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Sequential SEM imaging of microbial calcite precipitation consolidation treatment

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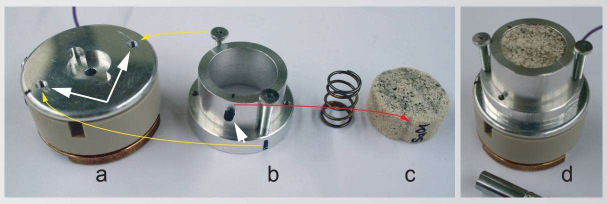
Abstract Cultural heritage built from limestone is prone to deterioration by chemical weathering, a natural process, that is enhanced by pollution. There are many historic monuments built from calcareous rocks suffering from deterioration and thus there have been a number of approaches over the last decades to consolidate these types of rocks and surfaces. Using natural biological processes by fostering the activity of calcite producing bacteria, referred to as biomineralisation, is one strategy that has been commercialised. The base of proving the effectiveness of any surface treatment is the observation of the surface at sequential stages before, after treatment, and after exposure to weathering. Due to the heterogeneity of natural materials and processes, our aim was to observe identical test areas at the micron scale throughout the observation period. To achieve this on a tungsten SEM, we employed a **beam deceleration accessory** that allowed low kV imaging on non-conductive surfaces at a sufficiently high image resolution, using a modified sample holder accommodating drill cores of 25mm diameter and up to 15mm height. The presented method is capable of producing time-sequenced images on the same test area on natural rock surface samples without manipulation for imaging purposes. This offers interesting perspectives for effective documentation of such processes in various fields.

Fig. 1. Modified stage bias single stub (12mm) accessory with a) additionally drilled and threaded holes, b) aluminium socket with top lid and added grub screw to accommodate 25mm diameter rocksamples of up to 15mm thickness. d) Fully assembled setup. With the aluminium socket mounted, the field lines emerge from the top lid for effective beam deceleration on the sample surface.



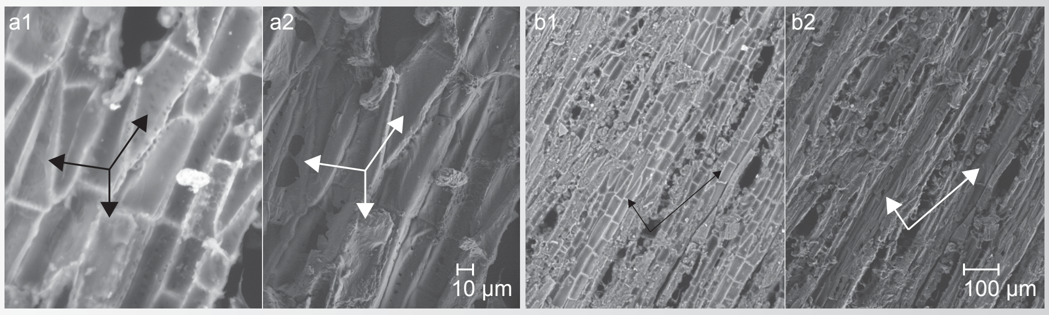


Fig. 2. SEM-BSE images of uncoated wood-cell structures of lindenbast. a1 and b1 were acquired in low vacuum (40Pa) at 20kV and 100pA. a2 and b2 were generated in high vacuum mode at 5kV, 70pA and 4000V beam deceleration (1kV landing energy). Series A focuses on the low penetration and improved surface detail on very thin organic structures such as cell membranes using beam deceleration (a2) on non-coated and non-conductive surfaces. When the structure rather than the surface of biological organisms is of interest, then the penetrating power of the higher kV is capable of delivering an X-ray-like image of the structure, in this case emphasising the cell walls (higher density of organic material) and strongly improving the recognition of the cell pattern (b1 vs b2).

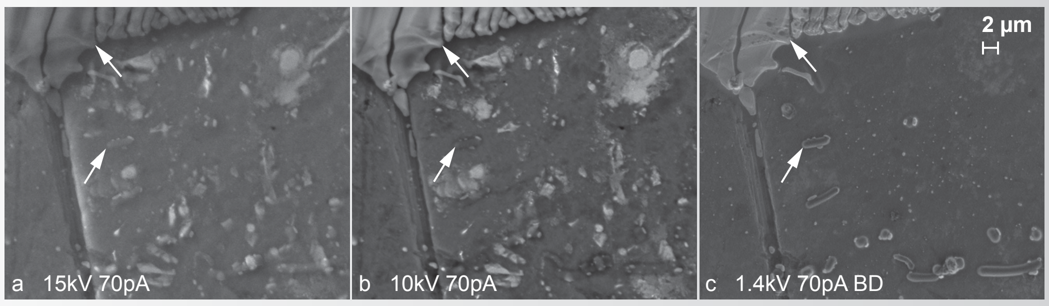


Fig. 3. SEM-BSE images on carbon coated samples of a dried drop of KBYO nutrient solution on a glass slide after interaction with an immersed rock sample. The image series compares surface sensitivity at a) 15kV, b) 10kV and at c) 5kV with 3600V beam deceleration (i.e. 1.4kV landing energy). Arrows point at surface details and at bacteria on the surface, which are best imaged and recognised using beam deceleration (c).

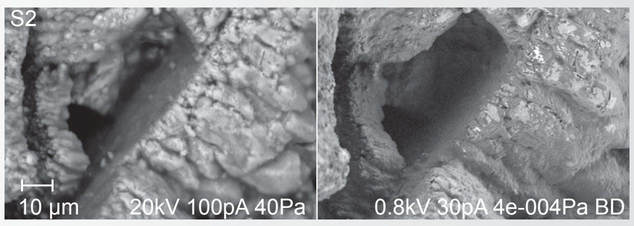


Fig. 4. Comparison of imaging at 40Pa chamber pressure (low vacuum) and 20kV (left) versus high vacuum mode and 3kV acceleration voltage with 2200V beam deceleration (0.8kV landing energy) on a non-conductive and charging surface (right). The biofilm formation of EPS encapsulating emerging bacteria upon KBYO nutrient application and conditioning, is only visible in the righthand image as a dark grey cover (organic) on the lighter grey calcite substrate (higher density rock).

Conclusion Beam deceleration is a viable option to study sequential states of surface conditions on non-conductive materials. It offers interesting applications beyond the presented case wherever coating for conductivity is no option: e.g. due to subsequent treatments or additional techniques, on non-conductive open structures, when searching traces of organic substances on inorganic surfaces.

While microbial calcite formation has been documented in several publications, the presented approach is new since identical test fields at the micron scale were imaged at different stages of the process, weeks and months apart, including outdoor weathering, to tell the true story.

The consolidating effect of a treatment with KBYO nutrient solution on Barrois-Oolith from Savonnières-en-Perthois was documented with sequential images acquired with the beam deceleration technique. To achieve a sustainable effect with this treatment north of the Alps, the procedure may require adjustments to the one practised in southern Europe in order to achieve a balance between production and erosion of microbial calcite. Documentation with the beam deceleration technique to accompany the treatment will help to better understand and influence the process for a successful outcome.

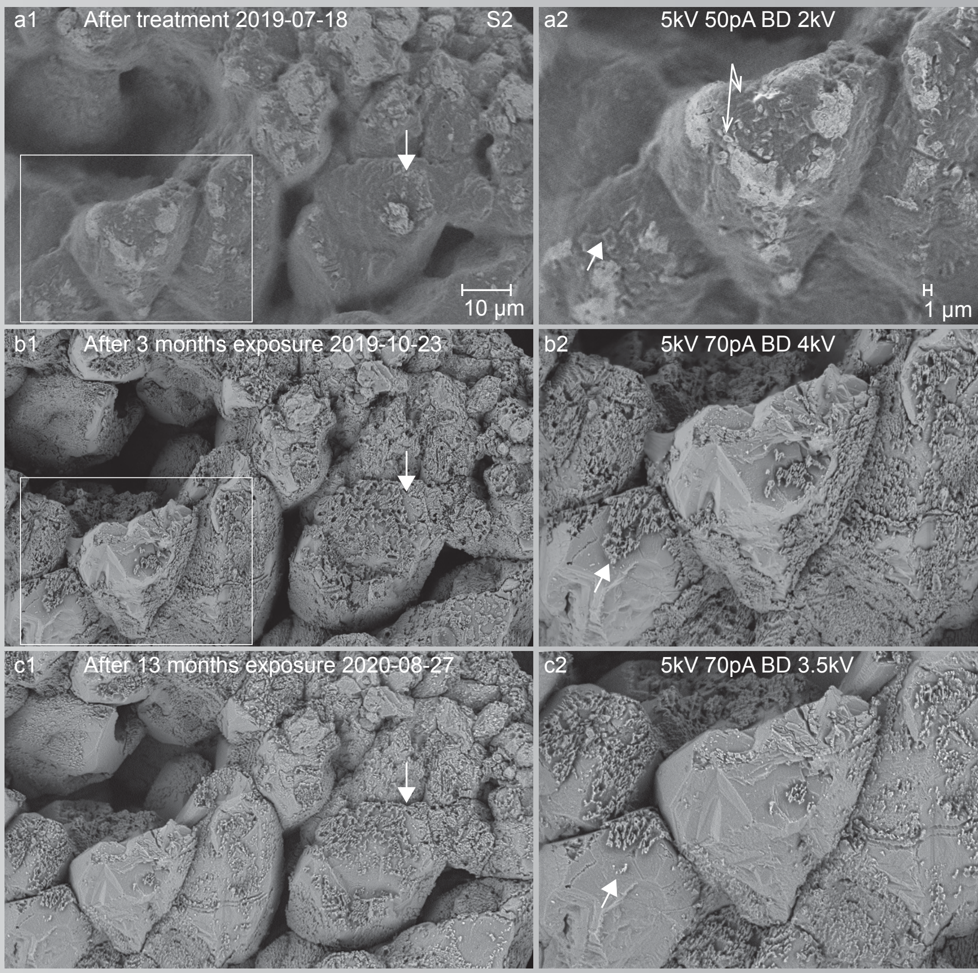


Fig. 5. SEM-BSE image sequence produced with the beam deceleration accessory on a non-conductive rock sample surface (Savonnières) at the identical locality at 3 different stages (months apart) of the microbial calcite formation and weathering process. a) upon completion of the KBYO-treatment and conditioning over 1 month; b) 3 months later after outdoor exposure (rooftop, 45° inclination, SSE aspect, 47.5° latitude); c) 13 months later after additional outdoor weathering. Simple arrows in a2) point at mobilisation of bacteria (oval body) from the inside of the rock sample to the surface upon application of the KBYO nutrient (dark, organic layer represents biofilm of EPS) within the 30 days of conditioning. The open arrow in b1) points at a cavity within the microbial crust, following the death of the organism. Filled arrows (sequence a-c) point at the development (b) and beginning, renewed erosion of the microbial calcite crust (c) within a year of exposure to weathering in this climatic environment (450m a.s.l., yearly avg. T 9.2°C, min. avg. January 0°, max. avg. July 18.2°C; annual rainfall 1049mm). Images b) and c) are multifocal compilations of 3-4 focal levels (greater depth of focus).

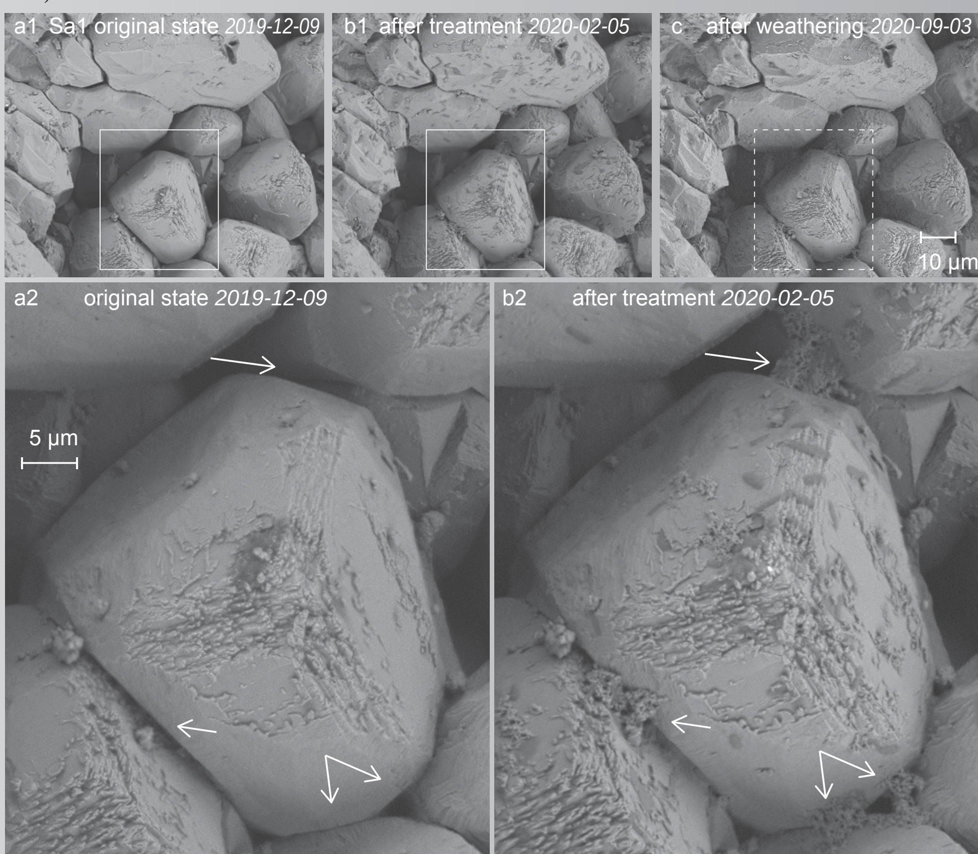


Fig. 6. SEM-BSE image sequence acquired with beam deceleration on a second and later series of Savonnière samples. These samples were imaged with identical conditions (5kV 70pA BD4000V), including the pre-treatment condition: a) original state before the treatment, b) ~1 week after the 30-day KBYO-treatment with conditioning, c) 7 months later with ~3 months of outdoor exposure to weathering as in (Fig. 5). Darker grey elongate dots in b) are interpreted as activated bacteria, not visible in a) and neither c). Arrows in a2 versus b2 point at microbial calcite formation, bridging the interstices between neighbouring calcite grains and as such initiating a consolidating effect. These bridges are still recognisable in c1 upon weathering, though already slightly reduced. Compared to Fig. 5, conditions obviously did not lead to a similar microbial crust formation.

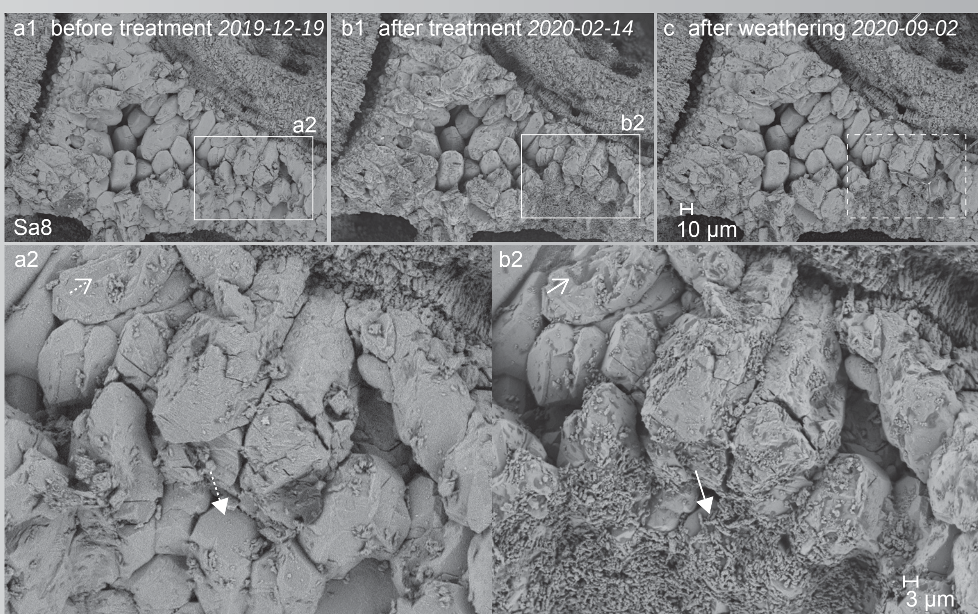


Fig. 7. Another Savonnières sample of the later treatment series imaged with beam deceleration before (a), after KBYO-treatment (b) and after a short exposure period (c) of 3 months (June-August 2020) at the same site. Simple arrows in b2 point at clusters of bacteria mobilised to the rock surface upon the application of the KBYO nutrient solution. Full arrows point at fresh deposits of microbial calcite, which are – shortly after completion of the treatment – at an initial state with heterogenous distribution (b2). Comparing b1) with c) suggests that microbial calcite crust formation was incomplete prior to exposure and thus susceptible to rapid erosion.