Supplement of:

Fluorescent double labelling of F-actin in Foraminifera: evaluation of granular pattern F-actin organisation in reticulopodia

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1 Granuloreticulopodia tend to lose the granular appearance during the fixation

The most common fixation artefact is so called beading response (Travis and Allen 1981). When conducting preliminary experiments for this study we realised, however, that it is not the only way in which pseudopodia may lose their intact structure after fixation. Pseudopodia tend to lose their granular appearance, the longer of the fixation time, the stronger the effect is. We were able to record time-lapse illustrating this phenomenon *Amphistegina* sp., however, other species, including *Quinqueloculina* sp., also display a strong tendency to lose the granularity of pseudopodium after fixation. The granules start to burst one by one several minutes after application of the fixative. It results in almost complete smoothing of pseudopodium appearance after a few minutes (see Figs. S1-S2 and Movie S1 in Supplementary Materials). Finding the exact cause of this process goes beyond the scope of this paper and should be addressed in a dedicated study. As our intention was to test hypotheses concerning the nature of the ALGs, we had to implement such a procedure that would not affect the natural granular appearance of the pseudopodia. Damaging any kind of the granules during fixation would result in losing the objects of our study, and therefore, producing experimental artefacts. Thus it was crucial to find an optimal procedure that would preserve intact granular structures.

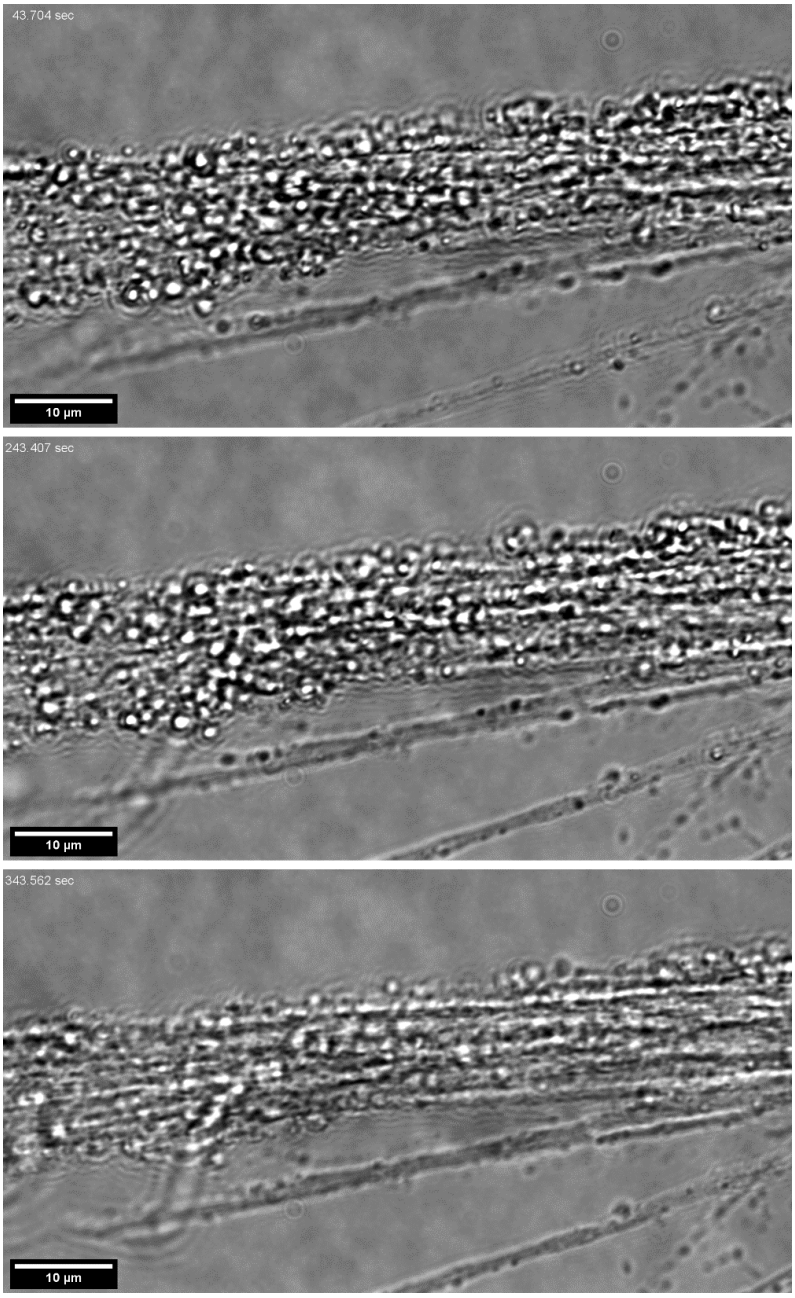

Figure S1. Three frames form the time lapse demonstrating fixation of the granuloreticulopodia of *Amphistegina lessonii* d'Orbigny. Top: Image of pseudopodium immediately after applying the fixative solution, when the motion of the pseudopodium ceased (43.704 s since start of recording). At this point the pseudopodium contains a large number of granules. Middle: image of pseudopodium after c. 200 s after application of fixative. Pseudopodium still displays its granular appearance. Bottom: image of pseudopodium after c. 300 s after the addition of fixative. Pseudopodium is almost completely devoid of clear granular objects. The time elapsed since the start of recording is displayed in the upper left corner. Images were captured with Zeiss Axio Observer Z1. The entire time lapse can be seen in the Movie S1 in the Video Supplement

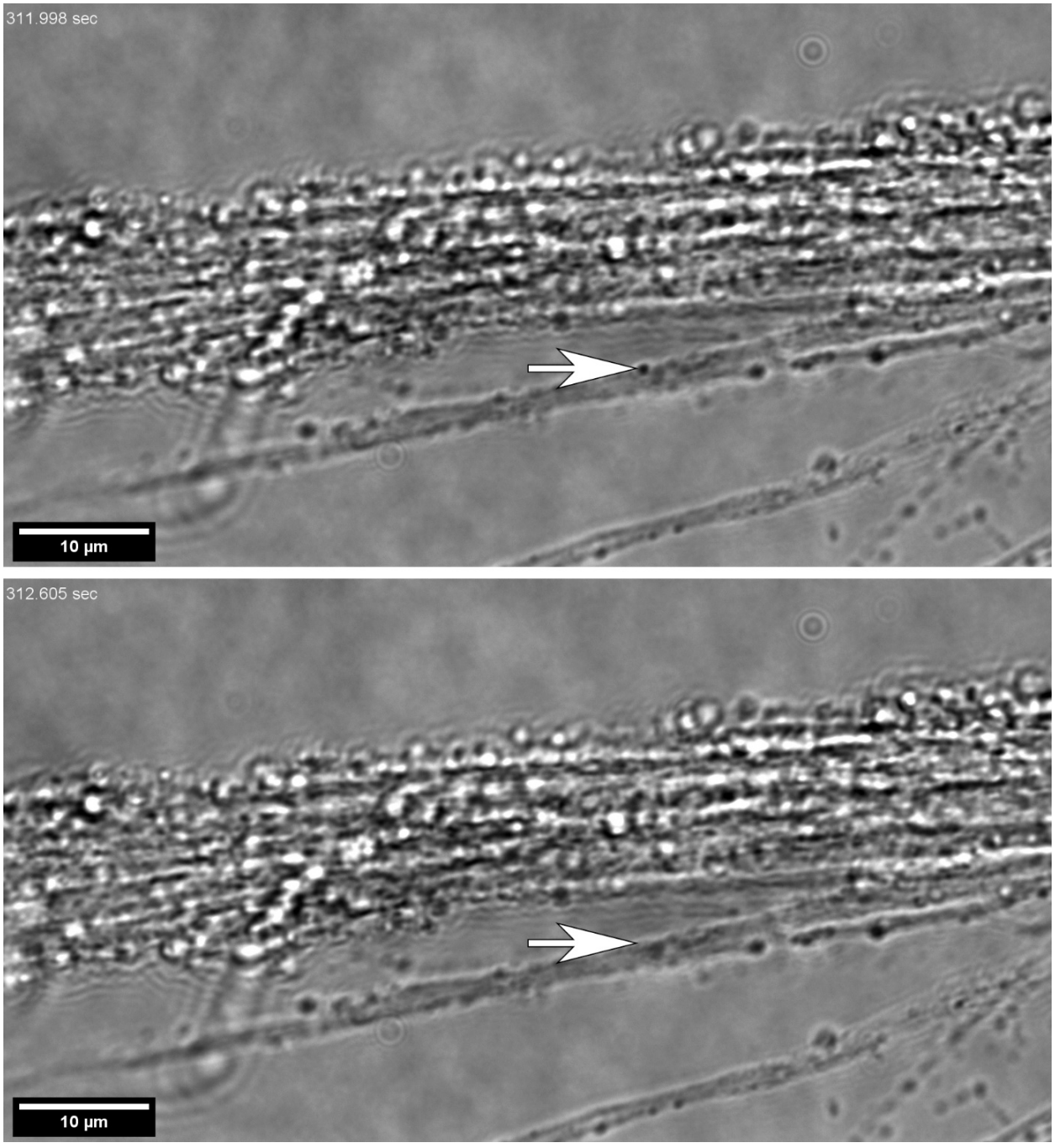

Figure S2. Two subsequent time lapse frames demonstrating fixation of the granuloreticulopodia of *Amphistegina lessonii* d'Orbigny (captured 311.998 s and 312.605 s from beginning of recording, c. 270 s after addition of fixative). Arrows indicate the position of a granule still visible at 311.998 s (upper) and missing from the lower image captured at 312.605 s. The time elapsed since the start of recording is displayed in the upper left corner. Images were captured with Zeiss Axio Observer Z1. The entire time lapse can be seen in the Movie S1 in the Video Supplement.

2. Comparison of the fluorescence intensity between control and experimental individual

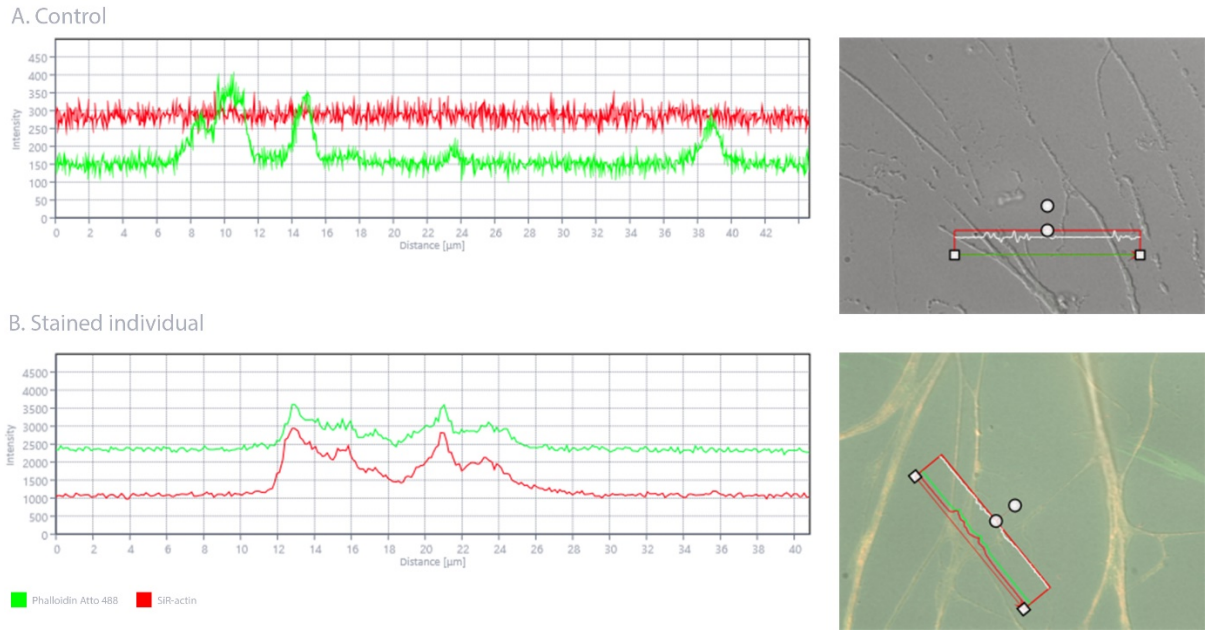

Figure S3. Intensity of fluorescence or transmitted light within the fixed pseudopodia of two individuals of *Quinqueloculina* sp. Comparison of fluorescence intensity in the control and experimental individual. In left column: fluorescent intensity graphs unstained (top) and stained (bottom) individuals across the lines indicated on the images on the right side. On the graphs the white line indicates the intensity of the transmitted light, red line indicates the intensity in the fluorescent channel for the SiR-actin fluorescent probe, and green line indicates the light intensity in the channel for Phalloidin Atto 488 probe. Note that overall intensities for the fluorescent channels are much higher for the experimental individual. Within the experimental individual, the fluorescent intensity is much more variable than in the control one, showing a significant peak in the same position. Images were captured with Zeiss Axio Observer Z1.

3. Shift between two fluorescent channels

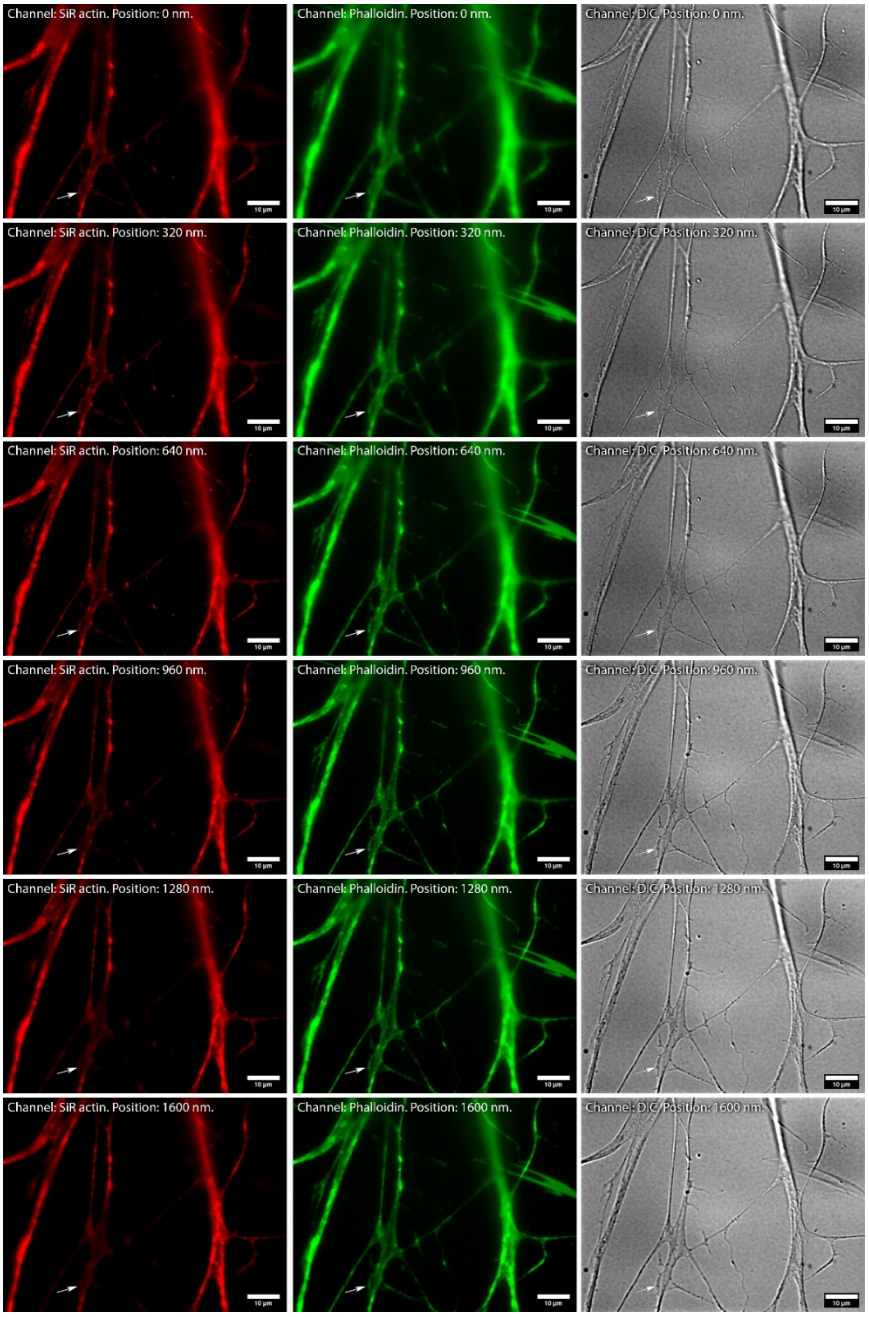

Figure S4. Z-stack of granuloreticulopodia of *Quinqueloculina* sp. stained with SiR-actin (red) and Phalloidin Atto 488 (green) fluorescent channels. Note the shift between two fluorescent channels. The arrow indicates the position of the SiR-actin-labelled granule (ALG) stained with both fluorescent probes. The image of this granule is in focus in the z-position 0-640 nm in the SiR-actin fluorescent channel. From the z-position 960 nm it becomes to be out of focus. For the Phalloidin Atto 480 channel the image of the same object is well focused in z-position 640-1280nm. This shift applies to other areas of the image. All images were captured with Zeiss Axio Observer Z1.The animation of the entire z-stack can be seen in the Movie S2 in the Video supplement.

Reference:

Travis, J. L., and Allen, R. D.: Studies on the motility of the foraminifera. I. Ultrastructure of the reticulopodial network of *Allogromia laticollaris* (Arnold). *J. Cell Biol.*, 90(1), 211-221, doi:10.1083/jcb.90.1.211, 1981.