Bruker microCT

Chemical drying of specimens to enhance contrast

Method note

Goal

Enhancing the contrast in specimens by chemical drying.

Intoduction.

Often, biological samples are wet, either as a fresh specimen, or when they are preserved in EtOH or formaldehyde. Scanning these samples is difficult, especially when there is little to no contrast available. For calcified samples, there is enough difference between the Ca and the surrounding liquid, allowing direct scanning. However, for soft tissues, if preserved in EtOH or formaldehyde the density difference is very limited. This method note describes the sample preparation steps and scanner settings to enhance the contrast using chemical drying.

Chemical drying.

Chemical drying is a procedure also used when preparing samples for other imaging technologies. In the May 1997 issue of Microscopy today, 97-4, p16, Oshel et al already describe the use of Hexamethyldisilazane (HMDS) for specimen preparation for SEM. The same technique has been applied in microCT and has already been reported during the microCT User Meeting by several of our users:

Hayes et al, microCT User Meeting 2010

Tercedor et al. microCT User Meeting 2011, 2012, 2013, 2014

Willems et al. microCT User Meeting 2011

Faulwetter et al. microCT User Meeting 2012 and 2013

Verleden et al. microCT User Meeting 2013

The full abstracts can be downloaded from:

http://www.skyscan.be/company/usersmeeting2014A.htm

Chemical drying has several advantages:

- It's an easy procedure where no additional equipment is required. The drying of specimens is very reproducible and straightforward.
- It's a reversible procedure; so after scanning, the sample can still be embedded in paraffin for sectioning and H&E staining. Even imunno histochemical staining can still be possible. It is advisable to test this first as the epitopes can be destroyed due to the fixation and/or drying.

Before the sample is dried, first make sure it is properly fixed using formaldehyde or glutaraldehyde. For some samples, if stored for a sufficient time in 70% EtOH, no additional fixations may be necessary.

For fresh samples, follow these steps:

- Fixation
  - using formaldehyde (FA) or glutaraldehyde (GA)
  - typical 4% is used, for 12 hours or more
- dehydration in alcohol:
  - 70% EtOH, 2h
  - 80% EtOH, 2h
  - 90% EtOH, 2h
  - 100% EtOH, 8h
- immerse in Hexamethyldisilazane (HMDS), 2h
- Air dry, minimum of 2h

The duration of 2h per step can vary, and depends on the type of sample and the size (samples with higher permeability e.g. lungs, will require less time to dehydrate), however, 2 to 3 hours per step is a good starting point.

Fig 1. Hexamethyldisilazane (HMDS) can be purchased from Aldrich

Allow the HMDS to completely evaporate before proceeding to scanning the sample!

After air drying, the sample can have a thin white layer, and is quite fragile, so be careful when handling and mounting the sample.

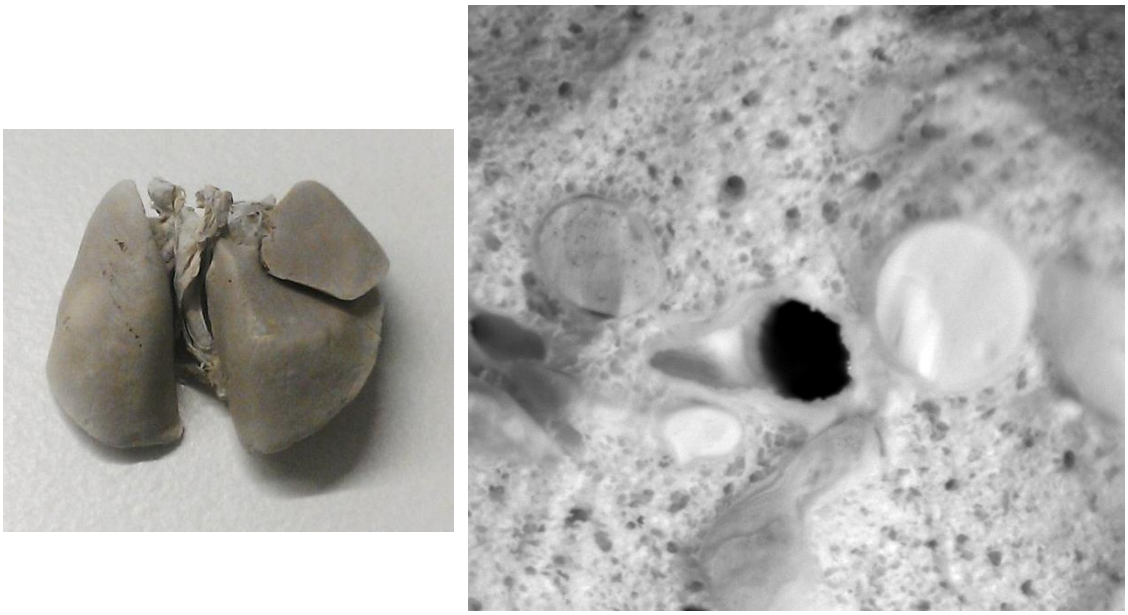

Fig 2. Macroscopic image of a mouse lung after HMDS drying. Left: entire lung. Right: higher magnification showing a large airway in the center and the alveoli.

Fig 3. Macroscopic image of an insect (left) and snake head (right) after HMDS drying. Note the white layer on the surface.

Mounting and scanning parameters.

As the majority of the objects that will be subjected to the chemical drying will be low dense, the mounting of the sample inside the scanner should be done is such a way that the sample is surrounded by a low dense container. Often used are small polystyrene tubes, which can easily be cut into any desired shape. The use of dental wax to fix the sample on a stage can also be done, however, keep in mind that the sample is fragile, so avoid the use of too much pressure as you might damage your sample.

Fig 4. Example of mounting a mouse lung lobe (left) and a snake head (right) in a tube of polystyrene.

Scanning conditions are typical without a filter (for e.g; the dried lung), or a small filter (0.125mm Al) for the insects and bigger samples. Adjust the pixel size, exposure time. If the scanning time has to be limited, and a choice has to be made between increasing the frame averaging or decreasing the rotation angel, then a smaller rotation angle is the preferred choice.

Cross sections showing the increased contrast due to chemical drying.

The first example shows the difference between a mouse lung scanned at 1.9um pixel size after fixing in formaldehyde, but without chemical drying vs the same mouse lung dried using HMDS as described above.

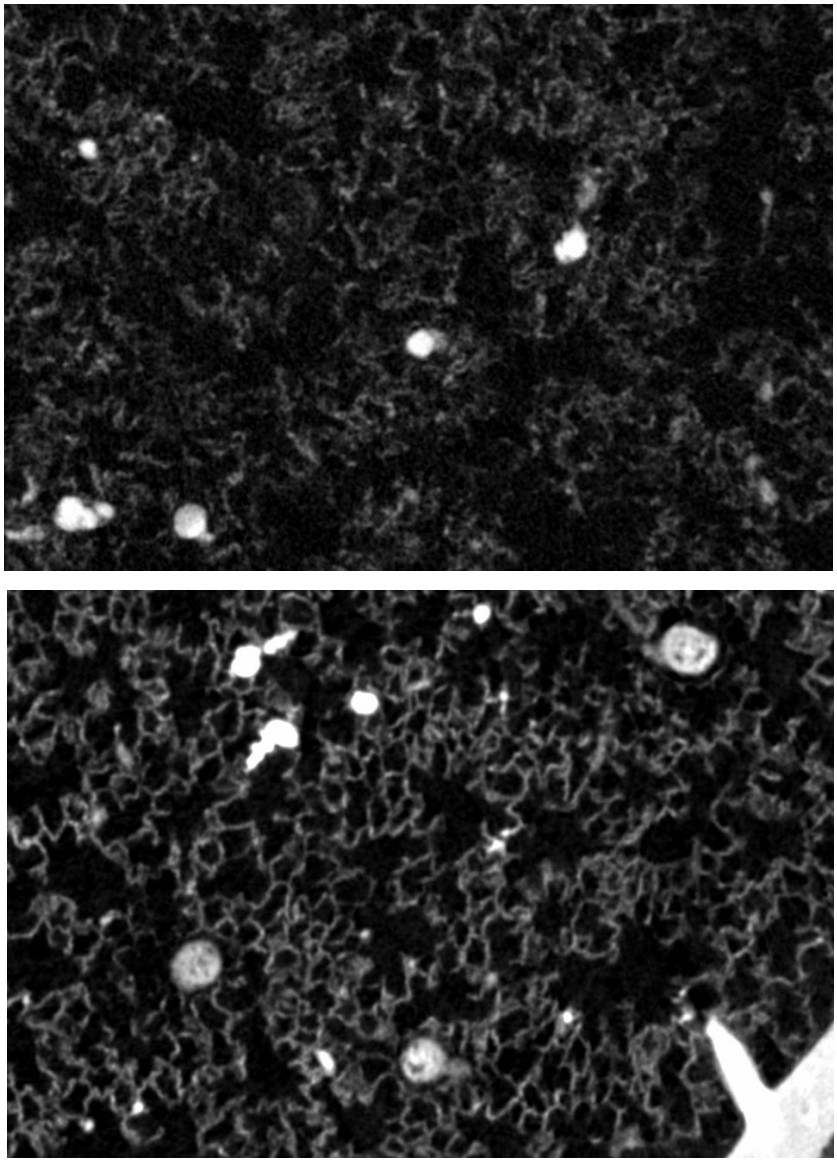

Fig 5. Mouse lung scanned at 1.9um pixel size with fixative still present in the lung (top) vs HMDS dried (bottom). Note the increase in contrast between the alveolar membranes vs the airspace between them.

The second example shows the result of a chemical drying procedure on the head of an insect scanned with 44kV, 0.125mm Al, 5um pixel size. Note the accumulation of the HMDS in the ganglia cells enhancing the contrast.

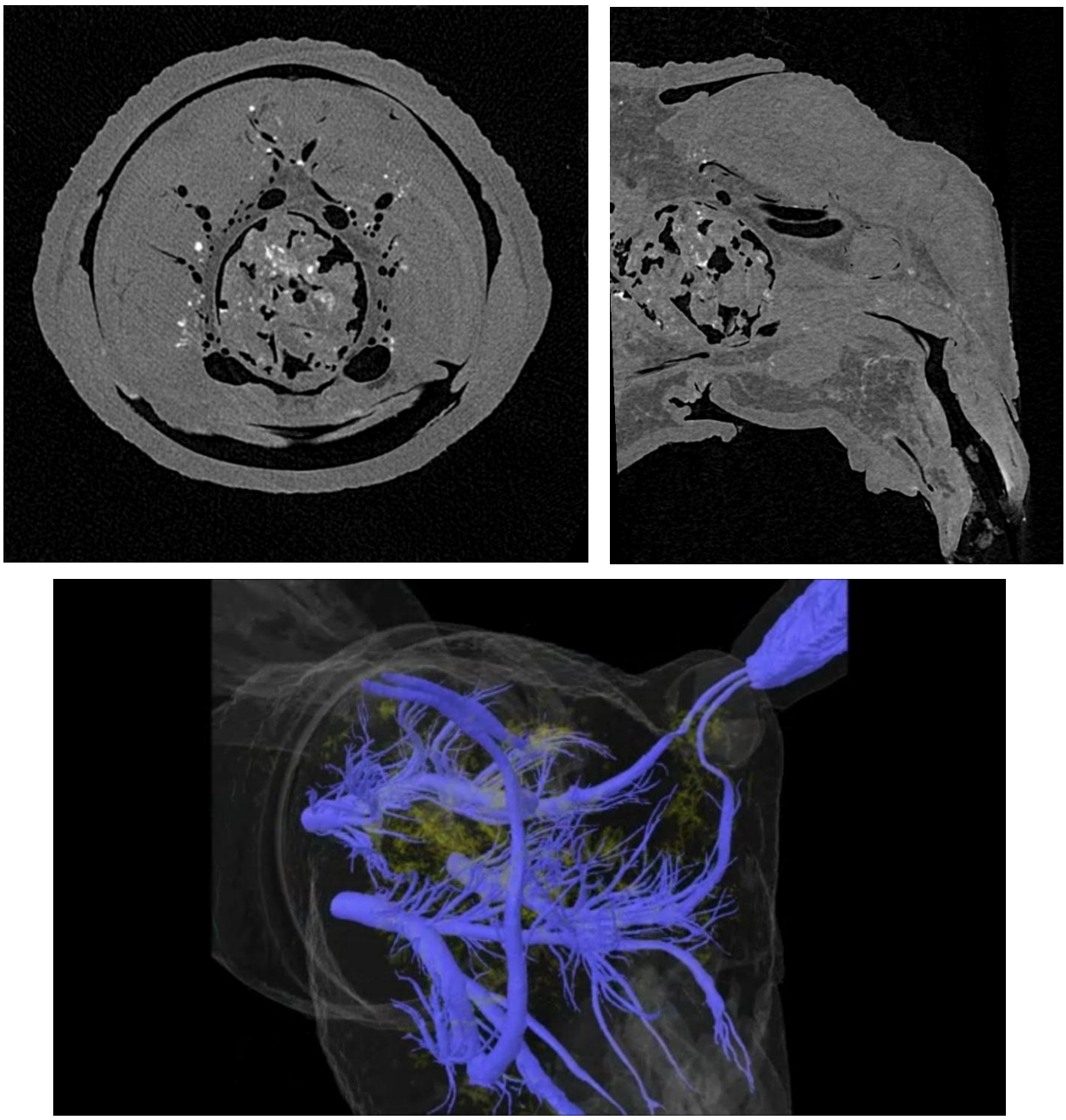

Fig 6. A cross section (top left) and a sagittal section (top right) through the head of an insect chemical dried using HMDS. 3D surface rendering visualization of the airway tubes in blue and the ganglia in yellow (bottom).

A third example showing the use of chemical drying of small vertebrates. A snake was scanned at 60kV, 0.5mm Al filter, 8um pixel size.

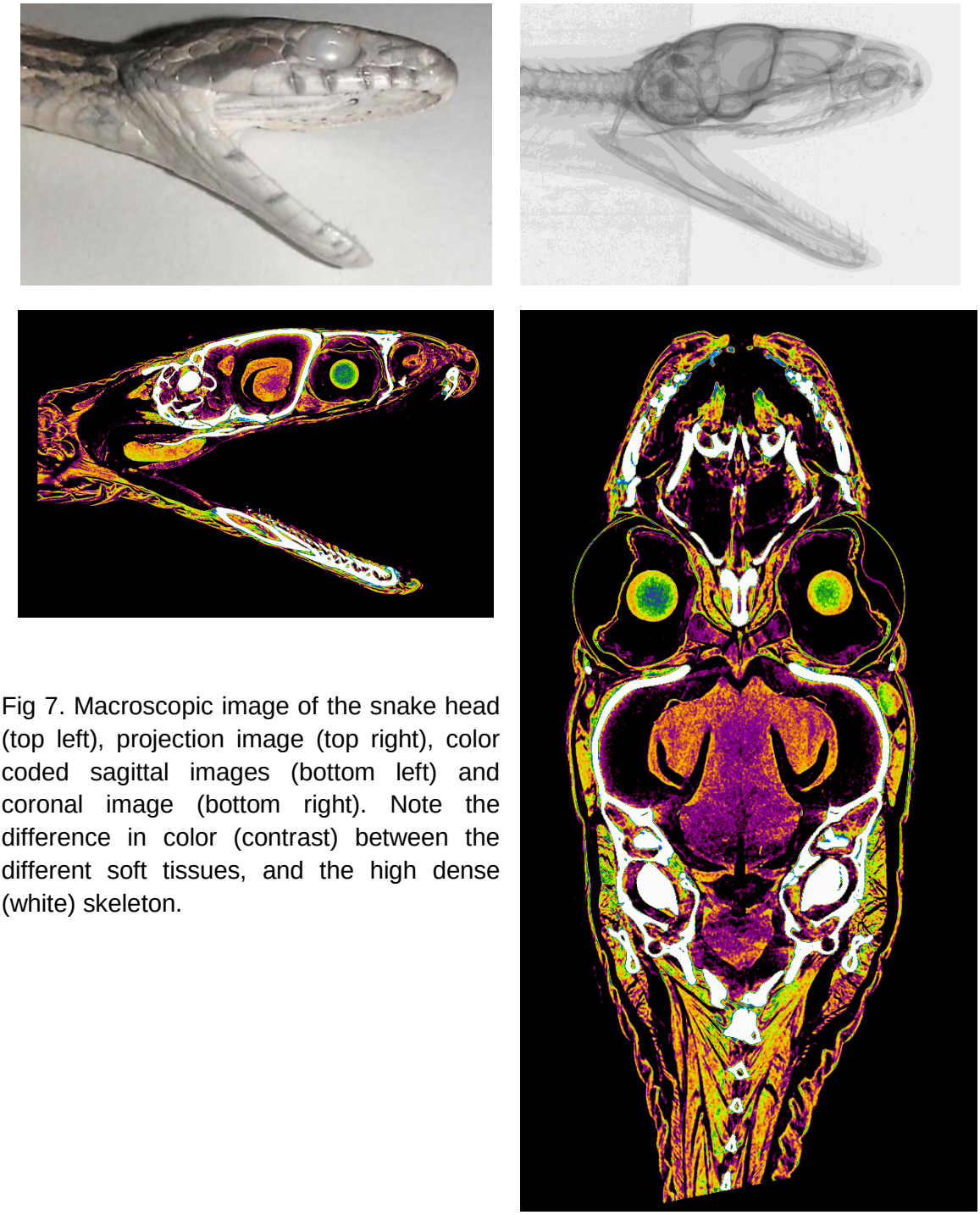

Fig 7. Macroscopic image of the snake head (top left), projection image (top right), color coded sagittal images (bottom left) and coronal image (bottom right). Note the difference in color (contrast) between the different soft tissues, and the high dense (white) skeleton.