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# Synchrotron Radiation Nanotomography of Biological Soft Tissues

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Biological soft tissues are composed of micrometer- to nanometer-scale structures of cellular and subcellular constituents. In order to visualize tissue structures with nanotomography, each constituent must keep its structure at nanometer precision throughout the image acquisition. Since soft tissues are easily deformed, samples should be pretreated taking account of a number of factors that affect the image. Here, we report an application of synchrotron radiation nanotomography to biological soft tissues.

Soft tissues are made of light elements, such as carbon and oxygen, which produce little contrast in a hard x-ray image. Hence, the contrast of the target structures must be enhanced to visualize them. The contrast enhancement should reproduce the tissue structure at a precision finer than the spatial resolution of the nanotomography. In this study, neurons of human brain tissue were labelled with silver by Golgi impregnation, as described previously [1]. Post-mortem human tissues were collected with informed consent from the legal next of kin using protocols approved by ethical committees of the related organizations.

Biological samples themselves are labile to x-ray irradiation. At the BL37XU beamline of SPring-8, a sample area of $50 \times 50$ μm$^2$ can be illuminated with a photon flux of $10^{13}$ photons/s by using an x-ray guide tube [2]. This corresponds to a heat load of 1 mW to 1 μg of tissue. Therefore, in order to visualize nanometer-scale structures, samples must be pretreated to make their structures tolerant to the heat load for typically 1000 seconds of the image acquisition. In this study, brain tissues were embedded in Petropoxy 154 epoxy resin (Burnham Petrographics, ID, USA), which is available as a petrographic embedding medium. Although we prepared samples with several other methods, including tissue lyophilization [3], the following procedure is best for synchrotron radiation nanotomography.

The assumed tissue size in this procedure is $5 \times 5 \times 5$ mm$^3$. First, we soaked the tissue in 10 mL of ethanol for one hour at room temperature (20–25°C). This process was repeated twice in total. Then the tissue was soaked in another 10 mL of ethanol for overnight. Next, it was transferred to 10 mL of *n*-butyl glycidyl ether and incubated for several hours or overnight at room temperature. This process was repeated twice in total. The tissue was then soaked in a 2 mL aliquot of Petropoxy 154 resin overnight at 4°C. This process was repeated twice in total. If the tissue floated to the surface of the resin aliquot after the two cycles of resin soaking, we performed an additional soaking using another resin aliquot. The obtained tissue was cut into pieces of approximately $0.5 \times 0.5 \times 5$ mm$^3$ and transferred to borosilicate glass capillaries filled with resin. The capillaries were incubated at 90ºC for 72–96 h to cure the resin. Limitation of this method is that tissue shrinkage can be introduced as in the case of paraffin sections for

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light microscopy or epoxy-resin sections for electron microscopy.

In nanotomography, thermal drift is the major cause of image blurring. Although the thermal expansion of instrument components can be minimized by using materials with a low linear expansion coefficient, such as invar ($2 \times 10^{-6}$ /K at room temperature), the thermal expansion of the sample itself, i.e., epoxy resin ($\sim 60 \times 10^{-6}$ /K) is unavoidable. For example, an epoxy sample with a 5 mm length deforms by 30 nm with a 0.1 K fluctuation. Therefore, the stability of the ambient temperature is essential in nanotomography. The heat balance of the experimental hutch must be held constant throughout the experiment. Turning equipment on or off in the hutch, including the lighting, should be kept to a minimum. Samples should be placed in the hutch at least one hour before data acquisition to equilibrate them with the hutch temperature. Mounting the sample in advance will also prevent drifts due to mechanical creep caused by securing the sample with a set screw.

Figure 1 shows nanotomography images taken according to the methods described above. A square-wave pattern with a pitch of 120 nm was resolved in a nanotomographic section of an aluminum test object (Fig. 1a). Figure 1b shows the three-dimensional structure of a spiny dendrite of a pyramidal neuron of the human cerebral cortex. A number of dendritic spines composed of bulbous heads and thin necks with diameters down to 100 nm are visible. The spatial resolution of the tomographic cross section of this structure was estimated to be 134 nm from the logarithmic plot [4,5]. This is comparable to the resolution of the test object image. These results indicate that nanometer scale structures of soft tissue samples can be visualized with synchrotron radiation nanotomography.

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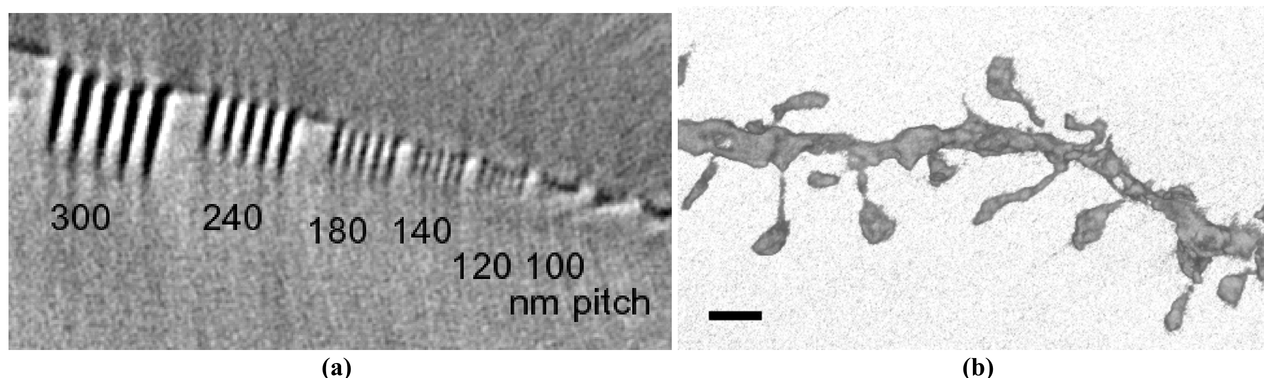

(a) (b)

**Figure. 1.** Tomographic images visualized with 8 keV x-rays at the BL37XU beamline of SPring-8 using an x-ray guide tube [2]. A total of 900 sample frames were taken with 800 ms exposure per frame. (a) Tomographic cross section of square-wave patterns with pitches of 300, 240, 180, 140, 120, and 100 nm on an aluminum wire. (b) Three-dimensional rendering of a spiny dendrite of a human pyramidal neuron. Scale bar: 1 μm.

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