ELECTRONIC SUPPORTING INFORMATION (ESI)

Identification of Nanoparticles and their Localization in Algal Biofilm by 3D-Imaging Secondary Ion Mass Spectrometry

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EXPERIMENTAL SECTION

Optical Light Microscopy

Algae cell growth and the development of algal biofilm were monitored with a binocular microscope (M205FA, Leica Microsystems, Wetzlar, Germany). Long working distance (20 mm) and about 1 µm resolution achieved upon acquisition in polarized light allowed for clear imaging of algae cell directly on the polymer-coated carrier in growth medium through the lid of Petri dish without disrupting the cell growth by opening the dish or removing the medium. After the chemical fixation, the samples of algal biofilm were imaged with circular differential interference contrast (C-DIC mode, Axio Scope.A1 microscope, Carl Zeiss Microscopy GmbH, Jena, Germany) providing the details of algal biofilm morphology with the lateral resolution of about 200 nm.

Scanning Electron Microscopy (SEM)

The efficiency of NP deposition was evaluated with Scanning Electron Microscopy (SEM, Merlin VP Compact, Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Elemental mapping of sample composition was done with an Energy Dispersive X-ray detector (EDX, XFlash FlatQUAD 5060 Detector, Bruker Nano GmbH, Berlin, Germany) allowing for elemental analysis with low current of the primary electron beam (50-200 pA to minimize the e-beam induced sample damages) due to the efficient collection of element-specific X-ray fluorescence in the solid angle of about 1 sr.

Dynamic Light Scattering (DLS)

DLS measurements of TiO2 NPs (1mg/mL) were performed with a DyanoPro Nanostar (Wyatt Technology Europe GmbH, Dernbach, Germany). The sample were analyzed in water and let equilibrate for 2 min prior to each measurement. Measurements were performed 10 times in triplicate. Afterwards, the average and deviation standard was calculated for the size and polydispersity index (PDI) (Figure S-4).

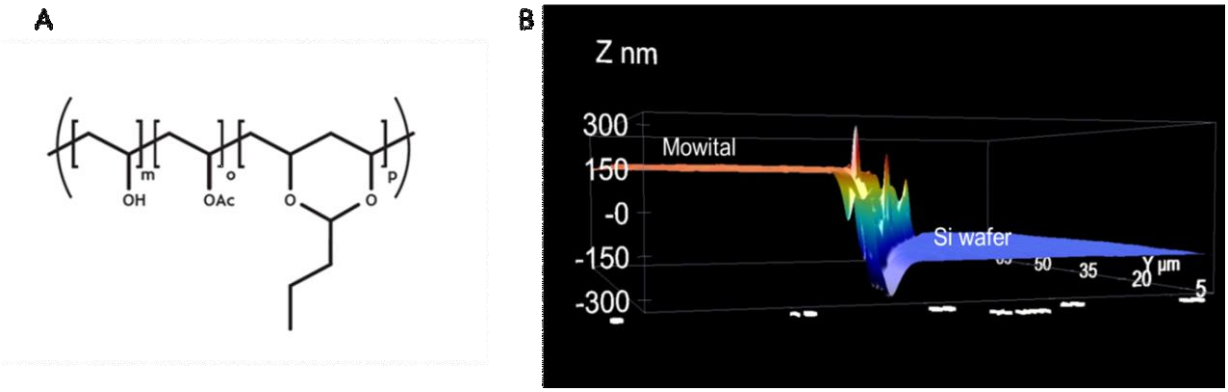


Figure S-1: (A) Mowital B family structure (Kuraray GmbH). (B) Thickness of Mowital layer (300 nm) measured *via* optical profilometer after a scratch was applied to reach the Si wafer support.

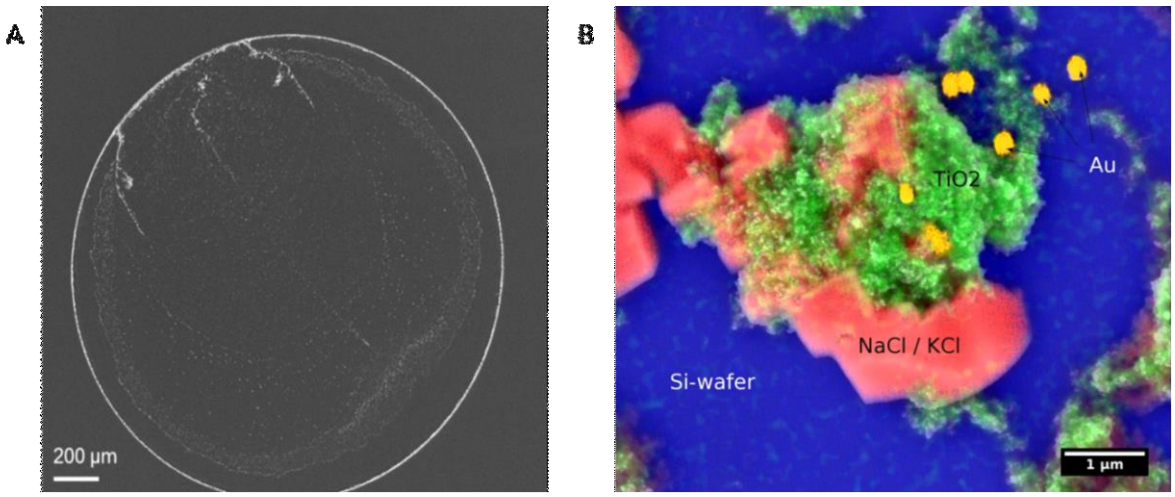


Figure S-2: Scanning electron microscopic images (SEM) of (A) a drop of NPs on the Mowital coated Si wafer showing a clear salt crystals "ring" and (B) aggregates of Au and TiO_2 NPs around salt crystals revealed by EDX analyses.

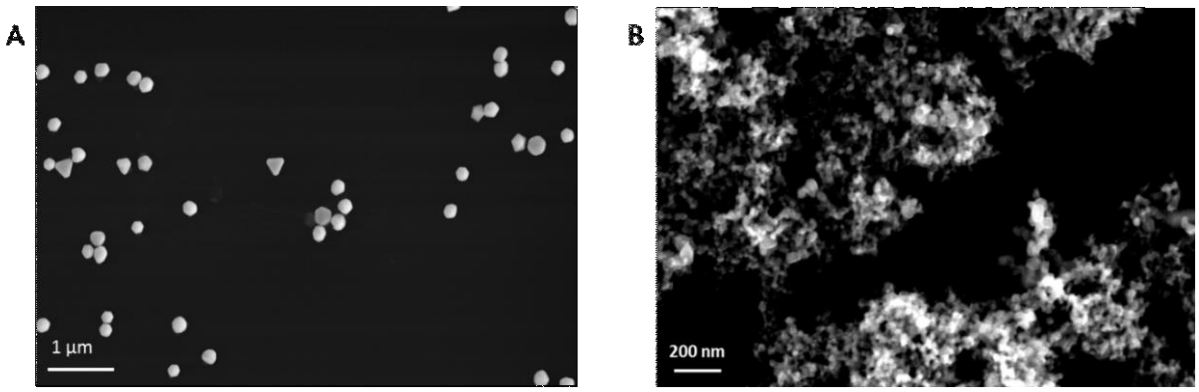
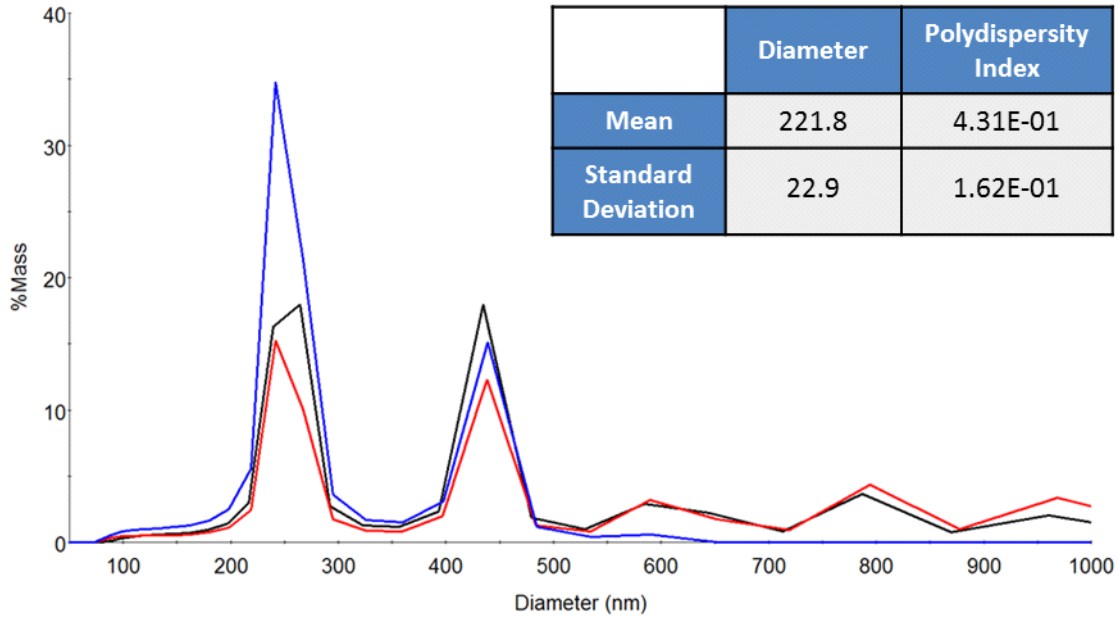


Figure S-3: Scanning Electron Microscopy (SEM) of cleaned Au NPs (A) and TiO_2 NPs (B) with Multilayered Filter Stack (MFS) method.



	Diameter	Polydispersity Index
Mean	221.8	4.31E-01
Standard Deviation	22.9	1.62E-01

Figure S-4: Dynamic Light Scattering (DLS) distribution of TiO_2 NPs suspended in water.

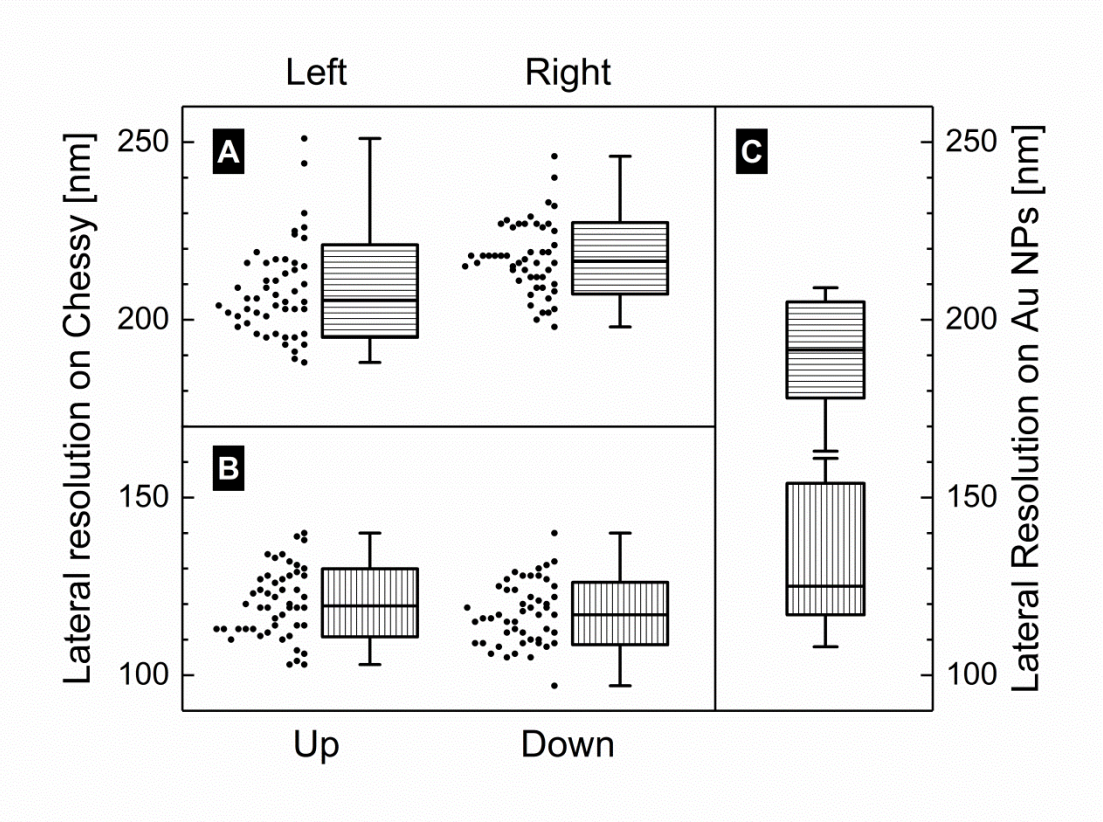


Figure S-5: Box plot of lateral resolution evaluated with linescans acquired on reference chessy sample (A-B) and on Au NPs (C). The boxes represent the 16-84% interquartile range, the horizontal line within a box shows median, the horizontal whiskers represent minimum and maximum values. Linescans in vertical direction (vertical box filling pattern) and in horizontal direction (horizontal box filling pattern) were evaluated showing considerably different values of lateral resolution. On the standard Chessy sample, the lateral resolution was evaluated on the left and right slopes of horizontal linescans (A) and up and down slopes of vertical linescans (B). The difference in lateral resolution values derived from left and right slope (A) was shown to be significant ($p<0.001$, $n=50$). This difference can be explained by the topography of the chessy structure (step of gold field 200 nm) imaged with the primary ion beam in 45° incidence geometry.

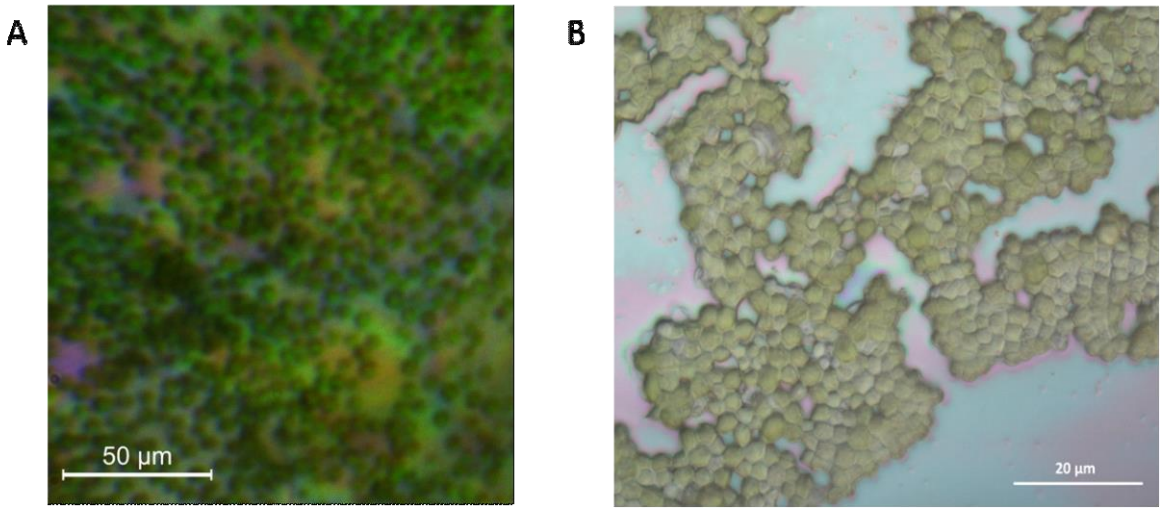
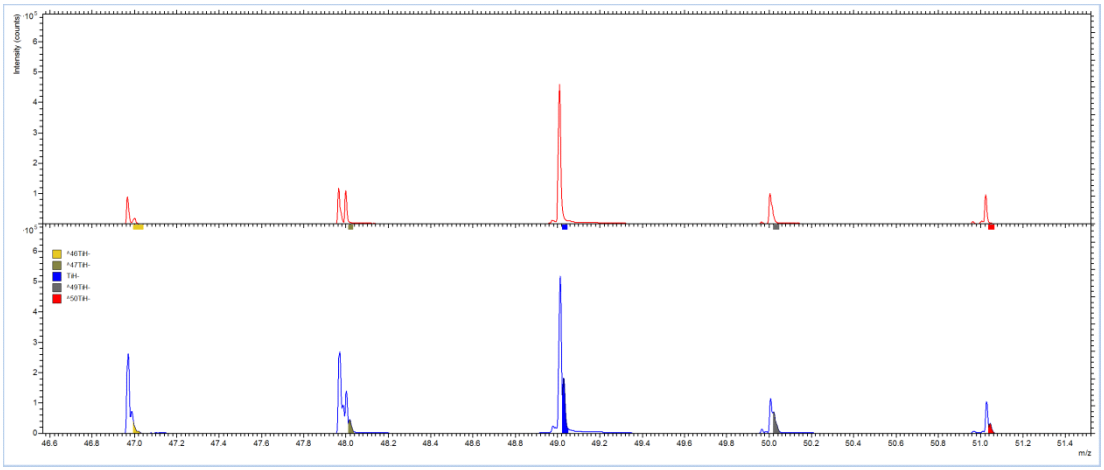
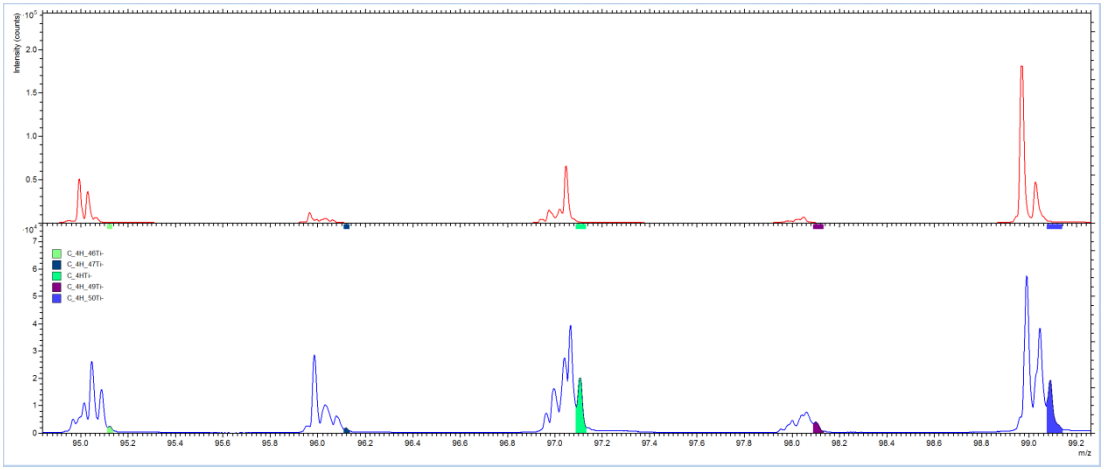


Figure S-6: (A) Binocular microscopy images of *C. vulgaris* before NPs incubation. (B) Optical images with DIC (differential interference contrast) of treated algae fixed after incubation of 1 µg/mL TiO_2 for 24 h.

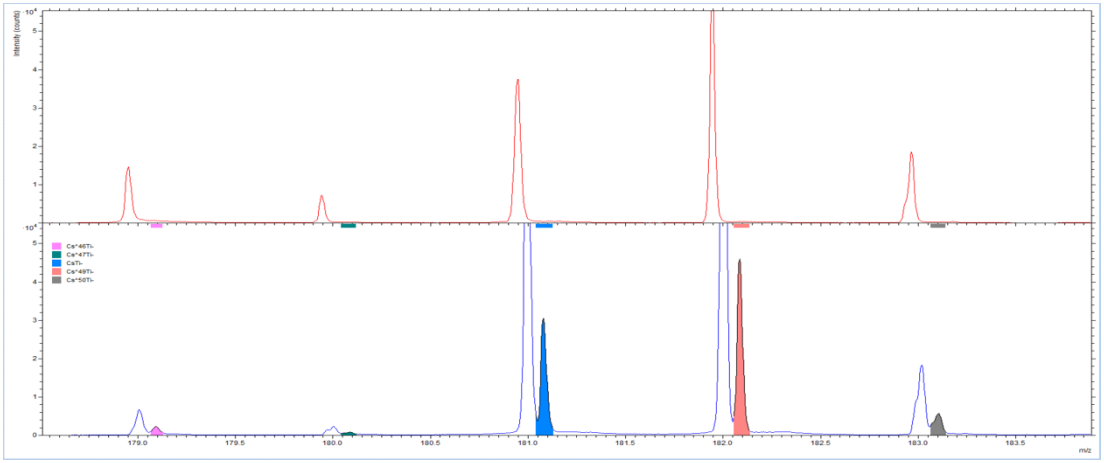
A


Intensity (counts)
*46TH-
*47TH-
TH-
*49TH-
*50TH-
m/z

B


Intensity (counts)
C_4H_46TI-
C_4H_47TI-
C_4HTI-
C_4H_49TI-
C_4H_50TI-
m/z

C


Intensity (counts)
Cs*46TI-
Cs*47TI-
CsTI-
Cs*49TI-
Cs*50TI-
m/z

D

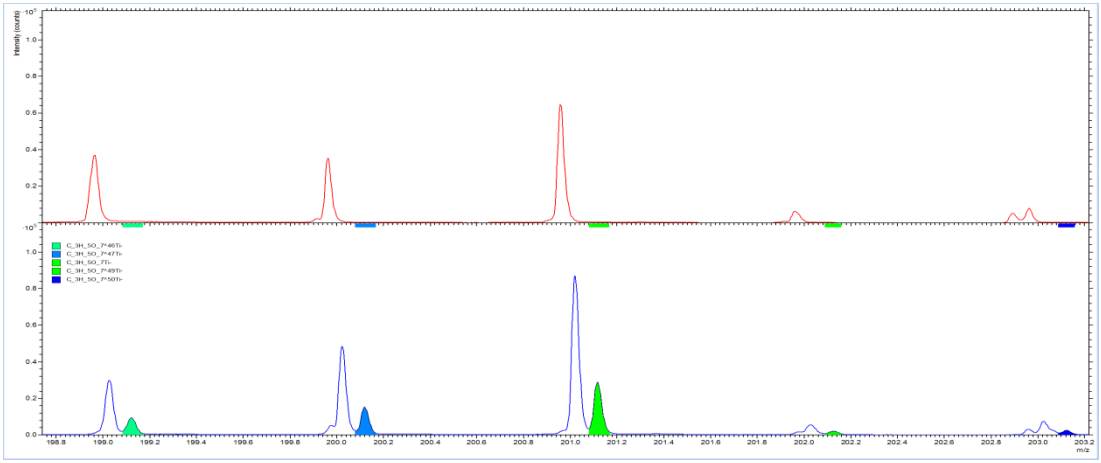


Figure S-7: Mass spectra of *C. vulgaris* biofilm exposed to TiO_2 NPs (bottom) and non-exposed biofilm (top) as an evidence for the association of mass peaks with TiO_2 NPs. A) Ti isotopic pattern for TiH^- ion. B) Ti isotopic patter for C_4HTi ion^-. C) Ti isotopic pattern for $CsTi^-$ ion. D) Ti isotopic pattern for $C_3H_5O_7Ti^-$.

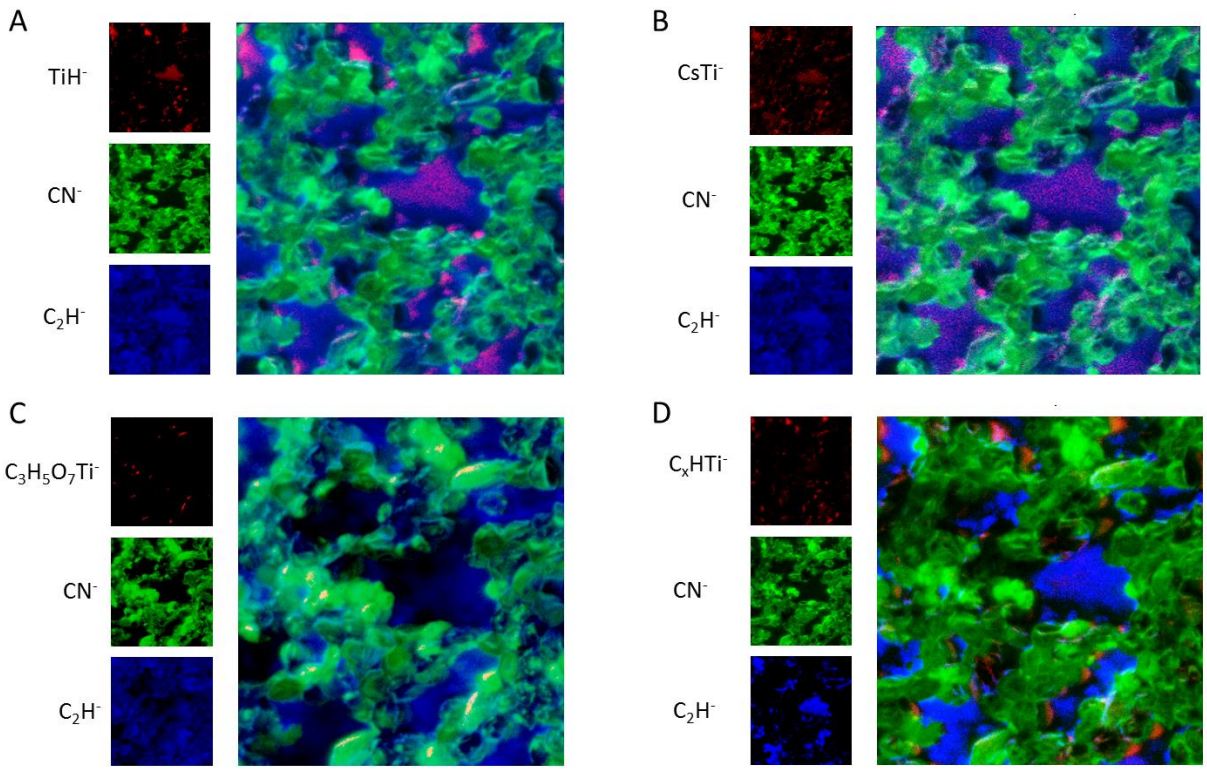


Figure S-8: RGB overlays displaying distribution of (A) TiH^- ion, (B) $CsTi^-$ ion, (C) $C_3H_5O_7Ti^-$ ion and (D) $CxHTi^-$ ion presented in Fig. 5 as 3D overlay. In addition, CN^- and C_2H^- are presented to represent cells and polymer respectively. All areas RGB overlays have an area of 52x55 μm^2.

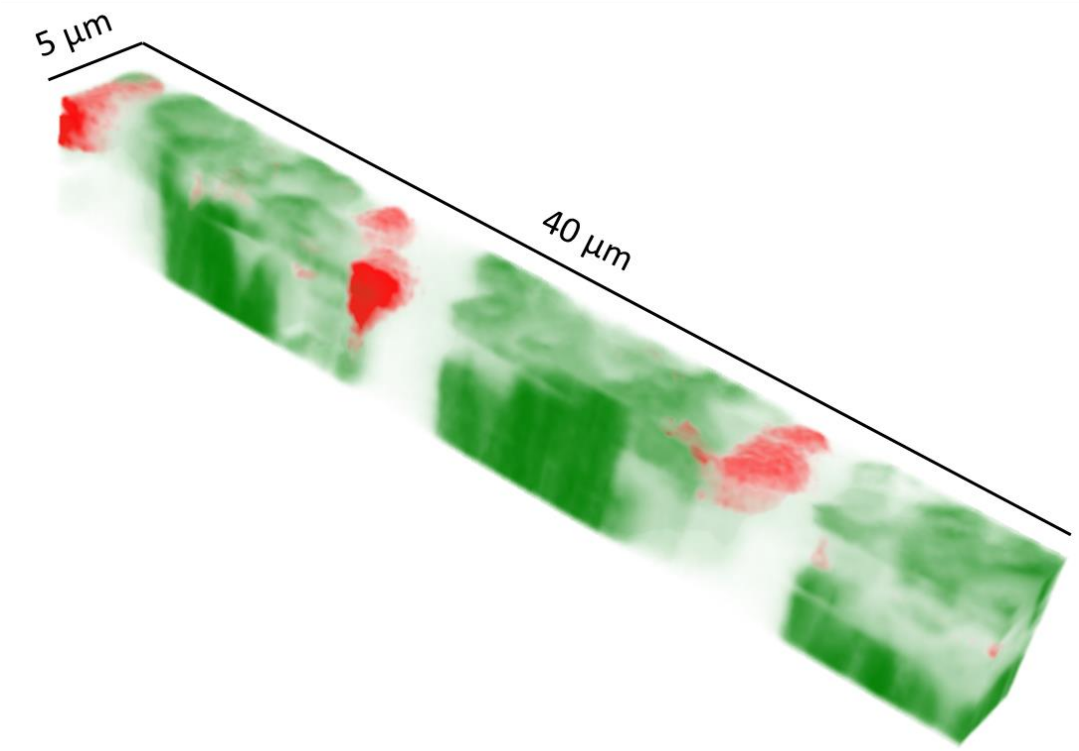


Figure S-9: XZ cross section of the 3D reconstruction showing the presence of C_xHTi^- secondary ions only at one side of the cells facing the x-direction where the primary ions are impinging the sample. Due to the height of the cells and the beam configuration (impinging the sample at 45 °) shadowing and displacement effect are clearly visible.

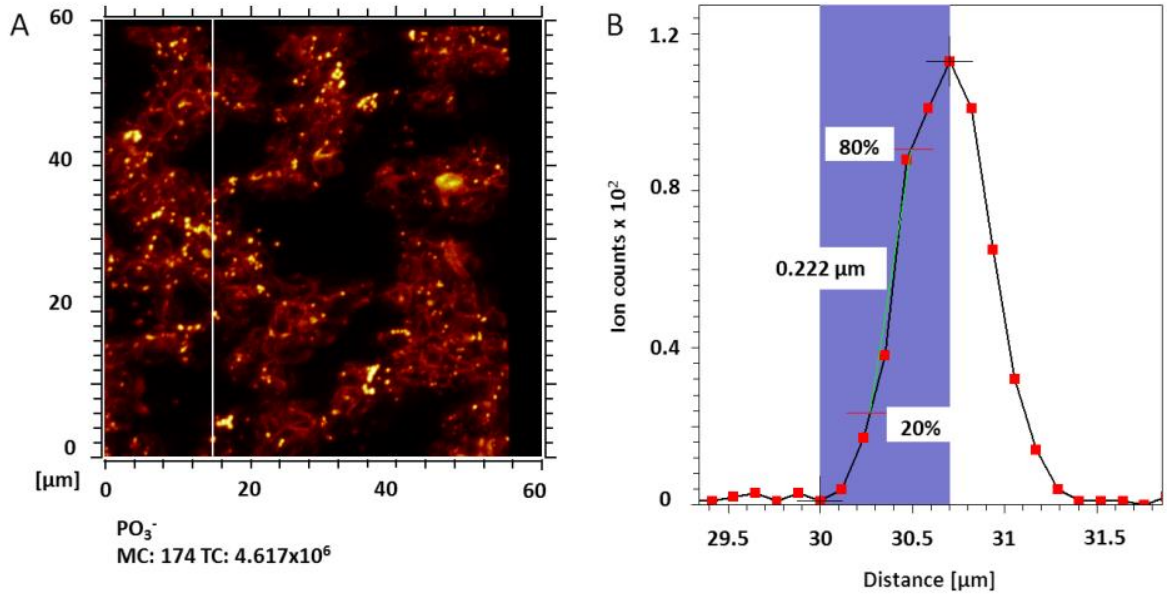


Figure S-10: ToF-SIMS image of phosphate phase in PO3- ion signal acquired on the algal biofilm in delayed extraction mode (A). Solid line indicates the position for the determination of the lateral resolution in vertical direction. (B) The linescan in vertical direction (Y-Profile) reveals the lateral resolution of 222 nm determined according to the 80%-20% slope. Red squares in (B) indicate individual pixels.