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Measurement of *Auxenochlorella protothecoides* (UTEX 250) Chlorophyll Fluorescence Kinetics (OJIP) and Photosynthetic Efficiency (Fv/Fm) using the Aqua Pen (AP110/C).

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We use this protocol and it's working

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Disclaimer

This protocol was adapted from the Photon Systems Instruments AquaPen-C AP110-C manual linked below: https://handheld.psi.cz/documents/AquaPen_Manual-verze_02_2021.pdf

Abstract

This protocol describes a method for examining the chlorophyll fluorescence induction kinetics, "Kautsky effect," of *Auxenochlorella protothecoides* (UTEX 250) cells. Photosynthetic efficiency (Fv/Fm) measurements are also acquired during the OJIP protocol. This protocol details the optimization of the super pulse intensity setting for a control sample and the application of those settings to probe experimental samples.

Guidelines

Please read the entirety of the protocol before attempting. A summary of the step is highlighted in bold at the beginning of each step, where applicable. Detailed descriptions are provided in the rest of the step.

Normalize the sample input by cell density, chlorophyll content, or optical density (O.D. 750) to ensure relatively equal and complete light penetration of the samples. We recommend using 10^6 cells / mL in 3 mL.

Materials

Aqua pen Model: AP110/C, Photon Systems Instruments (firmware: 1.2.1.6)
Aqua pen cuvettes and caps, cat. no. PSI16107, Photon Systems Instruments
Box for dark adaptation
Green safelight
Computer
Installed FluorPen Software: **https://psi.cz/support/downloads/hd001/ap001/**
RAININ P1000 and tips
RAININ P20 and tips
Hemocytometer
Microscope
Kimwipes

Safety warnings

The cuvettes are not disposable and should not be used for any other purpose.
Do not use acetone or alcohol to clean cuvettes.
Do not wash cuvettes in a dishwasher.
Do not scratch cuvettes.
Wash cuvettes with soapy water and air dry.

Take extreme caution when connecting the charging/ data transfer USB cable to the aqua pen. The connector is prone to irreversible damage. For this reason, we recommend keeping the cable attached and coiled.

If possible, keep the AquaPen in a protective case.

Super pulse intensity optimization of control sample

1 Optimize the super pulse intensity based on the wild type, nutrient replete, or other untreated control samples of your experiment.

Wild-type *Auxenochlorella* (UTEX 250) cells cultured in replete TAP medium typically have an optimal super pulse setting of 70% during the exponential phase of growth with 100 µmol photons · m^{-2} · s^{-1} light. If you are using cells that have been cultured in other conditions, follow the steps in this section to achieve the optimal super pulse intensity for your sample type.

Normalize the sample input by cell density, chlorophyll content, or optical density (O.D. 750) to ensure relatively equal and complete light penetration of the samples. We recommend using 10^6 cells / mL in 3 mL.

2 Count the cell density of your control sample using a hemocytometer. See (Camacho & Merchant, 2024) protocol for detailed instructions on how to count *Auxenochlorella protothecoides cells* using a hemocytometer.

3 Dilute the control sample to 10^6 cells / mL using the medium in which the cells were cultured.

4 Pipette 3 mL of the diluted cell suspensions to 10 cuvettes. You will test different super pulse light intensities on these samples to determine the optimum super pulse light intensity, also known as "saturating pulse".

> Note
>
> Hold the cuvettes at the top 1 cm. Do not touch the cuvette in the light path area.

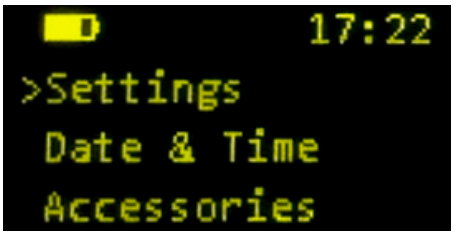
5 Place the cuvettes in a dark cuvettes' holder/ box.

Move the box of cuvettes to a dark room with a green safelight installed.

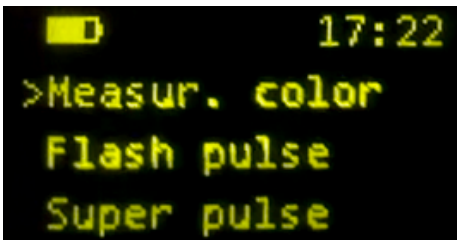
Turn the green safelight off and incubate the control samples in the dark for 15 min.

6 **Set the pulse color to blue (455 nm).**
Press and hold the "**SET**" button for 3 sec to turn the aqua pen on.

Make sure that the date and time are correct and that the battery is sufficiently charged.
Press the “**MENU**” button to toggle through the options until the curser points to “**Settings**”



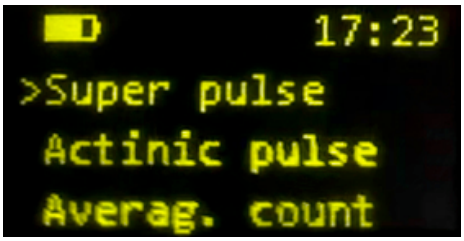
Press the “**SET**” button to enter into the settings menu
Press "**MENU**" until the “**Measur. color**” option is selected



Press “**SET**” to enter the “**Measur. color**” setting.
Press “**SET**” until **455 nm** (Blue) is selected.

7 **Change the super pulse intensity.**

Press the “**MENU**” button to exit the “**Measur. color**” setting and back to the settings page.
Press "**MENU**" until “Super pulse” is selected. Press the "**SET**" button to enter into the "**Super pulse**" settings.



Set the super pulse intensity to 10%

In the later steps of this protocol, you will increase the super pulse intensity by increments of 10%.
The percentage indicated is the percentage of the maximum super pulse intensity of 3000 µmol photons · m^{-2} · s^{-1} blue light.

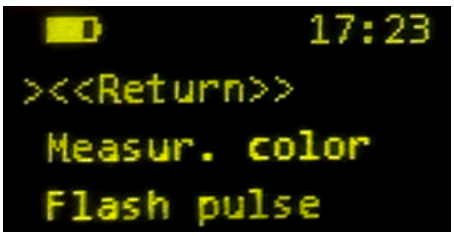
	A	B
	%	µmol photons / m^2 / s
	10	300
	20	600
	30	900
	40	1200

	A	B
	50	1500
	60	1800
	70	2100
	80	2400
	90	2700
	100	3000

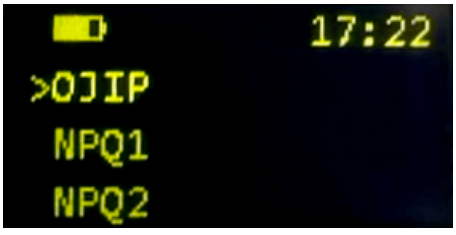
8 **Obtain an OJIP measurement of one control sample at a super pulse setting of 10%**

Make sure that you are in a dark room. Turn the green safelight on and minimize the exposure of the sample to the green light.

Press MENU until you reach the **<<Return>>** option to exit the settings page.



Press "**MENU**" until you reach the "**Measurement**" option.
Press "**SET**" to access the measurement options.
Press "**MENU**" until you reach the "**OJIP**" option.



Mix the cells by pipetting up and down 5 times or by inverting the capped cuvette 5 times. Cells will settle at the bottom of the cuvette and will yield inaccurate results if not resuspended properly right before the measurement.

Wipe the outside of the cuvette with a Kimwipe.
Make sure that the cuvette is dry and clean before inserting it into the Aqua Pen.

Press the "**SET**" button to acquire the OJIP measurement. The results will not be displayed. You will see a percentage indicating the progress of the measurement. The measurement will take a few seconds. Record the counter number and the time that the measurement was taken.

9 **Obtain an OJIP measurement of a new control sample at a super pulse setting of 20%. Repeat and increase by increments of 10% until all 10 samples have been measured.**

After the measurement of the first sample, remove the cuvette from the AquaPen and set it aside.

Note

Make sure to remove the sample from the Aqua Pen before changing the settings. Holding the AquaPen at a weird angle, while a cuvette is still inside, may cause the sample to leak into the AquaPen's internal electrical components.

Press "MENU" to until the **<<Return>>** option is selected.

Repeat step 7 to change the super pulse intensity to 20%.

Use a new dark adapted control sample for the OJIP measurement.
Do not reuse samples that have just been measured.

Repeat step 8 to obtain the new OJIP measurement.

Repeat steps 7-9, increasing the superpose intensity by 10% until all 10 cuvettes from step 4 have been measured.

10 **Export the data using the FluorPen Software.**

Connect the USB cable to the AquaPen and your computer.

Make sure that the FluorPen software is installed. See materials for a link to download the software.
You may need to download and install a driver (also included in the link).
The FluoroPen software will only work on windows computers.

Open the FluorPen program and connect to the device.
If it is your first time using this software on your computer, you will need to register the AquaPen's serial number into the program.

Click "**Help**" in the toolbar then select "**Register**".
Enter the serial number into the dialogue box.

Each time you open up the software, the aqua pen will not be recognized and you will have to set up the connection.

Click on **Setup** in the tool bar and click on "Device ID" (see image below).

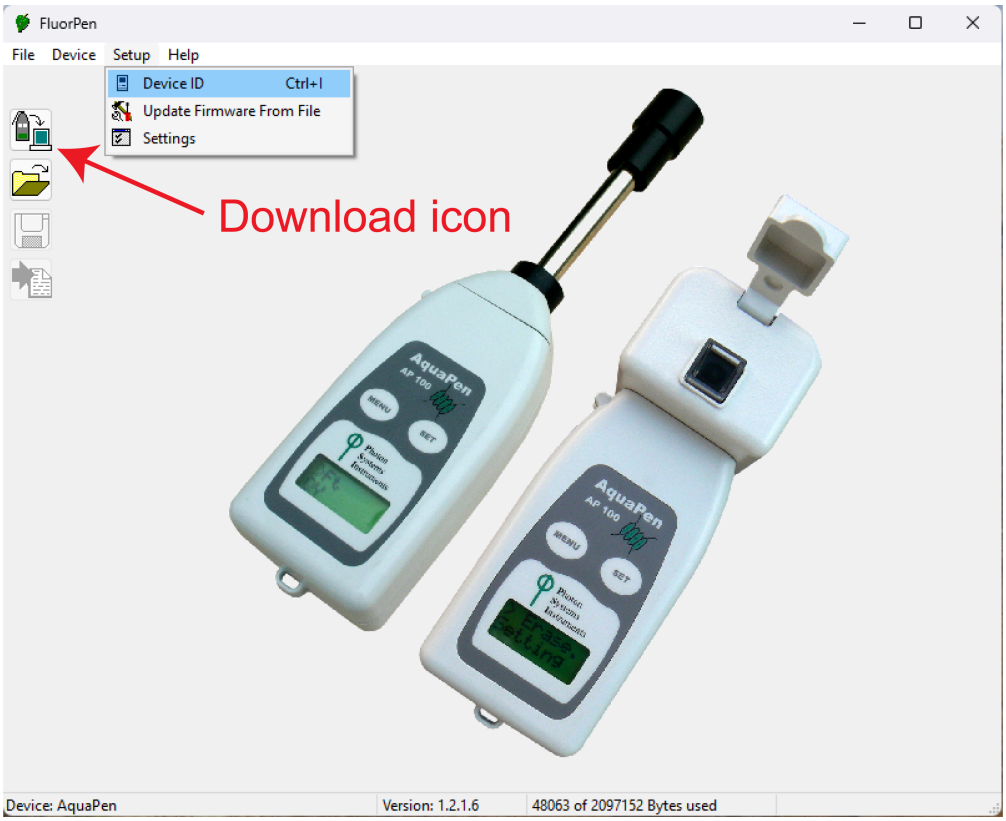


Image of FluorPen software and Setup tab opened.

The AquaPen will connect and the download icon should become visible.

Click on the Download icon.

A table with all data will automatically display. See image below. Each column is an independent measurement.

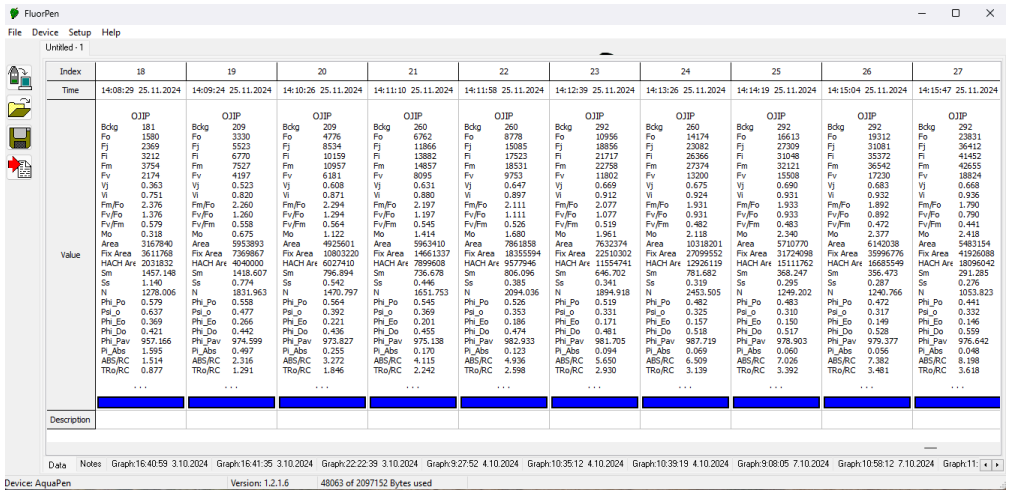
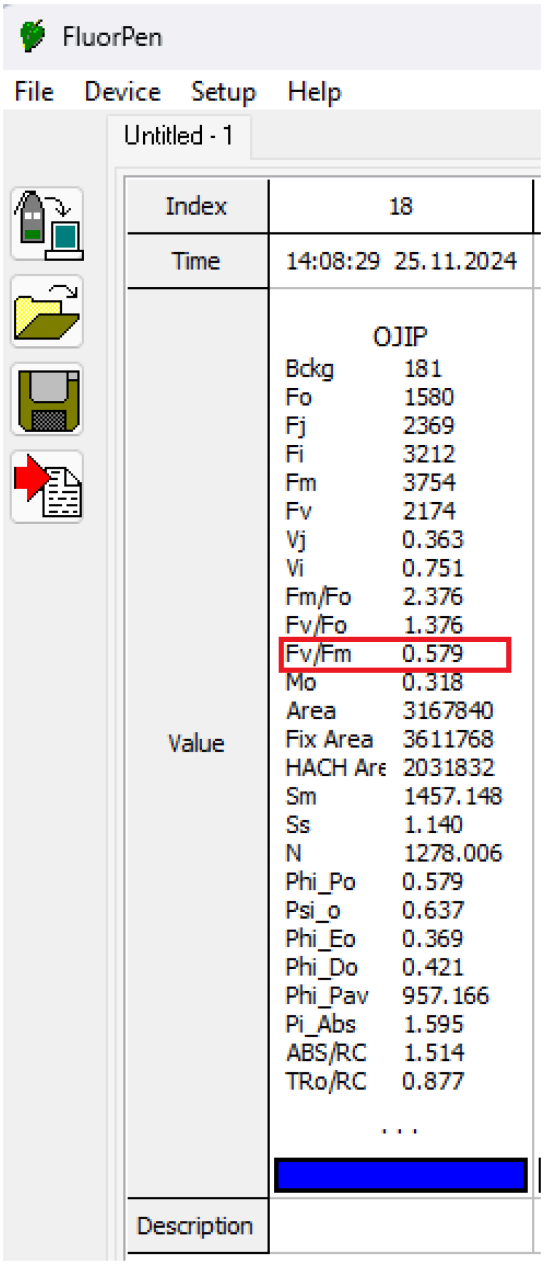


Table with data is automatically displayed after data is downloaded.

The index corresponds to the counter value from step 8. Verify that the index and time of the measurement matches.



Data from a single measurement. Fv/Fm value is highlighted in a red box.

11 **Determine the optimal super pulse intensity.**

Record the Fv/Fm values for each cuvette and their corresponding super pulse light intensities.

Plot the Fv/Fm values (y axis) against the super pulse intensity (x axis).

The super pulse intensity with the highest Fv/Fm value is your optimal super pulse intensity.

If the highest Fv/Fm value is measured at 100% super pulse intensity, it is possible that the sample is too dense and will need to be diluted.

Measurement of OJIP and Fv/Fm from experimental samples.

12 Change the super pulse intensity to the optimal intensity (See step 7).

13 Determine the cell density of your samples (see steps 1 and 2).

Dilute each sample to 10^6 cells / mL in 3 mL of medium and transfer to a cuvette (same as step 3).

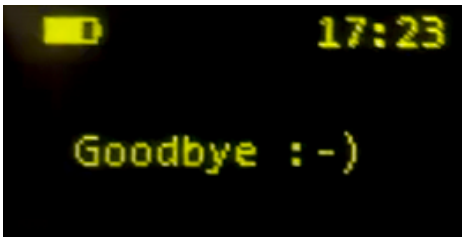
14 Dark adapt the samples for 15 min (see step 5).

15 Obtain the OJIP measurement using the optimized super pulse intensity (similar to Step 8).

16 Export the data (see step 10).

Clean up

17 Turn the AquaPen off by pressing and holding the "**MENU**" button.
You may also turn the instrument off by navigating back to the home page and selecting "**Turn off**"



18 Remove the final sample from the AquaPen.

Empty the cuvettes into an autoclavable liquid waste container and autoclave or kill the cells with 10% bleach.

19 Clean the cuvettes and caps by soaking in a soapy water solution.
Do not use bleach, acetone, or any alcohol solution to clean the cuvettes.
Do not wash cuvettes in a dish washer.

Air dry the Cuvettes and caps and store them safely in a box.

Protocol references

Dimitrios Camacho, Sabeeha Merchant 2024. Determination of *Auxenochlorella protothecoides* Cell Density With a Hemocytometer. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzjk12lx1/v1