doi:10.1017/S1431927618014125
Microsc. Microanal. 24 (Suppl 2), 2018

Bismuth-Oxo-Clusters for Soft-Tissue Staining

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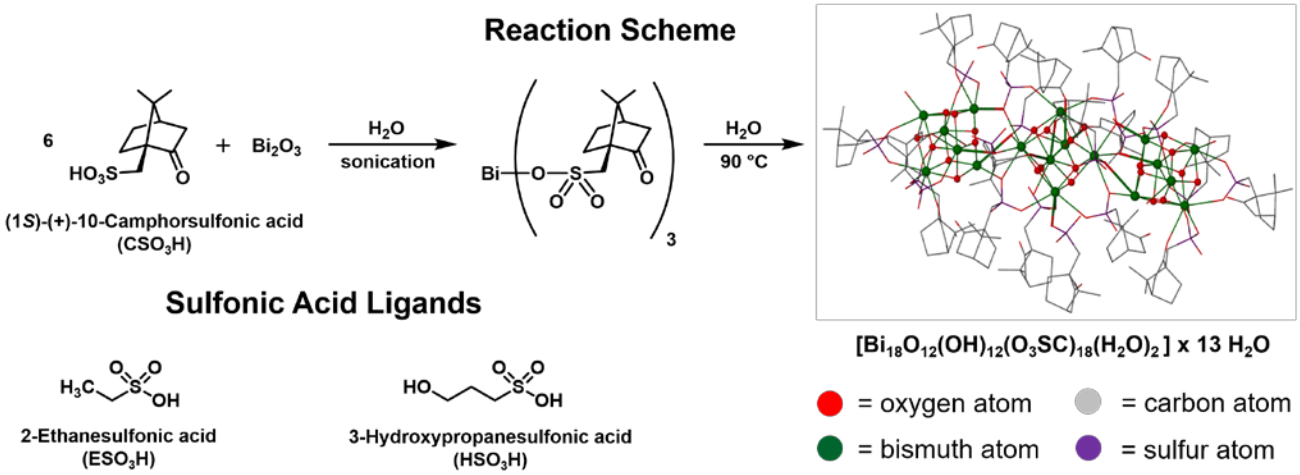
Currently, X-ray absorption-based micro-computed tomography (MicroCT) is successfully used in material science investigations as well as in non-destructive testing enabling 3D imaging of these materials with micrometer resolution [1]. However, the application of MicroCT for medical and biological sample screening (e.g. *ex vivo* screening of biopsies) is limited by the missing contrast of soft tissue [2-4], which is important to visualize microscopic structures [5]. Novel specific and functional contrast agents (stains) and staining protocols are essential to achieve the 3D visualization of specific cellular structures (e.g. nucleus or cytoskeleton).
To overcome these limitations, we developed a novel staining method in order to enhance the contrast in soft-tissue X-ray imaging. Furthermore, the staining with bismuth-oxo-clusters is compatible with conventional 2D histological techniques highlighting the potential for an application in modern histopathology.

The bismuth-oxo-clusters were synthesized following the procedure shown in the reaction scheme below [6]. The obtained bismuth-sulfonates were completely dissolved in dist. water at 90 °C and filtered hot. The filtrate was cooled to room temperature and kept for a minimum of 6 hours to allow cluster formation. Fixated mouse liver samples were stained for 24 hours with the prepared bismuth-oxo-clusters. The stained tissue samples were investigated with the ZEISS Xradia Versa 500 (commercially available machine) above ethanol vapor. Furthermore, the staining protocol is compatible with conventional histological methods not degrading morphological structures of the tissue sample.



Scheme 1: Reaction scheme of the bismuth-oxo-cluster synthesis with (1*S*)-(+)-10-Camphorsulfonic acid (CSO_3H) [6]. Two further sulfonic acid ligands are shown, which were used for synthesis. On the right the single X-ray crystal structure of the bismuth-oxo-cluster derived from CSO_3H is displayed [6].

Currently available ex vivo staining agents for X-ray microscopic imaging techniques are often toxic or inhibit subsequent histological treatment. The bismuth-oxo-clusters as novel contrast agents overcome these challenges and allow for a fast and homogeneous penetration of the stain within 24 hours through the soft tissue. This study demonstrates the effect of contrast enhancement through localized concentration of the element bismuth (Z = 83) within one molecule. The morphological structures within the soft tissue were preserved, despite applying the bismuth-oxo-clusters. This was validated on a cellular level (cytoplasm and cell nucleus) through histological investigations using subsequent staining procedures (H&E) [7].

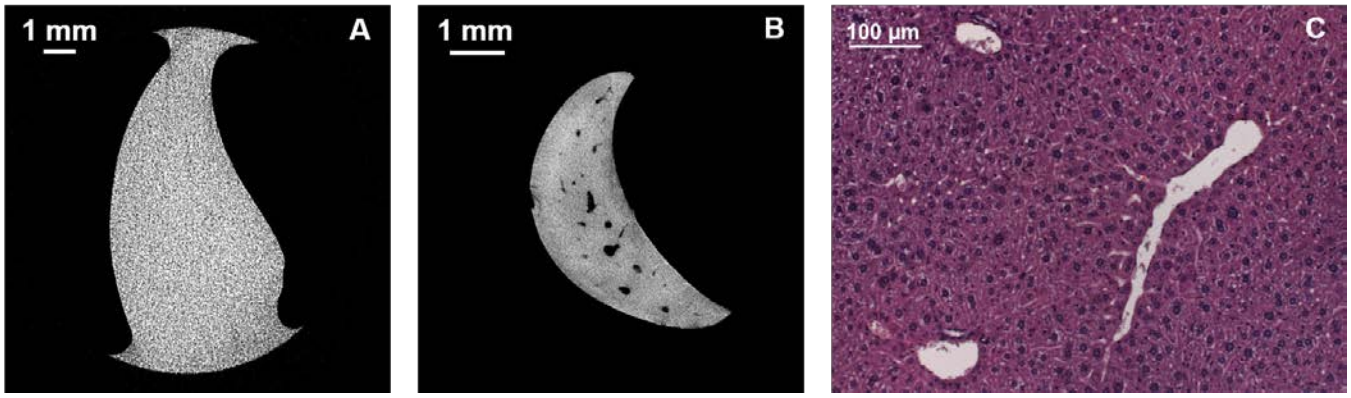


Fig. 1: MicroCT slices in comparison with histological microscopic slides. Both microCT data sets were acquired with the Xradia Versa 500 microCT using identical acquisition parameters. The voxel size is *ca.* 13.8µm. (**A**) CT slice of an unstained mouse liver lobule, which has been fixated in a 1% formaldehyde solution. Structural features are missing, due to the limited contrast of soft tissue. (**B**) The bottom right displays a CT slice of the fixated mouse liver lobule after staining. Here, the bismuth-oxo-cluster of ESO_3H was used for staining [6]. Contrast enhancement was observed, and excellent delineation of the vasculature was achieved. (**C**) The stained mouse liver was further investigated by conventional histological methods. Here, the standard H&E staining protocol was applied. Despite the staining for microCT, morphological structures were preserved, and no disturbance of classical histological stains was observed.

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[7] The authors acknowledge funding from the DFG Cluster of excellence Munich Center of Advanced Photonics. Furthermore, this project received funding from the European's Union Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie Grant agreement H202-MSCA-IF-2015-703745-CONSALT.

https://doi.org/10.1017/S1431927618014125 Published online by Cambridge University Press