Innovation with Integrity

BRUKER MICRO-CT ACADEMY

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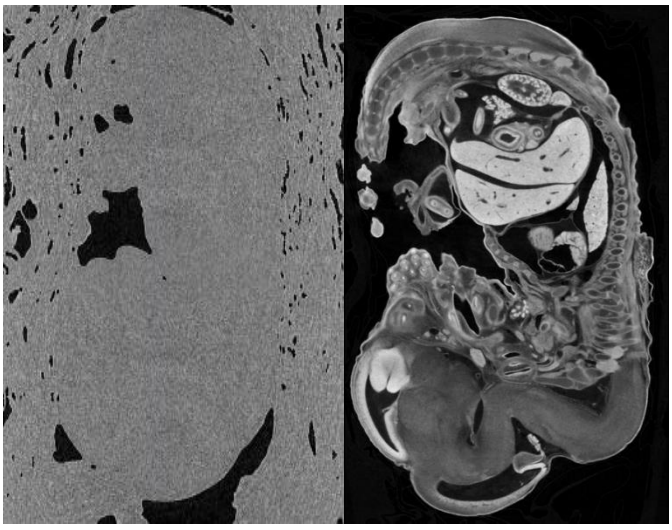
In this issue:

Welcome

Welcome to the first Bruker microCT Academy Newsletter of 2016! Best wishes for a successful year of imaging and research! This issue's topic could be called "microCT histology". It will focus on the staining of biological tissues with chemicals to enhance X-ray absorption contrast, where tissues are imaged by microCT *ex vivo* or *in vitro*, that is, not in the living animal. This represents part of the maturation of microCT as an imaging method for life sciences. By analogy, the development of visual colored stains for microscope histology of biological tissues began with Anton van Leeuwenhoek in 1673 who used a dye of saffron crocus bulb extract (in brandy) to enhance the color of specimens!

When are stains needed for microCT?

It is when biological tissues lack contrast in X-ray absorption allowing visualization by microCT. Conventional X-ray imaging (leaving aside for now phase contrast techniques) requires different structural components to have differing X-ray absorption for structures to be contrasted; microCT is no exception. Figure 1 shows the difference between microCT images of a mouse embryo obtained without staining (a) and after staining in phosphotungstic acid or "PTA" (b).



(a) (b)

Figure 1. MicroCT scans of a mouse embryo, pre-ossification, (a) without staining and (b) after staining in a phosphotungstic acid in ethanol solution.

Whereas in microscope histology stains confer visual color, for microCT stains – or "contrast agents" – confer X-ray contrast. Thus the stain must contain a heavy element with high atomic number (Z) for strong X-ray attenuation. Tungsten in PTA (Z=74) fits the bill for this. Stains must also have differential tissue specificity. Some classic histology stains also work for microCT. PTA is an example; it has long been used as both fixative and histology stain [i, ii, iii] for collagen and fibrin. Metscher [iv] has showed PTA also to be an effective microCT stain. His paper has become a classic reference for microCT study of soft tissue with its helpful instructions on use of PTA and other contrast agents (such as iodine). PTA can be used dissolved either in water or in ethanol. Lendrum *et al.* [v] observed "that a water-soluble dye (e.g. PTA) dissolved in alcohol then behaves as if it were of a smaller molecular size." Thus when in ethanol PTA may pervade into finer tissue structures. The group of Dr Mary Barbe in Temple University, Philadelphia, USA have added important refinements, such as to thoroughly wash out buffer (with water) from samples prior to staining to ensure optimal tissue pervasion of PTA [vi, vii].

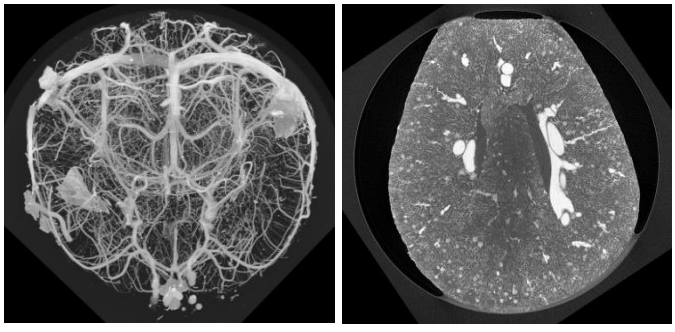
Staining mouse embryos with PTA can be found in the method note "*MN007_Embryo staining with PTA for exvivo micro-ct imaging*". Beyond PTA, Lugol's iodine is also an effective microCT stain; Degenhardt [viii] carefully optimized this method to maximize penetration

while minimizing shrinkage. They found that "staining with 25% Lugol for 48 hours resulted in complete and uniform tissue penetration with minimal shrinkage"

Pauwels *et al.* [x], from 28 chemicals tested, found mercuric chloride $HgCl_2$ to be promising for microCT imaging of soft tissues (as well as PTA and iodine). A previous newsletter (1:10, Dec2014) described a critical point drying method using HMDS (hexamethyldisilizane) which also enhances tissue contrast due to differential desiccation and some contrast provided by silicon (Z=14). The method note on HMDS: "*MN070_Chemical drying of specimens to enhance contrast*"

- Blood vessels: contrast and casting

Finally, contrast agents are available for infiltrating blood vessel networks for studying vascularization and angiogenesis. A popular one is MicroFil (FlowTech Inc.), a casting resin containing lead chromate; its use was pioneered by the Mayo Clinic, Minnesota, USA [ix]. Figure 2a shows mouse brain vasculature contrasted by MicroFil. Another contrast agent effective for the smallest capillaries is the iodine based Angiofil (Fumedica AG, Switzerland) as shown in figure 2b for mouse renal vasculature; such results were presented by Dr Ruslan Hluschuk (Bern, Switzerland) at the 2015 user meeting of Bruker microCT. Aside from commercial products, barium sulphate ($BaSO_4$) mixed in agar gel also works well for blood vessel contrasting [xi].



(a) (b)

Figure 2. MicroCT blood vessel contrast provided by (a) MicroFil for the mouse brain, and (b) Angiofil for the mouse kidney. Both scans were by the SkyScan1172.

i Hall (1949) *Journal of Biol.Chem.179*(2): 857-864

ii Greenlee et al. (1966) *The Journal of cell biol. 30*(1): 59-71.

iii Altshuler et al. (1949) *The Amer.journal of pathol.25*(5):1061

iv Metscher (2009) *BMC physiology 9*(1): 11

v Lendrum (1962) *Journal of clinical pathology 15*(5): 401-413.

vi Vegesna (2012) *World journal of gastroenterol.: 18*(32):4317.

vii Vegesna (2013) *Neurogastroenterology & Motility 25*(1): 53.

viii Degenhardt (2010) *Circulation: Cardiovasc.Imaging 3*(3):14.

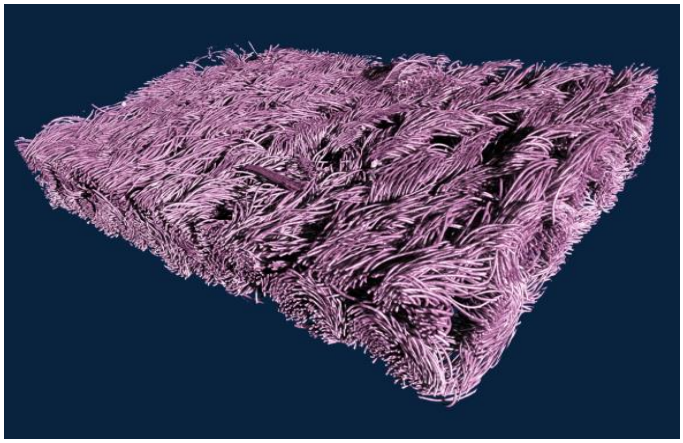
ix Jorgensen (1998) *American Journal of Physiology-Heart and Circulatory Physiology* 275(3): H1103

x Pauwels E *et al.* (2013) *J Microscopy* 250(1): 21-31

xi Aragones J et al. (2008) *Nature genetics*, 40(2): 170-180

- Image of the Month

MicroCT scan of a woven fabric, SkyScan1272, 1.25 micron pixel. Volume rendered using CTVox.



- Bruker microCT News

- Find out more information about the 2016 Bruker microCT user meeting! Please don't forget to submit your paper before March 18 to earn up to three free nights of accommodation.
- Bruker microCT is proud to announce our worldwide installation base has overcome 1000 X-ray microtomography systems, and also more than 50 optical projection tomography systems above that!

- Upcoming Events

Bruker microCT will participate with an exhibit in the forthcoming conferences. Please click the link below for more information. We hope to see you there!

iCT	Feb. 09 – Feb. 12	Wels, Austria
IBMS	Feb. 28 – Mar. 01	Bruges, Belgium
EMIM	Mar. 08 – Mar. 10	Utrecht, Netherlands
AACR	Apr. 16 – Apr. 20	New Orleans, USA
ATS	May 13 – May 18	San Francisco, USA
ECTS	May 14 – May 17	Rome, Italy
WBC	May 17 – May 22	Montreal, Canada