Published in final edited form as:

J Biophotonics. 2020 November ; 13(11): e202000223. doi:10.1002/jbio.202000223.

Optogenetic cardiac pacing in cultured mouse embryos under imaging guidance

Andrew L. Lopez III¹, Shang Wang², Irina V. Larina^{1,*}

¹Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA

²Department of Biomedical Engineering, Stevens Institute of Technology, 1 Castle Point Terrace, Hoboken, NJ 07030, USA

Abstract

The mouse embryo is an established model for investigation of regulatory mechanisms controlling cardiac development and congenital heart defects in humans. Since cultured mouse embryos are very sensitive to any manipulations and environmental fluctuations, controlled alterations in mouse embryonic cardiac function are extremely challenging, which is a major hurdle in mammalian cardiac biomechanics research. This manuscript presents first optogenetic manipulation of cardiodynamics and hemodynamics in cultured mouse embryos. Optogenetic pacing was combined with 4D (3D+Time) OCT structural and Doppler imaging, demonstrating that embryonic hearts under optogenetic pacing can function efficiently and produce strong blood flows. This study demonstrates that the presented method is a powerful tool giving quick, consistent, reversible control over heart dynamics and blood flow under real time visualization, enabling various live cardiac biomechanics studies toward better understanding of normal cardiogenesis and congenital heart defects in humans.

Graphical Abstract

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*Corresponding Author: larina@bcm.edu.

Keywords

optogenetics; mouse; optical coherence tomography; embryo; heart

1 Introduction

Live imaging of development is a powerful approach when it comes to understanding processes in embryonic development. In contrast to static methods, where samples are fixed and stained for analysis, live imaging provides information on the dynamics of embryonic development in space and time. As live imaging methods improve, new technologies are emerging to enhance our live imaging capabilities and our understanding of biology. In particular, the implementation of optogenetics is becoming a promising avenue in development and biophotonics research. Previously, most work done with optogenetics was conducted to study neuronal circuits—the ability to activate action potentials in subsets of neurons in an intact nervous system has revealed valuable information on how different types of neurons interact, develop, and regulate behavior and activity[1-4]. Now, optogenetics is gaining traction as a modality to regulate cardiomyocyte function in different model organisms. In this work, for the first time we present the use of optogenetics to control mouse embryonic heartbeat.

The heart is a mechanical organ, and a large effort is dedicated to understand how mechanical forces regulate heart development. Initial investigations have focused on altering

hemodynamics and heartbeat by physical means, and have provided clear evidence that physical forces are required for heart development[5-12]. Furthermore, molecular investigations of cardiovascular development cell signaling has shown that heart development, in part, is regulated by mechanical forces [13-16]. Lacking in this endeavor, is a more precise definition for the role of mechanical forces during development. Both contractile force and hemodynamic force are believed to play essential roles in cardiogenesis [17, 18]; it is thus imperative to investigate their specific roles for a more complete understanding on normal cardiogenesis and congenital heart defects.

Live embryonic imaging and optical cardiac pacing methods could provide a great opportunity to discriminate the roles of cardiac contraction and hemodynamics in development. In 2010, Jenkins et al. first demonstrated optical pacing as a non-contact approach to control embryonic heartbeat in chicks. This new approach served to bridge optical methods with embryonic assays by giving an experimenter control over cardiodynamics while leaving the embryo whole, intact, and unperturbed. While optical pacing does provide exogenous control of the embryonic heartbeat, optogenetics allows investigators to target distinct cell populations, and activation is achieved at much lower power densities. For example, by inserting the light sensitive channel ChR2 into a distinct class of cardiomyocytes that have a neuroendocrine role and localize to specific regions of an adult mouse heart, Wang et al. potentially identified a mechanism to regulate cardiac contraction through endocrine regulation using optogenetics [19].

The use of genetic engineering methods in model organisms to selectively insert photosensitive channels at high levels in tissues that have a unique genetic profile, is particularly useful in embryology because an embryo is constantly changing, and cells are actively migrating and differentiating. Using this approach, research into the cardiodynamics of the developing embryo is emerging. Studies in zebrafish have identified that cardiac contraction is regulated by a select group of cells and that those cells are dynamic and migrate across the embryonic heart tube through development [20]. Alex et al. in 2015 used optogenetics to determine how cardiodynamics changes through development from embryo to adulthood in *Drosophila* and described cardiodynamics through the entire life of an organism [21]. These studies pioneered the optogenetic control of embryonic heart with novel system designs and provided important insights into cardiodynamics over development. However, whether optogenetics can be used to manipulate the hemodynamics remains unknown, and optogenetic control of embryonic cardiodynamics in the mammalian model has yet to be explored.

Toward this gap, this manuscript presents a novel approach for optogenetic regulation of cardiodynamics and hemodynamics in live cultured mouse embryos under OCT imaging. The method builds upon our previously established live imaging protocols and 4D (3D +Time) OCT imaging methods for mouse embryonic cardiodynamics and quantitative Doppler OCT blood flow analysis [22-24]. We generated mouse embryos with specific expression of photosensitive protein ChR2 in cardiac cells for efficient excitation of cardiac contractions with low energy pulses of blue (473 nm) light. The optogenetic excitation beam was coupled with a lab built spectral domain OCT system for optogenetic beam guidance and detailed volumetric analysis of cardiodynamics and hemodynamics under optogenetic

control. The study revealed the pacing regimens for efficient periodic stimulation at higher frequencies as well as those resulting in arrhythmic contractions. 4D OCT analysis revealed that the hearts under optogenetic pacing can function efficiently to produce strong blood flows. Hemodynamic analysis performed at different optogenetic stimulation conditions demonstrated that the flow can be increased or decreased by tuning the pacing frequency, which suggests a potential for blood flow manipulation toward biomechanical analysis. This is a first demonstration of efficient non-invasive manipulation of cardiodynamics in mammalian embryos combined with live imaging. Taking advantage of genetic manipulation tools and numerous available mouse models, this method sets a basis for variety of live studies investigating the role of mechanics in cardiogenesis, providing better insight into the mechanism of congenital heart defects in humans.

2 Materials and Methods

2.1 Integrated setup for OCT imaging and optogenetic stimulation

We utilized a house built spectral-domain OCT system with a central wavelength of 810 nm and a bandwidth of ~110 nm [22, 24, 25]. Fiber-based Michelson interferometer is used to obtain interference fringes from light back-reflected from the reference and sample arms. Fringes are spatially resolved using a lab-built spectrometer that combines a diffraction grating with a focusing lens and a line-field camera (spL4096-140km, Basler). An A-line imaging rate was set to ~68 kHz. The axial resolution was ~5 μm in tissue, and the transverse resolution was ~4 μm . The system sensitivity was measured at ~97dB and a sensitivity drop of ~4dB over ~1mm in depth. Two galvanometer mirrors in the sample arm were used to obtain two-dimensional (2D) and three-dimensional (3D) scans.

For optogenetic stimulation, we used a 473 nm diode-pumped solid-state laser. The open-space laser beam was coupled into a single-mode fiber, delivering the light to the OCT sample arm. A longpass dichroic mirror with a 650 nm cut-on wavelength (DMLP650, Thorlabs) was utilized to combine the paths of optogenetic light with the OCT imaging beam. The dichroic mirror was placed between the OCT galvanometer mirrors and the scan lens, thus to have the positioning of the optogenetic beam independent from the light scanning for OCT imaging. For a spatial control over the optogenetic beam, the dichroic mirror was mounted into a right-angle kinematic mirror mount (KCB1C, Thorlabs), which allowed for transverse positioning of the optogenetic beam relative to the OCT imaging beam at the focal plane. For spatial adjustment of the optogenetic stimulation light, a laser beam profiler (LBP2-HR-VIS2, Newport) was used to guide the process in real time prior to all experiments. Specifically, the optogenetic beam was aligned to overlap with a reference position of OCT beam that is created with a known galvanometer setting. This allowed us to know the location of optogenetic beam relative to the OCT imaging volume, which was kept the same during experiment. The beam size was measure with the beam profiler as ~30 μm in diameter. The 473 nm laser was controlled by a function generator that was used to adjust the pulse width and the repetition frequency. For all experiments, the light pulse width was set at 20 ms, and the frequency was in the range of 1.8-4.0 Hz. The power of the optogenetic light was maintained around 0.7 to 1.0 mW out of the imaging lens. The sample arm of the

OCT system with the integrated optogenetic stimulation setup was placed within an incubator (37°C, 5% CO₂) for live embryonic imaging.

2.2 4D OCT imaging of cardiodynamics and hemodynamics in live embryo culture.

For 4D cardiodynamic reconstruction, OCT data sets were acquired as single dense volumes covering the heart (~400 μm X 400 μm) with 15,000 B-scans at 100 Hz B-scan rate. The raw OCT data were converted into structural intensity and corresponding Doppler frame series. The Doppler images of axial velocity were built using windowed Kasai autocorrelation function with a window size of 10x10 pixels [26]. Each data set of 15,000 frames contained approximately 150-300 heartbeat cycles, depending on the heartrate. The data set was split into individual heartbeats, as defined through periodicity analysis of structural images, and each heartbeat was assigned to a corresponding spatial location within the volume. The number of frames for each special location was doubled to yield two complete cycles, and the time lapses were sequentially synchronized to their neighbors based on structural images following previously established methods [27], leaving the original number of frames covering one heartbeat cycle for each position. This resulted in a synchronized 4D structural and corresponding Doppler OCT data set covering one complete heartbeat at 100 Hz volume rate. The volumetric rendering and visualization for all OCT data sets was performed in Imaris (Bitplane) software.

2.3 Mouse embryo manipulations

All animal manipulation procedures have been approved by the Institutional Animal Care and Use Committee at the Baylor College of Medicine. To generate embryos expressing ChR2 in the heart for optogenetic pacing experiments, we crossed homozygous *Ai32(RCL-ChR2(H134R)/EYFP)* mice, which express ChR2::EYFP fusion protein following exposure to Cre recombinase (JAX, Stock No. 024109), to homozygous *Nxk2.5^{IRESCre}* mice (JAX, Stock No: 024637), expressing Cre recombinase in cardiac crescent and heart tube progenitor cells. Mating pairs were set overnight, and the females were checked for a vaginal plug every morning. Upon confirmation of copulation—visually and with a vaginal probe—female mice were separated and recorded as embryonic day (E) 0.5. On E8.5, embryos were dissected and placed in static culture following previously established protocols [28]. Briefly, a pregnant female is sacrificed, and the uterine horn is surgically removed and quickly placed in 37°C medium containing 95% DMEM/F12 and 5% fetal bovine serum. Individual embryos are separated from the uterus with the yolk sac intact under microscopic guidance, transferred into individual dishes in the same media as mentioned before, and placed in a 37°C / 5%CO₂ incubator for recovery before imaging. All embryos were imaged within 1 to 5 hours after the dissection.

3 Results and Discussion

3.1 Optogenetic stimulation allows control of embryonic heartbeat

To enable manipulation of cardiodynamics with light, we generated embryos that express light-sensitive transmembrane protein Channelrhodopsin 2 (ChR2) in cardiac cells. ChR2 is a light activated non-specific cation channel. Upon activation with blue light, the channel opens to allow Na⁺ ions to pass through the membrane inside the cell, triggering action

potential (Figure 2A). In neurons, action potential causes neuron firing, while in cardiac and skeletal muscle cells, it initiates contraction. To express ChR2 in the embryonic hearts, we used the early cardiomyocyte specific Cre line, *Nkx2.5-Cre*, expressing Cre recombinase in cardiac crescent and heart tube progenitor cells with strong expression in cardiomyocytes[29]. The mice from this cross are viable and fertile, suggesting that *Nkx2.5-Cre* driven expression of ChR2 does not affect embryonic cardiac function.

We previously established a series of protocols to maintain live dissected mouse embryos in static culture for up to 24 hrs and dynamic imaging of beating hearts with OCT. In this study, we coupled blue (473 nm) pulsed laser beam for optogenetic stimulation into the OCT imaging system and co-registered the positions of the OCT imaging and optogenetic stimulation beams. This allowed to precisely image-guide the blue laser beam at the embryonic heart with OCT and then image embryonic cardiodynamics under optogenetic stimulation (Figure 2B). Figure 2C shows an M-mode OCT recording at the position indicated with red dashed line in Panel B during optogenetic stimulation. As one can see from the figure, before the blue laser was turned on, the heart was beating at a resting heartrate of 1.36Hz. Once the blue light was turned on at 2.6 Hz pulse frequency, the heartbeat immediately responded, matching precisely the pulse frequency of the optogenetic stimulation light. Once the pulsed blue light was turned off, the heartbeat immediately returned to its resting heart rate. This result demonstrates that optogenetic stimulation under OCT imaging can be used for quick noninvasive reversible manipulation of cardiodynamics in mouse embryos, setting it as a promising research tool for live investigation of mammalian cardiac biomechanics with light.

The blue line in Figure 2B indicating the position and the size (~30 μm) of the optogenetic beam was based on the measurement using the beam profiler, where neutral density filters were utilized to attenuate the light. The actual area of optogenetic stimulation could be larger and is dependent on several factors, some of them might be hard to control for: (1) the power density threshold for ChR2 channel opening by 473-nm light; (2) the attenuation of 473 nm light by the culture medium above the embryo; (3) the attenuation and scattering of 473 nm light by the embryonic yolk sac, blood cells, and epicardium in the beam path. Potentially, experiments in combination with the Monte Carlo simulation might be helpful to assist the evaluation.

3.2 Embryonic hearts can be paced within a range of frequencies

We next investigated the range of optogenetic stimulation frequencies, which produced efficient cardiac pacing with the heart rate exactly matching the frequency of stimulation (Figure 3). Figure 3 (A-D) shows M-mode OCT recordings of cardiodynamics under different pacing regiments. Without optogenetic stimulation, the heart contracted at 1.6 Hz resting rate (Figure 3A). At 2.2 and 3 Hz optogenetic stimulations, the heart rate exactly matched the frequency of stimulation (Figure 3B,C). However, just slight increase in stimulation frequency to 3.2 Hz resulted in arrhythmic beating (Figure 3D). Figure 3E summarizes heartrate response as a function of pacing frequency in the same embryos with measurements taken in increments of 0.2 Hz. The heart contraction rate exactly matched the rate of optogenetic stimulation in the range from the resting rate of 1.6 Hz to the maximum

of 3.0 Hz. Optogenetic pacing at higher frequencies resulted in arrhythmic cardiac response or contraction frequency not matching the stimulation. To define whether these parameters change as the heart develops, we performed similar analysis in embryos of different stages (Figure 3F). Since embryonic hearts undergo rapid, dramatic structural and functional changes, we organized the data based on somite stages rather than embryonic days, which is a more precise embryonic staging approach. As seen from the graph, with an increase of the somite stage, the resting heartrate overall increases and the maximum pacing heartrate also increases.

3.3 4D OCT cardiodynamic imaging under optogenetic control

The major role of the embryonic heart is to pump blood through the developing organism. Since efficient pumping requires coordinated movement of different cardiac regions, the next important aspect to investigate was whether the optogenetic pacing preserves or disrupts the overall pumping function of the heart. To answer this question, we performed 4D structural and Doppler OCT analysis of cardiodynamics under optogenetic control (Figure 4 and Supplementary Video 1). While structural OCT imaging reveals volumetric information about heart wall dynamics, Doppler OCT (color-coded) allows visualization and quantification of blood flow velocity in the embryonic heart. Figure 4A shows equally spaced time frames at the cross-section through the 4D OCT reconstruction of the embryonic heart beating at 1.59 Hz without optogenetic stimulation. For comparison, Figure 4B shows time frames at the same location of the heart under optogenetic pacing at 2 Hz. As one can see from the figure and the videos, the heart under optogenetic pacing functioned efficiently producing strong flows. In fact, the blood flow velocities produced in the heart under optogenetic pacing were higher than those without the pacing, which is seen as brighter magenta and cyan colors of Doppler OCT signal. These results demonstrate that optogenetic pacing triggers cardiac contractions without disruption of its pumping function.

3.4 Optogenetic cardiac pacing produced non-linear response in hemodynamics.

Hemodynamics have been implicated to be an important regulator of cardiac development. Defining how biomechanical stimuli, including blood flow and heart contraction, coordinate mammalian heart tube function and morphogenesis, depends on availability of research tools for manipulation of blood flow and blood flow induced shear stress. As shown in Figure 5, optogenetic pacing can be used to control blood flow velocity in cultured embryos. We performed optogenetic cardiac pacing at regiments from 2 to 3 Hz in increments of 0.2 Hz and studied the hemodynamic response to these stimulations. Figure 5A shows a region of interest within the ventricle where the blood flow measurements were conducted. The axial component of the peak blood flow velocity in the region of interest as a function of pacing frequency is summarized in Figure 5B. Interestingly, pacing the hearts from the resting rate of 1.6 Hz to 2 Hz increased the blood flow velocity by ~20%, while pacing at higher frequencies resulted in progressively smaller peak flow velocity. This result demonstrates that optogenetic cardiac pacing can produce upregulation as well as downregulation of blood flow velocity at specific regiments, which can be highly beneficial in studying the regulatory role of shear stress in vascular and heart development.

4 Discussion

This manuscript presents a novel approach to manipulate mouse embryonic cardiodynamics with light under OCT imaging guidance. We demonstrated that optogenetic pulsed stimulation produces an immediate response in cardiodynamics, and the heartbeat returns to the resting rate right after the light stimulation stops. Mouse embryonic hearts can be paced within a wide range of frequencies with a maximum pacing frequency of about twice their resting rate, which increases as the heart matures. Optogenetic stimulation at frequencies over the maximum pacing frequency can result in arrhythmia or contractions at a frequency different from the one of pacing.

The observed limitation in pacing frequency can be explained based on general knowledge of cardiomyocyte physiology. The cycle of cardiomyocyte contraction and relaxation is regulated by the membrane potential, which is maintained by voltage gated ion channels and active pumps. In the resting state, the cardiomyocyte membrane potential is negative (-90 mV), with a higher concentration of Ca^{++} and Na^+ outside the cell and a higher concentration of K^+ inside the cell. When a cardiomyocyte is stimulated by an influx of positive ions from pacemaker cells or neighboring cardiomyocytes, its membrane potential rises to the threshold value of -70 mV, which opens Na^+ channels, allowing for Na^+ influx and a sharp increase in membrane potential, called depolarization. Na^+ channels quickly inactivate, while K^+ and Ca^{++} channels open, resulting in a small decrease (called early repolarization) and a plateau phase in membrane potential. The plateau phase is associated with Ca^{++} induced Ca^{++} release from the sarcoplasmic reticulum, causing cardiomyocyte contraction. During the repolarization phase, the K^+ and Ca^{++} channels close, while the resting ion concentration and membrane potential are restored by active ion pumps. The absolute refractory period, a phase when another action potential is inhibited, is maintained by Na^+ channel inactivity. It ensures that the muscle can relax before next contraction and serves to preserve cardiac physiological function. Right panels in Figure 3 (A-D) illustrate cardiomyocyte membrane potential as a function of time at different pacing frequencies. The absolute refractory period, when the stimulation of an action potential is inhibited by the inactive state of Na^+ channels, is shaded brown. When the optogenetic pulse falls outside of the absolute refractory phase, it causes contraction, while stimulation during the absolute refractory period does not produce the response. Specifically, since we observed efficient pacing at 3.0 Hz and arrhythmic response at 3.2 Hz stimulation, the absolute refractory period for this heart is expected to be between 313-334 ms. This explains the observed limit to the pacing rate and arrhythmic contractions at higher stimulation frequencies. This also clarifies why at some pacing regiments, the heart responded at exactly half of the optogenetic stimulation frequency (brown dots in Figure 3E), as every first pulse out of two would fall within the absolute refractory period, while every second pulse would initiate the action potential. Since the maximum optogenetic pacing frequency is indicative of the length of the absolute refractory period, these observations also provide a new potential for optogenetic measurement of absolute refractory period, which might be implemented in physiological studies as a quick cardiomyocyte functional assessment tool.

This manuscript only presents optogenetic stimulations at frequencies exceeding the resting heart rate. Optogenetic pacing at frequencies below the natural beat frequency through ChR2

is not an efficient way to manipulate cardiodynamics because it would result in alternating natural contractions with optogenetically triggered contractions, bringing inconsistency in the results. To slow down or inhibit the contractions through optogenetic control, we are currently exploring the use of different light sensitive protein HR3, which allows inhibition of contraction by illumination with yellow light.

Optogenetic pacing was successfully combined with 4D OCT and Doppler OCT imaging of cardio- and hemodynamics, demonstrating that hearts under optogenetic pacing can function efficiently producing strong flows. 4D OCT reconstruction of cardiodynamics relies on the periodicity of the cardiac cycle and retrospective gating to combine time lapses recorded sequentially as parallel slices into a single beating volume. The fact that we have been able to successfully use this approach for dynamic visualization of optogenetically paced hearts suggests that the cardio- and hemodynamics was consistently periodic during the total acquisition time of about 2.5 min, setting optogenetics as a robust cardiodynamic manipulation tool for cardiac biomechanics studies.

Implementation of optogenetic pacing was also beneficial for the 4D reconstruction approach. Since naturally beating hearts always exhibit slight variations in heartrate, combining all the time lapses acquired in parallel slice geometry into a beating volume requires the implementation of retrospective synchronization method. This is performed by synchronizing each time lapse to its neighbors recursively from one plane to the next, which makes it susceptible to error accumulation. Implementation of optogenetic pacing results in a highly stable and defined heartrate, which allowed us to completely bypass the retrospective gating step and directly arrange the frames in the 4D data set, which significantly reduced the time required for synchronization and allowed to avoid the error accumulation.

We demonstrated that optogenetic pacing can be used to control hemodynamics. Furthermore, the blood flow can be either increased or decreased by tuning the pacing frequency. This result is logical since efficient cardiac pumping is achieved through coordinated movement of the heart wall and flows, and faster contractions would not necessarily result in increased cardiac output, which our data also suggest. Both contraction and blood flow induced shear stress have been implicated in the regulation of the developing heart [30]. However, because contractility and blood flow are interdependent, dissociating their individual roles is very challenging. According to our results, pacing hearts at higher frequency produces a non-linear response in blood flow, where regiments that produced a higher contraction frequency was accompanied by decrease in blood flow. Potentially, this dependence can be used to define specific roles of regional contractility and blood flow induced shear stress in cardiogenesis. This could be done by introducing different pacing regiments into different groups of embryos in culture and correlating the degree of alteration in the outcome to the deviations in contractility and shear stress, defining which of these two stimuli is dominant, providing new potential to uncouple the effects of contractility and blood flow shear stress in cardiogenesis.

5 Conclusion

Together, presented results demonstrate that optogenetic pacing under OCT imaging guidance is a unique novel approach for noninvasive, quick, flexible and efficient manipulation of cardiodynamics, which cannot be achieved with any other current methods. Taking advantage of numerous available mouse models and genetic manipulation tools, the presented approach combining 4D OCT, Doppler analysis, and optogenetic pacing in mouse embryo culture sets a platform for investigation of the interplay between genetic and mechanical factors in normal mammalian cardiogenesis as well as poor heart function and failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the American Heart Association Predoctoral Fellowship 19PRE34380240 to ALL and the National Institutes of Health with grants R01HD096335, R01EB027099, R01HD099026 to IVL, and R21EB028409 to SW. We would like to acknowledge technical support from the Optical Imaging and Vital Microscopy (OIVM) Core at the Baylor College of Medicine.

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Figure 1. Setup for optogenetic cardiac pacing under OCT imaging guidance.

The optogenetic stimulation beam is coupled with the OCT beam between the galvanometer scanners and the focusing objective.

Figure 2. Optogenetic cardiac pacing in embryonic mouse hearts.

(A) Illustrations depicting how ChR2 is activated to cause membrane depolarization when illuminated with blue laser light. (B) A representative frame from an OCT time lapse capturing the dynamics of the E8.5 embryonic heart under optogenetic stimulation. The position of the pulsed blue laser beam on the heart is labeled with blue line. (C) Corresponding M-Mode OCT recording at the position indicated with red dashed line in (B), showing cardiac contractions at the rate of 1.36 Hz, immediate response to blue laser stimulation matching the laser pulse frequency of 2.6 Hz, and immediate return to the resting heart rate once the laser was turned off.

Figure 3. Optogenetic cardiac pacing regimens.

(A-D) M-mode OCT recordings through the same embryonic heart at different stimulation frequencies (left) and corresponding interpretations of cardiomyocyte electric activities (right). The absolute refractory period (ARP), when cardiomyocytes are not sensitive to pacing pulses, is shaded brown. The pacing pulses are represented by blue notches. (A) Periodic cardiac contractions at a resting heart rate (RHR) of 1.6 Hz without optogenetic stimulation. (B, C) Periodic cardiac contractions following the pacing frequencies of 2.2 and 3 Hz, respectively. (D) Arrhythmic contractions at the pacing frequency of 3.2 Hz. (E) Heartbeat frequency as a function of pacing frequency in the same heart with measurements taken in increments of 0.2 Hz. The resting heart rate of 1.6 Hz (red dot) and the maximum pacing frequency of 3 Hz (blue dot) define the region for efficient optogenetic pacing, while pacing beyond 3 Hz produced arrhythmic response or beat at frequencies different from the optogenetic stimulation. (F) Summary of efficient pacing regimens for embryos of different somite stages. The pacing regimens were measured in 15 embryos from multiple litters.

Figure 4. 4D OCT cardiodynamic imaging under optogenetic control.

The axial blood flow velocity captured with Doppler OCT is color-coded according to the scale. (A) Representative frames from volumetric time lapses acquired in E8.5 embryonic heart beating at natural frequency of 1.59 Hz without optogenetic stimulation. (B) Representative frames from the same location of the same heart under 2 Hz optogenetic pacing. (Supplementary Video 1)

Figure 5. Optogenetic control of hemodynamics.

(A) A representative frame from an OCT time lapse of the E8.5 beating heart under optogenetic control. The axial component of blood flow recorded with Doppler OCT is color-coded. (B) Axial peak blood flow velocity measured with Doppler OCT at different pacing frequencies in the region (ROI) labeled in panel A. The measurements are acquired from the same embryo over 10 heartbeat cycles for each pacing regiment and shown as Average $\pm$ Standard Deviation. * $p < 0.05$; *** $p < 0.001$