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IMAGING VIGNETTE

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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WE OBTAINED NATIONAL HEALTH SERVICE ETHICAL APPROVAL TO IMAGE CONGENITALLY MALFORMED hearts from the Alder Hey archive, Liverpool, United Kingdom. Consented samples that were archived in the 1970s were scanned by Iodine-enhanced micro-computed tomography (CT) (1), producing 3-dimensional (3D) datasets with isometric voxels of 27 to 38 μm.

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 3D orientation and contain information that allows us to segment and visualize the cardiac conduction system (**Figures 1 to 3 and 6**) (Online Videos 1, 2, 3, 4, and 5) and display the alignment of cardiomyocytes (**Figure 4**). This represents a stepwise change for investigation of archived samples. Data can be distributed as digital files or 3D prints (**Figure 5**) and can be viewed with commonly available software. Thus, anatomists, surgeons, cardiologists, and educators benefit from this new information.

The samples were free of residing blood, and were probably perfused before preservation. They were immersed in 3.75% iodine/potassium iodide in phosphate buffered formal saline for 2 weeks, refreshing solutions at 1 week (1).

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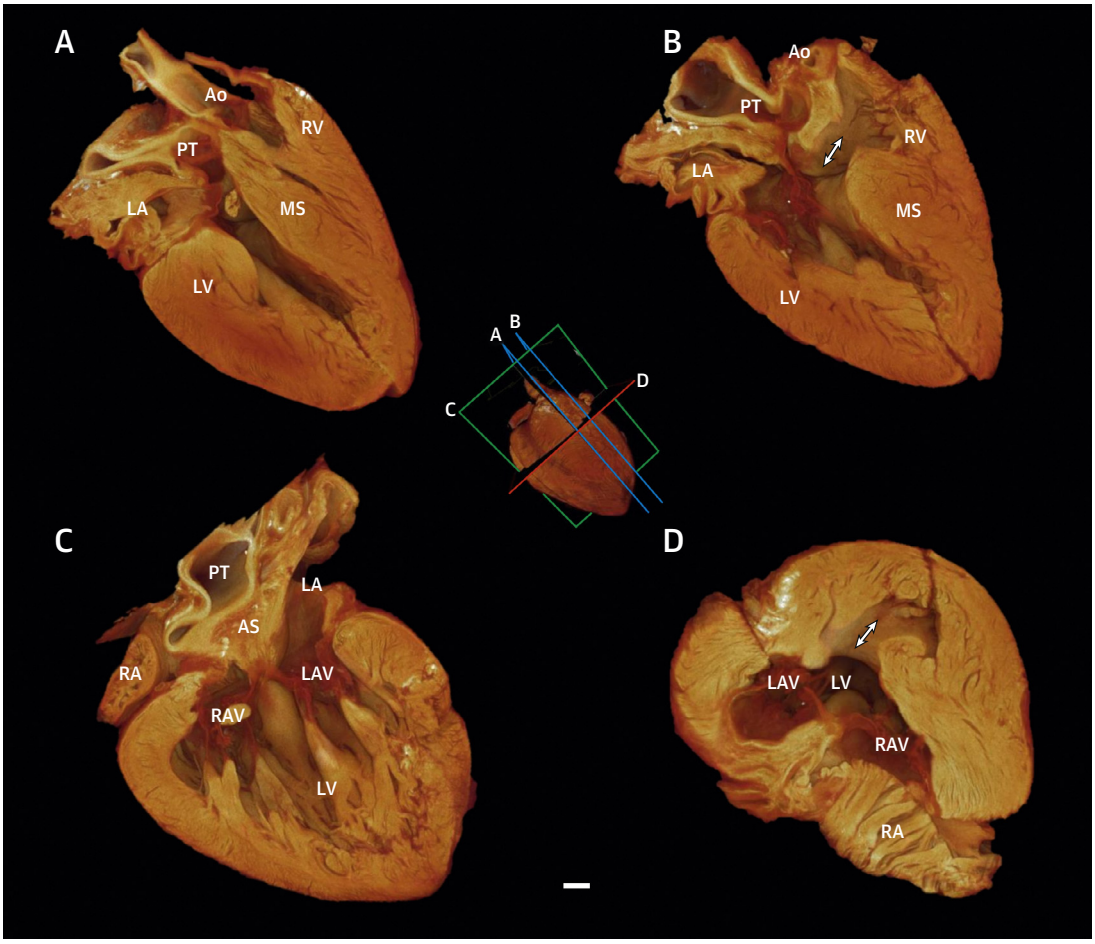
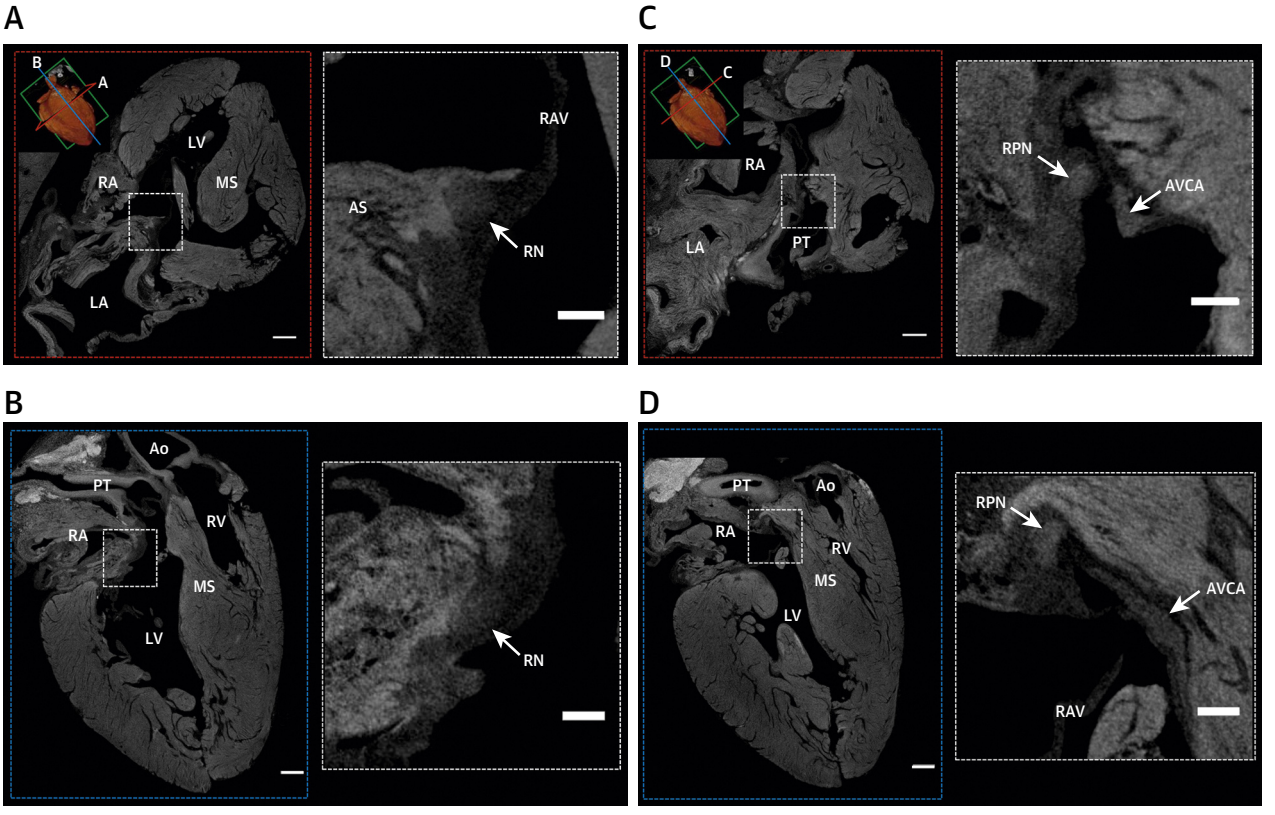
FIGURE 1 Sample From a 5-Day-Old Neonate Scanned in Nikon XTEK Custom Bay Micro-CT System



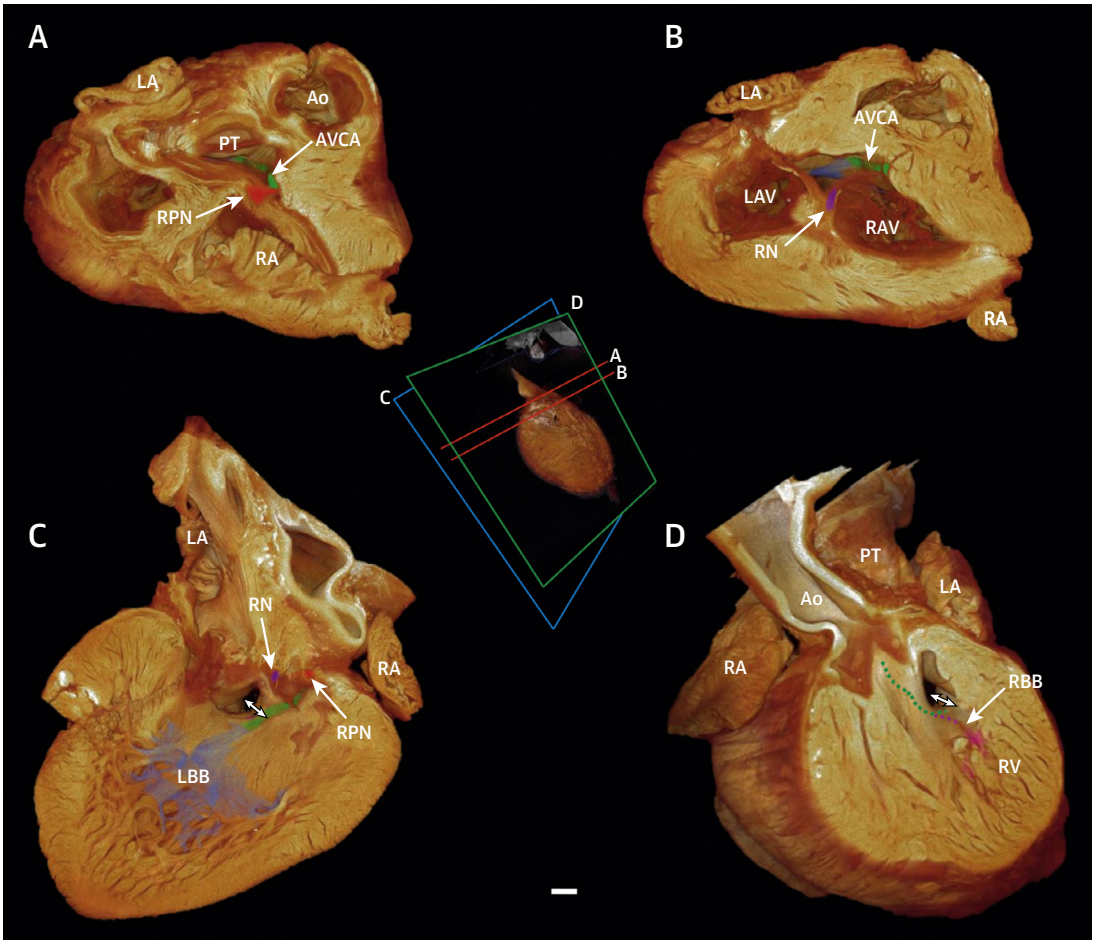
Sample scanned in Nikon XTEK Custom Bay micro-CT system, MXIF (University of Manchester). Scan conditions were previously described (1). Differential iodine absorption discriminates fat, working myocardium, and conducting and fibrous tissues with decreasing levels of attenuation. Before scanning, the sample was saline rinsed, contained in plastic wrap, then after scanning was returned to formaldehyde to remove iodine. Datasets examined using Amira 5.3.3 (Thermo Fisher, Waltham, Massachusetts), and objective segmentation (1). The sample shows usual atrial arrangement, double inlet atrioventricular connection to a dominant left ventricle (LV), and discordant ventriculoarterial connections. The anteriorly-positioned hypoplastic right ventricle directly supplies the aorta. **(A to D)** Cross-sectional volume renderings with cutting planes shown in the **center panel**. **(A)** Aorta (Ao) from right ventricle (RV); **(B)** pulmonary trunk (PT) from LV and VSD **(double arrow); (C)** dual inlets to LV; **(D)** short-axis view of VSD **(double arrow)** and common atrioventricular communication. ~27 μm isometric spatial resolution. Scale bar 3 mm. LA = left atrium; LAV = left atrial valve; MS = muscular septum; RA = right atrium; RAV = right atrial valve; VSD = ventricular septal defect.

FIGURE 2 Virtual Histology From Micro-CT of Heart With Double Inlet Left Ventricle



Virtual histology from micro-CT of heart with double inlet left ventricle as in Figure 1. Short-axis **(A and C)** and 4-chamber **(B and D)** views showing specialized tissue of the regular atrioventricular node (RN) **(A and B)**, and the retropulmonary node (RPN) and atrioventricular conduction axis (AVCA) **(C and D)**. We previously validated the use of micro-CT to identify the CCS with subsequent histological confirmation (Stephenson et al. [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 AVCA in double-inlet LV. 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-min scan. Scale bars 3 and 1 mm. Abbreviations as in Figure 1.

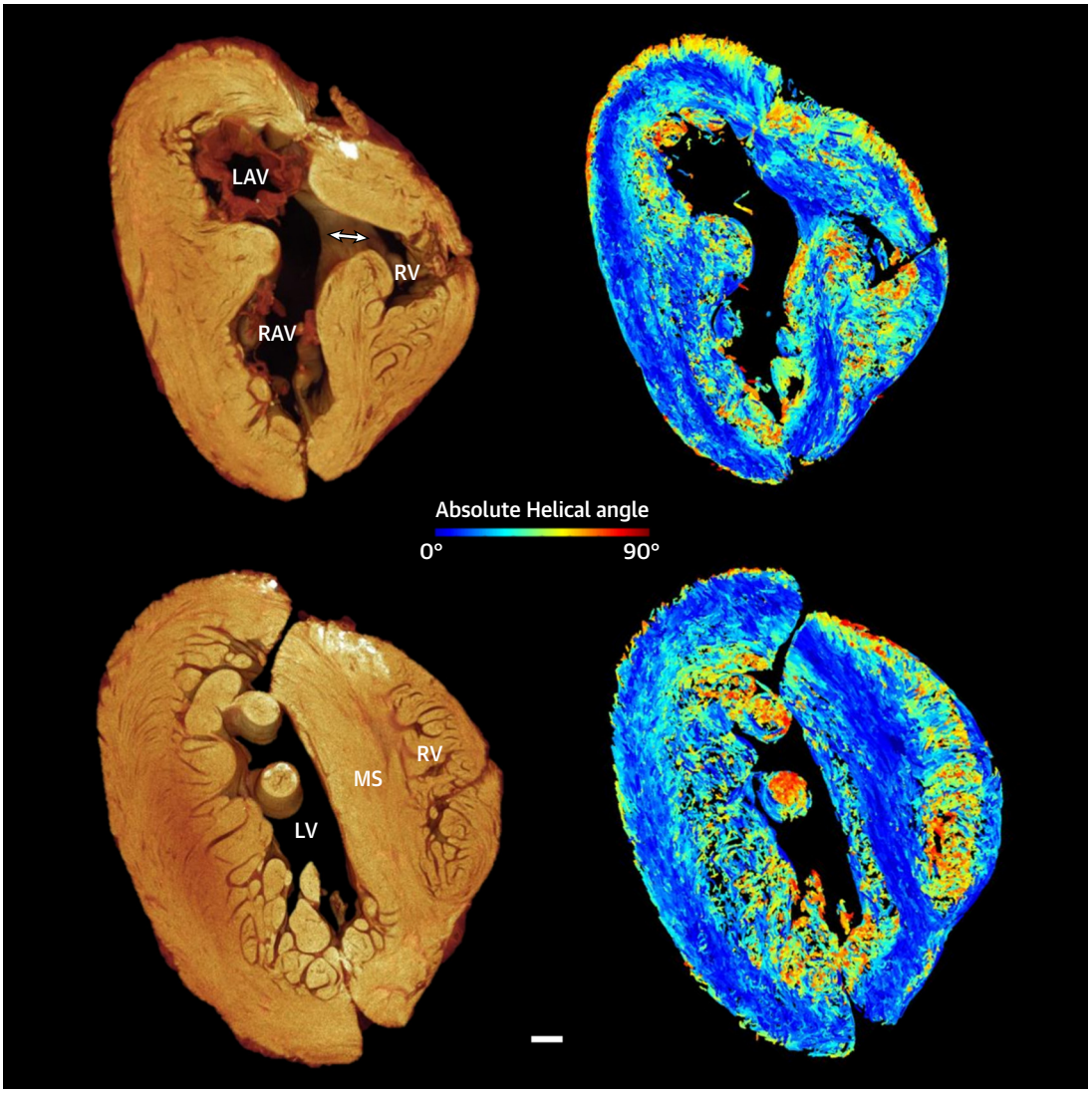
FIGURE 3 Volume Renderings Showing the 3D Disposition of the CCS in Double-Inlet LV With Discordant Ventricular Arterial Connections



We made virtual sections and "marked" regions of interest in 2-dimensional micro-CT slices and tracked their 3-dimensional course using segmentation techniques as previously described (1). **(A)** Short axis, retropulmonary node **(red)**, AVCA **(green)**; **(B)** short axis, regular node **(purple)**; **(C)** LV long-axis view, left bundle branch (LBB) **(blue)**; **(D)** RV long-axis view, right bundle branch (RBB) **(pink)**. **Dotted line** shows position of AVCA behind incomplete septum. Cutting planes shown in **center panel**. Scale bar 3 mm. See Online Videos 1, 2, 3, 4, and 5. Abbreviations as in **Figures 1 and 2**.

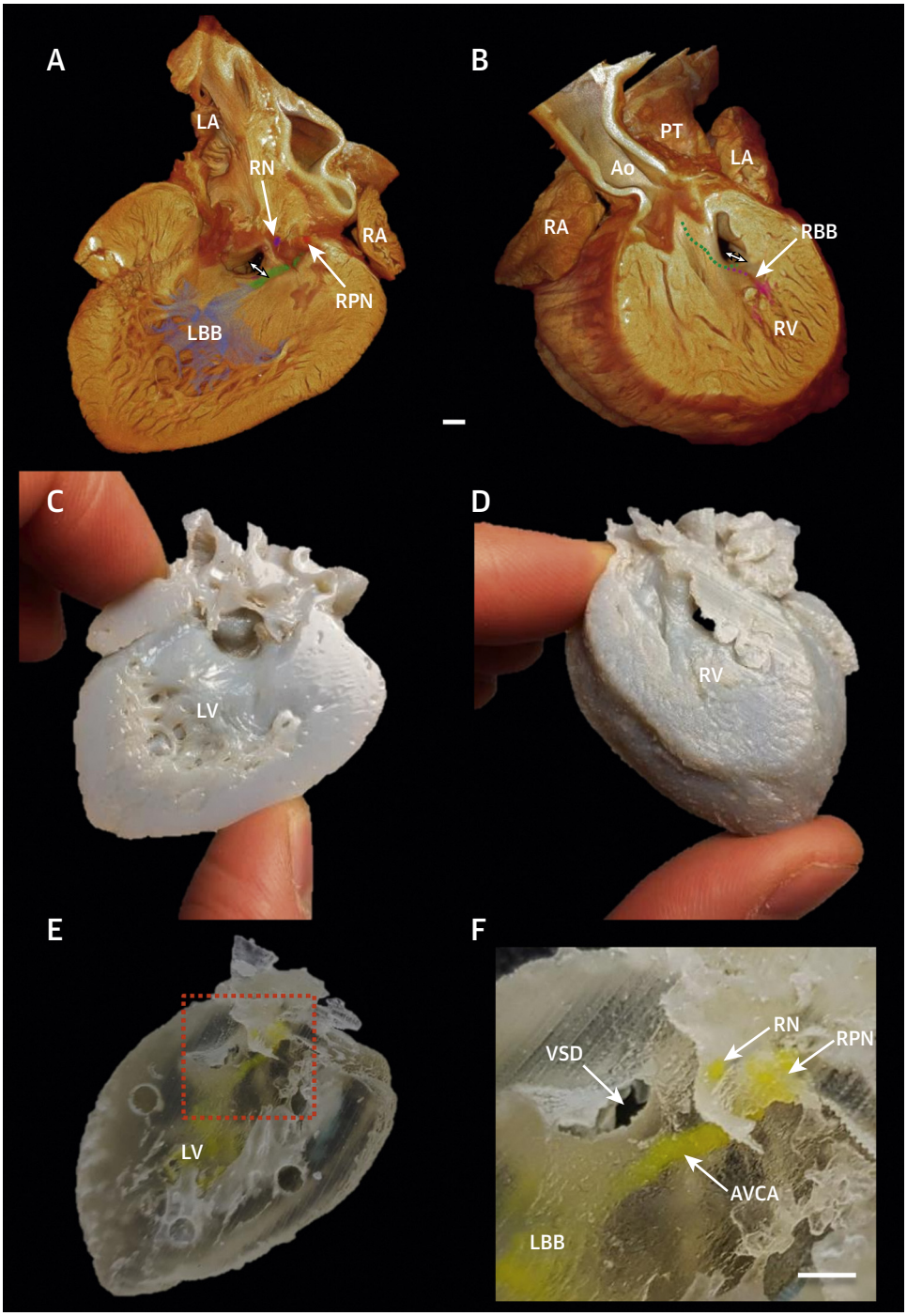
JACC: CARDIOVASCULAR IMAGING, VOL. 11, NO. 11, 2018
NOVEMBER 2018:1706–12

FIGURE 4 Volume-Rendered Cross Sections at Basal and Equatorial Levels With Corresponding Myocyte Orientation Maps



Volume rendered cross sections at basal **(top)** and equatorial **(bottom)** levels with corresponding myocyte orientation maps color coded according to the absolute helical angle. Zero degrees **(blue)** represents myocyte chains running in the plane of section (circumferential orientation) and 90° **(red)** 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 color maps confirm the "helical" organization within the walls of the LV, septum, and also the hypoplastic and incomplete RV. Scale bar 3 mm. Abbreviations as in Figure 1.

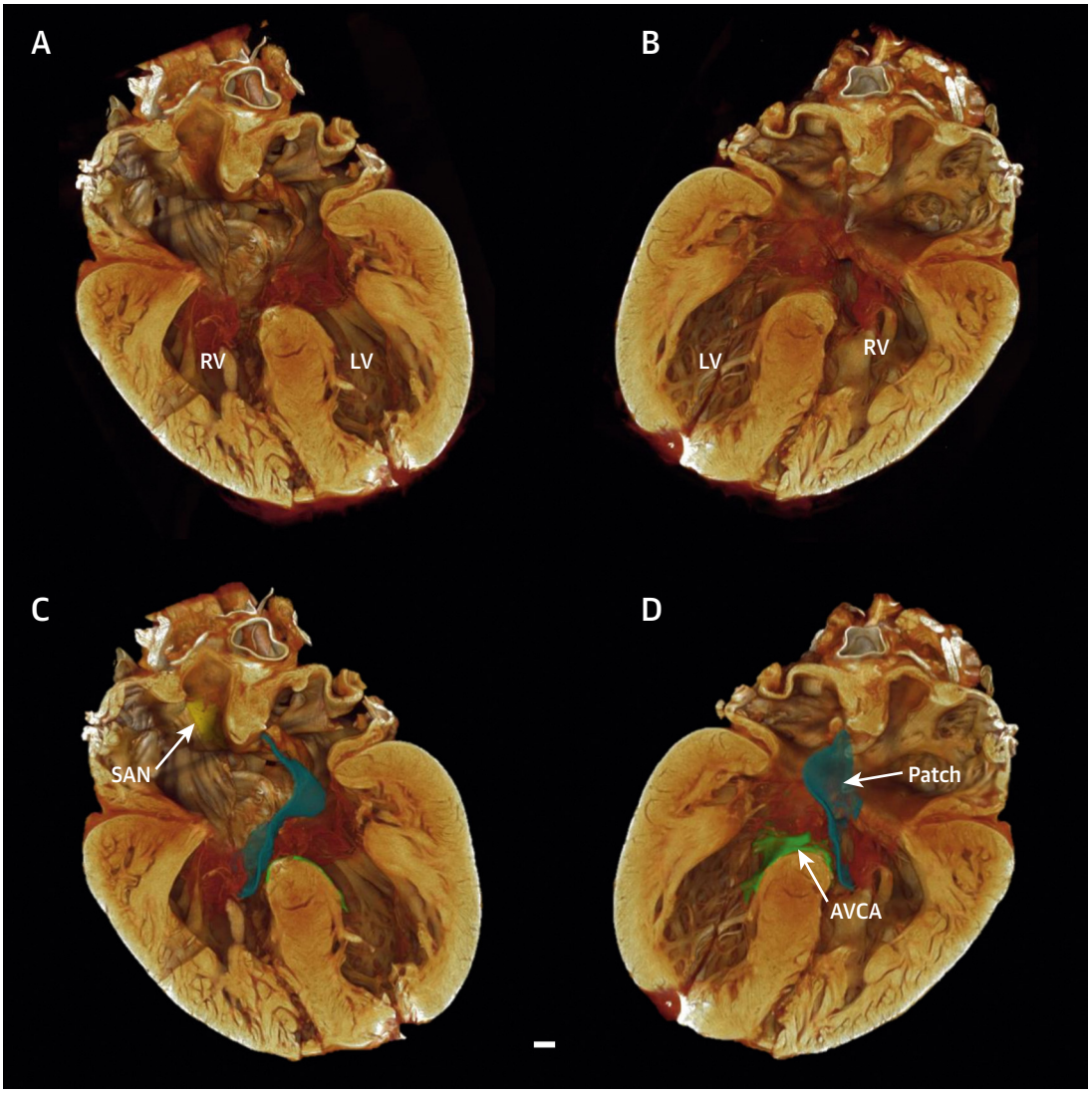
FIGURE 5 High-Resolution 3D Prints Derived From Micro-CT Data



Sectioned volume renderings of double-inlet LV as presented in Figure 3; LV **(A)** and RV **(B)** long-axis views showing the 3-dimensional (3D) disposition of the cardiac conduction system. **(C and D)** Corresponding life-sized semiflexible single-color 3D print. **(E and F)** A corresponding 3D print with transparent and flexible working myocardium and solid colored **(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. Abbreviations as in Figures 1 and 3.

JACC: CARDIOVASCULAR IMAGING, VOL. 11, NO. 11, 2018
NOVEMBER 2018:1706–12

FIGURE 6 Sample From a Second Case, a 5-Month-Old Male Patient With Atrioventricular Septal Defect



Sample processed and scanned as in **Figure 1** and as described in detail previously (1). Sectioned volume renderings in 4-chamber view, viewed from anterior **(A)** and posterior **(B)** aspects. **(C and D)** Corresponding volume renderings showing the sinus node in **yellow (C)**, and the atrioventricular conduction axis in **green (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 μm. Local tomography was performed on all samples giving isometric spatial resolutions of ~15 to 24 μm; these data are not presented here, but imply that imaging of smaller fields of view may be imaged nondestructively at near-cellular resolution. Scale bar 3 mm.

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APPENDIX For supplemental videos, please see the online version of this paper.