Getting Started

Creating an Account

Before you can start using PhotosynQ, you will need to create a free 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 Desktop app from PhotosynQ and select "sign up."
   - Using the Mobile app: Download the Mobile 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 let’s 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 – Desktop or Connect an Instrument – Mobile.

**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.
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 on Connect an Instrument – Desktop or Connect an Instrument – Mobile

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.
  1. These are sometimes the only source of communication between the project creator and you.
  2. 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.

Creating a Project

Before You Start...

Before you go on the PhotosynQ website to create your project, first you have a few things to think about...

1. “What is it I am trying to learn from this project?”
2. “What are the different factors I am testing?”
3. “How can I break down and categorize each sample I am taking”

Imagine you are working on the field in the picture below. Your goal is to figure out which varieties of your crop grow best using which treatment. What types of questions would you ask in this scenario? In order to know which ones to ask, ask yourself the three questions above and build from there.

1. I am trying to learn how different varieties respond to treatments.
2. Each individual plot will have a certain variety of my crop, treatment and other project design questions paired with it, like leaf position, or replication.
Field with crops grown under five different treatments

New Project

Log into PhotosynQ and select Create from the menu bar at the top of the page or from the menu underneath your picture on your user page. Give it a good name that relates to your project. Naming your project just “crop testing” would be a poor idea, as there are dozens of projects already about crops or with just the name “test” (you know who you are). Something like “Crop Response to Unique Treatments October 2017” will make your project distinct and easily searchable.
Select the category that best fits your project

![Category Options]

Crop Response to Unique Treatments October 2017

Make sure to pick a descriptive Project title. You can make changes later.

**Good:** "Screen for drought resistant bean varieties in Melawi"  **Not Good:** "Bean test 2"

Start a new Project by choosing a category and a descriptive name

**Protocols**

**Pick the protocol** that best suits your device/needs, for 99% of people the pre-selected protocol will do. If you have an Instrument other than a MultispeQ v1.0, or maybe small leaves, this protocol will be different. In this case select a protocol from the advanced list. Also, be sure to read the protocol description to make sure that it measures the parameters you are interested in and is compatible with your device and firmware version.

Carefully read the Protocol descriptions, before making your selection. Unless you have very specific needs, you don’t have to select any of the advanced protocols. Make sure, the Protocol works with your device!

Select a Protocol. The standard protocol will be sufficient for most users.

**Questions**
Here is where you need to take your answers to the aforementioned questions and turn them into questions and answers that will help you achieve your learning goals. We decided we would have questions for...

1. Variety, we have to know which variety we tested
2. Treatment, we have to know how the crop was managed
3. Leaf Position, maybe the treatment will affect lower leaves differently than top leaves
4. Replication, every good experiment has multiple reps after all!

Project questions are answered before every measurement and help to document, categorize, and analyze the data collected for your project. To avoid inconsistency, you should add all project questions before you start data collection!

Add questions to collect additional information

Tips and Tricks for Choosing the Best Project Questions

Knowing how answers work is key for fast data collection and successful data analysis later on.

1. **Multiple Choice**, is the safest and quickest option, provided you know all the possible answers.
2. **Short Answers** can used to provide answers to questions, but be careful! You might answer “red corn” once and then “Red corn.” Both may be the correct, but they will be sorted as different answers by the data explorer, since the answers are case sensitive.
3. **Multiple Choice with Pictures**, is useful if you want to provide visual guidance, other than that, it is the same as a Multiple Choice Questions. Pictures cannot be analyzed in PhotosynQ.
4. **Take a Picture** questions can be cumbersome on both the measurer and when analyzing data later on. If a picture is only sometimes needed, use the **notes function** with the [Desktop](#) or [Mobile](#) Applications instead.
You might notice there is a question for uploading questions/answers via a CSV, and this can be a powerful tool, especially if you have a large, well thought out experiment.

1. We only have a few varieties of one crop to test, but if we had many varieties, and we knew how our field was laid out, we could preload exactly how we plan to walk through the field.
2. Interested in CSV upload for a larger field? Check out our Guide [here](#).

**Location**

In the next screen, you will be asked to provide a Project Location. This is just so others have an idea of where the project is taking place. A project can have multiple locations, if you, for example, have a group of collaborators around the world.

![Map of East Lansing, MI, United States](#)

*Add locations to your project*

**Description**

The Description Page is next, and here you will fill in information about your project so that others can have a better understanding of your project.

![Project Description](#)

*Add locations to your project*

1. You can fill out a full description of what your learning objectives are, how and where the experiment will be conducted, and maybe how others can get involved.
and help out if it is a more open, collaborative project. Use the preview button to see what the text looks like if you use formatting.

2. You will also need to categorize your project, so that it can be properly sorted, maybe you are doing your research project, and so you would pick the research category, or maybe you are working with students and the education selection would be more appropriate.

3. Finally add any tags you want, these are like hashtags and allow other users to find your project through these keywords. For example you could look up “trees” or “nematodes” and see all the other projects working on those, and maybe connect and share with those researchers.

**Review**

The review step allows you to see all the information about your project up to this point. If all information is the way you want it, continue to the next step, otherwise navigate directly to the previous steps to make your changes.

**Settings**

**Who can contribute data to your project?**

- Anyone
- Collaborators only

**Invite collaborator**

- Invite as
  - Collaborator
  - Administrator

**Invitations**

- Invitations
  - No invitations

**Project Settings**

The last step is to select who can contribute to your project and who you would like to invite or collaborate or be an administrator for the project besides you.

If you select “Collaborators only”, you have to invite collaborators who are allowed to contribute data to your project. Otherwise anybody can contribute. Invited users can either be collaborators, who can contribute data or Administrators, who can contribute data and also edit a project. These settings have no impact on viewing the data. Any member of the PhotosynQ community can view your Project data.

Finally, after you have created or edited your project, you need an internet connection on your mobile or desktop device to update your project in the app.

**Connecting and Collecting Data on Your Project**
You will need to use the PhotosynQ mobile or desktop app to collect data, only the PhotosynQ apps can connect to the MultispeQ Instrument, go here for connection tips.

Once your project is updated and you have connected to your Instrument like the MultispeQ, you can collect a large volume of data and store it locally on the app, even if you do not have internet connection. Once you have finished collecting data, you will need to upload your data to the PhotosynQ server.

When you collect data in your project, the way it is collected, and later interpreted/displayed is dependent on the protocol and the associated macro selected in project creation. If you chose Leaf Photosynthesis V1.0 like most, the measurement should take about ~15 seconds and return data on your phone that looks something like this:

![Measurement Screen]

Measurements are either cached on the phone, or uploaded automatically to your project. Your results can be viewed and analyzed online by logging into PhotosynQ and finding your project in your profile. While you can see individual measurements on your phone, logging on to PhotosynQ will allow you to look at your whole dataset.

For more help on how to collect high quality data, best measurement practices, uploading cached data or some tricks for data collection day, check out our [Data]
**Collection Tutorial!**

**Viewing Project Data**

Data can be viewed using the PhotosynQ data explorer on the PhotosynQ website.

After your day of data collection, viewing PhotosynQ data is quick and easy! Log in to your PhotosynQ online account and go to your profile page by clicking your name on the home-screen. After this, find your project on the dashboard, click and allow your landing page with your data to load up. For a more in depth look into data viewing, series creation, making graphs or downloading your data, check out our tutorial here. If you need some more help understanding the online data analysis tools, we have a guide for that too, check it out here.

**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 Measurement 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.

**Tip**

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 Measurement Practices**

- Do not position your body so you are shading the leaf or the light sensor (A)
- 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 (B). 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. Let's 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>About</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phi2</td>
<td>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. <em>Typical range is 0 – 0.82</em></td>
</tr>
<tr>
<td>PhiNPQ</td>
<td>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 photo damage. <em>Typical range is 0 – 0.85</em></td>
</tr>
<tr>
<td>PhiNO</td>
<td>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. <em>Typical range is 0.15 – 0.55</em></td>
</tr>
<tr>
<td>Parameter</td>
<td>About</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Relative Chlorophyll Content</td>
<td>The concentration of chlorophyll in the leaf. It ranges from 0–80 and is a relative value so it has no units.</td>
</tr>
<tr>
<td>ECSt, vH+, gH+</td>
<td>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.</td>
</tr>
<tr>
<td>Leaf Temp Differential</td>
<td>The difference between leaf temperature and ambient temperature in degrees Celsius. The typical range is from -5 to +10</td>
</tr>
<tr>
<td>Light Intensity (PAR)</td>
<td>Photosynthetically Active Radiation in the 400 - 700 nanometer wavelengths that is used for photosynthesis. Typical ranges 0 to approximately 2000 microeinsteins (under full sun)</td>
</tr>
</tbody>
</table>

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**.

![Image of the Measurements tab](image)

**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.
Methods of Data Collection
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).

---

**Dashboard**

**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

---

To add multiple Series
1. Make your filter selections.
2. Select ▲ and choose **Import as separate 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 heat-map 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 heat-map by selecting the parameter of interest in the upper left hand corner of the 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 measurement and flag if necessary to indicate an insufficient quality, labeling error, etc.

Data Quality

Identifying Measurements with Issues

As we explained in the Data Collection tutorial, the best way to keep your data set clean is to discard low quality measurements before they get submitted to PhotosynQ. To help users filter out bad measurements, we have added a series of issue warnings to the default Leaf Photosynthesis MultispeQ v1.0 and Photosynthesis RIDES protocols. These issue warnings are displayed in red on the results screen of both the android and desktop apps. We recommend that users discard measurements with warning message’s, unless they are certain the measurement is accurate. If a poor quality measurement does get submitted to the website you will be able to Flag the data in the data explorer (see below).

Tip

The Leaf Photosynthesis MultispeQ v1.0 protocol also has yellow issue warnings as well. These are for information only and you should NOT discard measurements for yellow warnings unless you are sure the measurements is bad.

Issue warnings on the android (left) and desktop (right) apps
Common reasons for poor quality measurements

- The device or leaf was not held steady throughout the measurement. This can be due to taking measurements in windy conditions or the data collector's hand shaking.
- The leaf did not fully cover the light guide. This is especially problematic for absorbance measurements such as relative chlorophyll content. This often results in an issue warning stating that the relative chlorophyll content is either very low or out of the expected range.
- The leaf was dead or dying. Leaves in this condition can cause the Phi2, PhiNPQ or PhiNO values to be out of the expected range. This may be a completely valid measurement, or a measurement that should be discarded.

Identifying Measurements with "issues"

When viewing your Project data you can use the Data Quality - Issues panel on your Dashboard that provides information about how many of the non-flagged measurements have issue warnings. Alternatively you can also view the measurements in the Spreadsheet to identify measurements with issues.

Dashboard

If the panel is not visible, you can add it to your dashboard by:

1. Select Add Panel in the dashboard
2. Navigate to the Dashboard menu on the side bar and select the Show Issues button below Data Issues
1. Add panel to the dashboard. 2. Open the Data Quality tab from the Dialog and select the Show Issues button. 3. The panel will be added to the dashboard.

Note

Only non-flagged measurements with issues will be displayed on the dashboard.

Spreadsheet

1. In the Spreadsheet view, there is an Issues column that will display how many issues exist for a given measurement (left).
2. In the single datum view, any issues will be present as a red bar above the measurement results (right).
1. Identifying issues in the spreadsheet (left) 2. Click on the ID in the first column to select bring up the data view. 3. See the details in the single datum view.

Flagging data

If you submit data with issues to the website, you will still have the opportunity to remove those measurements from future data viewing and analysis. This is accomplished by Flagging the measurements that have issues. Flagging hides measurements, so that they are not added to filtered series or downloaded as a csv for data analysis. However, flagging data DOES NOT delete the measurements from the website. Flagged data can always be viewed by clicking the **Include flagged datasets** box in the **Add Series** tab.
1. Select the **add** button to create a new Series. 2. Check **Include flagged datasets** to add flagged datasets into your Series as well.

You can flag any measurement, for any reason. However, you have to provide a reason for flagging data, which will be visible to the community. Our hope is that data is only flagged for significant issues, such as the measurement issues mentioned above or because a measurement was mislabeled, etc, and not simply because the measurement is an outlier.

**Who can flag Measurements**

Within a given Project, the only people who can flag a measurement are:

- The Project lead
- Anyone who has administrative rights for that Project
- The individual who collected the measurement of interest

**How to flag a measurement**

Once you have identified the measurement that you wish to flag you need to:

1. Open the single datum view for that measurement by either selecting the datum id in the spreadsheet view or by clicking on the measurement in the plotting tool or
2. Select the Issues tab on the right side of the screen.
   a. If you have permission to flag that measurement, a text box will appear under the heading **Reason for flagging**.
   b. If you do not have permission to flag that measurement, you will get a message stating: Click here to report data issues in the Project discussion forum.
3. Enter the reason for flagging the measurement into the text box.
4. Click **Submit**

1. **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. Phi2) 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. $\Phi$) 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. $\Phi$) 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.

**Chi Square Test**

A chi-square test for independence compares two parameters in a Project to see if they are related. In a more general sense, it tests to see whether distributions of categorical variables differ from each another.

**Advanced Analysis**

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

<table>
<thead>
<tr>
<th>Tutorial</th>
<th>Python</th>
<th>R-Studio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Import PhotosynQ Data</td>
<td>View</td>
<td>View</td>
</tr>
<tr>
<td>Anova and Multivariate Analysis</td>
<td>×</td>
<td>View</td>
</tr>
<tr>
<td>Correlation and Mixed Effects</td>
<td>×</td>
<td>View</td>
</tr>
</tbody>
</table>

**Building a Protocol**

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.
The steps involved in taking a measurement

How do Protocols work

Protocols are written in the JavaScript Object Notation 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 it 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. Navigate to File → New Protocol... to open the Protocol Editor and start a new Protocol.
2. Check out the documentation on individual commands in the here.
3. Make sure you have your Instrument connected properly, so you can click on ► Run or use the shortcut Ctrl/⌘ + ↵ 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 can be divided up 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.

Pulses

A measurement is divided into pulses. Pulses can be grouped into pulse sets. The example below shows a total of 90 pulses grouped into 3 pulse sets. Most of the following parameters require you to define those 3 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 μs.

```json
{
    "pulses": [
        20, 50, 20
    ],
    "pulse_distance": [
        10000, 10000, 10000
    ],
    "pulse_length": [
        [ 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].

```
{
  ...
  "pulsed_lights": [
    [ 3 ], [ 3 ], [ 3 ]
  ],
  "pulsed_lights_brightness": [
    [ 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.

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

**Detectors**

Next we have to define the detector we want to use to record the fluorescence 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.

```
{
  ...
  "detectors": [
    [ 1 ], [ 1 ], [ 1 ]
  ]
  ...
}
```
Environmental Parameters

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.

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

Starting the 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.

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

The final Protocol

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

```json
[
  {
    "pulses": [ 
      20, 50, 20 
    ],
    "pulse_distance": [ 
      10000, 10000, 10000 
    ],
    "pulse_length": [ 
      [ 30 ], [ 30 ], [ 30 ]
    ],
    "pulsed_lights": [ 
      [ 3 ], [ 3 ], [ 3 ]
    ],
    "pulsed_lights_brightness": [ 
      [ 2000 ], [ 30 ], [ 30 ]
    ],
    "nonpulsed_lights": [ 
      [ 2 ], [ 2 ], [ 2 ]
    ]
  }
]
Save the Protocol

Now it is time to save the Protocol so you can use it in the future. Select Save from the File menu or use the shortcut Ctrl/⌘ + S to save the Protocol to the PhotosynQ platform. Provide a meaningful name, description and select a category your Protocol falls into. You don't have to select a Macro at this point, since you don't have one created yet.

Take a Measurement

When you have saved your protocol, go ahead and take a Measurement using a plant, so you get realistic data. Once the Measurement is done, save it to your Notebook, so you can use it to create your Macro.

Add a Macro

In order to process the Measurement and calculate you need to associate a Macro with your Protocol. See the tutorial Building a Macro on how to build a Macro and process the data coming out of the protocol you have just created.

Building an Advanced Protocol

In the previous tutorial we showed you how to write a Protocol for a simple Phi2 measurement. Make sure you are familiar with this first example, before you tackle this next tutorial. Here we want to explain to you how to build a complex Protocol, which combines simple Protocols like the Phi2 from the previous example into one set of Protocols, a Protocol Set.
Tip

The advantage of using a Protocol with a Protocol set, rather selecting multiple Protocols to be executed one after another is, that your Macro has access to all protocols inside that set.

Protocol Sets

The key to chain multiple Protocols together into one is the _protocol_set_ command. In the example below, you see how the command is used inside a Protocol. All Protocols you would like to execute as one you can put into the _protocol_set_ array. For example you can copy and paste existing protocols into the _protocol_set_: [...]. Make sure you remove the square brackets {{Protocol}} before you add the Protocol into the Protocol Set.

```
[1]
[2]
[3]
[4]
[5]
[6]
[7]
```

Macro required!

In contrast to regular Protocols, when using Protocol Sets you have to use a Macro in order to output Parameters. That way, you have full control over the output of complex measurement Protocols (see Building Advanced Macros).

Special Commands

There are a couple of commands to allow Protocols inside a Protocol Set to communicate with each other, meaning information like light intensity can be transferred from one Protocol to the next. This is not possible if regular Protocols are chained together (select multiple Protocols during Project creation).

Labels

The command label allows to give every Protocol within a Protocol Set a name, so it is easier to identify when creating a Macro.

Previous Light Intensity

The command previous_light_intensity allows to measure the light intensity in the first Protocol and re-use it in all the subsequent Protocols instead of measuring it each time.
The light intensity used and displayed in the subsequent Protocols will be the same as in the first one.

Building a Protocol Set

```json
[...
{
  "_protocol_set_": [  
    {
      "label": "Phi2",
      "pulses": [  
        20, 50, 20
      ],
      "pulse_distance": [  
        10000, 10000, 10000
      ],
      "pulse_length": [  
        [ 30 ], [ 30 ], [ 30 ]
      ],
      "pulsed_lights": [  
        [ 3 ], [ 3 ], [ 3 ]
      ],
      "pulsed_lights_brightness": [  
        [ 2000 ], [ 2000 ], [ 2000 ]
      ],
      "nonpulsed_lights": [  
        [ 2 ], [ 2 ], [ 2 ]
      ],
      "nonpulsed_lights_brightness": [  
        [ "light_intensity" ], [ 4500 ], [ "light_intensity" ]
      ],
      "detectors": [  
        [ 1 ], [ 1 ], [ 1 ]
      ],
      "environmental": [  
        [ "light_intensity" ]
      ],
      "open_close_start": 1
    }
  ]
...
]  
```

Building a Macro

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](https://developer.mozilla.org/en-US/docs/Web/JavaScript). After a measurement has been taken, the data is send from the Instrument to your device and the Macro is processing the data before showing all the calculated parameters. Not every measurement requires post processing (e.g. a simple temperature measurement), but if you want to calculate a parameter from the measurement [Trace](https://developer.mozilla.org/en-US/docs/Web/API/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).

**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. Navigate to **File → New Macro...** to open the Macro Editor and start a new Macro.
2. Select your measurement by searching your **Notebook**.
   - In case you don't have a measurement, take a measurement with the Protocol you are creating the Macro for.
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**

```javascript
/**
 * Macro for data evaluation on PhotosynQ.org
 * by: John Doe
 * created: June 4, 2018 4:00 PM
 */

// 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 \( F_s \) (steady state fluorescence) and \( F_{mp} \) (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

```javascript
1 var fs = MathMean(json.data_raw.slice(1, 5));
2 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.

Equations

\[(1) \phi_{II} = \frac{F_{m'} - F_s}{F_{m'}} \]
\[(2) LEF = \phi_{II} \times PAR \times 0.4 \]

Equations as Code

```javascript
1 var phi2 = (fmp-fs)/fmp;
2 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.

```javascript
1 output['Fs'] = fs;
2 output['Fmp'] = fmp;
3 output['Phi2'] = phi2;
4 output['LEF'] = lef;
5 output['PAR'] = json.light_intensity;
```

The Final Macro
Building an Advanced Macro

When building a Macro for a Protocol using the _protocol_set_ command, you start of the same way as building a macro described in the previous example (Building a Macro). But since a protocol set was used, accessing the retuned Parameters has changed a little.

The _json.set_ Object

The object _json_ is no longer holding all the measured Parameters as shown in the previous tutorial. Instead it has a key called _set_ with all Protocols from the Protocol Set. Since _json.set_ is an array of Protocols, you have to provide the index as well. If you want to access the third Protocol for example, you simply use _json.set[2]_. This can get a bit confusing, when you are using multiple Protocols inside your Set. To make it more accessible, the Macro Editor provides a dropdown menu in the top menu bar with all available Protocols inside a Set numbered starting from 0. If you use the _label_ command inside each protocol, you will see the label in the dropdown menu making the access easier. After selecting the Protocol from the Set, use _Variables_ from the top menu, to see and access all Parameters in the selected Protocol.
Use the dropdown menu to select a specific Protocol Set. You can use the Variables from the menu, as well as the selector for the Raw Traces.

**Simple Protocol Set Example**

```javascript
/**
 * Macro for data evaluation on PhotosynQ.org
 * by: John Doe
 * created: June 4, 2018 4:00 PM
 */

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

// Check if the key time exists in the third protocol of the set
if (json.set[2].time !== undefined){
  // Add key time and value to output
  output.time = json.set[2].time;
}

// Return data
return output;
```

**Multiple Detectors**

In the previous example for a simple for (Building a Macro) only one detector was used. But it can happen, that a measurement requires multiple detectors. This can be accomplished in two different ways as described. The number of data-points within the `data_raw` element in both examples the same, but the way the data is collected is fundamentally different (see Detecting - Output Examples).

**One Detector per Pulse-Set**

The detector readings in this example are sequential, so the first 20 pulses are recorded with `detector 1`, the next 20 pulses are recorded with `detector 3`. In the `data_raw` array, the first 20 values are the readings from `detector 1`, the next 20 from `detector 3`. Using the JavaScript `Array.slice() Method` the two readings can be easily separated in the analysis. Keep in mind, that the first value has the index `0`.

```javascript
//
pulses: [ 20, 20 ],
detectors: [ [1], [3] ],
```

```javascript
```
Two or more Detectors per Pulse-Set

In this scenario, the detector readings alternate between detector 1 and detector 3. The total number of pulses will be 20, but since the detectors now alternate, the returned values in `data_raw` alternate as well. To separate the output of the two detectors you can use the provided function `ArrayNth`. The alternative is to loop through the `data_raw` array and extract the values yourself.

```javascript
javascript
...
pulses: [20]
detectors: [[[1, 3]]],
...
```

```
```javascript
// ArrayNth( <array>, <step size>, <start> )
var detector1 = ArrayNth(json.data_raw, 2, 0);
var detector2 = ArrayNth(json.data_raw, 2, 1);
```