**Arrhythmia Caused by a *Drosophila* Tropomyosin Mutation Is Revealed Using a Novel Optical Coherence Tomography Instrument**

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**Abstract**

**Background:** Dilated cardiomyopathy (DCM) is a severe cardiac condition that causes high mortality. Many genes have been confirmed to be involved in this disease. An ideal system with which to uncover disease mechanisms would be one that can measure the changes in a wide range of cardiac activities associated with mutations in specific, diversely functional cardiac genes. Such a system needs a genetically manipulable model organism that allows in vivo measurement of cardiac phenotypes and a detecting instrument capable of recording multiple phenotype parameters.

**Methodology and Principal Findings:** With a simple heart, a transparent body surface at larval stages and available genetic tools we chose *Drosophila melanogaster* as our model organism and developed for it a dual en-face/Doppler optical coherence tomography (OCT) instrument capable of recording multiple aspects of heart activity, including heart contraction cycle dynamics, ostia dynamics, heartbeat rate and rhythm, speed of heart wall movement and light reflectivity of cardiomyocytes. We applied this OCT instrument to a model of Tropomyosin-associated DCM established in adult *Drosophila*. We show that DCM pre-exists in the larval stage and is accompanied by an arrhythmia previously unidentified in this model. We also detect reduced mobility and light reflectivity of cardiomyocytes in mutants.

**Conclusion:** These results demonstrate the capability of our OCT instrument to characterize in detail cardiac activity in genetic models for heart disease in *Drosophila*.


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**Introduction**

Dilated cardiomyopathy (DCM) is a progressive pathological cardiac condition characterized by an enlarged heart with impaired contractility, defects that often lead to heart failure. To date a wide range of genes, from those involved in mechanobiochemical signaling to components of the contractile architecture, have been confirmed to cause DCM when defective [1–4]. This suggests that a large molecular genetic network regulates the development of this disease. Therefore, in order to shed light on disease mechanisms, evaluation of each gene cardiac impact is necessary. With this ultimate goal in mind, one method allowing us to move forward is to use a genetically tractable model organism to investigate cardiac function of individual genes. Such an approach requires a model organism with two properties, a methodology that allows individual genes to be knocked-down specifically in the heart, and a heart that is easily accessible to imaging. In addition, an imaging system is required that can accurately and easily measure subtle changes in multiple parameters of cardiac function.

The fruitfly, *Drosophila melanogaster*, is an ideal model for this experimental approach. It has an open circulatory system, a cardiovascular organ called the dorsal vessel driving hemolymph flow around the body [5,6]. The dorsal vessel is a simple tube formed by a single layer of cardiomyocytes. It is divided into two morphologically distinct functional domains: the thin anterior aorta serving as an outflow tract and the broad posterior heart acting as a rhythmic pump. It resides next to the body surface so that it allows easy observation *in vivo*. Despite the obvious morphological differences between the *Drosophila* and vertebrate hearts there is a remarkable degree of conservation between the two, not only in the characteristic rhythmic cardiomyocyte contraction, but also in the genetic networks that regulate early heart development [7–13] and aspects of heart physiology [14,15]. Indeed in both *Drosophila* and vertebrate hearts mutations in homologous genes cause DCM-like phenotypes [16,17]. Moreover, genetic tools available in *Drosophila* provide the ability to knockdown more than 90% of the genome using RNAi specifically targeted to the dorsal vessel [17].
Key to the potential of this *Drosophila*-based heart model is an imaging technique that can accurately record cardiac dynamics. Several approaches have been reported. In the embryo cardiac dynamics can be recorded in situ using fluorescence microscopy combined with cardiac specific expression of GFP [10]. This has the potential to identify genes involved in the development and initial activity of the dorsal vessel, but as contractile activity only starts an hour before hatching and in larvae the dorsal vessel becomes partially obscured by the overlying fat body, this technique is limited in its ability to detect genes involved in progressive heart disease. An alternative and powerful technique pioneered in adult cardiac imaging, utilizes a digital video camera with differential interference contrast optics to image the edge of the heart wall [19]. However, pigment in the adult cuticle limits light penetration, thus imaging has to be performed in dissected animals. This technique has recently been applied to whole *Drosophila* larvae [20], however issues with the obscuring fat body remain. The final approach is to use optical coherence tomography (OCT) [21]. This is a real time label-less imaging technique with mm range of depth penetration that generates an image by interfering a reference light with light back-scattered from the specimen. Tissues and cells are distinguished from surrounding tissue fluid due to their different light reflectivity [22]. OCT has been applied to the study of cardiac dynamics in intact adult *Drosophila* [16,23], as well as in mouse [24] and avian embryos [25]. However, real time recording using OCT has been limited to 2-dimensional (lateral × depth) cross sectional views of the heart, thus sampling contractile activity at a single point rather than along the full length of the organ. An OCT instrument that could image a plane along the longitudinal axis of the heart, producing an en-face image oriented as in conventional microscopy, could provide further functional information.

Here we describe an OCT instrument which can operate in two regimes. In the imaging regime, the system can acquire OCT images, with either cross sectional or en-face orientation, of the *Drosophila* larval heart. In the Doppler regime, movement of the heart wall is measured. We demonstrate the utility of this instrument in recording larval heart dynamics by applying it to the analysis of a mutation in the *Drosophila* muscle specific Tropomyosin gene previously shown to cause a DCM phenotype in the adult fly heart [16]. We show that a similar phenotype pre-exists at larval stage and demonstrate that this Tropomyosin defect also causes an arrhythmia characterized by increased length and frequency of heart pacing. Moreover, the OCT instrument uniquely enables assessment of cellular mobility and light reflectivity *in situ*. In combination with the genetic tools available in *Drosophila*, this instrument provides an excellent platform for future detailed study of the molecular mechanisms of DCM and arrhythmia.

**Results**

**En-face imaging of wild type larval heart**

Initially we applied en-face OCT (details are described in Materials and Methods section) to heart imaging of wandering stage 3rd instar *w*¹¹¹º control larvae. Previous studies have shown that the fly heart contracts in peristaltic waves that propagate along the heart [5,18,26], but there is discrepancy regarding the directionality of hemolymph flow. Slama and Farkas [26] reported that the larval heart beat is always uni-directional from the posterior towards the anterior, while Rizki [3] reported occasional reversals. This reversal contraction has also been observed in semi-dissected larval heart [27]. In all the *w*¹¹¹º larvae (n = 60) imaged in this work, the dominant form of contraction was a peristaltic wave of caudal origin (see Movie S1). In 4 larvae (~7%) peristaltic contractions were occasionally interspersed by short periods of twitching. In 13 larvae (~22%) peristaltic contractions were interspersed with periods when the heart chamber shortened along its anterior-posterior axis, these longitudinal contractions appearing to initiate from the anterior.

The larval heart chamber possesses three pairs of laterally located ostia along its axis [28,29], and they divide the heart chamber into four sub chambers [5], as indicated in Fig. 1A, 1B. These ostia are heart gates, each of them a pair of specialized cells that serve as valves to regulate the inward flow of hemolymph into...
the heart [5,6,28]. Ostia dynamics have been described in the embryonic heart [18], the valves opening in a coordinated fashion as the heart relaxes, drawing hemolymph in, and closing at systole to push hemolymph forward into the aorta. Using our en-face OCT system similar dynamics in the larval heart were imaged (Fig. 1C, 1D).

En-face imaging of the heart in Tropomyosin mutant larvae

Adult Drosophila homozygous for the Tropomyosin II null mutant TM2<sup>3</sup> exhibit a DCM-like heart [16]. Using our OCT instrument we examined whether this mutation causes a heart defect at an earlier developmental stage. Compared to control w<sup>1118</sup> larvae, TM2<sup>3</sup> mutants showed a decreased shortening fraction (SF) (Fig. 2), indicating reduced cardiac contractility. In adult TM2<sup>3</sup> mutants, the dilated heart chamber results from enlargement at both maximal systole and maximal diastole [16]. At the earlier larval stage, heart chamber dilation is mainly due to failure of the heart to fully contract, the enlargement of chamber diameter being statistically significant only at maximal systole. This demonstrates that the tropomyosin mutation causes a DCM-like phenotype as early as the larval stage. Interestingly the degree of the dilation defect is consistent between larval and adult stages [16], in both cases the shortening fraction being decreased by 19% of the control value.

In addition, although the same proportion of w<sup>1118</sup> and TM2<sup>3</sup> larvae exhibited chamber shortening contractions (Table 1), there was a noticeable increase in the frequency at which these contractions occurred in the tropomyosin mutant larvae. An example is shown in movie S2 (see Supplement Information). The cause of this phenomenon is not clear.

Heart rate of control and Tropomyosin mutant larvae

It has been noticed previously that short periods of heart pausing are associated with eating activity and with preparation for crawling in Drosophila larvae [5]. We noticed a similar association of body movement with heart pausing in our en-face imaging observations. This slowed heart rate prior to and post body crawling contraction was confirmed (an example of w<sup>1118</sup>, Audio Recording S1) by our Doppler recordings (as described in Materials and Methods). Typical recordings of control and TM2<sup>3</sup> mutant hearts are shown in Fig. 3. To take this kind of pausing into consideration, heart beat in both control and TM2<sup>3</sup> mutant larvae was analyzed by two methods, counting the number of heart beats over either periods of 10 seconds or 1 minute. With the 10 second period, average heart rates of mutants and controls were similar, but using the 1 minute period a drop of 14% in heart rate was seen in the mutants (Table 1), heart pausing increased to 7% of larvae, n = 27, heart pausing increased to ~26% (n = 27) in TM2<sup>3</sup> mutants (Table 1). An example is shown in Movie S3 and Fig. 3. In addition, pauses were more frequent and lasted longer, up to 30 seconds. These data suggest that the dilated hearts of TM2<sup>3</sup> mutants can have similar pumping capacity as wild type over short time periods, but over longer periods there is a trend towards reduced pumping capacity. Further studies are needed to confirm this finding.

Arrhythmia in the heart of Tropomyosin mutant larvae

Heart arrhythmia is associated with DCM-causing tropomyosin mutations in humans, but has not been apparent in the adult Drosophila TM2<sup>3</sup> mutant. Taking into consideration the natural irregularity of fly heartbeat, we therefore screened Doppler audio recordings for larval heart pausing of longer than 2 seconds. Doppler data was cross-examined with en-face OCT imaging from the same animal to confirm the occurrence of contractile pauses.

| Table 1. Heart rate analyzed using the OCT imaging and audio recording data. |
|-----------------------------|-----------------------------|-----------------------------|
|                             | Control (w<sup>1118</sup>, %) | TM2<sup>3</sup>, %          |
| Imaging analysis            | n<sup>+</sup> = 38          | n = 47                      |
| Wavy contraction            | 64                          | 62                          |
| Occasional chamber shortening| 28                          | –                           |
| Dominant chamber shortening | –                           | 27                          |
| Chamber shortening only     | 0                           | 4                           |
| Pauses                      | 8                           | 34                          |
| Audio recording analysis    | n = 27                       | n = 27                       |
| Regular rate                | 82                          | 63                          |
| Irregular rate              | 11                          | 11                          |
| Pause (>2 sec)              | 7                           | 26                          |

*: number of animal used. doi:10.1371/journal.pone.0014348.t001
seconds in mutants compared to less than 10 seconds maximum in control animals.

Reduced cellular mobility and light reflectivity in the heart of Tropomyosin mutant larvae

As shown in the bottom panels of Fig. 3A and 3B, the Doppler frequency was not constant throughout one heartbeat cycle. According to the Doppler shift principle, a higher frequency is produced when the distance between the detecting point and the moving heart wall diminishes and a lower frequency is produced when the distance increases. Therefore, a higher frequency is recorded when the heart wall moves toward the instrument. In order to compare the velocities of heart wall motion between $TM2^3$ mutants and control larvae, only the part of the tracing showing the highest frequency within a heartbeat (boxed in Fig. 3A, 3B, bottom panels) was used in calculating the velocity (as described in Materials and Methods). Results showed that the velocity of movement of the heart chamber wall decreased by 31% in $TM2^3$ mutants compared to controls (Fig. 4).

Amplitude recorded by the Audicity plot (Fig. 3) represents the intensity of the Doppler signal and is a measure of the optical density of the tissue, in this case the heart wall. The cardiomyocytes that make up the heart wall are densely packed with myofibrils, the highly developed ultrastructural units of contraction. Thus, signal amplitude can be taken as a rough measure of the integrity of the myofibril ultrastructure. Analysis of the maximum amplitude of the Doppler signal observed in each animal shows a significant reduction in the $TM2^3$ mutant heart (Fig. 4), indicative of a reduced ultrastructural density in this mutant cardiomyocyte.

Discussion

In order to improve the sensitivity of the methodologies used to dissect the genetic networks underlying dilated cardiomyopathy, we built a dual-regime OCT instrument dedicated for the in situ measurement of multiple cardiac parameters in the Drosophila larval heart. Measurements from this instrument allow the organ level phenotype to be assessed, including heart size, mode of heart contraction, ostia dynamics, heart rate and heart rhythm. In addition, the Doppler regime uniquely provides measurements for assessing the mobility of the cardiomyocyte in situ. The light

Figure 3. Larval heart rate and arrhythmia in Tropomyosin mutant. A: a typical control heart audio tracing. B: a representative Tropomyosin mutant heart audio tracing. Boxed double spikes of the tracing in top panels of both A and B are one heart beat; both bottom panels are the enlarged trace of each of the indicated heart beat in the top panels. The higher frequencies indicated in the boxed part in each heart tracing (both bottom panels) were used in calculating the velocity of Doppler signals.

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signals, representing cardiomyocyte's mobility. Mean in both counting groups. Middle panel is the max. velocity of Doppler

6

p = 0.012. Bottom panel is the amplitude, representing cardiomyocyte's

TM23
doI:10.1371/journal.pone.0014348.g004

SEM for controls is 196

6

for control group is 726

6

240 (n = 23), for

TM23

is 185

6

SEM (dB) for the control

6

12.09 (n = 25), for

TM23

6

10 (n = 12), p

6

SEM (m/sec)

6

0.05

The following information was generated from the Doppler audio recording. Top panel is heart rate in 1 minute. In 10 sec heart rate
counting group, Mean ± SEM for controls is 154±12.09 (n = 25), for

TM23

is 129±10.4 (n = 15). In 1 min heart rate counting group, Mean ± SEM for controls is 196±8 (n = 27), for

TM23

is 185±10 (n = 12), p>0.05 in both counting groups. Middle panel is the max. velocity of Doppler signals, representing cardiomyocyte’s mobility. Mean ± SEM (μm/sec) for control group is 726±240 (n = 23), for

TM23

group is 502±293, p = 0.012. Bottom panel is the amplitude, representing cardiomyocyte’s light reflectivity, of Doppler signals. Mean ± SEM (dB) for the control group is 0.44±0.04 (n = 13) and for the

TM23

0.32±0.03 (n = 17), p = 0.0214.
doi:10.1371/journal.pone.0014348.g004

reflectivity of the cardiomyocyte, as indicated by the amplitude of the Doppler signal, is another unique parameter provided by this
detecting instrument. Application of our instrument to larvae homozygous for a Tropomyosin mutant known to cause DCM in
the adult fly reveals for the first time, to our knowledge, that an
arrhythmia is associated with this mutation in this organism. Both
mobility and light reflectivity of cardiomyocytes were also found to
be affected in this mutant. In addition recording of ostia dynamics
provides an avenue for studying the impact of hemolymph
dynamics on cardiac physiology and on fly heart development. Such a role for fluid dynamics has been demonstrated in early
chick heart development [25]. These results demonstrate that

application of our OCT instrument in this model organism
enables detailed characterization of cardiac gene activities across
multiple hierarchical structures in situ. In comparison, a recent Drosophila genome wide screen identified a number of candidate
cardiac genes by screening for adult lethality associated with a
combination of RNAi knockdown and cardiac stress [17]. Surprisingly, muscle specific Tropomyosin was not identified by
this screen despite its known adult cardiac phenotype. Thus, while
more time consuming, a screening approach in which detailed
characterization of cardiac function is performed using our OCT
system is likely to be much more comprehensive in identifying
potential cardiac disease genes.

The reduced light reflectivity in the

TM23

mutant found in this
work suggests a reduced density of cellular ultrastructure content.
Supporting evidence for this suggestion comes from the observa-
tion that a mutant in Tropomyosin’s partner protein, Troponin I
exhibits disorganized cardiac ultrastructure [16]. Furthermore,
a massive reduction of Doppler signal amplitude is associated with
substantial loss of myofibrils in larval hearts depleted of the βPS
integrin subunit (our unpublished data).

Our data reaffirms that, predominantly, heart contraction takes
the form of a peristaltic wave of posterior origin. In embryos [18]
and larvae (this work), these contractile waves are coordinated with
ostia dynamics and the opening of the aortic valve to generate an
anterior-ward flow of hemolymph through the aorta. In the normal
heart, these peristaltic heart beats are interspersed with short
periods of either shortening contractions, where the heart
chamber shortens along its anterior-posterior length, or fast
twitching. Because the twitching contractions appeared to be
coordinated or synchronized in the whole heart, they could be
caused by interference between the caudal cardiac pacemaker and
neural activity from the anterior aorta directing retrograde
contractions. Such interference has been suggested in the adult
heart where retrograde contractions are directed by neural
connections to the anterior aorta. In this scenario, the shortening
contractions we observed would be larval retrograde contractions
described by some observers [5,18], but not others [26], where

arrhythmia associated with defects in ion channels have been
reported in Drosophila [29,32]. Abnormal irregular heart beat can
also be induced by drugs, different ionic solutions and by physical
injury of the heart in the semi-dissected fly [33]. Investigating the
mechanisms underlying these affects in combination with
electrophysiology studies, is a further potential application of our
dual OCT imaging and Doppler instrument. In particular, in
injured cells combining monitoring of structure damage with OCT
imaging with measurement of electrophysiology and contractile
functionality may identify similarities between the model injury
and infarct in heart-attack patients. Moreover, a comparative
study of arrhythmias caused by functionally diverse genes is
expected to generate a better understanding of the causative
mechanism. The phenotypic resemblance between Drosophila and

Figure 4. Summarised heart rate, velocity and light reflectivity of cardiomyocyte deduced from Doppler OCT-based audio
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Arrhythmias occur in one third of the patients suffering from
DCM associated with mutation in the α-tropomyosin gene [30]. An altered sensitivity of myofilaments to calcium ion concentration
has been observed in an animal model with a mutation of this gene
[31]. Arrhythmia associated with defects in ion channels have been
reported in Drosophila [29,32]. Abnormal irregular heart beat can
also be induced by drugs, different ionic solutions and by physical
injury of the heart in the semi-dissected fly [33]. Investigating the
mechanisms underlying these affects in combination with
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dual OCT imaging and Doppler instrument. In particular, in
injured cells combining monitoring of structure damage with OCT
imaging with measurement of electrophysiology and contractile
functionality may identify similarities between the model injury
and infarct in heart-attack patients. Moreover, a comparative
study of arrhythmias caused by functionally diverse genes is
expected to generate a better understanding of the causative
mechanism. The phenotypic resemblance between Drosophila and
human in tropomyosin-associated arrhythmia in DCM suggests a conserved disease mechanism between species. Thus our OCT instrument provides a powerful tool for further investigation of the molecular mechanisms of the DCM coupled arrhythmia.

Materials and Methods

En-face OCT imaging and Doppler instrument dedicated for in situ analysis of cardiac function in Drosophila larvae

The ideal instrument for recording cardiac dynamics in Drosophila requires the following attributes. It must be capable of imaging the heart in the intact animal, to do so it must be capable of penetrating the overlying cuticle and be capable of imaging at depth. Ideally orthogonal images of the heart should be acquired so that the full length of the organ can be observed. In addition, as the Drosophila larval heart beats between 3.5 and 4 times a second [26], the rate of image capture must exceed this frequency.

OCT has several of these attributes. It is a label-less imaging technique that generates an image from interference between an internal reference beam and light reflected by the target. It is capable of imaging at depth through the cuticle.

Previous studies that applied OCT to imaging of the adult Drosophila heart have produced cross-sectional images to depict the dynamics of heart diameter, and therefore heart rate, in real time [16,23]. However, the specific technique used in these studies, spectral domain OCT, cannot generate en-face oriented images of the Drosophila heart at the required frame rate, (our initial studies on a SD-OCT system operating at 29 kHz rate still, in spite of its high line rate, required 5 seconds to acquire the whole OCT data volume [34]). To resolve this issue we turned to en-face time domain (TD)-OCT that can, in principle, achieve high speeds of frame acquisition by using resonant scanners [35]. Another reason for opting for a TD-OCT method is due to its compatibility to dynamic focus, the ability to move both the coherence gate and the focus gate together. This makes the TD-OCT method better suited to microscopy applications. Using a resonant scanner at 2 kHz, our TD-OCT imaging system can produce en-face images at up to 5 Hz rate, slightly faster than mean heart rate in Drosophila larvae.

Although the 5 Hz acquisition rate has largely improved our capability of following the heart rate, we are still missing important parts of the heart wall motion during each beat. To address this deficiency, we complemented the information acquired during the imaging process with that acquired during Doppler signal recording. In this regime, the heart wall motion is recorded in real time. This technique exploits the fact that a moving light-scattering object imposes a Doppler shift to the frequency of the scattered light that is proportional to the velocity of the movement of the object. In our instrument a Doppler shift was produced when the distance between fixed detecting focal point and heart wall changes due to the contraction/relaxation movement of the latter. The wavelength of the SLD light source, together with the speed of movement of the heart wall, place the Doppler frequency within the audio range; hence our instrument performs like a stethoscope and the Doppler signal may be plotted as a sound signal using Audicity software. A typical heartbeat audio recording from a control larva is shown in the supplement material (Audio Recording S1), and the plot is shown in Fig. 3A. Closely neighboring double peaks of high-density tracings (Fig. 3A, top panel) represent a single heartbeat.

A schematic diagram of the dual en-face imaging/Doppler signal recording OCT system is shown in Figure 5. The technical details of the system are as follows. A pigtailed super-luminescent diode (SLD, SuperLum, Moscow) emitting at 1300 nm and having a spectral bandwidth of 65 nm is used which determines an OCT longitudinal resolution of around 17.3 μm in the sample. Light
from the SLD source is injected into the system via a first directional coupler (DC1) that splits the light towards the probing and the reference arm of the interferometer. The probing beam is sent via the galvanometer scanners SX and SY to the specimen (Drosophila larval heart). Two telescopes conveniently alter the diameter of the beam in order to match the aperture of different elements in the probing path and convey a probing beam of around 8 mm in diameter through the microscope objective MO’s pupil plane. The two transverse scanners, SX and SY, are separated here using a telescope in order to project a flat wavefront on the target under high numerical aperture (NA). Lenses L1, L2 and L4 have a focal length of 7.5 cm, while lens L3 has a focal length of 3 cm. The MO is a scan lens (focal length 1.8 cm) specially designed by ThorLabs to prevent image degradation and distortion during scanning. Hence, a lateral resolution better than 4.3 μm in the en-face OCT images is obtained (determined by imaging a USAF test target). To maintain this lateral resolution throughout the whole depth of the specimen, tracking of coherence gate and focus position (dynamic focus) has been implemented by simultaneously adjusting the two arms of the interferometer via the computer driven translation stages TS1 and TS2.

Light back-scattered by the specimen passes a second time through the object arm, guided via the first directional coupler towards the second single mode directional coupler where it interferes with that coming from the reference arm. Both output fibers from the second coupler are connected to two pin photodetectors, PD1 and PD2, in a balanced photo-detection unit constructed using a differential amplifier, DA. The OCT signal is rectified and low pass filtered in the demodulator DMOD. A computer-driven translation stage, TS1, is used to alter the reference path length to select different depths for C-scan while acquiring stacks of C-scans as well as scanning the depth in the B-scan acquisition mode. The scanning procedure is similar to that used in any confocal microscope, where the fast scanning is en-face (line rate, using the scanner SX) and the frame scanning is much slower (at the frame rate, using the scanner SY). The frame grabber in Figure 5 is controlled by TTL signals from the generators driving the SX-scanner and the SY-scanner. The SX resonant scanner is driven with a ramp at 2 kHz and the SY galvoscanner with a ramp at 5 Hz. In this way, an en-face image, in the plane (x, y) is generated at constant depth. The next en-face image at a new depth is then generated by moving the translation stage, TS1, in the reference arm of the interferometer and repeating the (x, y) scan. En-face images with a size of 1.5 : 1 in width over height in this report are obtained using suitable amplitudes for the voltages applied to the X and Y-galvoscanners.

To switch the system to the audio (stethoscope) regime, the scanning of the beam across the specimen is interrupted using switches SWX and SWY. Hence, the recorded interferometric signal, low-pass filtered by the DMOD block, is exclusively due to switches SWX and SWY. Hence, the recorded interferometric scanning of the beam across the specimen is interrupted using switches SWX and SWY. The heartbeat tracing in Fig. 3A (bottom panel) also displays the frequency of the Doppler signal produced by the moving wall of the heart chamber. As described in the review by Podoleanu [37], the frequency (f) of the Doppler signal is related to the velocity (v) of movement of the heart wall according to the equation \( f = \frac{2v}{\lambda} \), where \( \lambda \) is the wavelength of SLD light source (1.3 μm).

**Statistics**

Student’s t test was used in the statistic analysis.

**Supporting Information**

**Audio Recording S1** Sound of Drosophila heartbeat. Found at: doi:10.1371/journal.pone.0014348.s001 (3.00 MB WAV)

**Movie S1** Heart beat in a wild type Drosophila larva. The speed of the movie is 5 frames / sec. Found at: doi:10.1371/journal.pone.0014348.s002 (5.23 MB AVI)

**Movie S2** The speed of the movie is 5 frames/ sec. Cardiac chamber-shortening contraction in a Tropomyosin mutant larva. Found at: doi:10.1371/journal.pone.0014348.s003 (6.51 MB AVI)

**Movie S3** Cardiac pausing in a Tropomyosin mutant larva. The speed of the movie is 5 frames / sec. Found at: doi:10.1371/journal.pone.0014348.s004 (5.09 MB AVI)

**Author Contributions**

Conceived and designed the experiments: LM AB AGP. Performed the experiments: LM AB. Analyzed and interpreted the data: LM. Drafted and edited the manuscript: LM. Contributed analysis tools: AB. Participated in the writing and editing of the manuscript: AB AGP JWB.
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