Solving the Challenges of Submicron MicroCT (NanoCT) for Delicate samples.

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Aims

Current bench top microCT instrumentation (CT) is able to image samples at sub-micron pixel resolution. This allows ever-finer 3D details to be acquired in a non-destructive manner from both dry and fully hydrated samples. Realizing sub-micron pixel resolution in soft tissues, however, brings added challenges for accurate 3D reconstruction. The hours required to collect the large number of individual x-ray projections for reconstruction requires great stability of both the source and sample, particularly at high magnifications. To achieve the 350 nm pixel resolution in the case of a SkyScan 1272, the sample is placed 12 mm from the source. The total energy dose, to which samples are exposed when close to the source, is however often neglected. A simple method to assist in successful imaging at sub-micron pixel resolution is proposed.

Method

In CT, magnification is the geometrical relationship of source to sample and source to camera. Positioning the sample very close to the source and the camera far away gives the greatest magnification and thus the smallest pixel resolution.

<table>
<thead>
<tr>
<th>INSTRUMENT</th>
<th>0.35</th>
<th>0.4</th>
<th>0.5</th>
<th>1.0</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1272</td>
<td>12 mm</td>
<td>15 mm</td>
<td>18 mm</td>
<td>37 mm</td>
<td>*45 to 150 mm</td>
</tr>
<tr>
<td>1172</td>
<td>-</td>
<td>-</td>
<td>21 mm</td>
<td>99 mm</td>
<td>121 mm</td>
</tr>
</tbody>
</table>

*Camera position: close to far.

Table 1. Source to sample distances.

Small biological tissues samples are extremely delicate and dry out very quickly. Mounting in a dissimilar amorphous supporting medium (fat) was found to be an advantageous method to support this type of sample without damage when imaging. Nevertheless a stained rat embryo mounted in palm fat (MP 27 °C) in a 2 mm diameter straw was not stable as the mounting medium had softened considerably by the end of a 2.5 hour scan at 1.0 µm pixel resolution. Tempered cocoa butter (MP 37 °C) was then tested as a mounting medium which improved the sample stability but still partly softened when scanning at 0.5 µm pixel resolution. The visual difference of heat on mounting media is illustrated in fig 1. Cocoa butter tube on the right and palm fat on the left, which becomes more translucent when heated.

Fig 1. Softening of mounting medium
Calculating, or determining, heating caused by x-ray ionizing interaction would be difficult and complex and would have to be on a specimen to specimen basis. It is felt the ionization effect of x-rays is not a substantial factor in heating tiny samples\(^1\) although beam damage in the form of bond breaking is known to occur in synchrotron imaging\(^2\). It is more likely heating comes from the generation of x-rays in the source as \(1\%\) of the input energy generates x-rays whereas \(99\%\) is lost to heat\(^3\) even if microfocus sources have the advantage of radial heat transfer\(^4\). Moving from a pixel resolution of \(1\ \mu m\) to \(0.5\ \mu m\) requires halving the source-sample distance but this quadruples any radiant heat load on the sample from the x-ray source. Heating causes drying and dimensional changes in delicate samples. Furthermore, small volumes and low mass samples have limited capacity to absorb radiated energy without a rise of temperature.

To better understand radiant heating effects on samples from a heat source, bench experiments were carried out using a 10 W halogen lamp at 7.5 V. This source is not intended to be equivalent to heat output from the x-ray microfocus source. Changes of temperature were measured in an air-filled 2 mm diameter straw at 1 minute intervals from 37 mm to 12 mm (Fig 2). There is relatively minimal temperature change until 17 mm from the heat source is reached where a distinct increase is noted. At 12 mm from the source, a sharp increase occurred. In the Bruker Skyscan 1272 a 37 mm distance corresponds to \(1\ \mu m\) pixel resolution and 12 mm to 0.35 \(\mu m\) pixel resolution. Samples which have a greater thermal heat capacity will take longer to reach a stable temperature than if measured in air.

Furthermore the percentage of the 39 degree incident x-ray beam the sample is exposed to increases as the sample moves closer to the source. To illustrate this, taking the area that filled the field of view at \(1\ \mu m\) pixel resolution (4.9 x 3.2 mm), and calculating the percentage of the beam irradiating this same area at smaller pixel resolutions the following numbers were obtained. At \(1\ \mu m = 3.0\%\), \(0.5\ \mu m = 12.1\%\) and \(0.35\ \mu m = 24.6\%\) of the beam (Fig 3a). There is a substantial increase in area of the beam impinging on the sample as the source to sample distance decreases. The graph (Fig 3b) clearly shows how significant this factor becomes below \(1\ \mu m\) pixel resolution.
Cooling the sample by means of a cold stage, or modifying the instrument to provide cooling air, are costly and could limit the sample distance to the source thus becoming problematic for work at sub-micron pixel resolutions. Mounting the sample in a medium that has greater heat capacity is certainly an option as has been shown. Nevertheless this proved not to work consistently as the heat flux could still be too great when working below 1.0 µm pixel resolution.

Self-adhesive Mylar tape reflects light & heat and is transparent to low energy x-rays. Wrapping tape around the sample tube and repeating the temperature vs. distance experiment limited the rate of temperature rise & peak temperature observed (Fig 4). The rate of cooling when removed from the heat source suggests the 20 µm aluminum layer of the Mylar tape is also acting as a heat sink as it has high thermal diffusivity thus dissipating heat away from the zone being heated by the source.

Results

As an example a 10.5 day old rat embryo, fixed in 4% PFA in PBS for 30 hours and stained with 0.3% PTA in 70% ethanol for 43 hours, was mounted using tempered cocoa butter in a 2 mm diameter straw. It was initially imaged with two layers of Mylar® tape around the outside of the straw in a Bruker SkyScan 1272 at 0.45 µm pixel resolution (Plate 1).

Plate 1.
Projection image (embryo length = 1.5 mm). (b) Trans-axial reconstruction in heart area. (c) Heart area enlarged. Excellent resolving of fine structures (arrowed), in particular, the single cell layer of forming endocardium. (E). (d) Good alignment profile. Scale bar = 100 µm.

The Mylar® tape was then removed and the sample imaged again using the same parameters. A 4 layer Aluminum foil filter was placed in front of the camera to approximate the filtration by the Mylar tape. Images were recorded at the beginning and end of the scan to
monitor sample movement. Initially the reconstructed trans-axial plane appears to be acceptable however on detailed inspection there is reduced resolution and quality (Plate 2).

Plate 2.
(a) Overlay of start and finish projection images to show 27 µm sample movement during the 2.5 hour scan. Green = start. Red = end. (b) Trans-axial reconstruction. (c) Enlarged area: 1. Collapsed amniotic sac. 2. Reduce sharpness. 3 Forming endocardium layer appears thicker. (d) Poor alignment profile. There is a distinct loss of fine structural detail. Scale bar = 100 µm.

Visualizing in 3D the dataset obtained with the sample in a Mylar covered straw illustrates the structural elements of an embryo.

Plate 3.
3D visualization. (a) Anatomy: Hd-Head, H-heart, A-amniotic sac, T-tail, (b) Planar cutaway to show internal structure. S-somite. Length of embryo = 1.5 mm.
Plate 4.
(a) An effective 3D thin slice in the area of heart. (b) Enlargement showing single cell layer of forming endocardium- E. (c) To fully appreciate the fine level of detail achieved, viewing the stereo image using red – green stereo glasses is required. Scale = 25 µm.

Conclusion
In this study, we show that the temperature rise in a sample will be dependent on the heat capacity of the sample plus mounting medium, as well as the specific heat capacity, heat load and ionization cross-section from impinging x-rays. The heat capacity of an object is more or less proportional to its size, or mass, and a characteristic of the material known as the specific heat. Simply stated, the heat load on the sample is the sum of the fraction of the beam impinging on the sample, the distance from the source and the power of the source to which is added the exposure time. Work in progress is to develop continuous recording of sample temperatures in the MicroCT instruments.

We have shown that a simple, inexpensive and effective method to reduce the sample heating effects in order to obtain higher quality results at sub-micron pixel resolution of delicate samples in microCT is to wrap the outside of the sample tube with layers of Mylar tape. This reduces the unwanted radiated heat from the x-ray source which otherwise results in sample movement or change in sample shape. To successfully image at sub-micron pixel resolutions, it is not only necessary to optimize the imaging parameters: kV, mA & ms but also to select the source-to-sample distance to manage heat load. Finally, it is important to mount in a manner which increases heat dissipation, thereby increasing the stability of the sample.

References:
1. Pers comm. Professor Leslie Allen, Melbourne University, Melbourne, Australia.