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Depth-resolved 3D visualization of coronary microvasculature with optical microangiography

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Abstract

In this study, we propose a novel implementation of optical coherence tomography-based angiography combined with *ex vivo* perfusion of fixed hearts to visualize coronary microvascular structure and function. The extracorporeal perfusion of Intralipid solution allows depth-resolved angiographic imaging, control of perfusion pressure, and high-resolution optical microangiography. The imaging technique offers new opportunities for microcirculation research in the heart, which has been challenging due to motion artifacts and the lack of independent control of pressure and flow. With the ability to precisely quantify structural and functional features, this imaging platform has broad potential for the study of the pathophysiology of microvasculature in the heart as well as other organs.

Keywords

Optical coherence tomography; Optical microangiography; Coronary vasculature; heart imaging

1. INTRODUCTION

Coronary circulation plays an important role in delivering oxygen and nutrients to the myocardium as well as removing deoxygenated blood from the heart muscle (Komaru *et al.*, 2000; Reese *et al.*, 2002). Mis-regulation of coronary circulation can cause cardiac dysfunction in various pathological contexts, e.g., hypertension, atherosclerosis, and myocardial infarction (Wei and Kaul, 2004). In the past century, advances in angiographic imaging technologies have greatly strengthened the understanding of the structure of coronary vasculature (van den Wijngaard *et al.*, 2013). This is of great significance as the

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vascular networks are one of the main determinants of perfusion distribution within the heart.

In vivo imaging techniques have been developed to visualize the cardiovascular networks. The most successful one is the micro-computed tomography (μ -CT), which requires injection of radiopaque agents (e.g. iodinated compounds) (Jorgensen *et al.*, 1998; Ritman, 2011). Although capable of 3D imaging of the coronary vascular tree *in vivo* with high resolution, it is radiotoxic and thus usually doesn't allow multiple scans in live animals (Ritman, 2011). This limits the application of μ -CT in the research of coronary circulation. Recently, optical imaging modalities have also been proposed for *in vivo* imaging of coronary capillaries at microscopic resolution (Lee *et al.*, 2012). A custom-designed stabilizer was utilized to immobilize the local heart tissue, and real-time image acquisition was triggered at appropriate phases based on retrospective electrocardiogram gating to further eliminate artifacts induced by respiratory and cardiac motion. This technique has been successfully applied to confocal and multiphoton microscopy to image microvasculature in a beating mouse heart *in vivo*. However, the imaging depth was relatively shallow, and the use of the stabilizer may alter the superficial blood flow due to the physical contact with the pericardium.

In fact, all the *in vivo* imaging technologies have a common limitation in coronary circulation research, which is a lack of independent control of coronary blood supply. Since coronary pressure is autoregulated by myocardial oxygen consumption, it is difficult to study metabolic blood flow adaption and autoregulation independently in hearts of live animals (van de Hoef *et al.*, 2012). From this point of view, *ex vivo* imaging can be useful to add values to the understanding of the heart function in the absence of autoregulation. *Ex vivo* imaging of coronary blood vessels was initially carried out with X-ray angiography. In 1938, Schlesinger first visualized coronary collateral vessels with X-ray angiography by injecting a radiopaque contrast medium into *ex vivo* coronary vessels (Schlesinger, 1938). Subsequently, it was extended to study various animal models (Schaper *et al.*, 1979a; Schaper *et al.*, 1979b) and the human heart (Fulton, 1956, 1964) which yielded important morphological information. Nevertheless, the two dimensional (2D) representation of the X-ray angiography essentially limits its application in 3D vascular tree modeling. Vascular corrosion casting technique was developed to visualize the coronary microvasculature in three-dimensions by creating high-quality physical replicas of the vasculature and removing the surrounding tissue with acid corrosion (Bassingthwaighte *et al.*, 1974; Baroldi *et al.*, 1956). It has been combined with scanning electron microscopy (SEM) to clearly illustrate ultrastructural capillary bundles (Hossler and Douglas, 2001). A main drawback is the one-time replica doesn't allow longitudinal investigations on the same heart. In addition, it cannot provide information on spatial relations between the tissue bed and embedded blood vessels. Cryomicrotomy recently overcame the latter issue in cooperation with fluorescence imaging (Spaan *et al.*, 2005; van den Wijngaard *et al.*, 2010). In principle, it successively removes sections that have been imaged and numerically produces a 3D data volume containing both structural and functional information. But this technique is time-consuming; it typically takes several days for a single 3D reconstruction. Also, similar to corrosion casting, cryomicrotomy is limited to static studies without the ability to monitor dynamic changes of the coronary circulation.

Optical coherent tomography (OCT) based angiography has emerged in recent years as one of the most powerful and clinically useful imaging modalities owing to its ability to visualize volumetric microvascular networks innervating tissue beds *in vivo* (Wang *et al.*, 2007; Wang and Hurst, 2007; An and Wang, 2008; Wang and An, 2009; An *et al.*, 2010; Wang *et al.*, 2010; Zhi *et al.*, 2011a; Zhi *et al.*, 2011b; Qin *et al.*, 2015a; Zhang and Wang, 2015; Zhi *et al.*, 2015; Zhang *et al.*, 2015; Makita *et al.*, 2006; Yasuno *et al.*, 2007; Fingler *et al.*, 2009; Yu and Chen, 2010; Jia *et al.*, 2012). Amongst successful OCT based microvascular imaging techniques, Optical microangiography (OMAG), first demonstrated in 2007 (Wang *et al.*, 2007), has been extensively applied to study dynamic microcirculation in various animal models (Wang and Hurst, 2007; Zhi *et al.*, 2011a; Marcu *et al.*, 2015; Qin *et al.*, 2015b) as well as in human skin (An *et al.*, 2010; Baran *et al.*, 2016) and the human eye (An and Wang, 2008; Wang *et al.*, 2010). Many other derivatives of OCT-based angiography technologies have also been developed subsequently and provided advantages for clinic purposes, e.g., low cost, fast acquisition, high resolution, noninvasive and depth-resolved imaging ability (Zhang *et al.*, 2015). For example, Yasuno *et al.* in 2007 proposed an intensity-threshold-based angiography by virtue of the low-signal nature of blood vessels in choroid, which is a simple but effective method for choroid vasculature imaging (Yasuno *et al.*, 2007). In the same year, Fingler *et al.* proposed the use of phase variance between adjacent B-scans to visualize transverse flow (Fingler *et al.*, 2007) and applied it to volumetric microvascular imaging of human retina in 2009 (Fingler *et al.*, 2009). Yu and Chen later developed a method using Doppler variance with histogram-based bulk motion compensation and successfully imaged human retina and choroid (Yu and Chen, 2010). More recently, Jia *et al.* proposed a split-spectrum amplitude decorrelation angiography that calculates the decorrelation values between two consecutive B-scans from narrowed spectral bands to visualize blood vessels.

Although OCT-based angiography is an attractive angiographic technique, imaging coronary microvasculature with OCT-based angiography either *in vivo* or *ex vivo* remains blank. Because the contrasting signal of OCT-based angiography is produced intrinsically from flowing particles, particularly flowing red blood cells, and the heart beat is essential to drive the blood flow, *in vivo* imaging has not been possible due to severe motion during ventricle contraction. *Ex vivo* imaging has also not been achieved in the absence of cardiac blood flow. In this paper, we demonstrate a novel implementation of OMAG for depth-resolved 3D visualization of coronary microvasculature in rat hearts fixed in diastole, combined with retrograde perfusion. Intralipid solution is perfused through the aorta into the coronary vasculature so that flowing scatterers in the Intralipid perfusate can generate OMAG contrast to visualize blood vessels. This is, to the best of our knowledge, the first demonstration of coronary vascular imaging with OCT-based angiography. In addition to eliminating the motion problem, the proposed *ex vivo* architecture also provides more degrees of freedom, and potentially benefits studies of coronary blood flow that require independent control of the coronary pressure.

2. METHODS

A. OMAG imaging system

Figure 1 shows the schematic of the OMAG imaging system utilized in this study, which is similar to the one described in our previous reports (Zhi *et al.*, 2011b; Qin *et al.*, 2015a). Briefly, it was in a typical fiber-based spectral domain (SD) OCT configuration. The light source was a broadband supercontinuum laser (SuperK Versa, Koheras A/S, Denmark) with a bandwidth of 120 nm centered at 850 nm wavelength, yielding an axial resolution of ~3 μ m. The sample arm of the OCT system employed a pair of galvo-scanners and a 10 $\times$ objective lens for 2D scanning. OCT interferograms were captured by a custom-built high speed spectrometer equipped with a fast line-scan CMOS camera (Basler SPL 4096-140km, Germany). The scanning protocol was as follows: each B-frame was composed of 400 A-lines; 5 repeated B-scans were performed at each of 400 frame locations (2000 B-scans in total) to achieve high sensitivity. The data acquisition rate was set at 250 frames per second so that acquisition of each 3D data volume took 8 seconds.

B. Heart preparation

All animal procedures used in this study were performed in accordance with US NIH Policy on Humane Care and Use of Laboratory Animals and approved by the University of Washington Institutional Animal Care and Use Committee (protocol #2225-04). Normal hearts were harvested from healthy athymic rats, ~2 months old at the time of sacrifice. Animals were euthanized with an overdose of pentobarbital/phenytoin solution (Beuthanasia; 1.5 mL intraperitoneal injection). Once the animals achieved deep anesthesia while the heart was still beating, the chest was opened and 50U Heparin was intravenously infused via the inferior vena cava and allowed to circulate for 1–2 minutes to prevent thrombosis in the coronary vessels. Intravenous infusion with supersaturated potassium chloride was then used to arrest the heart in diastole followed immediately with excision of the heart. The aorta was cannulated followed by retrograde perfusion with a vasodilator buffer (PBS containing 4 mg/L Papaverin and 1 g/L adenosine) followed by fixation with 4% paraformaldehyde for 10 minutes. By arresting the heart in diastole combined with vasodilation prior to fixation, the coronaries were fixed in an open state to ensure efficient flow *ex vivo*. Perfusion pressure was maintained at 80–120 mmHg. After fixation, the hearts were immersed in buffer and transferred on ice to the OMAG imaging setup.

C. Retrograde perfusion for OMAG imaging

During OMAG image acquisition, the heart was placed on a custom-built housing plate that is illustrated in Fig. 2(a). The cannula was secured in a slot to prevent tissue movement. The heart holder was composed of two parts, a fixed part and an adjustable part, which allowed flexible adjustment of heart position as well as adaptive support to individual hearts with different size and shape. The housing plate was fixed in a 60 mm petri dish that served as a reservoir of waste perfusate. The perfusion system was established based on Langendorff's method (Bell *et al.*, 2011; Skrzypiec-Spring *et al.*, 2007), illustrated in Fig. 2(b). Perfusion pressure was manually exerted with a 60 mL syringe and monitored by a pressure gauge. Since the perfusion setup was utilized for both heart preparation and OMAG imaging, two reservoirs were employed to store buffers and Intralipid. Two 3-way stopcocks were used

downstream; one was for switching perfusate and another one for bubble removal. During imaging, the heart, by cannulating the aorta, was perfused with Intralipid solution (Sigma-Aldrich, St. Louis, USA) under constant hydrostatic pressures. This constant pressure perfusion mode prevented the risk of coronary artery damage from overdose of perfusate or excessive shear stress. The direction of flow during retrograde perfusion through the aorta was opposite normal physiological blood flow and forced the aortic valve to close. The applied pressure therefore forced the Intralipid into the coronary arterial vasculature through the two coronary ostia located at the left and right aortic sinuses. This situation mimicked a heart immediately after systole where the aortic pressure remains relatively constant for the remainder of diastole. Passing through the vascular bed, the Intralipid was transported via the coronary veins to the coronary sinus in the right atrium, before exiting through the vena cava.

3. Results and Discussion

A. 3D Visualization of Coronary Microvasculature

1. High-resolution 3D imaging of coronary microvasculature—Visualization of coronary microvasculature in 3D is essential for coronary circulation research. Figure 3 shows the OMAG imaging results of a fixed rat heart perfused with 10% Intralipid under a constant pressure of 110 mmHg. Data were captured over a $2\text{ mm} \times 2\text{ mm}$ region in the heart at 20 minutes after Intralipid perfusion. Figure 3(a) is a representative cross-sectional structure image of the heart reconstructed from the 3D OCT raw data. By applying the ultrahigh-sensitive OMAG (UHS-OMAG) algorithm to the structure images, corresponding functional blood vessel images were obtained, exemplified as Fig. 3(b). Volumetric vasculature was visualized through 3D rendering from the 400 cross-sectional OMAG images, as shown in Fig. 3(d). It provided detailed morphologic information as a part of the coronary vascular tree. Figure 3(c) is the color-coded microvasculature image of maximum intensity projection (MIP) from the top view of the 3D OMAG image. Depths from the heart surface to deep myocardium were coded with colors ranging from blue to orange. Notably, very dense capillaries were visualized and well resolved at different depths. We can clearly see that most of the capillaries run parallel with interconnections. This demonstrates that Intralipid solution instead of blood flowing through coronary blood vessels can serve as a good alternative to produce OMAG contrast. Based on the *ex vivo* preparation of Intralipid perfusion, OMAG was capable of imaging coronary microvasculature in 3D with high resolution, which may represent a key aspect in understanding heart function.

2. Co-registration of volumetric tissue structure and blood vessel images—

One of the unique features of the OMAG is the ability to co-register both functional blood vessels and heart tissue bed in a single volumetric image. It is owing to the fact that both blood vessel and tissue bed signals are derived from a same 3D OCT raw data, and their spatial dimension remains the same. This feature brought us the convenience to assess the relation between the heart tissue and the coronary vessels at any location by slicing the 3D volume. Figure 4 shows two orthoslices at random locations from *en face* and side directions, respectively, in a single 3D rendered volume merged with the tissue bed and the embedded blood vessels. As is demonstrated by these results, OMAG can provide more

informative details within the 3D data volume compared to conventional angiographic techniques.

3. Highly depth-resolved imaging without cross-talk between frames at different depths—OMAG has been demonstrated as a depth-resolved angiographic imaging technique in various animal models as well as human skin and eyes (Wang *et al.*, 2007; Wang *et al.*, 2010). In this study, when implemented in conjunction with extracorporeal perfusion of Intralipid instead of intrinsic blood flow, OMAG was able to resolve information along depth even better. In conventional OCT based angiography that is utilized for *in vivo* blood vessel imaging, tailing artifacts appear below vessels in cross-sectional images due to multiple scattering of photons before the photons are collected by OCT detector. The multiple scattering pathway of a scattered photon increases its optical path length and thus leaves a “tail” below the original scatterer (e.g., red blood cell) in the cross-sectional OCT image. In contrast, the use of Intralipid perfusate in our approach can effectively suppress tailing artifacts. Because the typical size of particles in Intralipid ranges from several nanometers to several hundred nanometers which are much smaller than red blood cells (5–8 microns). According to Mie theory, the probability of forward scattering of nanoparticles is much lower than that of micro-particles. Therefore, reduced multiple scattering suppressed tailing artifacts in our OMAG imaging results. Moreover, by virtue of the broad bandwidth of the supercontinuum light source, the OMAG imaging system can yield a high axial resolution of $\sim 3\ \mu\text{m}$. Consequently, highly depth-resolved imaging was achieved in rat heart. Figure 5 shows the OMAG imaging results of a region different from Figs. 3 and 4 in the same heart where the vascular network varied significantly along depth. Single *en face* OMAG frames at 6 different depths ($z = 60\ \mu\text{m}$, $100\ \mu\text{m}$, $140\ \mu\text{m}$, $180\ \mu\text{m}$, $220\ \mu\text{m}$, and $260\ \mu\text{m}$) were selected to demonstrate the depth-resolving capability of the proposed imaging technique. The perfusion pressure was still at 110 mmHg, and the concentration of Intralipid was 10%. Because the frames at different depths have minimal cross-talk, we can see clearly that the alignment orientation of capillary bundles varied at different depth of the tissue bed. The depth-resolving capability is an important feature of the proposed imaging method. It enabled us to observe capillaries at every depth. As is known, there are very dense capillaries distributed in myocardium. The ability to measure capillary orientation along the depth of the heart would be very useful to provide additional structural information when studying the orientation of myocardial fibers, since the myocardial capillaries are confined by and mostly in parallel with the myocardial fibers. Understanding myocardial fiber architecture is of importance because it is essential to study and accurately interpret cardiac electrical and mechanical performance (Taccardi *et al.*, 1994; Streeter *et al.*, 1969). Furthermore, improper remodeling of both myocardial fibers and coronary vasculature is a key pathological feature of heart disease (Burchfield *et al.*, 2013).

B. Changes in coronary flow in response to coronary pressure change

Coronary pressure is one of the main determinants of driving coronary blood flow. Many coronary heart diseases are related to abnormal coronary pressure (van de Hoef *et al.*, 2012). *In vivo* imaging does not allow independent control on coronary pressure because it is difficult to break the cardiovascular autoregulation in the living body. The proposed imaging technique can overcome this difficulty and easily vary perfusion pressure, which is

equivalent to coronary pressure, with an external perfusion system, providing an opportunity to investigate coronary circulation in response to changes in coronary pressure. To demonstrate this point, we acquired two datasets at the same region under different perfusion pressures, 110 mmHg and 80 mmHg, of which the MIP images are shown in Figs. 6(a) and 6(b), respectively. Qualitatively, both pressures led to highly dense-packed microvasculature. We selected four regions of interest (ROIs), as depicted with the four red dashed boxes in Fig. 6(a), and enlarged them to display in Fig. 6(c). The four images in the top row of Fig. 6(c) were extracted from Fig. 6(a) (110 mmHg) while the other four from Fig. 6(b) (80 mmHg). Images in each column of Fig. 6(c) represent a same region of interest with different perfusion pressures. Through comparison of the OMAG images of the four representative ROIs, we see pressure-dependent recruitment of microvascular branches at many locations, highlighted by yellow arrows in Fig. 6(c).

To quantitatively analyze vascular flow with changes in pressure, we evaluated the microangiograms in terms of fractal dimension (FD) and vessel area density (VAD) on the two MIP images (Figs. 6(a) and (b)). An FD is a value that indicates how an image fills space as one zooms into smaller scale, and thus it can be employed to describe the vessel tortuosity (a higher FD value correspond to more tortuosity). VAD is defined as the area of the blood vessels in the MIP image divided by the full area of the image. The method and algorithm we utilized to calculate FD and VAD can be found in Ref (Reif *et al.*, 2012). The quantification results are shown in Figs. 6(d) and (e). The FDs of the two microangiograms were calculated to be 1.828 ± 0.004 (pressure 110 mmHg) and 1.824 ± 0.005 (pressure 80 mmHg), respectively, and the VADs were 0.401 ± 0.001 (pressure 110 mmHg) and 0.388 ± 0.001 (pressure 80 mmHg), respectively. Increased applied-pressure led to better visualization of perfusion through a greater density of vessels, which possibly led to higher FD and VAD values. The observed higher vessel density could be a result of vessel compliance and widening resulting in higher flow and greater OMAG signal in regions that were previously not detectable. Alternatively, it is possible that increased pressure led to the opening of small vessel subnetworks that were not perfused at the lower perfusion pressure. In addition, OMAG signal at a cross section has been reported to be proportional to total number of particles flowing through the cross section per unit time, which is flux (Yousefi *et al.*, 2013; Yousefi and Wang, 2014). As a result, OMAG offers convenient measurement and comparison of relative flux between different conditions or locations. By summing up the pixel values of each image in Fig. 6(c) divided by the area, we measured the relative flux of the four ROIs at the two different perfusion pressures and normalized them with the average of the four flux values at the pressure of 110 mmHg, as shown in Fig. 6(f). All four ROIs exhibited a decrease in flux when the perfusion pressure was dropped from 110 mmHg to 80 mmHg which confirmed the monotonic relationship between flux and perfusion pressure. These imaging results and quantitative measurements validated our imaging technique as an effective tool for cardiovascular research, particularly enabling independent control of coronary pressure for studies on coronary circulation.

C. Observe perfusion dynamics in low and high flow regions

Ischemic heart disease is the leading cause of death worldwide usually caused by a partial or complete blockage of coronary arteries (Prinzmetal *et al.*, 1961). Reperfusion of coronary

flow is necessary to resuscitate the ischemic myocardium. However, reperfusion from severe myocardial ischemia may also exacerbate the initial injury caused by ischemia, resulting in reperfusion injury (Buja, 2005). This is of importance in the investigation of the pathology of ischemic heart disease as well as in the development of effective treatments. The developed imaging technique could be utilized to study disease states, such as ischemia and reperfusion, by dynamically monitoring coronary flow at different stages of perfusion and injury.

As a proof of concept, we studied the dynamics of coronary flow by evaluating OMAG signal over time as it approaches steady state. Prior to perfusion, the coronary vessels were filled with buffer that does not generate an OMAG signal. At the onset of Intralipid perfusion, the perfusate filled the myocardial vasculature through the coronary arteries with perfusion pressure maintained at diastolic pressure (80 mmHg) throughout. We monitored the OMAG signal accumulation at three time points after starting the infusion of Intralipid (post perfusion minute 5, 15, and 25) and observed changes in the visible microvascular structure as the Intralipid perfusion reached steady state. The results are shown in Fig. 7. At post perfusion Min 5, the myocardial vasculature was partially perfused with Intralipid and regions of low flow were easily distinguishable by low OMAG signal intensity and low vessel density compared to high flow regions which showed high OMAG intensity and high vessel density (Fig. 7(a)). This time point was considered to be the baseline of perfusion, where maximal Intralipid perfusion and OMAG signal has not been achieved in all regions of the myocardium. The two regions, as depicted by the orange and red dashed boxes in Fig. 7(a), were selected to demonstrate that OMAG imaging can be used to identify regions of both low and high flow. Subsequently, another two acquisitions were performed at post perfusion Min 15 (Fig. 7(b)) and Min 25 (Fig. 7(c)). We can see that Intralipid perfusion increases over time and reaches steady state by Min 25 with myocardial microvasculature almost completely perfused.

Normalized flux was consecutively measured over the low and high flow regions and shown in Fig. 7(d). The values of flux were normalized with the flux of the low flow region at baseline. In the low flow region, normalized flux slightly rose at Min 15 compared to the baseline, and dramatically increased thereafter. By contrast, flux of the high flow region did not increase continuously. At the baseline, the flux of this area was about 2.8 times of that of the low flow area. 10 minutes later, flux in the high flow region reached maximum, with an 18% increase compared to the baseline. At Min 25, it decreased by an imperceptible percentage. It can be inferred that a mild hyperperfusion occurred at Min 15. After that, the perfusion approached steady state. We also calculated VAD values over the low and high flow regions at the three time points, as can be seen in Fig. 7(f). Vessel density of the low flow region was progressively increased from 0.1598 at Min 5 to 0.1818 at Min 15 and reached its peak, 0.3324, at Min 25. In the high flow region, the vessel density was initially 0.3344 at Min 5. As perfusion continued, the vessel density was 0.4002 at Min 15 and dropped slightly to 0.3984 at Min 25, indicating the hyperperfusion at Min 15. These results demonstrated the capability of the proposed imaging technique to study the perfusion dynamics of physiologically different regions with potential to study the physiology and pathology of cardiac ischemia and reperfusion. Although the model utilized here used fixed

hearts and as such may not be clinically valuable, it validated that our imaging technique is highly feasible to adapt for this purpose.

4. Conclusions

In summary, we have demonstrated a novel *ex vivo* implementation of OCT based angiography, specifically OMAG, combined with retrograde perfusion of Intralipid for 3D visualization of coronary microvasculature with high resolution. The tailing artifact in conventional OCT based angiography was suppressed by virtue of the nanoscale particulate matter in Intralipid, which is much smaller than red blood cells. Consequently, truly depth-resolved angiographic imaging was achieved without cross-talk between depths. The extracorporeal perfusion scheme provided feasible convenience to independently control perfusion pressure and blood supply. As it offers precise morphological visualization of microvasculature and quantitative measurements, we expect this imaging technique will open up new opportunities for the investigation of microcirculation in not only the heart but also other organs (e.g., kidney), particularly 3D modeling of the vascular tree, biophysical modeling of perfusion distribution within the organ, effectiveness of drug/treatment to various pathological models, and evaluation for tissue engineering.

It should also be noted that there are some limitations to the proposed imaging technique. The imaging field view was $2\text{ mm} \times 2\text{ mm}$ in this study. Although it can be expanded to some extent, it is still not comparable to conventional X-ray angiography and impossible to image an entire heart with a single acquisition. To image an entire heart, several improvements are required: advanced heart housing plate allowing arbitrary rotation of heart for imaging every location, multiple acquisitions at every location, and sophisticated post-processing algorithms for stitching all the acquisitions into one final image. Another limitation is that the imaging depth is very shallow, $< 500\text{ }\mu\text{m}$. It could be enhanced to $\sim 2\text{ mm}$ by replacing the light source with longer wavelength to have better penetration depth and optimizing the optics. However, this is still insufficient to model a complete coronary vascular tree because the thickness of myocardium can be up to 1 cm . More importantly, in many cases, flow analysis of a fixed heart in a blood-free environment provides a “snapshot view” of the coronary circulation at one moment of vasoregulation. This prevents studies on real time physiological flow modulation and blood vessel response, like vasoconstriction, vasodilation, or the dynamic recruitment and closure of vascular subnetworks on a beat to beat basis. To perform OMAG imaging in a live sample, certain features such as temperature control, nutrient and oxygen infused intralipid solution, and a mechanically arrested heart would be required. In order to obtain the most accurate representation of coronary blood flow in our current study, the heart was arrested in diastole prior to fixation. Since most coronary blood flow occurs during diastole when the coronaries are able to dilate and circulate blood as the ventricle relaxes, flow measurements taken within diastolic pressure range ($80\text{--}90\text{ mmHg}$) are the most biologically relevant (Ramanathan and Skinner, 2005). Despite these limitations, *ex vivo* analysis of the fixed heart allows assessment of some of the key structural and functional properties of the coronary circulation that cannot be obtained in the living organ *in vivo*. Furthermore, we demonstrated as a proof of concept that changes in flow rate as a result of increased pressure are detectable through increased OMAG intensity suggesting this technique could be adapted to study vascular function. We

think this will be useful for studies of coronary microvascular disease and adaptation, e.g. in tissue engineering-based cardiac repair.

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Fig 1. Schematic of the OMAG imaging system equipped with a custom-designed heart housing plate and an external perfusion system. SC source: supercontinuum laser source; SM fiber: single mode optical fiber.

Fig 2.
 (a) Schematic 3D views of the parts (upper panel) of the heart housing plate and the assembly (lower panel). (b) Photograph of the perfusion system.

Fig 3.
 (a) Cross-sectional OCT image of the heart. (b) Corresponding OMAG flow image derived from (a). (c) *En face* maximum intensity projection view of the 3D flow image. Depths were coded with color: colors from blue to orange represent depths from heart surface to deep. (d) 3D microangiogram of the heart reconstructed with 400 consecutive cross-sectional OMAG images. Scale bars: 500 μm .

Fig 4.
Orthoslices at random locations from (a) en face direction and (b) side direction in a 3D rendering of merged tissue bed (gray color) and embedded vessels (golden color)

Fig 5. Single *en face* OMAG frames at different depths, z , in the 3D microangiogram. (a) $z = 60 \mu\text{m}$, (b) $z = 100 \mu\text{m}$, (c) $z = 140 \mu\text{m}$, (d) $z = 180 \mu\text{m}$, (e) $z = 220 \mu\text{m}$, (f) $z = 260 \mu\text{m}$. Scale bar: $500 \mu\text{m}$.

Fig 6.

(a) *En face* MIP image obtained under a perfusion pressure of 110 mmHg. Red dashed boxes indicate four regions of interest (ROIs). (b) *En face* MIP image obtained under a perfusion pressure of 80 mmHg. Scale bar: 500 μ m. (c) Enlarged images of the four ROIs under different perfusion pressures. Yellow arrows indicate places where vessel recruitment happened. Scale bar: 50 μ m. (d) Fractal dimension analysis upon the two MIP images. (e) Vessel area density calculated from the two MIP images. (f) Normalized flux of the four ROIs at different pressures. Relative flux values were normalized with the average of the

four flux values at the pressure of 110 mmHg. Error bars in (d) – (f) indicate standard deviations over three measurements.

Fig 7.

(a) *En face* MIP image of post perfusion minute 5. Orange and red dashed boxes indicate representative low and high flow regions, respectively. (b) *En face* MIP image of post perfusion minute 15. (c) *En face* MIP image of post perfusion minute 25. Scale bar: 500 μ m. (d) Normalized flux of the low flow and high flow regions at the three time points, Min 5, Min 15, and Min 25. (e) Normalized flux of whole areas of Figs. 7(a)–(c) at the three time

points. (f) Line chart to display trends of VAD change over the two regions. Orange line: low flow region; red line: high flow region.