Supplementary Materials for

Correlated Cryo-SEM and CryoNanoSIMS Imaging of Biological Tissue

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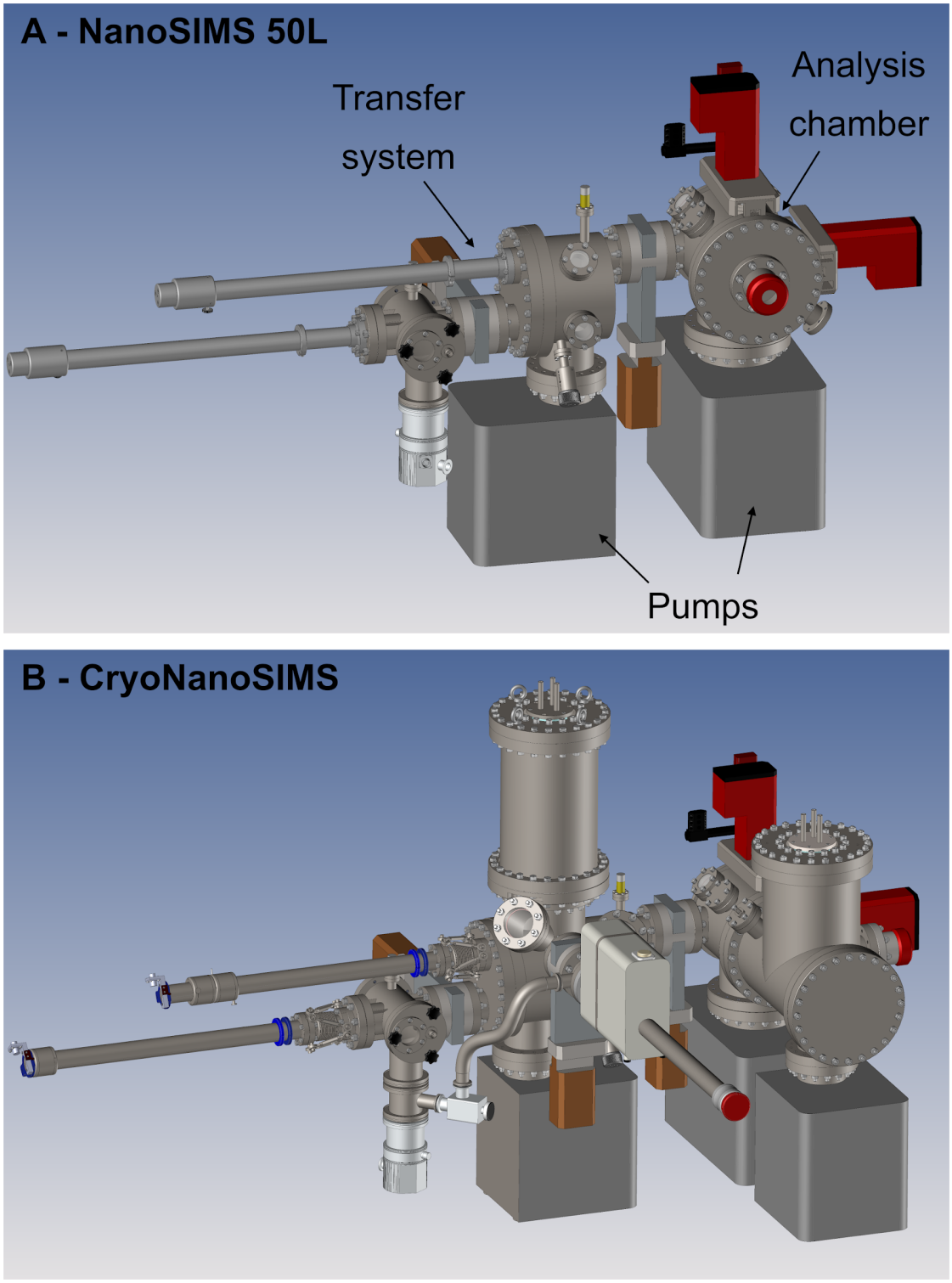

Figure S1. 3D renderings comparing the transfer system and analysis chamber of the conventional NanoSIMS instrument (**A**) with those of the CryoNanoSIMS (**B**).

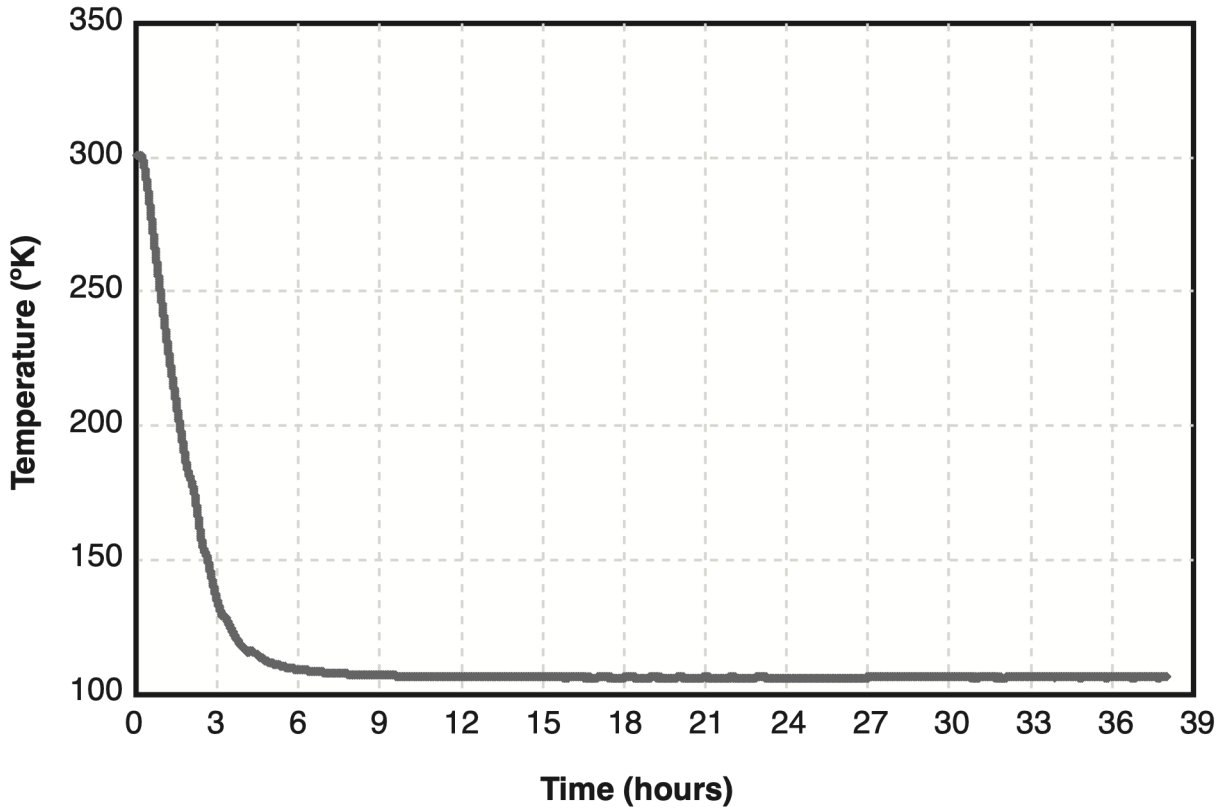

Figure S2. Cooling curve for the CryoNanoSIMS. The temperature is measured with a thermocouple on the sample stage a few mm from the sample itself. Starting at room temperature, a stable temperature of 106.2 K is reached in about 10 hours, after which the temperature variation is within ± 0.1 K (1 standard deviation) for as long as liquid N_2 is supplied to the instrument.

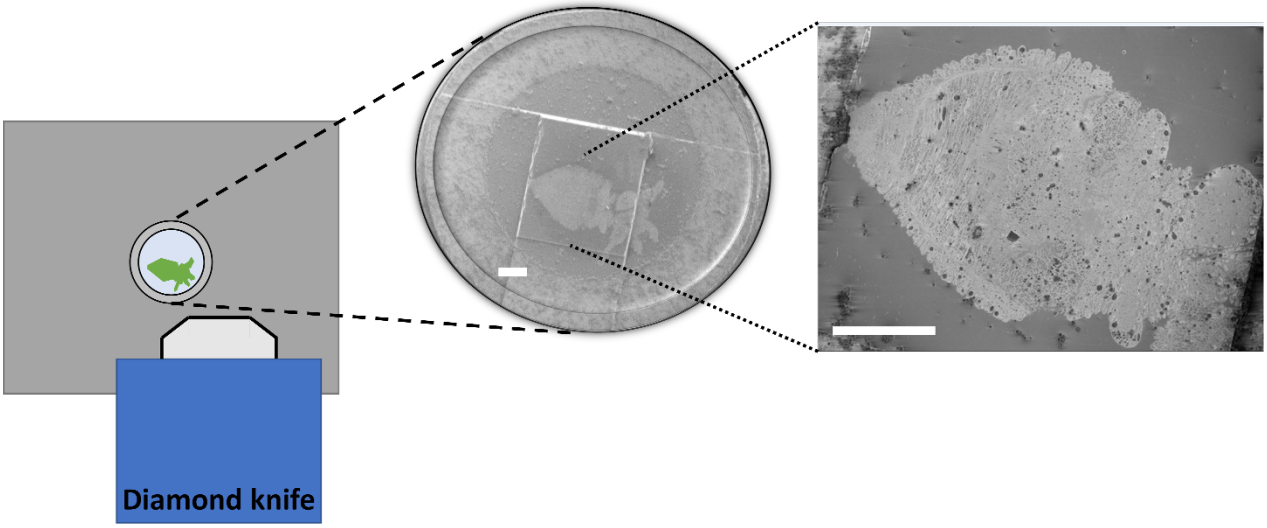

Figure S3. Schematic drawing illustrating ultramicrotome cryo-planing. The diamond knife trims and planarizes the sample (left) creating a rectangular pyramid with a very flat block-face surface as shown in the cryo-SEM image (middle). Right: Cryo-SEM micrograph of a Green Hydra sample. Scale bars are 250 µm.

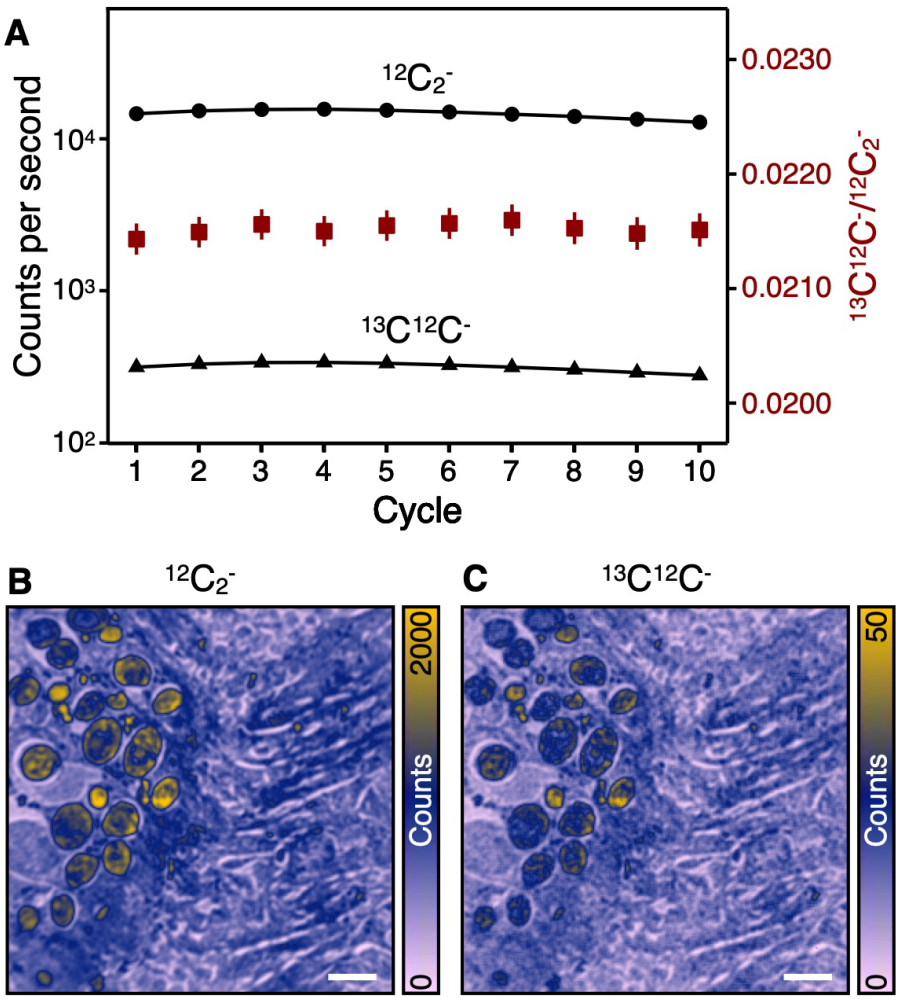

Figure S4. CryoNanoSIMS analysis of carbon isotope ratios in Green Hydra: count rates, isotope ratio, and ion images obtained from analysis of isotopically normal tissue of Green Hydra. **(A)** Average $^{12}C_2^-$ and $^{13}C^{12}C^-$ ion count rates and corresponding isotope ratio over 10 imaging cycles for the entire image. Each cycle consisted of a 256x256 pixel raster with a beam dwelling time of 5 milliseconds, for a total imaging time of ca. 60 minutes. Isotope ratio error bars are ±2 std error. **(B** and **C)** Drift-corrected and accumulated ion-maps for $^{12}C_2^-$ and $^{13}C^{12}C^-$, respectively. Scale bars are 5 µm.

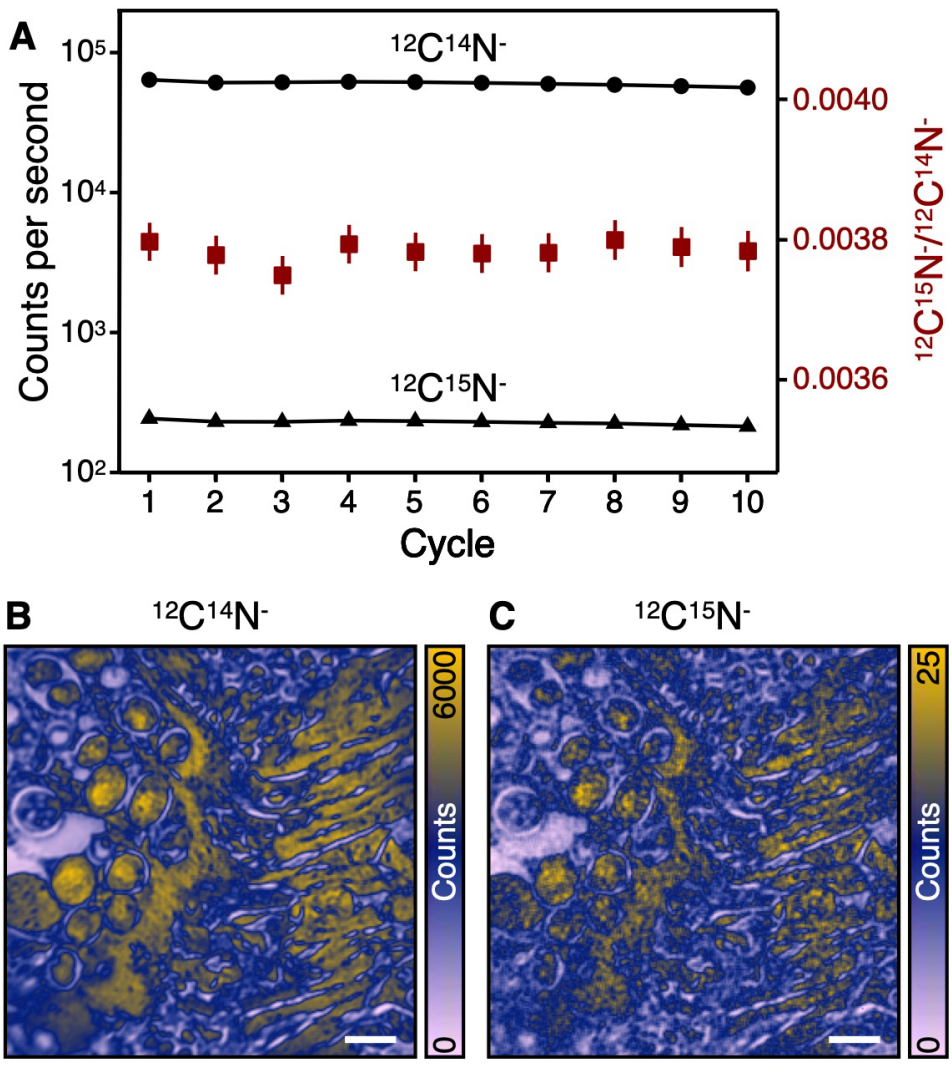

Figure S5. CryoNanoSIMS analysis of nitrogen isotope ratios in Green Hydra: count rates, isotope ratio, and ion images obtained from analysis of isotopically normal tissue of Green Hydra. (**A)** Average $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$ ion count rates and corresponding isotope ratio over 10 imaging cycles for the entire image. Each cycle consisted of a 256x256 pixel raster with a beam dwelling time of 5 milliseconds, for a total imaging time of ca. 60 minutes. Isotope ratio error bars are ±2 std error. (**B** and **C)** Drift-corrected and accumulated ion-maps for $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$, respectively. Scale bars are 5 µm.

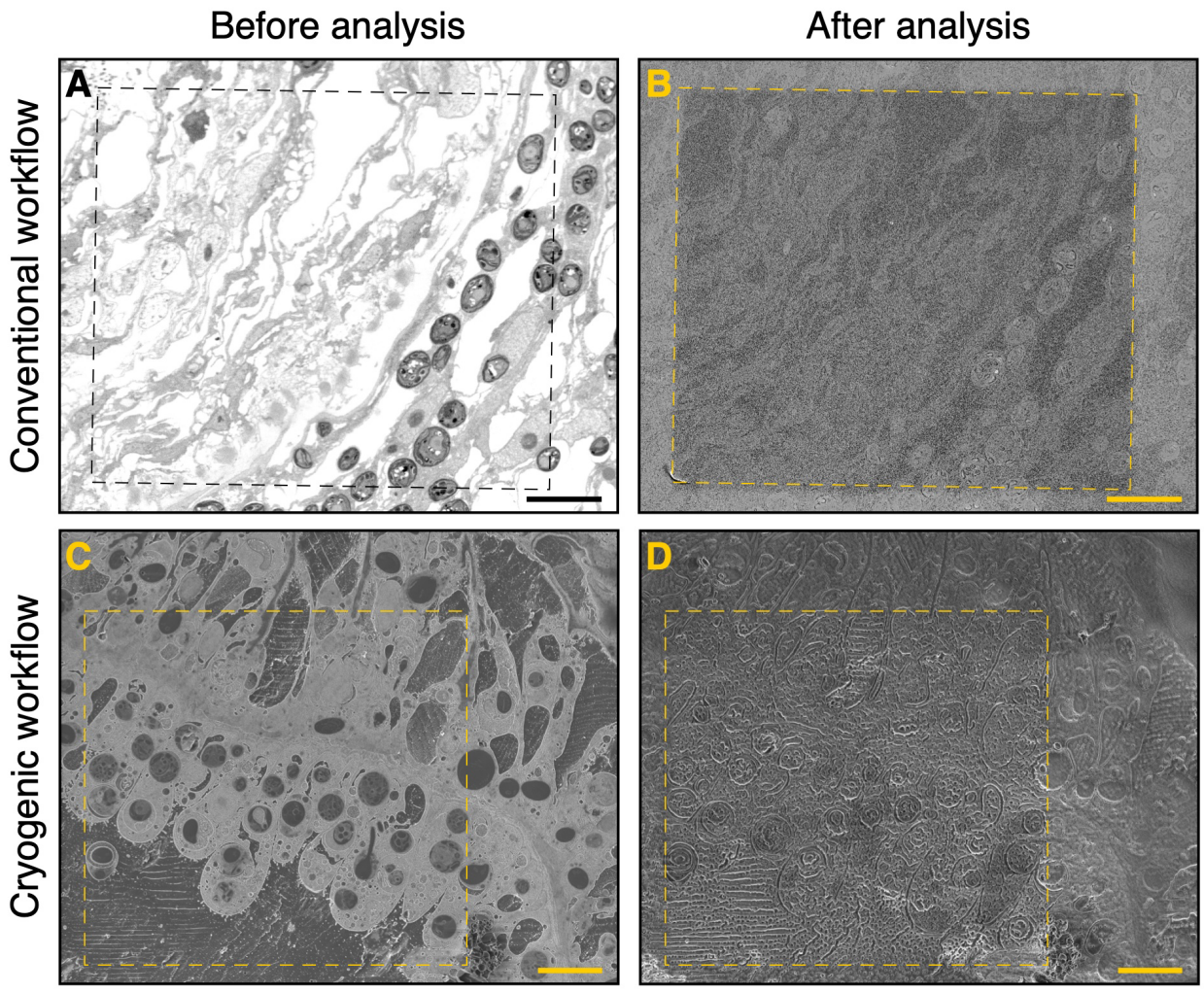

Figure S6. Sample surface before and after NanoSIMS imaging. SEM images of sample surfaces before and after NanoSIMS analysis with conventional (**A** and **B**) and cryogenic (**C** and **D**) workflows, respectively. Squares indicate the areas of NanoSIMS analysis. In A and C the sample surfaces were coated with about 3 nm metal, whereas in B and D the surface was coated with about 20 nm metal, except for the areas of NanoSIMS imaging, where this coating had been removed by pre-sputtering. Scale bars are 10 µm.