Table of contents

This test concerns only the microscopes that can acquire fluorescence emission spectra.

I. SPECTRUM ACQUISITION PROCEDURE

The ***"spectral response"*** analysis is associated with the ***"target"*** (Pattern A), ***"logo"*** (Pattern J), or ***"repositioning cross"*** (Pattern H) patterns (see Figure 1).

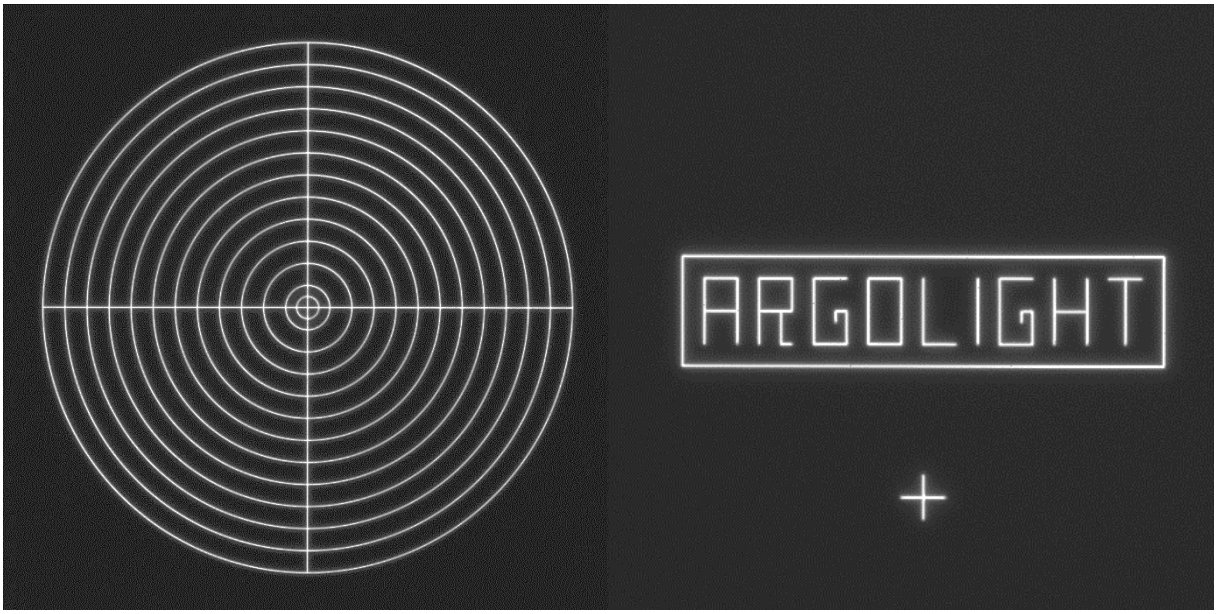

Figure 1: Image example of the target, logo and repositioning cross.

1. ACQUISITION RECOMMENDATIONS

This test concerns only the microscopes on which the spectrum measurement mode is available.

- **Recommended file type**

Z stack	No
Multi-channel	Recommended but not mandatory: acquire emission spectra with specific excitation wavelengths (365, 375, 405, 488, 514 and 543 nm)
Tiles	No
Lambda-scan	Yes

Do not zoom in, this could damage the pattern.
The area of the scanned zone should not be smaller than the area of the pattern.

- **Data organization in the "*.csv" file**

The data in the "*.csv" or "*.txt" file should be organized as follow:

- Data should be organized in columns:
  Wavelength (in nm);
  Intensity (in arbitrary units).
- Data should be separated by a comma or a semicolon.
- Rational numbers should be written with dots (no comma).
- There can be several header lines (text format).

```
wave_length,measured_intensity
430;6.727938307449222e-05
435;0.00015864457236602902
440;0.00022089271806180477
445;0.00024471088545396924
450;0.000414153968449682
455;0.0007094984757713974
460;0.0014022840186953545
465;0.0029159511905163527
470;0.0061706011183559895
```

Figure 2: Example of a correct file organization.

A spectrum example acquired following the acquisition recommendations can be found in your Daybook folder, located here: C:\Program Files\Daybook\Daybook-Analysis\trial images
We encourage you to process this spectrum to start being familiar with the use of the software.

2. HOW TO ACQUIRE THE SPECTRUM?

1- Find the patterns

a) Start with a low mag objective (such as 10× or 20×). Set the DAPI (405 nm) or GFP (488 nm) channel.
b) Make coincide the center of the slide with respect to the objective.
c) Adjust focus through the eyepieces.

d) Switch to the objective you'd like to use. Move the slide to the pattern.

2- Adjust your setup

a) Match the center of the pattern with the center of the field of view.
b) Adjust the focus.
   The best focus usually corresponds to the Z-plane for which the central cross looks the clearest (qualitative approach) and/or for which the intensity histogram is the broadest (quantitative approach).

3- Acquire the spectrum

a) Image the pattern by following the acquisition recommendations.
b) Save spectra into a "*.csv" or "*.txt" files.

II. SPECTRUM ANALYSIS PROCEDURE

1. HOW TO LAUNCH AN ANALYSIS?

a) Select “Spectral response” in the “Select analysis” list.

b) Click on “Upload file” to upload the “*.csv” or “*.txt” file of the acquired spectrum.
Click on “Display data” to select the spectrum.

c) Set the required and optional settings (see chapter 2 “Analysis Settings”).

d) Click on “Start the analysis”.

e) Results are displayed and can be saved as “*.csv”, “*.pdf”, or transferred into Daybook Data Manager (if available in your package).

2. ANALYSIS SETTINGS

1- <u>Required settings</u>

- **Argolight type of glass**
  There are 2 types of ArgoGlass®; they both have slightly different spectral features (excitation and emission) which can influence the analysis.

  Select the type of glass according to your slide type:
  For an Argo-POWER, Argo-HM, Argo-LM, Argo-SIM and Argo-Check → Select AG02.
  For an Argo-Z → Select AG01.

- **Central excitation wavelength**
  The central excitation wavelength is usually the wavelength of the laser used when acquiring the image.

III. RESULTS PAGE DESCRIPTION

The picture below shows the results interface for this analysis (*cf.* Figure 3).
Results are displayed in the form of graphs and tables.

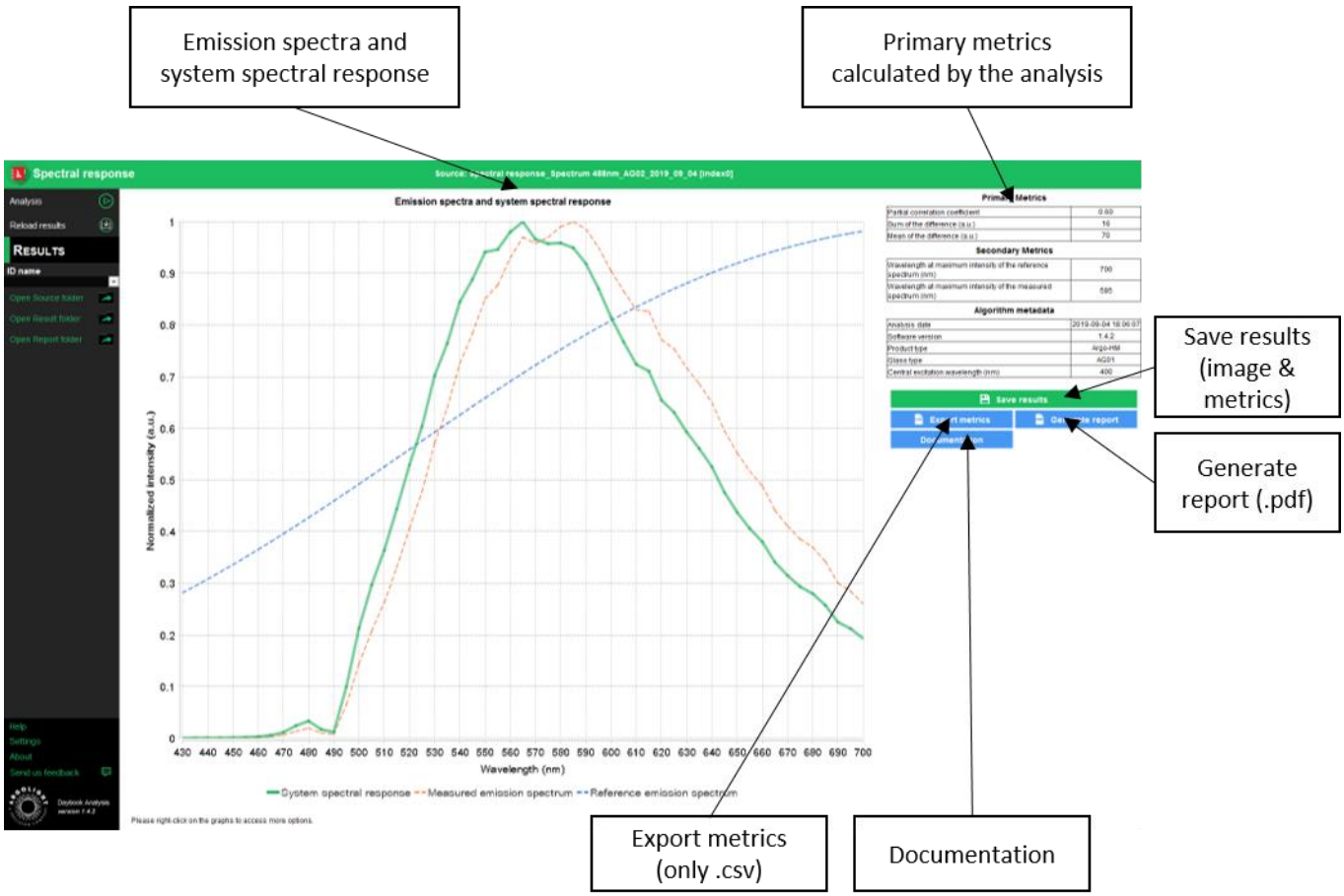

Figure 3: Results page.

When Daybook Data Manager is disabled, the results can be saved into a "*.csv" file thanks to the "Save results" or "Export metrics" buttons.

When a valid Daybook Data Manager license is registered, the results are transferred into Daybook Data Manager thanks to the "Save in Data Manager" button.

Reports (in a "*.pdf" format) containing the results (maps, graphs, metrics) can be generated and saved by clicking on the "Generate report" button (*cf.* Figure 9).

By default, the results will be saved in the "/Daybook results" folder, located within the Daybook directory. To modify the default folder, go to the "Settings" menu at the bottom left corner.

- **Graph options:**

- Zoom in and out: Hold the left or right button of the mouse and move it towards the bottom right to create a selection rectangle. To go back to the initial size, hold the left or right button of the mouse and move it towards any direction.
- Optional features. Right click on the graph to have access to:
  - "Properties": Edit the chart properties.
  - "Save as": Save an image into a "*.png" file, or the graph values into a "*.txt" file.
  - "AutoRange": Adjust automatically the ranges of the axes.

IV. ANALYSIS ALGORITHM DESCRIPTION

The diagram below describes the algorithm that allows the extraction of the spectral response from one spectrum of a target, logo or repositioning cross (*cf.* Figure 4).

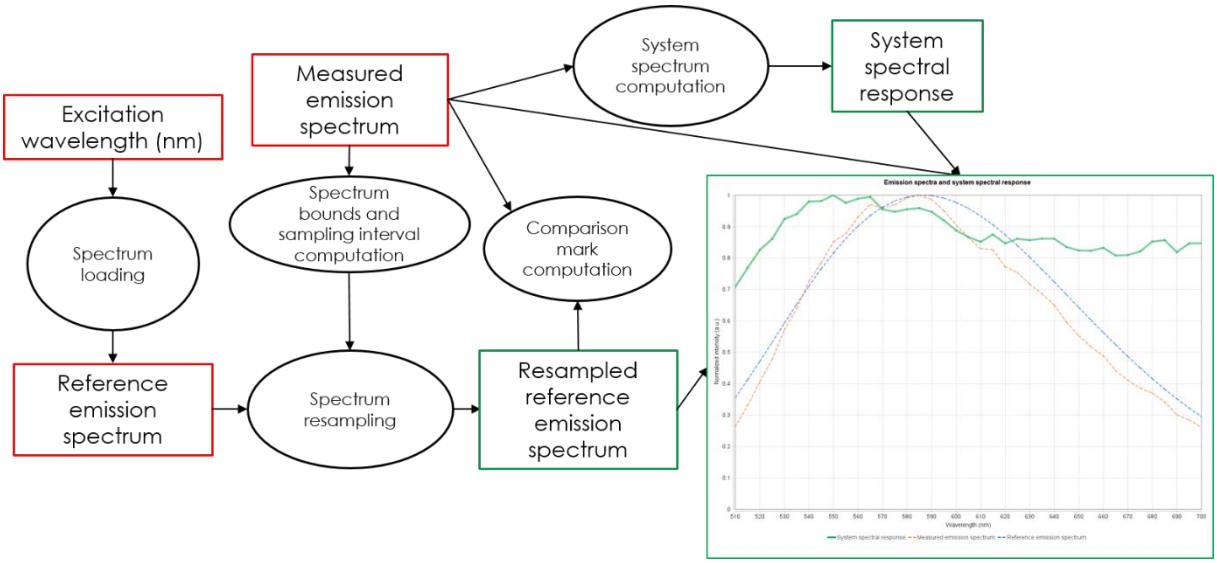

Figure 4: Schematic description of the different steps of the analysis algorithm.

In short, the algorithm works as follows:

- It resamples, if needed, the measured spectrum.
- It divides the measured spectrum by the reference spectrum to deliver what is called the spectral response of the system.
- It displays the spectral response with the measured and reference spectra into the same graph.

V. OUTPUT METRIC DESCRIPTION

- *Spectral response* is the ability of a device to detect photons within a given spectral range. It is expressed as the ratio between a measured reference spectrum by a "true" reference one, both acquired at the same excitation wavelength. It is unitless.

1. PRIMARY METRICS

- *Partial correlation coefficient (r')* provides information about how close the measured spectral response is from the reference one, stored in Daybook database. It is unitless, and is given by the following formula:

$$r' = \frac{mean(Intensities_{ref} \times Intensities_{mes}) - mean(Intensities_{ref}) \times mean(Intensities_{mes})}{\sigma(Intensities_{ref}) \times \sigma(Intensities_{mes})}$$

  Where *mean* denotes the mean (average) value and σ the standard deviation.

- *Sum of difference* is the sum of the absolute value of the difference between the measured and the reference spectra. It is expressed in arbitrary unit, and is given by the following formula:

$$Sum\ of\ difference = \sum |I_{measured} - I_{reference}|$$

  Where $I_{measured}$ is the measured spectral intensity values and $I_{reference}$ the reference spectral intensity values.

- *Mean of difference* is the mean of the absolute value of the difference between the measured and the reference spectra. It is expressed in arbitrary unit, and is given by the following formula:

$$Mean\ of\ difference = \frac{1}{n}\sum |I_{measured} - I_{reference}|$$

  Where *n* is the number of experimental points, $I_{measured}$ is the measured spectral intensity values and $I_{reference}$ the reference spectral intensity values.

2. SECONDARY METRICS

- *Wavelength at maximum intensity for the reference spectrum* corresponds to the wavelength at which the intensity of the reference spectrum is maximal. It is expressed in nm.

- *Wavelength at maximum intensity for the emission spectrum* corresponds to the wavelength at which the intensity of the measured spectrum is maximal. It is expressed in nm.

3. ALGORITHM METADATA

- *Analysis date* is the date at which the analysis has been performed.
- *Software version* is the version of the software.
- *Product type* is the type of Argolight product selected in the panel settings.
- *Glass type* is the type of ArgoGlass® selected in the analysis settings.
- *Excitation wavelength* corresponds to the wavelength of the illumination source used to acquire the fluorescence spectrum. It is pre-set by the user. It is expressed in nm.

4. IMAGE METADATA

- *Acquisition date* is the date at which the acquisition of the image has been performed. If this information is not contained in the metadata of the image, then the note “unknown” is displayed.

Encountered an issue or a question when running this analysis?
Please send a screenshot and your issue description at
customer@argolight.com