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I. INTRODUCTION

The optical power measured at the sample plane is a key parameter in microscopy setups, either to troubleshoot any issue or to perform quantitative microscopy.

Measuring the optical power at regular interval using the same illumination settings allows to troubleshoot, for example, changes in light sources power, drops in filters transmission, or laser-to-fiber coupling misalignment.

Measuring the optical power prior and after an imaging campaign ensures that the light source power has not fluctuated in the meantime, evidencing it has not affected the intensity values in the images.

Measuring the optical power prior an imaging campaign using the same illumination settings as for a previous imaging campaign ensures that the same optical power is applied on the sample, so that the illumination conditions are reproduced.

Measuring the optical power allows to report this value in scientific publications, so that other researchers can reproduce these illumination conditions, thus contributing to the combat against the replication crisis.





The “power meter” analysis allows to perform live ***optical power***, live ***irradiance***, ***time-lapse*** and ***power vs setting*** measurements.





II. OPERATING THE POWER METER

1. INSTALLATION

- Download the Daybook software here: <http://argolight.com/measure-microscopes-performances-detect-issues-with-daybook/>
- Download the ARGO calibration file of the sensor here: <http://argolight.com/knowledge-center/get-my-calibration-file-for-argo-power/>
- Install Daybook on the computer that will be used to read the optical power measurements.
- Plug the USB cable to the control unit and to the computer.
- Open Daybook Analysis. A settings panel will open.
- In the settings panel, choose “Argo-POWER^{HM}” in the “product type” list (cf. Figure 1).

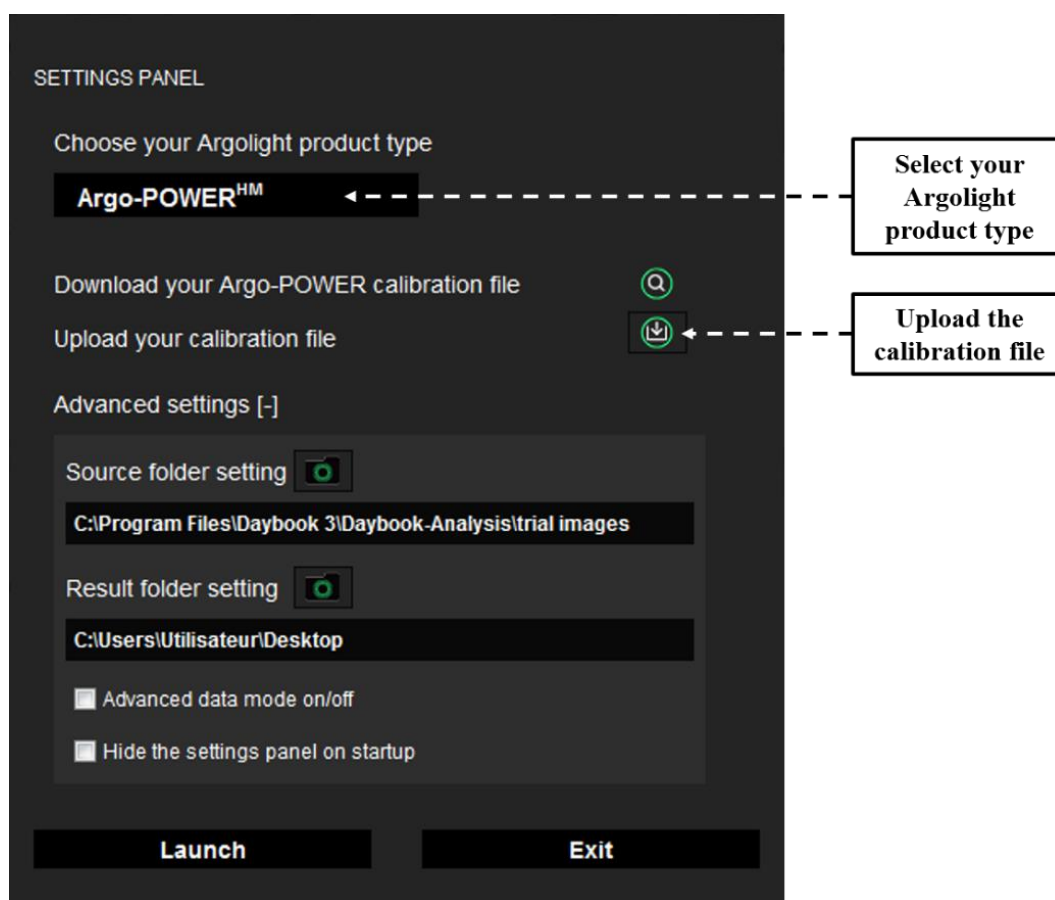


Figure 1: Settings panel.

- In the settings panel, upload the ARGO calibration file and click “OK” (cf. Figure 1).
- Click on the “Power meter” button on the top-left corner (cf. Figure 2).

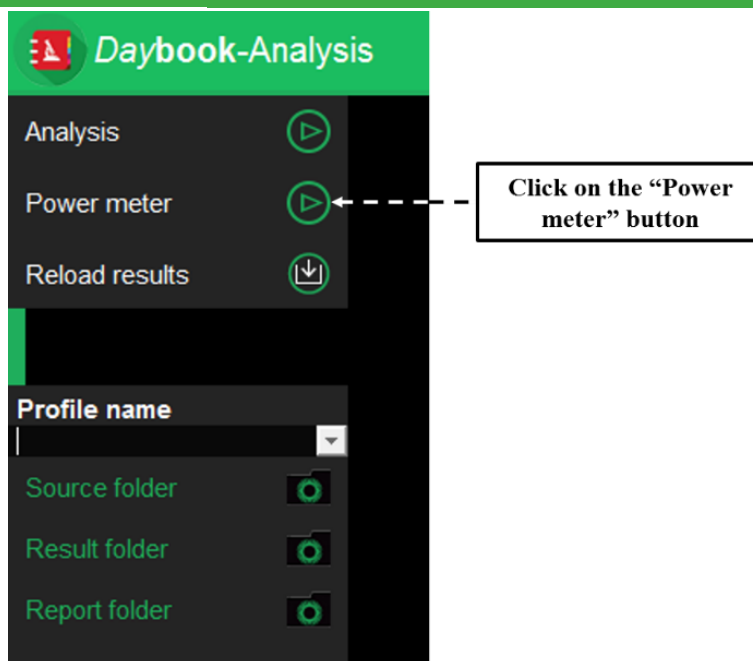


Figure 2: Daybook Analysis starting-up window.

- After the connection between the device and the computer is established, enter the light source wavelength and the sampling period, and click on the “Power measurement” button.

2. LIVE MEASUREMENT

- Select a microscope objective without immersion (dry objective).
- Set up the device in the microscope sample holder.
- Plug the cable from the power meter head to the control unit.
- Illuminate the sensor, starting at relatively low optical power.
- Align the light beam to the center’s sensor, using the XY translation stages (*cf.* Figure 3). For inverted microscopes, use the target etched at the back of Argo-POWER^{HM} head for centering the beam on the sensor (*cf.* Figure 4).



Figure 3: Argo-POWER^{HM} head inserted in the sample holder of an upright microscope.

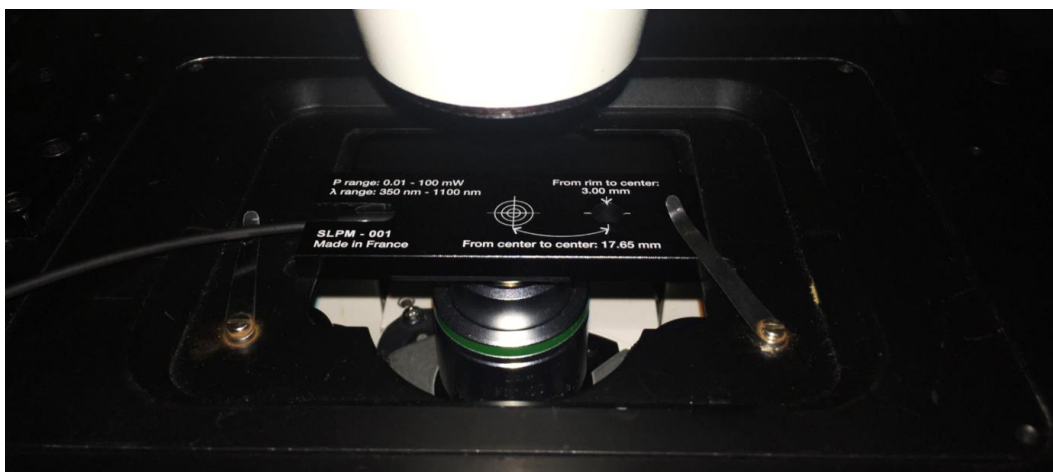


Figure 4: Argo-POWER^{HM} head inserted in the sample holder of an inverted microscope.

- The Argo-POWER^{HM} head can also be used like a “traditional” power meter, to measure the optical power of a collimated (or slightly converging / diverging) light beam (*cf.* Figure 5).



Figure 5: Argo-POWER[™] head inserted across the optical path of a collimated light beam.

- Fill out the “Excitation wavelength” and the “Sampling period” boxes (cf. Figure 6).
- Click on the “Run power measurement” button (cf. Figure 6).



Figure 6: User interface for optical power measurement.

- Adjust the focus until the highest optical power is measured. This indicates that the sensor is at the focal plane of the microscope objective (cf. Figure 7).

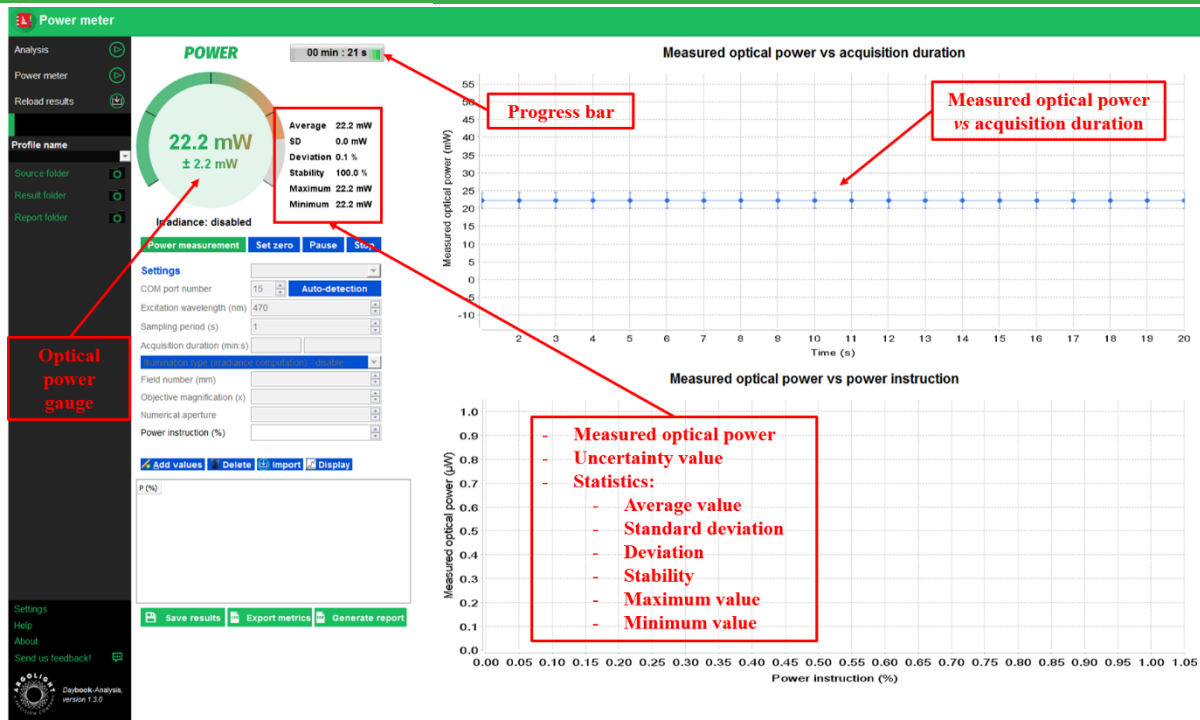


Figure 7: Live optical power measurement.

- Start the optical power measurements.

3. STATISTICS/METRICS

Statistics/metrics are displayed on the right of the gauge (cf. Figure 7):

- The average (in μW or mW) for n measurements of power P_i :

$$\text{Average} = \frac{1}{n} \sum_{i=1}^n P_i$$

- The standard deviation (in μW or mW) for n measurements of power P_i :

$$SD = \sqrt{\frac{1}{n} \left(\sum_{i=1}^n P_i^2 \right) - \text{Average}^2}$$

- The deviation (in %):

$$\text{Deviation} = 100 \times \frac{P_{\max} - P_{\min}}{\text{Average}}$$

- The stability (in %):

$$\text{Stability} = 100 \times \left(1 - \frac{P_{\max} - P_{\min}}{P_{\max} + P_{\min}} \right)$$

- The maximum is the maximum power P_{\max} measured during the acquisition.
- The minimum is the minimum power P_{\min} measured during the acquisition.



4. TIME-LAPSE POWER ACQUISITION

To investigate the fluctuations of a light source over a limited duration (for example 10 minutes, 1 hour, or 1 night), it is possible to monitor the evolution of the optical power versus the acquisition duration (cf. Figure 8), *i.e.* to perform time-lapse measurements.

To carry this out, fill out the acquisition duration, and click on the “Power measurement” button. Once the indicated time is passed, the acquisition is automatically stopped.

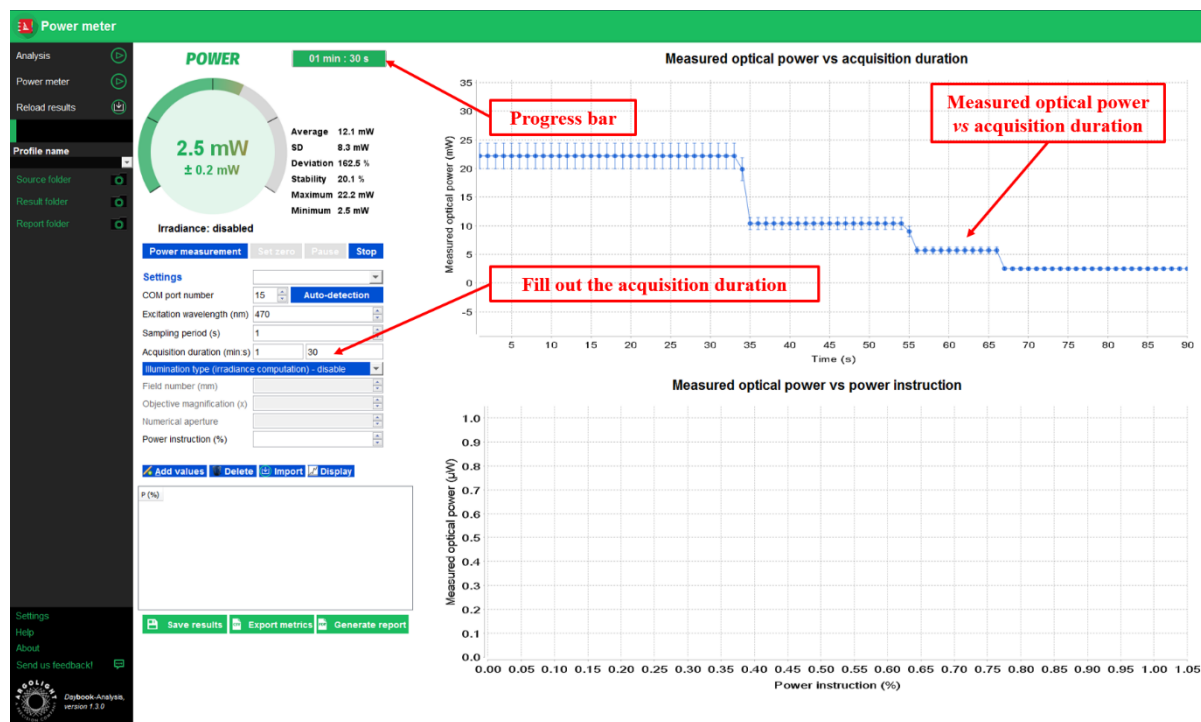


Figure 8: Example of evolution of the optical power versus the acquisition duration.

5. IRRADIANCE CALCULATION MODE

In order to reproduce experimental images and data independently from the used imaging system, it is important to know how much light a biological sample receives on a given surface, through the calculation of the surface power density, *i.e.* the irradiance.

In general, the irradiance (in $\text{W}\cdot\text{cm}^{-2}$, watts per square centimeter) is defined as the ratio of the optical power (in W, watts) divided by the illuminated surface (in cm^2 , square centimeter). The optical power can be measured with a power meter at the focal plane of the objective. The surface, however, is difficult to measure, but can be evaluated from calculation, depending on the microscope acquisition mode (wide-field, confocal laser-scanning and confocal spinning disk microscopy).

For **wide-field microscopy**, the irradiance of the illumination circular area is given by the following formula:



$$I_{WF} = \frac{10^{-1} \times P_{av}}{\pi \times \left(\frac{FN}{2 \times Mag} \right)^2}$$

Where I is the irradiance (W.cm^{-2}), P_{av} the average power (mW) at the focal plane of the objective, FN the objective field number (mm) and Mag the objective magnification (unitless).

Note: Sometimes, the field number FN and the field of view FOV can be mixed-up. They are related through the following equation: $FOV = FN/Mag$.

For **confocal laser-scanning microscopy**, the irradiance of the focused laser beam is given by the following formula:

$$I_{CLS} = \frac{10^{11} \times 4 \times \ln(2) \times P_{av}}{\pi \times \left(\frac{0.52 \times \lambda}{NA} \right)^2}$$

Where I is the irradiance (W.cm^{-2}), P_{av} the average power (mW) at the focal plane of the objective, λ the illumination wavelength (nm) and NA the objective numerical aperture (unitless). The factors $4 \times \ln(2)$ comes from the fact that the spatial distribution of the laser beam is considered as Gaussian.

Notes:

- The irradiance for confocal laser-scanning microscopes can be several orders of magnitude higher than that for wide-field microscopes.
- The measured average optical power for confocal laser-scanning microscopes is the same whether the laser illumination spot is raster-scanned or held in a fixed position.

Once the type of illumination is chosen, and the associated parameters are filled-out, Daybook Analysis provides the live irradiance (*cf.* Figure 9), based on the measured optical power and the formula described above.





Figure 9: Example of irradiance live measurement for wide-field illumination.

6. MEASURED OPTICAL POWER VERSUS POWER SETTING

In most of the acquisition software of fluorescence microscopes, the adjustment of the light sources' optical power is performed in fractions of the full power (in %) and not in optical power units (in W).

The measurement of the evolution of the optical power in Watts versus the power setting in % can be useful to check the linearity of the evolution, the absolute power values, or the compliance with the light sources power specifications after a system installation or maintenance.

To display values in the “Measured optical power vs power setting” graph, proceed as follows:

- Measure the live optical power, by clicking on the “Power measurement” button.
- Enter a “Power setting”, from 0 to 100 %, corresponding to the setting you use in your microscope acquisition software.
- Click on the “Add values” button. A new value of the power setting and of the measured optical power appears in the table.
- Change the setting in your microscope acquisition software, enter this corresponding value in “Power setting”, wait for the measured optical power to stabilize, and click on the “Add values” button. An additional value of the power setting and of the measured optical power appears in the table.
- If necessary, to delete erroneous values, you can click on the “Delete” button.
- After adding two or more values in the table, click on the “Display” button to show those values in the graph, as well as a linear regression curve.
- Change the “Excitation wavelength” and repeat the previous steps if you want to display values from several light sources.



Figures 10 and 11 show the evolution of the optical power versus the power setting for one and two light sources, respectively.

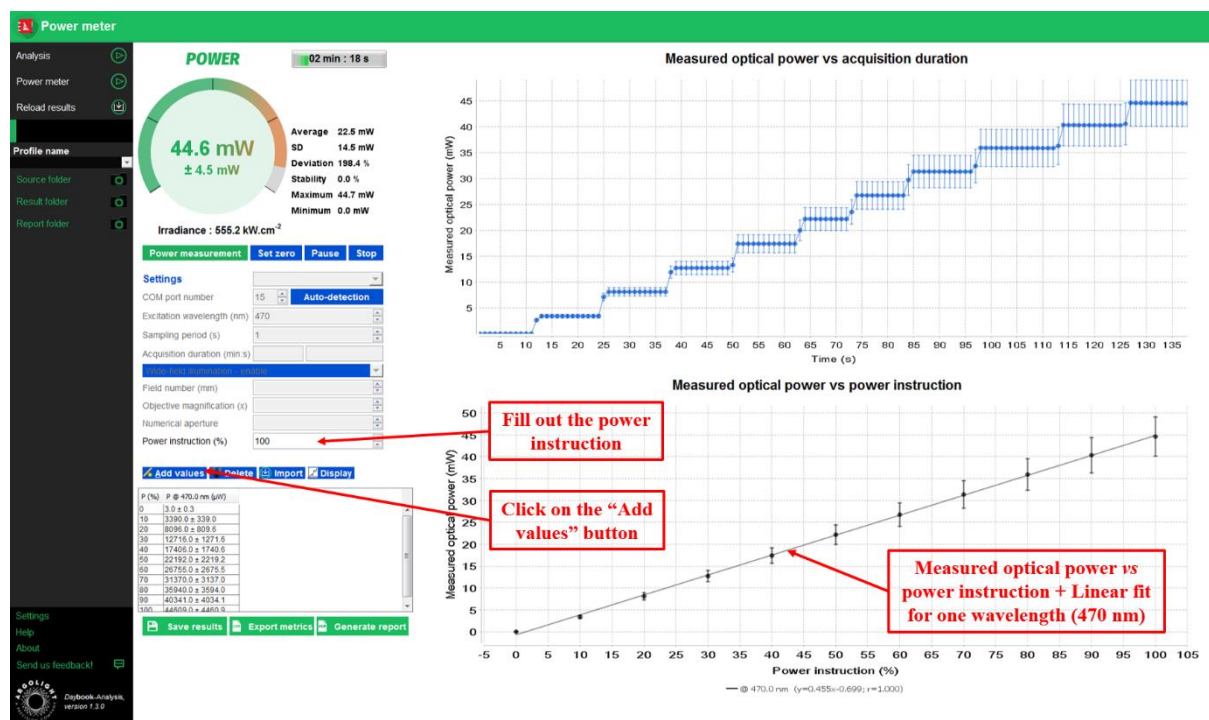


Figure 10: Example of measured optical power versus power setting for one light source.

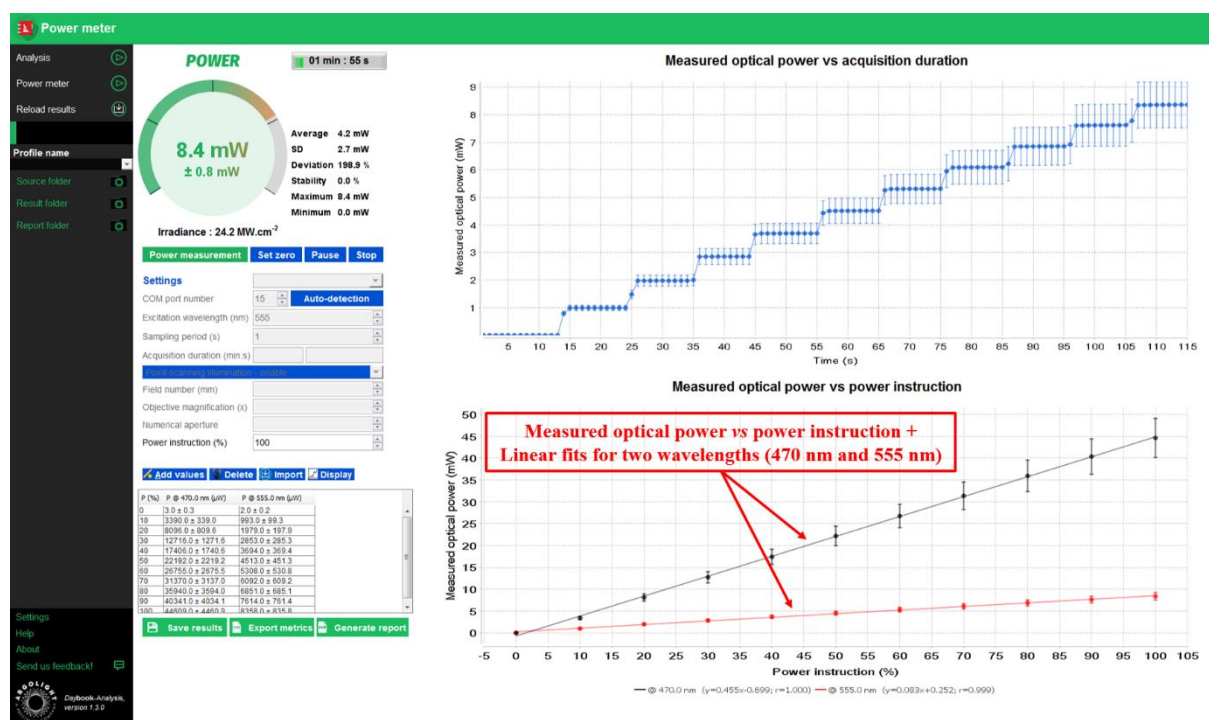


Figure 11: Example of measured optical power versus power setting for two light sources.

7. SAVING OPTIONS



There are different options to save the results (cf. Figure 12):

- Right click on the graphs to save the images into a PNG or JPEG file, or the graph values into a TXT file.
- Click on the “Save results” button to save the graphs into JPEG files and the metrics into a CSV file.
- Click on the “Export metrics” button to save only the metrics into a CSV file.
- Click on the “Generate report” button to edit and save a report into a PDF file.

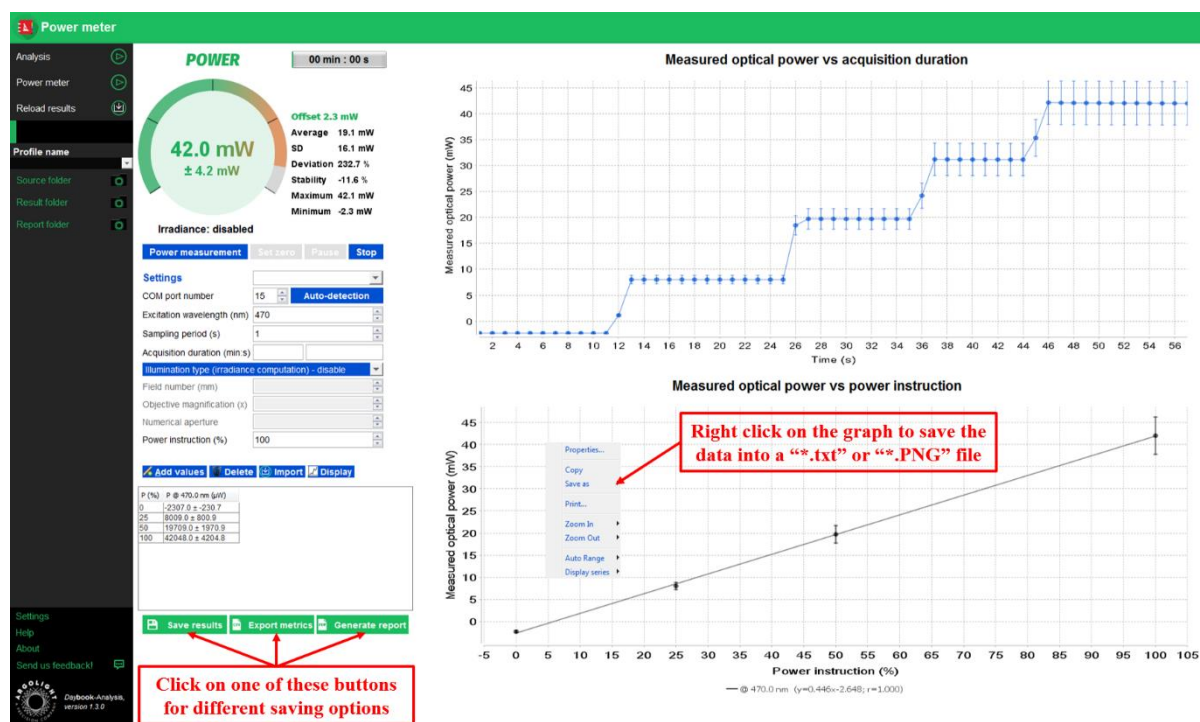


Figure 12: Different options to save the results.

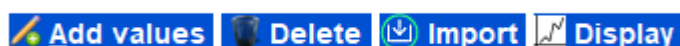
The saved CSV files can be used afterwards to reload your results and display them again in the power meter interface.

8. IMPORT RESULTS

Previous results of a power meter measurement can be imported, through the “Power meter” interface itself.

To import power meter results:

- Go to the “Power meter” interface.
- Click on the “Import” button and load your CSV file.



- The last power value shown in the gauge, the statistics on the right of the gauge, as well as the two graphs “Measured optical power vs acquisition duration” and “Measured optical



power vs power instruction” are reloaded (cf. Figure 13). Please note that the acquisition settings are not reloaded but are available within the CSV file.

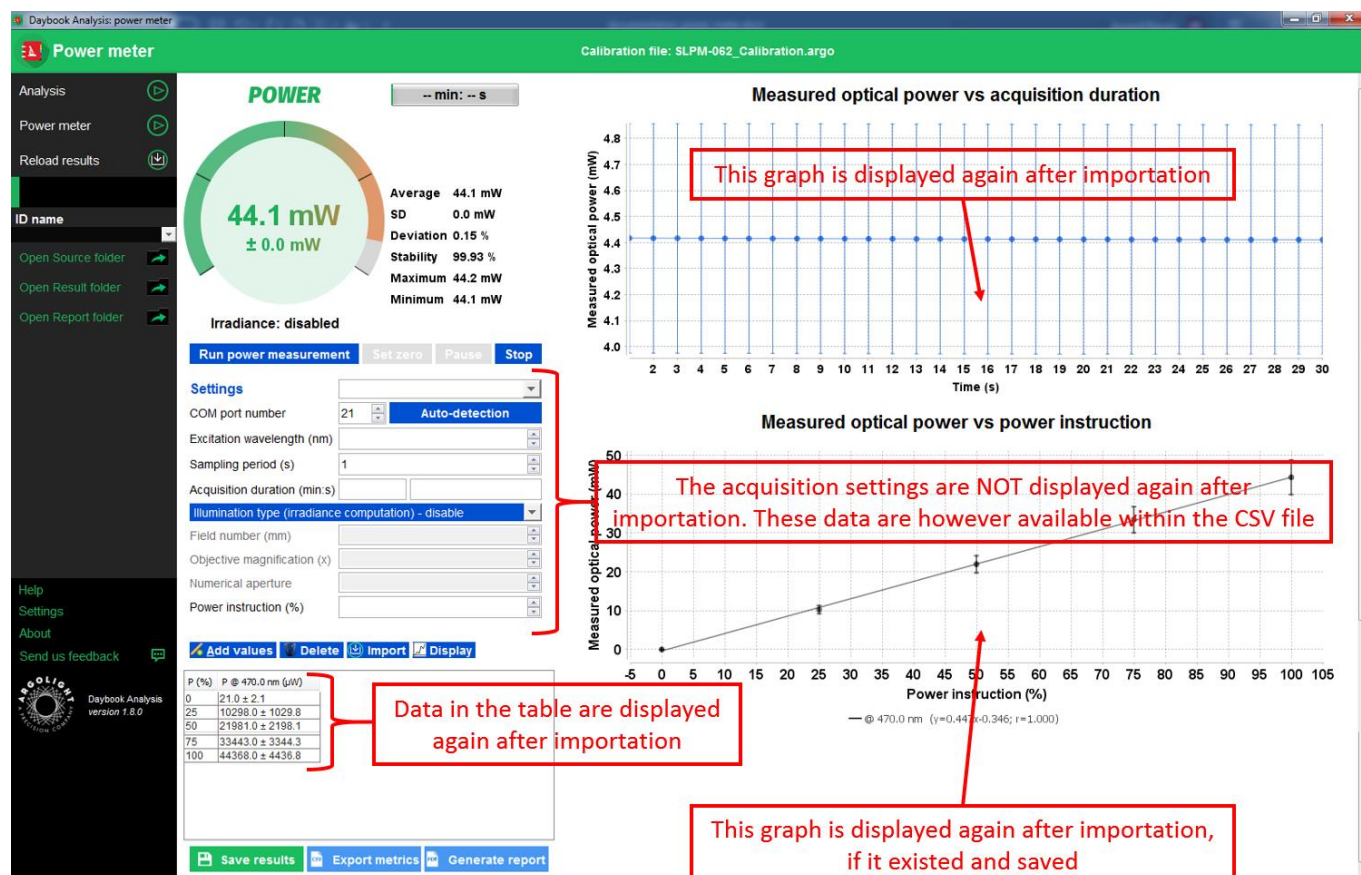


Figure 13: Power meter interface showing which data are displayed again after importation.



III. FREQUENTLY ASKED QUESTIONS

1. I HAVE CONNECTION ISSUES

When the control unit is not recognized by the computer, a warning message is displayed (cf. Figure 14). When this occurs, check that the unit control is correctly connected to the computer through the USB cable, and that the device is properly recognized by the computer in the Device Manager window (cf. Figure 15).

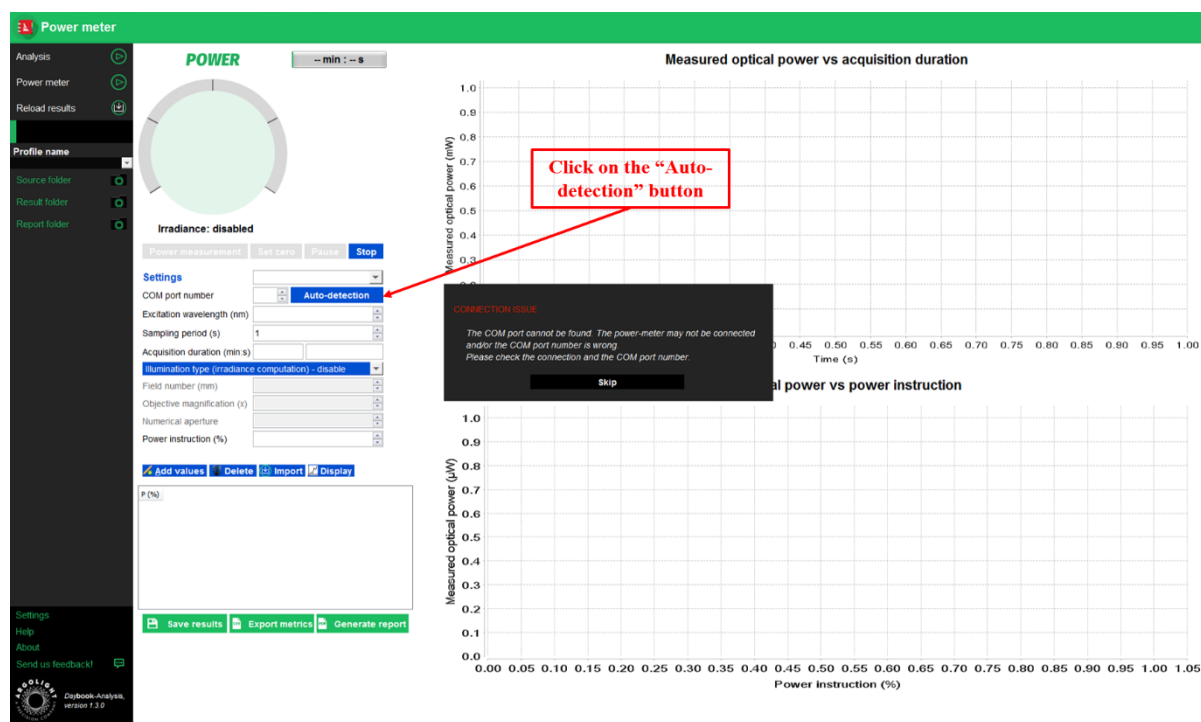


Figure 14: Displayed warning message for connection issues.

When facing connection issues, try to unplug and connect again the USB cable, or to use another USB port of the computer. Then click on the “Auto-detection” button.

If the device is still not recognized, try to turn the computer off and on. Open Daybook Analysis and repeat the operation again.

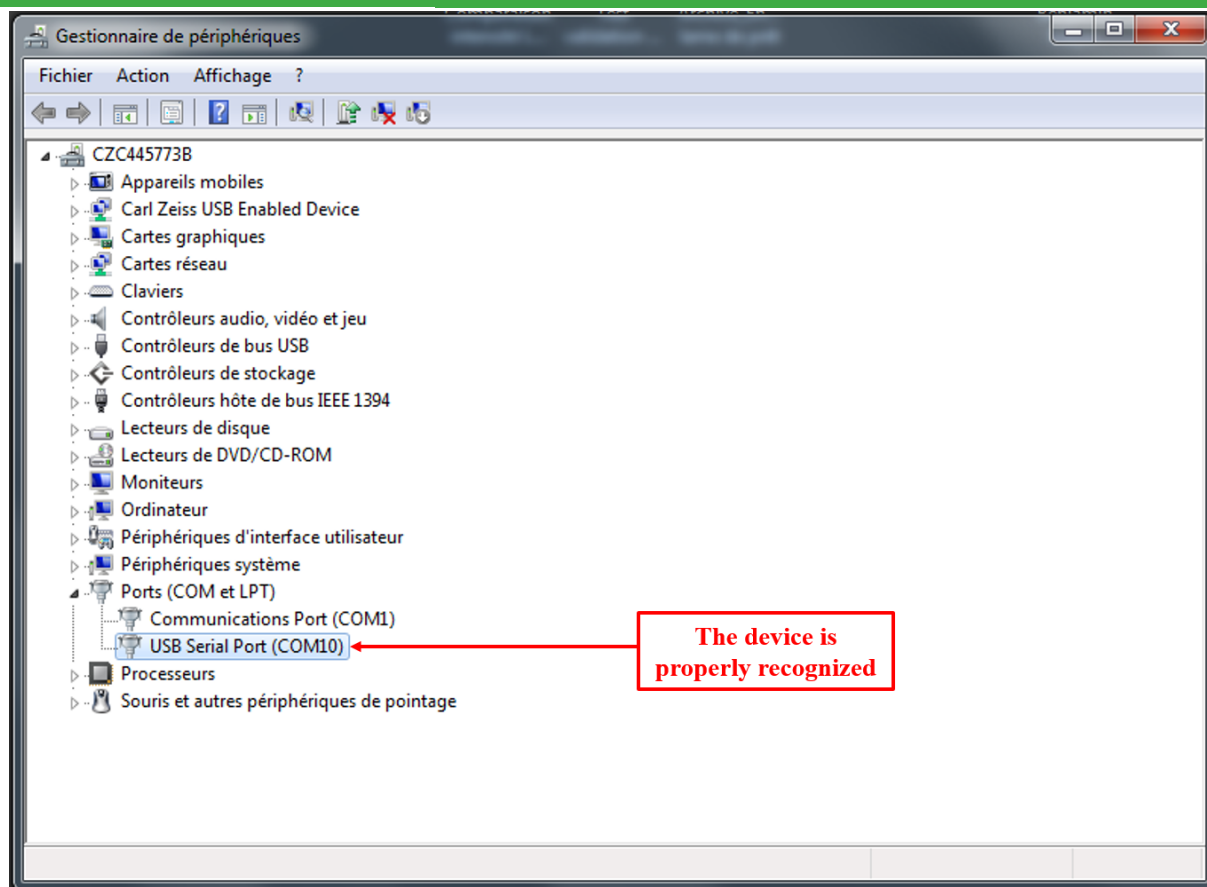


Figure 15: Device Manager window showing the device properly recognized by the PC.

2. I CANNOT START MEASURING THE OPTICAL POWER

When the excitation wavelength and/or the sampling period are not filled out, a warning message is displayed (cf. Figure 16). When this occurs, fill out the “Excitation wavelength” and/or the “Sampling period”, and click on the “Power measurement” button again.



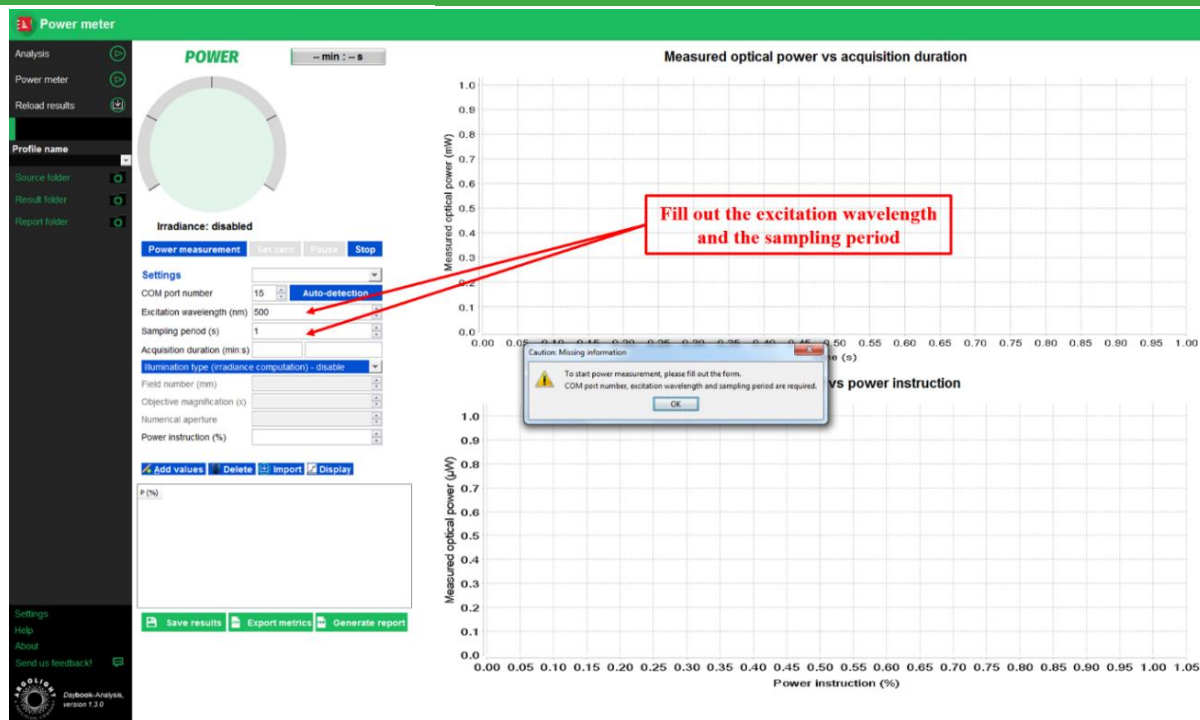


Figure 16: Displayed warning message for missing parameters.

3. I CANNOT MEASURE ANY OPTICAL POWER VARIATION

If no optical power variation can be measured as the actual power of the light source is changed, please contact Argolight technical support, as there may be a problem with the electronics.

4. THE MEASURED VALUES SEEM ODD

If the optical power meter does not seem to provide accurate measurements, please do the following actions:

- Check that the uploaded calibration file is the correct one.
- Check that the filled-out excitation wavelength is the correct one.
- Check that the sensor, or any other part of the power meter, is not damaged.
- If you work in a room with ambient light, click on the “Set zero” button to set the measured power at 0, before proceeding to optical power measurements of light sources (cf. Figure 17).



Figure 17: Offset option to subtract the optical power from any other contribution than the light source to be measured.

5. I CANNOT IMPORT MY DATA “MEASURED OPTICAL POWER VERSUS POWER SETTING”

When I click on the "Import" button and select a CSV result file, I get the following message: *"Please check the CSV result file", as it may be compromised.*

This error message appears when the data in the CSV file have been compromised, e.g. modified non-intentionally, or no longer compatible with the version of the Daybook software you are using. If this happens, please contact our technical support at customer@argolight.com.





Encountered an issue or a question when using Daybook Analysis?

**Please send a screenshot and your issue description at:
customer@argolight.com**

