ARGOLIGHT
A PRECISION COMPANY

USER GUIDE

Argo-WP Plate

Argo-**WP**

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1. Introduction

The Argo-WP plate is specifically designed to assess and follow the performance of fluorescence high-content screening (HCS) and high-throughput screening (HTS) 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 plate has a 96-well plate format. It is compatible with objective magnifications ranging from 5 up to 60× and contains the last generation of ArgoGlass®.

This product is composed of:

- A mechanical plate that includes four pieces of ArgoGlass® with embedded fluorescent patterns inside them.
- The Daybook 2 companion software, including the image analysis (Daybook Analysis) and data manager (Daybook Data Manager) modules.

The pieces of ArgoGlass® consist of a special glass substrate with different fluorescent patterns embedded inside. The Argo-WP glass is designed to quality-control many aspects of a fluorescence HCS or HTS imaging system: field non-uniformity, field distortion, co-registration inacurracy, system intensity and spectral responses, stage motion control, stage drift during Z-stacking, etc.

The Daybook Analysis software allows users to analyze and extract data (maps, graphs, and metrics) from images of the patterns, in order to measure significant parameters of your fluorescence HCS or HTS imaging system.

The Daybook Data Manager software allows users to visualize the data generated by Daybook Analysis, monitor the results and manage the quality control reports.

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

2. First use

2.1. Package checking

Inside the package, you will find:

- 1 Argo-WP plate,
- 1 storage suitcase,
- 1 user guide,
- 1 certificate of inspection.

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

2.2. Quick starting procedure

- In your imaging system, select a low magnification microscope objective, typically a 10× or 20×.
- Illuminate the Argo-WP 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-WP glass, using the XY translation stage.
- 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 Argo-WP glass.
- Start your imaging session.
- Save the image(s) of your pattern(s) of interest from your usual acquisition software.
- Run the image analysis through Daybook Analysis to get meaningful results.

3. General handling and care

3.1. Handling

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

- The Argo-WP glass is compatible with any immersion liquid (oil, glycerol and water). For the particular case of water immersion, continuous exposure higher than five minutes should be avoided. When longer continuous exposures are required, use an oil 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.
- Store in its suitcase (after having removed entirely the immersion liquid) at ambient temperature (10 – 40 °C) and normal relative humidity (20 – 70 %). Avoid ultraviolet irradiation.

3.2. Cleaning

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

3.3. Operating environment

The ArgoGlass® has been designed to be used at room temperature (10 – 40 °C) and normal relative humidity (20 - 70 % RH). Both the glass and the metal carrier composing the Argo-WP have a low thermal expansion coefficient, so that temperature variations will not significantly affect the imaging of the patterns.

3.4. Warranty

Each plate is warranted for 3 years, provided that the conditions of handling and storage are respected.

4. Technical specifications

4.1. Physical specifications

The plate has the same format and dimensions as 96-well plates, as shown in Figure 1. The plate contains four pieces of ArgoGlass®, which is a special glass substrate produced at the Argolight facility to ensure homogeneity and purity.

The weight of the Argo-WP plate is approximatively 180 g. The dimensions of the plate comply with the ANSI SLAS 1 to 4 - 2004 (R2012) standards.

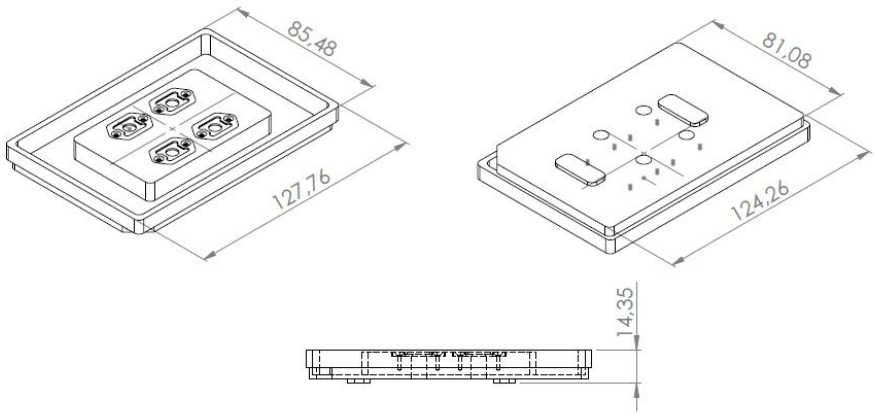

Fig. 1: Schematics of the Argo-WP plate with dimensional specifications. All dimensions are in mm.

4.2. Glass refractive index

The dispersion of the glass refractive index is shown in Figure 2. The measurement uncertainty is ± 7.10^{-4}. The ArgoGlass® features the same refractive index as microscope coverslips.

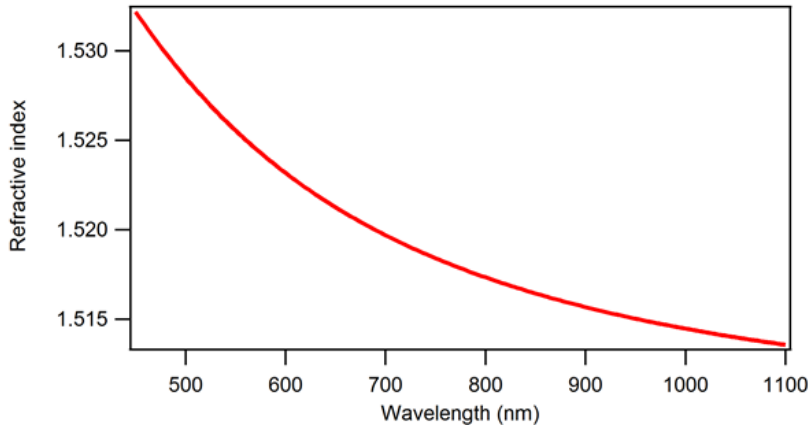

Fig.2: Dispersion of the refractive index of the glass.

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 1.

Sellmeier coefficient	Value
A	-31.204
B	33.798
C	823.160 nm^2
D	-0.317
E	35743.000 nm^2

Table 1: Sellmeier coefficients for the refractive index dispersion of the glass.

4.3. Patterns overview

The fluorescent patterns, depicted in Figure 3, are positioned (170 ± 5) µm below the top glass surface, on a horizontal plane which flatness is within ± 5 mrad. This emulates 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. The maximum relative positioning error within each individual pattern is ± 110 nm in X, Y and Z. The thickness (in the Z direction) of these patterns is about 8 µm FWHM (Full Width at Half Maximum).

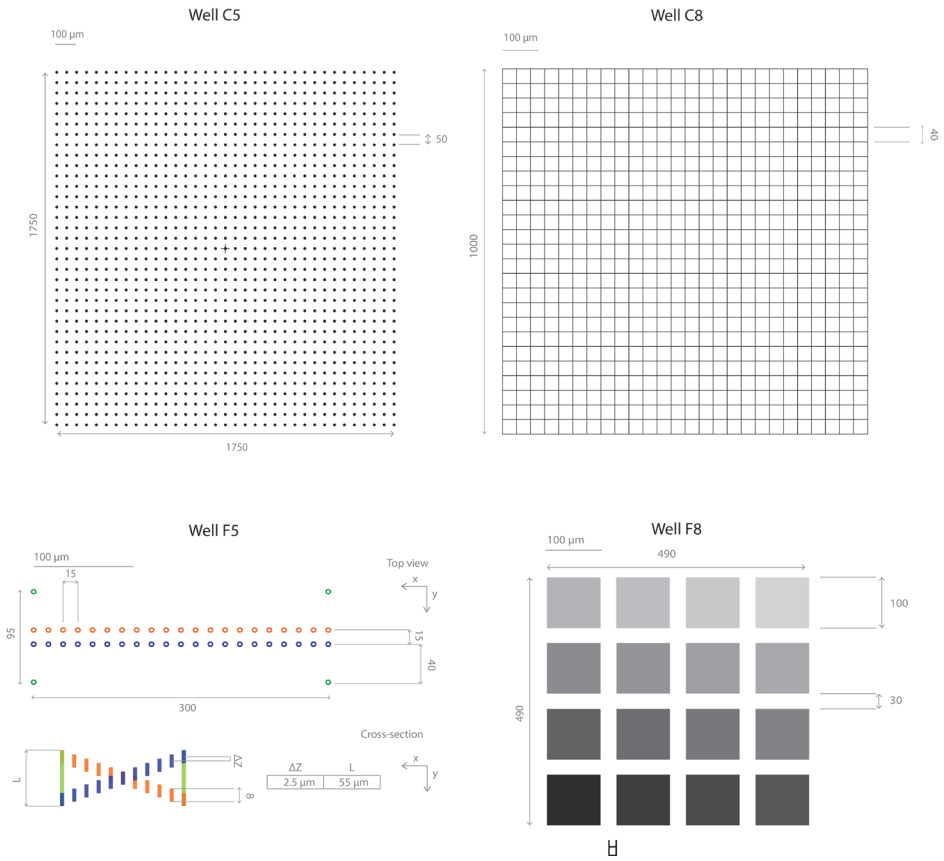

Fig.3: Schematics of the patterns inside the four wells of the plate.
All dimensions are in µm.

4.4. Fluorescence spectral features

The patterns exhibit the following fluorescence spectral features.

- Excitation:

The excitation ranges from 280 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 4.

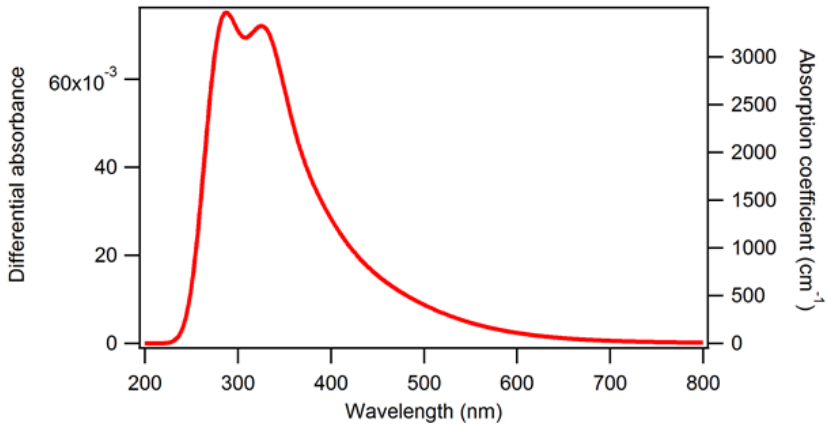

Fig.4: Typical absorption spectrum of the patterns.

- Emission:

The emission is a continuum starting from slightly above the excitation wavelength up to 800 nm. Typical emission spectra are shown in Figure 5 for UV-blue excitation wavelengths and in Figure 6 for visible excitation wavelengths.

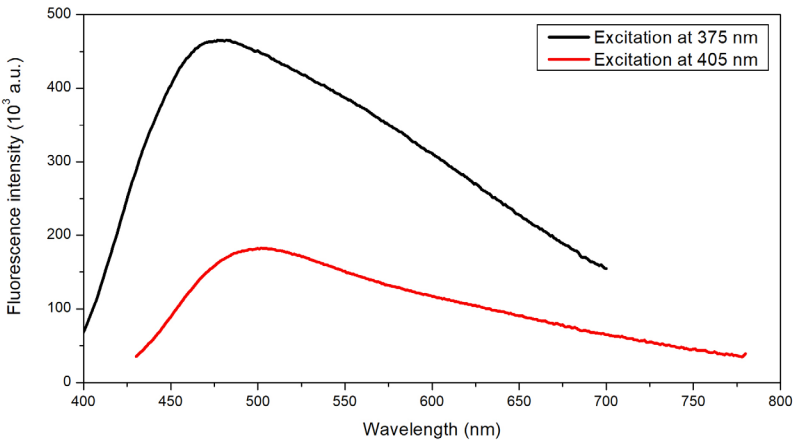

Fig.5: Typical emission spectra of the patterns for excitation wavelengths at 375 nm and 405 nm.

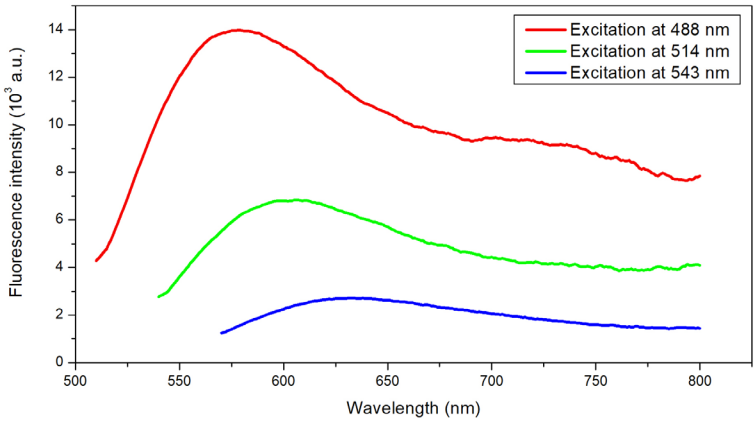

Fig.6: Typical emission spectra of the patterns for excitation wavelengths at 488 nm, 514 nm and 543 nm.

- Lifetime:

Using FLIM (Fluorescence Lifetime Imaging Microscopy), two main decay components of (0.25 ± 0.05) ns and (2.50 ± 0.50) ns have been measured. These values are provided for information and are not guaranteed. A typical fluorescence decay is shown in Figure 7.

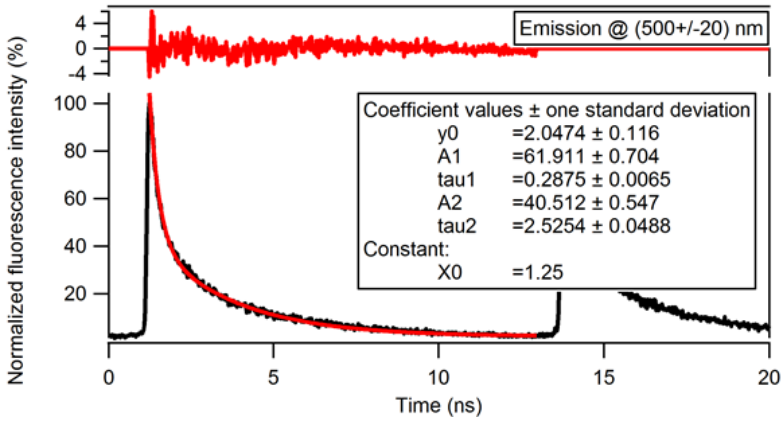

Fig.7: Typical fluorescence decay of the patterns for λ_{exc} = 400 nm, $\Delta\lambda_{em}$ = 500 ± 20 nm, 10×/0.25 objective.

- 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 depends on the irradiation conditions (power density, wavelength, pixel size, exposure time). A typical fluorescence intensity recovery signal is shown in Figure 8.

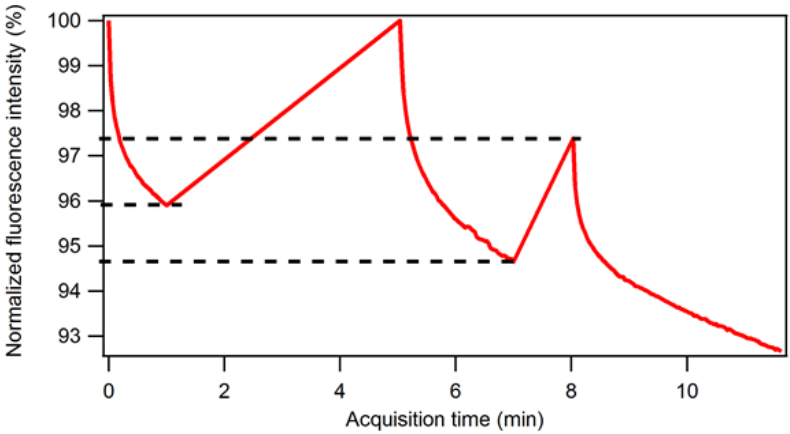

Fig.8: Typical fluorescence intensity recovery signal of the patterns. The power density was about 10 W.cm^{-2}, the excitation wavelength was (470 ± 20) nm and the collection window was (525 ± 25) nm. After one minute of acquisition and four minutes of waiting time, the fluorescence fully recovers for 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 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 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 tests

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

- Within Daybook software

  - Field non-uniformity
  - Field distortion
  - Lateral co-registration inaccuracy
  - Lateral stage repositioning repeatability
  - Lateral stage drift during timelapse
  - Lateral stage drift during Z-stacking
  - Line spread function
  - Intensity response of the system
  - Spectral response of the system

- By other means

  - Stitching performance
  - Axial co-registration inaccuracy
  - 3D reconstruction precision
  - Axial stage drift
  - 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 (well C5)

This pattern, depicted in Figure 9, consists in a matrix of 35 × 35 rings, separated by 50 µm, with a cross in its center, on a total field of 1750 × 1750 µm². It is located in the well C5.

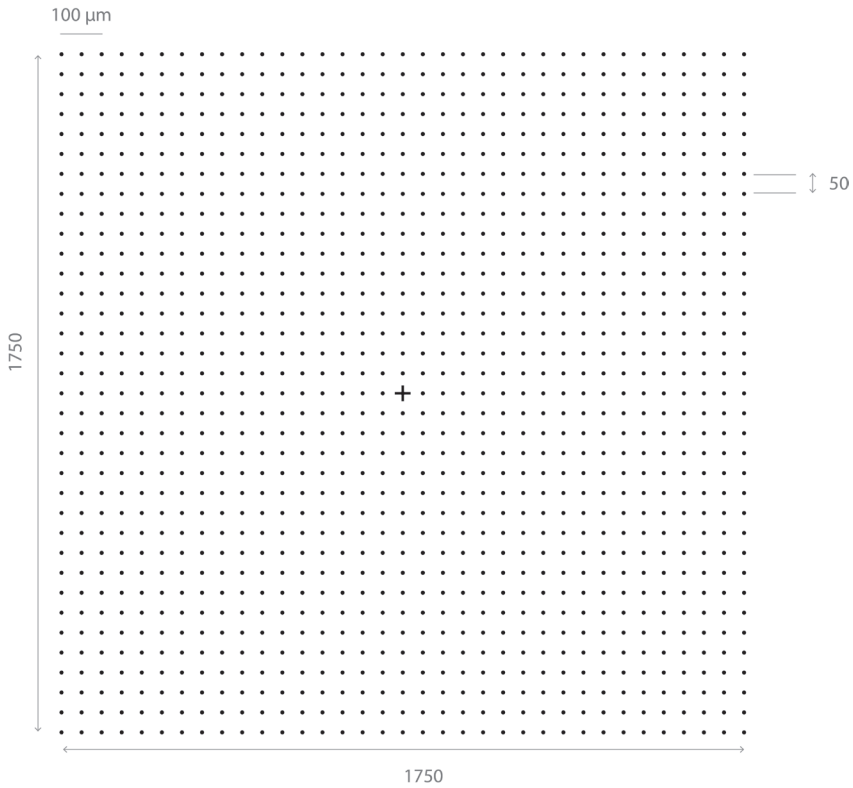

Fig.9: Schematics of the field of rings. All dimensions are in µm.

5.2. Grid (well C8)

This pattern, depicted in Figure 10, consists in a grid with a size of 1000 × 1000 μm² and a step of 40 μm. It is located in the well C8.

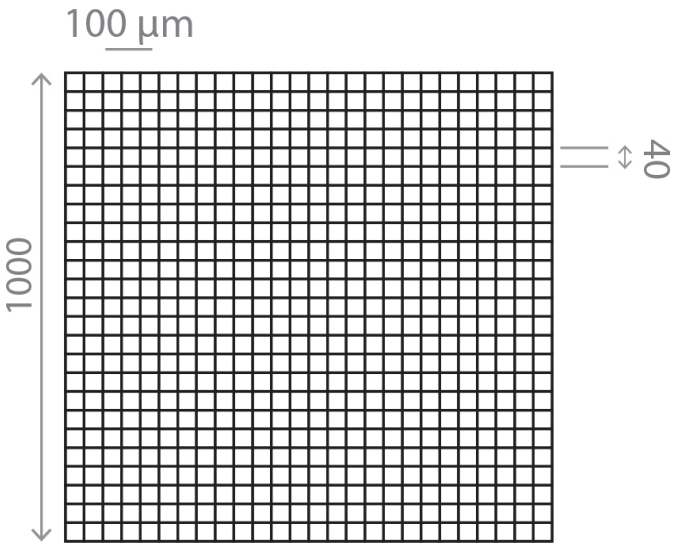

Fig. 10: Schematics of the grid. All dimensions are in μm.

5.3. 4×4 intensity gradation (well F8)

This pattern, depicted in Figure 11, 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.

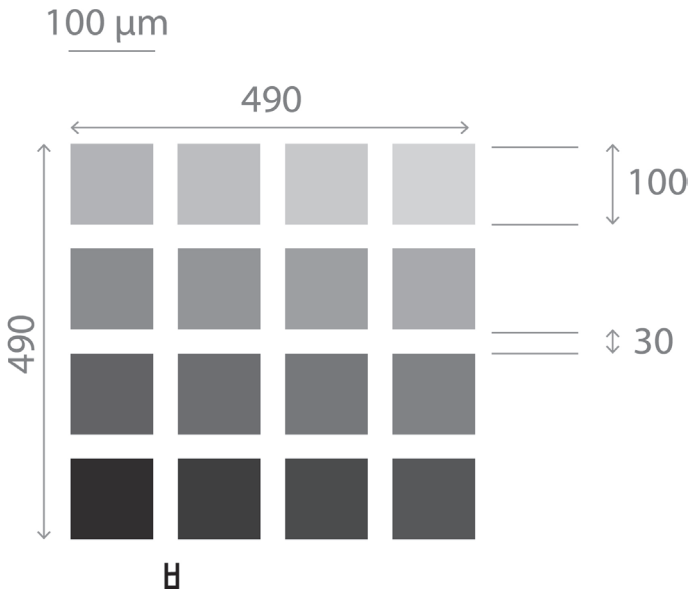

Fig.11: Schematics of the 4×4 intensity gradation. All dimensions are in µm.

5.4. 3D crossing stairs (well F5)

This pattern, depicted in Figure 12, 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.

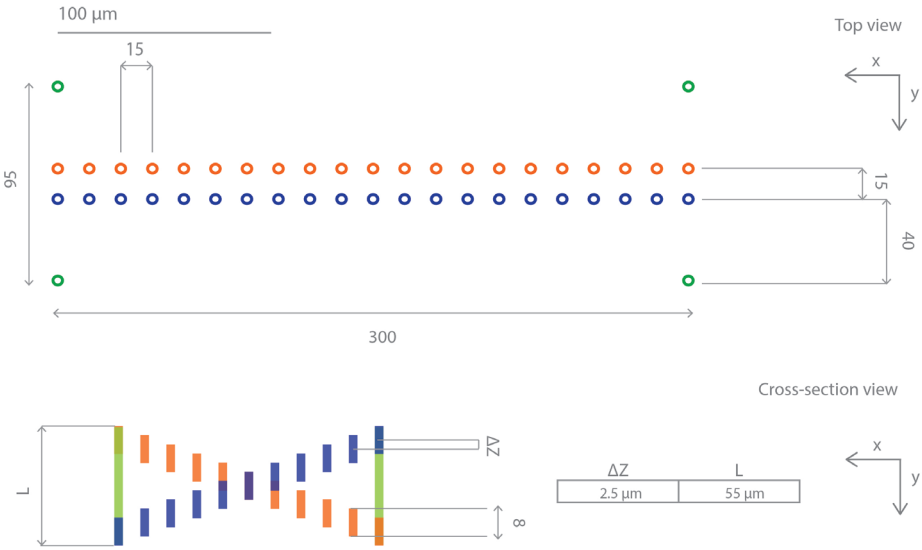

Fig.12: Schematics of the 3D crossing stairs. All dimensions are in µm.

5.5. Logo (well F5)

This pattern, depicted in Figure 13, consists in letters forming the company name “Argolight”, and surrounded by a 220 µm × 50 µm frame. It is located in the well F5.

Fig.13: Schematics of the Argolight logo. All dimensions are in µm.

5.6. Coordinates of each pattern

Table 2 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	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	Logo	(-13530;-28530)

Table 2: XY coordinates of the center of each pattern relative to the center of the plate.

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Contact information

Argolight SA

Cité de la Photonique, Bât. Elnath

11 Avenue de Canteranne

33600 Pessac

France

Tel: (+33) 5 64 31 08 50

Website: www.argolight.com

Support: customer@argolight.com

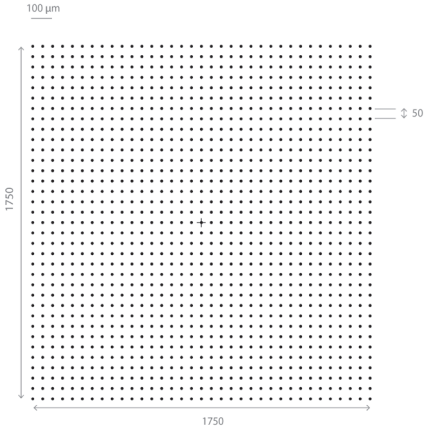
Note for readers

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

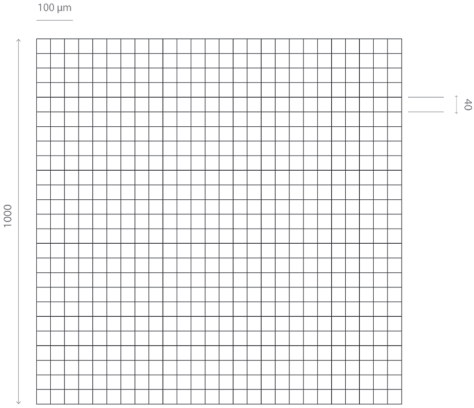
A word about waste management

Argolight policy is to offer robust 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 plates with common waste. The composition of the glass requires specific recycling. Thank you.

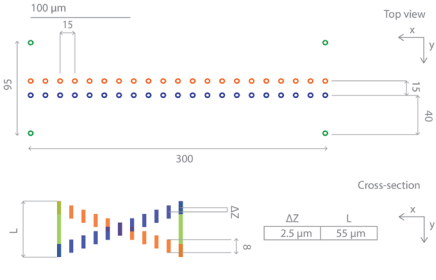
Well C5



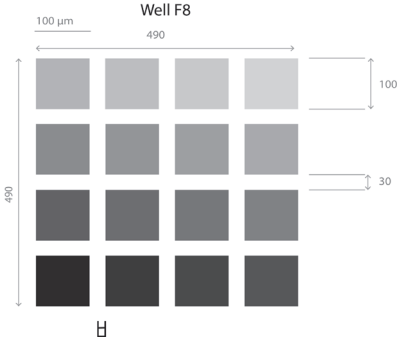
Well C8



Well F5



Well F8



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