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High resolution contrast enhanced micro-computed tomography to identify the cardiac conduction system in congenitally malformed hearts: valuable insight from a hospital archive

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Introduction:

We obtained National Health Service ethical approval to image congenitally malformed hearts from the Alder Hey archive, Liverpool, UK. Samples consented and archived in the 1970s were scanned by iodine-enhanced micro-computed tomography (micro-CT) (1), producing 3D datasets with isometric voxels of 27-38 microns.

The morphology of these important samples is preserved permanently. Digital micro-CT images do not degrade. They can be viewed in any sectional plane or 3-dimensional orientation and contain information that allows us to segment and visualise the cardiac conduction system (CCS). This represents a stepwise change for investigation of archived samples. Data can be distributed as digital files or 3-D prints, and viewed with commonly available software. Thus anatomists, surgeons, cardiologists, and educators benefit from this new information.

The samples were free of residing blood, and probably perfused before preservation. They were immersed in 3.75% iodine/potassium iodide (I₂KI) in PBFS for two weeks, refreshing solutions at one week (1).

Figure 1:

Sample from 5 day-old neonate scanned in Nikon XTEK Custom Bay micro-CT system, MXIF (University of Manchester). Scan conditions were previously described (1). Differential iodine absorption discriminates fat, working myocardium, conducting and fibrous tissues with decreasing levels of attenuation respectively. Before scanning the sample was saline rinsed, contained in plastic wrap then after scanning returned to formaldehyde to remove iodine. Datasets examined using Amira (5.3.3), and objective segmentation (1). The sample shows usual atrial arrangement, double inlet atrioventricular connection to a dominant LV and discordant ventriculo-arterial connections. The anteriorly-positioned hypoplastic right ventricle directly supplies the aorta. A-D show cross-sectional volume renderings with cutting planes shown in centre panel. A) aorta from RV, B) pulmonary trunk from LV and VSD (double arrow) C) dual inlets to LV, D) short axis view of VSD (double arrow) and common atrioventricular communication. ~27 microns isometric spatial resolution. Scale bar 3 mm.

Figure 2:

Virtual histology from micro-CT of heart with double inlet left ventricle as in Fig 1. Short axis (A, C) and 4-chamber (B, D) views, showing specialised tissue of the regular AV node (A, B), and the retropulmonary node and atrioventricular conduction axis (C,D). We previously validated the use of micro-CT to identify the CCS with subsequent histological confirmation (1 and papers therein). The micro-CT technique has many advantages, especially when destructive sectioning is not possible. Analogous samples from this archive were used in 1974 (2) to study the AV conduction axis in double inlet left ventricle. Two blocks from each heart, one containing the interatrial septum and another the ventricular septum and ventricular septal defect were required for histological analysis taking many days. By contrast, we identified the CCS in relation to the intact cardiac anatomy in minutes following approximately 50 minute scan. Scale bars 3 mm and 1 mm.

Figure 3:

Volume renderings showing the 3D disposition of the CCS in double inlet left ventricle with discordant ventricular arterial connections. We made virtual sections and 'marked' regions of interest in 2D micro-CT slices and tracked their 3D course using segmentation techniques as previously described (1). A) short axis, retropulmonary node (red), B) short axis, regular node (purple), C) left ventricular long axis view, left bundle branch (blue) D) Right ventricular long axis view, right bundle branch (pink). Cutting planes shown in centre panel. Scale bar 3 mm.

Figure 4:

Volume rendered cross sections at basal (upper panel) and equatorial (lower panel) levels with corresponding myocyte orientation maps colour coded according to the absolute helical angle. Zero

degrees (blue colour) represents myocyte chains running in the plane of section (circumferential orientation) and 90 degrees (red colour) represents myocytes running approximately in the long axis of the ventricles. Double arrow indicates ventricular septal defect. As seen in the normal adult human heart (1), the colour maps confirm the 'helical' organisation within the walls of left ventricle, septum and also the hypoplastic and incomplete right ventricle. Scale bar 3 mm.

Figure 5:

High resolution 3D prints derived from micro-CT data. Sectioned volume renderings of double inlet left ventricle as presented in Fig. 3; left (A) and right (B) ventricular long axis views showing the 3D disposition of the cardiac conduction system. (C, D) Corresponding life-sized semi-flexible single colour 3D print. (E, F) a corresponding 3D print with transparent and flexible working myocardium and solid coloured (yellow) material incorporated into the print to depict the CCS according to the segmentation of the micro-CT data. (F) illustrates the resolution and fidelity of such a printing process to locate in 3-dimensions the components of the conduction system. The print presented in (E) can be cut and sutured. Such printed models can facilitate surgical planning and training, patient consultations, and medical education. Scale bar 3 mm.

Figure 6:

Sample from a second case, a 5 month old male with atrio-ventricular septal defect. Sample processed and scanned as in Fig 1 and as described in detail previously (1). Sectioned volume renderings in 4-chamber view, viewed from anterior (A) and posterior (B) aspects. (C, D) corresponding volume renderings showing the sinus node in yellow in panel C, and the atrioventricular conduction axis in green in panels C and D. In this case some remaining surgical repair material was in place. An unfinished septal patch was segmented and is highlighted in blue. Imaged at a whole heart isometric spatial resolution of ~38 microns. Local tomography was performed on all samples giving isometric spatial resolutions of ~15-24 microns, these data are not presented here, but imply that imaging of smaller fields of view may be imaged non-destructively at near cellular resolution. Scale bar 3 mm.

Movie with commentary from Professor Robert Anderson illustrating the ability in a specialist 3D graphics program such as Amira to 'fly through' the dataset to achieve even better understanding of the anatomical relations.

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Abbreviations used in Figures: Ao, aorta: AS, atrial septum: AVCA, atrio-ventricular conduction axis: LA, left atrium: LAV, left atrio-ventricular valve: LBB, left bundle branch: LV, left ventricle: MS, muscular septum: PT, pulmonary trunk: RA, Right atrium: RAV, right atrio-ventricular valve: RBB, right

bundle branch: RN, regular node: RPN, retro-pulmonary node: RV, right ventricle: VSD, ventricular septal defect.