Lateral resolution of the Re-scan Confocal Microscope 2 measured with the Argolight Argo-SIM slide

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Introduction

The resolution of an optical microscope is defined as the shortest distance between two point-sources on a specimen that can still be distinguished as separate entities[1]. Determining the resolution of an optical microscope accurately and objectively can be challenging. Beads smaller than the diffraction limit are often used to assess the resolution of a microscope. These beads are sometimes hard to focus on and bleach over time. In addition, since their size is not infinitely small they broaden the microscope Point Spread Function (PSF), therefore underestimating the resolution of the optical system.

The company Argolight offers an exclusive technology to calibrate and monitor fluorescence microscopes. Argolight solutions reproduce fluorescence cell-like patterns in slides, which are extremely stable and perfectly known in size. With these slides, microscopes can be monitored and the bias quantified and corrected accurately.

The Re-scan Confocal Microscope (RCM) is based on the re-scanning principle[2], which increases lateral resolution by a factor of 1.4 compared to standard confocal systems. RCM goes beyond the diffraction limit, reaching 170 nm lateral resolution at 488 nm wavelength and 120 nm after deconvolution.

Here we showed RCM2 resolution, proven by the “gradually spaced lines” pattern inside the Argo-SIM slide[3] (Fig. 1).

Figure 1. Image of the Argo-SIM slide.

Materials & Methods

RCM2 images of the Argo-SIM slide pattern were recorded using an Olympus IX83 microscope frame with a 100x/1.5 oil objective (UPLAPO100XOHR). The 405nm laser of an Omicron lighthub was used for excitation of the fluorescent patterns. Detection of the emission light was performed with a Tucsen FL20-BW camera.

The acquisition settings resulted in a pixel size in the specimen plane of 16.5nm/pixel. The z-stepper of the microscope was set to 100nm interval, taking a z-stack of 4 micrometers in total to be able to do proper deconvolution. Subsequently deconvolution was performed by Microvolution with a dedicated RCM point spread function.

The figures show the slice of the z-stack with the best focus. Subsequent analysis of the datasets was performed with Daybook 3 software from Argolight, using the Gaussian fit model and a contrast criterion of 7.3% (corresponding to the contrast value equal to the FWHM of the microscope’s PSF in ideal imaging conditions).

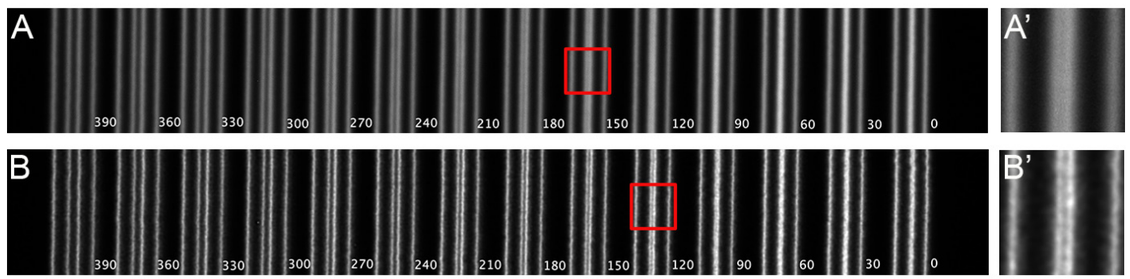

Figure 2. RCM2 images of the Argo-SIM slide pattern. A. Raw image. 2 lines can be discriminated by eye at the distance of 150 nm. A’ Magnification of the area inside the red square. B. Deconvolved image where the 2 lines can be resolved at the distance of 120 nm. B’ Magnification of the area inside the red square.
Numbers indicate line spacing in nanometers

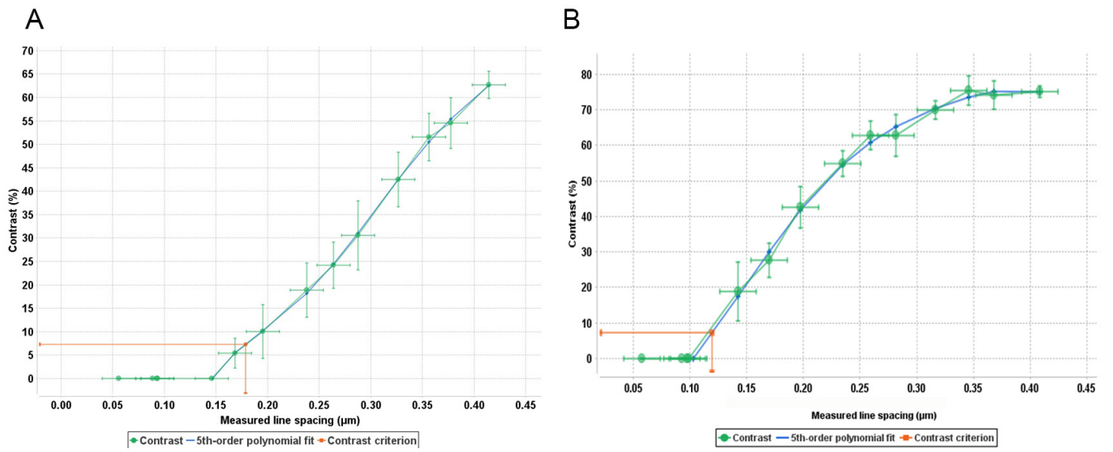

Figure 3. Graphs showing contrast vs measured line spacing.
A. Graph obtained with the raw image showing 170nm at 7.3% contrast level. B. Graph obtained with the deconvolved image which reflects the resolution improvement to 120nm.

Results

Argo-SIM slide allowed accurate determination of the lateral resolution of the re-scan confocal microscope, RCM2.

In this experiment, we used the Argo-SIM slide to evaluate the resolution of the RCM2 with the configuration described in the methodology.

The images acquired showed the pattern of the Argo-SIM slide which consists of vertically aligned lines, with 30 nm increment on the two center lines, starting at 0 nm and ending at 390 nm spacing. Looking at the raw images by eye, we were able to resolve the spacing of 150 nm (Fig. 2A).

“The line spacing at 120 nm was clearly visible in the deconvolved image”

Analysis of the images was performed using the Gaussian fit model and a 7.3% contrast criterion, corresponding to the microscope’s PSF in ideal imaging conditions. In the raw data, the 7.3% contrast criterion was reached at 178.5 nm line spacing (Fig. 3A, Table 1). These results corresponded exactly with the specifications previously assigned to the RCM2.

Deconvolution was performed on those images, leading to a significant increase of the resolution. Deconvolved images allowed us to resolve the pattern of 120 nm by eye (Fig. 2B), which was supported by the results of the image analysis (131.8 nm at 7.3% contrast level) (Fig. 3B, Table 1).

	Raw	Deconvolved
Contrast Criterion (%)	7.3	7.3
Lateral resolution (µm)	0.1785	0.1201
Lateral resolution at zero contrast (µm)	0.1446	0.1049
SNR	51.91	53.8

Table 1. Primary metrics of the resolution measurements. SNR - Signal to Noise Ratio.

Conclusions

The Argo-SIM slide allowed us to quantitatively and accurately determine the lateral resolution level of the RCM2 without introducing user-bias.

In the raw images RCM2 was able to achieve a resolution of 178.5 nm at a contrast level of 7.3%.

After deconvolution at 7.3% contrast level, the resolution was further increased to 131.8 nm. According to the primary metrics reported by the Daybook software the resolution of the images at zero contrast are 144.6 and 104.9 nm for the raw and deconvolved data, respectively. These results demonstrate that RCM2 achieves super-resolution in the images.

RCM
- Super-resolution imaging
- 120 nm lateral resolution

References

[1] https://www.microscopyu.com/microscopy-basics/resolution
[2] Giulia MR de Luca, Ronald MP Breedijk, Rick AJ Brandt, Christiaan HC Zeelenberg, Babette E de Jong, Wendy Timmermans, Leila Nahidi Azar, Ron A Hoebe, Sjoerd Stallinga, and Erik MM Manders. “Re-scan confocal microscopy: scanning twice for better resolution”. Biomed Opt Express. 2013. DOI 10.1364/BOE.4.002644
[3] https://argolight.com/products/argo-sim/