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Report:

The purpose of our proposal is to contribute the quantitative structural analysis of human cerebral micro-vascular networks. This experiment could allow us to investigate the spatial homogeneity of the micro-vascular network from different cortex regions as well as extract relevant structural parameters through statistical analysis. The purpose of this first set of experiments was to address the question of the feasability of imaging the vascular capillary structure with synchrotron X-ray, for given biological samples. The experimental procedure follows two major steps. The first one consists in the preparation of tissue samples extracted from post-mortem human brain. The second step consists in the imaging procedure involving X-ray tomography.

1. Samples preparation :

- Two fresh (between 12 hours and 24 hours post-mortem) human brains have been injected with a solution of iodine, gelatin, and Indian ink in collaboration with Pr. Fasel of the University of Geneva.
- Water soluble iodine is the radio-opaque marker currently used for angiography in living humans. Its concentration has been chosen equal to 200 mg/ml. Supposing that the iode is only present inside the vascular networks, this concentration give local absorption contrast at the Energy of 13kev close to 6. It should theoretically be largely sufficient for a good image contrast after reconstruction. Nevertheless because the volume of the vascular vessels representing only 3% to 5% of the total tissue volume the absorption of the iode relative to the total absorption is only a few tens of percent.
- The solution of gelatine and Indian ink is the classical marker used to visualize the brain micro-circulation by optic microscopy (Duvernoy et al. Brain Res Bull 1991; 7: 519-579). It was used to check the quality of the injection and to choose the adequate sample for synchrotron tomography. The concentration of the mixture of gelatin and Indian ink have been chosen in order to obtain rheological properties grossly similar to those of blood in order to facilitate injection.

The injected liquid is a non-Newtonian fluid which viscosity varies between 4 to 10 times the viscosity of water for shear rate between 0.3 to $6s^{-1}$.

- After fixation, the second brain appeared clearly as being better injected than the first one. Injected brains have be fixed in a formol bath during four weeks.
- Finally, up to 20 cylindrical samples whose diameter was 3mm and height 5mm have be cutted from the prepared brains, transfered in small tube of Plexiglas with inner diameter of 3.2mm. We used a gel (parafine with the first brain, gelatine for the second one) to include samples inside Plexiglas tubes.

2. X-ray measurements :
We performed three types of tomographic acquisitions : absorption, phase contrast and holotomography. Although the first two were largely unsuccessful for imaging, they gave us interesting insights to improve the samples preparation. The last holotomography measurements gave very interesting results. Let us briefly emphasize the main points of this on-going work:

- We did not obtained any contrasted structures on absorption measures either at 11kev and 33.2kev (the iode pic to get better local contrast). We nevertheless recovered the expected theoretical energy absorption rate. Hence it seems very possible, as suggested by some unpublished informations, that the iode has diffused through the hemato-encephalic barrier inside the tissue.
- Direct phase contrast acquisition (edge enhancement) at different distances with different wavelength has only permitted to identify very few large vessels. Nevertheless it also has indicated some instable micro-bubbles inside the gel used for the sample conditioning in Plexiglas tubes.
- Holotomographic phase reconstruction phase measurements have been carried out at 1.9μm resolution. The above figure display a 3D rendering and slice image of density contrast. They show a good contrast between the vessel structure and the tissue. The bifurcation of large vessel observed on the transmitted image is typical of cortical vessel structures.