ESRF	**Experiment title:** Imaging human brain micro-vascular networks	**Experiment number:** LS-2125
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Shifts: 9	**Local contact(s):** P. Cloetens	*Received at ESRF:*

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Report:

The results of our previous experiments number 1834 being encouraging we have decided to use the allocated shift left (3 of them) from this project to test different experimental improvements. In this process of defining an optimal experimental protocol we have preferred short beam line access (1 day) rather than three day long measurements. This important point has been possible with the help of line 19 direction that we would like to thank here.

The purpose of our proposal is to contribute to the quantitative structural analysis of human or animal cerebral micro-vascular networks. This first set of experiments have tested the feasibility of imaging the vascular capillary structure with synchrotron X-ray, for given biological samples. As will be clear from this short report, this objective is already reached. Let us summarize the main steps of the experimental protocol as well as the main improvements. The experimental procedure follows two major steps. The first one consists in the preparation of tissue samples extracted from post-mortem human brain, as well as monkey brain (Marmoset). The second step consists in the imaging procedure involving X-ray tomography.

1. Samples preparation :

- Two fresh (between 24 hours and 48 hours post-mortem) human brains have been injected with a solution of baryum-sulfate liquid powder (Micropaque, Guerbet) and gelatin. Local cerebral arteries as well as global carotid injections have been performed. After injection the brains have been plunged in a formol bath during two months for fixation.

- One newborn marmoset monkey has been lethal injected in the hearth left ventricle, first, with an isotonic Sodium-Chloride solution and, then, with a formol solution during half an hour. This in situ fixation has been closely followed with a baryum-sulfate powder (Micropaque, Guerbet) + gelatin injection for twenty minutes. After injection the monkey brain have been plunged in formol for few weeks.

- The baryum concentration has been chosen equal to 200 mg/ml. Supposing that it is only present inside the vascular networks, this concentration gives a local absorption contrast of about 14 at the Energy of 11 kev.

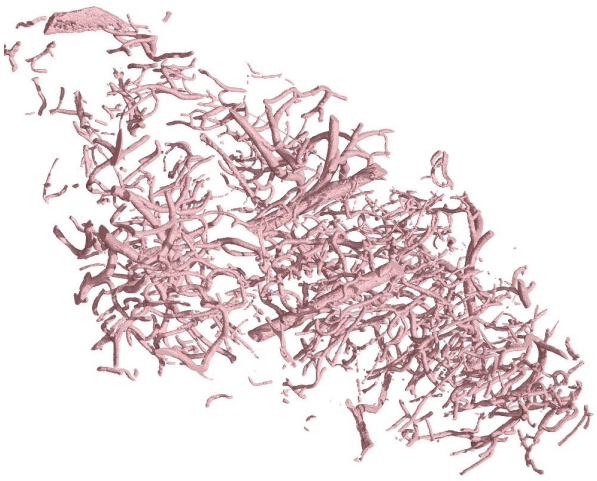
It should theoretically be largely sufficient for a good image contrast after reconstruction. Nevertheless because the volume of the vascular vessels representing only 3% to 5% of the total tissue volume the absorption of the baryum relative to the total absorption is only a few tens of percent.

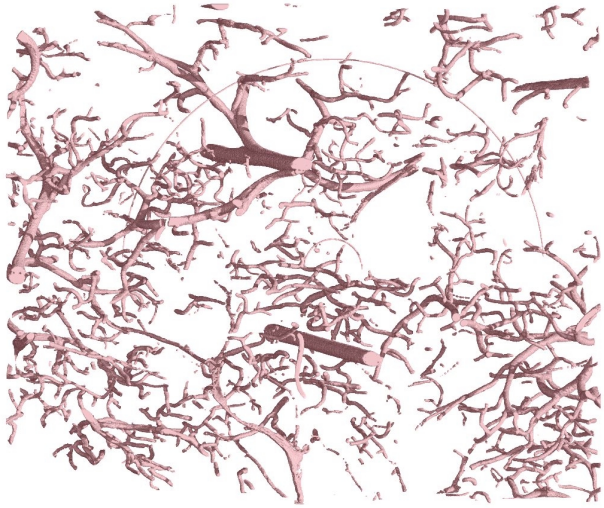
- 18 cylindrical samples with 3mm diameter and 4mm height have be cutted from the prepared brains. A diaphanisation (succession of alcohol and oxygen peroxide bath) protocol has been applied to all tissue sample before their inclusion in a Epon resin.

The animal lethal injection is, in principle, free from any post-mortem tissue degradation. It has the distinct advantage of a reproducible protocol as opposed to any human post-mortem injection were the post-mortem delay, the individual age, and other parameters are by definition, non-controlled.
2. X-ray measurements :
We performed two types of tomographic acquisitions : absorption and phase contrast. We choose the 2048×2048 camera scanning a 2.8 mm windows giving a $1.4\mu m$ resolution.

- We obtain a very contrasted signal for absorption measurements as illustrated in the images presented bellow. The quality of image contrast very strongly suggest that the baryum-sulfate powder does not penetrate the hemato-encephalic barrier, as already suggested from the particle micron typical size. This images represent almost one ten's of the total acquisition that has been performed on one human brain sample (almost $1.9mm^2$ horizontally and 300 μm in depth). These images show the preferential orientation of large vessels (as can also be observed with microscope) as opposed to the rather isotropic and complex structure of capillary networks. The quality of the acquisition already permits to state that classical segmentation algorithms can be applied to this data. It is nevertheless observed that the macroscopic vessel density is lower than expected. Moreover, a careful inspection of the acquisition data shows that an important percentage of small vessels disappear. This observation lead us to conclude that even if the baryum injection is very good in large vessels, there are some injection problems associated with small vessels. These characteristics have been observed on every humans and monkey samples (three tomography measurements of each type have been analyzed).

- The phase contrast acquisition also show interesting structures, very much correlated to vessel structure that are observed in the absorption images.





In conclusion to these on-going experiments, we have largely improve the quality of X-ray measurements from important modifications on the sample preparation. The interest of X-ray measure for imaging capillary vessels in biological tissues is now demonstrated for it provide high quality, high resolution images. There are nevertheless still some progress to be done on the biological sample injection protocol. We hope that future technical improvement could permit a good baryum injection in the smallest vessels.