

USER GUIDE

Argo-POWER^{HM}



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1. Introduction

The Argo-POWER^{HM} is specifically designed to assess and follow the performances of fluorescence-based imaging systems, such as wide-field, confocal, spinning disk and other types of microscopes, with high magnification (HM) objectives.

This product is composed of an optical power meter, an Argo-HM glass and Daybook, their companion image analysis software.

The optical power meter is designed to measure the optical power of laser light or other monochromatic or near monochromatic light sources at the sample plane in microscopy setups. This is important when one wants to perform quantitative microscopy (recording and recreating experimental conditions) and/or identifying fluctuations of light sources power, drops in filters transmission, fiber coupling misalignment, etc.

The Argo-HM glass is a special glass substrate with different fluorescent patterns embedded inside. This glass is designed to quality-control many aspects of a fluorescence microscope: illumination inhomogeneity, field distortion, chromatic shifts, lateral and axial resolving powers, system intensity and spectral responses, stage repositioning repeatability, 3D reconstruction accuracy, etc.

Daybook software allows to analyze and extract data (maps, graphs and metrics) from images of the patterns, in order to control significant parameters of your fluorescence imaging system.

Combining an optical power meter with the best of Argolight technology opens the path to complete, yet quick and easy, quality control of fluorescence-based imaging systems with a single device.

2. First use

2.1. Package checking

Inside the package, you will find:

- 1 Argo-POWER^{HM},
- 1 control unit,
- 1 USB 2.0 Type-A to Mini-B cable,
- 1 storage suitcase,
- 1 user guide,
- 1 certificate of conformity,

Before starting, check that all these items are present and control if the device has visible damages. If any damage is observed, please contact Argolight within one week (7 days) after delivery.

2.2. Quick starting procedure

Step 1 - Software installation:

- Download the Daybook software and the "*.argo" calibration file on the Argolight website: http://argolight.com/daybook-software/

- Install Daybook on the computer that will be used to read the optical power measurements. The operating system must be a 64 bits Windows 7, 8 or 10 version.

- Plug the USB cable to the control unit and to the computer.

- Open Daybook. A settings panel will open.

- In the settings panel, select the Daybook version compatible with the product: Argo-POWER^{HM}.

- In the settings panel, upload the "*.argo" calibration file and click on the "OK" button.

- Click on the "Power meter" button on the top-left corner in Daybook.

- 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.

Step 2 – Optical power measurements:

- In your imaging system, select a microscope objective without immersion.

- Set up the Argo-POWER^{HM} in the microscope sample holder.
- Plug the cable from the power meter head to the control unit.
- Illuminate the sensor.
- Align the light beam with the sensor's center, using the XY translation stages.
- Click on the "Power measurement" button.

- Adjust the focus until the highest optical power is measured. This means the sensor is at the focal plane of the microscope objective.

- Start the optical power measurements.

Step 3 – Patterns imaging and analysis:

- In your imaging system, select a low magnification microscope objective, typically a 10× or 20×.

- Illuminate the Argo-HM glass with UV-blue light (preferably at a wavelength between 350 nm and 500 nm).

- Coarsely align the center of the field of view with the center of the Argo-HM glass, using the XY translation stages, until observing white fluorescence coming out of the glass. This means the fluorescent patterns are excited by the excitation light (when a 405 nm excitation source is used for instance).

- Adjust the focus into the glass until observing the fluorescent patterns clearly through the eyepieces.

- Move the slide to observe the pattern(s) of interest.

- Switch to your working high magnification microscope objective.

- Re-adjust if necessary the position of the pattern(s) and the focus in the Argo-HM glass.

- Start your imaging session.
- Save the images of your pattern(s) of interest from your usual acquisition software.
- Run the images analysis through Daybook to get meaningful results.

3. General handling and care

3.1. Optical power meter

3.1.1. Handling

In order to make the optical power meter last for many years, we advise to respect the following handling and storage instructions:

- Do not put immersion liquids on the sensor.
- Do not shine more than 110 mW of average power on the sensor.
- Do not use with focused laser pulses.
- Do not drop out.
- Do not pull off the cables.
- Do not scratch the sensor surface.
- Do not push the sensor towards an objective.
- Do not expose to extreme temperature and humidity conditions.

- Store in its suitcase at ambient temperature $(10 - 40^{\circ}C)$ and normal relative humidity (20 - 70% of relative humidity).

3.1.2. Cleaning

For dust removal, use clean pressurized gas and lint-free soft cotton swabs (or lens tissue) dabbed in a reagent grade alcohol (ethanol or propanol). A minimum amount of alcohol is preferred to prevent from alcohol contamination below the sensor. As water tightness is not 100% warranted, the use of excessive liquid can infiltrate and damage the detector. Wearing gloves is advised. Do not use acetone.

3.2. Argo-HM glass

3.2.1. Handling

In order to make the Argo-HM glass last for many years, we advise to respect the following handling and storage instructions:

- The Argo-HM glass is compatible with water as an immersion liquid, but continuous exposure higher than five minutes should be avoided. When longer continuous exposures are required, use oils with the same refractive index as water as an immersion liquid.

- Do not use with near-infrared pulsed laser illumination for multiphoton microscopes.

- Do not illuminate with irradiances (peak or average) higher than 50 GW.cm⁻².
- Do not drop out.
- Do not scratch the glass surfaces.
- Do not push towards an objective.
- Do not expose to extreme temperature and humidity conditions.
- Clean after each imaging session.

- Store in its suitcase (after having removed entirely the immersion liquid) at ambient temperature ($10 - 40^{\circ}$ C) and normal relative humidity ($20 - 70^{\circ}$). Avoid ultraviolet irradiation.

3.2.2. Cleaning

Clean with lens tissue and alcohol (ethanol or propanol), as one would do for any regular optical component. Wearing gloves is advised. Do not use acetone.

3.2.3. Operating environment

The Argo-HM glass have been designed to be used at room temperature $(10 - 40^{\circ}C)$ and normal relative humidity (20 - 70% RH). Both the glass and the metal carrier composing the Argo-POWER^{HM} have a low thermal expansion coefficient, so that temperature variations will not significantly affect the imaging of the patterns.

4. Technical specifications: optical power meter

4.1. Physical specifications

Table 1 presents the physical specifications of the optical power meter.

Head dimensions	75.0 mm × 25.0 mm × 6.0 mm		
Control unit dimensions	100.0 mm × 50.0 mm × 25.5 mm		
Total weight	0.2 kg		
Head cable length	1.50 m		
USB cable length	1.83 m		
Head-to-unit connector	FFA Male		
PC-to-unit connector	USB 2.0 Type-A to Mini-B		
Post mounting	Universal 8-32 / M4 Tap, Post not included		

Table1: Physical specifications of the complete hybrid device.

4.2. Power meter specifications

Table 2 presents the technical specifications of the optical power meter.

Detector type	Silicon photodiode		
Active detector area	10 mm diameter		
Neutral density filter	Reflective (OD 2)		
Input aperture	9 mm		
Wavelength range	350 – 1100 nm		
Power range	10 µW – 100 mW		
Linearity	± 2%		
	< 3 µW (350 – 400 nm)		
Resolution	< 2 μW (400 – 460 nm)		
	< 1 µW (460 – 1100 nm)		
Calibration	± 10% maximum over the whole wavelength range		
uncertainty	Typically, $\pm 2\%$ at 633 nm		
Pooponoo timo	100 ms (minimum),		
Response time	3 s (to ensure stable reading)		
Objective compatibility	Air coupled objectives, with FOV* diameters less than 9 mm		

Table2: Technical specifications of the optical power meter. *FOV = "Field Of View".

4.3. Electrical specifications

Table 3 presents the electrical specifications of the control unit.

Head-to-unit connector	FFA Male	
PC-to-unit connector	USB 2.0 Type-A to Mini-B	
Maximum supply voltage	5 V	
Maximum supply current	500 mA	
OS compatibility	Windows 7/8/10 64 bits	

Table3: Electrical specifications of the control unit.

4.4. Environmental specifications

Operating conditions					
Ambient temperature 10 – 40°C					
Relative humidity	20 – 70%				
Atmospheric pressure 700 – 1060 hPa					
Altitude	2000 m (maximum)				
Transport and storage conditions					
Temperature	-10°C – 60°C				
Relative humidity 10 – 80%					
Atmospheric pressure 500 – 1060 hPa					

Table 4 presents the environmental specifications of the optical power meter.

Table4: Environmental specifications for operation, transport and storage of the optical power meter.

5. Technical specifications: Argo-HM glass

5.1. General description

The Argo-HM glass is a special glass substrate produced at the Argolight facility to insure its chemical homogeneity and purity. The glass includes the Argo-HM-like fluorescent patterns.

5.1.1. Glass refractive index

The dispersion of the refractive index of the glass is shown in Figure 1. The measurement uncertainty is \pm 7.10⁻⁴. The Argo-HM glass features the same refractive index as microscope cover-slips.



Fig.1: Dispersion of the refractive index of the glass.

The Sellmeier equation for the refractive index dispersion of the glass is (λ in nm):

$$n^2(\lambda) = A + \frac{B\lambda^2}{\lambda^2 - C} + \frac{D\lambda^2}{\lambda^2 - E}$$
, which coefficients are provided in Table 5.

Sellmeier coefficient	Value		
А	-31.204		
В	33.798		
С	823.160 nm ²		
D	-0.317		
E	35743.000 nm ²		

Table5: Sellmeier coefficients for the refractive index dispersion of the glass.

5.1.2. Patterns overview

The Argo-HM-like patterns are described in Figure 2. They are positioned $(170 \pm 5) \mu m$ below the top glass surface, on a horizontal plane which flatness is within ± 5 mrad. This emulates the presence of a microscope cover-slip, having a thickness of $(170 \pm 5) \mu m$ and a refractive index of (1.5255 ± 0.0015) at 546.1 nm. The maximum relative positioning error of ± 110 nm in XYZ within each individual pattern is certified by the manufacturing tools. The lateral (*i.e.* in the XY plane) thickness of the lines composing the patterns is about $(110 \pm 20) nm$ FWHM (Full Width at Half Maximum). The axial (*i.e.* along the Z direction) thickness of the patterns is about $(600 \pm 200) nm$ FWHM.



Fig.2: Schematics of the patterns inside the slide.

5.1.3. Fluorescence spectral features

The patterns exhibit the following fluorescence spectral features.

- Excitation:

The fluorescence emitted by the patterns is efficiently excitable from 300 to 650 nm, although above 550 nm, powerful light sources and/or sensitive sensors are required. The excitation efficiency is maximum at around 340 nm and drops towards the red wavelengths. A typical absorption spectrum is shown in Figure 3.



Fig.3: Typical absorption spectrum of the patterns.

- Emission:

The fluorescence emission spectrum is a continuum starting from slightly above the excitation wavelength up to 800 nm. Typical emission spectra are shown in Figure 4 for UV-blue excitation wavelengths and in Figure 5 for visible excitation wavelengths.



Fig.4: Typical emission spectra of the patterns for excitation wavelengths at 365, 375 and 405 nm.



Fig.5: Typical emission spectra of the patterns for excitation wavelengths at 488, 514 and 543 nm.

- Lifetime:

Using FLIM (Fluorescence Lifetime Imaging Microscopy), two main decay components of (0.25 ± 0.05) ns and (2.50 ± 0.50) ns have been measured. These values are only provided here for information as they depend on the excitation and emission acquisition configurations. A typical fluorescence decay is shown in Figure 6.



Fig.6:Typical fluorescence decay of the patterns for λ_{exc} =400 nm, $\Delta\lambda_{em}$ =500±20 nm, 10×/0.25 objective.

- Photo-stability:

The fluorescence intensity of the patterns may decrease. However, this decrease is transient. The fluorescence intensity recovers to its initial value after some time. The recovery time depends on the irradiation conditions (power density, wavelength, pixel size, exposure time). A typical fluorescence intensity recovery signal is shown in Figure 7.



Fig.7: Typical fluorescence intensity recovery signal of the patterns. The power density was about 10 W.cm-2, the excitation wavelength was (470 ± 20) nm and the collection window was (525 ± 25) nm. After one minute of acquisition and four minutes of waiting time, the fluorescence fully recovers for these irradiation conditions. When the waiting time is not sufficient, the fluorescence intensity does not restart at its original level.

The field or rings (section 5.2.2), the 4×4 (section 5.2.3) and the 2×16 (section 5.2.4) intensity gradations are patterns for which fluorescence intensity is important. They must therefore be imaged with a lot of care:

- First, move to a pattern for which intensity is not important, such as a repositioning cross or the Argolight logo.

- Second, set all the acquisition parameters (illumination power, sensor gain, exposure time, etc.) for one of these patterns.

- Third, move to the pattern of interest (field of rings, 4×4 or 2×16 intensity gradations) and image it in one shot.

Also, it is not recommended to image a single pattern by stitching overlapping image tiles.

By following this procedure, the transient fluorescence decay has barely the time to occur, making the recovery time much faster. This procedure allows a more frequent imaging.

5.1.4. Suggested tests

The Argo-HM glass can be used to assess the following characteristics of fluorescence imaging systems (non-exhaustive list):

- Inhomogeneity of illumination,
- Distortion of the field of view,
- Chromatic aberrations and shifts between channels,
- Stitching performance,

- Stage repositioning repeatability,
- Stage drift during stacking,
- Intensity response of the system,
- Spectral response of the system,
- Lateral resolving power,
- Axial resolving power
- Pseudo-point spread function,
- 3D reconstruction precision,
- Scanning performance,
- Parfocality and parcentrality between objectives,
- Optical axis determination,
- Objective issues,
- Distances in XY and Z.

5.2. Description of the patterns

To know more about the functions and applications of each pattern, you can consult the Applications guide, available on the Argolight website: http://argolight.com/how-to-use/.

The analysis of the patterns in Daybook software is described within Daybook's documentation, available directly in the software, under the Help menu.

5.2.1. Target

This pattern, depicted in Figure 8, consists in concentric circles with increasing radii from 25 μm to 300 μm with a step of 25 μm , plus an extra circle with a radius of 12.5 μm , featuring a target.





5.2.2. Field of rings

100 µm

This pattern, depicted in Figure 9, consists in a matrix of 39 \times 39 rings, separated by 15 μm , on a total field of 600 \times 600 μm^2 . The field of rings is surrounded by eight landmarks and exhibits a 7.5 μm long cross in its center.



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5.2.3. 4×4 intensity gradation

This pattern, depicted in Figure 10, consists in sixteen 8.5 μ m-wide squares having different fluorescence intensity levels following a linear evolution, organized in a 4×4 matrix.



Fig. 10: Schematics of the 4×4 intensity gradation. All dimensions are in µm.

5.2.4. 2×16 intensity gradation

This pattern, depicted in Figure 11, consists in twice sixteen 22.5 μ m × 1.5 μ m rectangles having different fluorescence intensity levels following a linear evolution, organized in a 2×16 matrix.



Fig.11:Schematics of the 2×16 intensity gradation. All dimensions are in μ m.

5.2.5. Gradually spaced lines

This pattern, depicted in Figure 12, consists in pairs of 50 μ m-long lines which spacing gradually increases, from 100 to 700 nm, with a step of 50 nm. Four sets of lines are present: one vertical, one horizontal, and two oriented at + and - 45°.





5.2.6. Matrix of crosses

This pattern, depicted in Figure 13, consists in a matrix of 6 × 6 crosses, having a length of 5 µm and separated by 8 µm, surrounded by a frame. The crosses are composed of vertical lines that are in the same plane, and by horizontal lines, going gradually deeper within the glass. The spacing between the vertical and horizontal lines gradually increases, from 0 to 3.5 µm, with a step of 100 nm.



Fig.13:Schematics of the matrix of crosses. All dimensions are in $\mu m.$

5.2.7. Meridians of a sphere

This pattern, depicted in Figure 14, consists in three circles of diameter 50 μm in different orthogonal planes, featuring the meridians of a sphere.



Fig.14: Schematics of the meridians of a sphere. All dimensions are in μm

5.2.8. 3D crossing stairs

This pattern, depicted in Figure 15, consists in empty cylinders embedded at different depths, like two crossing stairs, surrounded by four pillars. There are four stairs in the slide, with varying steps: 1, 0.75, 0.5 and 0.25 μ m.



Fig. 15: Schematics of the 3D crossing stairs. All dimensions are in μm .

5.2.9. Repositioning crosses

The repositioning crosses, depicted in Figure 16, are 20 μ m long and are positioned 500 μ m from one to another in the X direction, the Y direction, or both.



Fig.16:Schematics of one of the repositioning crosses. All dimensions are in µm.

5.2.10. Logo

This pattern, depicted in Figure 17, consists in letters forming the company name "Argolight", and surrounded by a 220 $\mu m \times 50 \; \mu m$ frame.



Fig.17:Schematics of the Argolight logo. All dimensions are in μ m.

5.2.11. Coordinates of each pattern

Table 6 presents the XY coordinates, relative to the central cross just below the Argolight logo, of the center of each pattern, in order to help for the automation of the image acquisition. The positioning precision of each pattern is within \pm 2 μm in both X and Y directions.

Pattern	Relative coordinates (X;Y) in µm		
Central cross (just below the logo)	(0;0)		
Logo	(0;85)		
Target	(-500;500)		
Field of rings	(500;500)		
4×4 intensity gradation	(-750;-200)		
2×16 intensity gradation	(-250;-200)		
Gradually spaced lines (horizontal)	(-750;-500)		
Gradually spaced lines (vertical)	(-250;-500)		
Gradually spaced lines (+45°)	(-750;-800)		
Gradually spaced lines (-45°)	(-250;-800)		
Matrix of crosses	(250;-200)		
Meridians of a sphere	(750;-200)		
3D crossing stairs ($\Delta Z = 1 \ \mu m$)	(250;-500)		
3D crossing stairs ($\Delta Z = 0.75 \ \mu m$)	(750;-500)		
3D crossing stairs ($\Delta Z = 0.5 \ \mu m$)	(250;-800)		
3D crossing stairs ($\Delta Z = 0.25 \ \mu m$)	(750;-800)		

Table6:XY coordinates of the center of each pattern relative to the central cross just below the Argolight logo.

6. Operating the power meter

6.1. Installation

- Download the Daybook software here: http://argolight.com/daybook-software/
- Download the "*.argo" calibration file on the website.

- 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. A settings panel will open.

- In the settings panel, select the Daybook version compatible with your device: Argo-POWER^{HM}.

SETTINGS PANEL	
Daybook settings profile (according to your Argolight product type)	Select your Argolight
Source folder setting	product type
argo-HM	
Export folder setting	
C:\Users\Desktop\result	
Upload calibration file for power meter 🕑 🗲 – – – – – – – – – – – – – – – – – –	Upload the calibration file
✓ Advanced data mode on/off	
✓ Hide the settings panel on startup	
Ok Cancel	

Fig. 18: Settings panel.

Daybook-	Analysis	
Analysis	\triangleright	
Power meter	⊳+-	 Click on the "Power meter" button
Reload results		
Profile name		
	*	
Source folder	0	
Result folder	0	
Report folder	0	

Fig. 19: Daybook 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.

6.2. Live measurements

- Select a microscope objective without immersion.
- 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 sensor's center, using the XY translation stages (*cf.* Figure 20).

For inverted microscopes, use the target etched at the back of the Argo-POWER^{HM} head to center the beam on the sensor (*cf.* Figure 21).



Fig.20:Argo-POWER^{HM} head inserted in the sample holder of an upright wide-field fluorescence microscope.



Fig.21:Argo-POWER^{HM} head inserted in the sample holder of an inverted fluorescence 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 22).



Fig.22: Argo-POWER^{HM} head inserted across the optical path of a collimated light beam.

- Click on the "Power measurement" button (cf. Figure 23).

Power meter		
Analysis 🕞	POWER -min:-s	Measured optical power vs acquisition duration
Power meter 🕞		1.0
Reload results 🛛 🕀		0.0 Click on the "Power
		s 94 measurement" button
Profile name		
Source folder		8 0.6
Result foldor 0		10 0.5
Report folder	Irradiance: disabled	§ 0.4
	Power measurement Set ters Pause Stop	0.3
	Settings	Fill out or modify these two
	COM port number 15 2 Auto-detection	mandatory parameters
	Bamping period (s) 1	0.0
	Acquisition duration (min s)	0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.80 0.95 1.00 Time (s)
	Remarkation type (madance computation) - disable	Measured optical power vs power instruction
	Objective magnification (c)	
	Numerical aperture	1.0
	2	0.9 5 0.0
	A Add values Delete & Import & Display	5 0.0 0 1 0 7
	100	
		3 0.5
		B 0.4
		5 0.3
		\$ 0.2
Hop	🖹 Save results 🖹 Export metrics 🛢 Generate report	0.1
About The Backbackt E		0.0
#81/e		0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.45 0.70 0.75 0.80 0.85 0.90 0.95 1.00 1.05 Power instruction (%)
Contraction of the local division of the loc		

Fig.23: 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 24).



Fig.24: Live optical power measurement.

- Start the optical power measurements.

6.3. Statistics/metrics

Statistics/metrics are displayed on the right of the gauge:

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

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

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

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

- The deviation (in %):

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

- The stability (in %):

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

- The maximum is the maximum power P_{max} measured during the acquisition,

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

6.4. Time-lapse optical 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 25), *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.



Fig.25: Example of evolution of the optical power versus the acquisition duration.

6.5. Irradiance calculation mode

For the sake of reproducing 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 W.cm⁻², watt per square centimeter) is defined as the ratio of the optical power (in W, watt) divided by the illuminated surface (in cm², 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 illumination mode.

For a wide-field illumination, the irradiance is given by the following formula:

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

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).

<u>Note:</u> 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 a point-scanning (e.g. confocal) illumination, the irradiance is given by the following formula:

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

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 is considered as Gaussian.

Note that the irradiance for point-scanning microscopes can be several orders of magnitude higher than that for wide-field microscopes.

Once the type of illumination is chosen, and the associated parameters are filled-out, Daybook provides the irradiance (*cf.* Figure 26) in live, based on the measured optical power and the formula described above.



Fig.26: Example of irradiance live measurement for wide-field illumination.

6.6. Measured optical power versus power instruction

In most of the acquisition softwares of fluorescence microscopes, the adjustment of the light sources optical power is performed in fraction 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 instruction in % should be regularly carried out to check the linearity of the evolution, the absolute power values, or the compliance with the specifications after a system installation or maintenance. Figures 27 and 28 show the evolution of the optical power versus the power instruction for one and two light sources, respectively.



Fig.27: Example of measured optical power versus power instruction for one light source.



Fig.28:Example of measured optical power versus power instruction for two light sources.

6.7. Saving options

There are different options to save the results (cf. Figure 29):

- Right click on the graphs and saving as a "*.txt" file the values or as a "*.png" file the picture.

- Click on the "Save results" button to save the graphs into "*.png" 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.



Fig.29: Different options to save the results.

7. Calibration, accuracy and limitations

7.1. Calibration

The power meter is calibrated by Argolight prior to shipping. An "*.argo" calibration file is provided, which must be uploaded into Daybook the first time the power meter is used. An Argolight certificate of calibration is provided with the product.

The power meter response can change or deteriorate with time, especially if it has been illuminated with excessive average and/or peak power. Argolight advises to recalibrate the power meter every 12 months to ensure accuracy in the measurements.

7.2. Calibration accuracy and measurement uncertainty

Argolight calibrates its power meters using secondary standards directly traceable to LNE (Laboratoire national de métrologie et d'essais, the official French metrology institute). The measurement uncertainty of the power meter is claimed to be \pm 10%, with k = 2. This means there is a 95% probability that the reported measurement from the power meter falls within \pm 10% of the LNE reference detector standard. The given uncertainty contains all possible contributions to the measurement error: The calibration accuracy, the non-uniformity of the detector response over its surface, and the light reflections from the components of the sensor.

7.3. Calibration limitations

The calibration stands as long as the power meter is operated within the environmental specifications (see section Environmental specifications), within the optical power range (10 μ W – 100 mW), and within the linear region of the detector response.

Special attention should be brought when non-focused laser pulses are shined onto the detector. Especially with laser pulses, saturation effects may occur, depending upon the wavelength, peak power, pulse shape, pulse duration and/or repetition rate.

8. Regulatory compliance

8.1. Product safety and electromagnetic compatibility

The Argo-POWER^{HM} devices have been tested and found to comply with product safety and electromagnetic compatibility requirements. For a complete list of tests and for certification details, please contact your Argolight representative.

8.2. CE marking

The "CE" logo (see on the right) etched on the control unit indicates that this equipment complies with the following directives

- Council Directive 2014/35/EU Low Voltage Directive
- Council Directive 2014/30/ECU EMC Directive
- Council Directive 2012/19/EU WEEE Directive
- Council Directive 2011/65/EU RoHS



<u>Note:</u> 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 when the equipment is operated in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, 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.

<u>Warning:</u> Changes or modifications not expressly approved by Argolight could void the user's authority to operate the equipment.

8.4. WEEE directive

The "crossed out bin" logo (see below) etched in the control unit indicates that this product should not be disposed of along with municipal waste, that the product should be collected separately, and that a separate collection system exists for all products that contain this symbol within member states of the European Union.

As required by the WEE (Waste Electrical and Electronic Equipment Directive) of the European Union (EU) and the corresponding national laws, Argolight offers all end users in the EU the possibility to return "end of life" Argo-POWER^{HM} without incurring disposal charges.

This offer is valid for Argolight Argo-POWER^{HM}:

- Sold after January 1st of 2018,
- Sold to a company or institute within the EU,
- Currently owned by a company or institute within the EU,
- Still complete, not disassembled and not contaminated.



If you wish to return an Argo-POWER^{HM} for waste recovery, please contact Argolight or your nearest distributor for further information.

If you do not return an "end of life" Argo-POWER^{HM} to Argolight, you must hand it to a company specialized in waste recovery. Do not dispose the device in a litter bin or at a public waste disposal site.

9. Frequently Asked Questions

9.1. I have connection issues

When the control unit is not recognized by the computer, a warning message is displayed (*cf.* Figure 30). 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 31).



Fig.30: Displayed warning message for connection issues.



Fig.31: Device manager window showing the device is properly recognized by the PC.

When facing connection issues, try to unplug and connect again the USB cable. Then click on the "Auto-detection" button.

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

9.2. I cannot measure the optical power

When the excitation wavelength and/or the sampling period are not filled out, a warning message is displayed (*cf.* Figure 32). When this occurs, fill out the excitation wavelength and/or the sampling period, and click on the "Power measurement" button again.



Fig.32: Displayed warning message for missing parameters.

9.3. 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 33).



Fig.33:Offset option to subtract the optical power from any other contribution than the light source to be measured.

Light power monitoring - Wavelength / Channel :

	Fill out your own scale of input power				
DATE					

Date of issue

27/09/18

Version

1.0

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Note for readers

The experimental data shown in this documentation are informative and not contractual. They may be different from one system to another.

A word about waste management

Argolight policy is to offer robust products that last. In the event our products become useless to you, please contact us so we can pick them up and recycle them. Please do not throw away the devices with common waste. The composition of the glass and electronic parts require specific recycling. Thank you.

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