1) Water is poured on a cold metal base and left until it is frozen.
2) The frozen tissue specimen is then placed on the base, where the ice layer serves to keep it firmly in place.
3) Using a cryostat, the frozen specimen is cryosectioned in 10μm wide slices. The tissue slices are placed on a glass slide, four/slide.
4) The DESI-MS spectrometer is used to acquire sequentially (row by row) the spectra of ~10μm sized square regions of the tissue. This works by applying a small jet of solvent to the tissue, and the molecules solvated are carried up to the detector tube, where they are read by the spectrometer. On the screen in the background can be seen the mass spectrum of the current pixel. The mass spectra from each pixel are entered into a database for future analysis.
5) A first step is to align the tissue sections. Here is an example of the affine registration of the optical H&E images onto the corresponding MS images (total ion current images). The optimal threshold for the identification of the tissue slices present in the current layer can be adjusted manually. Afterwards, the tissue slices are automatically identified and the images separated into different slices. If two or more identified slices are overlapping, the resulting binarised image is shown in order to identify the connected regions to this slice.
6) Data analysis using Deep Learning (a machine learning method) is used to construct a three-dimensional image of the tumour from the mass spectra, essentially the differences in chemistry of different regions of the tumour.
7) The reconstructed 3D volume of the tumour showing the 3 different clusters (in this case)` found. The clusters are shown in three colours (cluster 1 = red, cluster 2 = green, cluster 3 = blue). Before rendering the volume is smoothed applying a Laplace operator. Transparency is added to cluster 1 in order to visualise the internal tumour clusters.