Novel Techniques for a Quantitative Analysis of Processes in Liquids using Electron Microscopy

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Traditional electron microscopy provides important information on the topography, microstructure and composition of materials on the nano- or even the atomic scale but it is limited since it only allows to study these materials post mortem when dried or (in the case of cryo- electron microscopy) in a frozen state. The main reason for this limitation is the high vacuum required to avoid electron scattering severely affecting the electron detection and hence the spatial resolution. Clearly, these constraints strongly pose restrictions on the materials that can be studied and the information that can be obtained from these studies.

Our research makes use and enhances recent developments both in transmission electron microscopy and in scanning electron microscopy to directly image material systems in fluid environments using thin silicon nitride membranes separating the vacuum from the investigated specimens. These techniques provide powerful tools to image dynamic processes in fluids with high spatial resolution giving access e.g. to mineral formation and dissolution under controlled conditions in the microscope. We are using a novel atmospheric scanning electron microscope (ASEM – Jeol Clairscope) and a fluid cell transmission electron microscopy (TEM – Protochips Poseidon 200) holder, which gives access to different length scales and aspects of the dynamic processes involved. Our research concentrates on so called bio-inspired materials that means materials similar to those found in organisms such as bone, coral skeletons or sea shells.

Atmospheric Scanning Electron Microscopy (ASEM)

ASEM (Jeol Clairscope) is a powerful tool for observing materials and processes in fluids using backscattered electron detection. The principal setup is shown in *Figure 1*. The microscope consists of an inverted SEM on the vacuum side separated from the environment via petri dish with an integrated silicon chip which contains a thin silicon nitride membrane of 500 µm x 500 µm area. An optical microscope on the top-side allows for low resolution imaging. The fluid sample is inserted into the dish, an electron beam scans the membrane area and a back scattered electron (BSE) detector measures electrons scattered into the back half plane. This approach allows to study processes occurring in a depth of approx. 3 µm inside the liquid as we have previously shown for calcium carbonate precipitation in the presence of organic additives [1]. This limitation is due to the significant dynamic electron scattering in the fluid (e.g. water). A drawback of this method is that it gives poor control over the fluid composition, particularly if mixing and precipitation processes are studied. Therefore, we have combined a specially designed microfluidic device (MFD) with the ASEM petri dish allowing for a controlled flow of fluids and the investigation of reactions e.g. at laminar flow interfaces of alkaline earth metal ion solutions such as Ca^{2+}, Sr^{2+} and Ba^{2+} which are found to play important roles in many material systems such as shell and exoskeleton forming carbonates or gypsum ($CaSO_4$). The MFD consists of two inlets and one outlet with a cross-section of the main channel 600 µm x 100 µm, hence the main channel covered the membrane area and the laminar flow front could be detected using BSE as shown in *Figure 2*.

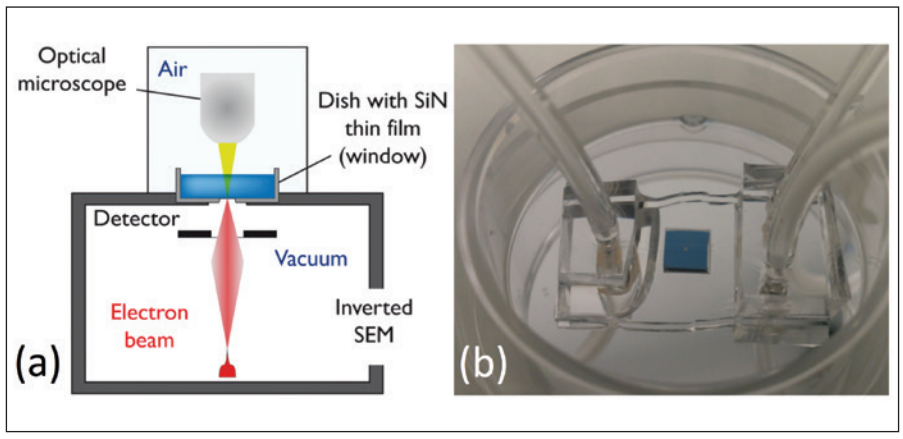

Figure 1. (a) Schematic of the atmospheric SEM, (b) integrated microfluidic channel in Petri dish used for fluid analyses in ASEM.

In these experiment *(Figures 2a and 2b)* we subsequently pumped (using a syringe pump) two different concentrations of barium chloride salt solutions (25 mM and 100 mM) and deionised water at a flow rate of 3000 µl/hr through the channels and studied the interface between the salt solutions and the water. The boundary between the water and the salt solutions is clearly visible in these images and also the impact of the different concentrations is apparent in the observed contrast. To study the precipitation of $BaCO_3$ we combined a 100 mM sodium carbonate (top channel) and a barium chloride solution of similar concentration *(Figure 2c)*. As a result we could identify the details of the $BaCO_3$ formation as a function of time.

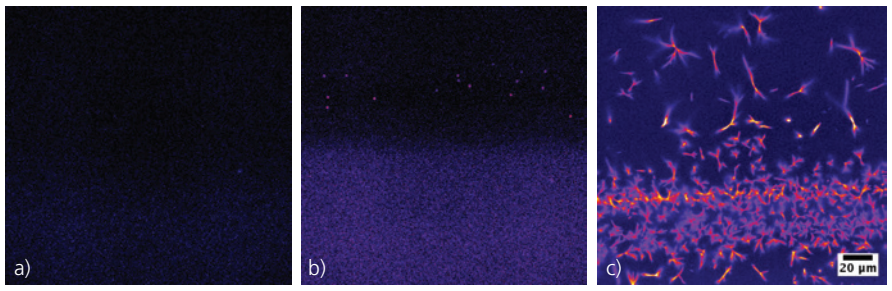

Figure 2. Fluid cell/ASEM studies of back-scattered electron intensity dependent on concentration. (a) top: water, bottom: 25 mM Ba^{2+} solution, (b) top: water, bottom: 100 mM Ba^{2+} solution, (c) top: 10 mM CO32- solution, bottom: 100 mM Ba^{2+} solution leading to precipitation of $BaCO_3$.

In a further experiment we compared different earth alkaline metal solutions and the BSE contrast in comparison to water and performed line scans across the laminar boundary *(Figure 3)*. The different atomic Z-numbers of Ca, Sr and Br are clearly reflected in the relative intensities between the salt solution and the water in line with the expected Z^2-dependence from Rutherford scattering. These are very encouraging results giving rise to a completely new quantitative approach of ion concentration measurement using electrons in liquids.

These results show that ASEM is a powerful tool for a quantitative study of ionic solution concentration when combined with MFD. There is currently no other technique available that allows for in situ concentration determination with such a spatial resolution.

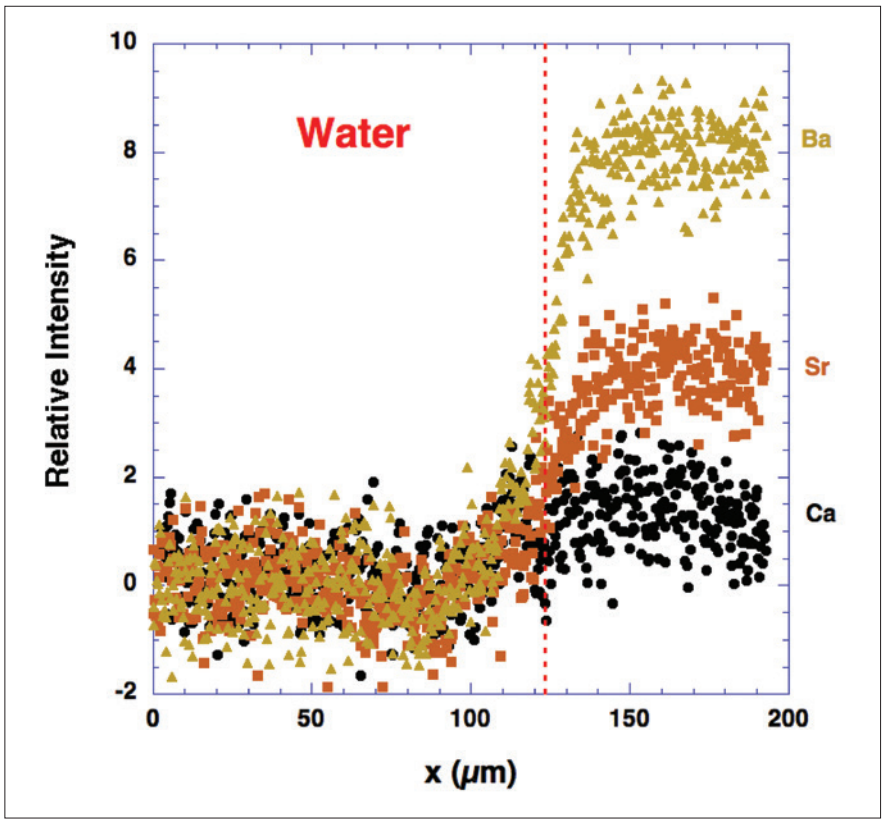

Figure 3. BSE contrast measured using line scans across the water/salt solution boundary for different earth alkaline metal ion solutions (Ba^{2+}, Sr^{2+} and Ca^{2+}) showing the impact of the atomic number Z on the contrast between water and solution.

Fluid Cell Scanning Transmission Electron Microscopy

Fluid cell scanning transmission electron microscopy experiments were performed using a three-port Poseidon 200 liquid flow cell holder (Protochips Inc) in an aberration corrected Jeol 2200 FS. The microscope was operated in STEM mode with a beam current of 225 pA using a high-angle annular dark-field (HAADF) detector. The geometry of scanning electron beam, fluid cell and HAADF detector is shown in *Figure 4*.

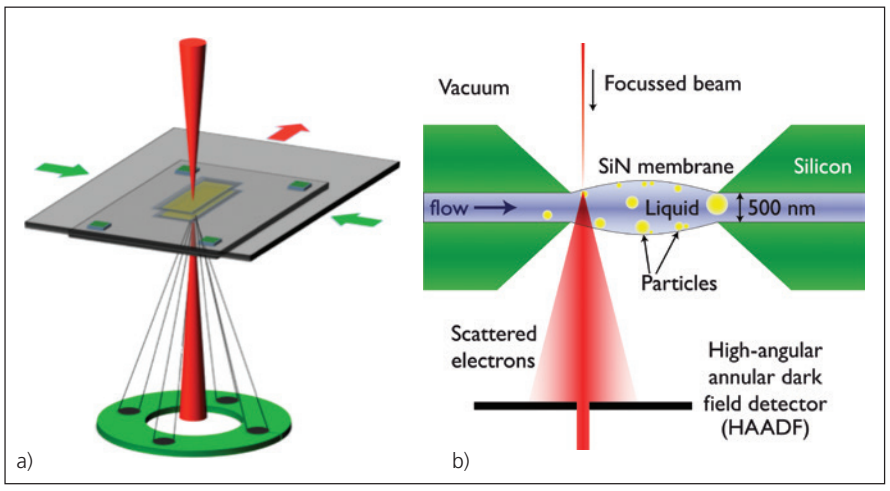

Figure 4. Schematic of the fluid-cell scanning transmission electron microscopy geometry. (a) The scanned electron beam passes through two SiN membranes which separated the fluid from the microscope vacuum. Two inlet ports and one outlet port allow for the independent introduction of fluids and the time resolved observation of dynamic processes. (b) A cross-sectional schematic of the arrangement of the SiN membranes.

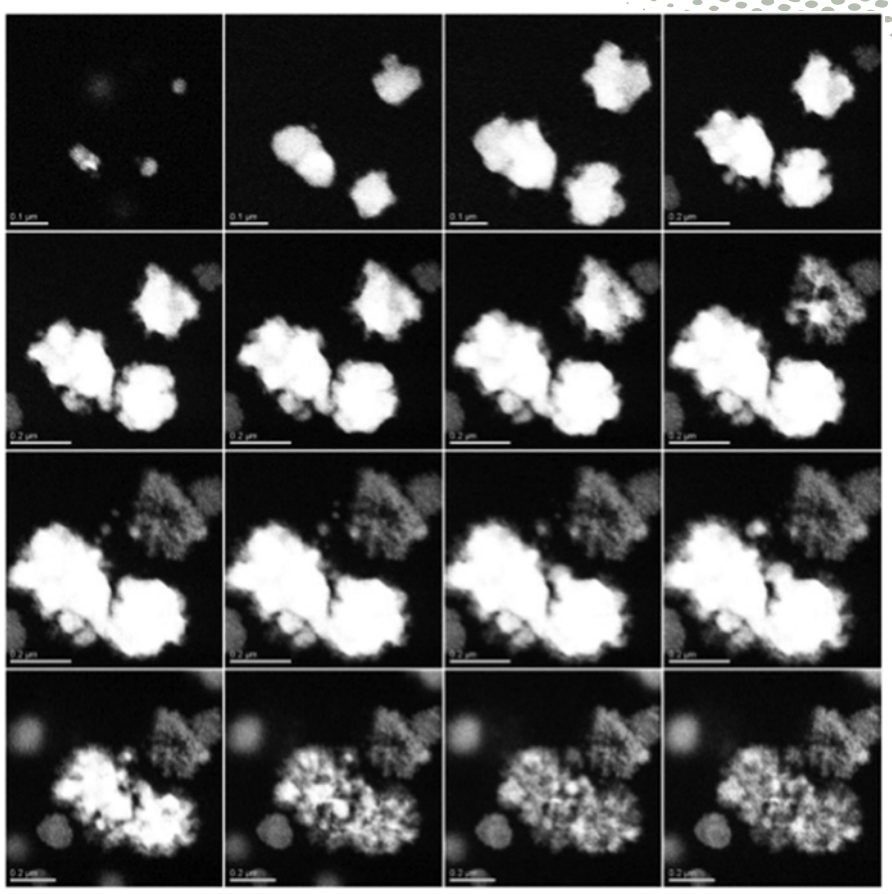

Figure 5: Investigation of $CaCO_3$ precipitation in the presence of the nacre protein AP7 by fluid cell STEM showing the time dependence of protein agglomeration, precipitation of calcite and subsequent dissolution under reduced electron beam irradiation. The time frame from the first to the last image of the sequence is 1200 s.

In collaboration with Professor John S. Evans (New York University) we have performed studies of calcium carbonate formation in the presence of the protein AP7 extracted from the nacre of Haliotis Rufensis known to play an active role in the nucleation and crystal growth. From our observations we conclude that AP7 has a significant impact on the precipitation process by acting as templates for Ca ion accumulation and subsequent nucleation and growth of the crystals. The calcium and carbonate containing solutions were prepared from 20mM CaCl2 dihydrate and 20mM $NaHCO_3$, respectively. A concentration of 0.1 mg/ml AP7 was combined with the carbonate solution. For the mixing experiment 500 nm spacers and flow rates between 90 and 180 µl/hr were used. Since STEM imaging is particularly sensitive to atomic mass we could directly observe the densification of the mineral/organic composite due to the formation of Ca rich domains in most likely amorphous particles and subsequently the growth of calcite crystals induced by the electron beam irradiation.

These results show exiting perspectives for high-resolution scanning transmission electron microscopy to be used to monitor precipitation dynamics on the nanometer level.

References

[1] Andreas Verch, Ian Morrison, Renee van de Locht, Roland Kröger: In situ electron microscopy studies of calcium carbonate precipitation from aqueous solution with and without organic additives. Journal of Structural Biology 08/2013; 183: 270-277.

[2] Iva Perovic, Andreas Verch, Eric P. Chang, Ashit Rao, Helmut Cölfen, Roland Kröger, and John Spencer Evans: An oligomeric C-RING nacre protein influences pre-nucleation events and organises mineral nanoparticles, submitted (2014).