VISUALIZING AWAKENING OF BACTERIAL SPORES USING RESCAN CONFOCAL MICROSCOPY WITH ANNULAR ILLUMINATION.

Ronald Breedijk[1], Juan Wen[1], Venkataraman Krishnaswami[1], Norbert Vischer[1], Tytus Bernas[3], Erik Manders[1,2] and Stanley Brul[1]
1 Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands
2 Confocal.nl BV, Amsterdam, The Netherlands
3 VCU Microscopy Facility, VCU School of Medicine, Richmond, VA, USA
E-mail: breedijk@uva.nl

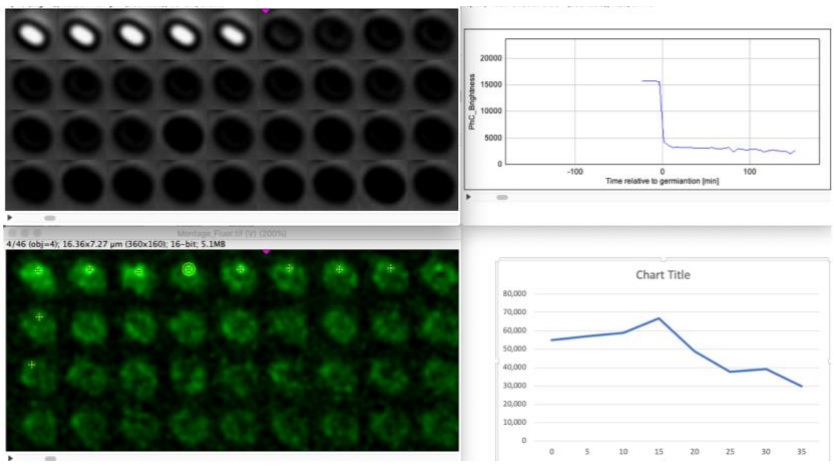
KEY WORDS: Rescan Confocal Microscopy, Super resolution microscopy, Germination of spores of Bacillus species

ABSTRACT

Germination of bacterial spores is central to the development of probiotics and food-processing techniques, but its underlying dynamics are yet to be visualized and understood, despite the growth of imaging technologies. Germinosomes are clusters of germinant receptors which trigger the awakening of spores. They are only present in low copy number, and their visualization is hampered by the highly auto-fluorescent coat layer. The growth of super-resolution microscopy has shown great promise for visualizing and studying germinosomes, however, time-lapse imaging of such living specimens still remain a challenge [1]. Here, we use a rescan confocal microscope with annular illumination [2]. Images with a lateral resolution of 110 nm are generated in a single scan without post-processing. Time-lapse images were recorded to give the first ever evidence for germinosomes in 'wild-type' spores and their spatio-temporal dynamics upon germinant addition, thereby visualizing spores coming to life.

REFERENCES

[1] J. Wen et all., Visualization of Germinosomes and the Inner Membrane in Bacillus subtilis Spores. J. Vis. Exp. (2019), doi:10.3791/59388.
[2] G. M. R. De Luca et all., Re-scan confocal microscopy: scanning twice for better resolution. Biomedical Optics Express. 4 (2013), p. 2644.



Montage of time-lapse images of phase contrast (top) and fluorescence images (bottom) of a single spore. The time point of germination is indicated by the magenta arrow. The leader spot is indicated by the yellow circle. Graph upper right, the brightness of the phase contrast image dropped upon spore germination. Graph at lower right, fluorescence intensity of a GerKB-sGFP spot increases prior to germination and drops rapidly after the phase transition.