Multimodality optical imaging of embryonic heart microstructure

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Abstract. Study of developmental heart defects requires the visualization of the microstructure and function of the embryonic myocardium, ideally with minimal alterations to the specimen. We demonstrate multiple endogenous contrast optical techniques for imaging the *Xenopus laevis* tadpole heart. Each technique provides distinct and complementary imaging capabilities, including: 1. 3-D coherence microscopy with subcellular (1 to 2 μm) resolution in fixed embryos, 2. real-time reflectance confocal microscopy with large penetration depth *in vivo*, and 3. ultra-high speed (up to 900 frames per second) that enables real-time 4-D high resolution imaging *in vivo*. These imaging modalities can provide a comprehensive picture of the morphologic and dynamic phenotype of the embryonic heart. The potential of endogenous-contrast optical microscopy is demonstrated for investigation of the teratogenic effects of ethanol. Microstructural abnormalities associated with high levels of ethanol exposure are observed, including compromised heart looping and loss of ventricular trabecular mass. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2822904]

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microscopy. The purpose of this study is to demonstrate these endogenous contrast microscopy technologies and elucidate the potential merits of high-speed and high-resolution imaging for the normal and abnormal embryonic heart.

2 Materials and Methods

2.1 Bench-Top Optical Coherence Tomography and Optical Frequency-Domain Imaging Systems

In time-domain OCT (TD OCT), axial ranging is performed by use of low-coherence reflectometry where the individual depth points are probed sequentially in time. A broad bandwidth [50 nm, full width at half maximum (FWHM)] source centered at 1.3 μm was used, providing an axial resolution of ~10 μm in tissue. The frame rate was 20 per second (2 kHz A-line rate, 100×500 pixels).

OFDI originates from a telecommunications ranging method termed optical frequency-domain reflectometry. The primary differences between OCT and OFDI are found in the system optics. With OFDI, the interferometer reference arm remains stationary and spectral interference is detected using a narrowband wavelength-tuning source. OFDI images, reconstructed by taking the Fourier transform of the spectral fringe pattern, are essentially identical to those obtained by OCT. OFDI provides a several-hundred-fold improvement in signal-to-noise ratio (SNR), compared to OCT, which can be utilized to increase imaging speed to nearly 1000 frames per second.

The wavelength tunable laser light source of our OFDI system was an extended cavity semiconductor laser employing an intracavity spectral filter. The laser featured a sweep repetition rate of up to 54 kHz, a wide tuning range of 111 nm centered at 1320 nm, and a high average output power of 30 mW (7 mW on the tissue). The axial resolution was 10 μm in tissue. The frame rate was 900 per second, resulting in a volumetric acquisition rate of 20 Hz (60×45×256 pixels per volume). The system further comprised an acousto-optic frequency shifter (25 MHz) to remove the depth degeneracy inherent in frequency-domain reflectometry.

Polarization-diversity detection was implemented to eliminate polarization artifacts in the fiber-based OFDI system. Dual-balanced photoreceivers were used to improve imaging sensitivity through the reduction of laser intensity noise. The photoreceiver outputs were digitized with a two-channel analog-to-digital converter at a sampling rate of 100 MHz with 14-bit resolution. TD OCT and high-speed OFDI were incorporated into a dissecting light microscope. The scanning system was comprised of a collimating lens (5-mm beam diameter), two synchronized galvanometric scanners for transverse scanning, a focusing lens (50-mm focal length), and a small mirror that deflected the beam downward toward the sample. The transverse resolution was 15 μm with a confocal parameter of 280 μm.

Displacements associated with local cardiac motion (Figs. 5(a)-(5d)) were estimated from the OFDI data by subtracting the heart surface locations at end diastole from those at end systole on a frame-by-frame basis. First, the heart was semi-automatically segmented using IPLab Spectrum (BD Biosciences, Franklin Lakes, New Jersey) in end diastole and end systole. Morphologic edge detection was then conducted on the segmented areas to determine the heart surfaces for end diastole and end systole. End diastole and end systole segmented areas were subtracted on a frame-by-frame basis. The subtracted image maintained its sign so that positive and negative displacements could be computed. For every point on the edge images, the thickness of the subtracted volumes was determined by summing the subtracted data within a local kernel centered at each edge point. The displacement value estimate for each surface position was then scaled according to the pixel dimensions and assigned a unique color that served as a color overlay on the 3-D volume rendering. Volumetric rendering and 3-D visualization was accomplished by using Osirix software.

High-resolution OFDI was performed using a novel laser source with 200-nm tuning range, centered at 1250 nm, in which two semiconductor optical amplifiers were utilized as the gain media. An axial resolution of 4 μm in tissue was measured. The transverse resolution was 2 μm with NA=0.2 objective lens. The imaging rate was 40 frames per second with an A-line rate of 20 kHz (500 A-lines per frame, 666 pixels per A-line).

Polarization-diversity and dual-balanced detection were performed and the photoreceiver outputs were digitized with a two-channel analog-to-digital converter at a sampling rate of 10 MHz with 12-bit resolution.

2.2 Full-Field Optical Coherence Microscopy System

FFOCM is an interferometric technique that utilizes 2-D parallel detection to provide subcellular resolution images of reflected light within biological specimens. The FFOCM system used spatially incoherent broadband light from a xenon arc lamp to illuminate the sample and the reference mirror of a Linnik interference microscope using two identical NA=0.3 water-immersion microscope objective lenses. Images were captured with a complementary metal oxide semiconductor (CMOS) area scan camera with a spectral response centered at 650 nm. The transverse resolutions were 2 μm and the axial resolution was 1.1 μm. Maximum penetration depth was typically 1 to 2 mm. Acquisition time was 2 s per frame for a transverse field of view of approximately 700×700 μm (512×512 pixels per frame, 800 frames per volume). Three-dimensional data was obtained by moving the sample through the focus at 1-μm increments. Volumetric rendering and visualization was accomplished by using Osirix software.

2.3 Spectrally Encoded Confocal Microscopy System

SECM is a reflectance confocal microscopy technique that uses near-infrared light, allowing deeper penetration into tissue, compared with confocal microscopes that utilize visible light. SECM differs from conventional laser scanning confocal microscopy in that it projects different wavelengths onto distinct locations on the sample. Rapid acquisition of spectra returned from the sample enables high-speed reconstruction of the image. In the SECM system, light from a rapid wavelength tuning source in the near-infrared (center wavelength=1.32 μm, instantaneous line width=0.1 nm, total bandwidth=70 nm, repetition rate up to 15.7 kHz) was
collimated onto a diffraction grating (1100 lines per mm) and
focused through a 1.2 NA, 60× objective (Olympus
UPlanApo/IR 60× /1.20 W). A multimode fiber was used for
signal collection, resulting in 0.9-μm transverse and 2.5-μm
axial resolutions. Images comprised of 500×500 pixels were
acquired at 1 frames per second. The maximum imaging
depth was limited to the 280-μm working distance of the
objective lens.

2.4 Resolution Measurements

For all imaging modalities (TDOCT, OFDI, SECM, and
FFOCM), transverse resolution was measured by imaging a
resolution target and calculating the full width at half max-
imum (FWHM) of the derivative of the edge response. Axial
resolution was measured by scanning a mirror along the opti-
cal axis and determining the FWHM.

2.5 Specimen Preparation, Ethanol Treatment, and
Histology

Xenopus laevis frogs were purchased from Nasco (Fort Atkin-
son, Wisconsin). Animal procedures were performed accord-
ing to the approved protocols of Massachusetts General Hos-
pital Subcommittee on Research Animal Care. Embryos were
obtained by in vitro fertilization, incubated in 0.1× Marc’s
modified Ringer’s medium (MMR),26 and staged according to
Nieuwkoop and Faber tables.27

Ethanol treatments were performed in 0.1× MMR (vol/
vol), from the mid-blastula transition (MBT, stage 8.5),28 until
the time of imaging, typically after 5 to 7 days at room tem-
perature. All embryos were imaged repeatedly during their
development. Aligning the embryos was performed by ob-
serving them through the dissecting microscope and TDOCT
cross sectional imaging of the heart area. Prior to in vivo
imaging, to prevent the embryos from swimming, they were
anesthetized using 0.02% 3-aminobenzoic acid ethyl ester (A-
5040, Sigma). For TDOCT and OFDI imaging, embryos were
positioned on a 1.5% agarose gel plate with their ventral side
facing up, covered by the anesthesia working solution. For
imaging with the SECM system, embryos were placed on a
cover slip, lying on their ventral side in an anesthesia buffer,
and imaged from below. FFOCM was conducted on fixed
embryos. Fixation was done in MEMFA [0.1-M MOPS
(pH7.4), 2-mM EGTA, 1-mM MgSO4, and 3.7% formalde-
yde] for more than one hour. Prior to imaging, the fixed
embryos were transferred into a Petri dish with 1× phosphate
buffered saline (PBS) (8-gr NaCl, 0.2-gr KCl, 1.44-gr
Na2HPO4, and 0.24-gr KH2PO4), with their ventral side fac-
ing up, supported by clay. Developmental stages and pheno-
types were determined by R. Yelin.

Plastic histology sections29 were obtained after additional
fixation in Karnovsky’s Fixative (KII) and embedding in
tEpox-812 (Tousimis). Sections 1 μm thick were cut on a
Reichert Ultracut Microtome and stained with methylene
blue/toluidine blue in borate buffer (Tousimis). Paraﬃn
sections (5 μm thick) were stained with hematoxylin and eosin.

3 Results

3.1 High-Resolution Three-Dimensional Imaging by
Full-Field Optical Coherence Microscopy in
Fixed Embryos

FFOCM offers the capability to image the microstructure of
the embryonic heart with nearly isotropic cellular level reso-
lution in a fixed embryo. Volumetric FFOCM images spanned
a transverse field of view of 700×700 and 1000 μm in
depth. The transverse and axial resolutions were 2 and
1.1 μm, respectively. Acquisition time was 2 s for a single en
face section, and 33 min for the entire volume. FFOCM sec-
tions of the heart in a fixed Xenopus tadpole (stage 49) allow
visualization of the ventricular trabeculae [Figs. 1(a) and
1(c)], the spiral valve [Figs. 1(b) and 1(d), arrows], and the
partial atrial septum [Fig. 1(d), arrow head]. Partially trans-
parent volumetric rendering of the heart [Figs. 1(e)–1(h)] re-
evals the looping-compression structure with the angled TA
[Fig. 1(e)], the aortic arches [Figs. 1(f) and 1(g)], and the thin
wall of the atrium [Figs. 1(g) and 1(h)], in their 3D context.
Cutaway views of Fig. 1(e) show fine 3-D internal structures,
including the trabeculae [Figs. 1(i) and 1(j)] and the atriovent-
tricular valve [Fig. 1(k)]. A magnified view of the atrioven-
tricular valve, shown [Fig. 1(l)] next to a corresponding his-
tology section of the same embryo [Fig. 1(m)], demonstrates its
cuspid morphology.

3.2 Heart Abnormalities Due to Ethanol Exposure

Cardiovascular malformation can be caused by genetic30
and teratogenic factors.31 Ethanol is a well-known teratogen; ex-
posure of human embryo during pregnancy to alcohol (etha-
nol) is associated with fetal alcohol syndrome (FAS).32,33 One
estimate suggests that 54% of the children with FAS have
heart defects.34 To study the teratogenic effect of ethanol on
Xenopus heart development, embryos were exposed to differ-
ent concentrations of ethanol (0.5 to 2.5% vol/mol) from the
mid-blastula transition (stage 8.5)28 until they were imaged.
Siblings developing under the same conditions, but not ex-
posed to ethanol, were used as controls. During the develop-
mental process, we screened the heart area of 121 live em-
byos in an attempt to identify and qualitatively evaluate the
extent of the teratogenic effect. To image the large number of
embryos repeatedly and reliably, we used a dedicated TDOCT
system that was incorporated into a dissecting microscope,
which allowed simultaneous imaging with the two systems,
with easy access for embryo manipulation (accurate orienta-
tion alignment). What appears as a complete maturation with
a substantial change in morphology compared to the controls
was found in a minority (25%) of embryos that were exposed to
1% ethanol (n=28), and in a majority (74%) of embryos that
were exposed to 1.5% ethanol (n=27, as in Video 1). Grossly
abnormal rotation of the heart tube and/or incomplete
maturation was found in all embryos in the 2.0 and 2.5%
groups (n=17 and n=7, respectively, as in Video 2). We did
not observe morphologic differences between the 0.5% etha-
nol treated group (n=16, as in Video 3) and the control group
(n=42, as in Video 4).

Cardiac motion was evident in all embryos, even those
with the most severe malformations. Using TDOCT, we se-
lected one tadpole (stage 48) from each of the control, 0.5,
1.5, and 2.0% ethanol treated groups to demonstrate typical
Fig. 1 3-D imaging of Xenopus heart (stage 49) using FFOCM in a fixed embryo. En face sections show trabeculae (a) within the ventricle and (b) the spiral valve (valve marked by arrow). Cross sections at different locations along the anterior-posterior axis show (c) the ventricular trabeculae and cavity, (d) the TA and spiral valve (marked by arrow), the thin atrium wall, and the partial atrial septum (arrowhead). Partially transparent volumetric rendering of the heart (e) through (h) is shown at different viewing angles, revealing (e) the characteristic looping-compression structure and the angled TA, (f) the TA splitting into the paired aortic arches, the carotid arches (ca), systemic arches (sa), the pulmocutaneous arches (pa), and (g) and (h) the thin walled atrium. (i), (j), and (k) Cut away views through the 3-D dataset. The atrioventricular valve is shown in a magnified view [(l), marked by star] next to the corresponding histological section [(m); resin-embedded, 1 μm sections, methylene blue stain]. v, ventricle; t, truncus arteriosus; a, atrium. Scale bars correspond to 100 μm.
phenotypes (Videos 1 through 4). We determined that the four tadpoles’ hearts were in advanced developmental stages by identifying the existence of a partial atrial septum and an atrioventricular valve (histology data not shown).

The TDOCT images provided the first indication of damaged looping in the 1.5 and 2.0% groups (Videos 1 and 2). We furthermore observed lower TDOCT signal from within the ventricle in those groups.

Photographs of the tadpoles, taken in vivo from the ventral aspect, are shown in Figs. 2(a)–2(d). The four embryos were then fixed and imaged with FFfCM to evaluate fine structural malformations. 3-D rendering of FFfCM data allowed evaluation of myocardial structure at high resolution, revealing the similarity between the control and the 0.5% tadpoles, and clearly showing defective heart tube looping in tadpoles from the 1.5 and 2.0% groups [Figs. 2(e)–2(h)]. Based on sections from the FFfCM datasets, we measured the TA dimensions in each of the four representative embryos and found a decrease in maximum diameter of approximately (20±9)% in the 1.5% embryo and (66±15)% in the 2% embryo, when compared to the control and 0.5% groups. The smaller distorted TAs and spiral valves (marked by arrows) are shown in the 1.5% [Fig. 2(k)] and 2.0% embryos [Fig. 2(l)] compared with the control [Fig. 2(i)] and the 0.5% [Fig. 2(j)] embryos.

Pericardial edema was present in the 1.5 and 2.0% groups [Figs. 2(k), 2(l), 2(o), and 2(p)] compared with the control and 0.5% groups. Ethanol also affected the ventricle. The developed trabeculae in the control [Fig. 2(m)] and 0.5% [Fig. 2(n)] hearts contrast the less developed trabeculae in the 1.5% group [Fig. 2(o)] and the large ventricular cavity with sparse, stunted trabeculae in embryos exposed to 2.0% ethanol [Fig. 2(p)]. Corresponding histological sections confirmed some of our findings, including the less developed trabeculae [Figs. 2(q)–2(t)] in embryos with the greater ethanol exposure.

3.3 High-Resolution Imaging of the Embryonic Heart with Spectrally Encoded Confocal Microscopy In Vivo

To obtain high-resolution images within the Xenopus heart in vivo, we have used SECM that allows the imaging of both structure and dynamics with microscopic resolution. SECM provides a transverse resolution comparable to FFfCM, but at much higher frame rates, enabling microscopy of the heart in vivo. The Xenopus myocardium (stage 49) was imaged with
SECM in vivo at a frame rate of 10/s, a transverse field of view of 220 × 220 μm, and transverse and axial resolutions of 0.9 and 2.5 μm, respectively. The maximum penetration depth was 280 μm. SECM images show the thin cusps of the atrioventricular valve [Fig. 3(a)], approximately 280 μm below the ventral surface, and parts of the ventricle and TA [Fig. 3(c)], containing individual blood cells within the trabecular spaces. SECM images correlated well with corresponding histological sections of paraffin, H and E. V, ventricle; t, truncus arteriosus; a, atrium; tr, trabeculae; frame width in (a) through (d) is 2.5 mm. Scale bars correspond to 100 μm.
that may represent nuclei and organelles can be observed.

3.4 Aneurismal Dilatation in the Xenopus Embryo

In one of the embryos (stage 47), we noticed a protrusion emanating from the TA wall. SECM sections obtained in vivo at two different depths [Figs. 4(a) and 4(b)], taken from Video 6 reveal its saccular shape, its location with respect to the spiral valve, as well as the flow of individual blood cells through the defect. This abnormality was also observed in vivo with TDOCT [Fig. 4(a), inset]. The embryo was then fixed and imaged with FFOMC. An FFOMC section [Fig. 4(c)] and a 3-D volumetric rendering of the dataset [Fig. 4(d)] shows the dilatation in the context of the entire heart. Difficult to see under conventional brightfield microscopy [Fig. 4(e)], but clearly visualized with TDOCT, FFOMC, and SECM, this protrusion may represent a saccular aneurismal dilatation of the TA in a heart that otherwise appeared to have a normal phenotype.

3.5 Optical Frequency Domain Imaging Allows Real-Time Four-Dimensional Imaging of the Xenopus Tadpole Heart

Direct imaging of heart dynamics in the whole mount embryo, in situ, including valve activity and myocardial motion, can help to elucidate heart function and the etiology of failure. While FFOMC and SECM are too slow, in their current implementation, to acquire the 3-D volume of the heart in real time, OFDI allows 4-D imaging without using gating or synchronization techniques. OFDI images of the Xenopus heart (stage 49) were acquired in vivo at a rate of 900 frames per second (fps), while the OFDI image plane was scanned through the heart at a rate of 20 Hz. Volumetric OFDI images were therefore obtained at a rate of 20 3-D datasets per second [Figs. 5(a)–5(d)]. The heart beat rate was measured to be approximately 2 Hz, resulting in ten volumetric datasets captured during a single cardiac cycle. A 4-D rendering of the myocardial dynamics is shown in Video 7. At end systole, OFDI demonstrated that the ventricle was at its smallest volume; the volumes of the atrium and truncus arte-

Figure 3 High-resolution confocal imaging in vivo using SECM. En face SECM images showing the atrioventricular valve [(a), marked by star], the ventricular trabeculae, and the TA [(c) and (d)], stained with methylene blue stain. The regions of interest in (b) and (d), marked by the dotted rectangles, correspond to the approximate fields of view of the SECM images in (a) and (c), respectively. (e)–(g) A series of three SECM images demonstrate the opening of the spiral valve, allowing the flow of blood cells to the aortic bifurcation. Arrows mark the flow direction. (h) Trabecular microstructure within the ventricle. Stars mark valve locations. v, ventricle; t, truncus arteriosus; s, spiral valve; b, blood cells. Scale bars correspond to 50 μm.
were conversely at their maxima [Figs. 5(a) and 5(c)]. At end diastole, the ventricle was dilated to its greatest volume, whereas the volumes of the atrium and TA were at their minima [Figs. 5(b) and 5(d)].

3.6 High-Resolution Optical Frequency Domain Imaging of the Embryonic Heart In Vivo

To increase OFDI resolution, a novel broadband (200 nm) wavelength-swept source\(^2\) was used to obtain cross sections of a stage 49 Xenopus heart, \textit{in vivo}. Compared to the 15-\(\mu\)m transverse and 10-\(\mu\)m axial resolutions of the previously described OFDI, the transverse and axial resolutions of high-resolution OFDI were 2 and 4 \(\mu\)m, respectively. Imaging speed was 40 fps.

Details within the three-chamber Xenopus heart can be clearly resolved in the high-resolution OFDI movie (Video 8), including atrioventricular valve dynamics [Figs. 5(e)–5(g)]. Individual blood cells can also be seen flowing from the...
Atrium to the ventricle through the atrioventricular valve [Fig. 5(g)]. The ventricular trabeculae [Fig. 5(h)] and the spiral valve [Fig. 5(j)] structure and dynamics are clearly resolved with the high-resolution OFDI. For comparison, we present FFOCM sections [Figs. 5(i) and 5(k)] of corresponding areas from embryos at similar developmental stages.

4 Discussion

A common paradigm in developmental biology research is to manipulate the genotype and monitor the phenotype. Morphology is an important aspect of the phenotype. In the heart, even slight morphological and dynamical abnormalities may be critical for proper myocardial function. An ability to identify subtle morphological and dynamical variations in two and three dimensions can significantly improve the sensitivity of this paradigm.

In the *Xenopus laevis* tadpole, heart structures such as the myocardial wall, septum, and valves may only be a few cells thick. Evaluating the morphological phenotype not only re-
quires resolving such fine structures, but also the capability to visualize these microscopic features within the beating heart, where typical displacement velocities are on the order of 1 mm/s (estimated based on the data from the high-speed OFDI, and assuming a beat rate of approximately 2 Hz). For cross sectional imaging, TDOCT at 20 frames per second is sufficiently fast to avoid motion artifacts in a 2-D image. With the several-fold increase in imaging speed enabled by OFDI, 3-D images of the embryo heart can be obtained at different times within the cardiac cycle.\textsuperscript{19,20}

High resolution and high speed are not the only requirements for effective imaging of the heart. In the \textit{Xenopus} embryo, the heart extends from between 200 and 800 μm beneath the ventral surface. An effective imaging method should therefore also be capable of imaging at these depths without substantial loss of signal and resolution.

Structural imaging of the heart \textit{in vivo} has been demonstrated using a variety of noninvasive imaging modalities such as micro-MRI in adult pigs\textsuperscript{35} and adult mice,\textsuperscript{92} micro-CT in adult pigs\textsuperscript{35} and adult mice,\textsuperscript{36} ultrasound in mouse embryos,\textsuperscript{49} and positron emission tomography (PET) in adult dogs\textsuperscript{20} and adult mice.\textsuperscript{41}

Optical techniques enable imaging of the embryonic heart at higher resolution. The morphology of the developing \textit{Xenopus laevis} heart has been studied in fixed embryos, using 3-D rendering of histology sections\textsuperscript{6} and confocal microscopy.\textsuperscript{7} Confocal microscopy has been also used for \textit{in vivo} heart imaging, including studying the role of intracardiac fluid forces\textsuperscript{42} and heart dynamics at very early developmental stages\textsuperscript{6} in zebrafish. Time-lapse study, which does not require optical sectioning, was conducted using high-speed video cameras, in chick embryos.\textsuperscript{10}

TDOCT was first applied to small animal heart imaging by Boppart et al.\textsuperscript{33,44} Doppler TDOCT allows quantitative velocity measurements under the tissue surface, and was used to demonstrate blood flow in the \textit{Xenopus} tadpole.\textsuperscript{35,46} Due to its limited speed, 3-D heart imaging using TDOCT has primarily been demonstrated in fixed embryos of \textit{Xenopus},\textsuperscript{4,45} mouse,\textsuperscript{2} and chick.\textsuperscript{47} The gating technique has been employed to circumvent imaging speed limitations, enabling the reconstruction of 3-D OCT heart images, at different stages in the cardiac cycle, of mouse and chick embryos.\textsuperscript{48} Post-acquisition synchronization techniques were used for 3-D \textit{in vivo} confocal microscopy in zebrafish embryos.\textsuperscript{49}

FFOCM was found to be capable of providing high quality 3-D imaging with cellular (1 to 2 μm) resolution. Compared to confocal microscopy, FFOCM has axial resolution that is independent of the objective lens numerical aperture, opening up the possibility of obtaining images with isotropic subcellular resolution. SECM was demonstrated with comparable transverse and axial resolution to those of FFOCM, but was capable of imaging at much higher speeds, enabling visualization of myocyte, blood, and valve motion \textit{in vivo}.

Owing to its high imaging speed, OFDI provided real-time 4-D imaging of a beating heart without requiring cardiac gating or synchronization.\textsuperscript{19,20} We showed that OFDI might be useful for assessing myocardial wall displacement during the cardiac cycle [Figs. 5(a)–5(d)]. The use of anesthesia for immobilizing the embryos during imaging also resulted with reduction in heart beat rate, from approximately 3 to 2 Hz, which helped to increase the number of volumetric samples acquired during a single cardiac cycle. While this demonstration is limited in scope, mainly due to the relatively low number of pixels in the dataset, and the anesthesia that was applied to the embryos, it still shows the potential of OFDI for 4-D imaging of the \textit{Xenopus} heart. Future technical developments on faster light sources (see, for example, Jenkins et al.\textsuperscript{29}) and more advanced image processing algorithms could extend this technology to allow reliable measurements of dynamic physiological parameters, such as stroke volume and ejection fraction, as well as valve opposition, stiffness, and nodularity, which all have close analogs in human pathophysiology. By modifying the OFDI light source, we were also able to conduct real-time cross sectional imaging with higher axial resolution (4 μm) while maintaining high frame rates (40 frames per second), enabling unprecedented cross sectional visualization of valve dynamics [Figs. 5(e)–5(g)] and single cell blood flow. Compared to FFOCM, the axial resolution of OFDI is limited due to the bandwidth of wavelength-tuning sources and its longer central wavelength (1.3 μm), which affects both transverse and axial resolution. The focal volume of FFOCM in this work was approximately four times smaller than that of OFDI, allowing higher resolution for visualizing the detailed microstructure of the developing heart. Table 1 summarizes the different capabilities of each technique, highlighting their complementary nature.

To demonstrate the potential of some of these techniques for evaluating teratogenic effects, we imaged \textit{Xenopus} embryos following the administration of different concentrations of ethanol. Different animal models are being used to study FAS, despite obvious differences in general structure and in the ethanol teratogenic concentrations.\textsuperscript{50} The teratogenic effect of ethanol on \textit{Xenopus laevis} was previously studied on the morphological and molecular levels,\textsuperscript{28,51,52} but to our knowledge, the teratogenic effect of ethanol on \textit{Xenopus} heart development has not been investigated. The ethanol concentrations that we used to obtain an observable effect on heart development were 1.5 to 2.5% (vol./vol.). These values are equivalent to 1.2 to 2 g/100 mL, which are about 6- to 10-fold higher than high intoxication levels in the human blood system (about 0.2 g/100 mL). While this value

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<td>Whole organ microscopic morphology</td>
<td>Subcellular dynamics</td>
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Table 1 Comparison of endogenous-contrast modalities for optical imaging of the embryonic heart. Bold text denotes the best imaging characteristics.
heart from different vantage points. Combining OFDI, SECM, and FFoCM could leverage their strengths (see Table 1) and provide a tool for obtaining a more comprehensive morphological and functional myocardial phenotype. This multimodality paradigm can be extended to other systems and animal models as well. Since these noninvasive imaging techniques do not alter the specimen, they can be used sequentially or in parallel. Furthermore, while we have used separate imaging systems in this work, there is no fundamental barrier preventing their combination into one imaging system that uses a single wavelength swept source.

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References

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