

Post-Procedure Micro-CT Analyses of Coronary Artery Stenting in Left Main Vessels of Human Hearts

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Abstract

Background: Percutaneous coronary interventions (PCIs) within left main coronary arteries are high-risk procedures that require optimization of interactions between stent(s) and diseased vessels. Optical Coherence Tomography (OCT) is a widely accepted tool that enhances physicians' ability to assess proper stent appositions during procedures. The primary aim of this study was to develop complementary post-procedure imaging methodologies to better assess and interpret outcomes of left main PCI procedures, utilizing reanimated and perfusion-fixed human hearts.

Methods: PCIs were performed while obtaining OCT scans within the left main anatomies of six human hearts. Following PCI, each heart was scanned with a micro-CT scanner with optimized parameters to achieve resolutions up to 20 μ m. Scans were reconstructed and imported into DICOM software to segment and generate models of implanted stents and coronary vessels. 2D images that were obtained during PCI from OCT were used to assess the accuracy of 3D models generated from micro-CT reconstructions. Additionally, the 3D models were utilized to create virtual reality scenes and enlarged 3D prints for "mixed reality" physician training and education relative to bifurcation stenting in human left main coronary arteries.

Results: We developed a reproducible methodology for post-implant analyses of coronary artery stenting procedures, and created 3D models down to 20 microns of resolution for reanimated and perfusion-fixed heart specimens.

Conclusions: These models can be used to obtain detailed measurements for further clinical insight on procedural outcomes. 3D models resulting from these scans are useful for generating virtual reality scenes and 3D prints for physician training and education.

Background

Coronary artery disease is defined as the narrowing of coronary vessels caused by buildup of sclerotic fatty deposits, otherwise known as plaque. In cases of right dominant coronary circulation, it has been estimated that the left main (LM) coronary artery can supply >75% of the left ventricular myocardium¹. It has also been reported that patients with LM stenoses ranging between 50-70% had a projected 3-year survival of 66%, whereas patients presenting with stenoses >70% only had a ~40% 3-year survival rate². Thus, in patients with significant LM disease, percutaneous coronary interventions (PCIs) with drug-eluting stents have been increasingly recognized as valid clinical revascularization procedures³. Because of such clinical severity, proper deployment of stents in the LM and resulting strut appositions play a critical role in patient outcomes.

Recently, intra-procedural coronary imaging technologies have been developed and optimized to allow interventional cardiologists the ability to view relative plaque depositions, lumen dissections, and/or stent appositions during various PCI procedures. A recent advancement has been the application of Optical Coherence Tomography (OCT) for such interventions. OCT uses a rotating glass fiber-optic system that

tracks over a 0.014" guidewire until it is positioned in the desired location within the patient's coronary anatomy. Once placed, a contrast flush is introduced into the OCT catheter, removing blood from the artery to allow for appropriate optical scanning; coherent infrared light is then directed and reflected within the tissue to create detailed images⁴. While OCT, in combination with fluoroscopy, continues to be a valuable tool in left main PCI cases, it is not universally available and is often reserved for the most complex cases.

For more than two decades, the Visible Heart® Laboratories have been dedicated to translational research and advancements in cardiac research, devices, and education. We routinely perform PCI procedures, in bifurcations specifically, on reanimated large mammalian hearts⁵. On rare occasions, we have the opportunity to conduct PCIs within reanimated human hearts⁶. The Visible Heart® Laboratories provide a unique research platform where PCIs can be performed using experimental devices, novel or approved procedural techniques, and/or multimodal imaging modalities, all with no risk to living patients. The primary aim of this study was to further develop complementary post-procedure imaging methodologies to better assess and interpret the outcomes of left main PCI procedures, utilizing both reanimated and perfusion-fixed human hearts.

Results

We successfully performed PCIs in six human LM coronary arteries employing Visible Heart® methodologies, in both reanimated and perfusion-fixed hearts. Both experimental approaches were deemed feasible and valuable for conducting preclinical research. We acquired OCT images immediately after each stenting procedure, then micro-CT scanned each perfusion-fixed heart using the same scanning parameters. The 2D OCT images were compared side by side with the 3D micro-CT reconstructions, and each micro-CT reconstruction accurately resembled its OCT counterpart as shown in **Figure 1**.

The three reanimated specimens were relatively healthy, and no significant calcification was observed through endoscopic visualizations, OCT imaging, or micro-CT-generated computational models. However, varying degrees of vascular calcifications were found in the three perfusion-fixed specimens, as we intentionally selected hearts with histories of coronary artery disease. This was most notable in HH115 which elicited large amounts of plaque buildup; it was not possible to fully expand the deployed stent into a desired cylindrical shape via multiple balloon expansions employing >20 atmosphere of balloon pressures. Despite the presence of vascular disease and previous perfusion fixation in formalin, these hearts still elicited significant increases, >60%, in LM cross-sectional areas after stent implantations (**Table 1**). These values were calculated by measuring the cross-sectional areas of the given vessel lumens at 5mm distances from the determined coronary ostia border, determined before and after PCI (**Fig. 2**).

In this study, we also sought to assess the relative effects of the formalin fixation process on a heart's vessel geometries. This was achieved by taking OCT measurements post-PCI, while the heart specimen

was <12 hours post-recovery (viable – fresh), to examine the LM lumen areas 2mm distal to the deployed stents. We obtained additional OCT scans of the same specimens after formalin fixation to evaluate lumen diameter changes induced by the fixation process. Additionally, the same lumen areas post-fixation were measured via micro-CT to enable comparisons between measuring modalities. Reviewing the lumen areas in **Table 2**, we observed larger tissue desecration after formalin fixation in the healthy heart specimens, compared to those with previously noted heart disease. In HH202, an increase in lumen area—from 5.04mm² to 5.22mm² (OCT) and 6.78mm² to 6.94mm² (micro-CT)—was observed; this may have resulted from residual expansion from the implanted stent. Some discrepancies in measurements between OCT and micro-CT were observed (**Table 2**), perhaps caused by methodological differences in how measurements were obtained. Ongoing investigations are currently underway in our laboratory to further assess the relationship between micro-CT and OCT measurements.

Finally, we used generated computational 3D models from micro-CT datasets to create “fly through” animations and develop virtual reality scenes (**Fig. 3b**). These scenes can enhance critical analyses of procedural outcomes and educate clinicians on methodological steps for bifurcation stenting. Furthermore, our laboratory has developed an approach to transform virtual reality scenes into anaglyph visualizations (**Fig. 3c**) in real-time, allowing multiple individuals to view PCI methodological scenes simultaneously (**Fig. 3a**). Free downloads of such scenes can be found in the Atlas of Human Cardiac Anatomy (<http://www.vhlab.umn.edu/atlas/device-tutorial/stents/index.shtml>). In addition, these models can be readily adapted for 3D printing as a unique tool that allows individuals to physically hold the same stent that they are flying through in virtual reality (**Fig. 3d, 3e**)—a “mixed reality” learning experience.

Discussion

Since 1997, the Visible Heart® Laboratories have performed novel preclinical research on reanimated hearts and have developed an expansive Human Heart Library with >600 perfusion-fixed specimens that can be accessed for anatomical research and device investigations^{7,8}. We utilize state-of-the-art imaging tools, such as OCT and micro-CT imaging, to expand our ability to develop novel educational experiences and materials. We firmly believe that both reanimated human hearts and perfusion-fixed hearts (at end-diastolic states) can be utilized to expand the current knowledge of methodologies in the bifurcation stenting of LM disease, where applicable.

Parameter Finalization

As previously stated, the Visible Heart® Laboratories routinely use reanimated swine hearts for a large variety of cardiovascular technological studies. We previously utilized these implanted animal specimens to optimize and finalize the scanning parameters for this study⁹. Hence, we knew the imaging, scanning, and modeling parameters needed to achieve approximate reconstructed stent resolutions of 20µm, to accurately depict the stent struts.

Through our initial attempts of scanning coronary stents in perfused tissue, it became apparent that we were not using enough voltage/power to penetrate through the struts, allowing it to be picked up by the scanner's detector and causing a "halo-ing" effect⁹. By increasing the power in these perfusion-fixed human heart specimens, we reduced halo artifacts but were still unable to penetrate appropriately through the struts, thus resulting in a hollowed core.

Other issues surfaced from employing too much radiation energy/power during these scans. In the fixed human hearts, radiation easily penetrated through the stent struts and through the tissue. This in turn led to significant streaking and shadowing artifacts of the struts, which yielded less than desirable 3D models. Once the optimized voltage and current values were determined, we were able to finalize additional parameters such as number of projections and scanning duration to further reduce imaging artifacts. We continue to improve this methodology by investigating how we can detect different cardiac anatomies with similar densities (i.e., cardiac valves, endocardium, myocardium) and we recently installed a micro-CT X3000 North Star Imaging scanner system in our laboratory.

Visible Heart® Methodology Applications in Coronary Artery Stenting

We continue to perform additional PCI studies to further validate the accuracy of our novel micro-CT imaging. This study focused primarily on achieving reproducible scans of implanted coronary stents, however our investigations continue to evaluate the final stent apposition via these micro-CT methodologies. We understand that having near perfect apposition long before the end of PCIs is vital, especially when addressing issues such as wiring across a lesion to validate procedures. Such studies are also ongoing within perfusion-fixed human specimens in our laboratory, and we have started to perform step-by-step bifurcation procedures with imaging employed for each recommended procedural step.

Finally, each perfusion-fixed specimen was repeatedly imaged with micro-CT until we achieved ~20-micron resolution scans of implanted devices, then high-resolution computational models were generated, analyzed, and further segmented to create various fly through videos, 3D prints, and rendered virtual reality scenes.

Extension of Application

We are conducting ongoing studies to further determine the effects of formalin fixation on diseased aortic and coronary tissues (i.e., uniaxial and biaxial testing). We observed here that diseased vessels lose their compliance, therefore the fixation process had little to no effect on the mechanical properties of the tissue that we studied. We believe it would be valuable to utilize the 600+ human heart specimens from our Human Heart Library to perform similar bench-top experiments in real human anatomies. Furthermore, the methodologies described in this study are not limited to coronary stenting procedures. Rather they can be utilized for procedural testing of other cardiac devices to study device-tissue interactions with extremely high (~20 micron) resolutions. For example, we conducted a study in which various post TAVR-PCI procedures were performed within reanimated hearts on the Visible Heart®

apparatus; hearts were subsequently perfusion fixed and later micro-CT scanned using the same parameters¹⁰.

Creation of Educational Stenting Modules

We envision that these described methodologies can generate high-resolution 3D models offering unique educational modules. For example, we have utilized these reconstructed models of bifurcation stenting procedures to create a variety of enlarged 3D prints, designed to better understand differences in various bifurcation techniques and their interactions with calcifications and/or complex anatomies. Further, mixed reality educational modules can couple 3D prints and virtual reality scenes¹¹ that allow individuals or multiple users to “fly through” the same scenes¹², while simultaneously performing physical inspections of the detailed models¹³. In other words, these mixed reality models can be used for unique educational endeavors such as anatomical analyses, identifying clinical plaque deposition trends, assessing coronary stent design, and/or developing computational fluid simulations (see free-access website “Atlas of Human Cardiac Anatomy” to visualize and download models and associated procedural videos; <http://www.vhlab.umn.edu/atlas/index.shtml>). It is critical to develop such educational materials as means to better engage and stimulate learning. Such approaches provide unique insights related to how interventional procedures may be applied in appropriate clinical cases, and are valuable to students, clinicians, and medical device designers.

Conclusions

In conclusion, we developed reproducible methodologies for post-implant analyses of coronary artery stenting procedures performed within reanimated and perfusion-fixed human hearts. We generated high-resolution 3D models to investigate device-tissue interfaces. These novel approaches for preclinical cardiac device testing can produce models that can be analyzed to gain insights on procedures and device designs. Further, the 3D models have been useful for generating virtual reality scenes and 3D prints that can be utilized for a variety of educational mixed reality training. Our methodologies are not limited to studying coronary stenting technologies, but could be applied to all types of cardiac device technologies.

Methods

Specimen Procurement and Selection

The Visible Heart® Laboratories received viable human hearts and heart-lung bloc specimens for research via LifeSource, a nonprofit organ procurement organization (Minneapolis, MN, USA). Donors gave consent for their organs to be used for scientific research purposes via LifeSource. The hearts used in this study were deemed non-viable for transplant due to advanced patient age, cardiac downtimes, identified poor cardiac function, and/or other reasons. All specimens were received as fresh viable tissues, along with donors’ relative cardiac-pulmonary clinical histories. We dissected lungs from the hearts while fresh,

and each specimen was carefully cannulated for immediate reanimation, if specific criteria were met. Otherwise, hearts were placed within a formalin fixation apparatus^{14,15} for a 24-hour fixation period. The perfusion-fixation apparatus preserved hearts in their end diastolic shape, importantly keeping the aortas and coronaries dilated. After being perfusion fixed (including hearts initially reanimated), each specimen was placed in its own container and stored for future studies.

We performed PCIs and micro-CT scanning utilizing six human heart specimens (see **Table 3** for detailed patient information). Three of the specimens exhibited adequate cardiac function prior to donation and were considered viable candidates for reanimation and eventual perfusion fixation, as described earlier. The remaining three hearts were received from LifeSource two to eleven years prior to this study. We selected these specimens due to noted histories of coronary artery disease and prior imaging indicating the LM coronaries were patent enough to perform PCIs.

Coronary Intervention in Isolated Hearts

We performed PCIs in three reanimated hearts (HH534, HH541, HH556) using Visible Heart® methodologies¹⁶; subsequently each heart was carefully removed from the apparatus and perfusion fixed so as not to damage the newly implanted stent(s). Previously fixed hearts (HH115, HH202, HH479) were rinsed for 24 hours, re-cannulated, and placed in an acrylic box where they were attached to a Langendorff static perfusion apparatus (**Fig. 4a**) which continuously perfused the aortas and coronaries with water. The advantages of utilizing Visible Heart® methodologies while performing PCIs were as follows: 1) endoscopic cameras enabled direct visualization of each procedural step; 2) there were no risks to any living patient, thus unlimited fluoroscopy could be used; and 3) OCT could be utilized as many times as desired as there was no need to expedite the procedures.

Each PCI procedure was guided and recorded simultaneously by 2.4mm and 4mm endoscopic cameras (Olympus, Tokyo, Japan), OEC Elite Fluoroscopy (GE, Boston, MA, USA), and episodic OCT imaging, as shown in **Figure 4b**. The combination of these imaging modalities nearly simultaneously would not be possible without the use of a clear perfusion solution (Krebs-Henseleit buffer for reanimated hearts and water for fixed hearts) that continuously circulated through the apparatus. All PCIs were conducted using Resolute Onyx drug-eluting stents, compliant and non-compliant Euphora balloons, and Cougar XT guidewires (Medtronic, Santa Rosa, CA, USA).

OCT Imaging during PCI

After stent implantations, we captured OCT scans using OPTIS Intravascular Imaging System and Dragonfly™ Imaging Catheters (Abbott Vascular, Abbott Park, IL, USA). The automatic pullback system captured 540 frames over a scanning trajectory of 54mm with a 5mm penetration distance, to capture the highest resolutions possible (~100µm). Since specimens were continuously perfused using clear solutions in both setups, no contrast injections were needed or administered during OCT scanning.

Following ex vivo stenting, the three reanimated hearts were perfusion fixed and then placed in formalin containers for long-term preservation. All OCT image datasets were post-processed to identify both distal and proximal portions of the implanted stents and then stored as 2D images. Since intracoronary OCT is a widely accepted method of imaging implanted stents, the images collected were later used to compare to the micro-CT reconstructions.

Micro-CT Scanning

Before micro-CT scanning each human heart, the specimens were rinsed in water for a minimum of 24 hours to remove traces of formalin before handling and/or transportation. Once thoroughly rinsed, each heart was placed within the specially constructed plexiglass container and then scanned using an X5000 micro-CT scanner (North Star Imaging, Rogers, MN, USA). All heart specimens were carefully perfusion fixed to elicit an end diastolic shape for all four chambers (maximal filled) prior to scanning because the internal space of the scanner did not readily allow for our perfusion system to be used during scanning. Each heart was placed in the scanner as shown in **Figure 5**, and imaging was performed utilizing the following parameters to achieve approximate isotropic voxel sizes of $20 \times 20 \times 20 \mu\text{m}$: 170kV tube voltage, 144 μA tube current, 24.5 isowatts, and 1,500 radiograph images captured throughout a ~15-min scanning duration. We selected these parameters after numerous iterations and scanning trials, all utilizing Resolute Onyx stents implanted in swine heart coronaries, to optimize scanning resolutions while minimizing streaking, shadowing, and/or scanning artifacts. **Figure 6** shows the progression of scanning parameters trialed, until we finalized the optimal parameters used in this study. Once scans were completed, each heart specimen was returned to the laboratory and placed in its respective formalin container. Imaging datasets were then reconstructed using North Star Imaging's reconstruction software, into 8-bit 2D images (*.tiff*), for future analyses as described below.

3D Reconstructions

We imported *.tiff* files from micro-CT scans into the DICOM analysis software Mimics (Materialise, Leven, Belgium), where they were computationally “stacked” to form 3D volumes from 2D images¹⁷, followed by further post-processing. Using Mimics, for each heart’s image dataset, we generated a high-frequency “mask” to segment out the higher density portions of the scan, i.e., the cobalt alloy shell and platinum iridium core of the Resolute Onyx. We manually created additional masks to segment out the vessel blood volumes and tissues. For subsequent analyses, each generated model consisted of a portion of the aortic wall, left coronary ostia, LM vessels, coronary stent(s), and proximal portions of left circumflex artery (LCX) and left anterior descending (LAD) coronaries. Once these models were generated, we used assessment tools in Mimics to measure the relative lumen areas. The 3D models were then exported from Mimics to be rendered as virtual reality scenes using a video game design software (Unity, Unity Technologies, San Francisco, CA, USA) which allowed for further visual inspections of LM stenting outcomes.

Declarations

Ethics approval: The University of Minnesota Institutional Review Board waives review and approval of research using waste tissue.

Consent to participate: Donors gave consent for their organs to be used for scientific research purposes via LifeSource.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Competing interests: PA Iaizzo has a research contract and educational consultation arrangement with Medtronic. TF Valenzuela is an employee of Medtronic.

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Authors' Contributions: PA Iaizzo and TF Valenzuela contributed equally to the planning, execution, and reporting of this work.

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Disclaimer: Approximately 5-7% of coronary angiography patients present with a left main lesion, with 60% of these patients eligible for PCI. The Resolute Onyx DES is currently CE marked for both left main and bifurcation stenting, however to date, it is not approved in the United States and the FDA considers any left main stenting to be off-label.

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Tables

Table 1. Micro-CT measurements of left main lumen using blood volume pre- and post-percutaneous coronary intervention (computational assessments using 3-Matic)

	HH115	HH202	HH479
Pre-PCI area	7.16 mm ²	10.54 mm ²	12.25 mm ²
Post-PCI area	11.89 mm ²	17.14 mm ²	19.80 mm ²
% increase	66%	63%	62%

Each heart exhibited varying areas prior to percutaneous coronary intervention (PCI) due to variability in heart sizes studied as well as extent of vessel disease, thus measurements were normalized by calculating overall percent increases in vessel lumen areas.

Table 2. Comparison of Optical Coherence Tomography (OCT) and micro-CT

		2mm Distal	2mm Distal
		OCT (mm ²)	micro-CT (mm ²)
HH541	Fresh Post-PCI	7.32	-
	<i>(healthy fresh)</i>		
HH556	Formalin-Fixed Post-PCI	6.67	7.64
	<i>(diseased fresh)</i>		
	Fresh Post-PCI	9.55	-
	Formalin-Fixed Post-PCI	9.47	10.48

OCT and micro-CT measurements were obtained of the lumen at distance 2mm distal to the implanted stents. Comparisons were made in specimens with and without disease (reanimated heart that was subsequently formalin fixed) to evaluate luminal changes associated with fixation process.

Table 3: Detailed patient information from donation records received from Lifesource

Heart #	Age/ Gender	Weight (kg)	Clinical Conditions	Cardiac Conditions	Date Received	Date of Additional Study	Procedure/ Location
HH115	62M	68.2	Diabetes, HTN	CAD	April 2009	11/2019	Stenting – Provisional/ Left main
HH202	68M	90	Diabetes, HTN, HLD	CAD, CABG, Stent (bare metal)	May 2011	2/2020	Stenting – Provisional/ Left main
HH479	74M	93	Diabetes, HTN, HLD	Mild aortic stenosis, CAD, Angioplasty (2004)	February 2018	2/2020	Stenting – Provisional/ Left main
HH534	54F	57.3	Asthma, HTN	Family history of CAD	June 2019	Reanimated June 2019	Stenting – Provisional/ Left main
HH541	73F	85.6	HTN	N/A	August 2019	Reanimated August 2019	Stenting – Provisional/ Left main
HH556	60F	61.5	HTN, CM	CAD	January 2020	Reanimated January 2020	Stenting – Provisional/ Left main

Clinical and cardiac conditions were obtained from next-of-kin interviews, pre-recovery imaging, and additional imaging performed after hearts were received. CABG=coronary artery bypass graft; CAD=coronary artery disease; CM=cardiomyopathy; HLD=hypersensitivity lung disease; HTN=hypertension

Figures

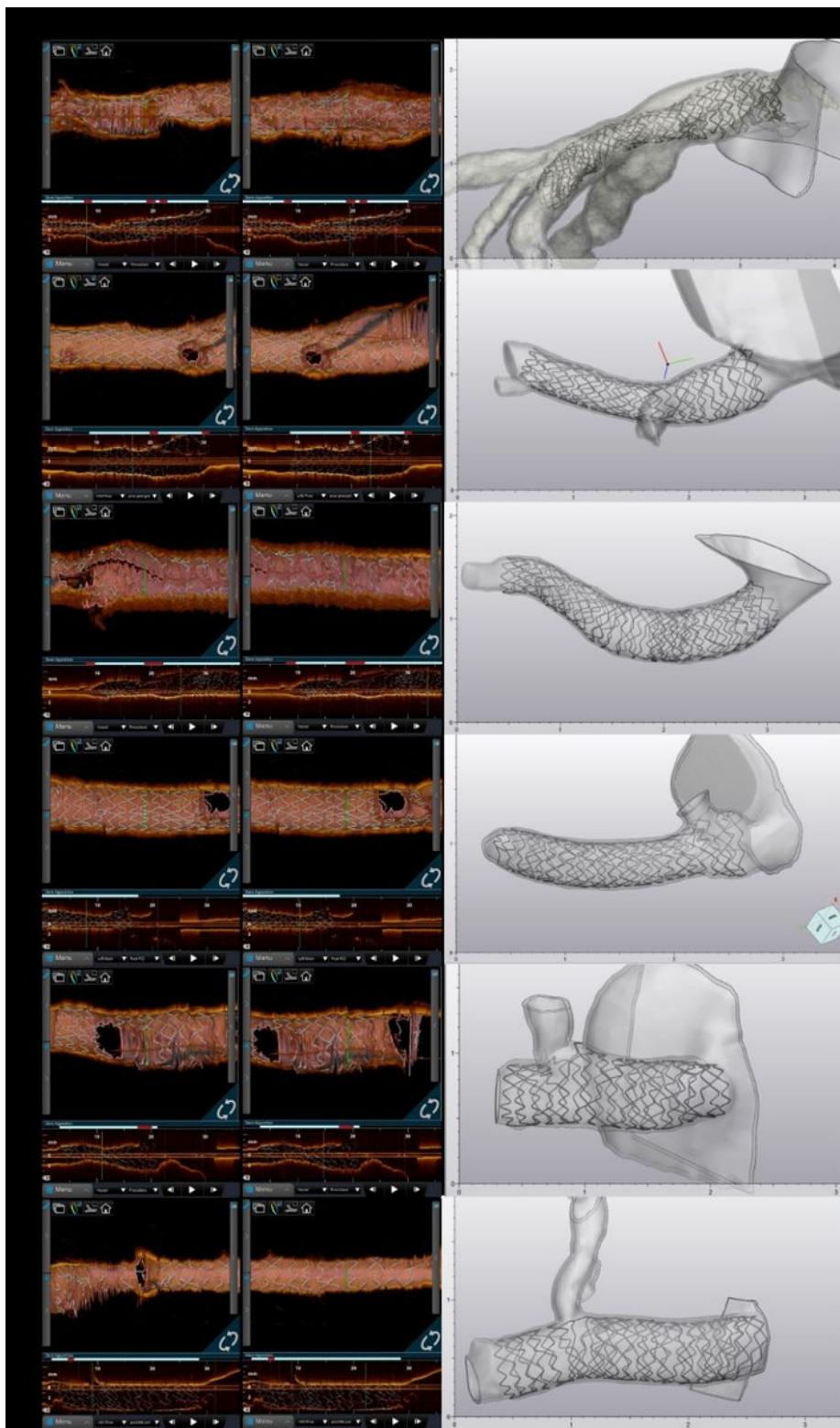


Figure 1

(Left) Series of 2D images showing distal and proximal portions of the stent obtained via Optical Coherence Tomography (OCT) imaging, using the Bifurcation Display, Stent Renderer, and Apposition Indicator function. (Right) Snapshots of the micro-CT 3D-generated computational model showing relative apposition analyses, to visually compare each stenting procedural outcome with OCT software.

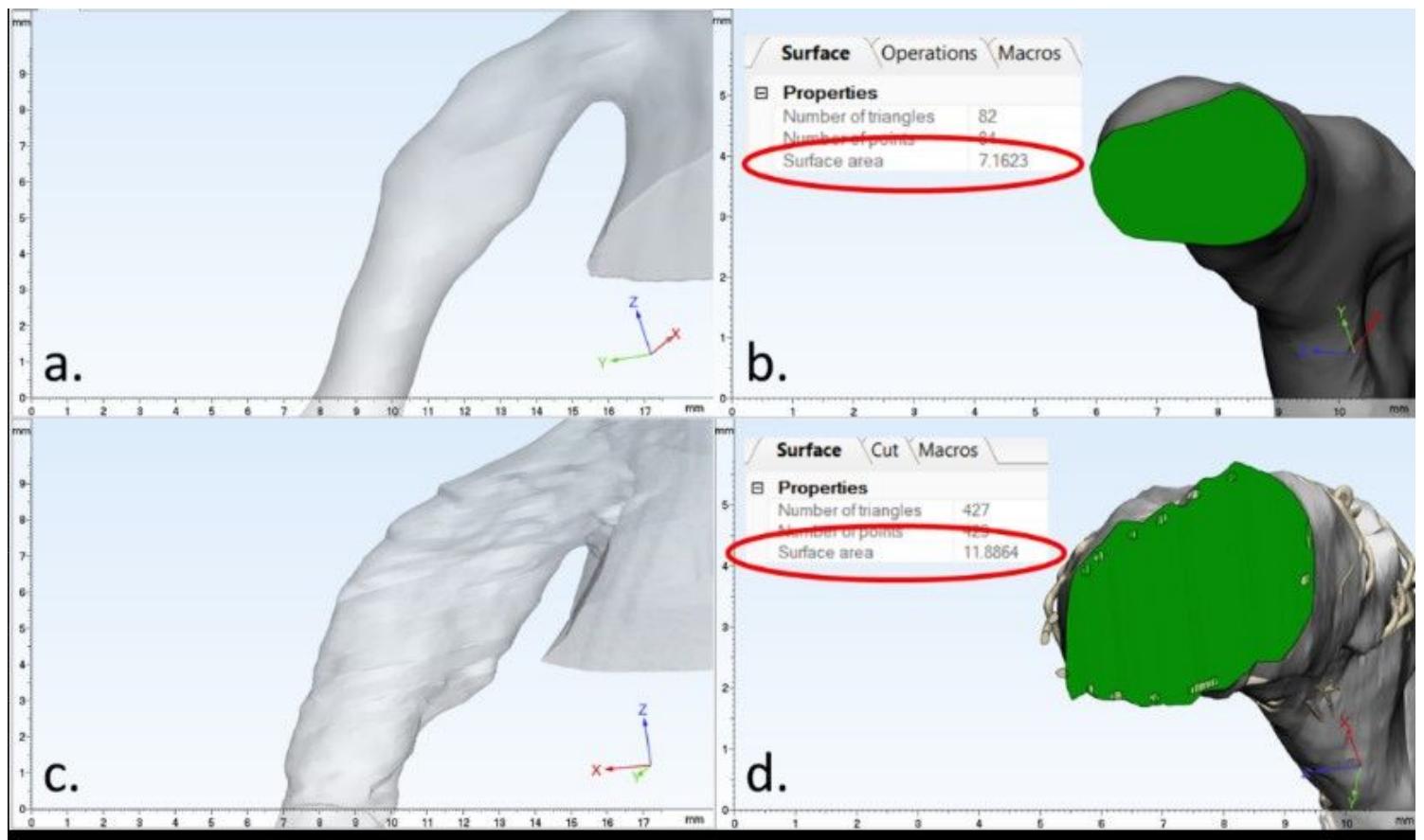


Figure 2

Additional changes in vessel dimension post-intervention (before and after PCI), determined by importing computational models into 3-Matic. Specific measurements were obtained at distances 5mm into the left main (LM) of each heart specimen, measured from the defined border of each coronary ostia. Once vessel distance was determined, a slice (normal to the centerline) was created to expose the face of the blood volume. 3-Matic was then used to measure area of the digitally determined vessel lumen.



Figure 3

Virtual reality and 3D printing have been used as effective educational tools to enhance knowledge of bifurcation stenting interventions. In one application, the instructor can “fly around” the anatomy of a bifurcation stent (a,b) while trainees follow along within anaglyph visualization scenes (c). Simultaneously, mentors and trainees can hold 3D prints of the same model as viewed in the visualizations (d,e).

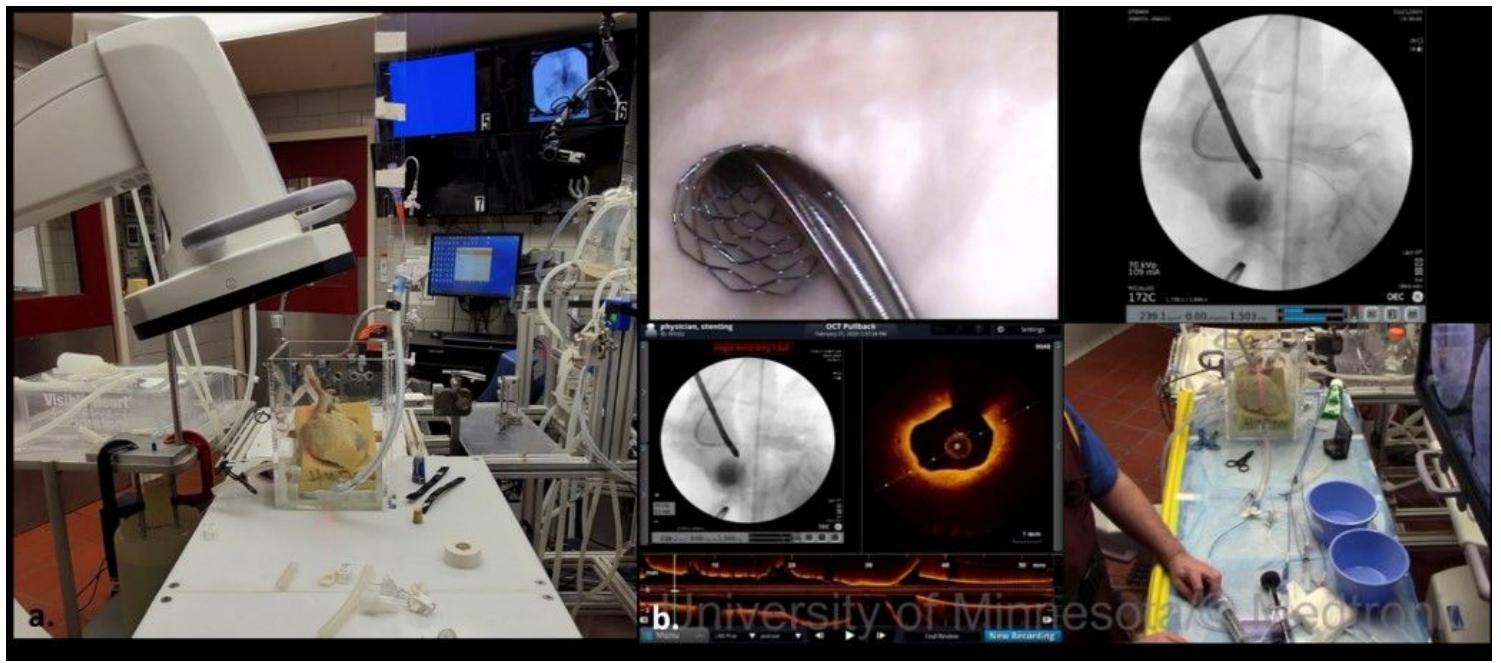


Figure 4

(a) Each perfusion-fixed isolated human heart was placed in a custom-made acrylic box for perfusion, utilizing fluoroscopy. (b) Quad-split of bifurcation showing endoscopic imaging, fluoroscopy, Optical Coherence Tomography images, and operator hand manipulations during percutaneous coronary intervention procedure.

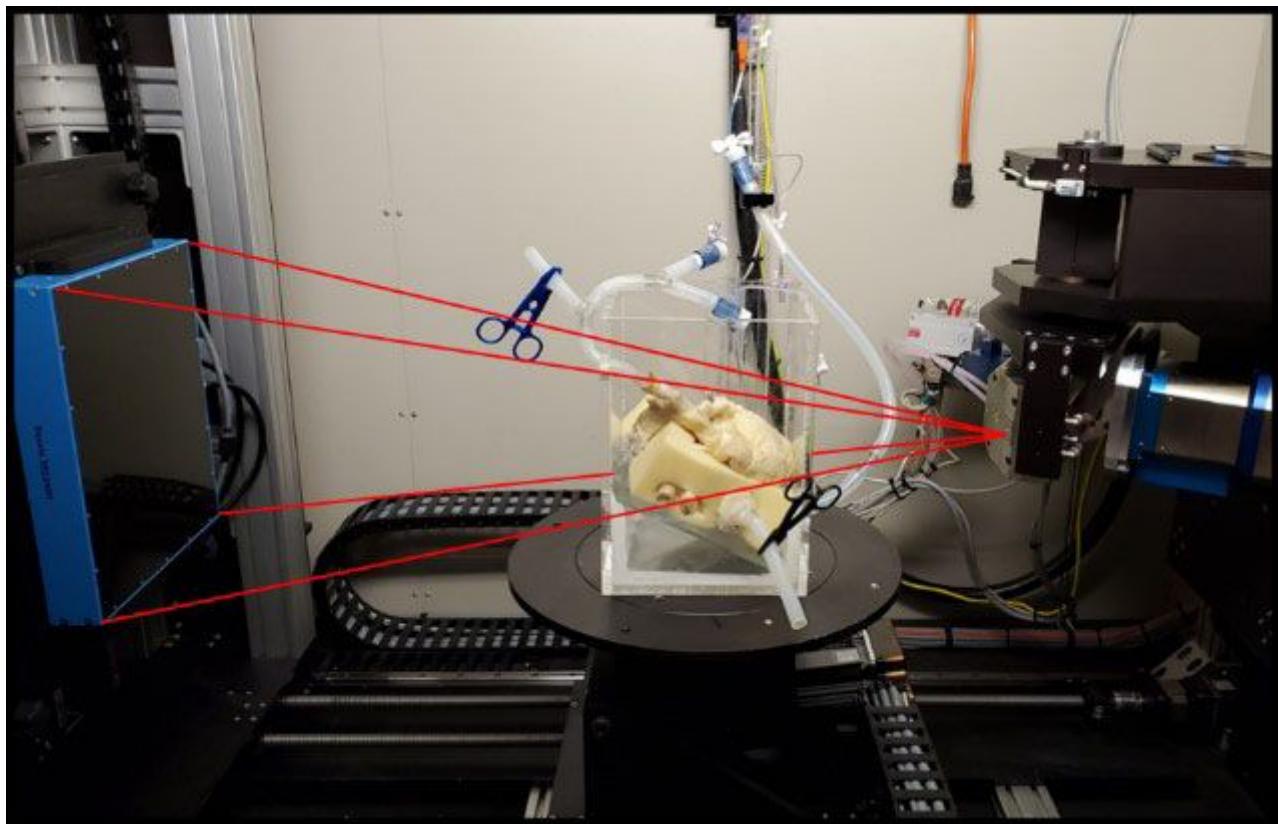


Figure 5

The same plexiglass case used for performing percutaneous coronary interventions was then used for micro-CT scanning. Tubes were disconnected from the perfusion apparatus and clamped so fluid was not spilled during scanning.

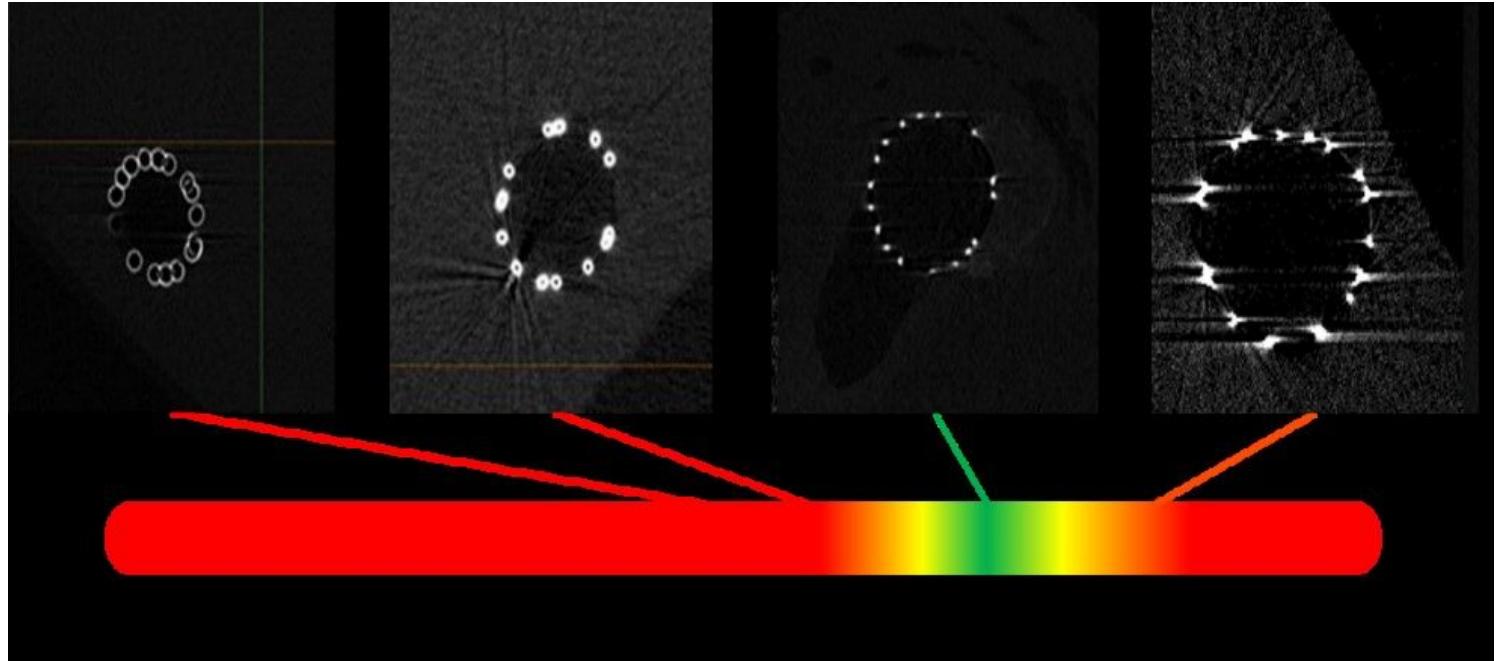


Figure 6

Initial attempts began with lower voltages and power that was unable to penetrate through the stent struts, resulting in outlining of the struts with black center cores. Too much voltage and power caused significant streaking in the stent struts, and further refinement of the parameters was needed to distinguish between the stent, tissue, lumen, and calcification.