

MicroCT of Biological Soft Tissue

A.E.Weston¹, A. Vezaro^{2§}, A. Yabe^{3§}, I. Orriss⁴, T. Arnett⁴, N.Corps⁵, L.M. Collinson¹

¹ Electron Microcopy Unit, Cancer Research UK London Research Institute, London, UK. anne.weston@cancer.org.uk

² Developmental Genetics, Cancer Research UK London Research Institute, London, UK.

³ Molecular Neuro Patho Biology, Cancer Research UK London Research Institute, London, UK.

⁴ Department of Cell and Developmental Biology, University College London, London, UK

⁵ e2v Scientific Instruments, Bucks, UK

§ These authors contributed equally to this work

Aims

The internal structure of large biological samples cannot be imaged at high resolution using traditional light and electron microscopes. The SkyScan 1174 and 1172 microCT systems were assessed for imaging of biological soft tissue. Sample preparation protocols were tested for X-ray imaging, and microCT was then applied to two important biological questions. Firstly, the development of body segments in vertebrates was investigated. Somites are precursors of the vertebral column that form in synchrony on either side of the embryonic midline in a reiterative manner¹. A genetic clock regulates this process controlling the timing of somite development². Disruption to this development can cause abnormal vertebral segmentation resulting in defects and disorders such as spondylocostal dysostosis^{1,2}. Secondly, the gross morphology of the heart and brain were investigated in kidins220 knockout (KO) mice. Kidins220 is a neurotrophin that can affect neuronal growth and survival and heart development^{3,4,5}.

Method

Embryos and isolated brains were prepared as for routine Scanning Electron Microscopy (SEM). Samples were fixed in 4% paraformaldehyde/ 2.5% glutaraldehyde in 0.1M phosphate buffer for 1 hour and dehydrated stepwise through ethanol to 100% prior to critical point drying from 100% acetone. Samples for the investigation of body segment development were post-fixed in 2% osmium tetroxide prior to dehydration to add contrast to the sample. MicroCT analysis was performed on whole chick and mouse embryos, mouse embryo torsos and isolated brains using the SkyScan 1174 and 1172 instruments (SkyScan, Kontich, Belgium) set at 50KV and 800µA and 40KV and 250µA respectively and at a resolution between 2.8-6.8µm. The images were reconstructed using the SkyScan NRecon program and analysed using SkyScan CTAn software. Volume rendering was performed using Disect software (Disect Systems Limited) and videos created from individual images using Quicktime Pro.

Results

Samples were prepared for microCT using standard SEM sample preparation protocols. Drying of samples increased contrast of internal structures compared to hydrated samples. Post-staining with osmium, which binds to lipids to produce an electron-dense stain, did not give a noticeable improvement in contrast over unstained dry samples, which had good ultrastructural detail of the embryonic tissue. In studies of the development of body segmentation in chick and mouse embryos, traditional scanning electron microscopy gave insufficient detail of somites as they

were masked by the skin of the embryo. This was a particular problem in larger, later stage embryos where somite definition was obscured in all regions other than at the very tip of the tail. Following reconstruction and volume rendering of microCT data, we were able to easily view and count somites in all embryo stages. Quantification indicated that development of the body segments is complete after 5 days in chick embryos and initial data suggests 13.5 days in mouse embryos (Fig.1), which gives a baseline to assess mutations in genes involved in vertebrate development.

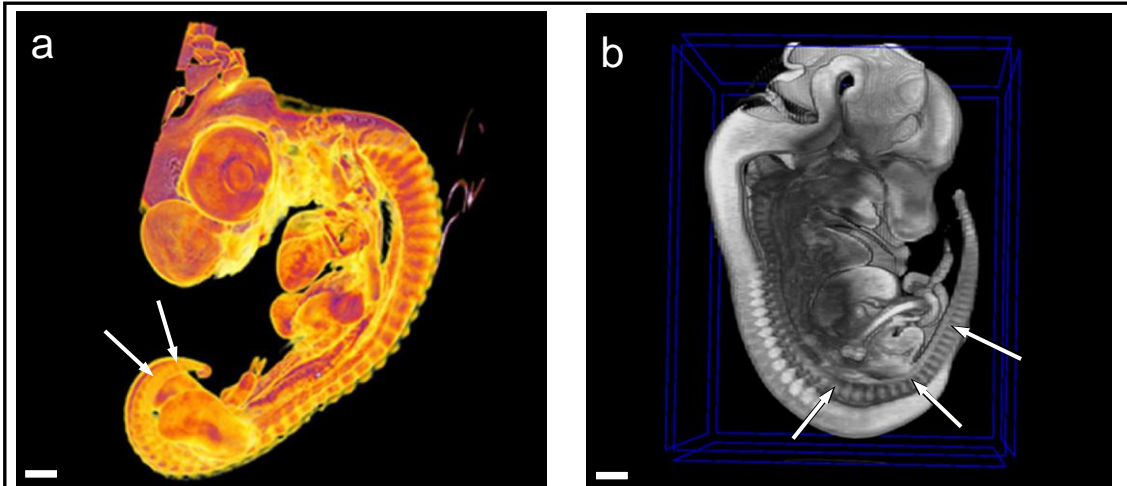


Figure 1. MicroCT reveals internal structure of chick and mouse embryos at high resolution. a) 3D rendered volume of an E4.5 chick embryo imaged with the Skyscan 1174. Somites, the smallest of which cannot be seen using SEM, are clearly visible (arrows). b) 3D rendered volume of an E13.5 mouse embryo imaged with the Skyscan 1172. Somites, which cannot be seen using SEM due to the thickness of the skin, are clearly visible (arrows). Bars = 1mm

When viewing reconstructed and volume rendered images of mouse embryos we were able to confirm previous observations of gross morphological changes in the brain and heart of kidins220 KO embryonic mice (Fig.2a,b).

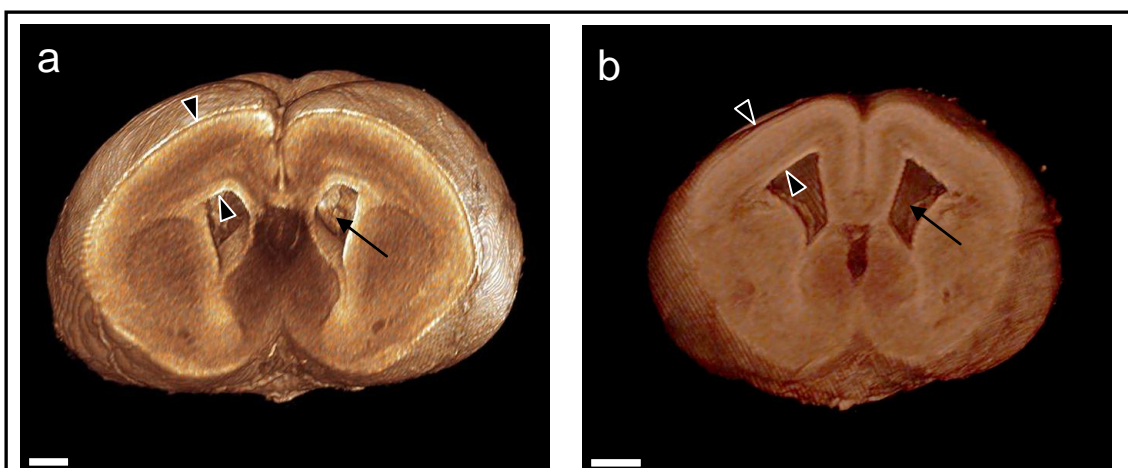


Figure 2. Analysis of soft tissue morphology in mouse mutants. Images taken from reconstructed Skyscan 1172 microCT scans showing 3D rendering of a) wildtype and b) kidins220 KO embryonic mouse brain. The structure of the kidins220 KO mouse brain is thinner than that of the wildtype (arrowheads) resulting in the ventricles within the brain being larger in the kidins220 KO mouse (arrows). Bars = 1mm

Conclusion

MicroCT has enabled us to quantitatively survey embryonic development in a manner that has not been possible using light microscopy and SEM. We have been able to view soft tissues *in situ* at high resolution and create virtual 3-dimensional models of entire embryos in a non-destructive manner. We have applied this technique to several important biological questions.

MicroCT has allowed us to effectively locate areas of interest within large intact biological samples. In the future, we plan to develop specific contrast agents targeted to cells and tissues of interest including components of complex systems such as the blood vascular and nervous systems of vertebrates. We believe that there is potential to use microCT as part of a multi-scale imaging protocol to analyse cellular structures at nanometre resolution within the context of whole organisms in healthy and diseased states, by linking light, X-ray and volume electron microscopy^{6,7}.

References:

1. Sparrow, D.B., Chapman, G., Turnpenny, P.D. & Dunwoodie, S.L. Disruption of the somatic molecular clock causes abnormal vertebral segmentation. *Birth Defects Res C*. 81, 2, 93-100, 2007.
2. Shifley, E.T. & Cole, S.E. The vertebrate segmentation clock and its role in skeletal birth defects. *Birth Defects Res C*. 81, 2, 121-33, 2007.
3. Iglesias, T., Cabrera-Poch, N., Mitchell, M.P., Naven, T.J., Rozengurt, E. & Shiavo, G. Identification and cloning of kidins220, a novel neuronal substrate of Protein Kinase D. *J. Biol. Chem.* 275, 51, 40048-56, 2000.
4. Skaper, S.D. The biology of neurotrophins, signalling pathways and functional peptide mimetics of neurotrophins and their receptors. *CNS Neurol Disord Targets*. 7, 1, 46-62, 2008.
5. Carporali, A. & Emanuelli, C. Cardiovascular actions of neurotrophins. *Physiol. Rev.* 89, 279-308, 2009.
6. Armer, H.E.J., Mariggi, G., Png, K.M.Y., Genoud, C., Monteith, A.G., Bushby, A.J., Gerhardt, H. & Collinson, L.M. Imaging transient blood vessel fusion events in zebrafish by correlative volume electron microscopy. *PLOS One*. 4, 11, e7716, 2009
7. Weston, A.E., Armer, H.E.J. & Collinson, L.M. Towards native state imaging in biological context in the electron microscope. *JOCB*, Nov. 15 (e-pub ahead of print).