PhotosynQ Tutorials

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Getting Started

Creating an account

Before you can start using PhotosynQ, you will need to create an account.

1. You can create an account from the website, the desktop app, or the mobile app.
    - Using the website: click on the ‘sign up’ button in the upper right corner of the website.
    - Using the desktop app: Download the **PhotosynQ** app from the chrome webstore and select "sign up."
    - Using the mobile app: Download the **PhotosynQ** app from the Google Playstore and select "no account? Register here."
2. Create a username and password for your account. This login will be used across the PhotosynQ platform.
3. Check your email for a confirmation.
    - If you do not see it, check your spam folder.
    - Once you confirm, your account will be been created!
4. Now go back to the website or app and sign in.

Connect an Instrument

You can use Bluetooth or USB to connect your Instrument with your device.

Depending on the instrument and device, some connection options may not be available.

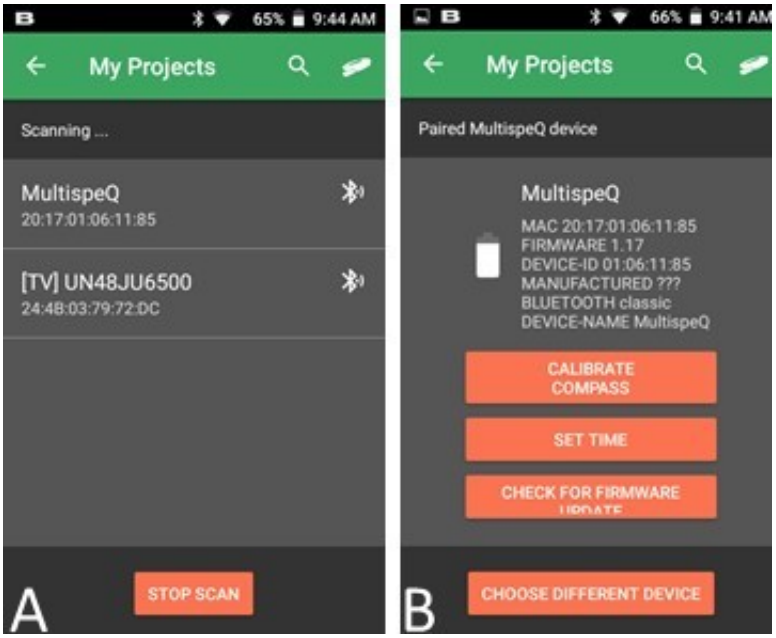
For data collection in the field, most people will use the mobile app. So lets focus on connecting the MultispeQ to your android phone. For tips on how to connect to the PhotosynQ desktop app please check out Connect an instrument in the Help Center.

Before connecting your MultispeQ to the Android or Desktop App you need to turn on the MultispeQ by pressing and holding the power button for 5 seconds. There is no indicator light to let you know if it is turned on.

Connect an Instrument: The arrow indicates the power and reset button.

1. In the app, select the instrument icon on the top right corner.
2. A list of available Bluetooth instruments will appear.
3. Below the Instrument name will be its ID. This should match the MAC address on your instrument (screen A, below)
    - If your instrument does not appear, click on **SCAN DEVICES**
    - You may have to click **SCAN DEVICES** multiple times before your instrument appears.
4. Select on the appropriate instrument.

5. A pop-up will appear asking to pair the device by entering the instrument PIN. **The PIN is 1234 and is the same for every MultispeQ.**
6. After pairing the MultispeQ, you will be taken back to the Device list. Select your MultispeQ from the list, if the screen B (below) appears your device is connected.



Android - Bluetooth: (A) Scanning for MultispeQ devices. (B) Information about the connected device.

You are now ready to take measurements with your MultispeQ!

*If you are having trouble connecting to the MultispeQ, please look for trouble shooting tips in the help center

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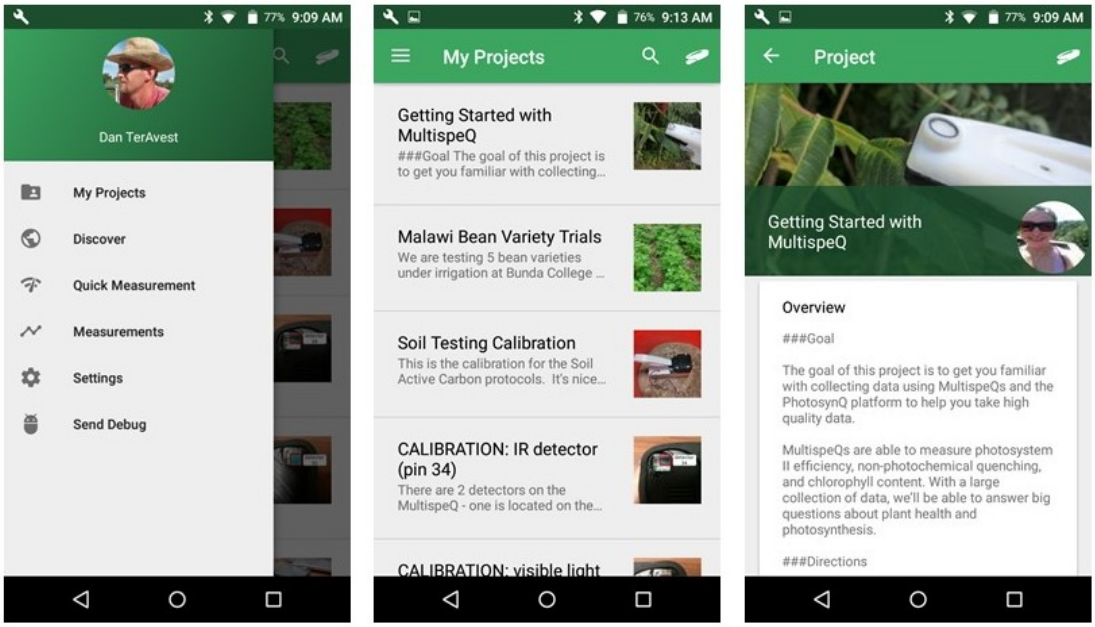
PhotosynQ Projects

Projects are the lifeblood of PhotosynQ, so it is important to understand what you are looking at!

- Inside the app, you can find all the projects you have either created or joined. You can do this by selecting the menu in the upper left corner of the app and then selecting **My Projects**.
- Everyone is automatically joined to the tutorial project, Getting Started with Multispeq
- Check out the overview and directions for the project.
    i. These are sometimes the only source of communication between the project creator and you.
    ii. Reading the directions is vital to taking proper measurements.
- Any additional questions about projects can be asked on the project discussion online.



My Projects: List of joined or created projects available for data contribution.

Take a few measurements using the **Getting Started with MultispeQ** project or create your own project.

Data Collection

Taking Quality Measurements

Once you have selected the project that you want to contribute measurements to, you can start taking quality measurements by following these steps:

1. Before clamping the leaf, answer all of the questions listed in the project
2. Select **measure.**
3. Clamp the leaf using the **Best Management Practices** listed below. The MultispeQ measures the leaf in its natural state. This means that changing the state of the leaf to take a measurement can affect your results!
4. The protocol will take ~15 seconds to complete. Once the measurement is complete, confirm that the measurement quality is good.
5. Select **ACCEPT** if you want to submit the measurement to the website or **DISCARD** if you want to discard the measurement and try again.

Tips: If you are using the default protocol **Leaf Photosynthesis v1.0** the measurement will automatically start once you have opened the clamp and closed it over the leaf. Other protocols the measurement may begin as soon as you select **Take Measurement**. - **Make sure you know when the protocol you are using begins!**

Best Management Practices

- do not position your body so you are shading the leaf or the light sensor
- do not pull the leaf out of the shade and into the sun or vice versa
- do not change the angle of the leaf, this will change how the leaf is intercepting light
- in order for the compass measurement to be accurate, clamp the leaf on the left side when facing the stem.

- Make sure the leaf completely covers the light guide. If the leaves you are measuring are too small, you may need to mask the light guides and recalibrate the MultispeQ

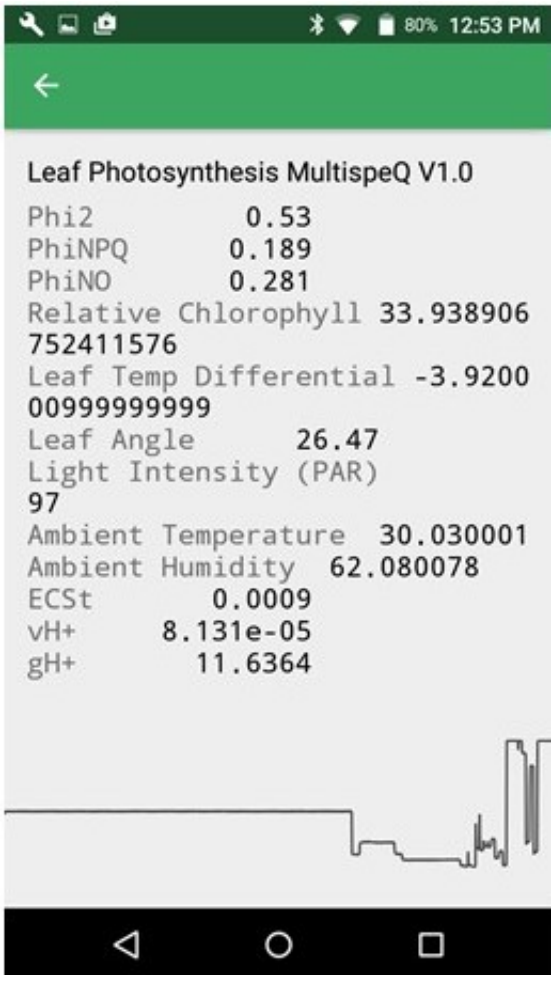
Best Measurement Practices

Understanding a Measurement

Once you have completed a measurement you will have the opportunity to examine it before submitting it to the website. Lets take a quick tour of your measurement!

Note: This section of the tutorial covers the default MultispeQ plant health protocol: **Leaf Photosynthesis v1.0**, and may not represent the results from other protocols.

The graphical representation of the measurement is called a **trace**. The parameters output by the PhotosynQ platform are generated from values within this trace.



Understanding a Measurement

Most Important Parameters

Here is a list of the most important parameters and their typical ranges. If your measurement is outside of the given ranges, your measurement may be bad and you may want to discard it and redo the measurement.

Parameter	About
Phi2	The fraction of light energy captured by Photosystem II which is directed towards Photochemistry to make ATP and NADPH and ultimately sugar for the plant to grow. `Typical range is 0 -`

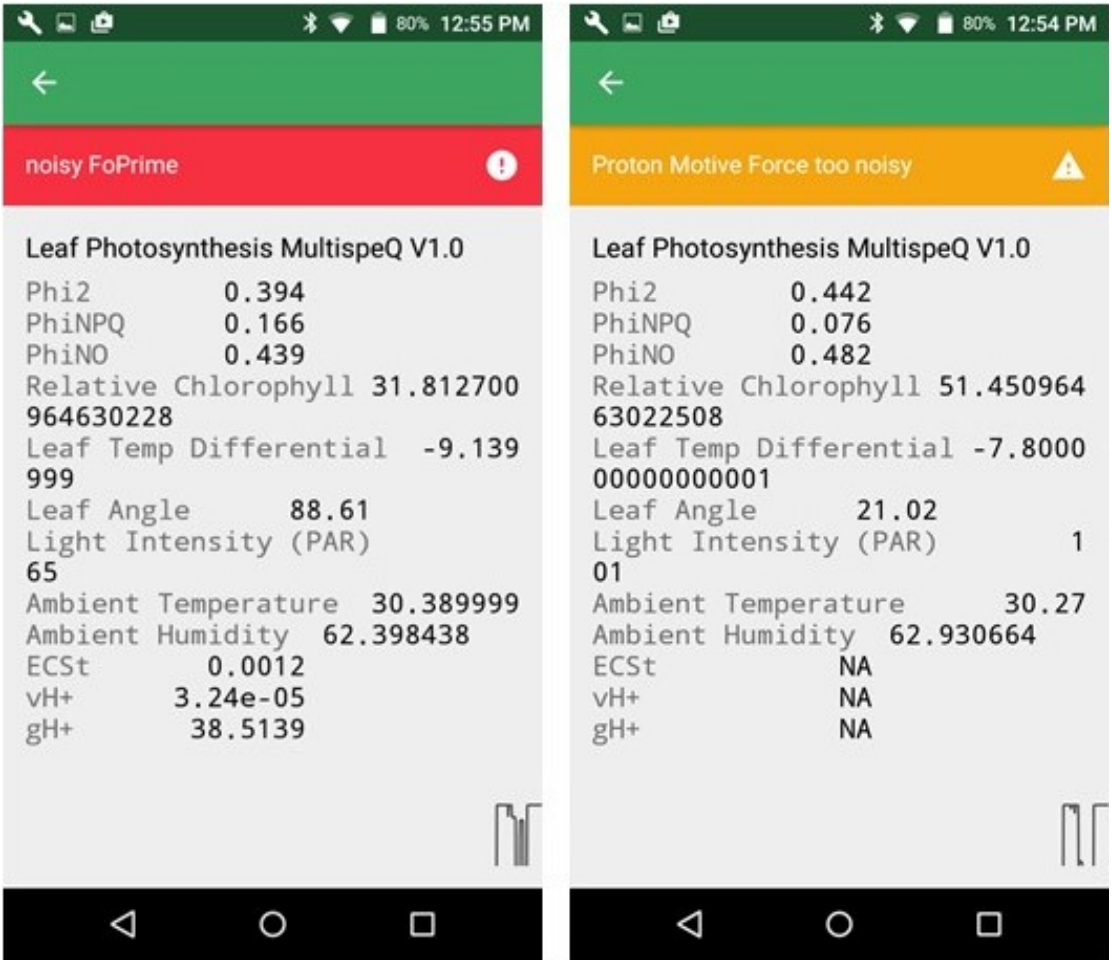
	`0.82`
PhiNPQ	The fraction of light energy captured by Photosystem II which is directed towards non-photochemical quenching and is dissipated as heat inside the leaf. The plant actively 'shedding' excess captured light to avoid photodamage. `Typical range is 0 - 0.85`
PhiNO	The fraction of light energy captured by Photosystem II that is directed...somewhere. This generally represents light energy lost to unregulated processes that can damage Photochemistry. `Typical range is 0.15 - 0.55`
Relative Chlorophyll Content	The concentration of chlorophyll in the leaf. `It ranges from 0-80 and is a relative value so it has no units.`
ECSt, vH+, gH+	These parameters describe the accumulation of protons in the thylakoid and their flow through ATP synthase which converts ADP to ATP, one of the main forms of transportable energy within the cell. This measurement often does not work well at low light intensities. Under these conditions it is common to get a pop-up message saying that the **signal is too low** or **too noisy** and you should accept the measurement. If you get this message under high light conditions, you may want to retake the measurement
Leaf Temp Differential	The difference between leaf temperature and ambient temperature in degrees Celsius. `The typical range is from -5 to +10`
Light	Photosynthetically Active Radiation in the 400 - 700 nanometer

Intensity (PAR)	wavelengths that is used for photosynthesis. `Typical ranges 0 to approximately 2000 microeinsteins (under full sun)`

If you click on **Show More** you can see many more details about the sensor readings. Additional information about PhotosynQ parameters can be found here.

Submitting Quality Measurements

Now that you are familiar with the parameters, you can check the quality of each measurement. If a measurement is out of the acceptable range or is too noisy a red **danger** or yellow **warning** notification will pop up describing the problem. Blue notifications are for information only.



Measurement Notifications

Tip: The easiest way to ensure quality data is to discard poor data before it gets submitted to the website!

One of the most common warning messages you will receive is that your data is too noisy. Noise can come from the sample shaking in the wind, the leaf slipping in the measurement chamber or a shaky hand. Stabilizing your hand and leaf stem often helps, but sometimes things are more complex. For example, if you measure a dead leaf, the app informs you that the values are very low, meaning that either you didn't measure a plant or something is probably wrong.

You can chose to keep the measurement from a dead leaf as a legitimate value or

discard it. It depends on your project goals.

If the measurement seems okay, values are in the reasonable range and there are no warnings you can go ahead and submit the measurement.

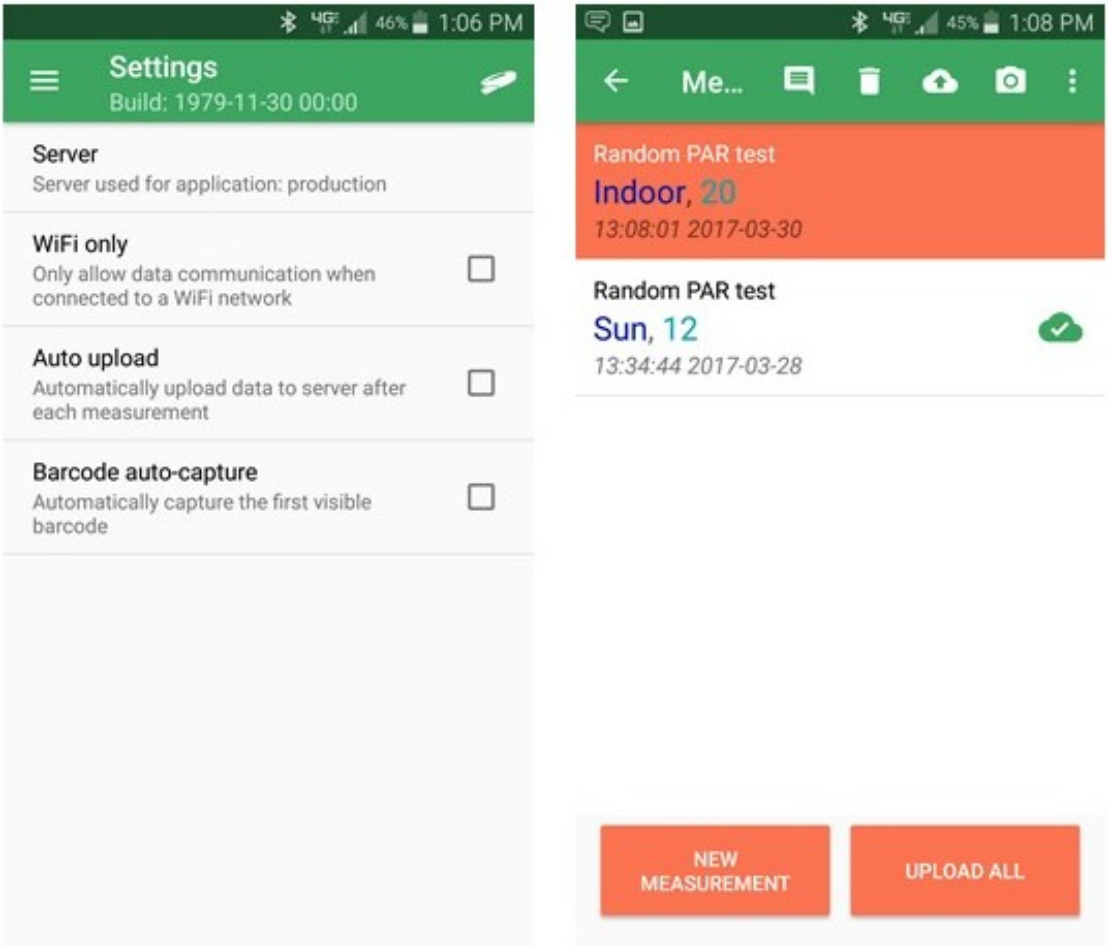
Once you submit the measurement you can see it in the **Measurements** tab, available in the menu on the android app. If there is a check next to the measurement, it has been submitted to the website.

To take another measurement, click on **new measurement**.

Submitting a Measurement

Submit Cached Data

If you would prefer to manually submit your data, or to limit the auto upload feature to when you have wifi connection only (to avoid using mobile data), go to the **Settings** tab in the mobile app menu. - This provides you more freedom to reconfirm all the measurements before submitting them to the website. - Before measurements are submitted to the website, you can add notes, pictures, or even delete measurements directly from the **Measurements** tab.



Settings

Methods of Data Collection

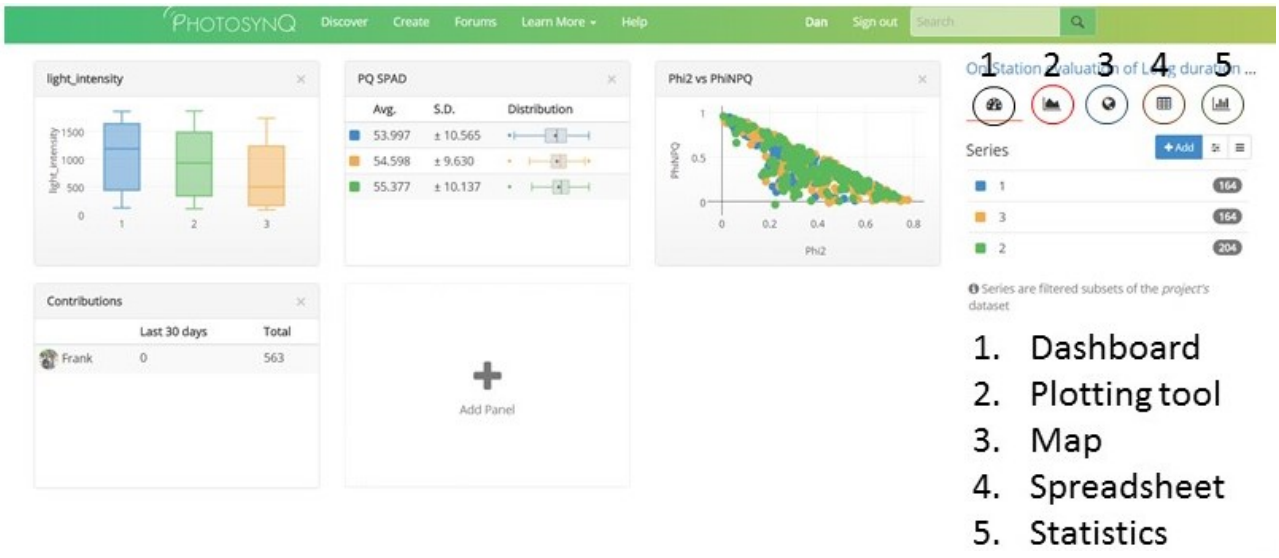
Data Viewing

Project Dashboard

As soon as you have uploaded your data from the mobile or desktop app to the website you check it out on the **Data Viewer**.

1. Go to your project page and click on **View Data** from the left side menu.
2. Wait for your data to load. This can take anywhere from a couple of seconds to a couple of minutes depending on the number of measurements in the project and the speed of your internet connection.
3. Once your project data has loaded you will land on your project's **Dashboard**.

From the **Dashboard** you can choose to graph your data, view it on a map, view it as a spreadsheet, or conduct some simple statistical tests by clicking on the appropriate icon (see below).



Filter Your Data

Looking at all of your data together may not be very informative. You can **Filter** your data to create separate **Series** that you can compare.

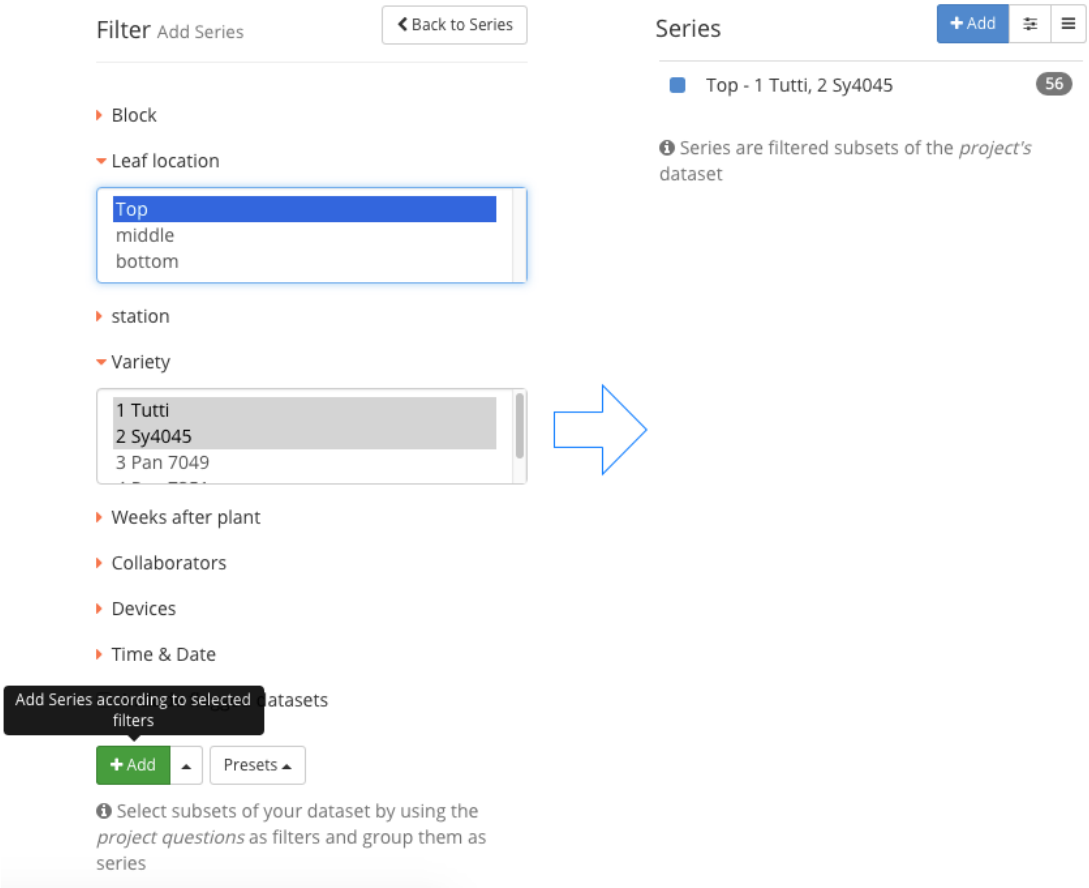
To start generating **Series** 1. Select

- **Add**

from the right site menu to show the filter dialog. 2. Expand the Project Question or other category that you want to filter by. 3. Select your answer or answers for each Question. 4. Choose whether you want to make a single series or multiple series - To add a single Series 1. Make your filter selections. 2. Select

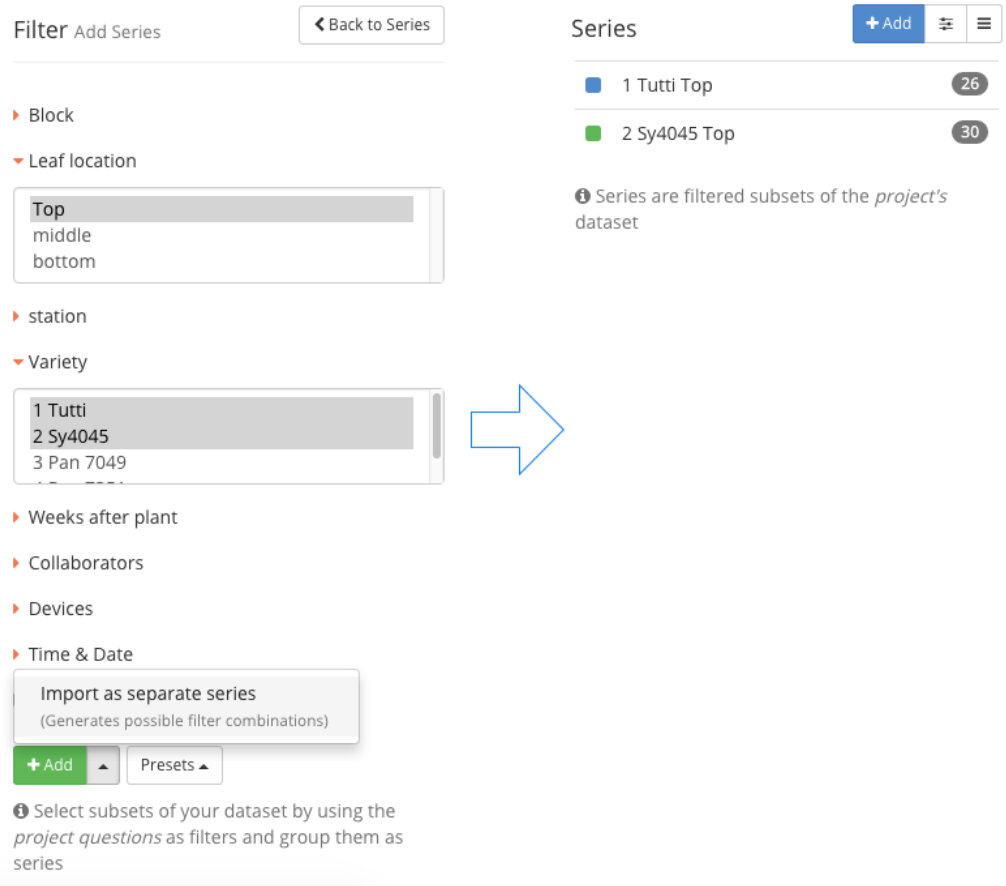
- **Add**

below the available filter options to create one series



Single Series

- To add multiple Series
  i. Make your filter selections.
  ii. Select ▲ and choose **Import as separate series**.



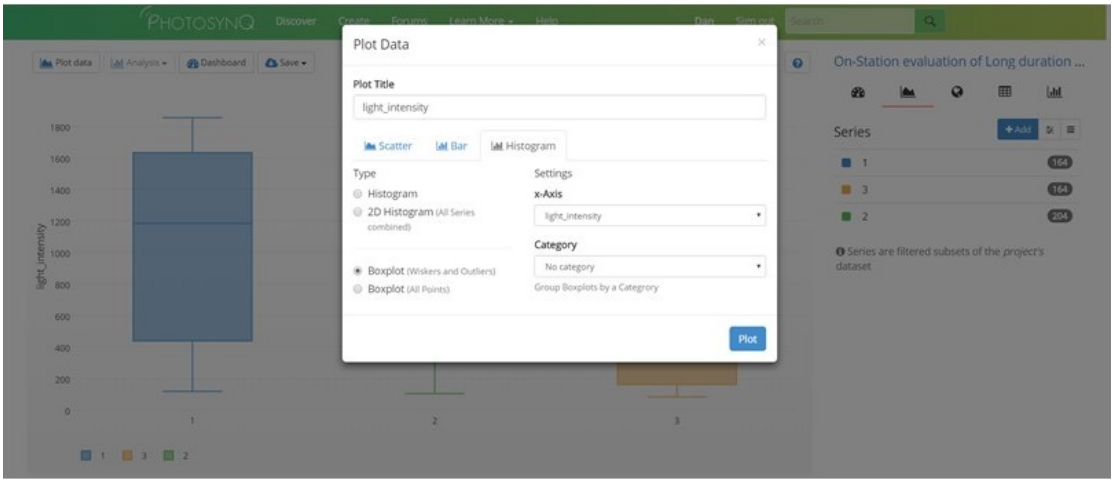
Multiple Series

Graph Data

1. Click on the graph creator icon in the data viewer.
2. Select the kind of graph that you want to create from the dialog box. You can choose between a variety of scatter, bar, and histogram charts.
3. Use the drop down menu's to choose which parameters you wish to graph.
4. After you have chosen the parameters to graph, select **Plot**.

Tip: The most important parameters will be listed as **Primary Parameters** and **Project Questions**. If the parameter you are looking for is not in these two categories, scroll to the bottom of the drop down menu and look under **Advanced**.

For more help with plotting data, please visit the Help Center.



Plotting tool

Map Data

To view your measurements on a map or generate a heatmap select the **Map** icon from the dashboard.

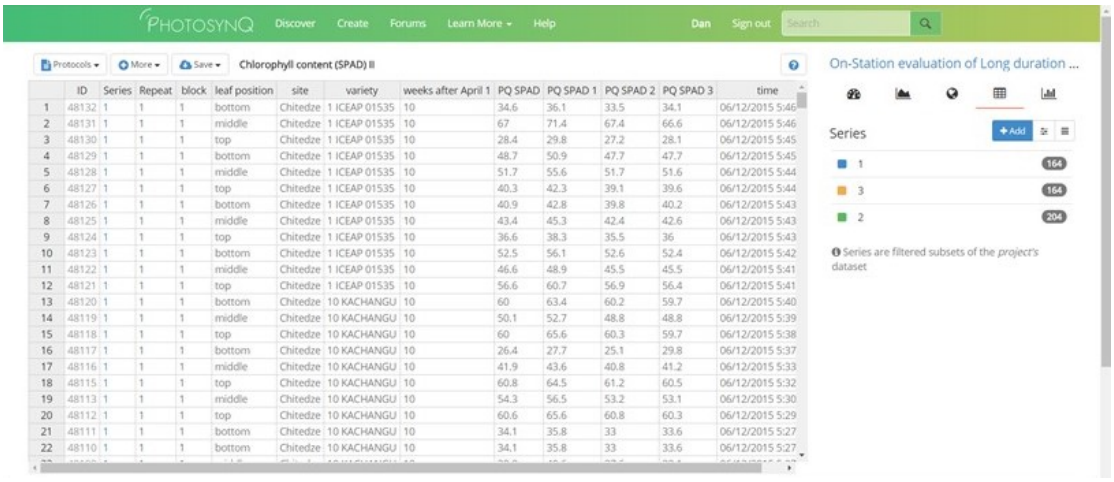
You can view your data overlaid on a satellite map or regular map and you can zoom in or out. You can also create a heatmap by selecting the parameter of interest in the upper left hand corner of the map.

Map

Data Spreadsheet

You can view your data as a spreadsheet by clicking on the **Spreadsheet** icon from the dashboard.

You have several options within the spreadsheet view: 1. Download the entire table as a csv or text file by selecting the **Save** dropdown menu. 2. Add more information to the table, including the Device ID, Latitude and Longitude, etc from the **More** menu. 3. Select which protocol you want to view from the **Protocols** menu. This only applies to projects with more that one measurement protocol.

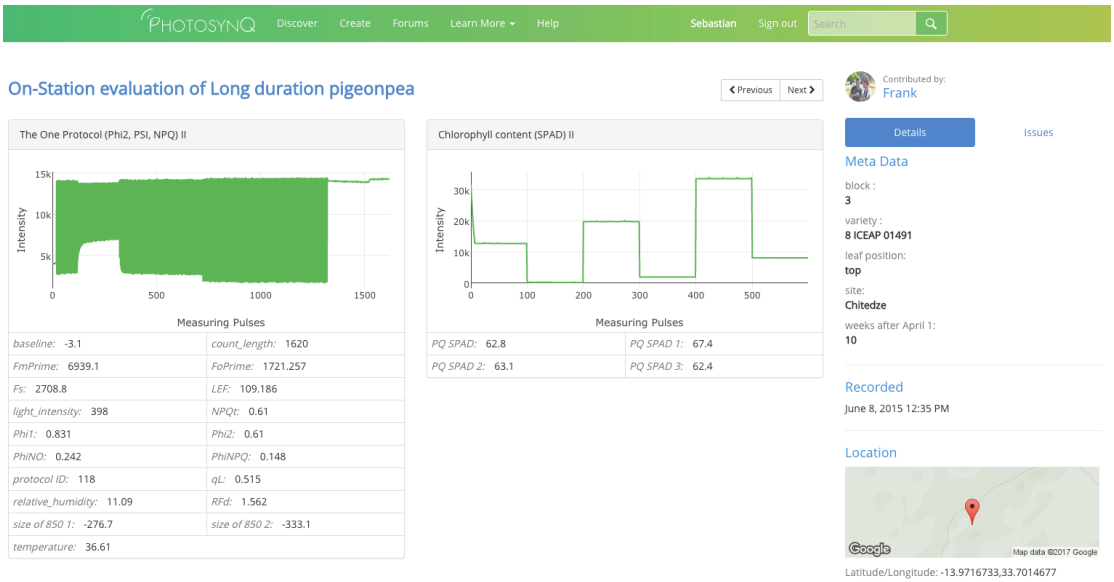


Spreadsheet

Single Measurements

In order to access a single measurement, you have multiple options:

1. Click on a marker in a scatter plot.
2. Click on a map marker and select [View Measurement] from the popup.
3. Click on an ID number in the ID column of the spreadsheet.



Single Measurement. Use the Next and Previous buttons to navigate

between measurements.

Tip: Viewing a single measurement allows you to verify a measurment and flag if necessary to indicate an insufficent quality, labeling error, etc.

Data Analysis

Analyzing your Data

Introduction

Many of the parameters measured by the MultispeQ (e.g. Phi2, PhiNPQ, and PhiNO) respond rapidly to changes in light intensity. For this reason, the analysis of PhotosynQ data often requires multivariate or more sophisticated analytical methods.

However, there are a number of simple tools available from the dashboard in the data viewer. These simple statistical tools include a summary, students t-test, and ANOVA.

Summary

A summary is created for one parameter (e.g. ΦII) at a time. A histogram to shows the distribution of values, as well as Sample Size, Median, Average, Confidence Interval of Average, Standard Deviation, Minimum, Maximum and Sum are calculated for each series. It provides a quick overview of your dataset.

Student's t-Test

A t-test compares the values of a single parameter (e.g. ΦII) between two series. If the sample size is the same for both series, a one tailed t-test can be selected. If the numbers are different a two tailed t-test. In case a one tailed t-test is picked and the sample size differs between the two series a two tailed test is performed

automatically.

ANOVA

Analysis of variance (ANOVA) compares a single parameter (e.g. ΦII) between more than two series. A One-Way ANOVA should be used when the series are created using one filter (e.g. Leaf #). This rule may not apply if the project is looking for several plant species and a second filter is used to select only one species.

Advanced Analysis

These are basic tutorials on how to do advanced data analysis outside the data viewer and use the available packages.

Tutorial	Python	R-Studio
Import PhotosynQ Data	View	View | PDF | .Rmd
Anova and Multivariate Analysis	×	View | PDF | .Rmd
Correlation and Mixed Effects	×	View | PDF | .Rmd

Protocols

Why do PhotosynQ measurements require Protocols and Macros?

On the PhotosynQ platform, we use **Protocols** to provide specific measurement instructions to the instrument, such as the MultispeQ. Every time a measurement is taken, the Protocol is sent to the instrument, and the results are sent back.

You can choose to attach a **Macro** to a Protocol. Macros are used to make calculations after a measurement has been taken. Not every measurement requires post processing (e.g. a simple temperature measurement), but if you want to calculate a parameter from the measurement **Trace** or want to compare parameters (e.g. ambient temperature vs. leaf temperature), a Macro will calculate the parameters of interest and display the results instantly on your mobile device (e.g. a phone).

The steps involved in taking a measurement

How do Protocols work

Protocols are written in the **J**ava**S**cript **O**bject **N**otation or JSON. It's important to note that most scripting languages have the capability to parse, modify and validate a protocol. If the Protocol is sent to the instrument, it needs to be *parsed as a string* before is gets sent. Unless you build your own application, the PhotosynQ apps will take care of that for you.

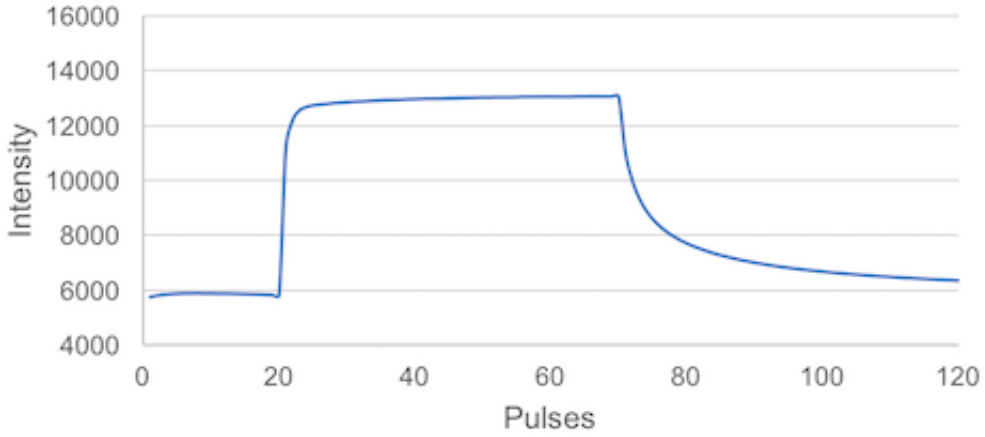
Before you Get Started

In order to build your first Protocol, make sure you have the Desktop App installed. You will also need an Instrument like the MultispeQ to test your protocol. 1. Select **Protocols** from the menu and click on `+ New` or select the **Protocol Editor** directly. 2. Check out the detailed documentation on how to create a protocol in our wiki on github 3. Make sure you have your instrument connected properly, so

you can click on ▶ Run to test your Protocol 4. Now you are ready to create your first Protocol...

Measuring Photosystem II efficiency

In this tutorial, we show you how to acquire a simple Phi2 value using the MultispeQ. Before we start, lets take a look at the measurement.



The measurement is divided into three parts:

1. **20** Pulses at ambient light intensity
2. **50** Pulses at a saturating light intensity
3. **20** Pulses at ambient light intensity

This is all we need to record the photosystem II quantum efficiency, or Phi2. The following protocol has another 4000 pulses prior to the above-mentioned protocol, to adapt the leaf to the ambient light intensity, which is recreated inside the MultispeQ instrument.

Pulses

A measurement is divided into pulses. Pulses can be grouped into pulse sets. The

example below shows a total of **4,090** pulses grouped into **4** pulse sets. Most of the following parameters require you to define those **4** groups. `pulses` defines those groups, `pulse_distance` defines how far apart each pulse is (in *µs*). The command `pulse_length` defines the pulse duration in *ms*.

Table View

pulses	pulse_distance	pulse_length
4000	1000	30
20	10000	30
50	10000	30
20	10000	30

Advanced View

```
[
    {
        "pulses": [
            4000,
            20,
            50,
            20
        ],
        "pulse_distance": [
            1000,
            10000,
            10000,
            10000
        ],
        "pulse_length": [
            [
                30
```

```
            ],
            [
                30
            ],
            [
                30
            ],
            [
                30
            ]
        ],
        ...
    }
]
```

Pulsed lights

Once we have defined are pulse groups, we need to define the lights we want to use to probe the fluorescence. `pulsed_lights` defines which lights are pulsed during each pulse set. `0` means that there is no light pulsing, `3` uses the 605 nm LED (amber), Lumileds LXZ1-PL01. `pulsed_lights_brightness` defines the light intensity of each pulse. Since multiple lights can be pulsed, lights or brightness are written like `[3]` this and not simply like `3`. Multiple light would be written in this way: `[2,3]`.

Table View

pulsed_lights	pulsed_lights_brightness
0	0
3	2000
3	2000

3	2000

Advanced View


```
[
    {
        ...,
        "pulsed_lights": [
            [
                0
            ],
            [
                3
            ],
            [
                3
            ],
            [
                3
            ]
        ],
        "pulsed_lights_brightness": [
            [
                0
            ],
            [
                2000
            ],
            [
                2000
            ],
            [
                2000
            ]
        ],
        ...
    }
```

```
]
```

Non Pulsed Lights

In this protocol we need an actinic light (which is not pulsed), so the plant has light available to continue doing photosynthesis during the measurement. To set the intensity we use the command `light_intensity` to reproduce the ambient light intensity, which is recorded by the PAR sensor. Light `2` is the 655 nm LED (red), Lumileds LXZ1-PA01.

Table View

nonpulsed_lights	nonpulsed_lights_brightness
2	light_intensity
2	light_intensity
2	4500
2	light_intensity

Advanced View

```
[
   {
       ...,
       "nonpulsed_lights": [
           [
               2
           ],
           [
               2
           ],
```

```
            [
                2
            ],
            [
                2
            ]
        ],
        "nonpulsed_lights_brightness": [
            [
                "light_intensity"
            ],
            [
                "light_intensity"
            ],
            [
                4500
            ],
            [
                "light_intensity"
            ]
        ],
        ...
    }
]
```

Detectors

Next we have to define the detector we want to use to record the fluorecence coming off the leaf. We use the command `detectors` to define which detector we will use for each pulse set. Since we can use multiple detectors per pulse set we use `[1]` instead of the `1` notation (using two detectors would look like this: `[1,2]`). When the detector is set to `0` no data is captured. Detector `1` is the 700 nm - 1150 nm, Hamamatsu S6775-01.

Table View

detectors
0
1
1
1

Advanced View


```
[
    {
        ...,
        "detectors": [
            [
                0
            ],
            [
                1
            ],
            [
                1
            ],
            [
                1
            ]
        ]
        ...,
    }
]
```

Environmentals

To record the ambient light intensity required for the non pulsed lights intensity, we have to add a command to include the PAR sensor using `light_intensity`. This is also where you could add other environmental parameters like temperature, relative humidity, etc, depending on the sensors available in your instrument.

Table View (Fixed)

```
environmental
[
    [
        "light_intensity"
    ]
]
```

Advanced View

```
[
    {
        ...,
        "environmental": [
            [
                "light_intensity"
            ]
        ],
        ...
    }
]
```

Start measurement

To start the measurement as soon as we have clamped the leaf, in order to perturb it as little as possible, we add the following command: `1` indicates the

measurement starts as soon as the clamp is closed and `0` starts the measurement as soon as you select `Start Measurement` on your device.

Table View (Fixed)

```
open_close_start
1
```

Advanced View

```
[
    {
        ...,
        "open_close_start": 1
    }
]
```

The final Protocol

Putting all the pieces together, the protocol to measure Phi2 looks like this:

Table View

pulses	pulse_distance	pulse_length	pulsed_lights	pulsed_lights_
4000	1000	30	0	0
20	10000	30	3	2000
50	10000	30	3	2000
20	10000	30	3	2000

```
environmental
[
    [
        "light_intensity"
    ]
]

open_close_start
1
```

Advanced View

```
[
    {
        "pulses": [
            4000,
            20,
            50,
            20
        ],
        "pulse_distance": [
            1000,
            10000,
            10000,
            10000
        ],
        "pulse_length": [
            [
                30
            ],
            [
                30
            ],
            [
                30
            ],
```

```
            [
                30
            ]
        ],
        "pulsed_lights": [
            [
                0
            ],
            [
                3
            ],
            [
                3
            ],
            [
                3
            ]
        ],
        "pulsed_lights_brightness": [
            [
                0
            ],
            [
                2000
            ],
            [
                2000
            ],
            [
                2000
            ]
        ],
        "nonpulsed_lights": [
            [
                2
            ],
            [
                2
```

```
            ],
            [
                2
            ],
            [
                2
            ]
        ],
        "nonpulsed_lights_brightness": [
            [
                "light_intensity"
            ],
            [
                "light_intensity"
            ],
            [
                4500
            ],
            [
                "light_intensity"
            ]
        ],
        "detectors": [
            [
                0
            ],
            [
                1
            ],
            [
                1
            ],
            [
                1
            ]
        ],
        "environmental": [
            [
```

```
                "light_intensity"
            ]
        ],
        "open_close_start": 1
    }
]
```

Tip: Continue with the Macro Tutorial to learn how to calculate Phi2 from the recorded measurement.

Macros

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You can choose to attach a **Macro** to a Protocol. Macros are used to make calculations after a measurement has been taken. Not every measurement requires post processing (e.g. a simple temperature measurement), but if you want to calculate a parameter from the measurement **Trace** or want to compare parameters (e.g. ambient temperature vs. leaf temperature), a Macro will calculate the parameters of interest and display the results instantly on your mobile device (e.g. a phone).

The steps involved in taking a measurement

How do Macros work

Macros are small snippets of code, which run calculations based on your measurements. They are written in the popular script language JavaScript.

Before you Get Started

In order to build your first Macro, make sure you have the Desktop App installed. You will also need a Protocol with an output that you want to analyze. In this example, we will take the Protocol from the Tutorial as a basis for this Macro.

1. Select **Macros** from the menu and click on `+ New`
2. Select your measurement by searching your `Notebook`
3. Now you are ready to start coding...

Calculating Photosystem II efficiency

In the previous tutorial we built a protocol to measure photosystem II efficiency. Now we can build a simple macro to automatically calculate it every time you take a measurement.

Initial Code

```
//============================================
// Macro for data evaluation on PhotosynQ.org
// created: 4/7/2017
//============================================
```

```
//Define the output object here
var output = {};

//Check if the key time exists in json
if (json.time !== undefined){

    //Add key time and value to output
    output["time"] = json.time;
}

//Return data
return output;
```

Accessing the recorded Trace

In order to calculate the parameters **Fs** (steady state fluorescence) and **Fmp** (maximum fluorescence), you have to access the recorded fluorescence trace. The Macro editor allows you to select the regions, by using the graph of the trace. In the example below, check range and select the region of interest. Then click on the ⟷ icon to add the selected range into your code, `json.data_raw.slice(63,68)` in this case. We use the already pre-defined method `MathMEAN( array )` from the Function Menu to calculate the mean of the values in the selected range.

Selecting a range of values using the Macro editor

```
var fs = MathMEAN(json.data_raw.slice(1,5));
var fmp = MathMEAN(json.data_raw.slice(63,68));
```

Deriving values and adding them to the output

Now we can calculate Phi2 and LEF. For LEF we also need the light intensity. We can insert the light intensity by selecting `light_intensity` from the variables in the top menu.

```
var phi2 = (fmp-fs)/fmp;
var lef = phi2 * json.light_intensity * 0.4;
```

Defining the Macro Output

Finally we can return the results by adding the calculated values to the `output` object.

```
output['Fs'] = fs;
output['Fmp'] = fmp;
output['Phi2'] = phi2;
output['LEF'] = lef;
output['PAR'] = json.light_intensity;
```

The Final Macro

```
//============================================
// Macro for data evaluation on PhotosynQ.org
// created: 4/7/2017
//============================================

//Define the output object here
var output = {};
```

```
var fs = MathMEAN(json.data_raw.slice(1,5));
var fmp = MathMEAN(json.data_raw.slice(63,68));

var phi2 = (fmp-fs)/fmp;
var lef = phi2 * json.light_intensity * 0.4;

output['Fs'] = fs;
output['Fmp'] = fmp;
output['Phi2'] = phi2;
output['LEF'] = lef;
output['PAR'] = json.light_intensity;

//Return data
return output;
```

Output

```
Fs = 5817.25
Fmp = 13056.6
Phi2 = 0.554
LEF = 3.770
PAR = 17
```

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Warning:
Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

NOTE:
This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

FCC Statement:
This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:
—Reorient or relocate the receiving antenna.
—Increase the separation between the equipment and receiver.
—Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
—Consult the dealer or an experienced radio/TV technician for help.