Science Park, 106. 1098XG Amsterdam. The Netherlands 2nd June 2021

The Re-scan Confocal Microscope (RCM) minimizes motion-blur when imaging fast germination proteins dynamics

Jeroen Kole, BASc, Mariliis Tark-Dame, PhD & Noelia Munoz-Martin, PhD

Introduction

Imaging highly dynamic proteins in cells is challenging. These proteins are sometimes in low quantities, form below-resolution clusters or are surrounded by autofluorescent structures. Therefore, high imaging speed is required, but also high sensitivity.

When imaging with a wide-field system, the exposure time can be increased to capture more light but this is associated with phototoxicity, which can alter cell behaviour. Alternatively, super-resolution imaging techniques based on image reconstruction can be used. However, those techniques are prompt to generate artifacts and also implicate high light exposure to the sample and phototoxicity. Some common artifacts associated with the super-resolution based on image reconstruction are non-native protein localization and motion-blur[1].

The Re-scan Confocal Microscope (RCM)[2] is a highly sensitive imaging system, with super-resolution and minimal light exposure for the sample. These make RCM a good imaging system to solve the limitations mentioned above.

Here, we imaged *Bacillus subtilis* sporulation and germination proteins using 2 imaging modalities, wide-field microscopy and RCM.

Materials & Methods

Imaging was performed by Erik Manders, Confocal.nl
Confocal.nl RCM1 equipped with Nikon TiE, Toptica CLE and Hamamatsu Flash4 V3 camera, was used to acquire the images. Bypass mode was used to change between wide-field and RCM modes. Field of view was reduced to 2 x 2 microns in order to adjust it to sample size and increase acquisition speed.
Image analysis was done by Desiree Salas, Confocal.nl using ImageJ.

Sample courtesy of J. Wen, S. Brul, University of Amsterdam. *Bacillus subtilis* with the SpoVAEa germination protein fused to GFP.

Results

The RCM showed superior performance than wide-field when imaging fast SpoVAEa protein dynamics in the germinosome of B. subtilis

Spores of *Bacillus subtilis* are metabolically dormant and resistant to harsh environmental conditions, but the presence of specific nutrients can trigger the process of germination. During germination, spores lose their dormancy and outgrow into vegetative cells. SpoVAEa is a protein essential for the uptake and release of dipicolinic acid during sporulation and germination, and is present in the inner membrane of the spore [3]. Here we used *Bacillus subtilis* with SpoVAEa protein fused to GFP to visualize the highly dynamic process of germination.

"RCM captures fast protein dynamics happening at the ms scale"

In order to image fast protein movement without motion blur, imaging speed in the range of 20ms is required. In wide-field, a decent image was acquired in a snapshot with exposure time of 2 seconds (Fig. 1A). This image revealed three distinct protein clusters but the resolution was very low and motion blur artifacts were evident.

When imaging with RCM, a higher temporal resolution was achieved. In this case, 45 frames per second were used, which translates to 1.35 ms exposure time at the molecular resolution (the time every single sub diffraction level molecule is excited during scanning). Due to faster scanning, the motion blur was minimized (Fig. 1B). In addition, the re-scanning principle improved resolution for a 1.4 factor.

Following the germinosome, highly dynamic protein structures moving continuously through the spore were identified (Fig. 2A). The average intensity projection based on these 1000 images shows preferential positioning of the SpoVAEa during this image acquisition time (Fig. 2B).

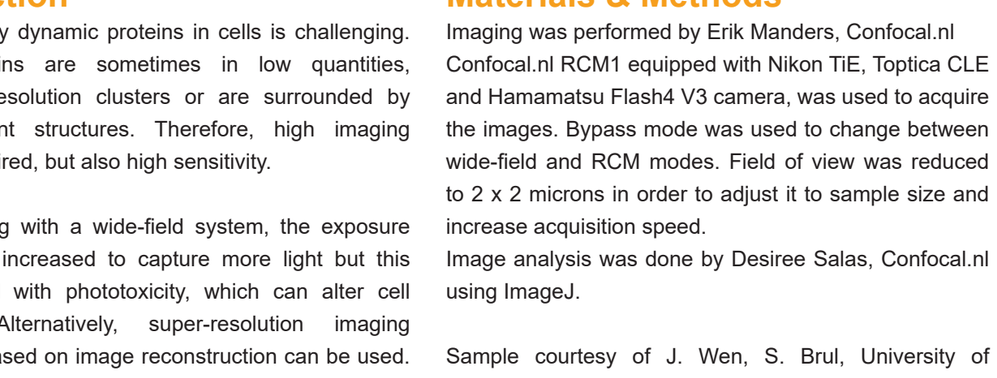


Figure 1. Comparison of wide-field and RCM image of *B. subtilis* SpoVAEa protein. SpoVAEa protein fused to GFP. A. Wide-field B. RCM. Scale bar: 500 nm.

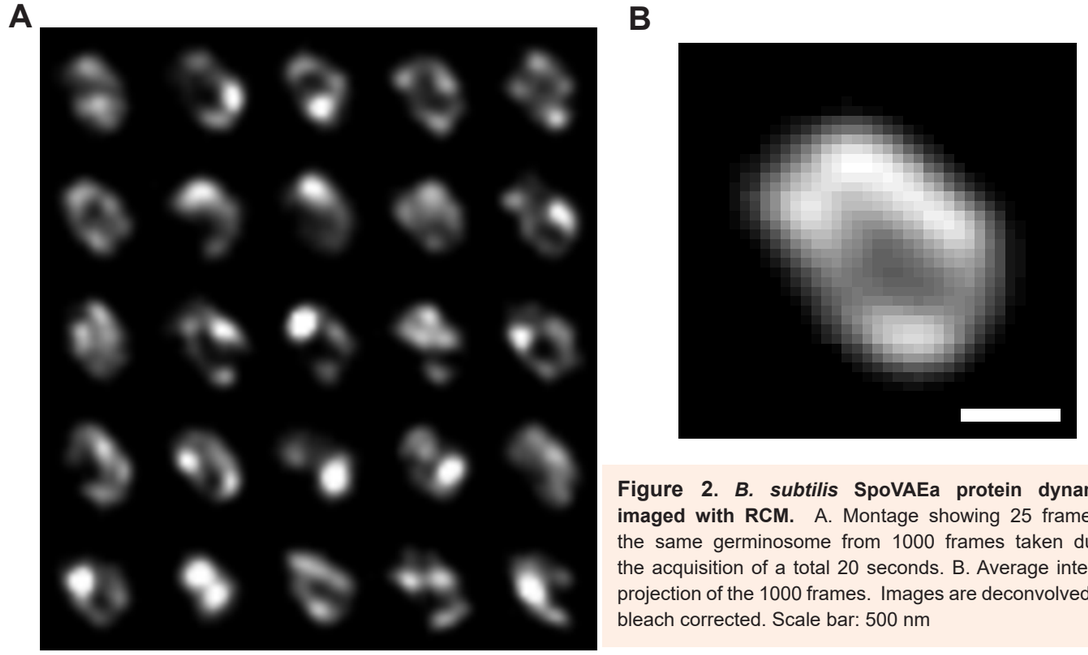


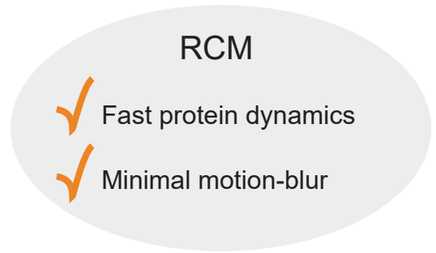
Figure 2. *B. subtilis* SpoVAEa protein dynamics imaged with RCM. A. Montage showing 25 frames of the same germinosome from 1000 frames taken during the acquisition of a total 20 seconds. B. Average intensity projection of the 1000 frames. Images are deconvolved and bleach corrected. Scale bar: 500 nm

Conclusions

The RCM showed superior performance than wide-field when imaging fast SpoVAEa protein dynamics in the germinosome of B. subtilis.

The higher sensitivity of RCM gave better signal to noise ratio and higher contrast images when compared to a wide-field imaging system. The resolution of RCM images was 1.4 times higher than wide-field. In addition, the fast-scanning speed of RCM drastically diminished the motion-blur artifacts.

Altogether, RCM provided more detailed and higher quality images that allowed for a better analysis of the SpoVAEa dynamics during germination.



References

[1] Heintzmann, R. & Huser, T. Super-resolution structured illumination microscopy. Chem. reviews. 2017. DOI 10.1021/acs.chemrev.7b00218
[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] Troiano, A, Zhang, J, Cowan, A, Yu, J & Setlow, P. Analysis of the Dynamics of a Bacillus subtilis germination protein complex during spore germination and outgrowth. J. Bacteriol. 2015. DOI 10.1128/JB.02274-14