Fast differential interference contrast imaging combined with autocorrelation treatments to measure the heart rate of embryonic fish

Jin-Tao Zhu, a Jia He, a Ji-Yao Chen, b,c Da-Ru Lu, c and Lu-Wei Zhou a

aFudan University, Surface Physics Laboratory (National Key Laboratory) and Department of Physics, Shanghai 200433, China
bFudan University, State Key Laboratory for Advanced Photonic Materials and Devices, Shanghai 200433, China
cFudan University, State Key Laboratory of Genetic Engineering, School of Life Sciences, Shanghai 200433, China

Abstract. To develop an accurate and convenient method for measuring the heart rate of zebra fish in vivo, a system combining fast differential interference contrast (DIC) imaging with an autocorrelation technique is established. The imaging correlation coefficient corr(i,j) between frame i, selected from the obtained time-lapse imaging series as the reference image, and any other frame j, is calculated as the time-dependent cycle course. Heart rate is determined by the cycle period of the corr with a high temporal resolution of 4 ms, achieved by fast charge-coupled device (CCD) imaging of 250 frames per second. With this high-resolution system, we find that 1-mg/L cadmium not only induces the slowing of the heart rate, but also caused signs of arrhythmia in treated fish. © 2008 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2904667]

Keywords: heart rate; differential interference contrast; imaging; correlation.

Fig. 1 The different states of the beating heart in a zebra fish. The DIC images were recorded from a fish on day 3 postfertilization.

important. However, in Burns’ method, the transgenic treatment for acquiring the GFP-expressed embryos must be carried out before the measurements. Moreover, the weak fluorescent signal of GFP does not allow for fast imaging, so the frame rate Burns et al. used was 512 frames per minute, resulting in a low temporal resolution of 177 ms. This system is probably not suitable for detection of a high heart rate (>300 BPM), and is also not able to discern heartbeat periods with variations smaller than 117 ms, such as in the case of arrhythmia. To improve the assay of heart rates in zebra fish, we report a new system that combines a fast differential interference contrast (DIC) imaging technique with autocorrelation treatments. Since a higher imaging rate of 250 frames per second was used in our system, the temporal resolution is as high as 4 ms, which ensures the precise measurements of heartbeat periods.

The zebra fish were raised at 28 °C according to the literature. Before the measurements, the embryonic fish were dipped in the culture liquid on the coverslip. The sealed sample was then put into a transparent, temperature-controllable cell (Olympus) on the measuring stage of the microscope. The Olympus IX71 microscope, containing the DIC function and equipped with a high-speed charge-coupled device (CCD) camera (480 × 420 pixels) (MotionScope PCI 8000 S), was used to acquire DIC images of a zebra fish continuously with a frame rate of 250 frames/s. In the DIC imaging measurements, the transmitted light was strong enough so that the imaging exposure time could be as short as milliseconds, resulting in a high temporal resolution. In fact, DIC images recorded the intensity in each pixel that correlated with the optical path length accordingly. With the heart beating, the optical path length of the transmitted light in the heart area correspondingly changed, leading to the imaging differences in time-lapse DIC images, as shown in Fig. 1. The heart beating is a cycle course. Figures 1(b) and 1(c) differ much from that of Fig. 1(a), showing that the imaging simi...
larity is poor between them. When beating heart finished a cycle reaching Fig. 1, the similarity between Figs. 1(d) and 1(a) became great. Such similarity could be defined as autocorrelation, and quantitatively described as the correlation coefficient $R(t_0, t_0 + t)$ in Eq. (1).9

$$R(t_0, t_0 + t) = \frac{\langle I(t_0)I(t_0 + t) \rangle}{\langle I(t_0) \rangle \langle I(t_0 + t) \rangle},$$

where, in our case, $I(t_0)$ is the intensity of a pixel in the frame of time $t_0$, $I(t_0 + t)$ is that of the same pixel in the frame of time $t_0 + t$, and $\langle \rangle$ denotes an ensemble average. Based on Eq. (1), the imaging correlation coefficient $\text{corr}(i, j)$ between frames $i$ and $j$, defined for our case, can be transformed as Eq. (2).

$$\text{corr}(i, j) = \frac{\sum_n I_i(n)I_j(n)}{(\sum_n I_i(n)^2)(\sum_n I_j(n)^2)^{1/2}}.$$  

where $I_i(n)$ is the intensity of pixel $n$ in frame $i$, and $I_j(n)$ is that of pixel $n$ in frame $j$. The value of $\text{corr}(i, j)$ should be from 0 to 1.

Selecting a reference image from an obtained imaging series, $\text{corr}(i, j)$ was calculated as the time-dependent function. Figure 2 shows the typical corr in the heart area (Fig. 1) of the fish, in which a reciprocal of the cycle period represents the heart rate. Thus, by combining the fast DIC imaging and autocorrelation treatment, a new modality of heart rate measurements for zebra fish was developed.

Referring back to the 28 °C, temperature alteration will change the heart rate of the fish as reported.7 With temperature increments from 22 to 34 °C, we found that the heart rate gradually increased from 120 to 285 BPM, which is in good agreement with the result in Ref. 7, but is a slight difference compared with other reports.5,6 Furthermore, as shown in Fig. 3, the variation of heartbeat periods also increased for fish at the abnormal temperature of 34 °C, compared to the distribution of heartbeat periods of fish at the normal temperature of 28 °C, indicating that abnormal temperature induced abnormal heartbeats.

Fig. 2 The time-dependent corr. The reciprocal of cycle periods represents the heart rate. Here the picture in Fig. 1(c) is selected as a reference image.

Cadmium (Cd) is a known toxic element that affects the cardiovascular function of fish with the typical phenomenon of slowed heart rates.10 After 1-mg/L cadmium was added to the fish dish for 2 days, we found that the average heart rates of these treated fish at the normal temperature of 28 °C indeed decreased. Moreover, the variation of heartbeat periods also increased in the treated fish, compared with that of the control fish (Fig. 4). Arrhythmia is defined as the variation range of heartbeat periods between the longest and shortest periods exceeding the common range of healthy groups. In contrast to the 24-ms variation range of heartbeat periods in the control fish [the right one in Fig. 3(b)], the 36-ms variation of heartbeat periods demonstrates the signs of arrhythmia in Cd

Fig. 3 (a) Heartbeats of the embryonic fish on day 3 postfertilization at 34 °C. (b) The distribution of heartbeat periods at 34 °C compared with that at the normal temperature of 28 °C.

![Fig. 3](image)

Fig. 4 (a) Heartbeat of the Cd-treated fish on day 3 postfertilization at 28 °C. (b) The distribution of heartbeat periods in the treated fish.
treated fishes. Since the variation of heartbeat periods is around 30 ms, our system was good enough to reliably measure such period changes, while the previous system with poor temporal resolution would not be reachable. In addition, since numerous commercial microscopes have the DIC function, this measuring system is easy to acquire and adapt.

Acknowledgments
We thank Professor Houyan Song for her fruitful discussion and supplying us zebra fish used in this study. J.T.Z. and L.W.Z. wish to thank the support from the National Natural Science Foundation of China under Grants No. 10334020 and No. 10574027, the Science and Technology Commission of Shanghai Municipality (05DZ19747), and the “973” Grant of Ministry of Science and Technology of China.

References