BRAIN TISSUE SEEN IN X-RAY VISION

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Abstract

A brain tissue sample of human cerebellum was analyzed using X-ray computed tomography. Data coming from transmission and fluorescent radiation were collected. This technique proved to be useful in 3D imaging of the inner structure of brain. Obtained resolution of images allowed to differentiate basic structures, e.g. cell nuclei in the sample. The problem of noise present in images was also discussed and dealt with. An algorithm correcting the effect of secondary absorption of X-ray fluorescence radiation was implemented and tested on obtained data.

Contents

1. Introduction

Computed tomography is a popular nondestructive technique which uses X-rays to produce cross-section pictures of examined sample and therefore allows to take a look inside. Thanks to the fact that the object is irradiated from every side, it can differentiate soft tissues of similar densities, what is impossible with radiography. Micro-CT is widely used in medicine for the purposes of diagnostics – for example tumors. In general, the X-ray source and detector are typically stationary during the scan while the sample rotates.

At DESY we examined a sample of cerebellum tissue. Two kinds of data were obtained – transmission scans and fluorescence radiation of different energies for the purpose of quantitative analysis of elemental content of the tissue. What we've done was:

- 3D imaging of inner sample structure
- spatial resolution assessment by power spectral density (PSD) analysis of obtained transmission images
- correction of sinograms of fluorescence radiation in tissue matrix.

2. Experiment conditions

The scans were performed at PETRA III/P06 beamline. The tissue sample was collected from a dead human brain. It was immediately shock-frozen with the liquid propane at the temperature of ca. -80 C. deg. and further kept in the atmosphere of liquid nitrogen. The experiment was taken in cryogenic chamber in vacuum.

Schematic illustration of experiment is presented in Fig. 1.

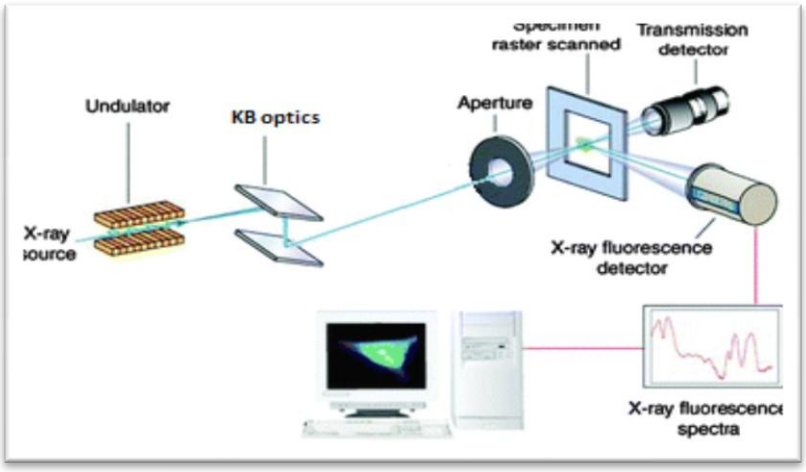

Fig.1. Setup used in the measurement.

2.1.Transmission experiment

The transmission images were obtained by PCO camera placed behind the sample. In this part of experiment 3600 projections were obtained. The basic parameters were:

- X-ray beam energy: 10.5 keV
- Rotation step: 0.05 deg.
- Rotation range: 180 deg.
- Exposure time: 700 ms
- Lens magnification: 5x
- Pixel size on transmission detector: 0.658 μm.

2.2. Fluorescence experiment

Focused X-ray beam of size 0.62 μm and an energy of 10.5 keV scanned the sample. The fluorescence radiation emitted by the tissue was collected using a Vortex SDD detector situated with an angle of 75 deg. relative to the beam. The basic parameters of experiment were:

- Translation step: 2 μm
- Rotation step: 1.6 deg.
- Rotation range: 360 deg.
- Exposure time: 100 ms
- Type of scanning: raster scan.

3. Results

From the measurement we obtained transmission images, which are shown below. They represent the attenuation of incident X-ray beam after it was transmitted through the sample. We got 3600 images, which were the starting point to make sinograms and further tomograms.

Sinogram is constructed by concatenating i-th row from each transmission image. It represents scans for all the rotations. Tomograms are obtained using an inversed Radon transform on sinograms. Simplified diagram of post-processing is showed in Fig. 2.

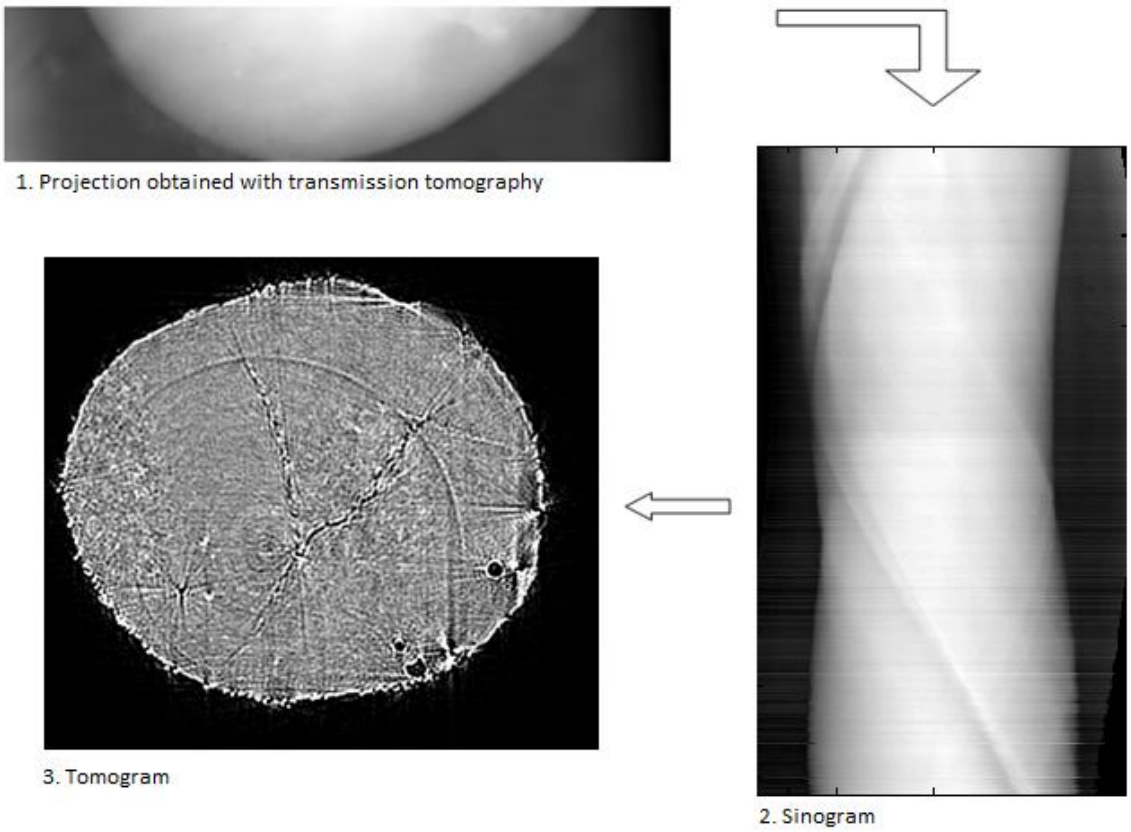

Fig. 2. Picture 1 represents a projection collected by transmission detector. Picture 2 illustrates a sinogram, which lets us make a tomogram (picture 3). For this inversed Radon transform was utilized.

Due to wiggling motors moving the sample, alternating rows of created sinograms were shifted – basically it means that the sample's mass center was moving when taking rotation scans. Therefore it was necessary to move the rows so that the mass center of the sample was placed in the middle of sinograms. Below the sinograms before and after the correction were shown.

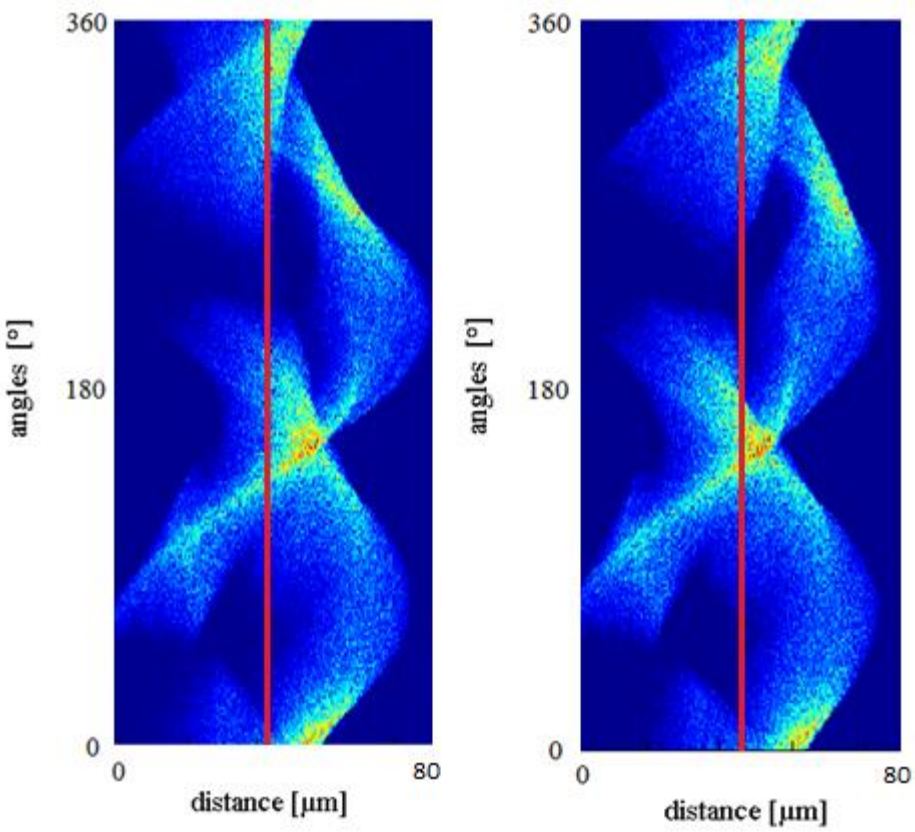

Fig.3. Sinograms of calcium Kα line; uncorrected sinogram is on the right, corrected on the left. The red lines represent rotation axis of the sample.

On the left side of each sinogram one can notice "holes", which the secondary attenuation of fluorescence radiation in matrix is responsible for.

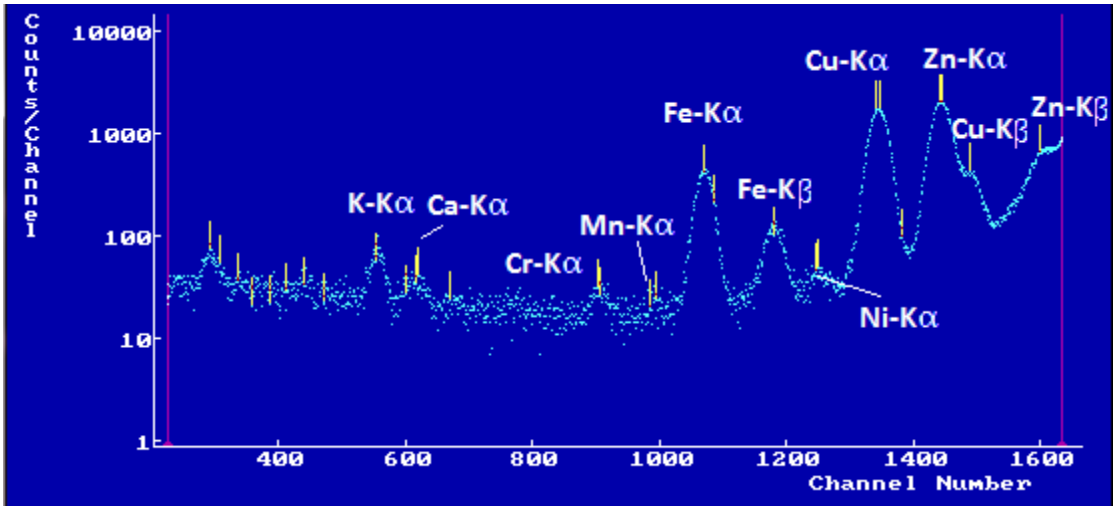

Fig. 4. The XRF spectrum of the examined sample.

From the emission experiment obtained the characteristic radiation spectrum, which is shown in Fig. 4. We are going to perform the quantitative analysis of the elements most relevant to biochemical processes, e.g. Fe, Cu, Zn.

4. The resolution issue

In order to tell how much can we see in obtained tomograms and how far we can draw the conclusions, the assessment of spatial resolution of the method was performed. To achieve that the power spectral density calculation algorithms were implemented in MatLab. This method is based on the Fourier transform of images. Spatial frequencies and their intensities tell us where the boundaries of recognizable structures are. This method can also evaluate the general noise level of an image.
The spatial resolution was estimated with two different approaches.

First approach[i]:

- 2D FFT of image was taken; an example is presented in Fig. 6.
- absolute values of the pixels were squared in order to obtain spectral power values
- concentric rings of the FFT image were cropped and pixels' values in each of them were summed and then normalized to the most inner ring
- these values were presented in the function of spatial frequencies
- the cut off frequency was finally converted into a resolution limit.

The result of this estimation is shown in Fig. 5. Assessed spatial resolution is about 1.67 μm, which is the approximate size of 2 pixels. Nucleus size in cerebellum is about 5 μm, what lets us differentiate them in obtained CT tomograms.

Second approach:

- 2D FFT of images are taken and spectral power values are calculated
- a line from the middle of the FFT image to the corner as shown in Fig. 7 represents growing frequencies from 0 to the maximal, according to the Kotielnikow - Shannon's theory.

The results are showed in Fig. 8. Approximate spatial resolution is about 1.25 μm. As it is seen, those methods do not allow us to conclude directly about the resolution of images, it only lets us to estimate the level.

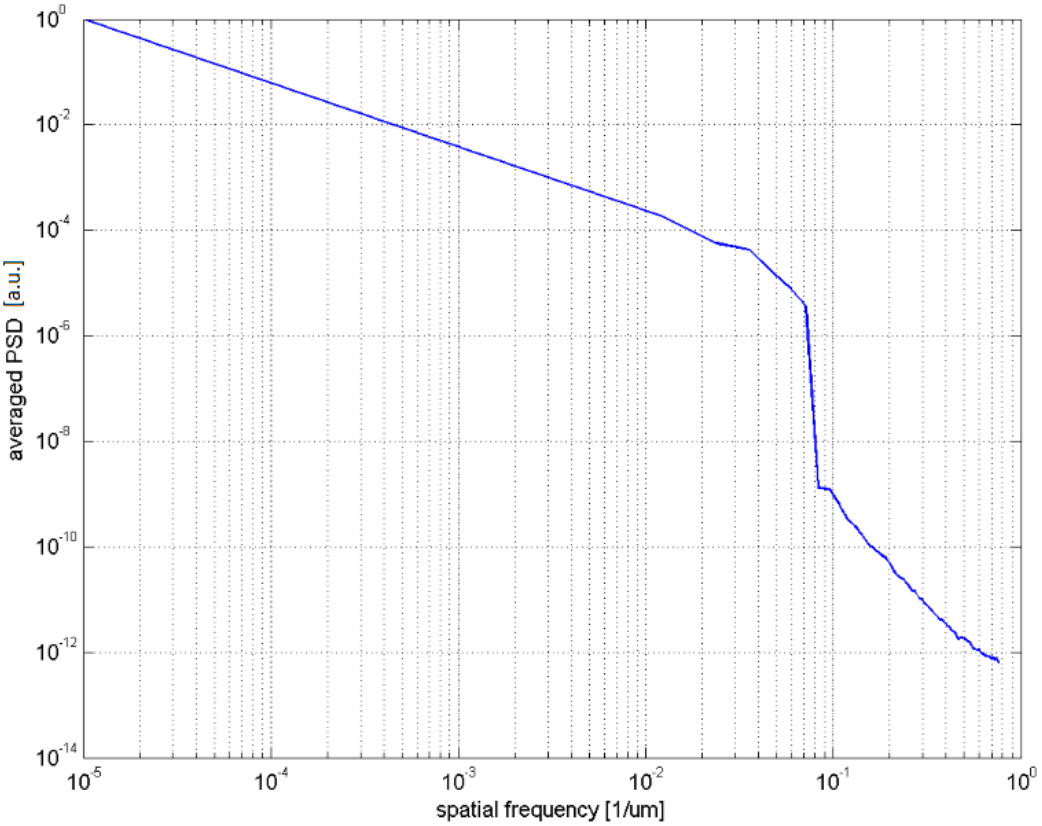

Fig. 5. Averaged power spectral density values in the function of spatial frequency for transmission data. The boundary frequency is 0.3/um. It corresponds to the resolution of 1.67 μm.

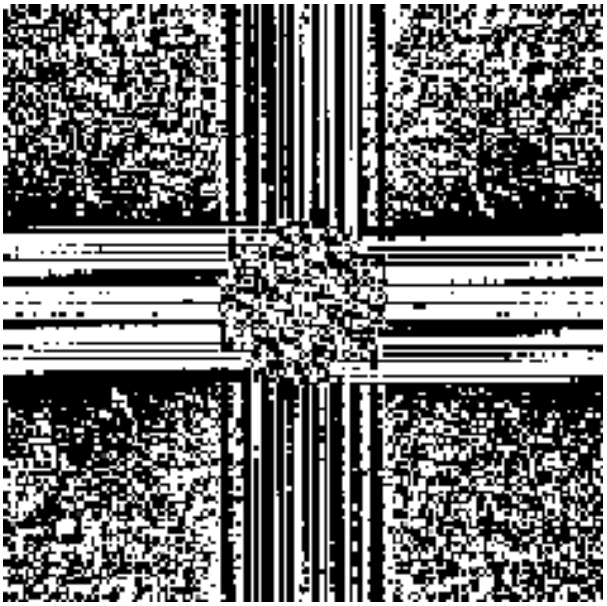

Fig. 6. 2D FFT of a tomogram fragment. Frequencies are growing from the middle of the picture to the sides.

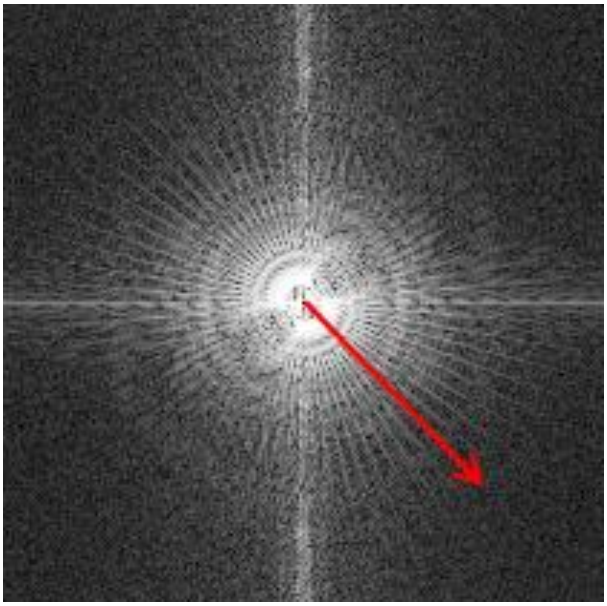

Fig. 7. Power spectrum of an image. Values of pixels under the red line are taken into account when preparing PSD in the function of spatial frequency - chart (Fig. 8).

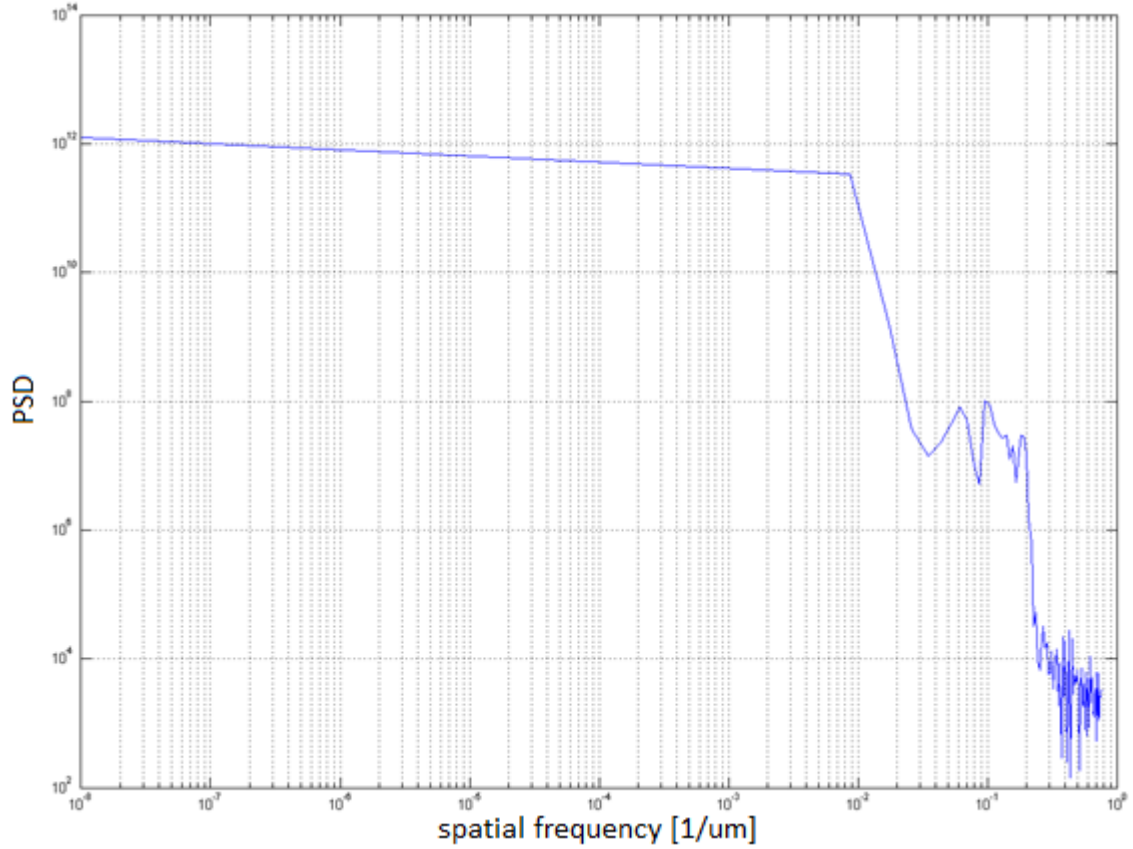

Fig. 8. Power spectral density values in the function of spatial frequency for transmission data. The boundary frequency is 0.4/um. It corresponds to the resolution of 1.25 um.

5. 3D imaging

Based on the images of transmission scans 3D visualisation of the inner structure of the tissue was prepared. Because the spatial resolution of obtained images was too poor to differentiate between different cytoplasmatic structures like axons or dendrites of the nerves, only the nuclei were presented. Cell nuclei are generally more dense than the rest of the tissue matter so the amount of radiation passing through them is different. The analysis of corresponding linear attenuation coefficients let us match an appropriate threshold value to extract only the nuclei. To obtain 3D images, which are presented below, we used Avizo 8 Fire, Amira and the MeVisLab.

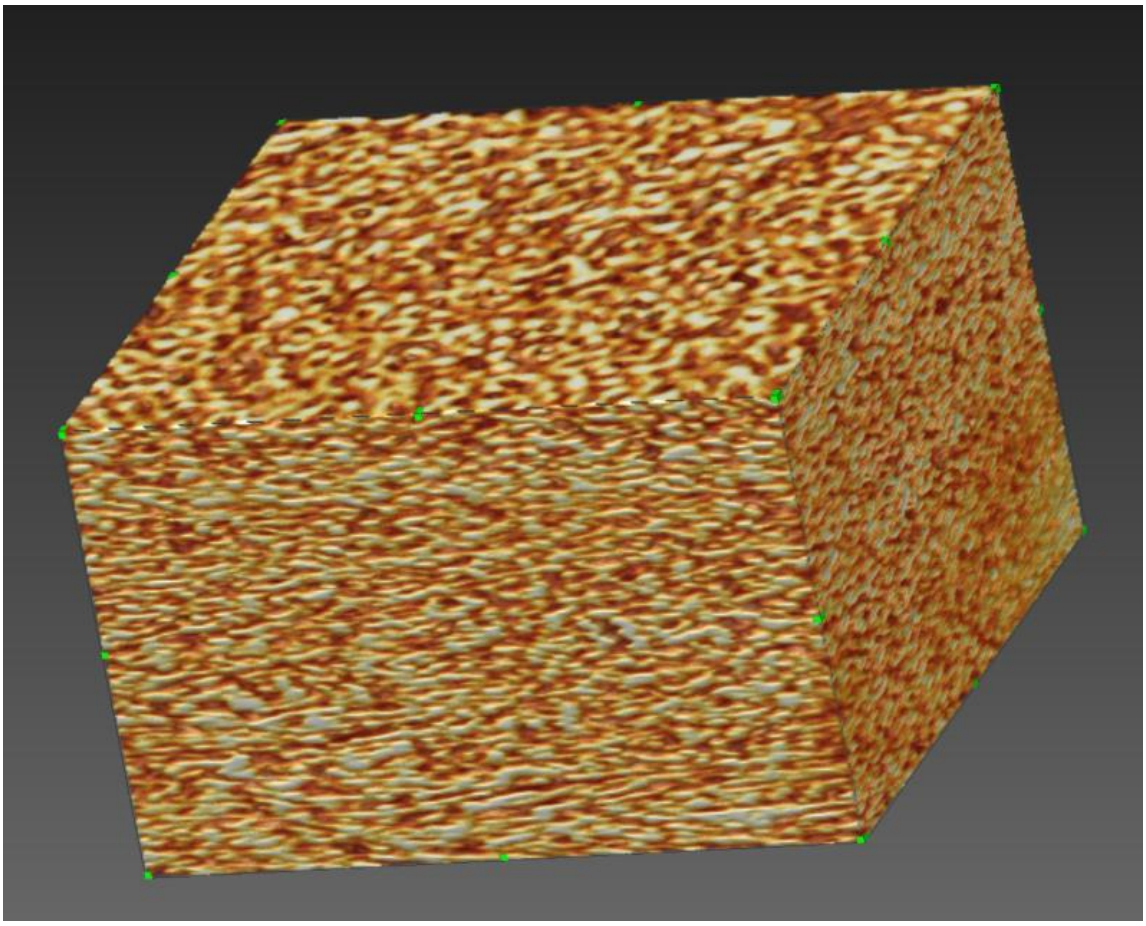

Fig. 9. 3D visualization of the interior part of examined sample. The brightest structures are nuclei.

6. Absorption correction in XRF tomography

X-ray fluorescence microtomography allows us to map element distribution inside the sample. This method provides high sensitivity and resolution in the micrometer range. Unfortunately, due to self-absorption effect the fluorescence data requires the correction for the attenuation inside the sample. The correction algorithm was implemented in Matlab[ii]. The algorithm allows to determine the weight fractions of detectable elements.

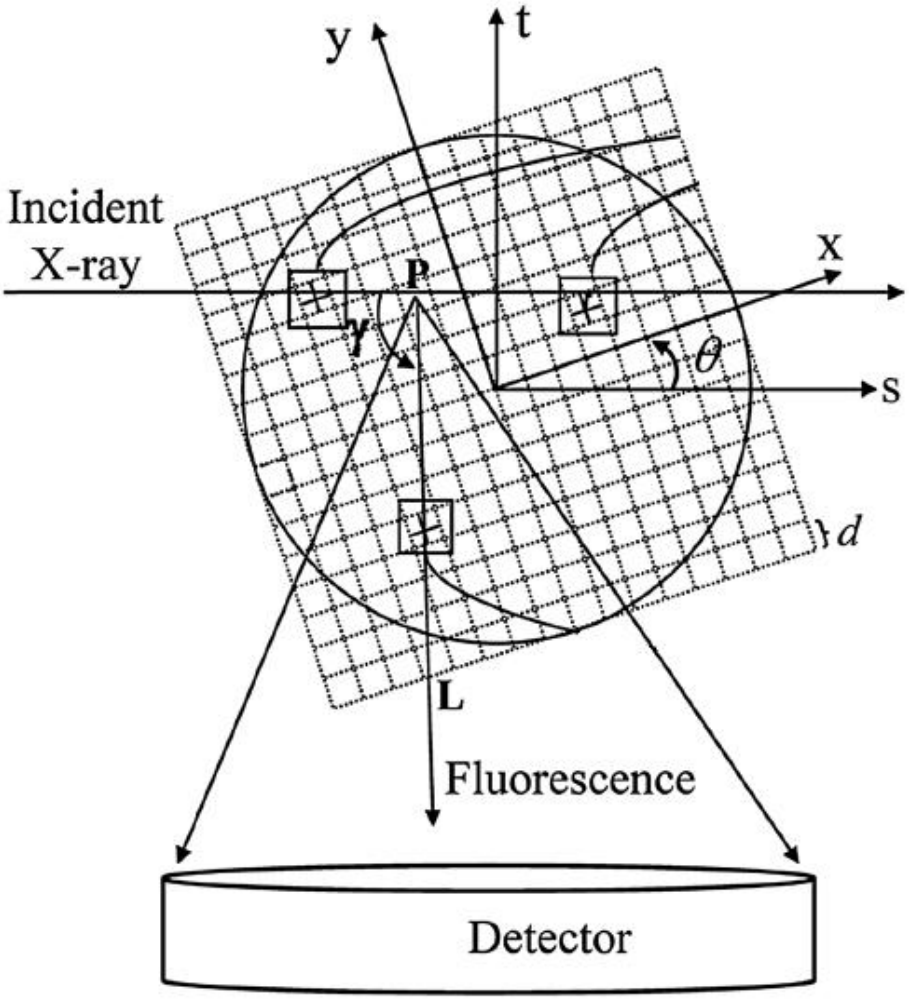

Fig. 10. Schematic diagram of the acquisition geometry of XFCT, where xy-coordinate and st-coordinate systems are fixed to the object and the laboratory, respectively. d is the size of the pixel. L denotes the distance from point P to the edge of the object.[ii]

The focused beam with intensity I_o excites the elements along the penetration path. They emit isotropically the X-ray fluorescence radiation, which is recorded by detector after escaping from the sample. The intensity of X-ray characteristic lines of the i-th element is given by:

$$I(\theta,t) = K \cdot I_0 \cdot \Omega \int_{-\infty}^{+\infty} [C(s,t) \cdot f(\theta,s,t) \cdot g(\theta,s,t)] ds \quad (1)$$

where

$$f(\theta,s,t) = \exp\left[-\int_{-\infty}^{S} \mu'(s',t) ds'\right] \quad (2)$$

$$g(\theta,s,t) = \int_{\gamma_{min}}^{\gamma_{max}} \exp\left[-\int_0^L \mu^F(s',t) dl'\right] d\gamma \quad (3)$$

C – weight fraction of the i-th element
K - scaling constant
μ' and μ^F – the incidental X-ray and X-ray fluorescence attenuation coefficients
$\gamma_{min} - \gamma_{max}$ – the range of solid angle Ω
$I(\theta,t)$ – the projection value.

Based on those equations we calculate the contribution K of every pixel for each particular rotation and translation to the intensity of X-ray fluorescence radiation that is collected by the detector:

$$K(i,j,m,n) = I_0 \cdot \Omega \cdot f(s,t) \cdot g(s,t) \quad (4)$$

Then it is only the matter of implementation of the obtained data to the ordered subset expectation maximization (OSEM) algorithm which is given by:

$$C^{l+1}(i,j) = \frac{C^l(i,j)}{\sum_{(m,n)\in S_l} K(i,j,m,n)} \sum_{(m,n)\in S_l} \frac{K(i,j,m,n)I(m,n)}{p^l(m,n)} \quad (5)$$

$$p^l(m,n) = \sum_{i,j} K(i,j,m,n)C^l(i,j), (m,n) \in S_l \quad (6)$$

$C^{l+1}(i,j)$ and $C^l(i,j)$ – the reconstructed weight fraction of element from the subset (l+1) and I, respectively
$I(m,n)$ – the projection value of the m-th rotation and n-th translation
S_l (l=1,2…L) – the chosen subset.

7. Summary

From data obtained in the transmission and emission experiments the sinograms were prepared, which were then corrected due to the instability of motors that occurred during the experiments. Another step was performing an inverse Radon transform on sinograms and getting tomograms as a result. Using several programs the 3D visualisation of examined sample was prepared. Also, the estimation of the spatial resolution of the method was performed. It appears, that the conditions of experiment and post processing of images allowed us to achieve the resolution level of about twice a size of PCO camera pixel size. Due to the self-absorption effect of fluorescence radiation within sample matrix it was necessary to implement a correction algorithm, what was done using Matlab. It has yet to be finished and tested on obtained data.

[i] https://misio.fis.agh.edu.pl/media/misiofiles/9bccaf2638f1770838de5a558b208944.pdf; Karolina Stachnik;

[ii] *X-ray fluorescence computed tomography with absorption correction for biomedical samples*; Qun Yang, Biao Deng, Guohao Du, Honglan Xie, Guangzhao Zhou,Tiqiao Xiao and Hongjie Xu; DOI 10.1002/xrs.2550;