

Spotxel[®] Reader 1.1

Smartphone-based Colorimetric Image Reader

Quick Start Guide

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Spotxel[®] Reader is only intended for research and not intended or approved for diagnosis of disease in humans or animals.

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Sicasys Software GmbH
Hans-Bunte-Str. 8
69123 Heidelberg
Germany

Phone +49 (62 21) 7 28 50 40
Fax +49 (62 21) 7 28 48 94
Email info@sicasys.de
Web www.sicasys.de

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1 Introduction

Spotxel® Reader enables analysis of colorimetric assays in microplates with a smartphone, taking advantage of the smartphone camera as the image capturing device and the touch-screen as the control interface. Typically, Spotxel® Reader supports:

- **Image capture:** Provide tools to captures images of microplates that could be optimized to the smartphone camera and the assay setup.
- **Signal detection and quantification:** Effectively detect and measure color signal in microplate wells, employing powerful noise processing and image analysis algorithms.
- **Assay analysis:** Annotate the microplate, specify the assay standards' concentration, and derive the unknowns' concentration with regression models, charts, and tables.

This document provides a brief explanation of the app's user interface and tools.

1.1 Installation

Spotxel® Reader runs on Android and iOS smartphones and tablets. The app is to be installed directly from the Google Play Store and the Apple App Store.

Android platforms

- Minimum Android version: 5.0 (Android Lollipop)
- Minimum hardware: Dual-core 1.2 GHz, 1 GB RAM, 16 GB SD Card.

iOS platforms

- Minimum iOS version: iOS 8
- Minimum hardware: Dual-core 1.2 GHz, 1 GB RAM, 16 GB SD Card.

1.2 User Interface

Technically, the tools are grouped into “**pages**”, each providing a set of related functions:

- **Camera:** Calibrates the image acquisition and captures images of microplates.
- **Image Analysis:** Detects and measures wells' color signal of microplate images.
- **Assay:** Assign name and notes to wells, set up the assay standards' concentration.
- **Quantified Data:** Provides a table showing the microplate wells' signal and concentration.
- **Standard Curve:** Show a chart with standard curve for deriving the unknowns' concentration.
- **Licenses:** Show the active license information and options for purchasing a license.
- **Info:** Display the build version and the links to privacy policy and manual.
- **Share:** Shortcut to email-clients or social medial tools for sharing the app.
- **Exit:** Safely quit the app.

Touch a page on its menus and drag left or right to move between pages. Alternatively, you can open the *main menu* (Figure 1, left) and select a page directly. The main menu can be opened either by double-tapping on a *page menu* (Figure 1, right) in each page, or swipe from the left edge of the smartphone screen inwards.

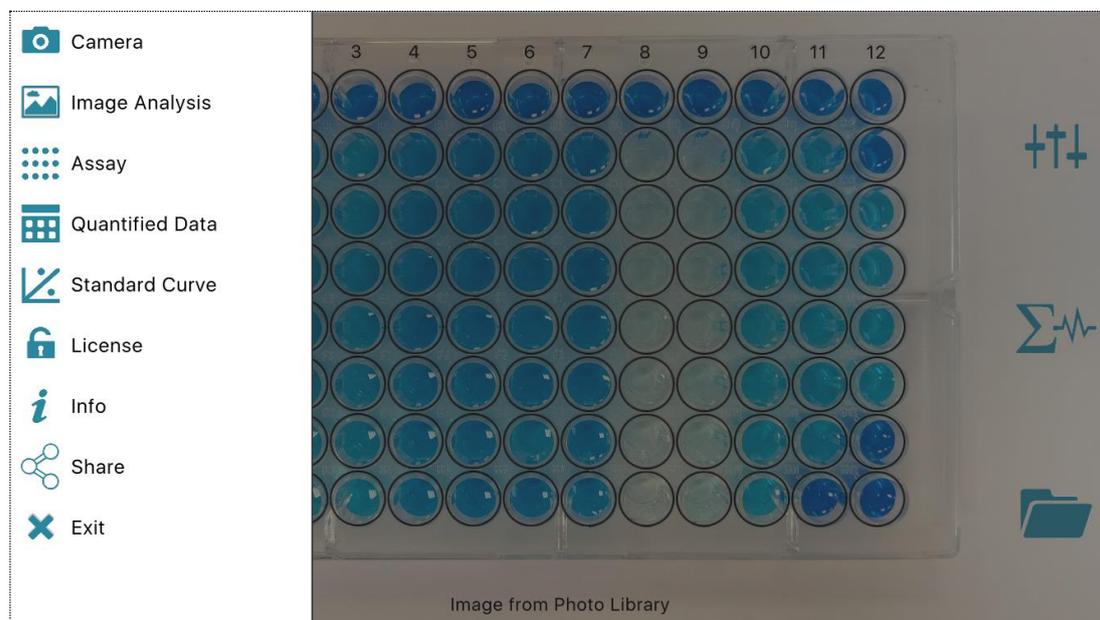


Figure 1: Main menu (left) and page menu (right).

2 Calibration

This step setups the capturing so that the acquired images are of good quality, enabling convenience and quality of the microplate image analysis. The tools are provided in the *Camera* page (Figure 2).

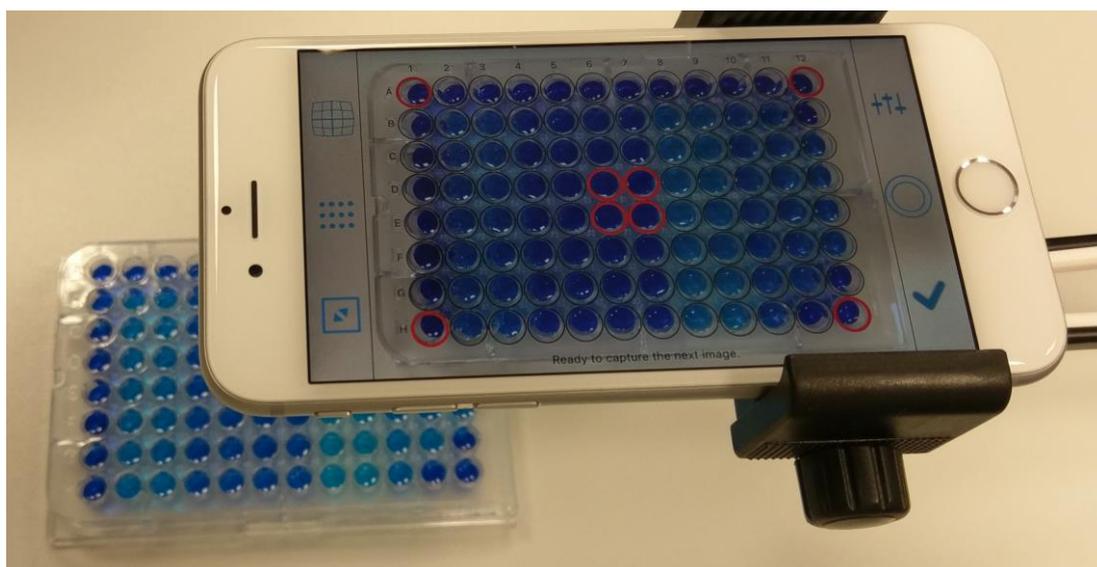


Figure 2: Camera Calibration.

Image capturing is calibrated with *Calibration Grid* buttons (Table 1) on the left menu.

Button	Name	Function when tapped in <i>Camera</i> page
	Distortion	When the calibration grid is visible: allows tuning the distortion factor of the camera lens.
	Calibration Grid	Show/hide the calibration grid.
	Resize	When the calibration grid is visible: allows adjusting the calibration grid's size to fit the wells' size and the camera's focus.

Table 1: Calibration Grid Buttons.

The calibration grid, with red wells at the corner and the center, may not align well with microplate in the camera's screen window in the first place. To adjust the grid's size or distortion factor, first tap on the *Resize* or the *Distortion* button, and then adjust the corresponding value by holding the calibration grid with two fingers and sliding them together or apart from each other.

Tips on grid calibration:

- First, position the (smartphone) camera or the microplate in such a way that in the camera screen (Figure 2), the four edges of the microplate appear (almost) parallel to those of the calibration grid (the black rectangle).
- Position the camera or the microplate so that the wells at the center align with the red wells of the calibration grid.
- Adjust the distortion factor to “un-squeeze” the calibration grid.
- Resize the grid to match its wells with those of the microplate in the camera screen.
- Finalize the alignment with the distortion factor.

Once the calibration grid is aligned, you can start capturing the images. The app saves the calibration grid's distortion factor and size after each image capture, so calibration needs to be done only once.

3 Image Capture

When calibration is finished, image capturing is straightforward with the *Image Capture* buttons (Table 2) on the right menu of the *Camera* page.

Button	Name	Function when tapped in <i>Camera</i> page
	Setting	Adjusting the camera parameters such as focus or color balance. Not yet supported.
	Capture	Take a picture of the microplate in the current camera screen and save it to a file. Show the acquired image as a “static” picture for review purpose.
	Accept	Send the captured image in the review screen directly to the image analysis page for signal detection and quantification.
		iOS devices: Since captured images can only be stored within the app's local data folder, this <i>Accept</i> button will also send the captured image to your Photo Library for archiving or sharing.

Table 2: Image Capture Buttons.

If the grid has been aligned with the microplate (Figure 2) during calibration, the captured microplate images could be analyzed directly without having to align the grid again.

4 Image Analysis

Image analysis (Figure 3, Figure 5, and Figure 7) detects the color signal in each well and reports the measured color intensity. In order to support different color scales and measures, the app reports the *normalized color intensity*, whose value is between 0 and 100.

Typically, you'll go through the following steps:

- Load the image (or have it sent directly from the *Camera* page).
- Align the grid with the microplate in the image. Note that this step can be saved if the capturing is well calibrated; the grid is already aligned with the captured image.
 - You can scale the image up/down.
 - With the grid you can scale up/down, adjust its distortion factor, and rotate.
- Assign some specific value to a well if necessary. This could be e.g. forcing a well's intensity to zero or setting the assay's maximum color intensity to a value like 70 (instead of 100).
- Start the signal detection and quantification and wait for the results.

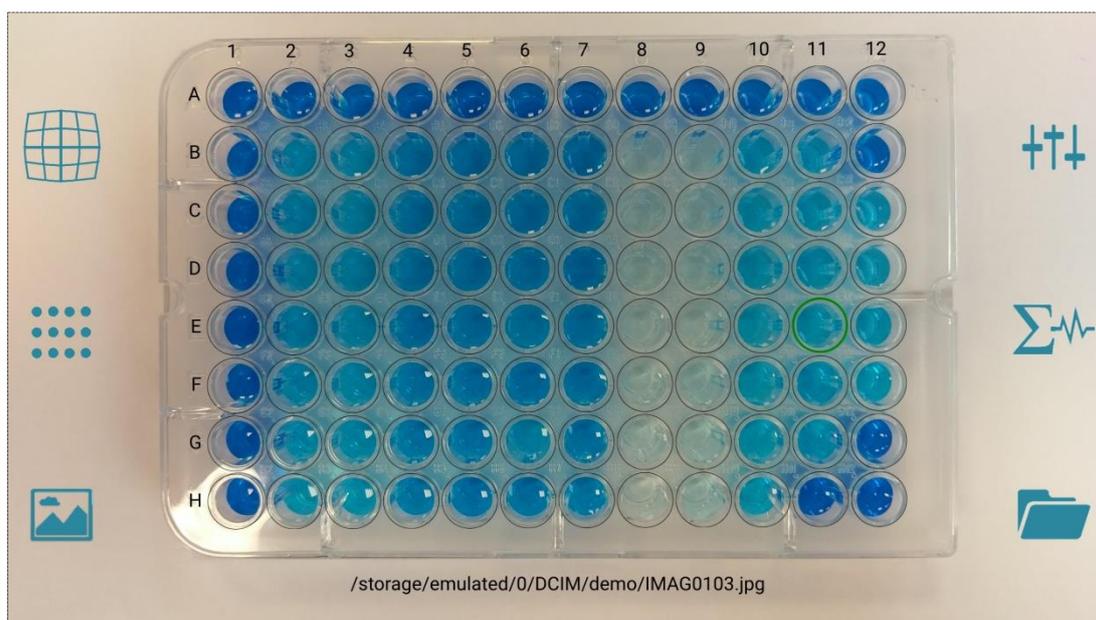


Figure 3: Grid Alignment in Image Analysis.

The buttons on the right menu (Table 3) control the image analysis procedure.

Button	Name	Function when tapped in <i>Image Analysis</i> page
	Setting	Show/hide the slider for assigning specific intensity value (Figure 5) to a well.
	Quantify	Start the signal detection and quantification process.

	Open	Tapping on the <i>Open</i> button will show the image explorer for selecting and loading an image.
		iOS devices: If you double-tap (two continuous taps) on the <i>Open</i> button, the device's Photo Library will be opened. This enables to analyze images stored in the Photo Library.

Table 3: Image Analysis Control Buttons.

Loading an image for analysis

Show the *Image Explorer* (Figure 4) with the *Open* button, **swipe** the list of images up/down to scroll to the selected image and then **tap** to load it.

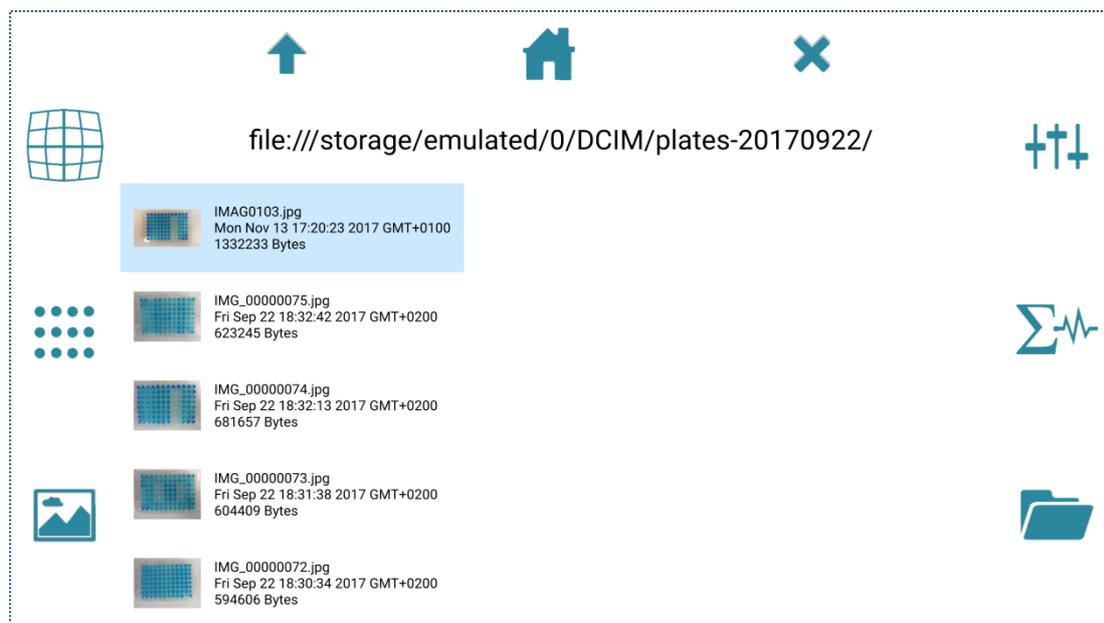


Figure 4: Image Explorer.

Assigning an intensity value to a well

- Show the slider by tapping the *Setting* button.
- **Select** the well; this highlights its border in **red** (e.g. Figure 5, well H12).
- Move the slider's handle up and down to change the value. You can also enter the value directly into the box at the slider's bottom. The value is then shown in the well.

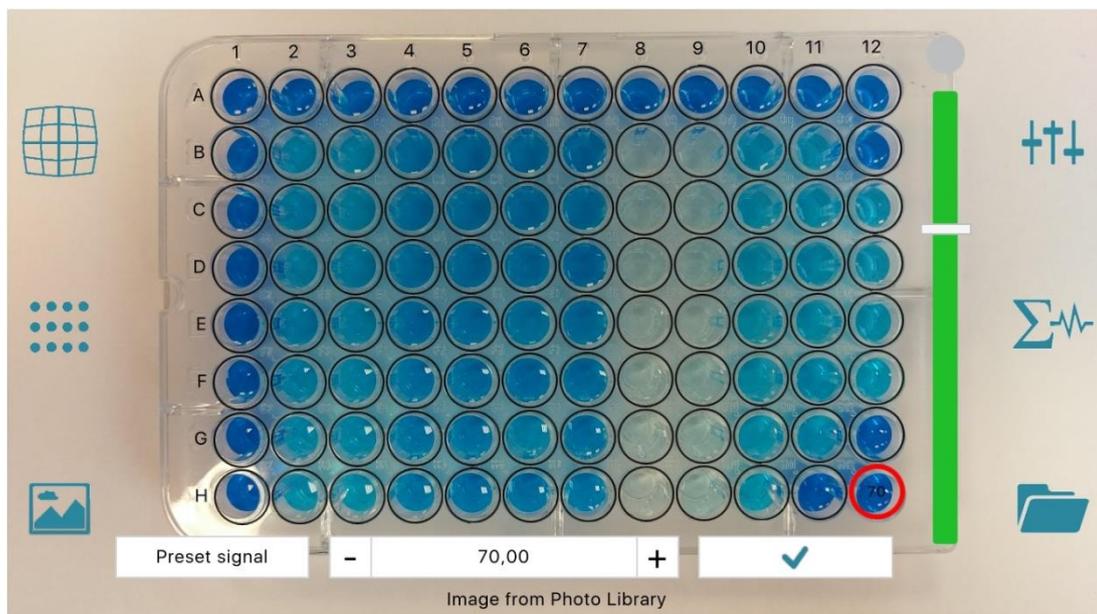


Figure 5: Setting Specific Value to Wells.

Detecting and quantifying wells' signal

Hit the *Quantify* button on the right menu to start the process. This may take some time depending on the processor and the image resolution. The progress is updated during processing (Figure 6). Since this is executed *asynchronously*, you can move to *work on another page*.

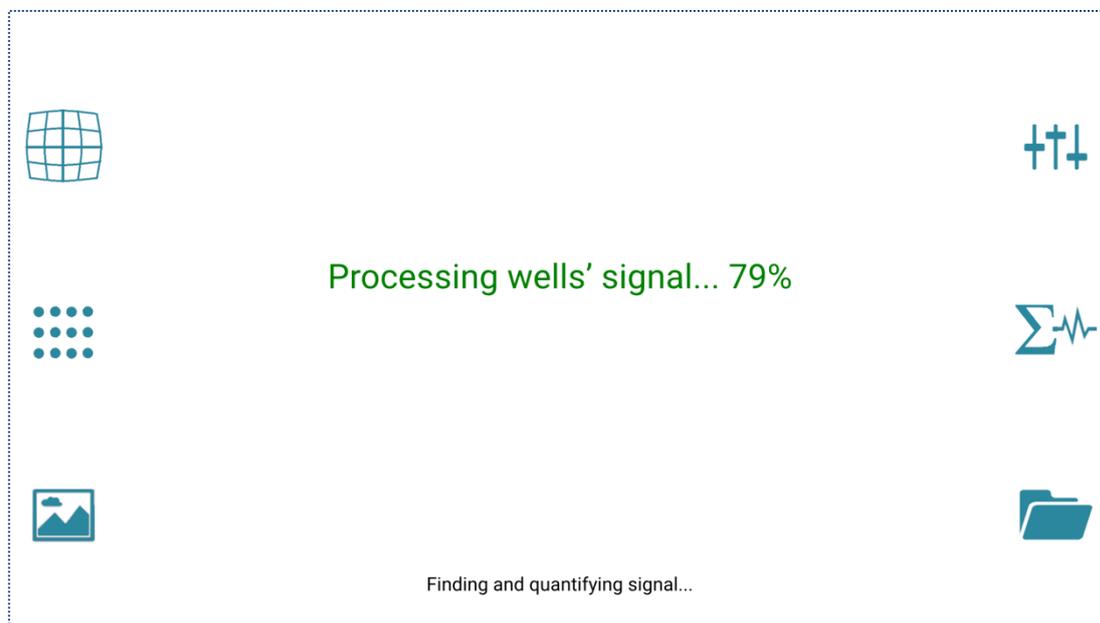


Figure 6: Signal Detection and Quantification Progress.

When finished, the detected signal is highlighted by showing its boundary in red (Figure 7). You can scale the image up and down, and/or move it, for better viewing of a certain well.

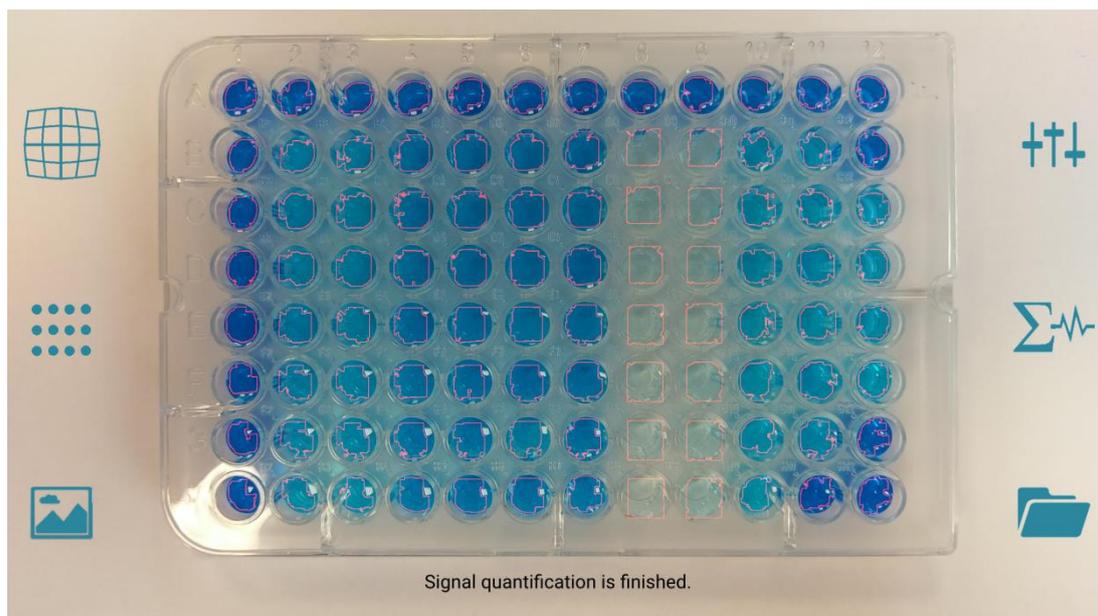


Figure 7: Detected Signal with Boundary in Red.

The results are reported in the *Quantified Data* page (Figure 11) and the *Assay* page (Figure 9).

Grid Alignment

As mentioned, if calibrated well the grid alignment step can be skipped. However it is necessary when loading an image not captured by the app, or when the calibration does not work as expected. This is accomplished with the *Grid Alignment* buttons on the left menu of the *Image Analysis* page.

Button	Name	Function when tapped in <i>Image Analysis</i> page
	Distortion	Switch to <i>distortion adjustment</i> mode.
	Grid	Switch to <i>grid adjustment</i> mode. Hide or show the grid.
	Image	Switch to <i>image adjustment</i> mode.

Table 4: Grid Alignment Buttons.

Depending on the selected mode (distortion factor, grid, or image), holding the grid or the image with two fingers and sliding them together or apart from each other will change the selected object:

- *Distortion adjustment*: Change the distortion factor of the current grid.
- *Grid adjustment*: Scale the grid up and down. In addition, *rotation* of the two fingers results in rotating the grid.
- *Image adjustment*: Scale the image up and down.

When visible, the grid can be moved at any time by tapping the grid with a finger and dragging it to the desired position.

Tips on scaling and rotating the **grid**:

- First, align the grid's top-left well with the top-left well of the microplate image.
- Hold the grid's top-left with a left-hand finger and the grid's right edge with a right-hand finger, then
 - move the right-hand finger *horizontally* to *scale* the grid, and/or
 - *rotate* the right-hand finger around the grid's top-left corner to *rotate* the grid.
- Finally, if necessary adjust the grid's position a bit so that all wells are equally aligned.

Tip: You can return the grid or the image to the **original size** by **double-tapping** on it (after selecting the right mode). This is useful when the object has been adjusted too much.

5 Assay Setup

In the *Assay* page (Figure 8, Figure 9, and Figure 10), you can give name to microplate wells, review the measured color intensity, as well as set the assay standards' concentration. Each function can be done in the corresponding *mode* which is selected by the buttons on the left menu.

Button	Name	Function when tapped in <i>Assay</i> page
	Annotation	Switch to <i>Annotation</i> mode.
	Color Intensity	Switch to <i>Color Intensity</i> mode.
	Concentration	Switch to the <i>Concentration</i> mode.

Table 5: Assay Setup Buttons.

Microplate Annotation

In the *Annotation* mode, you can give a name and add comments to a well. First, tap a well to select it (Figure 8, well H12). Then enter the information into the boxes at the bottom.

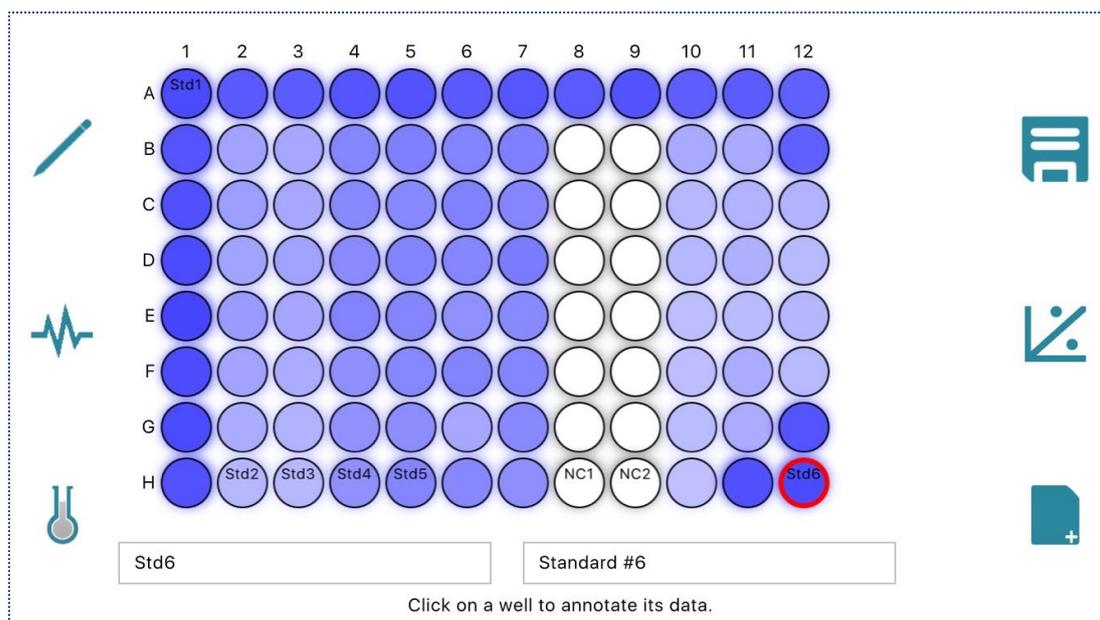


Figure 8: Microplate Annotation.

Reviewing the measured color intensity

If image analysis is done beforehand, the Assay page depicts the wells in the color according to those in the microplate image. The *Color Intensity* mode (Figure 9) also shows their quantitative value.

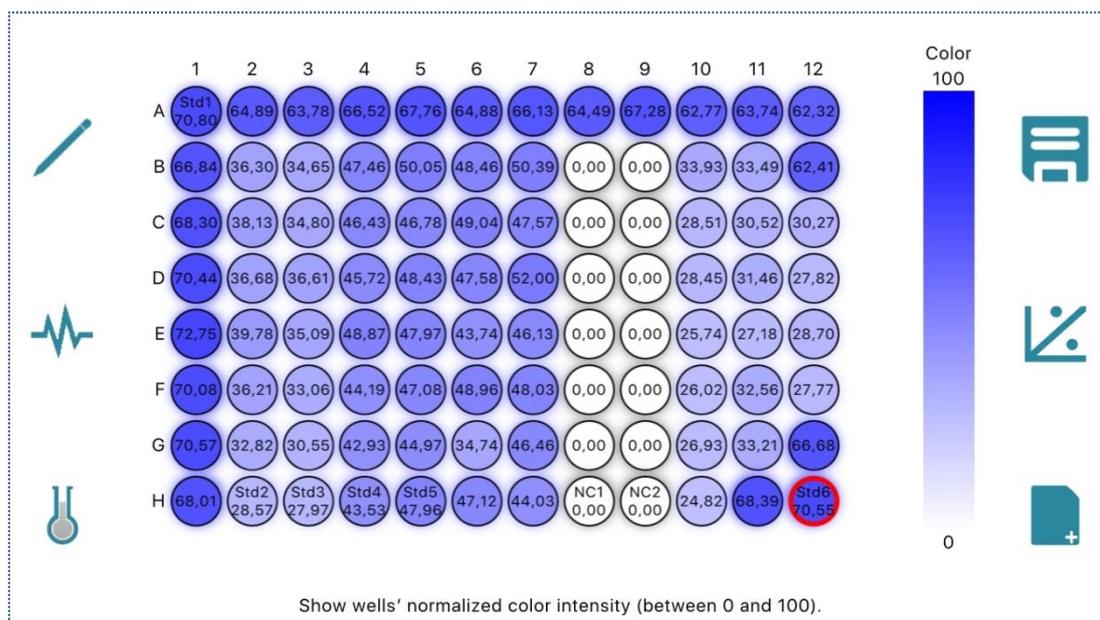


Figure 9: Measured Color Intensity.

Setting standards' concentration

The *Concentration* mode (Figure 10) provides tools to set the assay standards' concentration.

- First, define the assay's maximum concentration and select the concentration unit.
- To set a well as the assay's standard
 - Tap on the well to select it.
 - Use the slider to set a concentration value in the range from zero to the maximum value. You can also enter the value directly into the value box at the bottom.
 - The border of the standard well becomes red.
- To unset a standard well, select the well and click on the top of the slider (ND = Not Defined); its border becomes black again.

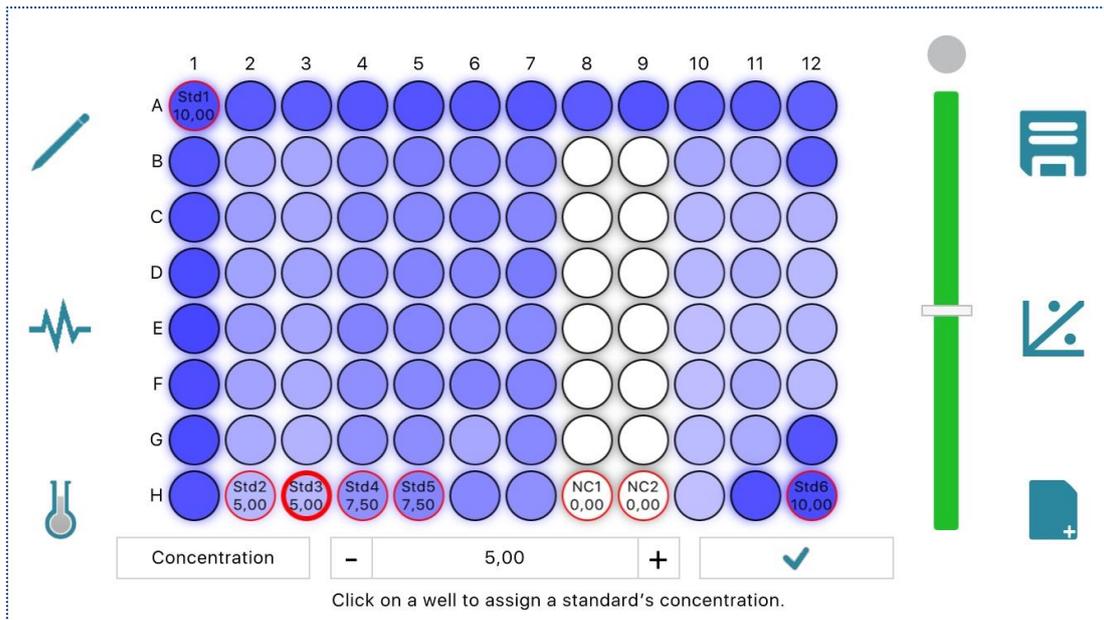


Figure 10: Setting Standards' Concentration.

Updates to the table of quantified data

Note that every update to the microplate is reflected in the *Quantified Data* page (Figure 11). This includes the wells' name and notes, their color intensity and concentration, if the data is available.

6 Assay Analysis with Standard Curve

When the microplate has been annotated, the assay standards' concentration is set, and the color intensity data is available, you can proceed to derive the assay unknowns' concentration with a standard curve.

Row	Col.	Name	Signal	Conc. (µg/µl)	Flag	Notes
H	12	Std6	70.55	10		Standard #6
H	5	Std5	47.96	7.5		Standard #5
H	4	Std4	43.53	7.5		Standard #4
H	3	Std3	27.97	5		Standard #3
H	2	Std2	28.57	5		Standard #2
A	1	Std1	70.8	10		Standard #1
H	9	NC2	0	0		Negative control #2
H	8	NC1	0	0		Negative control #1
A	2		64.89			
A	3		63.78			
A	4		66.52			
A	5		67.76			
A	6		64.88			
A	7		66.13			

Figure 11: Table of Quantified Data with Color Intensity.

The buttons on the right menu of the Assay page handle the analysis:

Button	Name	Function when tapped in Assay page
	New	Create a new assay project. Not yet supported.
	Analyze	Build the standard curve and use it to derive the assay unknowns' concentration.
	Save	Save the assay's setup to a project file. Not yet supported.

Table 6: Assay Analysis Buttons.

Analyzing the assay

Tap the *Analyze* button in the Assay page to start the analysis when the input data is ready. The app builds a regression model, currently a linear regression one, based on the standards' concentration. The derived concentration values are then shown directly in the Assay page (Figure 12).

In addition to the wells' name, you can distinguish the standard wells from the unknowns, or the samples, by the well border; the standard one is highlighted in red.

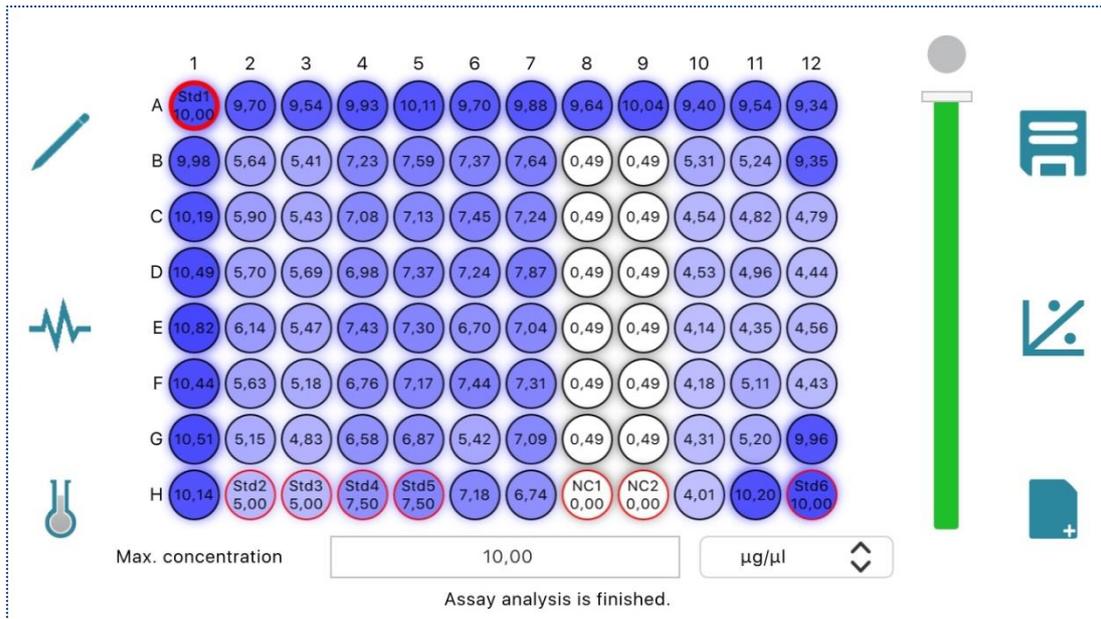


Figure 12: Derived Concentration of Assay Unknowns.

Table of quantified data

The *Quantified Data* page (Figure 13) again shows the microplate wells' address, name, notes, as well as the measured color intensity (the *Signal* column) and concentration (the *Conc.* column).

Row	Col.	Name	Signal	Conc. (µg/µl)	Flag	Notes
H	12	Std6	70.55	10		Standard #6
H	5	Std5	47.96	7.5		Standard #5
H	4	Std4	43.53	7.5		Standard #4
H	3	Std3	27.97	5		Standard #3
H	2	Std2	28.57	5		Standard #2
A	1	Std1	70.8	10		Standard #1
H	9	NC2	0	0		Negative control #2
H	8	NC1	0	0		Negative control #1
A	2		64.89	9.701275		
A	3		63.78	9.544266		
A	4		66.52	9.932976		
A	5		67.76	10.109426		
A	6		64.88	9.70005		
A	7		66.13	9.877544		

Figure 13: Table of Quantified Data with Derived Concentrations.

You can **sort** the table according to the values of a column by clicking on the column header. E.g., the table in Figure 13 is sorted according to the decreasing value of the *Notes* column.

Standard curve

The *Standard Curve* page (Figure 14) depicts the standard curve of the assay analysis. The standards, whose coordinates being concentration and color intensity, are shown on the chart, along with the fitting line (or the standard curve).

On top of the chart is the standard curve equation. Here Y means the concentration, X is the normalized color intensity, and R2 is the coefficient of determination.

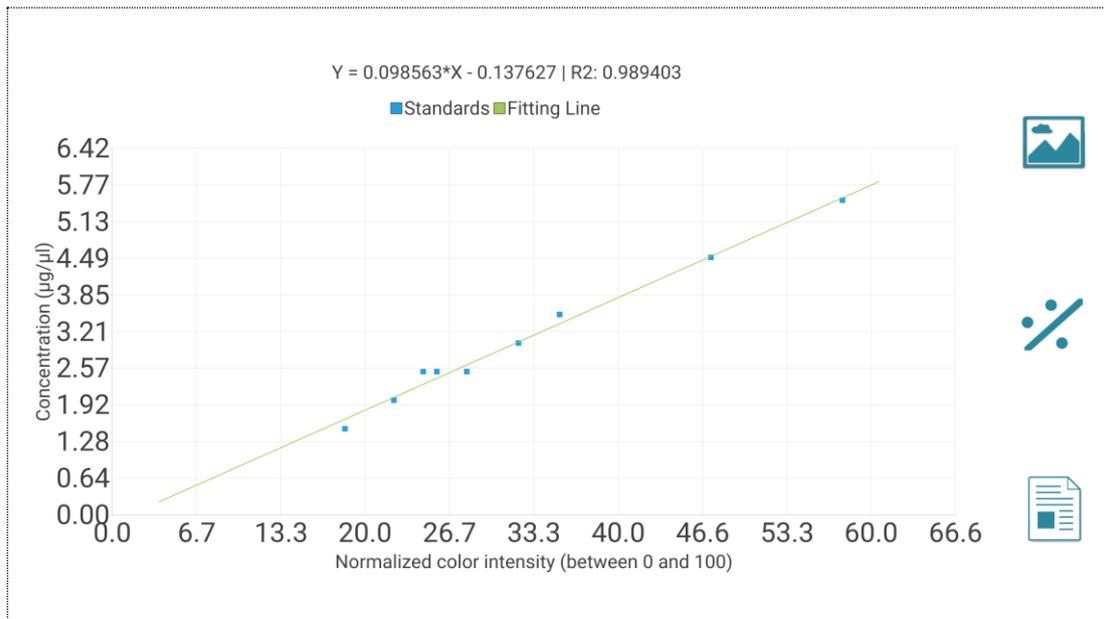


Figure 14: Standard Curve.

Tips on the chart:

- You can scale the chart up and down with two fingers.
- The chart is movable, so you can adjust its position for the best view.
- If the scaling is lost, *double-tapping* on the chart will scale it back to the original size.