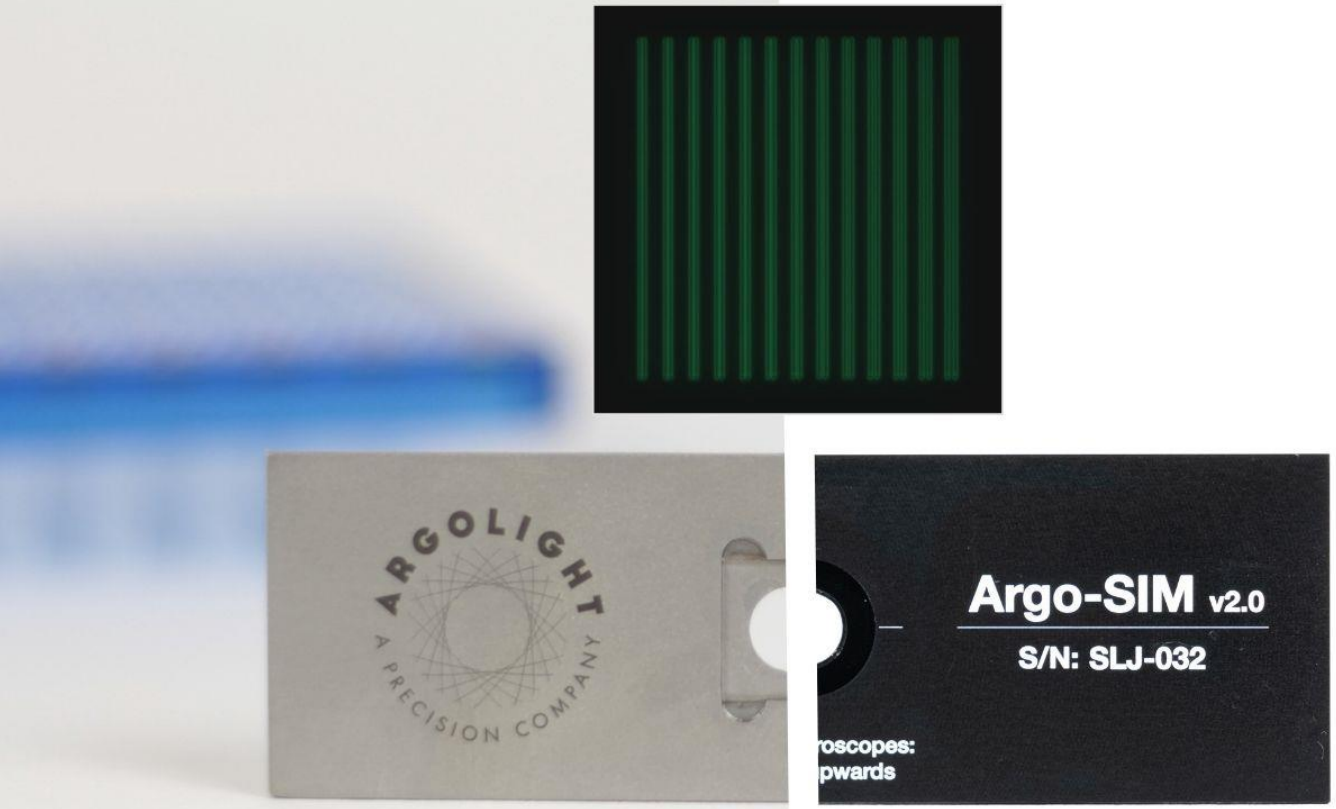
ARGOLIGHT
A Precision Company

TECHNICAL NOTE

Why V1 and V2 slides may provide different results in terms of lateral resolution



ARNAUD ROYON, PHD

Table of contents

1. Introduction

Argolight released two generations of slides. The V2 slides, released in 2021, exhibit a significant improvement of the emitted fluorescence intensity compared to the V1 slides, released in 2016. This improvement comes with an extended axial length of the patterns.

The larger axial length of the patterns in the V2 slides compared to those in the V1 slides influences the results of the analyses available in Daybook, the companion software of Argolight's slides. This is particularly true for the "lateral resolution" analysis, associated with the "gradually spaced lines" patterns.

In this technical note, we provide information about the reasons why the "gradually spaced lines" patterns inside the V1 and V2 slides may provide different results in terms of lateral resolution.

This manuscript aims to:

1. Remind the user of the spatial features of the V1 and V2 slides.
2. Describe the influence of the axial length of the "gradually spaced lines" patterns and the optical sectioning capability of the imaging system.
3. Discuss how these two parameters affect the lateral resolution measurement.

2. Spatial features of the V1 and V2 slides

The main difference between the V1 and V2 slides comes from the glass that they are composed of: the *V1 slides* contain the *second generation* of ArgoGlass®, while the *V2 slides* contain the *third generation* of ArgoGlass®. The general features of the V1 and V2 slides can be found in their respective user guides. Their spatial features are recapitulated in this section.

The elementary pattern that can be induced inside glass with Argolight's engraving process is a hollow cylinder (like a tube) in 3D (Figure 1b), whose section is a ring (Figure 1a).

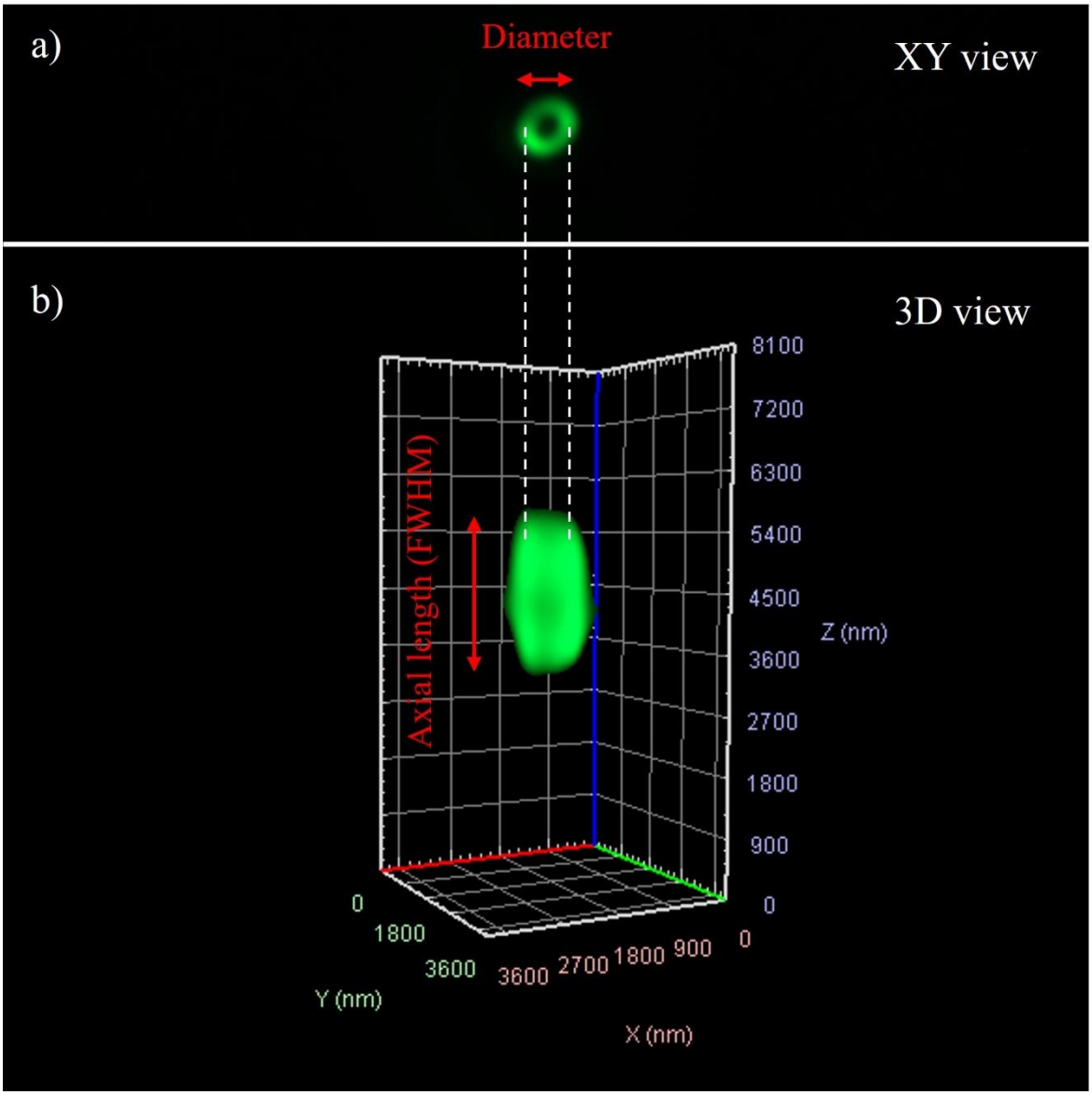


Figure 1: Image of the elementary pattern that can be induced inside glass with Argolight's engraving process. The XY view (a) of the elementary pattern is a ring, which is a section of a tube as observed in the 3D view (b).

For the Argo-HM, Argo-SIM, Argo-Check-Resolution (discontinued), and Argo-GSL slides (i.e., the slides containing the "gradually spaced lines" patterns used to measure the lateral resolution), the transverse diameter (in the XY plane) of the ring at the middle of the tube is about (0.70 ± 0.10) µm for the V1 slides, while it is (0.65 ± 0.10) µm for the V2 slides.

The typical axial length (in the Z-direction) at FWHM (Full Width at Half Maximum) of the tube is about (0.70 ± 0.30) μm for the V1 slides, while it is (2.30 ± 0.30) μm for the V2 slides.

The main difference between the spatial features of the V1 and V2 slides therefore relies on the axial length of the patterns. In the following section, it is discussed and illustrated how this parameter can affect the measurement of lateral resolution.

3. Impact of the axial dimension on the lateral resolution measurement

The "gradually spaced lines" patterns are used to measure the ***contrast transfer function*** of an optical imaging system, which ***determines its capacity to transmit a finite amount of information***. From the contrast transfer function, one can extract what is commonly called the "lateral resolution" (i.e., the resolvable distances associated with a given contrast, signal-to-noise ratio, and signal-to-background ratio). This approach is the essence of the analysis "lateral resolution" in Daybook Analysis.

3.1. Influence of the background on the contrast transfer function

In general, the background influences the lateral resolution measurement. There are different sources of background in the Argolight slides:

- The background coming from the ***auto-fluorescence*** of the glass in which the patterns are embedded.
- The background coming from the ***out-of-focus light*** due to the axial length of the patterns.

For most of the microscopes and imaging conditions, the auto-fluorescence of the glass is negligible compared to the fluorescence emitted by the patterns. For microscopes having optical sectioning capabilities, such as confocal fluorescence microscopes, the out-of-focus light is greatly reduced. The way that these two sources of background impact the measurement of the contrast transfer function is explained below.

<u>Auto-fluorescence:</u>

Figure 2 shows two simulated images of vertical "gradually spaced lines" of an Argo-HM slide without (Figure 2a) and with (Figure 2b) constant background, the latter emulating auto-fluorescence of the glass. The contrast transfer functions obtained from these two images superimpose almost perfectly (Figure 2c), evidencing that constant background such as auto-fluorescence does not influence on the contrast and, therefore, the resolvable distances.

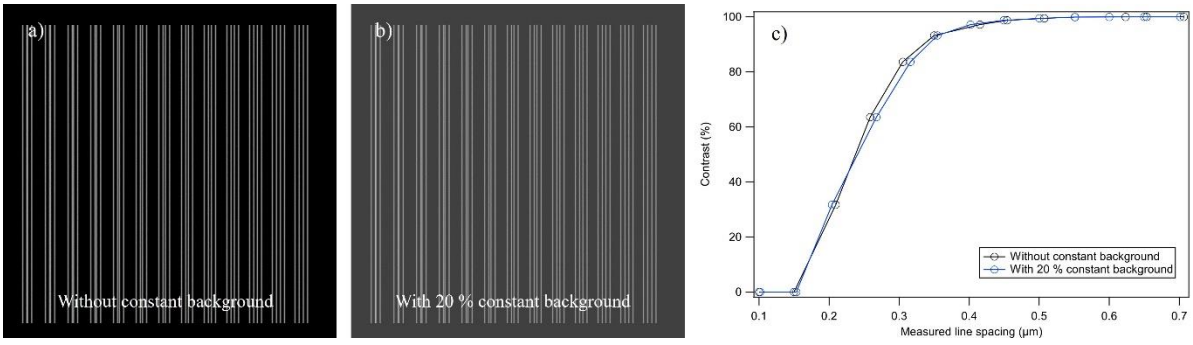


Figure 2: Simulated images of vertical "gradually spaced lines" of an Argo-HM slide (a) without and (b) with 20% of constant background. (c) Contrast transfer functions obtained from these two images using the analysis "lateral resolution" in Daybook.

Out-of-focus light:

Figure 3 shows four simulated images of vertical "gradually spaced lines" of an Argo-HM slide without (Figure 3a) and with (Figures 3b, 3c, and 3d) several levels of local background, the latter emulating out-of-focus light emitted from the lines in the planes above and below the focal plane. The contrast transfer functions obtained from these four images are different. As the level of local background increases, the contrast decreases, evidencing that local background such as out-of-focus light has an impact on the contrast and, therefore, on the resolvable distances.

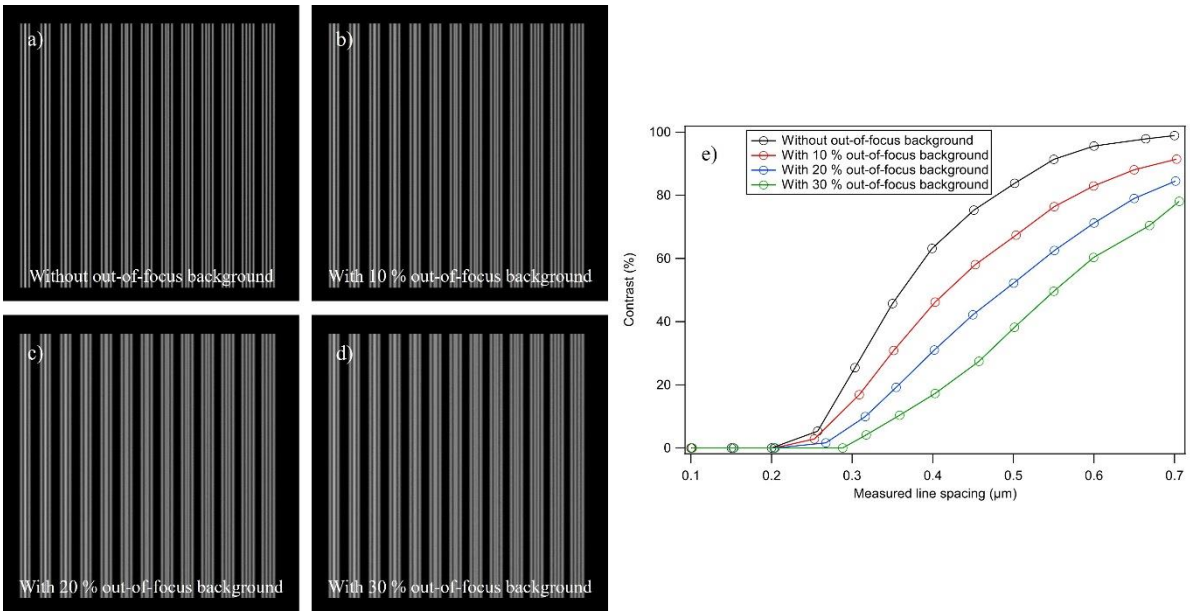


Figure 3: Simulated images of vertical "gradually spaced lines" of an Argo-HM slide (a) without out-of-focus background and with (b) 10%, (c) 20%, and (d) 30% of out-of-focus background. (e) Contrast transfer functions obtained from these four images using the analysis "lateral resolution" in Daybook.

Recap:

While the auto-fluorescence of the glass does not affect the contrast in the images of the "gradually spaced lines" patterns, the out-of-focus light decreases the contrast. As a consequence, the out-of-focus light decreases the amount of information that the imaging system can transmit. Distances are resolved with less contrast, affecting the "lateral resolution" of the microscope.

There are two origins of out-of-focus light, which are closely tied: the axial length of the sample (here the "gradually spaced lines" patterns) and the optical sectioning capability of the imaging system. There is no out-of-focus light if an infinitely thin sample is imaged with an imaging system having no optical sectioning capability. The same applies if a thick sample is imaged with an imaging system having an infinite optical sectioning capability. Between these two extreme scenarios, which appears to be the case in biological imaging whatever the used microscope, both contributions give rise to out-of-focus light.

3.2. Influence of the axial length of the sample on the contrast transfer function

As discussed in Section 2, the axial length (in the Z-direction) of the patterns in the V1 and V2 slides are different: ~0.70 µm for the V1 slides and ~2.30 µm for the V2 slides. Because the axial length of the "gradually spaced lines" patterns is larger in the V2 slides than in the V1 slides, the amount of light coming from the out-of-focus planes is more important for the V2 slides than for the V1 slides, ***for the same microscope***. As a consequence, for the same spacing between two lines, the contrast is lower for the V2 slides than for the V1 slides.

Figures 4a and 4b show two images of the horizontal "gradually spaced lines" of an Argo-HM V1 slide and of an Argo-HM V2 slide, respectively, captured with the same wide-field microscope (i.e., a microscope without optical sectioning capability). While the measured line spacings are the same for both slide versions (Figure 4c), within the measurement uncertainties, the contrast transfer function obtained from the Argo-HM V2 slide is lower than the one obtained from the Argo-HM V1 (Figure 4d).

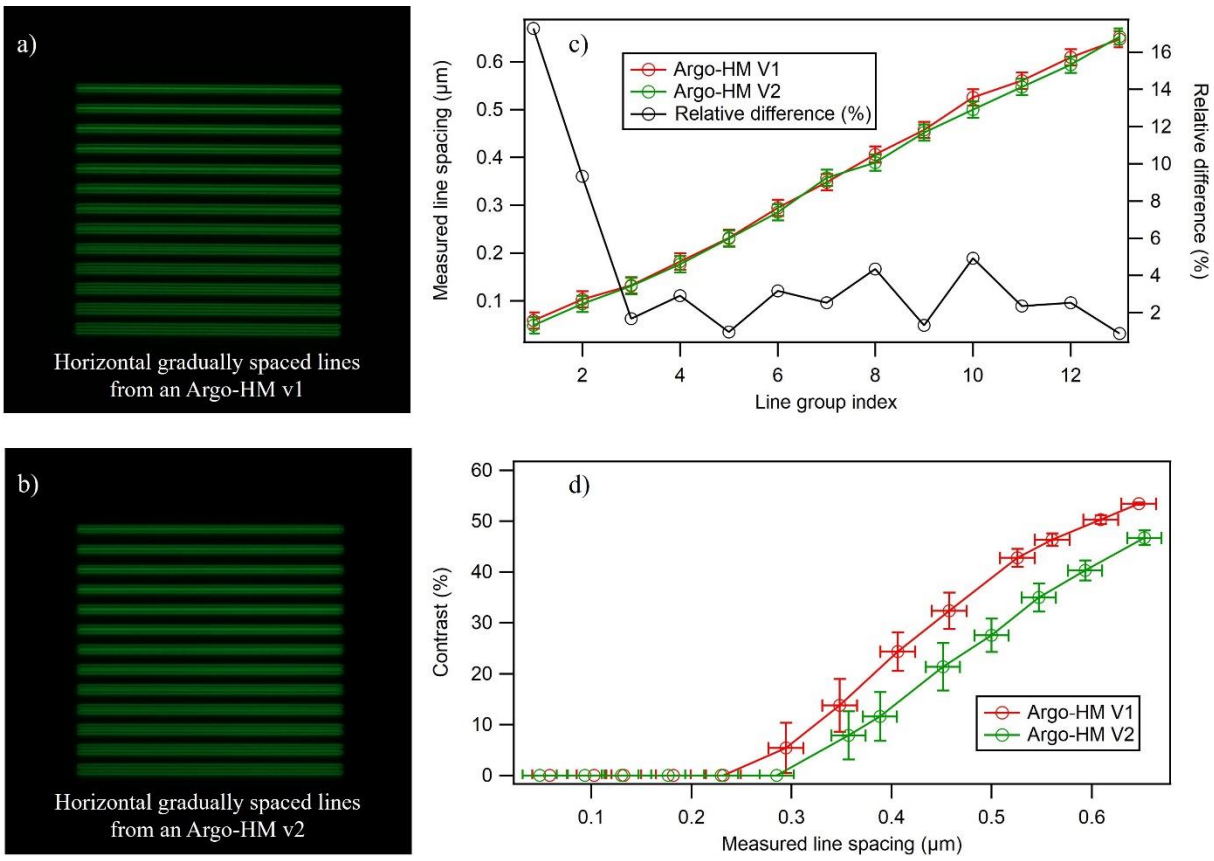


Figure 4: Images of the horizontal "gradually spaced lines" (a) of an Argo-HM V1 slide and (b) of an Argo-HM V2 slide captured with the same wide-field microscope, using a 100×/1.46 objective on the GFP channel, and applying distinct acquisition settings so that the histograms of the images are similar. (c) Evolution of the measured spacing versus the line group index from (a) and (b). (d) Contrast transfer functions obtained from (a) and (b) using the analysis "lateral resolution" in Daybook.

For microscopes having no optical sectioning capability or finite optical sectioning capability, the "gradually spaced lines" patterns in the V2 slides provide a decreased contrast

transfer function, and therefore a less good "lateral resolution," compared to the ones in the V1 slides.

3.3. Influence of the optical sectioning capability of the imaging system on the contrast transfer function

Figures 5a and 5b show two images of the horizontal "gradually spaced lines" of an Argo-HM V1 slide captured with two different confocal spinning disk microscopes (i.e., microscopes with optical sectioning capability). Figures 5c and 5d display the axial intensity profile of these images. The experimental data are fitted with a Gaussian function, whose FWHM is a metric of the optical sectioning strength of the microscope. The FWHM of the fitting Gaussian functions are about 1.25 µm and 1.14 µm, respectively. Figures 5e and 5f present the contrast transfer functions obtained from these images. For the Rayleigh criterion (26.5% of contrast), the measured resolvable distances are about 440 nm and 370 nm. The microscope having the largest optical sectioning strength ($FWHM_z \approx 1.25$ µm) presents the largest resolvable distance (~440 nm), while the microscope having the lowest optical sectioning strength ($FWHM_z \approx 1.14$ µm) presents the lowest resolvable distance (~370 nm).

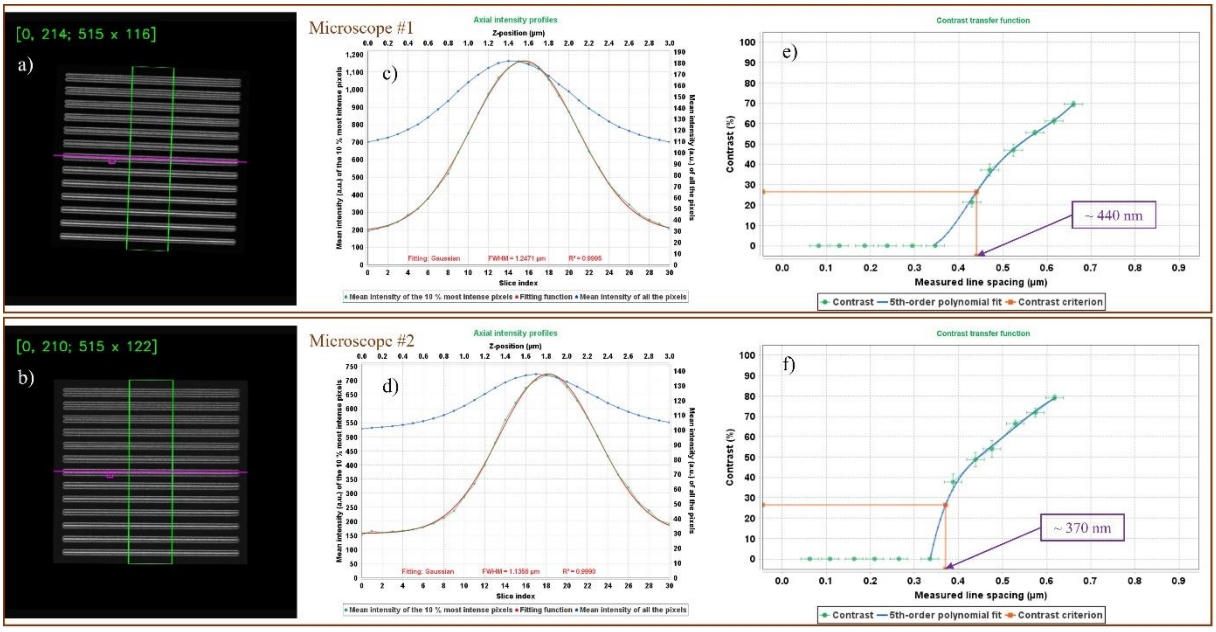


Figure 5: (a, b) Images of the horizontal "gradually spaced lines" of an Argo-HM V1 slide captured with two different confocal spinning disk microscopes having the same configuration. (c, d) Axial intensity profiles obtained from (a) and (b). The FWHM of the fitting Gaussian functions are about 1.25 µm and 1.14 µm, respectively. (e, f) Contrast transfer functions obtained from (a) and (b) using the analysis "lateral resolution" in Daybook. The resolvable distance associated with the Rayleigh criterion (i.e., 26.5% of contrast) is about 440 nm for the microscope having the highest optical sectioning strength ($FWHM_z \approx 1.25$ µm), while it is about 370 nm for the other one ($FWHM_z \approx 1.14$ µm).

When imaged with two microscopes of the same model but with different optical sectioning capabilities, the "gradually spaced lines" patterns provide a decreased contrast transfer function, and therefore a less good "lateral resolution," for the microscope having the lowest optical sectioning capability, compared to the other one.

Notes:

- A low optical sectioning strength means a high optical sectioning capability and a low measurable distance means a high lateral resolution.
- In this example, it is assumed that no other parameters than the optical sectioning capability of the imaging system (such as the objective features or camera noise) influence the measured contrast transfer function.

3.4. Discussion

We have seen that both the axial length of the "gradually spaced lines" patterns and the optical sectioning capability of the imaging system influence the measured contrast transfer function, because out-of-focus light decreases the contrast and, thereby, the lateral resolution.

Acquisition mode	**Difference in the contrast transfer functions between the V1 and V2 slides** *From "very important" (*****) to "almost identical" (*)*	**Comment**
Wide-field	*****	Wide-field microscopes have no optical sectioning capability
Confocal laser-scanning	**	The optical sectioning capability of confocal laser-scanning microscopes is, in general, better than the one of confocal spinning disk microscopes
Confocal spinning-disk	***	
Deconvolution	*	Depends on the deconvolution algorithm and coupling with a confocal microscope
Super-resolution	*	Depends on the super-resolution approach: interference-based SIM (for example 2D SIM and 3D SIM), single point-scanning SIM (for example Re-scan and Airyscan), and multipoint scanning SIM (for example instant SIM, or iSIM)

Table 1: Difference in the contrast transfer functions between the V1 and V2 slides for different acquisition modes. This difference ranges from "very important" (*****) to "almost identical" (*).

In theory, if the imaging system used to image the "gradually spaced lines" patterns has an infinitely small optical sectioning capability, both V1 and V2 slides will provide the same results in terms of lateral resolution.

In practice, the results obtained from the V1 and V2 slides depend on the imaging system's acquisition mode.

Table 1 presents the expected amount of difference in the contrast transfer functions between the V1 and V2 slides for different acquisition modes. As the optical sectioning capability of the imaging system increases, the difference in the results between the V1 and V2 slides decreases.

4. Conclusion

This technical note aimed to answer the questions raised by some of our customers concerning the difference of lateral resolution results obtained from the "gradually spaced lines" patterns inside the V1 and V2 slides.

This note described:

- The intrinsic differences between the V1 and V2 slides in terms of spatial features.
- The reasons why the lateral resolution measured with the "gradually spaced lines" patterns can be "less optimal" in the V2 slides than in the V1 slides.
- The relationship between the lateral resolution, the sample's axial length, and the optical sectioning capability of the microscope.

To sum up, although in theory, both V1 and V2 slides will provide the same results in terms of lateral resolution, in practice, the resolution results may vary between the two generations, depending on the type and features of the microscope used.

We trust that this note will provide the necessary information to answer all the interrogations and remove the possible misunderstanding about the lateral resolution measurements obtained from the "gradually spaced lines" patterns in the V1 and V2 slides.

Date of issue

16/05/2024

Version

1.1

Author

Arnaud Royon, PhD

Copyright notice

Contact information

Argolight
Cité de la Photonique, Building. 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.

Disclaimer

To the extent allowed by law, Argolight will not be liable for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including the use of it.

ARGOLIGHT

A Precision Company