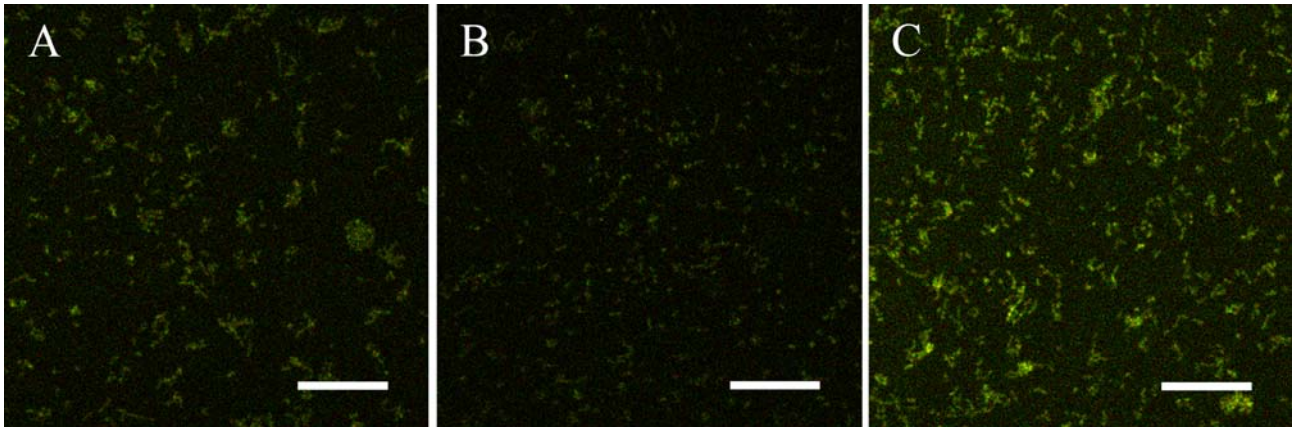

Figure S1. Cells of *S. mitis*, kept at pH 5.0, were stained with 20 µM **(A)**, 30 µM **(B)** or 50 µM **(C)** of C-SNARF-4 and imaged with a confocal microscope. Staining with 50 µM of C-SNARF-4 yields the best cell/background ratio. Bars = 20 µm.

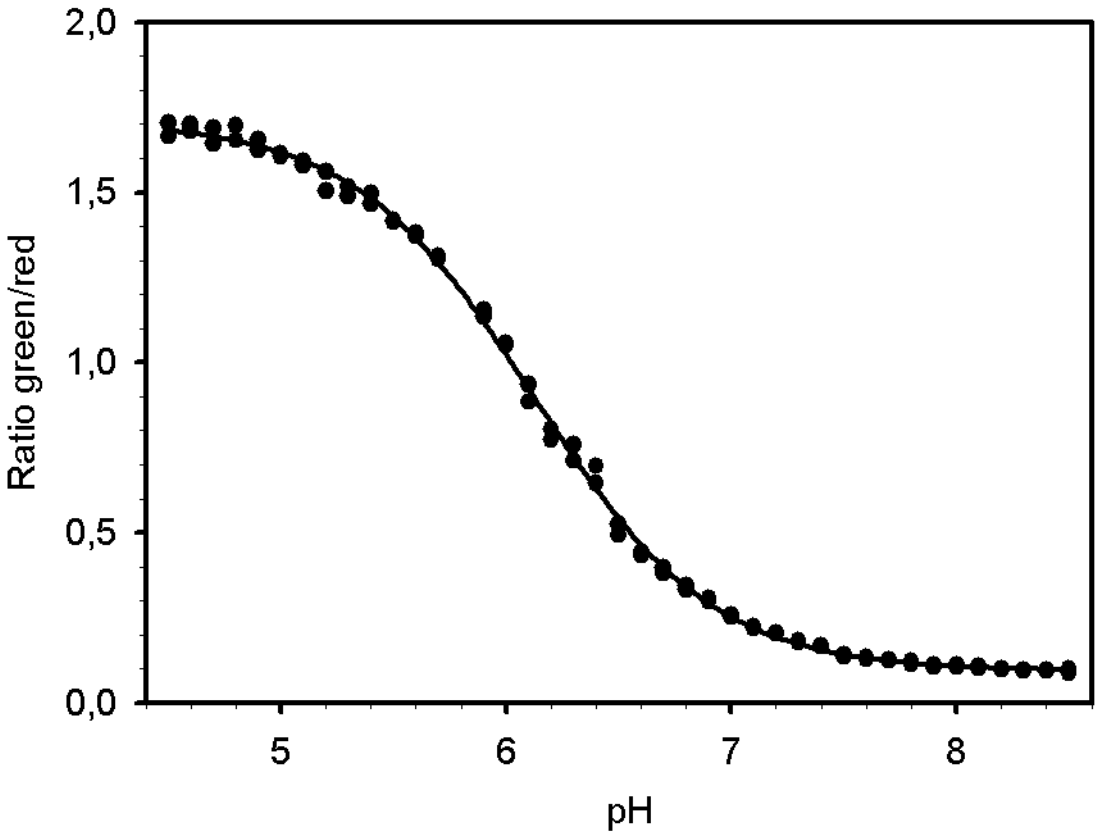

Figure S2. Calibration curve of C-SNARF-4. Fluorescence emission of C-SNARF-4, kept in HEPES buffer at different pH values (4.5 – 8.5), was monitored simultaneously within 576–608 nm (green) and 629–661 nm (red) intervals, and the fluorescence ratios (R) were plotted against pH and fitted to the function:

(1) $$pH = \ln\left(\frac{1{,}61}{R-0.0937} - 1\right) \cdot 0.397 + 6.12$$

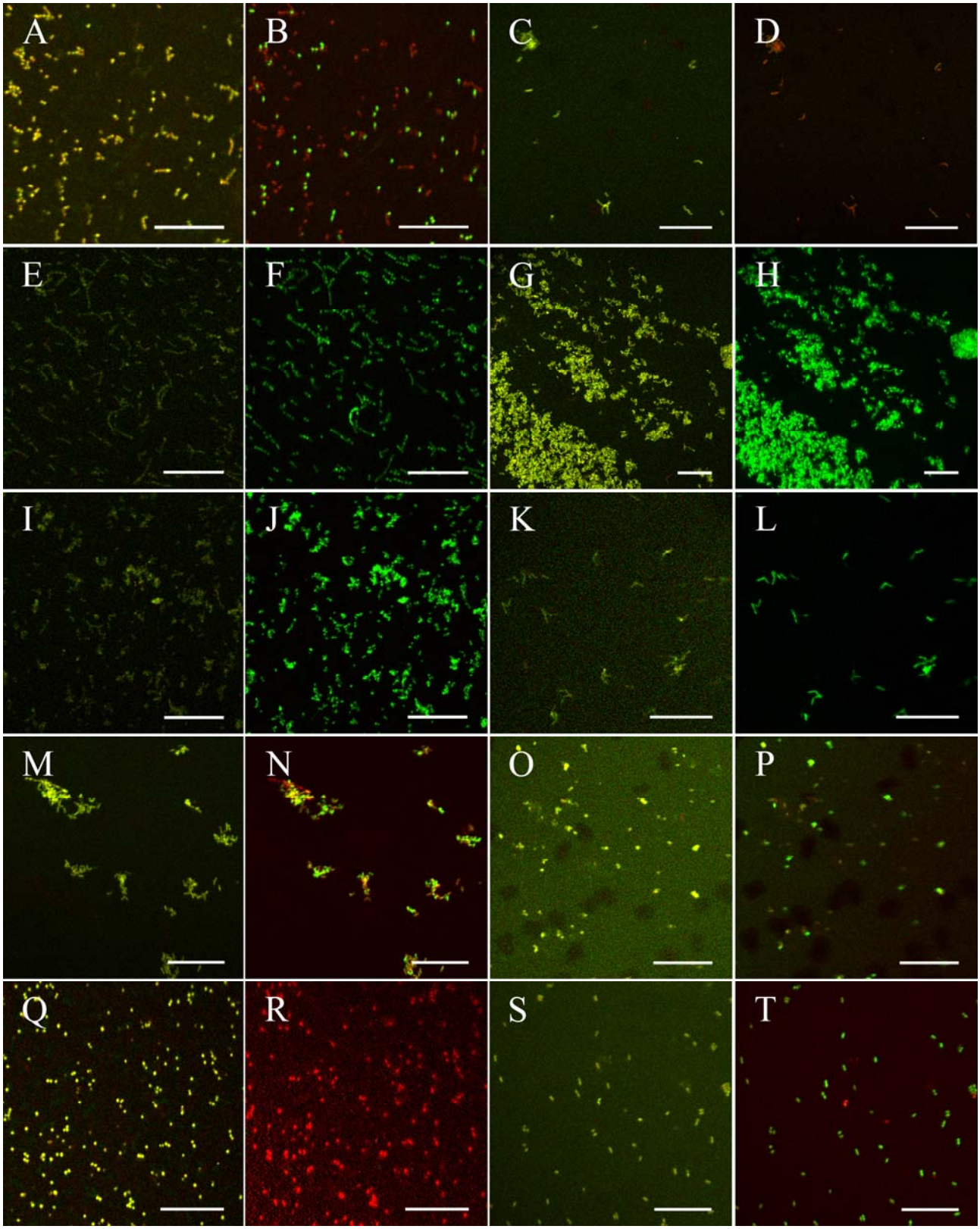
A
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Figure S3. Cells of different bacterial species were imaged with a confocal microscope after staining with C-SNARF-4 (A, C, E, G, I, K, M, O, Q, S) and counterstaining with BacLight (B, D, F, H, J, L, N, P, R, T). **A)** and **B)** *S. mitis*, kept at pH 5.0. **C)** and **D)** *S. mutans* , pH 4.0. **E)** and **F)** *S. gordonii*, pH 4.5. **G)** and **H)** *S. oralis*, pH 4.5. **I)** and **J)** *V. parvula*, pH 4.5. **K)** and **L)** *A. viscosus*, pH 4.5. **M)** and **N)** *A. naeslundii*, pH 4.5. **O)** and **P)** *N. subflava*, pH 4.5. **Q)** and **R)** *P. intermedia*, pH 4.0. **S)** and **T)** *E. faecalis*, pH 5.0. At low pH (4.0 – 5.5) all cells of all organisms were reliably stained by C-SNARF-4, as compared to the positive control stain. Both viable and membrane compromised cells (red in the BacLight image) were targeted by C-SNARF-4. Bars = 20 µm.

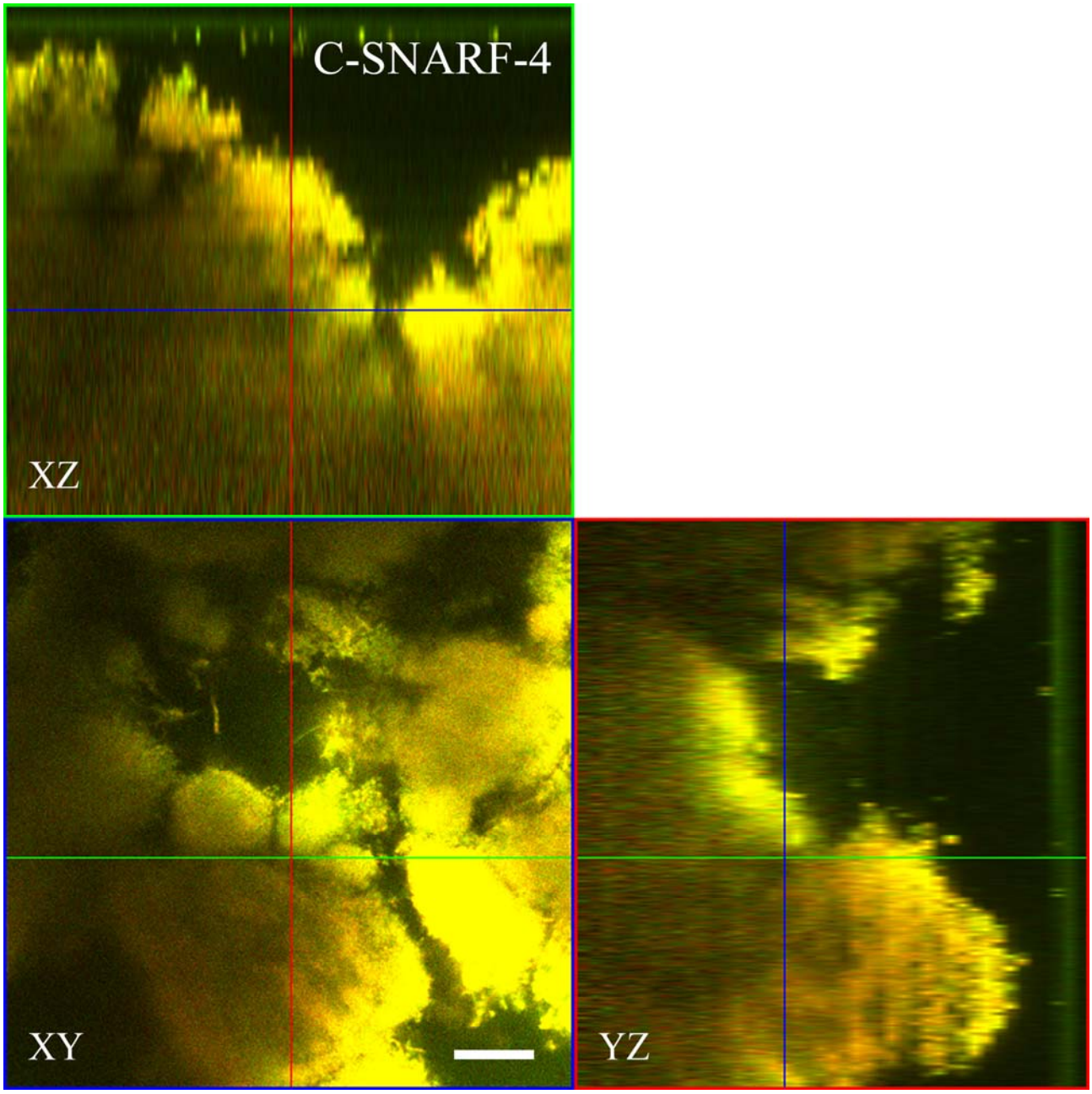

Figure S4. Orthogonal view of a dental biofilm incubated with sterile saliva containing 0.4 % (w V^{-1}) glucose and stained with C-SNARF-4. Bar = 20 µm. At a depth of 75 µm from the top of the biofilm, bacterial biovolume and extracellular matrix can be distinguished reliably (xy plane).

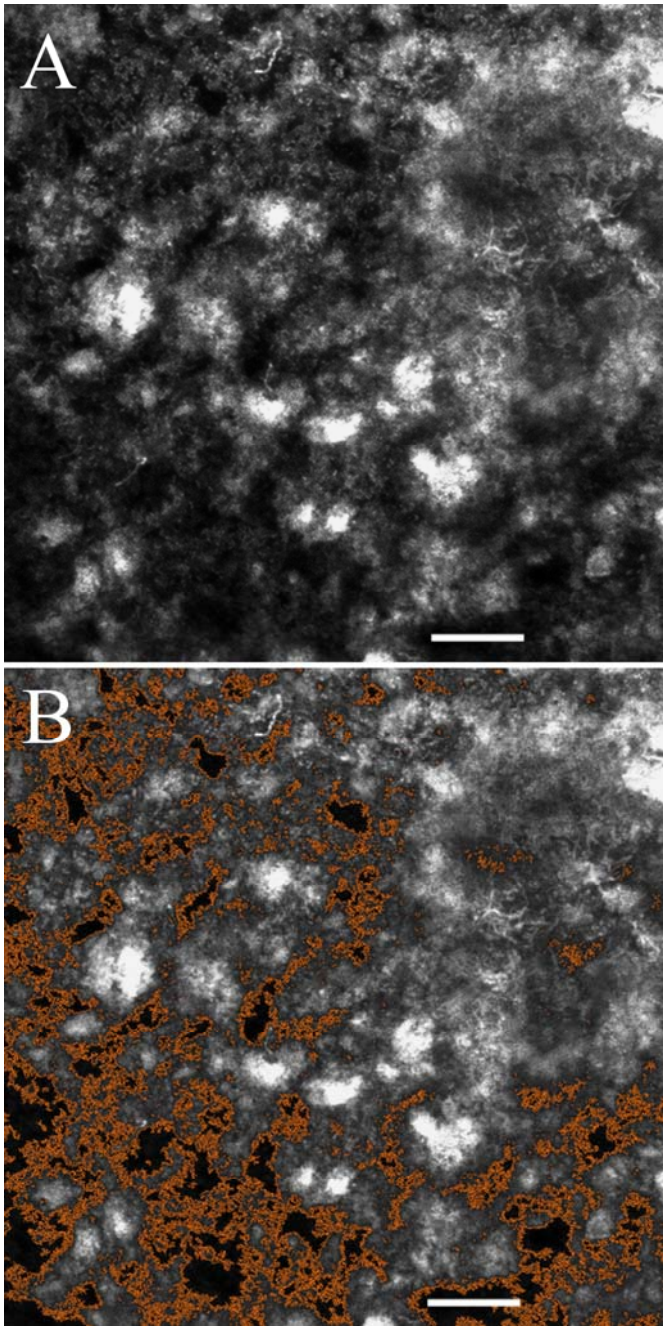

Figure S5. Biofilm images, acquired at the biofilm substratum interface, were subjected to digital image analysis. **A)** Biofilm image before segmentation. **B)** Segmentation in the software daime with individually chosen brightness thresholds allows detection of the entire bacterial biomass (contoured with orange lines). Thereafter it is removed and the extracellular pH in the acquired images can be determined. Bars = 20 µm.