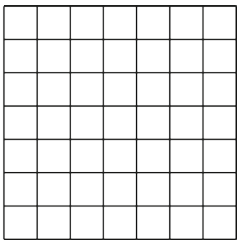
USER GUIDE

Argo-LM Slide



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1. Introduction

Argolight multidimensional slides are specifically designed for assessing and following the performances of fluorescence-based imaging systems, such as wide-field, confocal, spinning disk and other types of microscopes. They can be used as a human vision- or a software-assisted tool.

The Argo-LM slides are specifically designed for low-magnification systems, typically for magnifications from 5 up to 20×.

The slides consist in a special glass substrate, the ArgoGlass®, set on a metal carrier. Different fluorescent patterns are embedded inside the glass. They also exhibit a contrast in bright and dark fields, DIC (Differential Interference Contrast) and phase contrast. Each fluorescent pattern is intended and designed to respond to a particular performance assessment. The slides are manufactured at the highest level of precision to insure meaningful test results.

The patterns are accurately positioned and stable to light illumination, so that image acquisition can be automated. The degree of automation depends on the level of motorization of the assessed imaging system. The analysis of the acquired images can be simplified using Argolight software solutions.

2. First use

2.1. Package checking

Inside the package, you will find:

- 1 Argo-LM slide,
- 1 storage box,
- 1 user guide documentation,
- 1 certificate of inspection.

Before starting, check that all these items are present and control if the slide has visible damages. If any damage is observed, please contact Argolight **within three days after delivery.**

2.2. Starting procedure

- Remove the Argo-LM slide from its storage box and set it up in the microscope sample holder.
- Select a low magnification microscope objective, typically a 10× or 20×.
- Illuminate the slide with UV-blue light (preferably at a wavelength between 350 nm and 500 nm).
- Make coincide coarsely the center of the field of view with respect to the center of the slide, using the XY translation stages, until observing white fluorescence coming out from the glass. This means the fluorescent patterns are excited by the excitation light (when a 325-405 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 in order to observe the pattern(s) of interest.
- Start the acquisition of the images.

3. General handling and care

3.1. Handling

In order to make Argolight slides last for many years, we advise to respect the following handling and storage instructions:

- The slide 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.
- Store in its box (after having removed entirely the immersion liquid) at ambient temperature, avoid humidity and ultraviolet irradiation.

3.2. Cleaning

Clean with lens tissue and alcohol only. Wearing gloves is advised. If necessary, in order to clean both the glass and the metal carrier, the slide can be placed inside an ethanol ultrasonic bath for a few minutes, and then dried with pressured air. Do not use acetone.

3.3. Operating environment

The slides have been designed to be used at room temperature (10-40°C) and normal relative humidity (less than 70%). Both the glass and the metal carrier composing the slides have a low thermal expansion coefficient, so that temperature variations will not significantly affect the slides. They should however not be exposed to extreme conditions of temperature and humidity.

3.4. Warranty

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

4. Slide Features

4.1. Slide description

This slide consists in a special glass substrate produced at the Argolight facility to insure homogeneity and purity of the material. The glass is set on a metal carrier, featuring the same dimensions as a standard microscope slide (75 mm 25 mm), except for the thickness which is 1.5 mm, in order to satisfy the required rigidity for this kind of tool. The dimensional specifications of the metal carrier are shown in Figure 1.

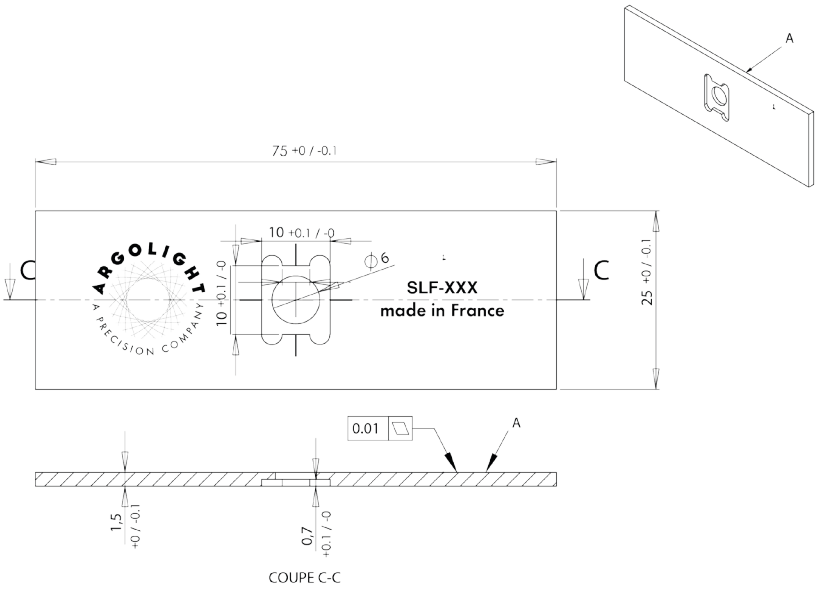


Fig. 1: Scheme with dimensional specifications of the metal carrier.

4.2. Glass refractive index

The dispersion of the refractive index of the glass is shown in Figure 2. The measurement uncertainty is ± 7.10^{-4}.

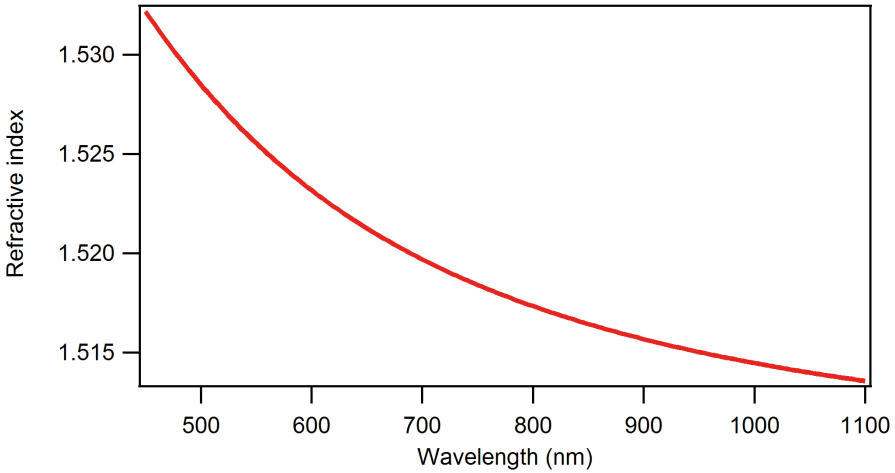


Fig. 2: Dispersion of the refractive index of the glass.

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

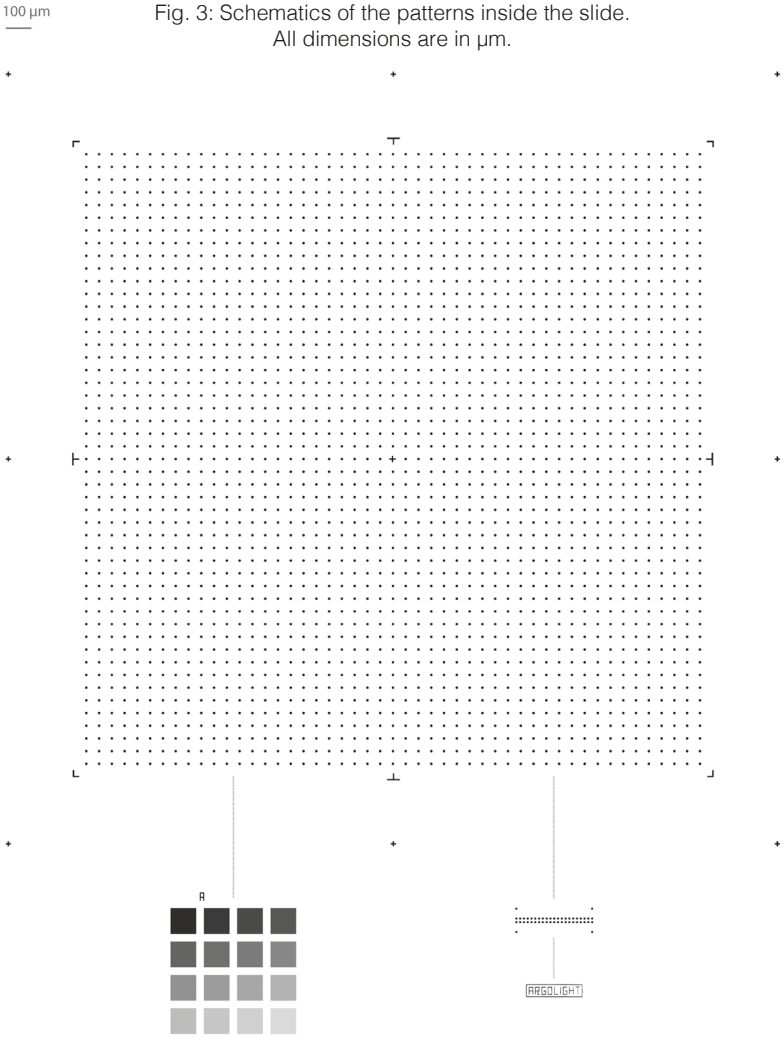
$$n^2(\lambda) = A + \frac{B\lambda^2}{\lambda^2 - C} + \frac{D\lambda^2}{\lambda^2 - E}$$

Sellmeir Coefficient	Value
A	-31.204
B	33.798
C	823.160 nm^2
D	-0.317
E	35743.000 nm^2

4.3. Patterns Overview

This slide includes the patterns described in Figure 3. They 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 cover-slip, 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 of ± 110 nm in XY and ± 110 nm in Z within each individual pattern is certified by the manufacturing tools. 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 slide.
All dimensions are in µm.



4.4. Fluorescence spectral features

The patterns exhibit the following fluorescence spectral features:

- Excitation:

The patterns can be efficienly excitable from 300 to 550 nm. Longest excitation wavelengths require higher optical power and/or longer exposure time.
The excitation efficiency is maximum at around 340 nm and drops towards the red wavelengths. A typical absorption spectrum is shown in Figure 4.

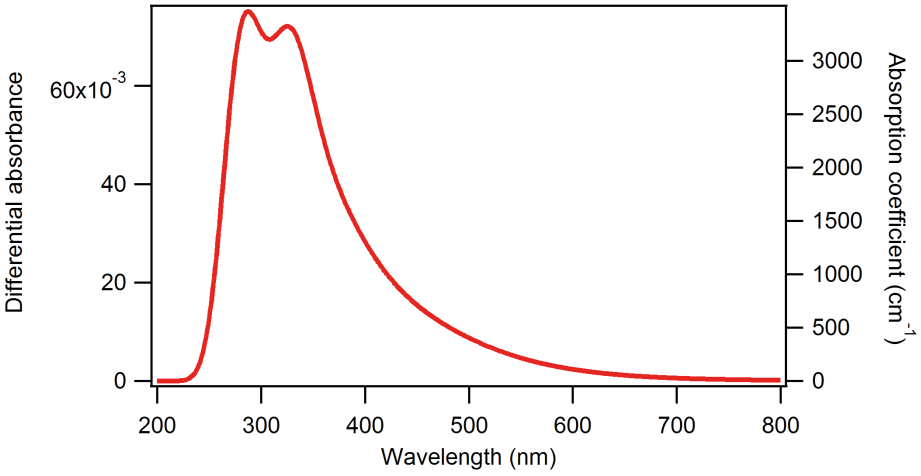


Fig. 4: Typical absorbance/absorption spectrum of the patterns.

- Emission:

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

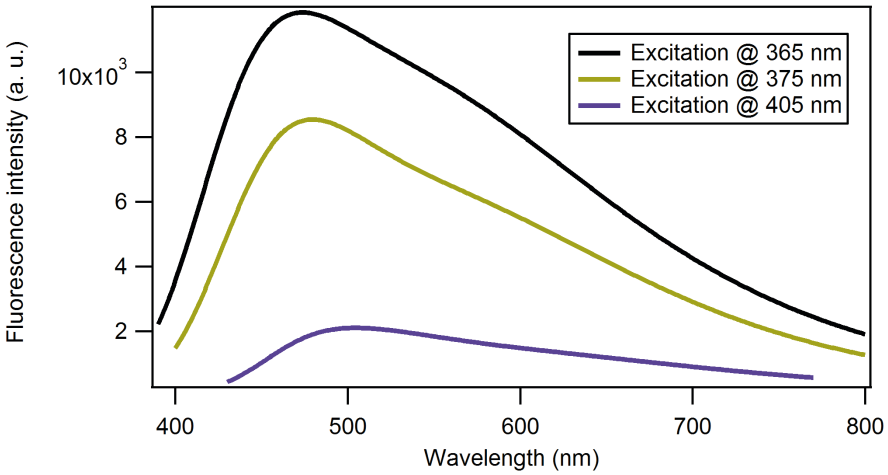


Fig. 5: Typical emission spectra of the patterns
for excitation wavelengths at 365, 375 and 405 nm.

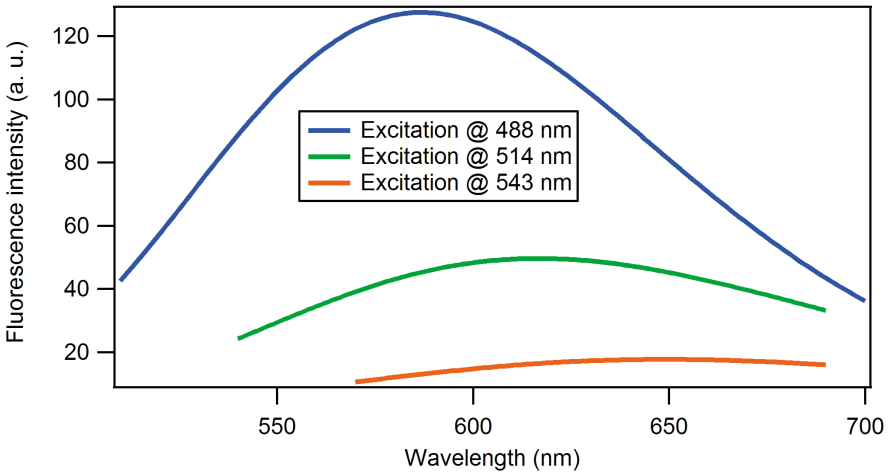


Fig. 6: Typical emission spectra of the patterns
for excitation wavelengths at 488, 514 and 543 nm.

- 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 provided for information and are not guaranteed. A typical fluorescence decay is shown in Figure 7.

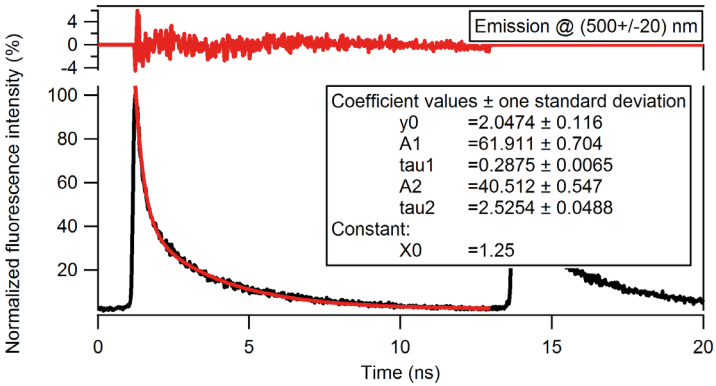


Fig. 7 : Typical fluorescence decay of the patterns for λexc=400 nm, Δλem=500±20 nm, 10×/0.25 objective.

- Photostability:

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.

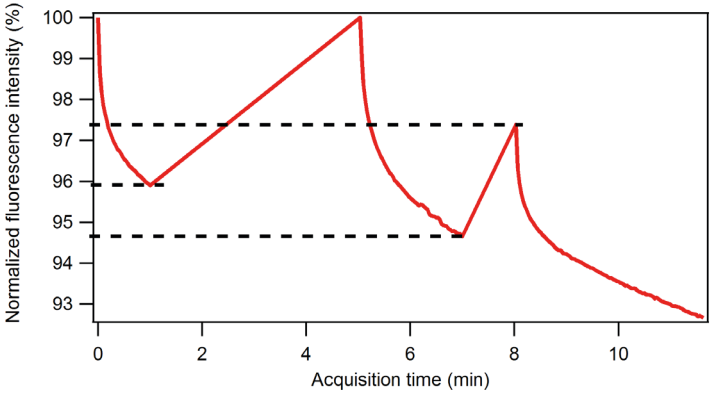


Fig. 8: Typical fluorescence intensity recovery signal of the patterns.

Fig. 8 (cont.): 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 of rings (section 5.1) and the 4×4 (section 5.2) intensity gradations are patterns for which fluorescence intensity is important. They must therefore be imaged with a lot of care.

We recommend to proceed as follows:
• first, move to a pattern for which intensity is not important, such as a 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 or 4×4 intensity gradations) and image it in one shot.

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

This slide can be used to assess non-exhaustively the following characteristics of fluorescence imaging systems:

- Evenness of illumination,
- Distortion of the field of view,
- Chromatic shifts,
- Stitching performance,
- Stage repositioning accuracy,
- Intensity response of the system,
- Spectral response of the system,
- 3D reconstruction precision,
- Parfocality and parcentrality between objectives,
- Objective issues,
- Distances in XY and Z.

5. Description of the patterns

5.1. Field of rings

This pattern, depicted in Figure 9, consists in a matrix of 49 × 49 rings, separated by 50 µm, on a total field of 2500 × 2500 µm². The field of rings is surrounded by eight landmarks, and exhibits a 25 µm long cross in its center.

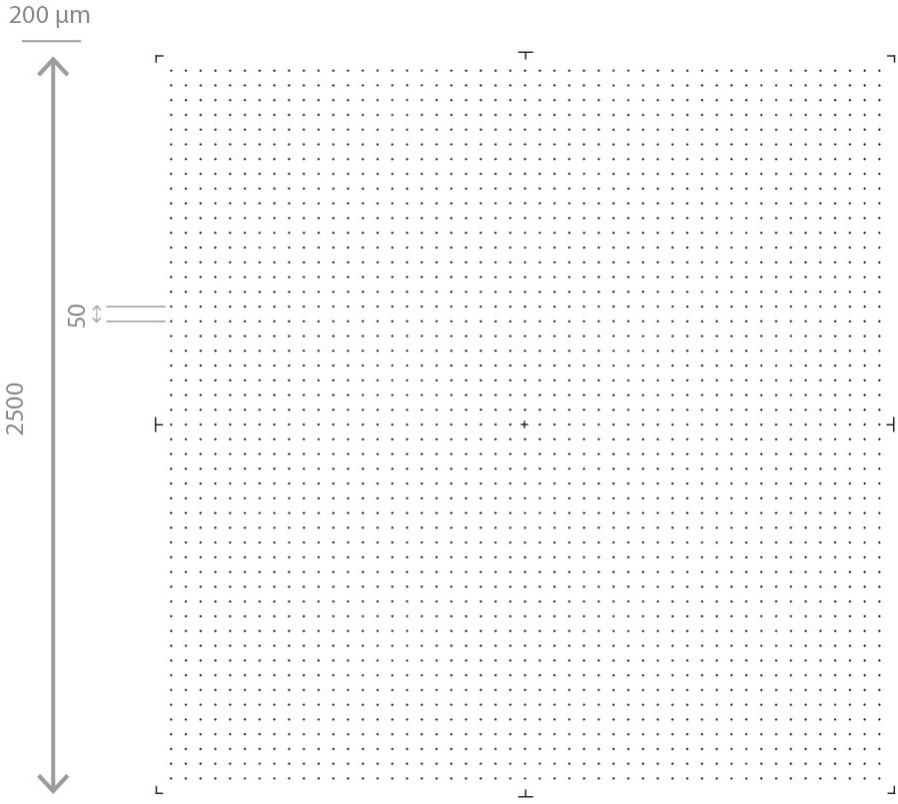


Fig. 9: Schematics of the field of rings. All dimensions are in µm.

5.2. 4×4 intensity gradation

This pattern, depicted in Figure 10, consists in twice 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.

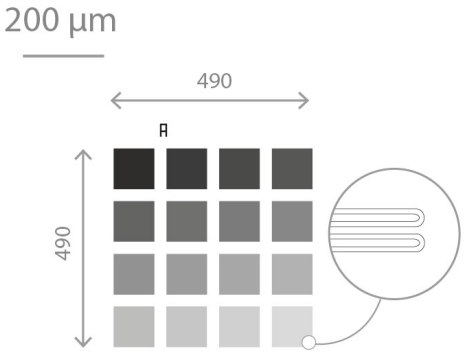


Fig. 10: Schematics of the 4×4 intensity gradation. All dimensions are in µm.

5.3. Repositioning crosses

The repositioning crosses, depicted in Figure 11, are 20 µm long and are positioned 1500 µm from one to another in the X direction, the Y direction, or both.

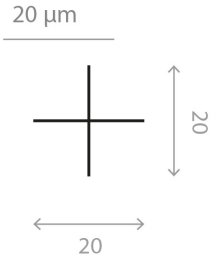


Fig. 11: Schematics of one of the repositioning crosses. All dimensions are in µm.

5.4. 3D Crossing stairs

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.

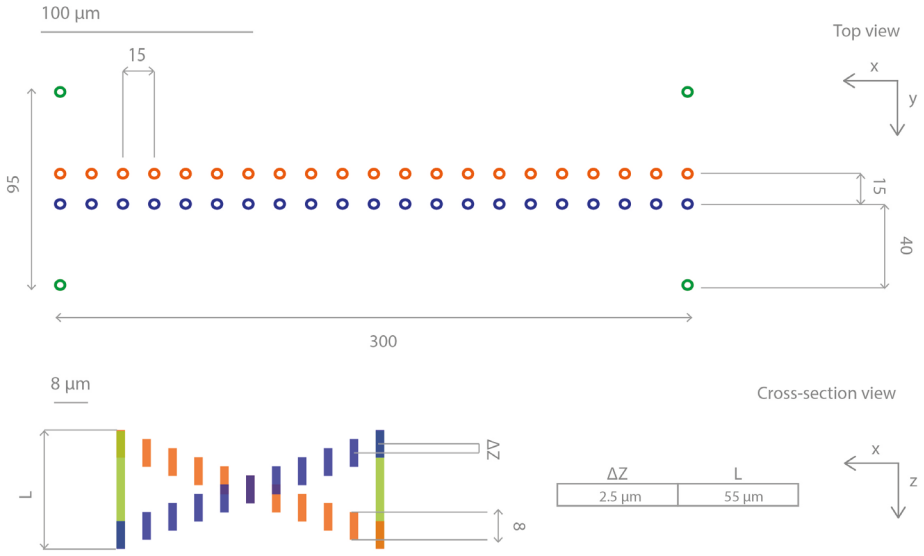


Fig. 12: Schematics of the 3D crossing stairs. All dimensions are in µm.

5.5. Logo

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

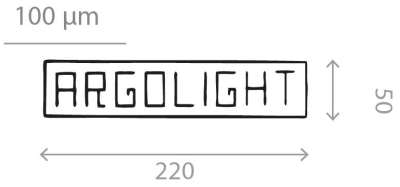


Fig. 13: Schematics of the Argolight logo. All dimensions are in µm.

5.6. Coordinates of each pattern

Table 1 presents the XY coordinates, relative to the central cross of the field of rings, 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 ± 2 µm in both X and Y directions.

Pattern	Relative coordinates (X;Y) in µm
Central cross of the field of rings	(0;0)
4×4 intensity gradation	(-625;-1995)
3D crossing stairs	(625;-1772.5)
Logo	(625;-2075)

Table 1: XY coordinates of the center of each pattern relative to the central cross of the field of rings.

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1.1

Contact information
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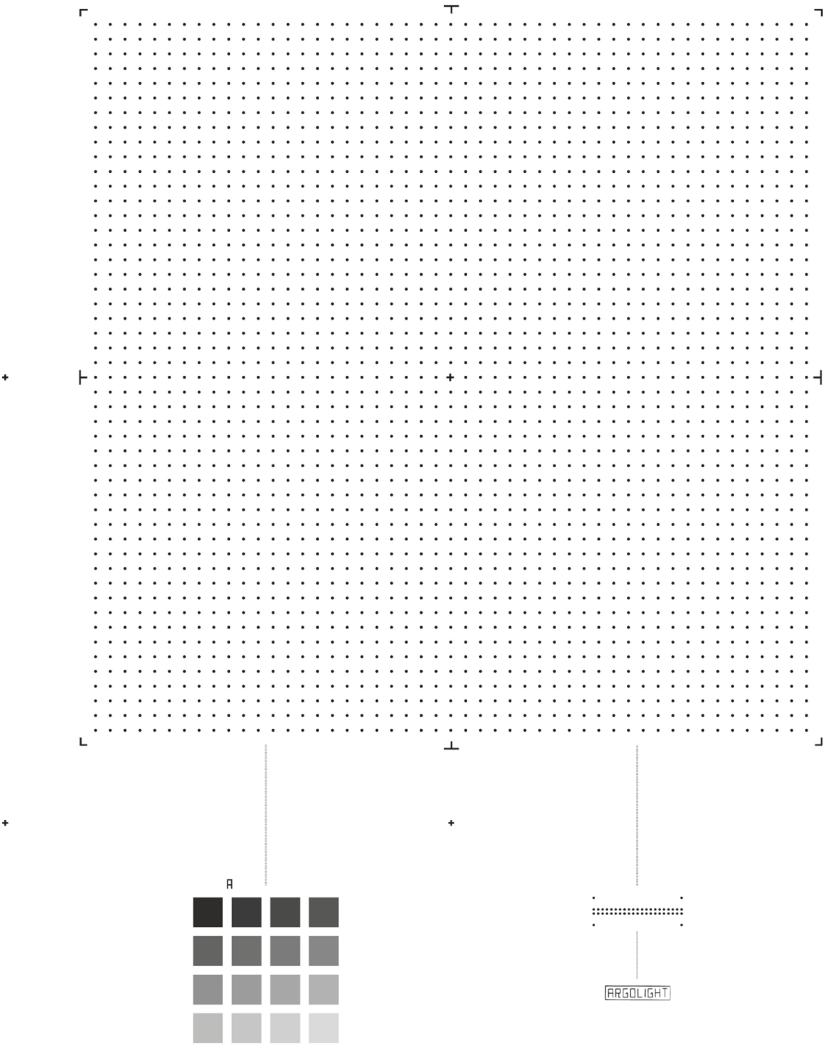
Note for readers
The experimental data shown in this documentation are informative and not contractual, and may be different from one system to another.

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 slide with common waste.** The composition of the glass requires specific recycling.

Thank you.

100 µm



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