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# Methods to assess *Drosophila* heart development, function and aging

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ABSTRACT

In recent years the *Drosophila* heart has become an established model for many different aspects of human cardiac disease. This model has allowed identification of disease-causing mechanisms underlying congenital heart disease and cardiomyopathies and has permitted the study of underlying genetic, metabolic and age-related contributions to heart function. In this review we discuss methods currently employed in the analysis of the *Drosophila* heart structure and function, such as optical methods to infer heart function and performance, electrophysiological and mechanical approaches to characterize cardiac tissue properties, and conclude with histological techniques used in the study of heart development and adult structure.

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## 1. Introduction: The *Drosophila* heart as a tool for investigating cardiomyopathies

The *Drosophila* heart or dorsal vessel is a linear tube that is reminiscent of the primitive vertebrate embryonic heart tube. Although the final heart structure in *Drosophila* is very different from that in vertebrates, the basic elements for cardiac development, function and aging are remarkably conserved [6,11,62]. Because of the simplicity in structure and availability of powerful genetic tools, the *Drosophila* heart has emerged as a pioneering model system for unraveling the basic genetic and molecular mechanisms of cardiac development, function and aging [7,61]. The *Drosophila* heart model has proven to be a valuable asset to elucidate the etiology of human cardiac disease, including dilated and restricted cardiomyopathy, channelopathies, diabetic and congenital heart disease, as well as cardiac senescence [4,8,51,56,64,70,81,91,93]. The *Drosophila* heart has also been used as a tool for the identification of novel genes and pathways potentially involved in heart disease (e.g. [35,36,58,71]). Of note, certain important ion channel gene functions are conserved between *Drosophila* and humans to maintain a regular heart rhythm, such as KCNQ [64]). Interestingly, some of these ion channels do not play a significant role in the (faster beating) adult mouse heart [59]. This suggests that in some regards the fly heart model may be more informative than the mouse model.

We will first discuss different methods to assess heart function that may change under different genetic and environmental conditions, as well as with age. Then we will summarize tools and markers for structural features of the heart during development, maintenance and aging. An overview of the different methods discussed in this article is presented in Table 1.

## 2. Optical-based analysis methods to measure heart function and performance

### 2.1. Heart rate and pacing

The *Drosophila* heart is a linear tube with a non-contractile aorta that extends from the posteriorly located heart to the head in both larva and adults. Rudimentary valve-like structures divide the heart into chambers and prevent back flow of hemolymph. In larvae the heart is suspended within the hemocoel and undergoes substantial remodeling during pupal stages prior to forming the four chamber abdominal heart of the adult fly [53,98]. Early efforts to examine heart function in intact *Drosophila* were performed on dissected larva [21] and early pupa, where the cuticle is nearly transparent [14]. For larval preparations the heart rate was determined visually. For the pupal preparation, light is passed

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**Table 1**
Comparison of the different methods for analyzing heart function in *Drosophila*.

| Method | Effect on animal | Heart rate | Systolic & diastolic intervals | Systolic & diastolic diameters | Rhythmicity | Structure | Stiffness | Stress response |
|---|---|---|---|---|---|---|---|---|
| Gross Δ intensity optical method | Stressful/ *in vivo* | + | | | + | | | |
| SOHA optical method | Invasive/ *in situ* | + | + | +/−1 μ | + | + | | |
| Atomic force microscopy | Invasive/ *in situ* | | | | | | + | |
| Optical coherence tomography | Stressful/ *in vivo* | + | | +/−10 μ | + | + | | |
| Electrical recordings | Invasive/ *in situ* | + | + | | + | | | |
| Immuno histochemistry/electron microscopy | *in situ* | | | | | ++ | | |
| Electrical pacing | Stressful/ *in vivo* | | | | | | | + |

through the heart and detected by a phototransistor in the microscope eyepiece. Changes in overall light intensity could be recorded and displayed as linear traces. Using custom software, Maximum Entropy Spectral Analysis (MESA) the overall heart rate could be determined [15,16]. In addition, heartbeat rhythmicity could be quantified by MESA as a correlation coefficient. Using genetic mutants and pharmacological manipulations these techniques provided evidence that the fly heartbeat is myogenic and that the cardiac action potential likely does not have a substantial $Na^+$ current since heartbeats were not affected by tetrodotoxin (TTX; [21,30]). These studies also showed that reduction of extracellular $Ca^{2+}$ stopped heart function suggesting that the cardiac action potential is $Ca^{2+}$ based, although each of these groups came to different conclusions concerning the specific type of $Ca^{2+}$ channel that was involved. In addition a prominent role for $K^+$ channels was suggested in that channel blockers such as TEA caused dramatic reductions in heart rate [21] and mutations in shaker, ether-a-gogo and slowpoke $K^+$ channels all affected heart rate albeit to different extents [30].

A variation on these optical detection methods has been developed for monitoring the adult hearts expressing GFP [17]. The GFP signal permits the heart walls to be visualized through the cuticle; however, since the fluorescent signal is usually very low, high frame rate recordings require specialized equipment that is costly. Another approach for optically monitoring heart function in adult makes use of infrared light and an array of sensors oriented along the abdominal cuticle [89]. Longer wavelengths are better able to penetrate the pigmented cuticle of the adult thus permitting heart rate and rhythmicity to be determined *in vivo* from tethered adult flies. However, the spatial resolution is limited and frame rate is still relatively low with this method.

In order to test cardiac performance, cardiac stress tests have been developed based on increasing or 'pacing' the resting heart rate. These assays can be used to compare the effects of different mutations as well as the effect of age on heart function. In one such assay, pacing of pupal or adult hearts to higher rates was achieved by increasing temperature [66,92]. In another pacing assay, the head and abdomen of adult flies are placed to touch two strips of electrode jelly (Fig. 1) and a square wave current is passed through the animal to pace the heart to 6 Hz (the upper end of the unperturbed heart rate range, which normally is 4–5 Hz) for a set amount of time (30 s; [90,91]). Immediately after pacing, heart function is monitored visually through the abdominal cuticle (A2-3) and flies are scored depending on whether the heart can still beat following the stimulus or whether it fails to contract or contracts in a spasmodic fashion. This assay has been used to show that insulin signaling plays a heart-autonomous role in cardiac senescence [91] and KCNQ channels are critical for cardiac repolarization in *Drosophila* [64].

### 2.2. Semi-automated Optical Heartbeat Analysis (SOHA)

The movement detection methodologies described above all rely on detecting overall changes in the intensity of light passing through the heart while it is beating ('darkness' profiles, Fig. 2A). A more sensitive method that relies on the combined use of two different computer algorithms has been developed for use with high speed, high resolution digital cameras [64]). This Semi-automated Optical Heartbeat Analysis (SOHA; detailed method in [19] uses two computer algorithms that were developed to combine information about overall darkness changes (Fig. 2A), which denote systolic periods, with a pixel-by-pixel analysis of intensity changes detecting only the regions in the movie frame that are moving (Fig. 2B). This combinatorial approach thus detects the individual inward and outward movements of the heart wall and uses the gross darkness changes to determine the "state" (i.e. contracted v. relaxed); permitting the quantification of large numbers of contractions in relatively long movie clips. This capability becomes significant with respect to rhythmicity measurements (see below). Camera setups that can record images at speeds of 150–500+ fps with pixel resolution in the 1 micron range allow very precise temporal and spatial measurements. In addition, the high spatial resolution enhances the ability to detect even subtle changes in contractility measured as Fractional Shortening (FS, e.g. decreased FS with age; [8]). Of particular usefulness, this program produces high-resolution qualitative records of heart wall movements (M-mode, kymograph) by excising a 1 pixel horizontal "slice" of pixels from each frame of the movie and aligning them horizontally to provide an edge trace displaying heart wall movements in the *X*-axis and time along the *Y*-axis (Fig. 2C; [19]).

This analysis program can also be used with lower speed and lower resolution movies, such as of GFP fluorescent-labeled hearts [39,54]. However, due to the lower resolution and speed, the output will be limited to heart rate (see discussion below). This analysis method also works with movies of intact (*in vivo*) preparations of larval, pupal and adult *Drosophila* hearts [64,78], as well as larval zebrafish and day 8–9 embryonic mouse hearts [19]. However, SOHA is most sensitive when using semi-dissected preparations to expose the beating heart and using direct immersion objectives. Although it takes some time to learn the dissection technique [86], the attainable speed of dissection is roughly equivalent to the time it takes to position the intact organisms and the light source for other optical methods, including OCT (see below). SOHA is not fully automatic because it is desirable to permit user input to verify and

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