

# Quality Control of Fluorescence Imaging Systems

A new tool for performance assessment and monitoring

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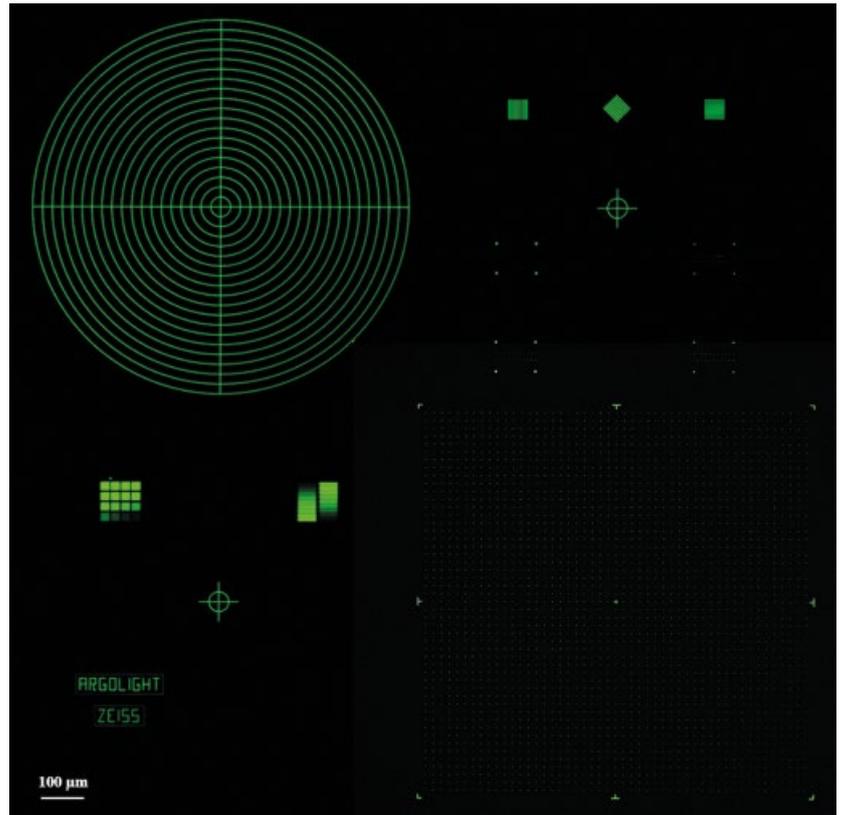
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We have developed a new tool for the assessment and monitoring of most of the performances of fluorescence microscopes. We believe it can advantageously be integrated in the quality control process of core facilities where a certain level of performance for the end users must be assured.

Although performance evaluation and quality control of fluorescence microscopes is a topic that appeared more than fifteen years ago in academic laboratories [1] and national regulatory agencies [2], it is still topical as it was in the program of the Core Facility Satellite Meeting of the 15th international ELMI meeting in 2015. Due to the increasing complexity of the instrumentation used for confocal and high-end wide-field fluorescence imaging microscopy, national metrology institutes [3], microscope manufacturers [4], and more recently core facilities [5] have gotten involved in identifying, manufacturing and / or testing different tools, both hardware and software, to assess the numerous aspects of fluorescence microscopes.

On the one hand, it has become obvious for the core facilities that quality control of fluorescence microscopes is important, as they provide a charged service to microscope end users. In this sense, they have to assure, up to a certain level, the performances of their instruments. Quickly identifying and solving microscope issues is therefore essential in order to prevent the acquisition of corrupted data and to minimize the machine downtime. That is why core facilities usually spend tens of thousands euros per year for the maintenance of their systems.

On the other hand, for the microscope manufacturers, maintenance is not as effective as it could be for two

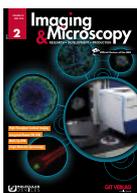


main reasons. First, in average, one intervention of the maintenance service over two is not justified, as it is based on a wrong (human misinterpretation) in situ diagnosis of the system while it performs correctly. Second, when the system is faulty, the identification and fixation of the problem can require several interventions. Knowing in advance what the microscope issue is allows optimizing the maintenance, if it is necessary. This would reduce the maintenance time and increase the technician availability for others systems / facilities.

For both these actors, a win-win opportunity could arise if an evaluation and monitoring tool, accepted from both sides, would allow to assess quickly and simply most of the performances of

a fluorescence microscope. Thus, the core facilities would get the assurance they provide the best possible service, so that the end users get reliable data, while the microscope manufacturers would reduce their maintenance intervention time and would improve their knowledge of the malfunction sources, so that they can correct them. Besides, after an installation or an intervention, both parties could validate the performances of a system no longer on the basis of a subjective image of a biological sample, but on objective and quantified parameters. This would be a huge step towards quality control of fluorescence microscopes.

Having perceived the significance of this issue, we have worked together to develop a new tool, for the performance



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assessment and monitoring of fluorescence microscopes. This tool aims to: first, validate a system at a  $t_0$  origin time (after an installation and / or a maintenance); second, monitor the performances of a system over time; and third, detect any malfunction of a system.

### Evaluation slide

The device, basically a slide, consists of a custom glass substrate, set on a stainless steel carrier (Fig. 1a). The carrier features the same dimensions as a standard microscope slide. Different fluorescent patterns (Fig. 1b) are embedded inside the glass, at a depth emulating the presence of a microscope cover slip. These patterns also exhibit a contrast in bright and dark fields, DIC (differential interference contrast) and phase contrast. Each fluorescent pattern is designed for one or several performance assessments.

Non-exhaustively, the slide allows to assess and monitor the following characteristics of a fluorescence microscope (confocal, spinning-disk and wide-field): evenness of illumination, distortion of the field of view, parcentrality, parfocality, optical axis determination, chromatic lateral shifts, co-localization issues, stitching performance, stage repositioning accuracy, intensity response of the system, spectral response of the system, lateral resolving power, objective issues, three-dimensional (3D) reconstruction precision, distances in  $xy$  and  $z$ , and scanning performance.

### Spectral features

The patterns exhibit the following fluorescence spectral features:

- **Excitation:** The excitation ranges from 300 up to 650 nm. The excitation efficiency is maximal at around 340 nm and drops towards the red wavelengths.
- **Emission:** The emission is a continuum starting from slightly above the excitation wavelength up to 800 nm.
- **Lifetime:** Using fluorescence lifetime imaging microscopy (FLIM), two main decay components of  $0.25 \pm 0.05$  ns and  $2.50 \pm 0.50$  ns have been measured.
- **Photo-stability:** The intensity of the patterns may decrease, but this decrease is transient. The fluorescence

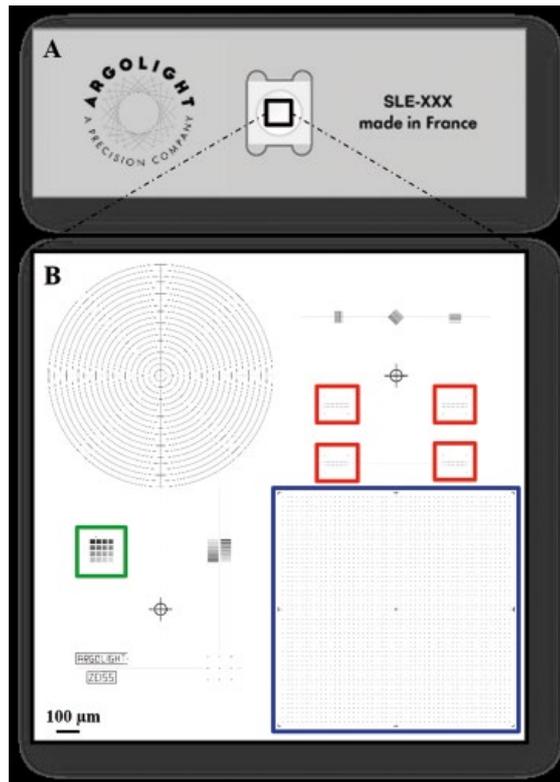


Fig. 1 (A) The slide, containing numerous fluorescent patterns (B).

intensity recovers to its initial value after some time. The recovery time depends on the irradiation conditions (power density, wavelength, pixel size, exposure time).

### Performance assessment examples

The tests that can be performed with the slide are too numerous to be described individually in the framework of the present paper. We will therefore limit ourselves to three examples, illustrating nevertheless the potential of this tool.

#### Lateral chromatic shifts

Because the patterns can be excited from the UV up to the red, lateral shifts between different channels can be measured. The  $1 \text{ mm}^2$  matrix of rings (blue inset in Fig. 1b) allows doing that, not only in the center of the field of view as one would do with a bead, but in its whole.

Fig. 2a – d depict the matrix of rings imaged with three different channels (DAPI, GFP, and Texas Red), and the superposition of these channels, respectively. Fig. 2f and 2g present the intensity profiles for the three channels for two rings of the image, one at the bottom left and the other one at the top right, respectively. One can see that the lateral shift between the three channels is no

more than 130 nm, less than the lateral resolution of the system (here about 200 nm) for the present plan-apochromat  $63\times / 1.4$  objective. Plan-apochromat means that the lateral shift between four different colors (dark blue, blue, green and red) must be less than the system lateral resolution. The results shown in Fig. 2 are in accordance with the manufacturer specifications, for the three present channels.

#### Objective issues and 3D reconstruction precision

An interesting aspect of this technology is its ability to induce patterns at different depths of different lengths. We have designed four 3D patterns (red inset in Fig. 1b), consisting of rings at different depths with different steps (5, 2, 0.5 and  $0.2 \mu\text{m}$ ) surrounded by four pillars, featuring two “crossing stairs”. On a single 2D image, it is therefore possible to have access to the spreading of the light in 3D from the rings at different planes, and to get a clue on the out-of-focus issues.

The “crossing stairs” with a  $2 \mu\text{m}$  step has been imaged with the same objective, in good conditions (Fig. 3a) and with a DIC slider partially inserted in the optical path in order to simulate a problem (Fig. 3b). On the image acquired with the partially inserted DIC slider (Fig. 3b), one can see that the light

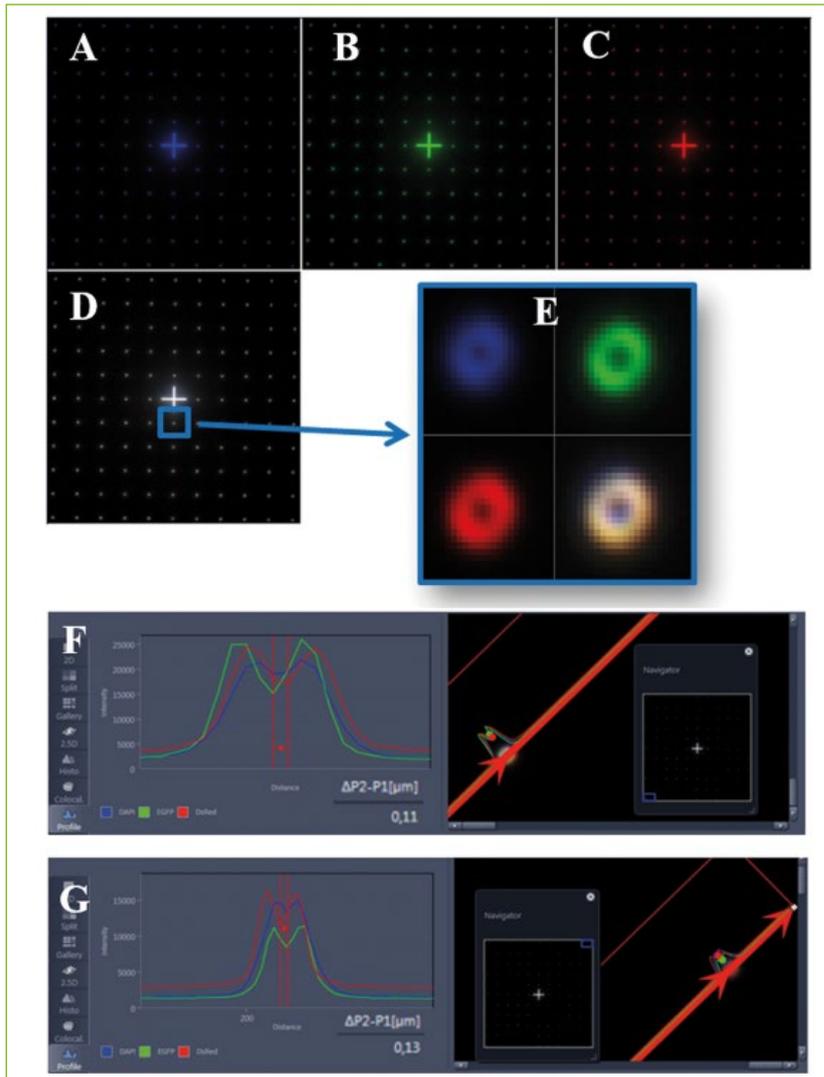
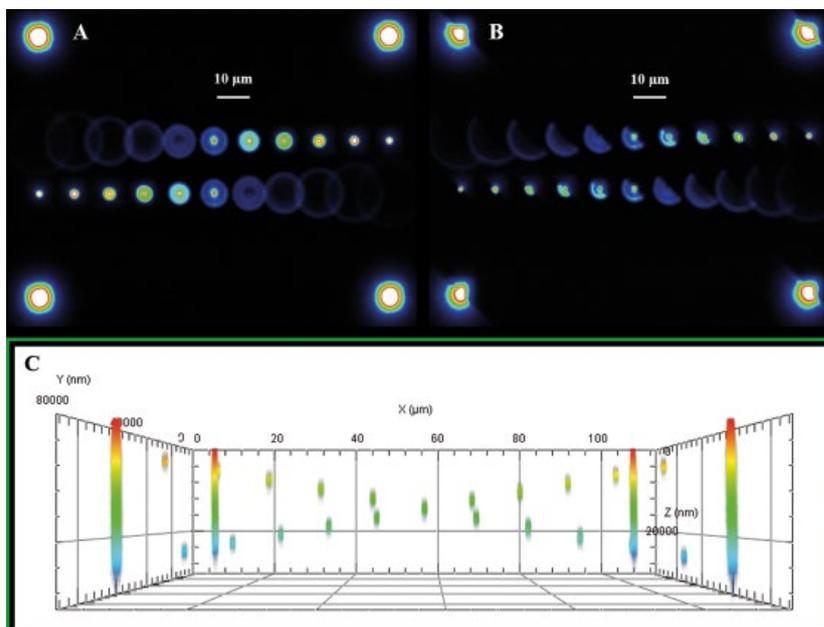


Fig. 2 Confocal images (plan-apochromat  $63\times/1.4$  objective) of the matrix of rings for three different channels (DAPI, A; GFP, B; and Texas Red, C), and the superposition of these channels (D). Inset: Zoom of one ring for the three channels, and their superposition (E). Lateral shift between two rings of the image, one at the bottom left (F) and the other at the top right (G).



spread by the in-focus rings looks correct, while the light spread by the out-of-focus rings does not have a circular symmetry, unlike in Fig. 3a, evidencing the presence of an issue. This is a simple and fast way to check the optical quality of a system. Besides this particular case, one can also observe with this method if a microscope objective is damaged or if there is dust or oil on it.

Such a pattern can also be used to evaluate the accuracy of its reconstruction in 3D, as it is illustrated in Fig. 3c. In this figure, we can clearly see that the reconstruction is accurate, and that the  $z$ -distances are those expected.

#### System intensity response

This technology also enables to control the fluorescence intensity of the patterns, up to 16 well-discriminated intensity levels following a warranted linear evolution. The pattern consists in 16 squares having different intensities (green inset in Fig. 1b). It can be used to characterize the intensity response of the system, in terms of linearity range, sensitivity, and limit of saturation.

Fig. 4a shows an image of these sixteen squares. The mean intensity of each square has been extracted and plotted on a graph versus the square number. The evolution of the intensity levels follows a linear trend, with a Pearson correlation coefficient better than 0.99, evidencing a good linear response of the actual camera (Fig. 4b). This analysis has been achieved with Daybook-Z, the companion analysis software of the Argo-Z slide, in less than a minute. Besides the intensity response of the system, Daybook-Z also allows to extract from images of the suitable patterns the illumination homogeneity, the field distortion, the spatial co-localization, the lateral resolving power, the stage repositioning accuracy and the spectral response of the system (Fig. 4c).

Fig. 3 Confocal images ( $40\times/1.3$  objective,  $\lambda_{exc} = 405$  nm,  $\Delta\lambda_{em} = 410 - 605$  nm) of the  $2\ \mu\text{m}$  step "crossing stairs" in good conditions (A), and with the DIC slider partially inserted (B). 3D reconstruction of the stairs (C).

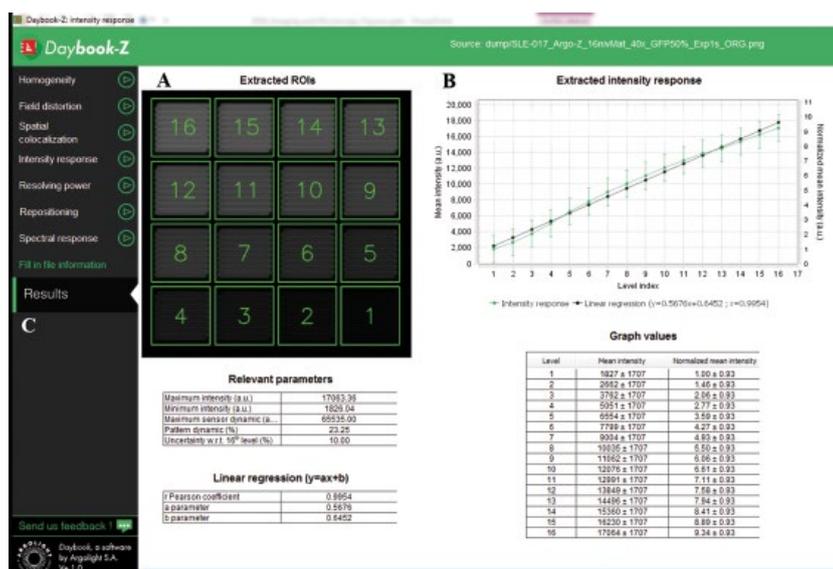


Fig. 4 Wide-field fluorescence image ( $40\times / 0.95$  objective, GFP channel, 1 s exposure time) of the 16 squares having different intensities (A). Evolution of the mean intensity of each square versus the square number. The linear regression curve has a Pearson coefficient better than 0.99, evidencing a good linear response of the actual camera (B). Screen shot of the Daybook-Z interface (C).

## Conclusion

For the first time to our knowledge, there exists a technology that allows to assess and to monitor most of the performances of fluorescence microscopes over the same time scale as their lifetime. The presented slide satisfies the requirements listed by the broad community of fluorescence microscopists and the National Metrology Institutes [3]. This is a huge step towards quality control of these instruments.

Besides, because all the patterns are accurately positioned, it is possible to fully automatize the assessment process, first in the acquisition of the images, secondly in the analysis through dedicated algorithms, and thirdly in the edition of quality management documents, including data, graphs, reports, et cetera.

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