USER GUIDE

Argo-WP

v2.0

Argo-**WP**

Table of contents

1. Introduction

The Argo-WP (Well Plate) is specifically designed to assess and monitor the performance of high-content screening (HCS) and high-throughput screening (HTS) fluorescence imaging systems that can image each well of 96-well cell culture plates with high resolution (to look at cell populations for instance). The Argo-WP has a 96-well plate format. It is compatible with system magnifications ranging from 10 up to 60× and contains the third generation of Argoglass®.

This product is composed of:

- An aluminum plate that includes four pieces of Argoglass® with embedded fluorescent patterns inside them.
- A dedicated software, Daybook, to generate, track and export quality control data.

The pieces of Argoglass® consist of a special glass substrate with different fluorescent patterns embedded inside. The Argo-WP is designed to quality-control many aspects of an HCS or HTS fluorescence imaging system: field uniformity, field distortion, lateral co-registration accuracy, system intensity response, stage drift during Z-stacking, etc.

The Daybook software has two modules:

- The “Analysis” module: it allows to analyze and extract data (maps, graphics and metrics) from images of the patterns, in order to measure significant metrics of HCS or HTS fluorescence imaging systems.
- The “Data Manager” module: it allows to visualize the data generated by the “Analysis” module, monitor the results, and manage the quality control reports.

The best of Argolight technology, adapted into a 96-well plate format, and combined with the Daybook software, opens the path to easy, yet reliable and complete, quality control of HCS or HTS fluorescence imaging systems.

2. First use

2.1. Parcel verification

Inside the package, you will find:

- 1 Argo-WP.
- 1 storage box.
- 1 user guide documentation.
- 1 certificate of inspection.

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

2.2. Quick start procedure

In order to quickly find and capture images of fluorescent patterns, we advise to follow the following steps:

- In your fluorescence imaging system, select a low magnification microscope objective, typically a 10× or 20×.
- Illuminate one of the four glasses (preferably the one containing the grid pattern, in the well C8) 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 glass, using the XY translation stages.
- Adjust the focus into the glass until clearly observing the fluorescent patterns through the camera(s).
- Move the plate to observe the pattern(s) of interest.
- Switch to your working microscope objective.
- Re-adjust, if necessary, the position of the pattern(s) and the focus in the glass. Once you are perfectly in focus and the pattern is centered with respect to the field of view, you can set to zero the X, Y and Z positions in the acquisition software, so that it will be easier to come back later to the pattern position.
- Start your imaging session.
- Save the image(s) of your pattern(s) of interest from your usual acquisition software.
- Run the “Analysis” module from Daybook to get meaningful results.

To know how to image your pattern(s) of interest properly, please refer to our documentation, available within the Daybook “Analysis” module, or online on our website (www.argolight.com).

3. General handling and care

3.1. Handling and storage

In order to make the Argo-WP last for many years, we recommend that you observe the following handling and storage instructions:

- The third generation of Argoglass® is compatible with any type of immersion medium (oil, water, glycerol and air). In the case of a water immersion, continuous exposure higher than 20 minutes in a row should be avoided. When longer continuous exposures are required, use oils with the same refractive index as water as an immersion liquid.
- Do not illuminate with pulsed lasers used in multiphoton microscopy.
- Do not illuminate with irradiances (peak or average) larger 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.
- Store in its suitcase (after having removed entirely the immersion liquid) at ambient temperature (10 – 40 °C) and normal relative humidity (20 – 70 % RH). Avoid ultraviolet irradiation.

3.2. Cleaning

To clean the glass surfaces, we recommend that you observe the following cleaning instructions:

- Remove dust using pressured air.
- Use lens tissue soaked with ethanol or isopropanol (alcohol degree higher than 90° for both), as one would do for any regular optical component.

Wearing gloves is advised. Do not use acetone.

3.3. Operating environment

The Argo-WP has been designed to be used at room temperature (10 – 40 °C) and under normal relative humidity (20 – 70 % RH). Both the glass and the aluminum plate composing the Argo-WP have a low thermal expansion

coefficient, so that temperature variations will not significantly affect the imaging of the patterns.

4. Technical specifications

4.1. Physical and dimensional specifications

The aluminum plate has the same format and dimensions as 96-well plates, as shown in Figure 1. The dimensions of the plate comply with the ANSI SLAS 1 to 4 – 2004 (R2012) standards. The weight of the Argo-WP is approximately 180 g.

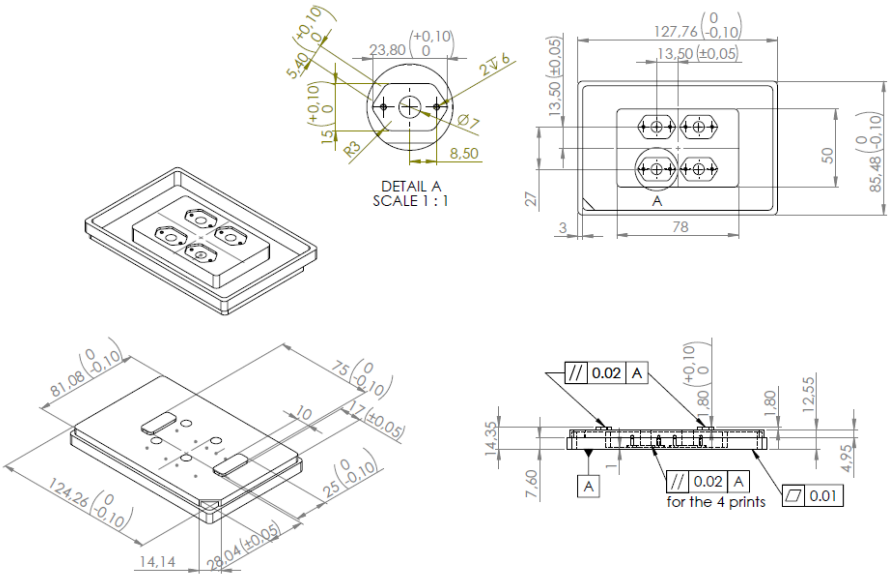


Figure 1: Scheme of the aluminum plate with dimensional specifications. All dimensions are in mm. General tolerances are ± 0.1 mm.

The acquisition software of most of HCS or HTS fluorescence imaging systems includes a plate configuration step which requires knowledge of some dimensions of the plate to be imaged (*cf.* Figure 2 and Table 1). Some of the labels and dimensions shown in Figure 2 and Table 1 may vary from one system to another.

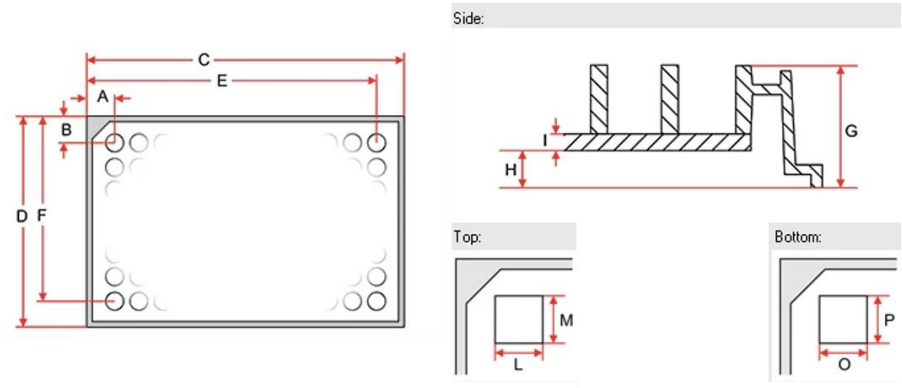


Figure 2: Plate and well dimensions required by the acquisition software of most of the HCS or HTS fluorescence imaging systems.

Label	**A**	**B**	**C**	**D**	**E**		**F**
Dimension (mm)	14.4	11.2	127.8	85.5	113.4		74.2
Label	**G**	**H**	**I**	**L**	**M**	**O**	**P**
Dimension (mm)	12.6	1.0	0.1	7.0	7.0	7.0	7.0

Table 1: Plate and well dimensions required by the acquisition software of most of the HCS or HTS fluorescence imaging systems.

4.2. Glass refractive index

The plate contains four pieces of Argoglass®, which is a special glass substrate produced at the Argolight facility to ensure homogeneity and purity.

The dispersion of the glass refractive index is shown in Figure 3. The measurement uncertainty is ± 0.001. The Argoglass® features the same refractive index as microscope coverslips, defined in the ISO 8255-1:2017 standard.

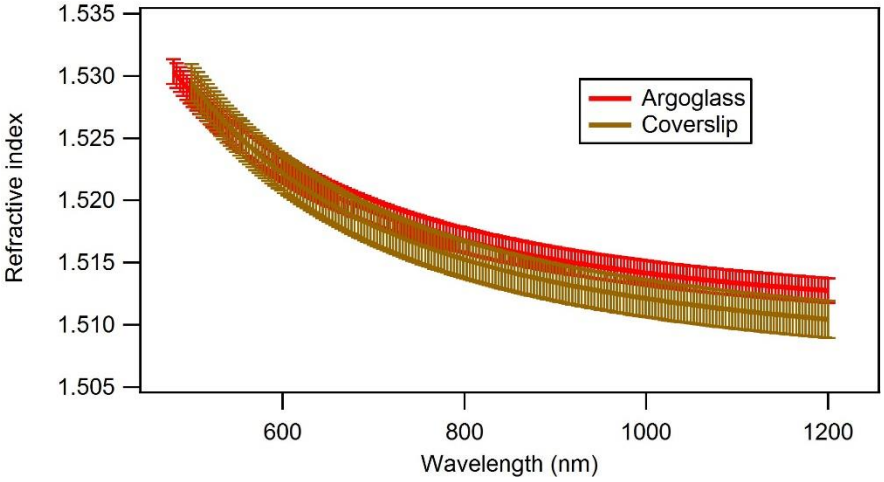


Figure 3: Dispersion of the refractive index of the Argoglass®, compared to the one of microscope coverslips.

The Sellmeier equation for the glass refractive index dispersion 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 2.

Sellmeier coefficient	**A**	**B**	**C**	**D**	**E**
Value	0.699	0.697	136.960 nm^2	0.883	15269.000 nm^2

Table 2: Sellmeier coefficients for the refractive index dispersion of the Argoglass®.

4.3. Patterns overview

The fluorescent patterns, depicted in Figure 4, are positioned (170 ± 5) µm below the top surface of the Argoglass®, whose optical flatness is typically 0.065 µm, on a plane whose parallelism with respect to the bottom surface of the aluminum plate is less or equal to 5 mrad.

These features emulate the presence of a microscope coverslip, having a thickness of (170 ± 5) µm and a refractive index of (1.5255 ± 0.0015) at 546.1 nm.

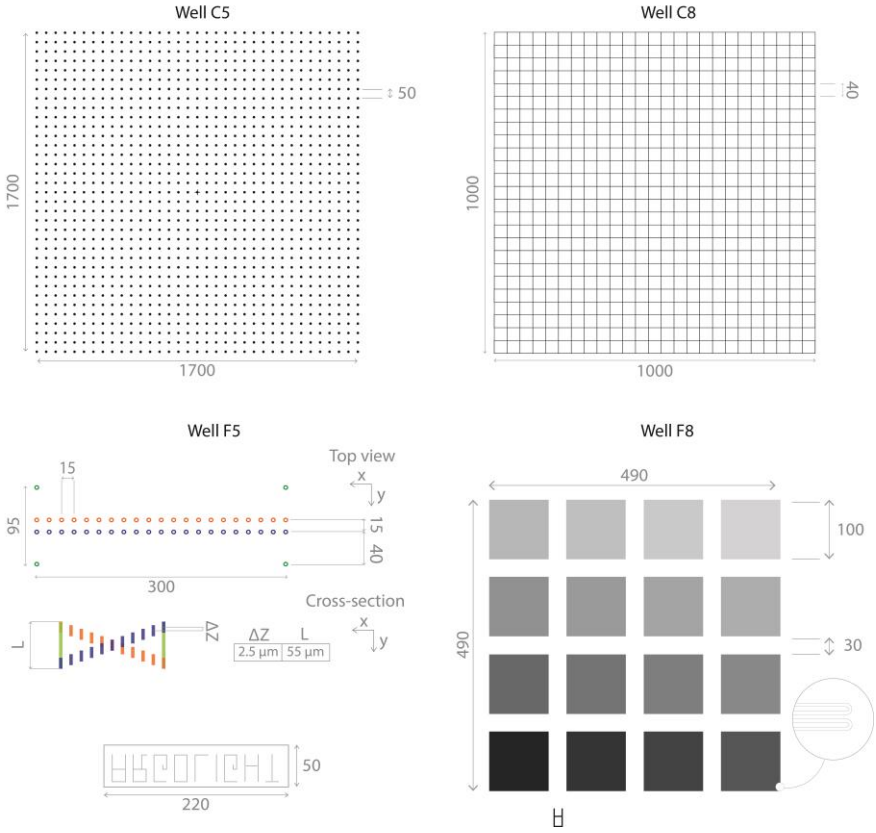


Figure 4: Schematics of the patterns inside the four pieces of glass of the plate. All dimensions are in µm.

The constituents within each individual pattern are positioned with a maximum relative error of ± 0.11 µm in XY and ± 0.15 µm in Z.

4.4. Fluorescence spectral features

The spectral features (excitation spectrum, emission spectrum and lifetime) of the patterns depend on the excitation and emission wavelengths, on the spatial scale at which they are measured, and on the illumination power density and duration.

Given the large range of possible irradiation conditions, Argolight only provides typical spectral features averaged on a scale of several micrometers, which are only valid for given excitation/emission conditions. Typical fluorescence spectral features are shown below.

- ***Excitation***

The excitation ranges from 250 nm up to 650 nm. The excitation efficiency is maximum at around 340 nm and drops towards the red wavelengths. A typical absorption spectrum is shown in Figure 5.

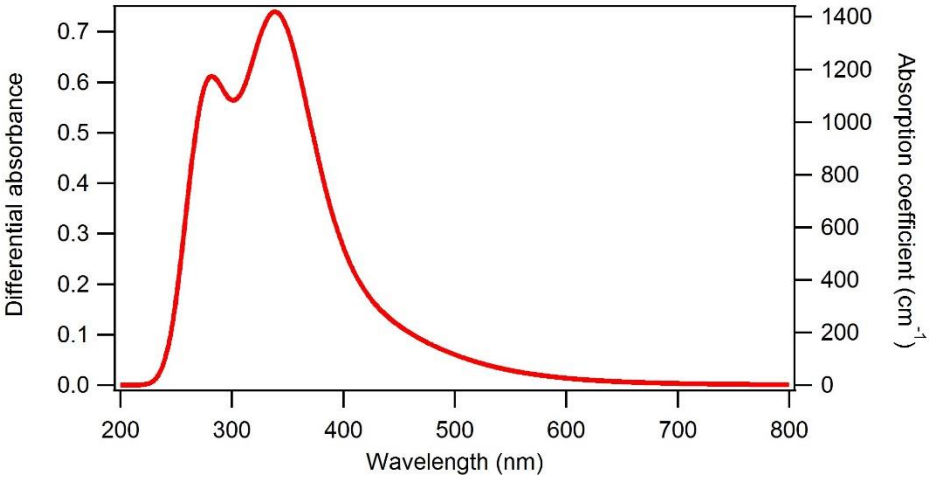


Figure 5: Typical absorbance/absorption spectrum of the patterns.

- ***Emission***

The emission is a continuum starting from slightly above the excitation wavelength up to 800 nm. Typical emission spectra are shown in Figure 6 for UV-blue excitation wavelengths and in Figure 7 for visible excitation wavelengths.

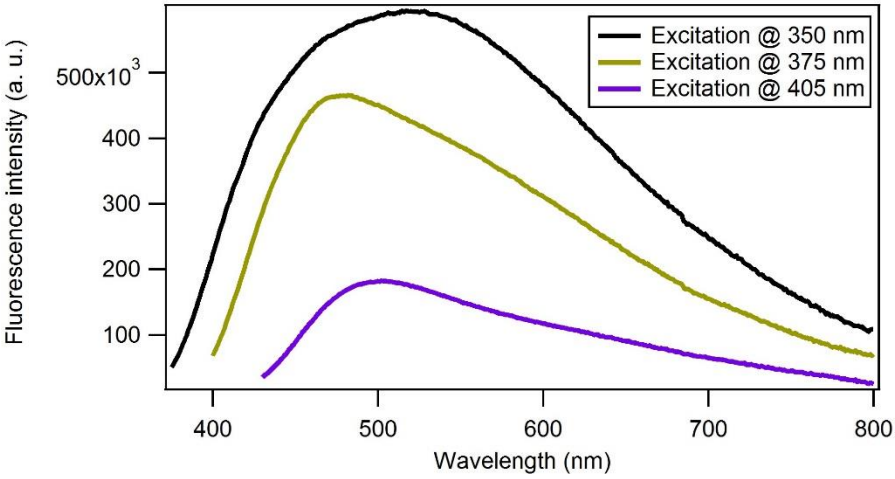


Figure 6: Typical emission spectra of the patterns for excitation wavelengths at 350 nm, 375 nm and 405 nm.

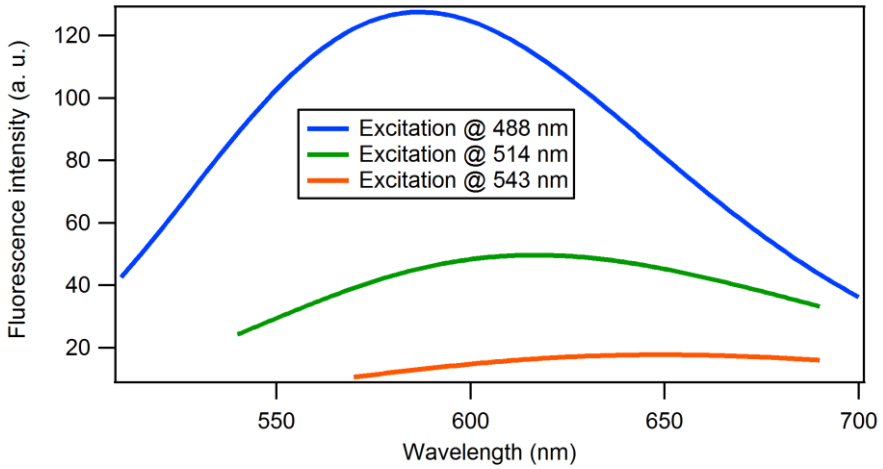


Figure 7: Typical emission spectra of the patterns for excitation wavelengths at 488 nm, 514 nm and 543 nm.

- ***Lifetime***

Using FLIM (Fluorescence Lifetime Imaging Microscopy), two main decay components of (0.29 ± 0.05) ns and (2.52 ± 0.50) ns have been measured. These values are provided for information and are not guaranteed. A typical fluorescence decay is shown in Figure 8.

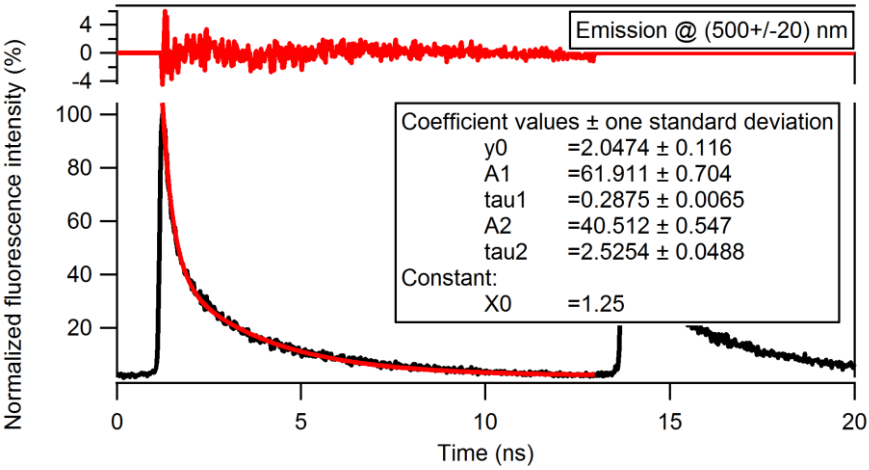


Figure 8: Typical fluorescence decay of the patterns for λ_{exc} = 400 nm, $\Delta\lambda_{em}$ = (500 ± 20) nm, 10×/0.25 objective.

- ***Photo-stability***

The 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, *i.e.* the time it takes to the fluorescence intensity to get back to its initial value, depends on the irradiation conditions (power density, illumination duration, excitation and emission wavelengths, zoom, etc.). The recovery time can be from seconds to months, with typical values in the minutes range. A typical fluorescence intensity recovery signal is shown in Figure 9.

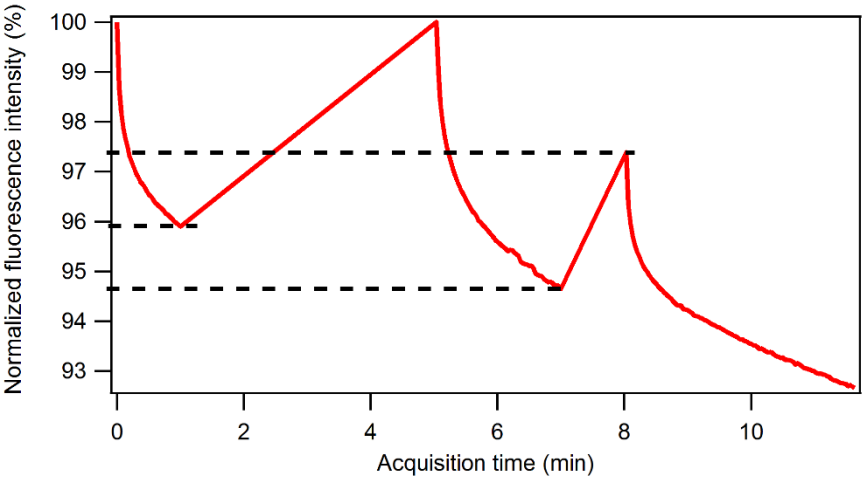


Figure 9: Typical fluorescence intensity recovery signal of the patterns. The power density was about 10 W.cm^{-2}, the excitation wavelength was (470 ± 20) nm and the collection window was (525 ± 25) nm. After one minute of acquisition and four minutes of waiting time, the fluorescence fully recovers for these irradiation conditions. When the waiting time is not sufficient, the fluorescence intensity does not restart at its original level.

Proceed with caution!

The field or rings (section 5.1) and the 4×4 intensity gradation (section 5.3) 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 the grid or the word ARGOLIGHT.
- 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 or 4×4 intensity gradation) and image it in one shot.

Do not image one of these patterns using a tiles acquisition mode.

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.

4.5. Suggested analyses

The Argo-WP can be used to assess non-exhaustively the following characteristics of HCS or HTS fluorescence imaging systems:

- ***Using the "Analysis" module (at the date of issue of this document)***
  - Field uniformity
  - Field distortion
  - Lateral co-registration accuracy
  - Stage drift during Z-stacking
  - System intensity response
- ***By other means***
  - Stitching performance
  - Line spread function
  - Ring spread function
  - Stage repositioning repeatability
  - Stage drift during timelapse
  - System spectral response
  - Axial co-registration accuracy
  - 3D reconstruction accuracy
  - Parfocality and parcentrality between objectives
  - Objective optical aberrations
  - Objective issues
  - Distances in XY and Z

5. Description of the patterns

5.1. Field of rings

This pattern, depicted in Figure 10, consists in a matrix of 35 × 35 rings, separated by 50 µm, with a cross in its center, on a total field of 1700 × 1700 µm². It is located in the well C5.

The typical transverse diameter (in the XY plane) of those rings is about (1.8 ± 0.3) µm.

The typical axial length (in the Z direction) of those rings is about (12.0 ± 3.0) µm FWHM (Full Width at Half Maximum).

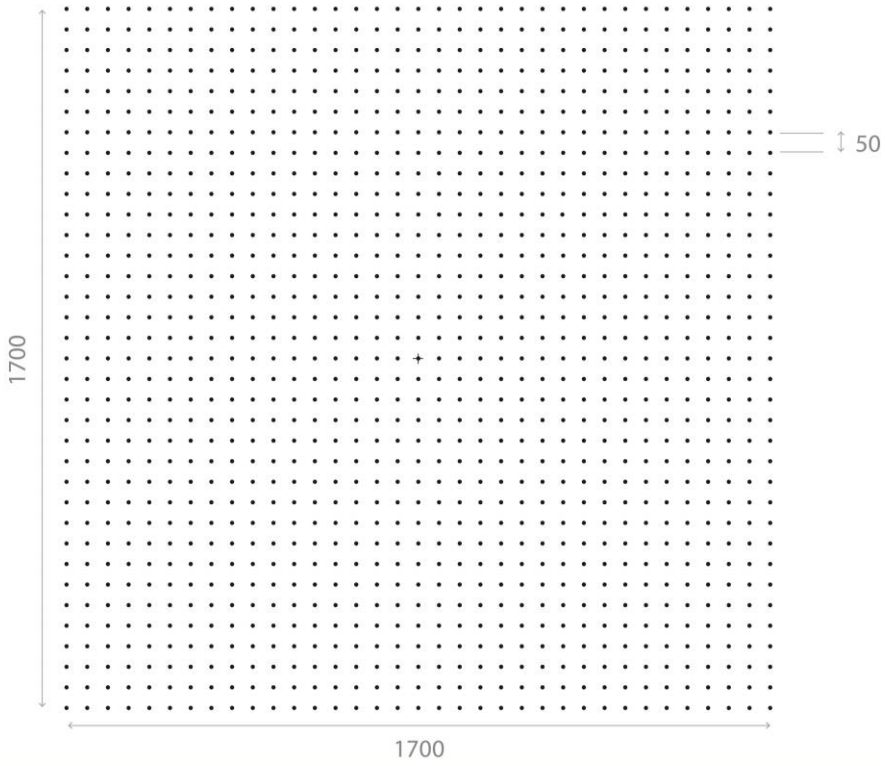


Figure 10: Schematics of the field of rings. All dimensions are in µm.

5.2. Grid

This pattern, depicted in Figure 11, consists in a grid with a size of 1000 × 1000 µm² and a step of 40 µm. It is located in the well C8.

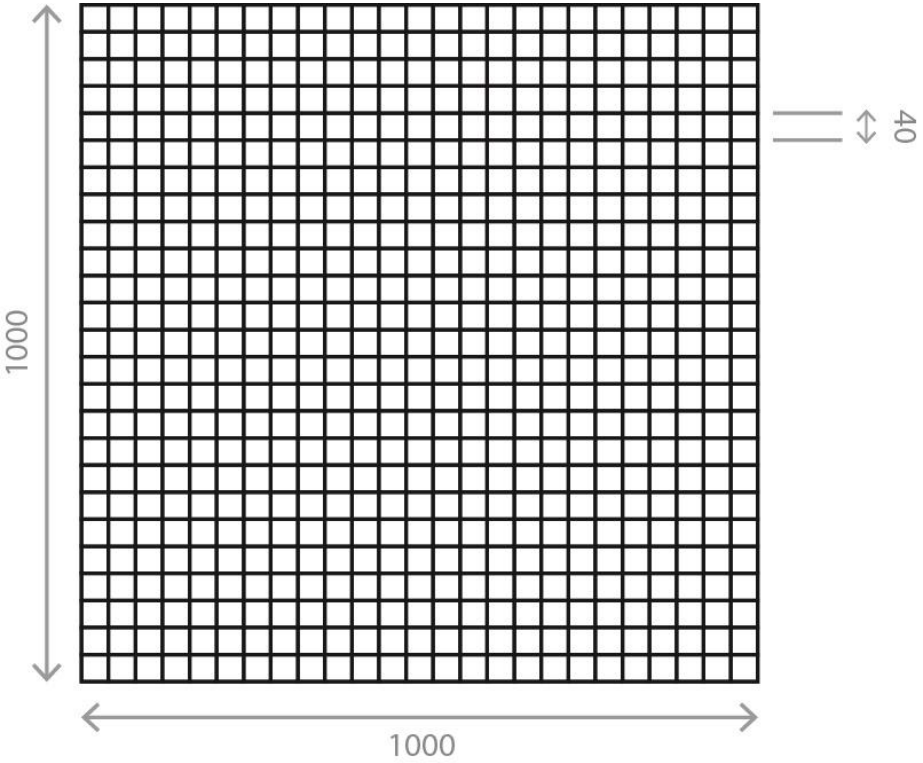


Figure 11: Schematics of the grid. All dimensions are in µm.

5.3. 4×4 intensity gradation

This pattern, depicted in Figure 12, consists in two layers of sixteen 100 µm-wide squares, on top of each other, having different fluorescence intensity levels following a linear evolution, organized in a 4×4 matrix. It is located in the well F8.

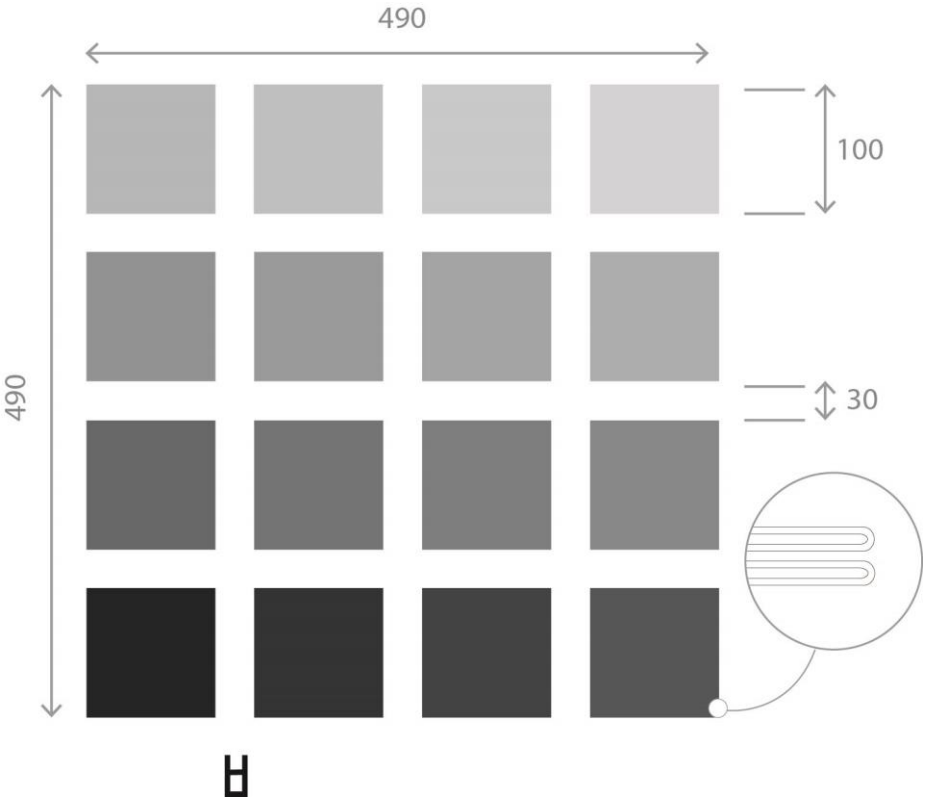


Figure 12: Schematics of the 4×4 intensity gradation. All dimensions are in µm.

5.4. 3D crossing stairs

This pattern, depicted in the Figure 13, consists in empty cylinders embedded at different depths, like two crossing stairs, with a step of 2.5 µm and surrounded by four 55 µm-long pillars. It is located in the well F5.

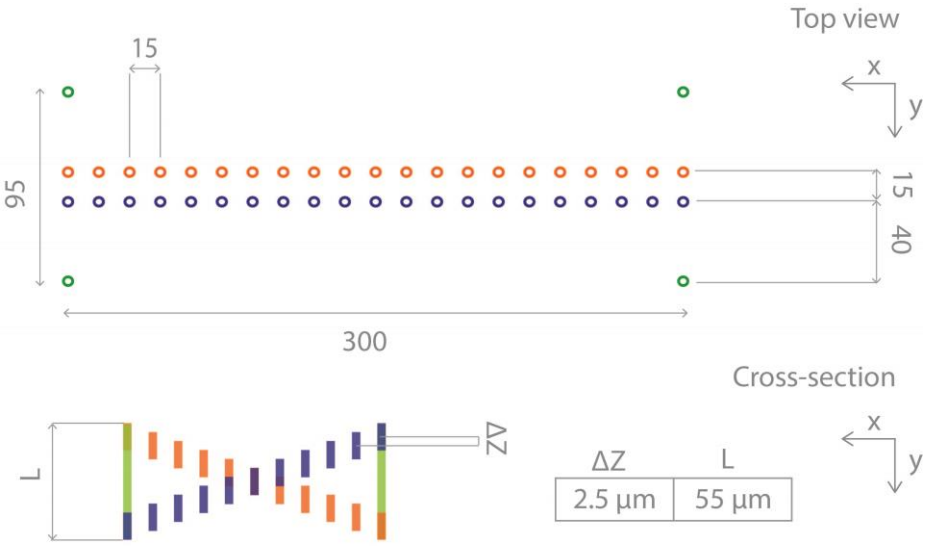


Figure 13: Schematics of the 3D crossing stairs. All dimensions are in µm.

5.5. Word ARGOLIGHT

This pattern, depicted in Figure 14, consists in letters forming the company name “Argolight”, and surrounded by a 220 µm × 50 µm frame. It is located in the well F5.

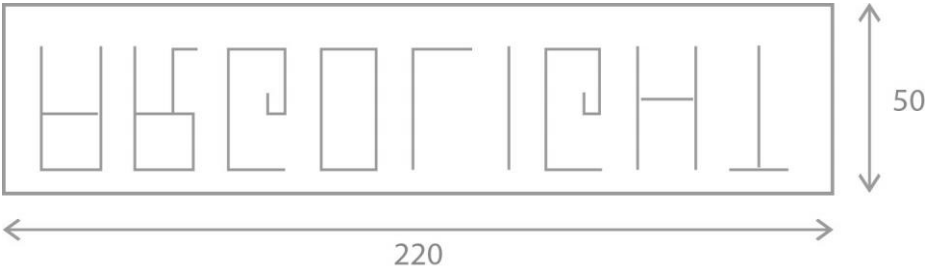


Figure 14: Schematics of the word ARGOLIGHT. All dimensions are in µm.

5.6. Coordinates of each pattern

Table 3 presents the XY coordinates, relative to the center of the Argo-WP plate, of the center of each pattern, in order to help for the automation of the image acquisition.

Well number	Pattern name	Relative coordinates (X ; Y) in µm
C5	Center of the field of rings	(-13530 ; 13530)
C8	Grid	(13530 ; 13530)
F8	4×4 intensity gradation	(13530 ; -13530)
F5	3D crossing stairs	(-13530 ; -13530)
F5	Word ARGOLIGHT	(-13530 ; -15030)

Table 3: XY coordinates of the center of each pattern relative to the center of the plate.

Date of issue: 01/01/2021 (1st January 2021)

User guide version: v1.0

Product name and version: Argo-WP v2.0

Copyright notice

Contact information

Argolight SA
Cité de la Photonique, Bdg. Elnath
11 avenue de Canteranne
33600 Pessac
France
Tel: (+33) 5 64 31 08 50
Website: www.argolight.com
Support: customer@argolight.com

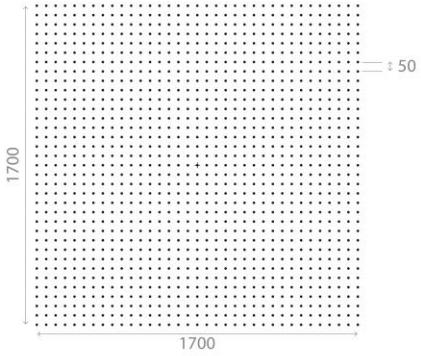
Note for readers

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

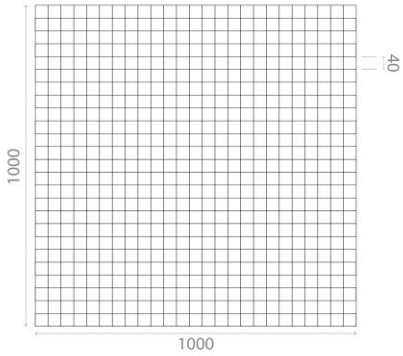
A word about waste management

Argolight policy is to offer robust and reliable products that last. In the event your product becomes useless to you, please contact us so we can pick it up and recycle it. Please do not throw away the plate with common waste. The composition of the glass requires specific recycling. Thank you.

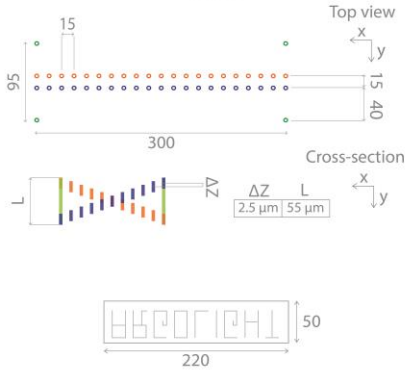
Well C5



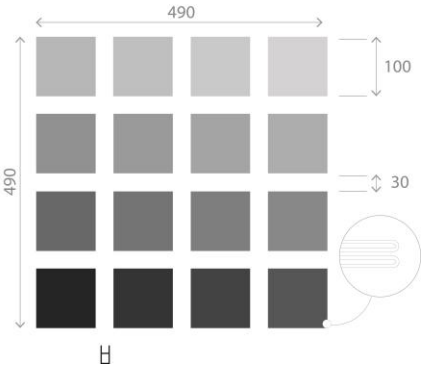
Well C8



Well F5



Well F8



ARGOLIGHT
A Precision Company