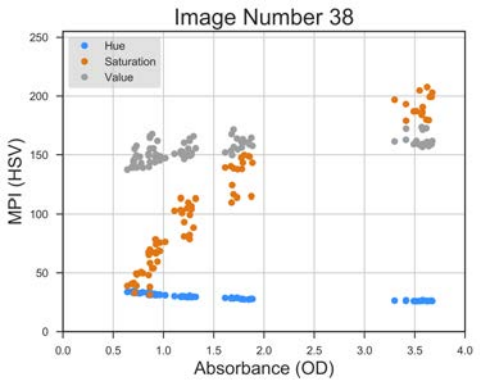
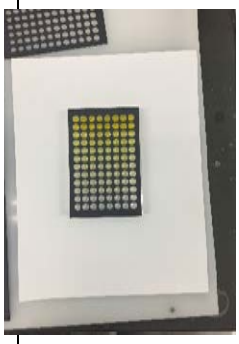


Image Number 38
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



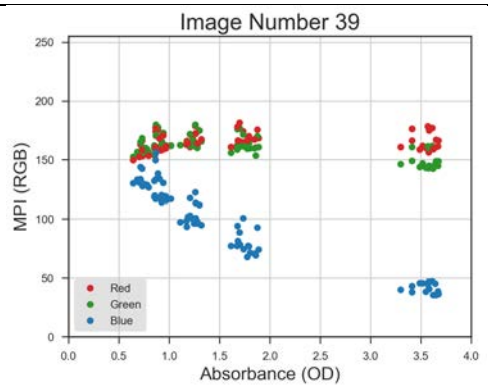


Image Number 39
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

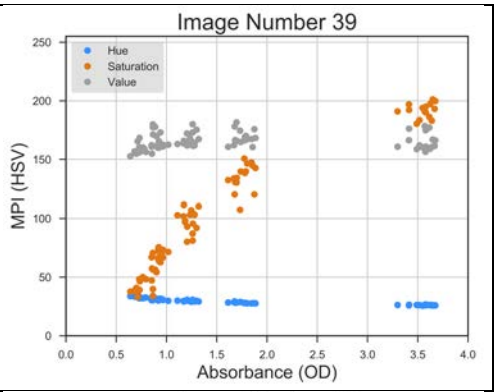
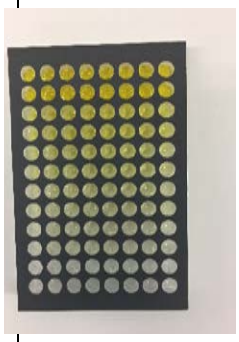


Image Number 39
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



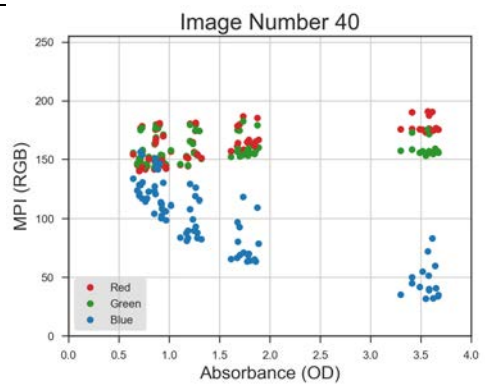


Image Number 40
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

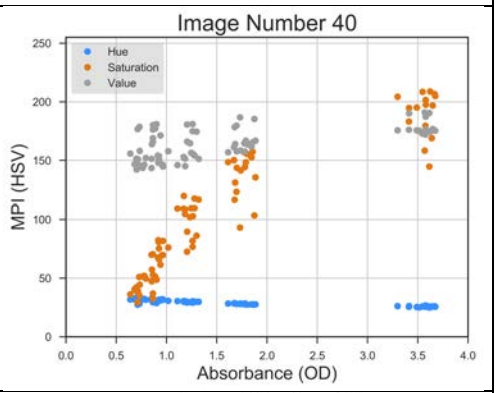


Image Number 40
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

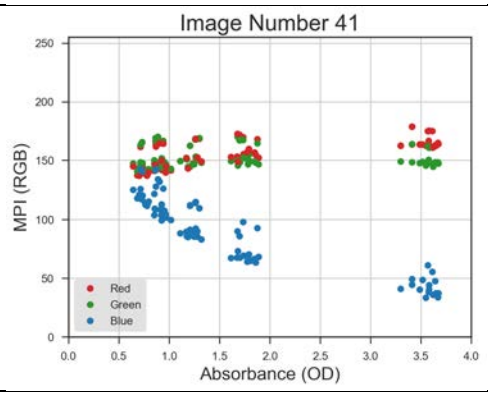


Image Number 41
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

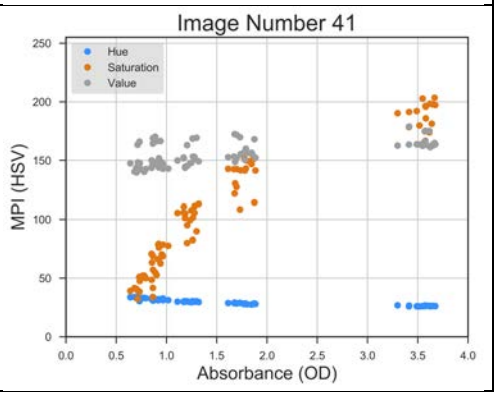
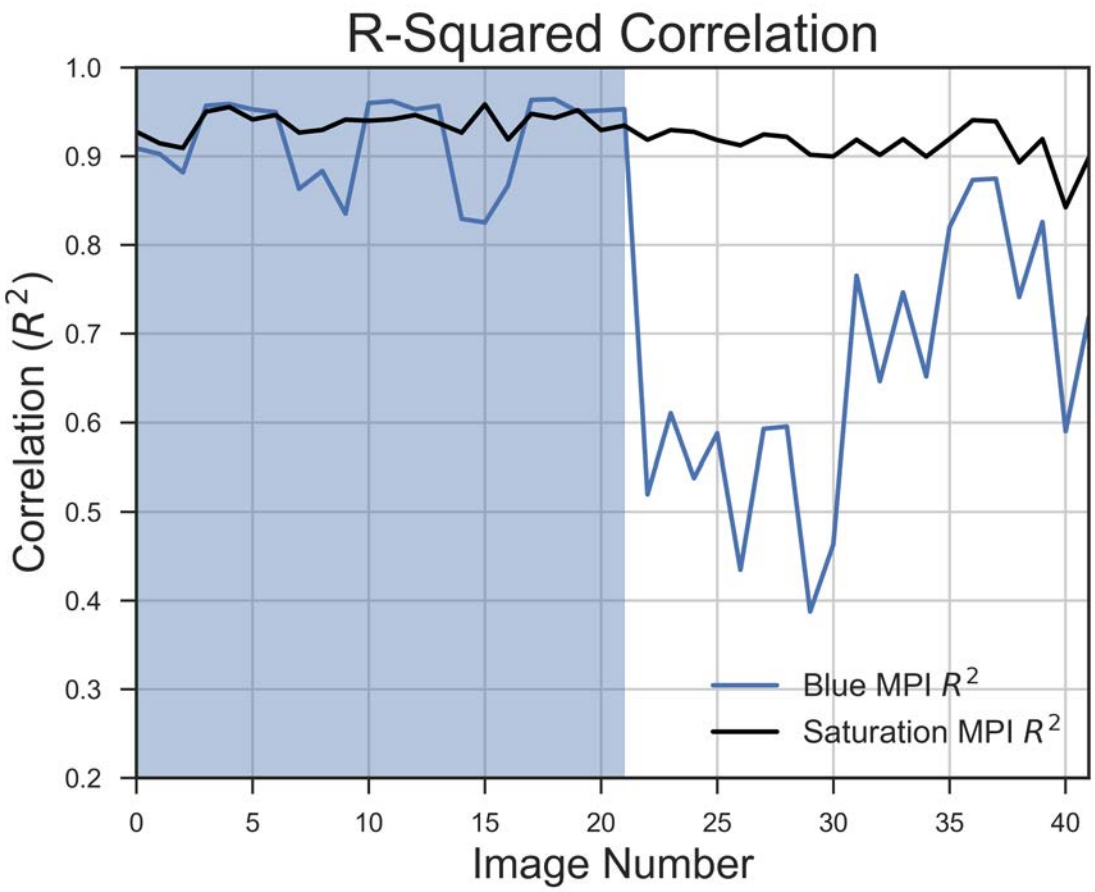
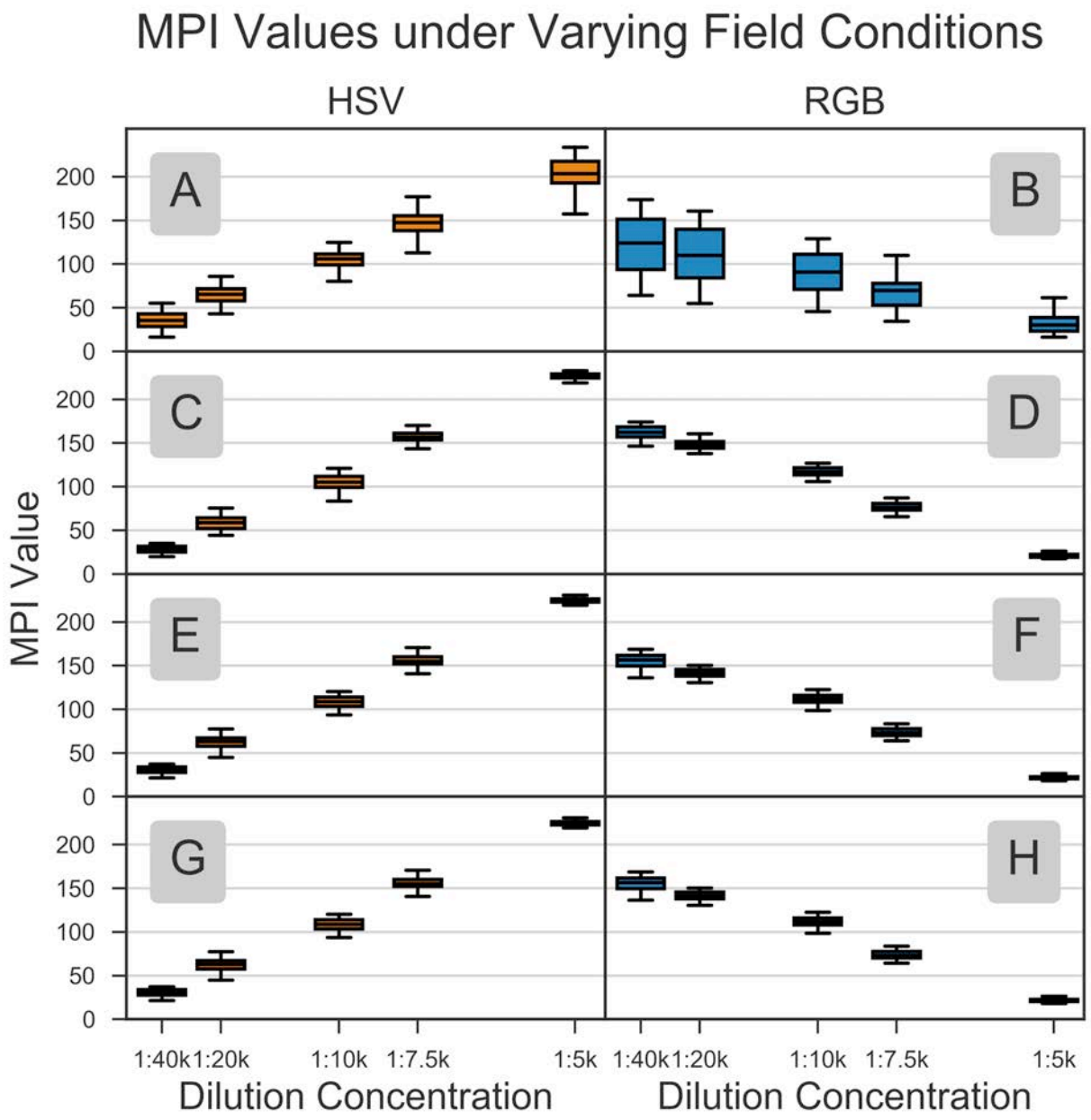


Image Number 41
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



Supplementary Figure 2: Linear regression model, R^2 for each image in dataset 2A for 12 MP camera. Only values within the linear response range were used for the model. Shaded region indicates use of capture equipment

In addition to the boxplot containing all the data, with wide variation in image capture conditions; we also considered subsets of the data.

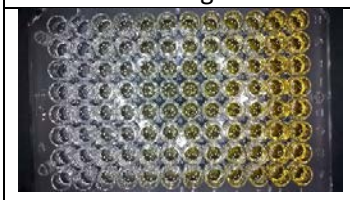
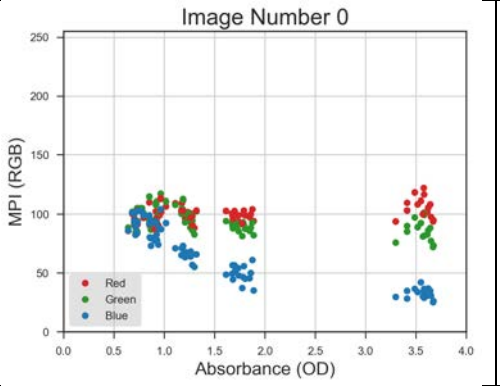
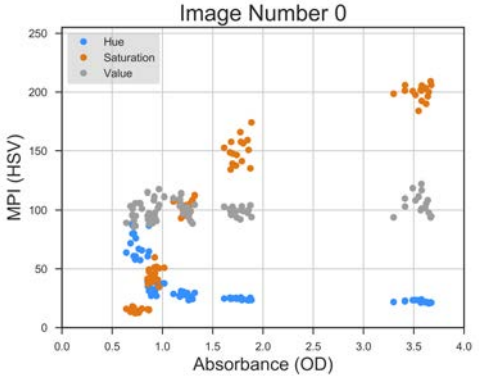
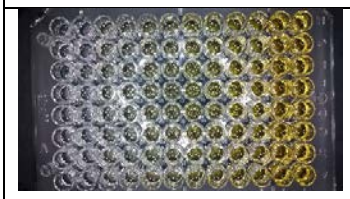
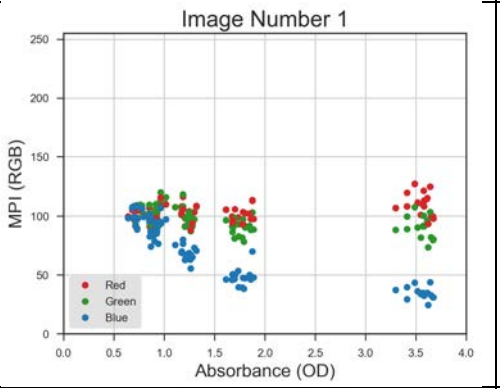
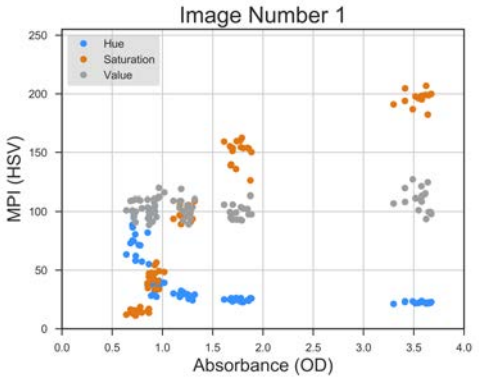
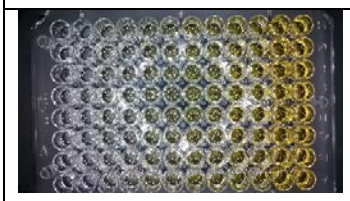
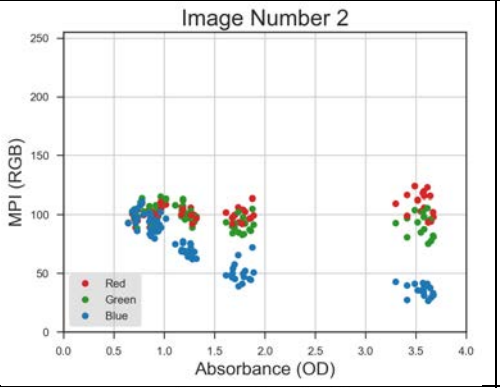
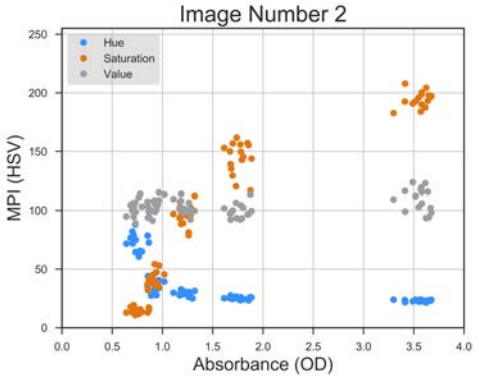
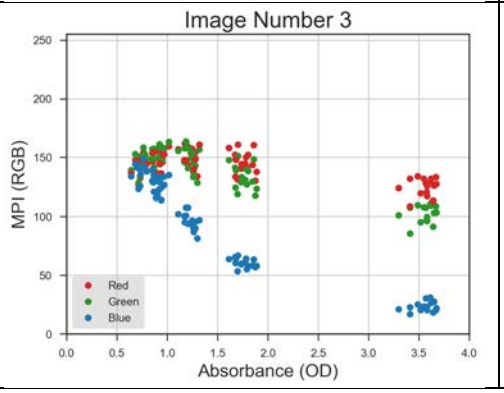
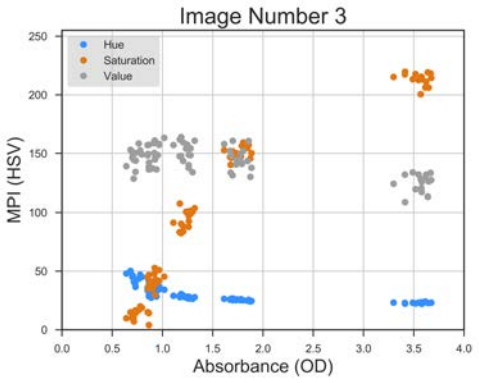


Supplementary Figure 3: Boxplots for subsets of MPI data in dataset 2A. All saturation MPI values (A), all blue MPI values (B), saturation values for bright lighting (C), blue values for bright lighting (D), saturation values for dark lighting (E), blue values for dark lighting (F), saturation values without capture equipment (G), blue values without capture equipment (H). For (A) and (B), N = 336. For (C), (D), (E), and (F), N = 24. For (G) and (H), N = 56

Dataset 2-B for Table 3: Android Images

This dataset consists of 46 images taken with the 5 MP camera of the Android Moto G. All 96 wells were used, and image capture conditions were the same as those from Dataset 2-A. The only difference between Dataset 2-A and Dataset 2-B is the resolution of the camera used to obtain the images.

Table 3: Android HSV and RGB Response Within Linear Range

Image	RGB MPIs vs. Absorbance	HSV MPIs vs. Absorbance
		
		
		
		

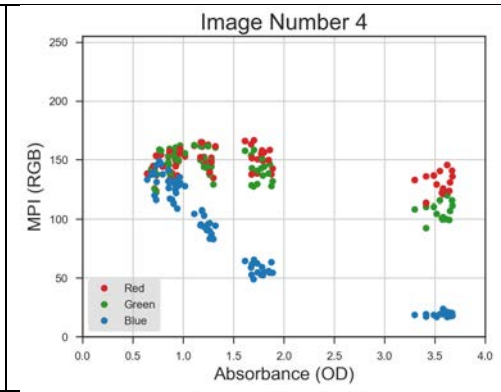


Image Number 4
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

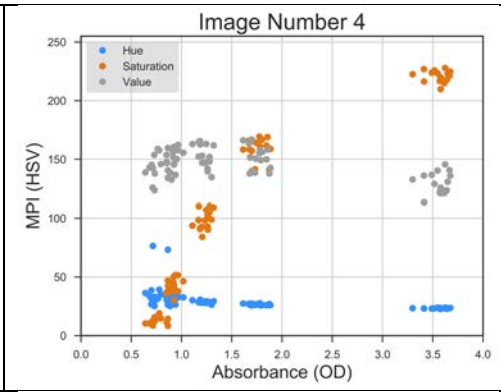


Image Number 4
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

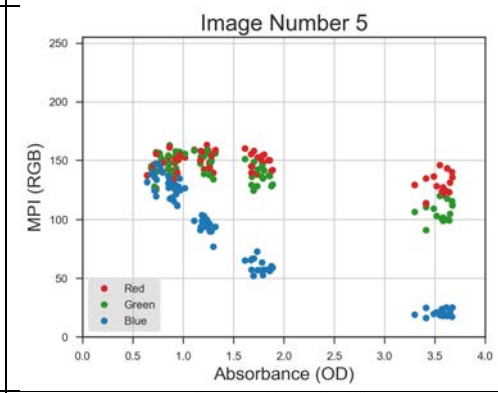


Image Number 5
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

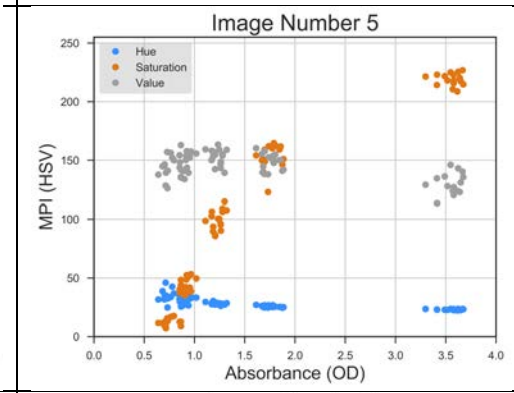
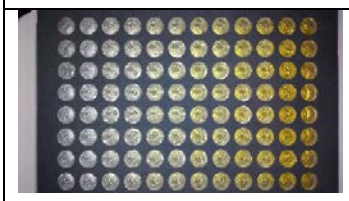


Image Number 5
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



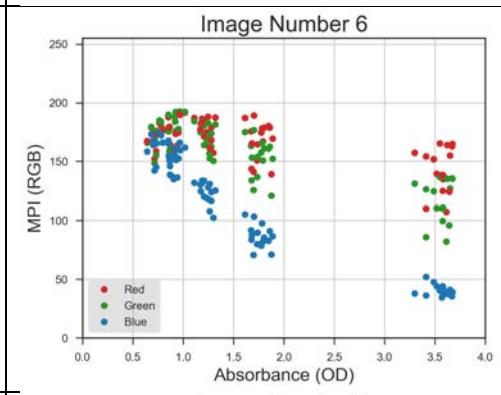


Image Number 6
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

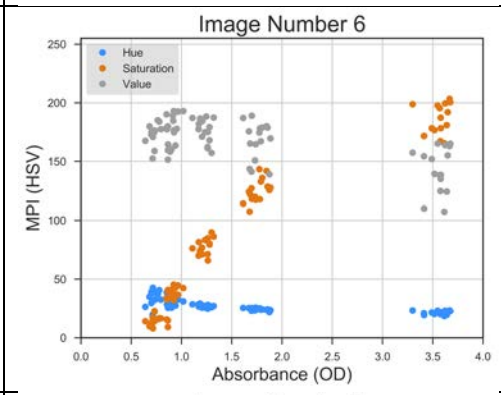
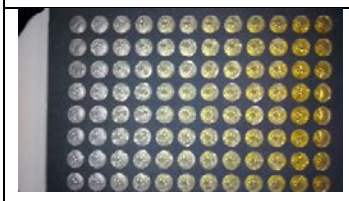


Image Number 6
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



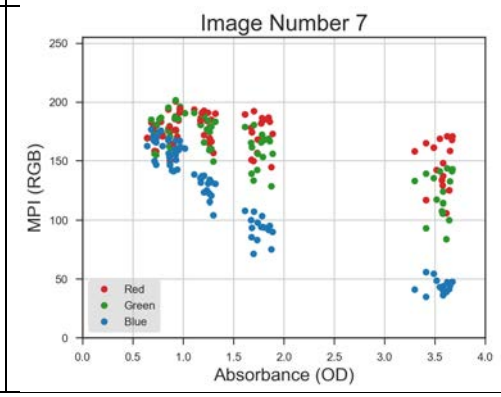


Image Number 7
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

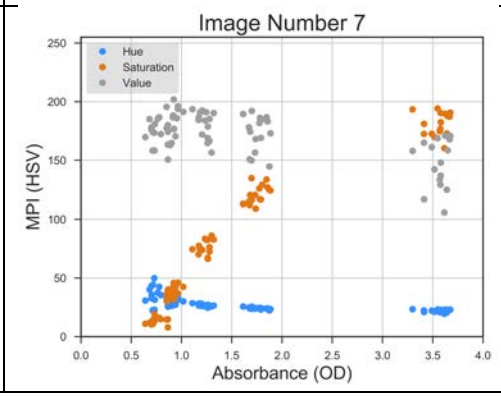
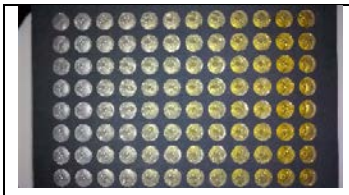


Image Number 7
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



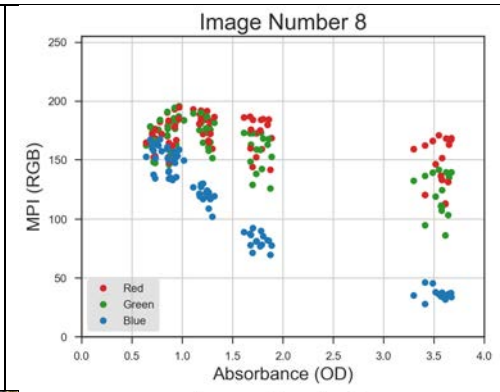


Image Number 8
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

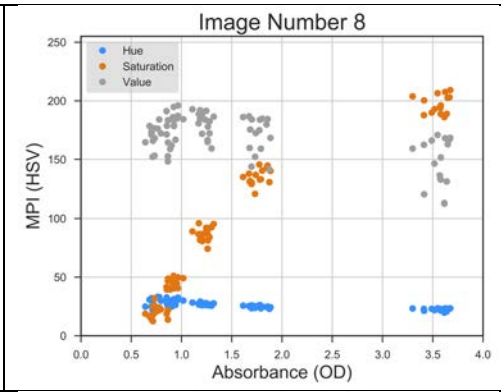
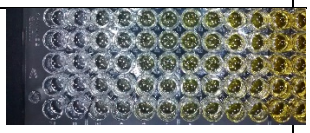


Image Number 8
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



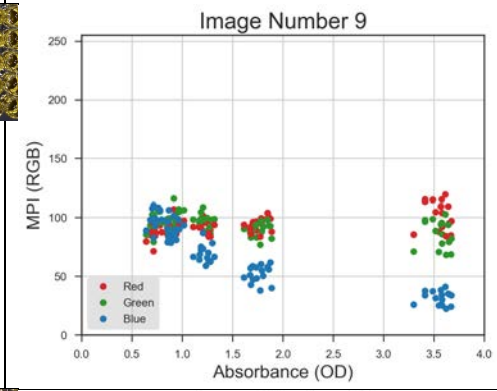


Image Number 9
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

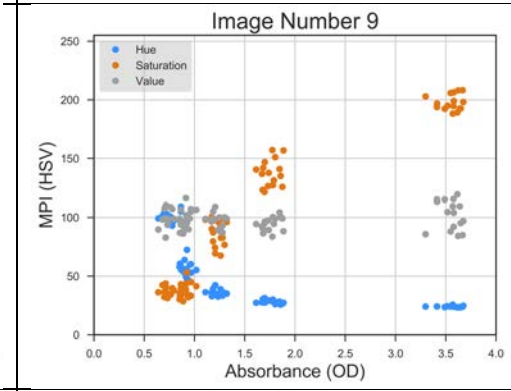
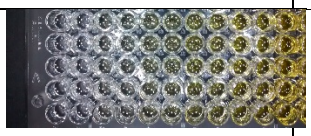


Image Number 9
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



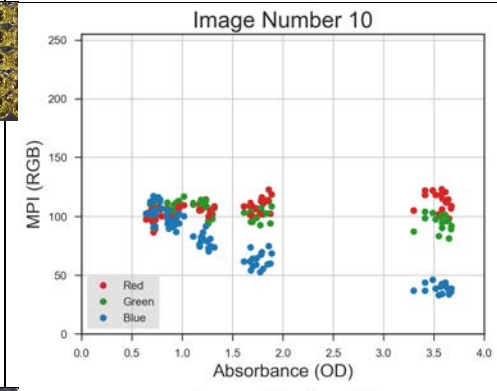


Image Number 10
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

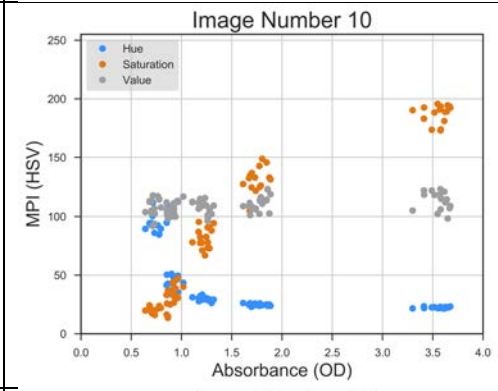
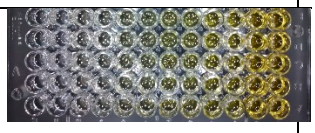


Image Number 10
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



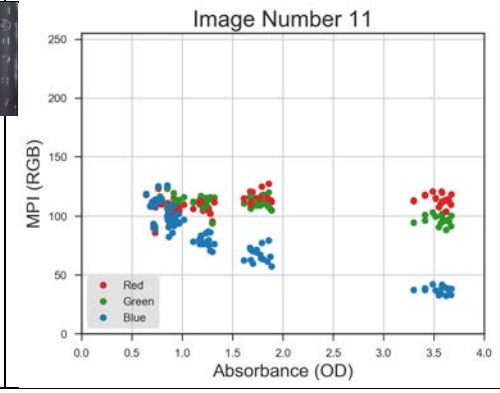


Image Number 11
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

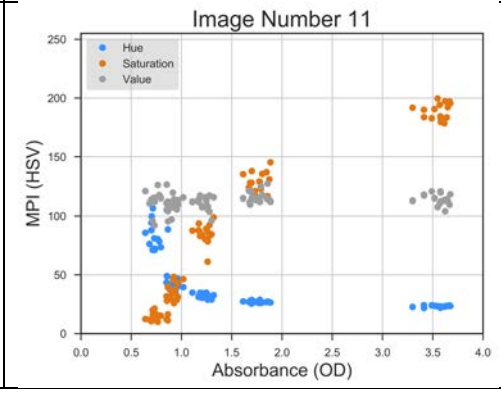


Image Number 11
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

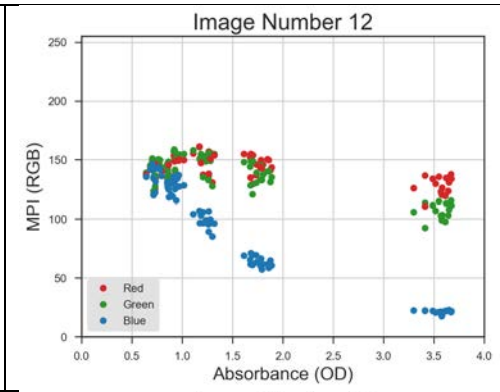


Image Number 12
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

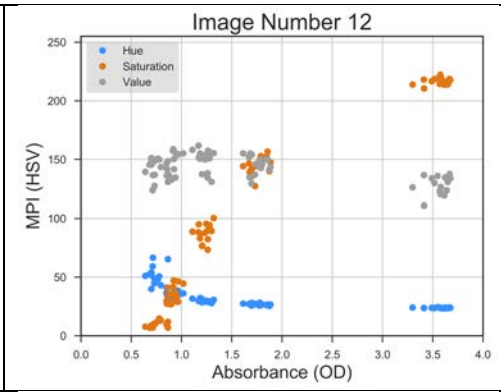


Image Number 12
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

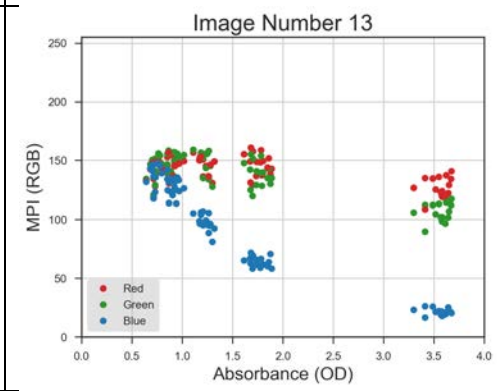


Image Number 13
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

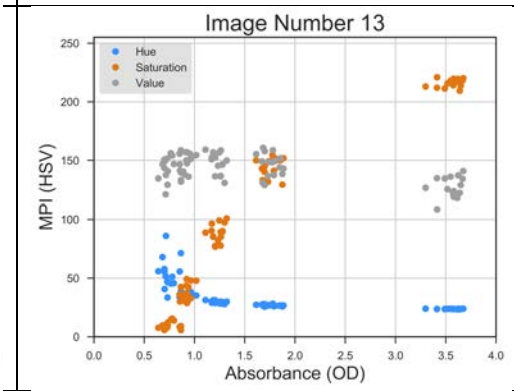


Image Number 13
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

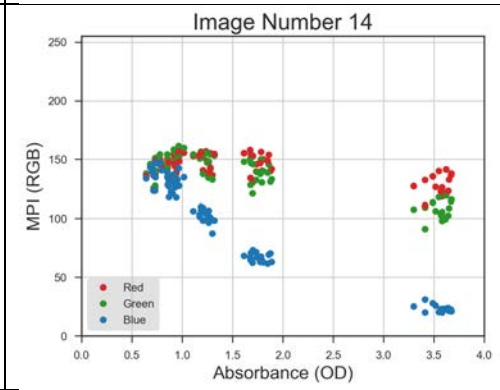


Image Number 14
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

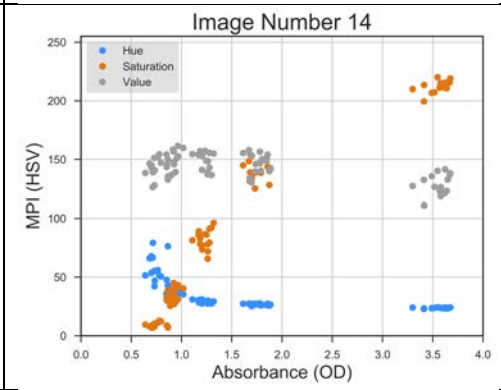
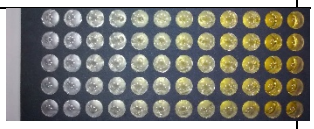


Image Number 14
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



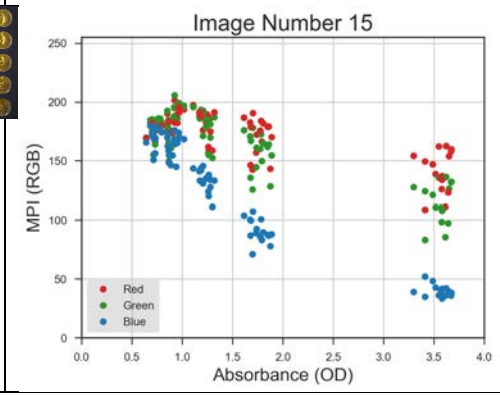


Image Number 15
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

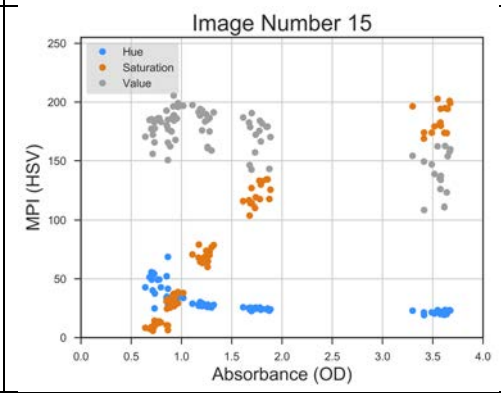
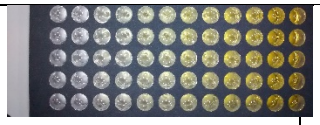


Image Number 15
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



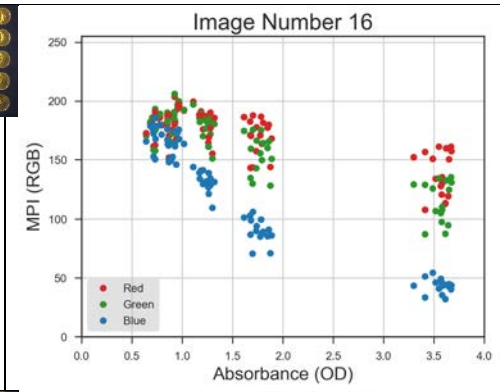


Image Number 16
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

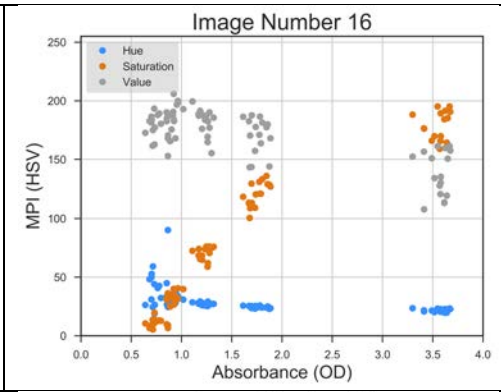
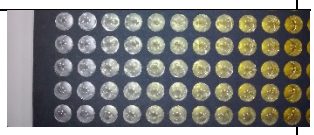


Image Number 16
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



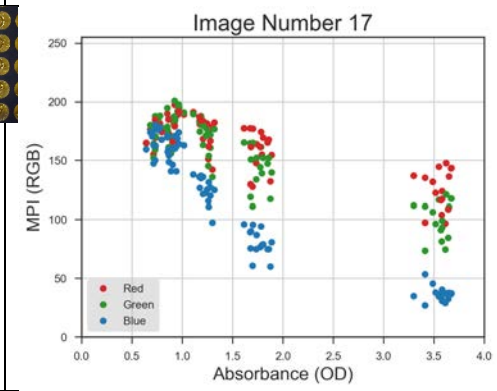


Image Number 17
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

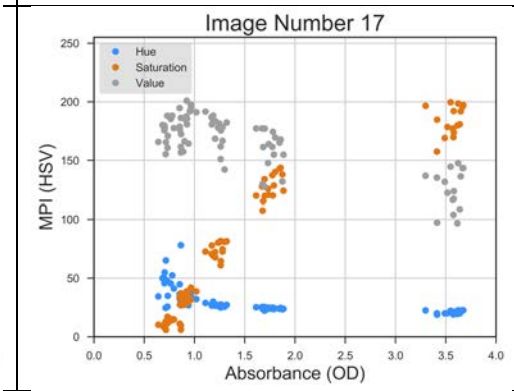
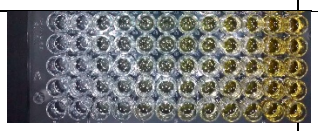


Image Number 17
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



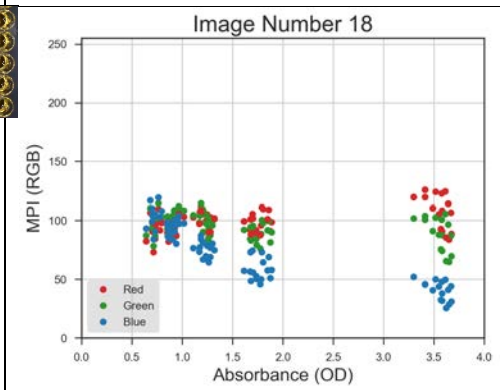


Image Number 18
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

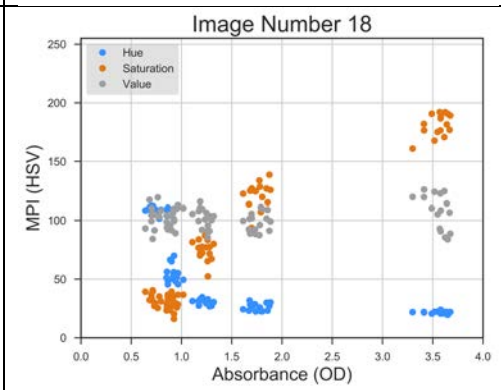
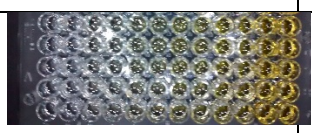


Image Number 18
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



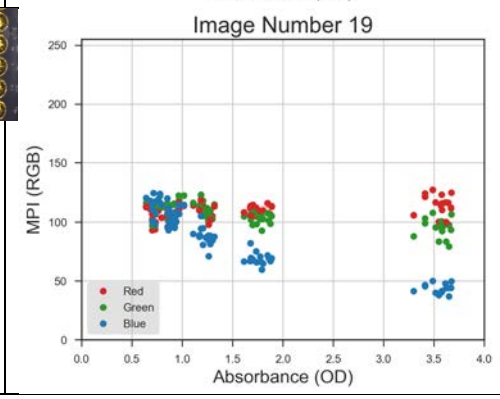


Image Number 19
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

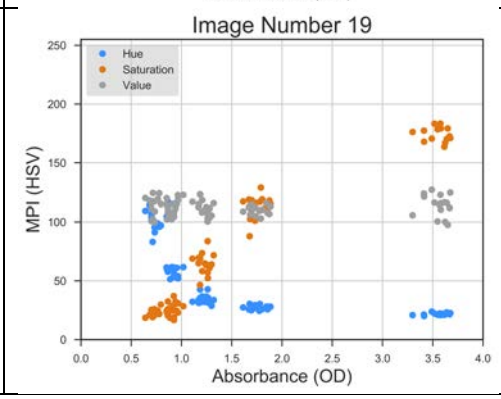
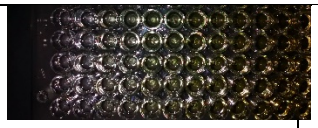


Image Number 19
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



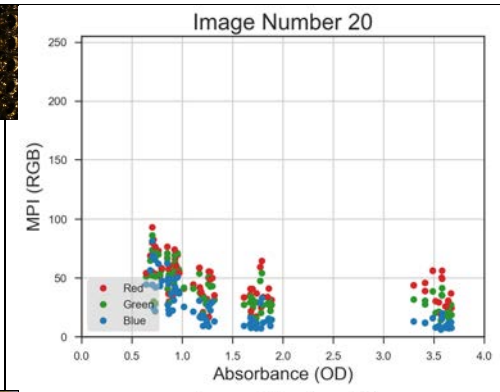


Image Number 20
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

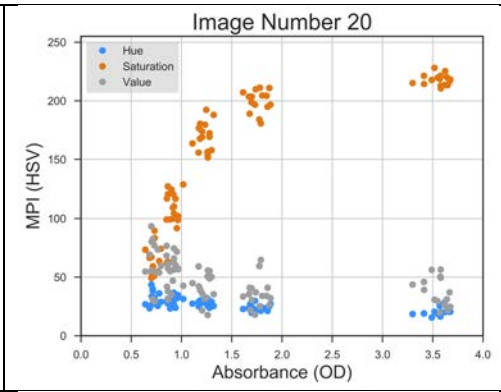


Image Number 20
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

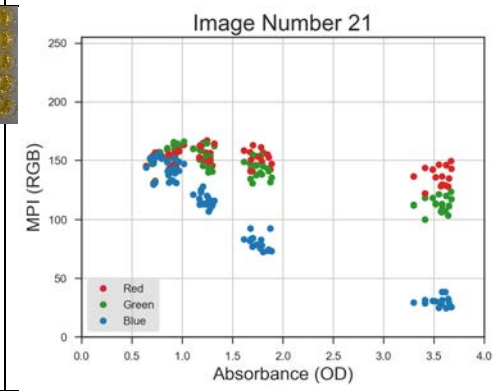


Image Number 21
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

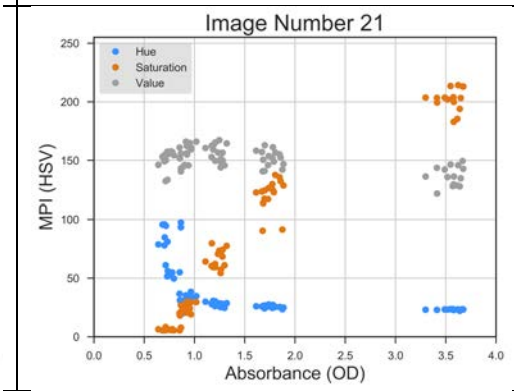


Image Number 21
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

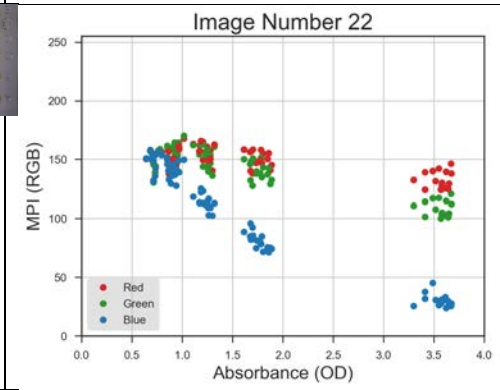


Image Number 22
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

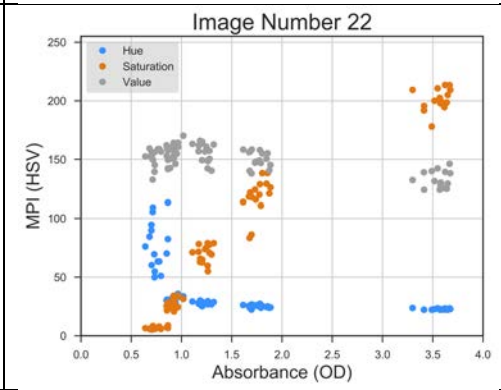


Image Number 22
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

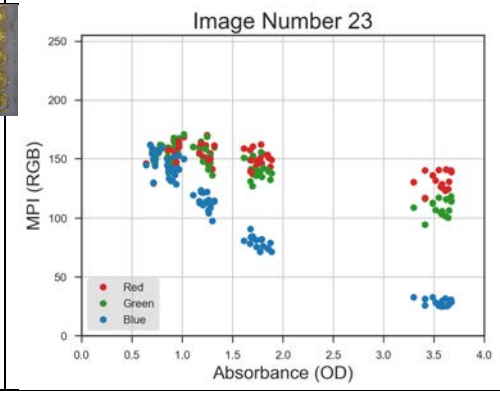


Image Number 23
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

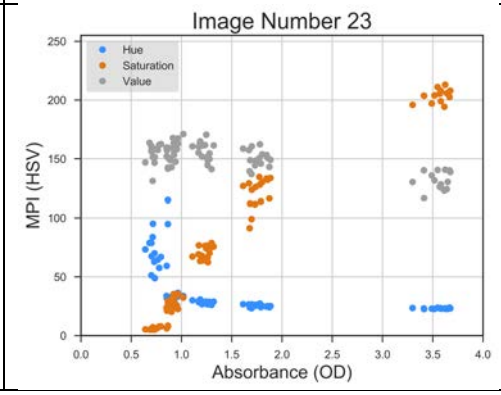
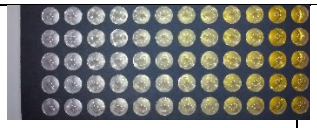


Image Number 23
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



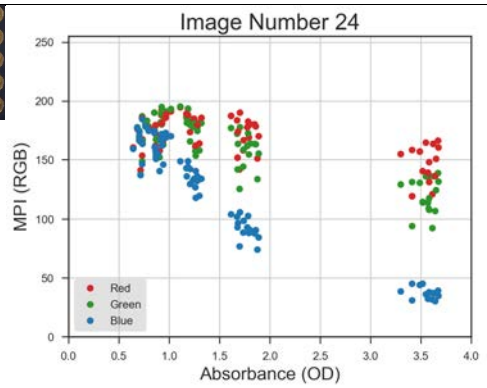


Image Number 24
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

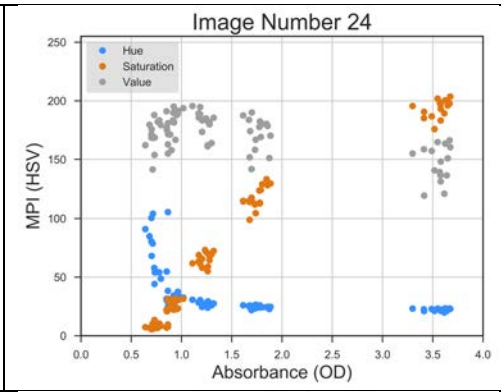
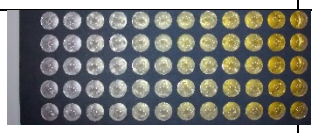


Image Number 24
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



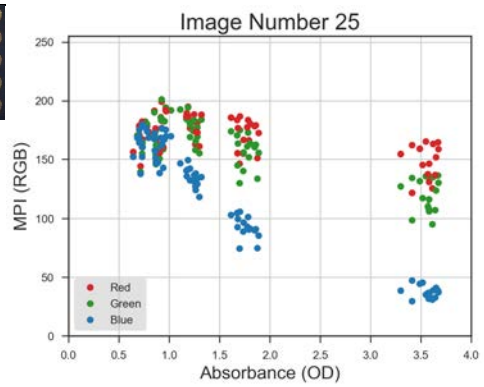


Image Number 25
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

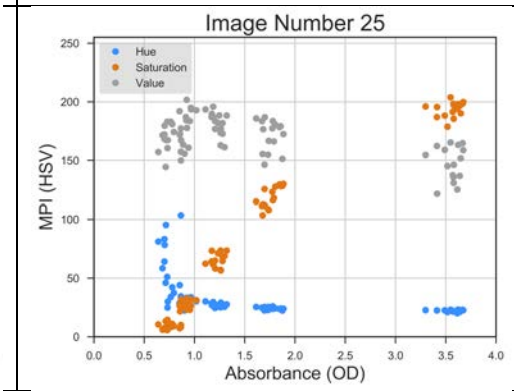
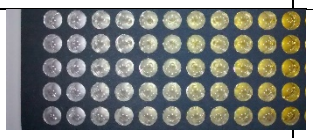


Image Number 25
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



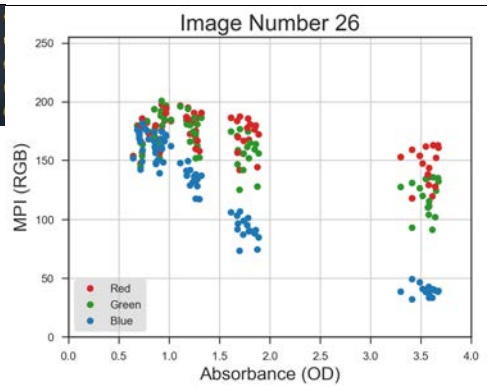
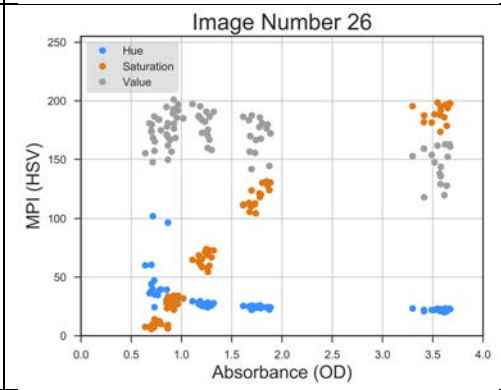


Image Number 26
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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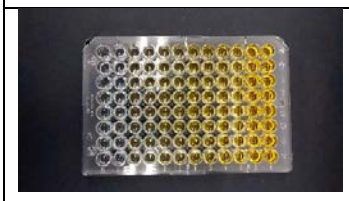
Absorbance (OD)
Hue
Saturation
Value



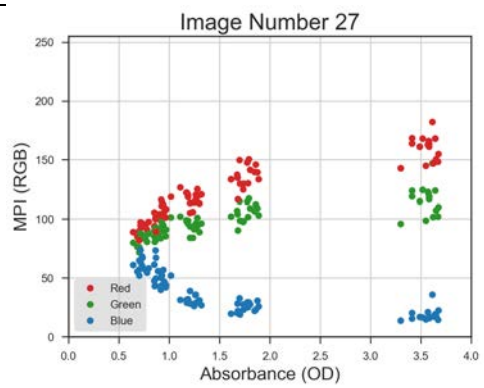
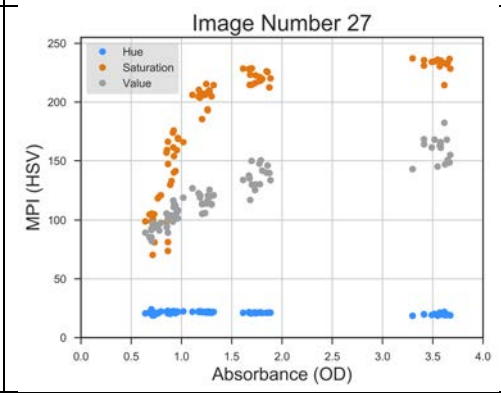


Image Number 27
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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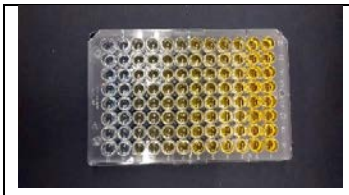
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



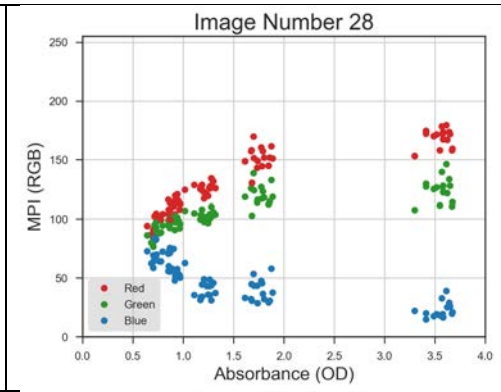
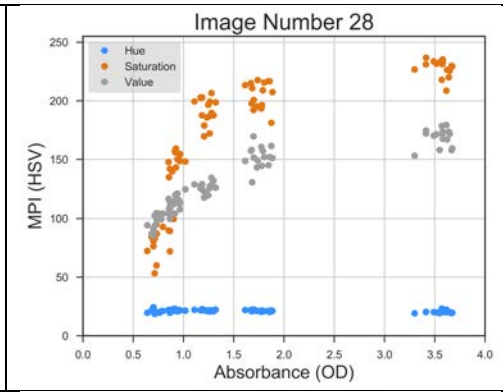


Image Number 28
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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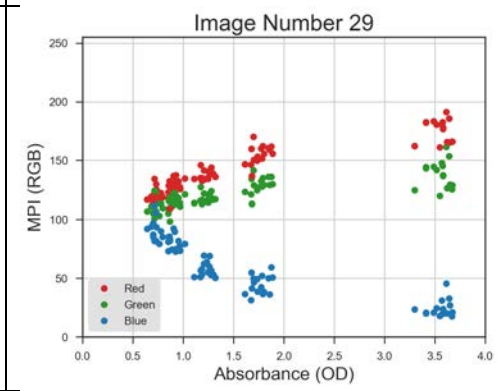
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value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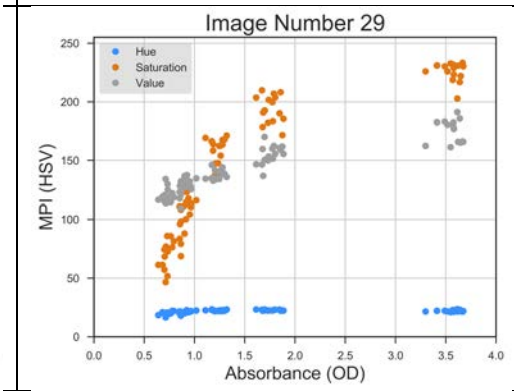
Absorbance (OD)
Red
Green
Blue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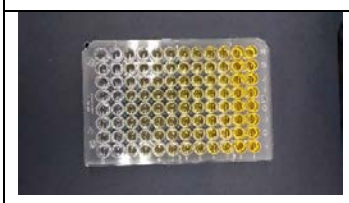
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



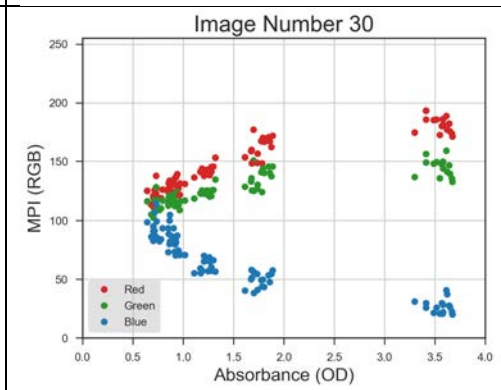


Image Number 30
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

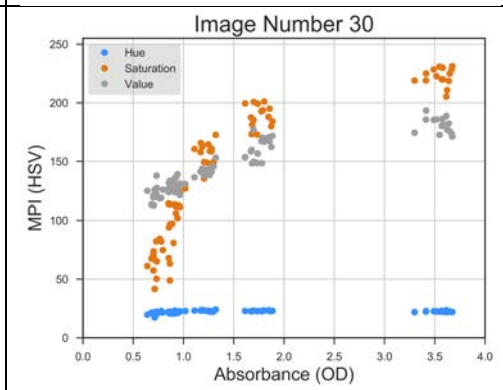


Image Number 30
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

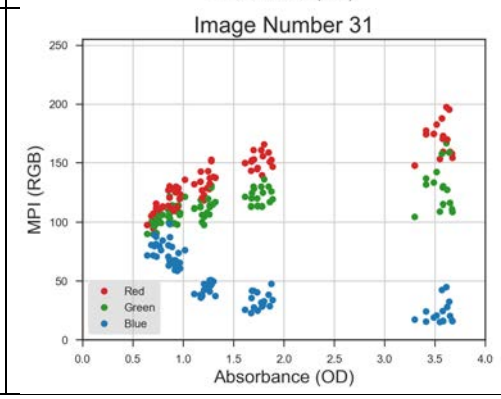


Image Number 31
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

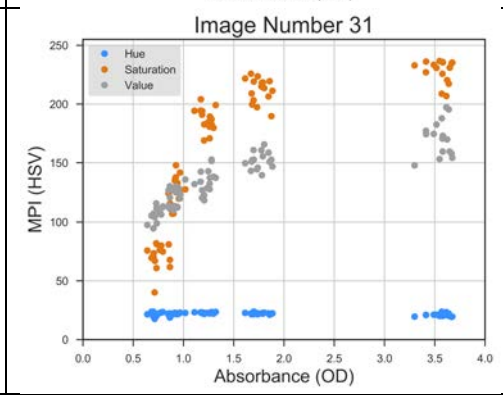
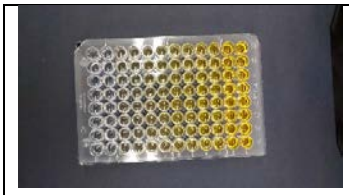


Image Number 31
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



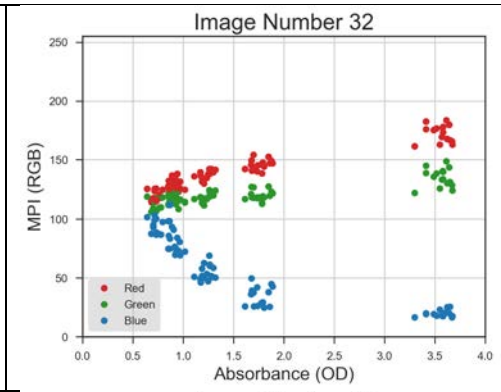


Image Number 32
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

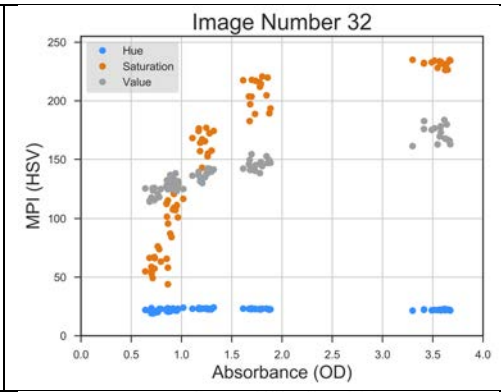
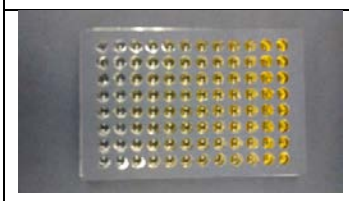


Image Number 32
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



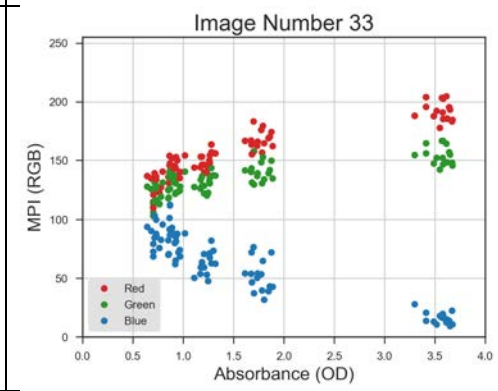


Image Number 33
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

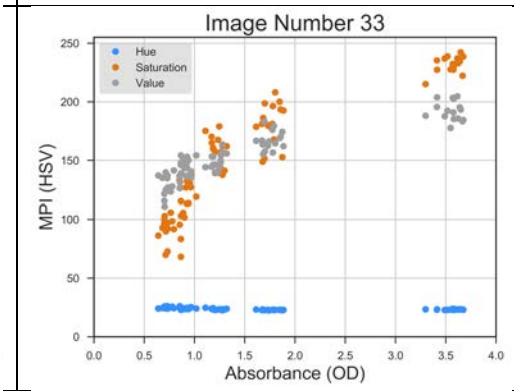
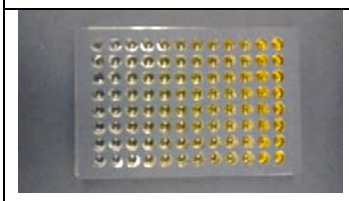


Image Number 33
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



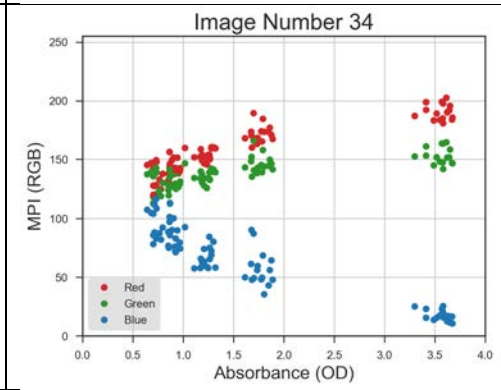
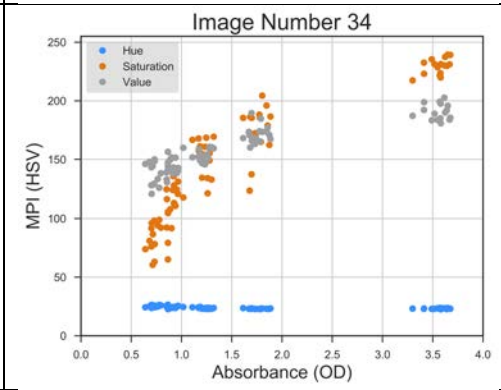


Image Number 34
MPI (RGB)
Absorbance (OD)
Red
Green
Blue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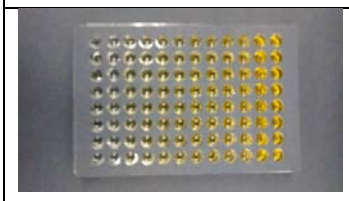
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



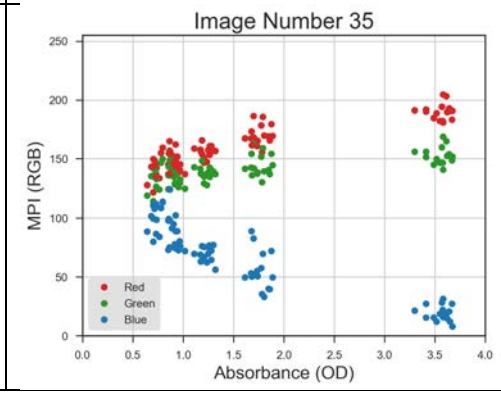


Image Number 35
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

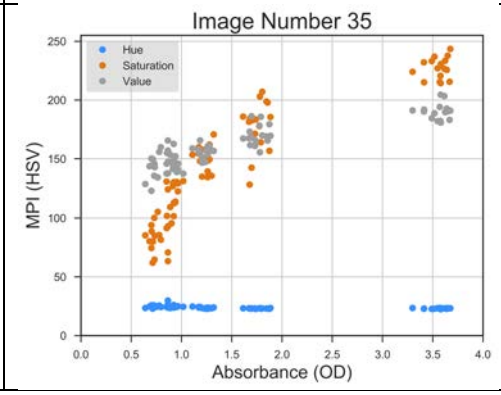


Image Number 35
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

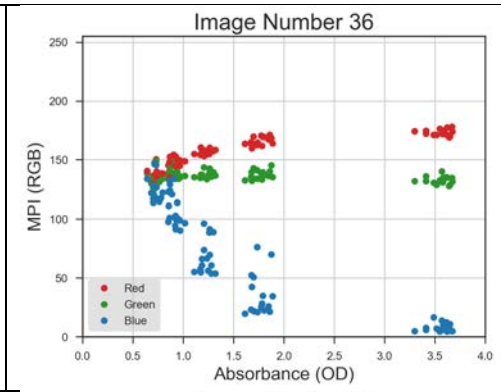


Image Number 36
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

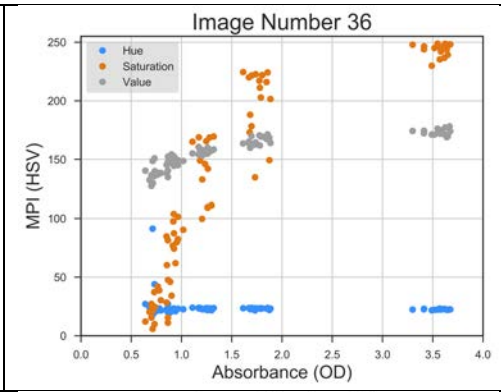


Image Number 36
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

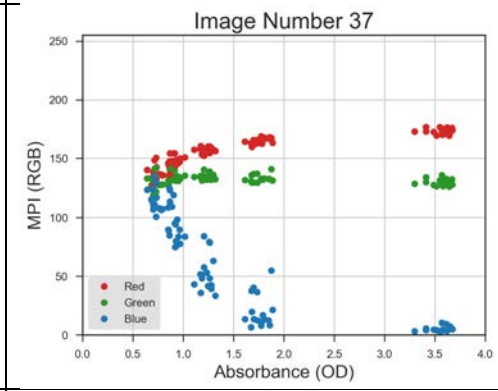


Image Number 37
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

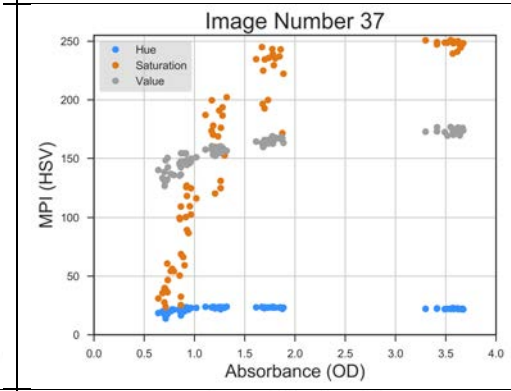


Image Number 37
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

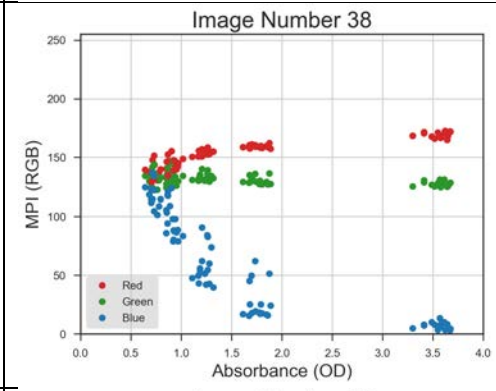


Image Number 38
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

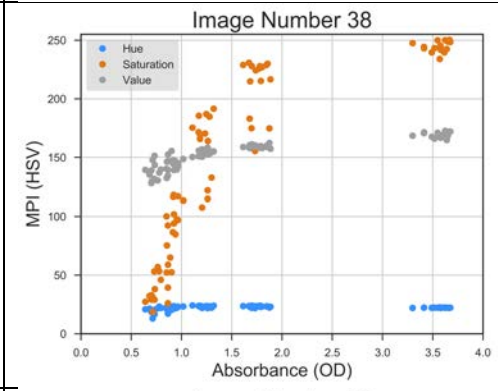


Image Number 38
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

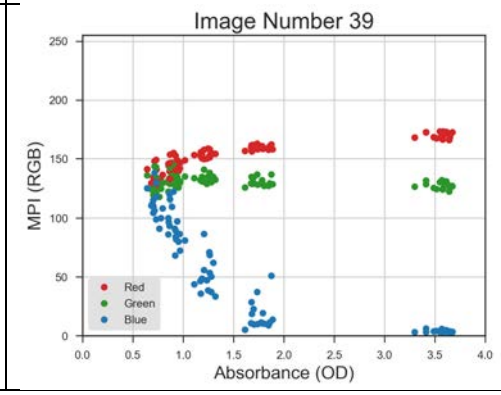


Image Number 39
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

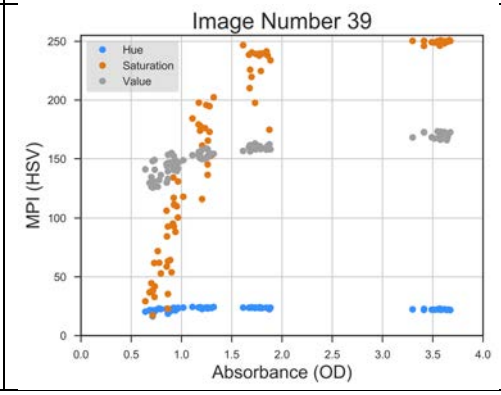


Image Number 39
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

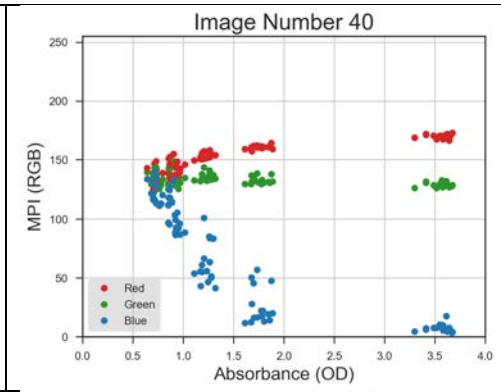


Image Number 40
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

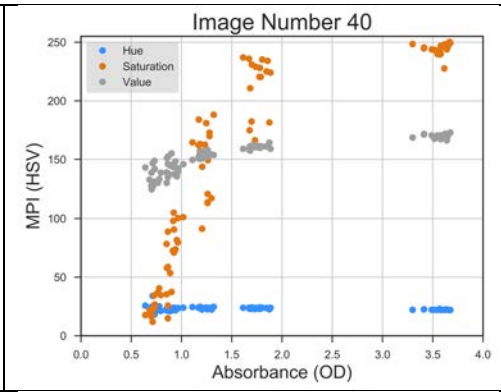


Image Number 40
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

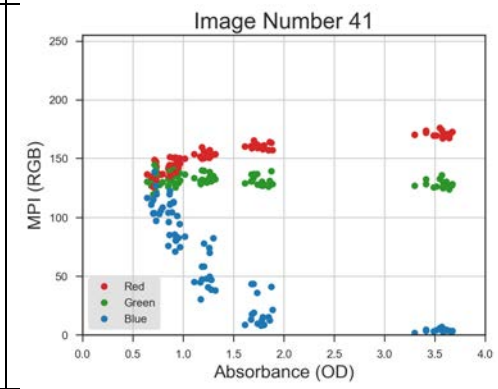


Image Number 41
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

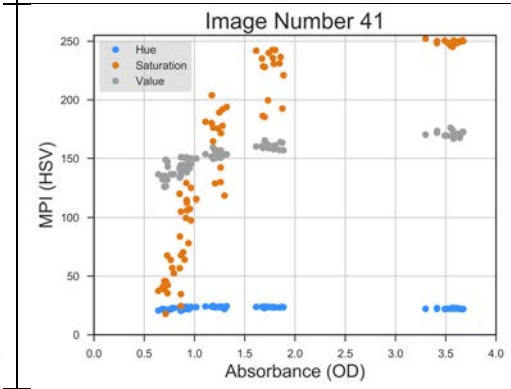
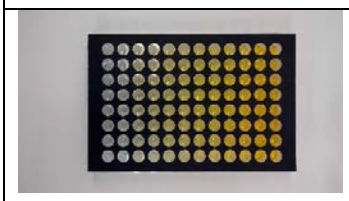


Image Number 41
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



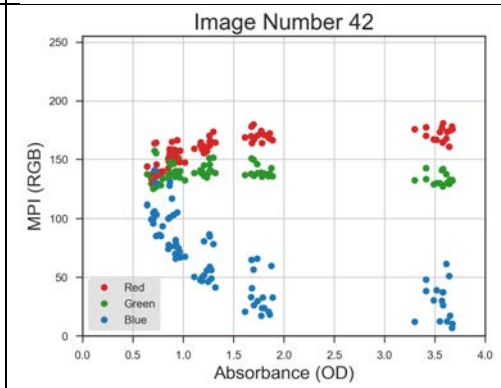


Image Number 42
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

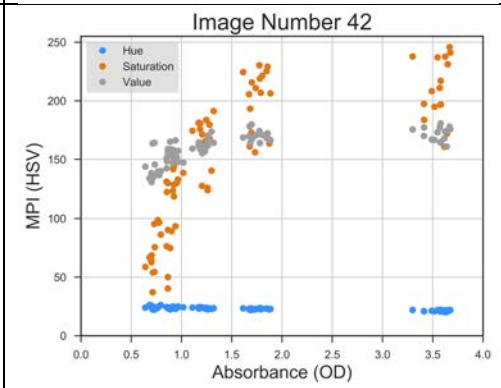


Image Number 42
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

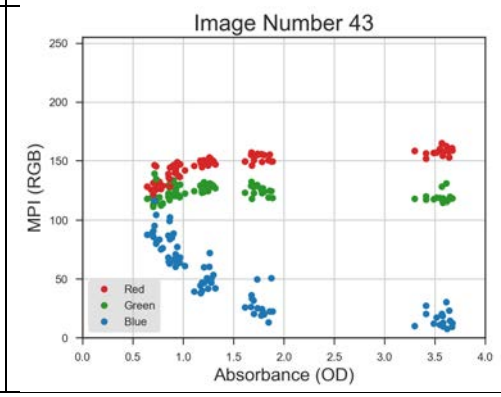


Image Number 43
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

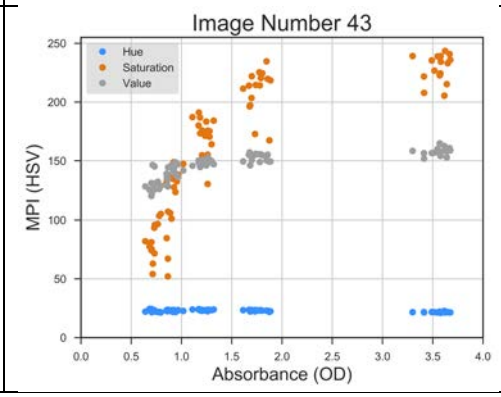
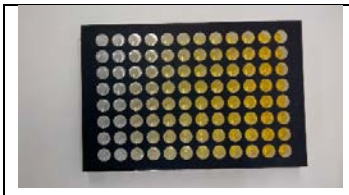


Image Number 43
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



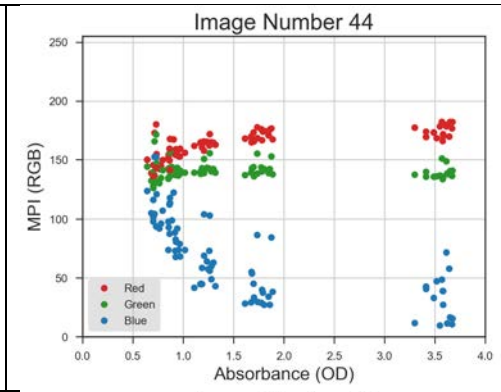


Image Number 44
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

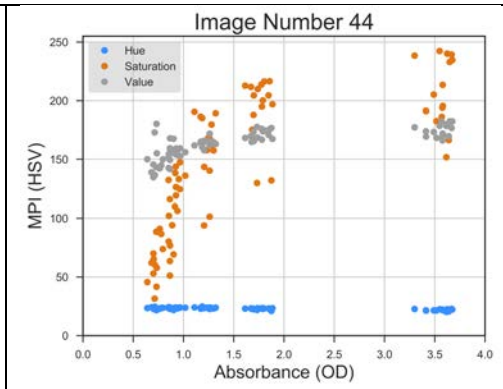


Image Number 44
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value

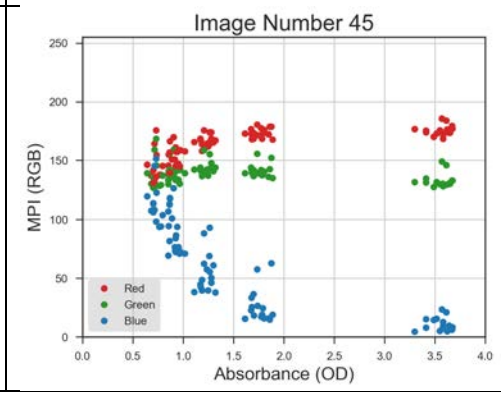


Image Number 45
MPI (RGB)
Absorbance (OD)
Red
Green
Blue

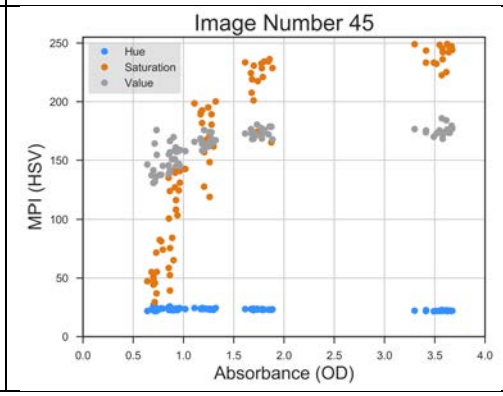
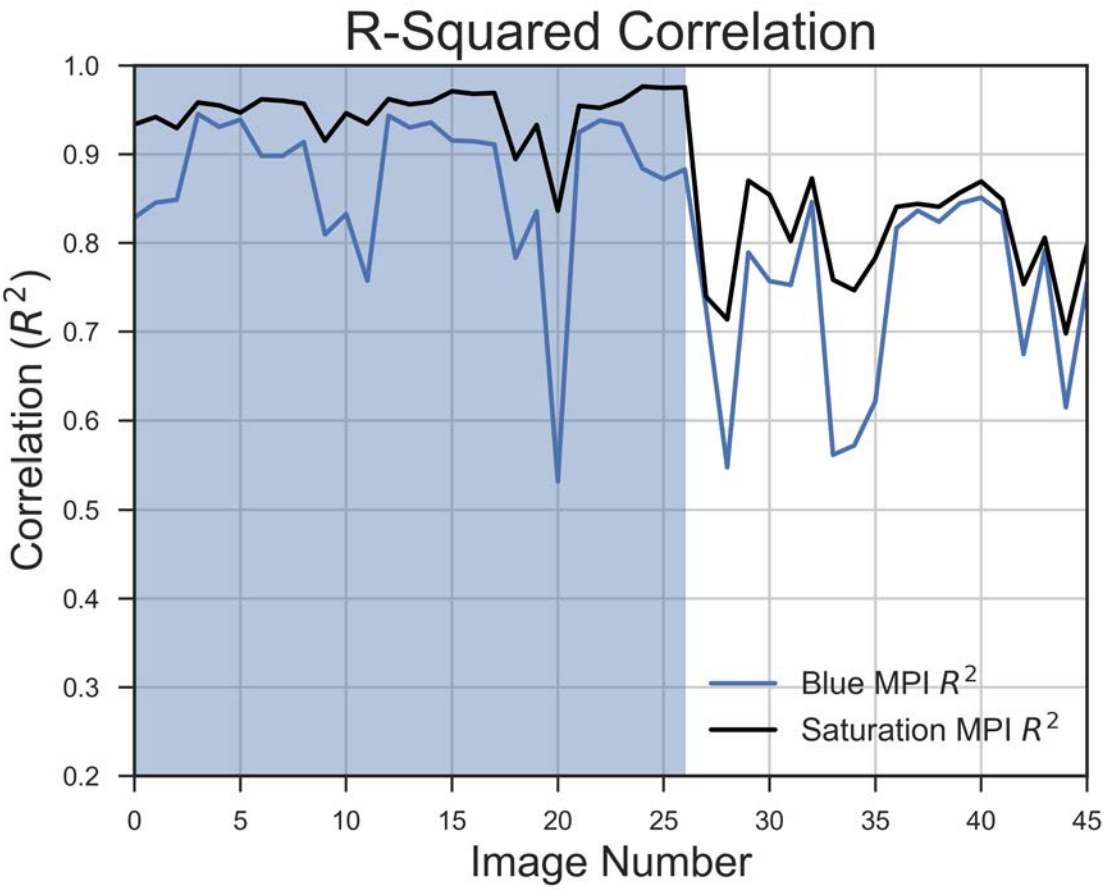
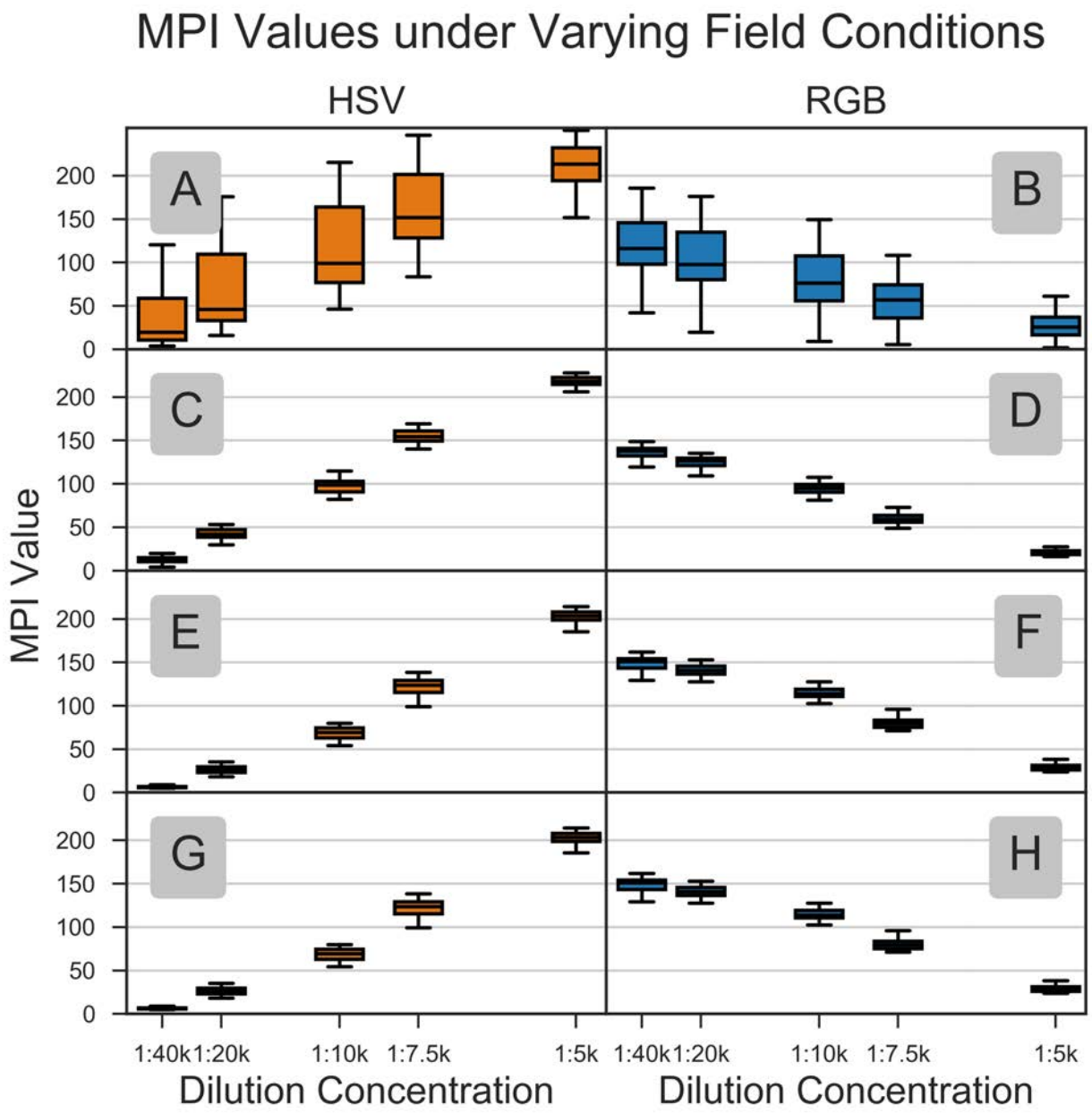


Image Number 45
MPI (HSV)
Absorbance (OD)
Hue
Saturation
Value



Supplementary Figure 4: Linear regression model, R^2 for each image in dataset 2B with 5MP camera. Only values within the linear response range were used for the model. Shaded region indicates use of capture equipment



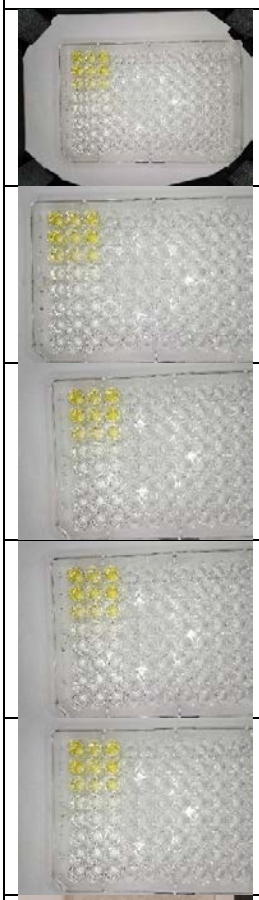
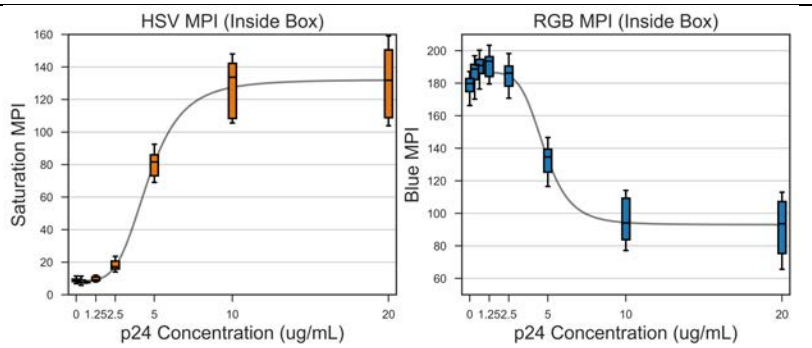
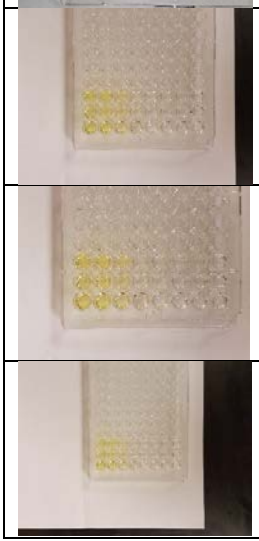
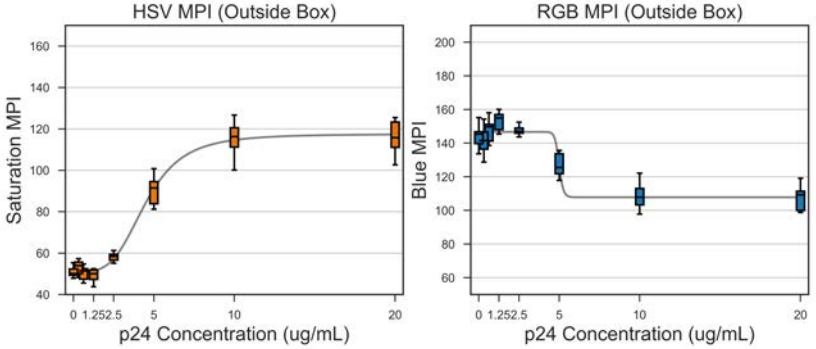
Supplementary Figure 5: Boxplots for subsets of MPI data in dataset 2B. All saturation MPI values (A), all blue MPI values (B), saturation values for bright lighting (C), blue values for bright lighting (D), saturation values for dark lighting (E), blue values for dark lighting (F), saturation values without capture equipment (G), blue values without capture equipment (H). For (A) and (B), N = 368. For (C), (D), (E), and (F), N = 24. For (G) and (H), N = 80

Dataset 3 for Table 4: p24 HIV capsid protein direct ELISA

This dataset was obtained using the 12 MP camera of the Galaxy Edge S7 and is used to show a possible application for the saturation method. A smaller range of capture conditions was considered – one subset with ideal conditions and the other subset with realistic conditions outside the enclosure. These images were used to obtain the concentration relationships, LOD values, and LOQ values for the HIV p24

assay. To compute the LOD, the variance of the blank (S_b) was found from the concentration curve. A 4-parameter logistic regression model (4PL) was formed from the MPI values and the concentration value corresponding to 3.3 S_b was found from the model. The LOQ was computed in a similar way, except 10 S_b was used.

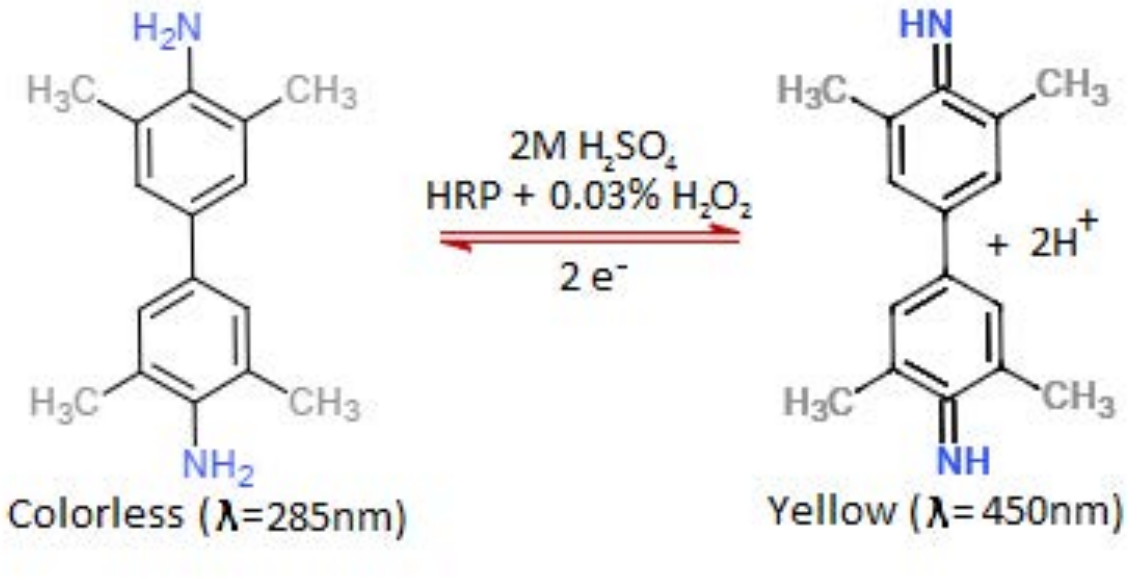
Table 4: Practical Application Images for detection of HIV p24 antigen

Image	Capture Conditions	Concentration Curve
	Within imaging box Constant, bright light from LED	
	Outside imaging box Variable distance to 96 well plate Ambient room lighting	

Assay Protocol 1: HRP Antibody-Conjugate Concentration Curve

The first dataset was obtained using a series of two fold dilutions of an anti-p24 HIV-1/horse radish peroxidase (HRP) conjugate. The initial concentration was 1 microgram/mL of conjugate, and this was diluted two fold using 200 microliters of conjugate solution into 1800 microliters of phosphate-buffered saline (pH 7.0). The HRP reacts with a colorimetric substrate, 3,3',5,5'-Tetramethylbenzidine (TMB) using 0.03% H_2O_2 (Thermofisher Scientific) to catalyze the oxidation of the substrate to a diimine, rendering a blue color. Using a 2M H_2SO_4 solution (pH -0.6), the reaction can be stopped by enzyme denaturation, and the diimine is formed under full deprotonation, forming a yellow color (450nm).

Overall Net Reaction:



Supplementary Figure 6: Net reaction used for data generation

A series of 5 dilutions in duplicates in a 96 well plate to achieve a sample color saturation curve to test the algorithm.

The amount of each reagent per well was: 50 µL of conjugate, 50 µL of TMB (Thermofisher Scientific) and 100 uL of 2M H_2SO_4.

Simultaneously, the duplicates of each serial dilution of the conjugate, respectively, were added to the plate rows using an eight-well multichannel pipette (Eppendorf), each well had the TMB added, which reacted for six seconds until the 2M H_2SO_4 was added.

The plate was read by spectrophotometer at (λ = 450nm), and images taken by a Samsung Galaxy Edge 7 within 10 minutes of color development.

Assay Protocol 2: p24 HIV capsid protein direct ELISA

Using a recombinant p24 antigen protein (Abcam), a series of seven (7) two-fold dilutions were used:

20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL, 0.3125 µg/mL

Each antigen target was diluted in a carbonate buffer (pH 10) (Fisher Scientific), and loaded into the 96-well plate. The plate was covered with parafilm wax, and left to incubate overnight at 4°C. The plate was washed with PBS (pH 7.0) three (3) times, and tapped on kimwipes (Fisher Science) between each rinse to empty the wells completely.

The anti-p24 antibody (Abcam) was diluted to 0.5 µg/mL (1:2000) in PBS (7.0), and 100 µL was added to each well, and incubated for one hour at room temperature. The plate was then washed with PBS (pH 7.0) three (3) times, and tapped on between each rinse to empty the wells completely.

The plate had 100 µL of blocking solution (Thermofisher) added to each well, and the plate was covered with parafilm wax, and left to incubate overnight at 4°C. The plate was washed with PBS (pH 7.0) four (4) times, and tapped on kimwipes (Fisher Science) between each rinse to empty the wells completely.

The anti-p24 conjugate (Abcam) was diluted to 0.2µg/mL (1:5000), and 100 µL was added to each well, and incubated at room temperature for 1 hour. The plate was washed with PBS (pH 7.0) three (3) times, and tapped on kimwipes (Fisher Science) between each rinse to empty the wells completely.

The plate was developed with the TMB substrate (Thermofisher) with 100 µL to each well, and stopped 100 µL with 2M H_2SO_4 after five minutes to each well.

The plate was read by spectrophotometer at (λ = 450nm), and images taken by a Samsung Galaxy Edge 7 within 10 minutes of color development.