Application Note:

VisiScope Cell Explorer: VS-CMM
Cardiac Myocyte Contraction Monitoring

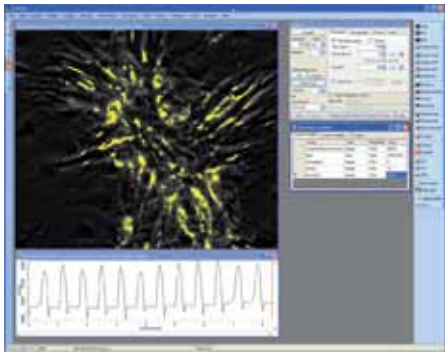
Introduction

The heart is our central organ and almost any disease or failure of it leads to severe illness or sudden death. Good indicators of a persons constitution are the heart rate and the blood pressure, which are the basis of any medical examination.

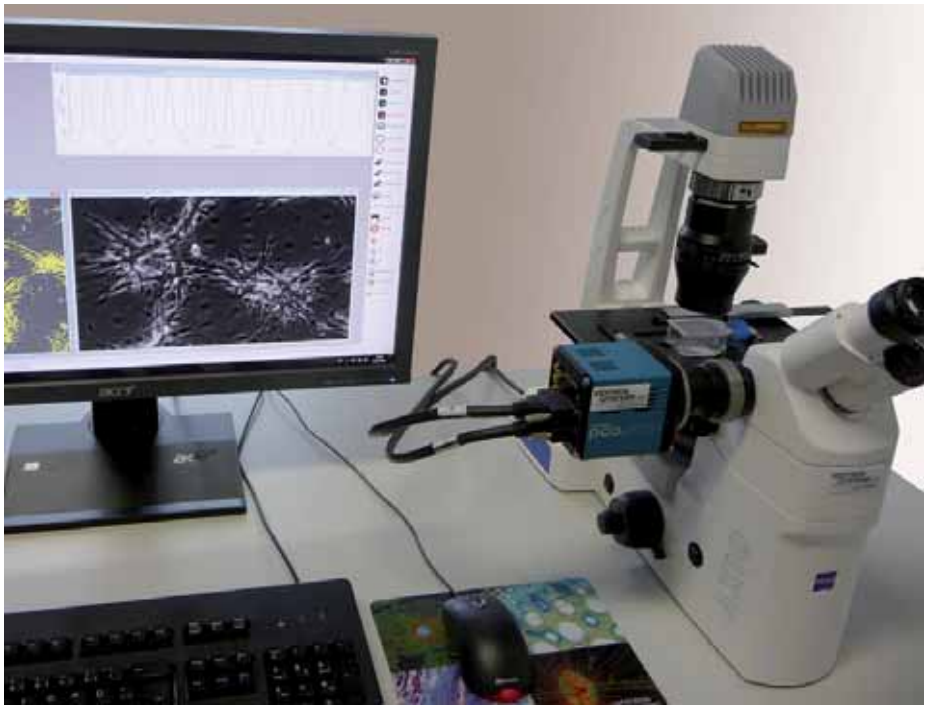
In similar way, but on the cell or tissue level, scientists analyse the excitation-contraction of cardiac myocytes to gain knowledge of our central organ's physiology.

The basic functional unit of the heart are cardiac myocytes. All types of cardiac myocytes can either be extracted from adult organs or can be grown from early embryonic stem cells.

These cell cultures can be used for easy microscopic examination of the effects drugs have on cardiac myocyte's electrical stability and contraction and hence on our heart.



VisiView Software with CMM Analysis and Intensity / Time Display



VisiScope CMM System with Zeiss Axiovert A1 and sCMOS PCO Edge camera

Methods

Since these cell cultures are barely visible in bright field, phase contrast is applied. Phase contrast is a ingeniously simple and cheap method of enhancing the contrast of thin and transparent objects which influence the light rather in phase than in amplitude. Positive phase contrast usually results in darker objects in front of brighter backgrounds.

Like for any other live cell observation the microclimate has to be stabilized to maintain viability of the cell culture and the responsivness of the cells.

For this purpose one can either build a closed incubation chamber around the microscope or at least keep the temperature constant with heatable inserts or heatable tables/objectives.

CMM works even with cells grown in cell culture flask or multi-well plates with 1 mm Polystyrene bottom using long working distance objectives.

Monitoring a high rate of contraction (up to 8 bpm (beats per minute)) requires an even higher frame rate for acquisition in order to get several data points per period and not to miss single contractions (figure 1). The new sCMOS camera PCO edge offers a very high frame rate (100 full frames per second) while keeping the spatial resolution and the field of view at the maximum level like no other camera.

Cardiac Myocyte Contraction Monitoring

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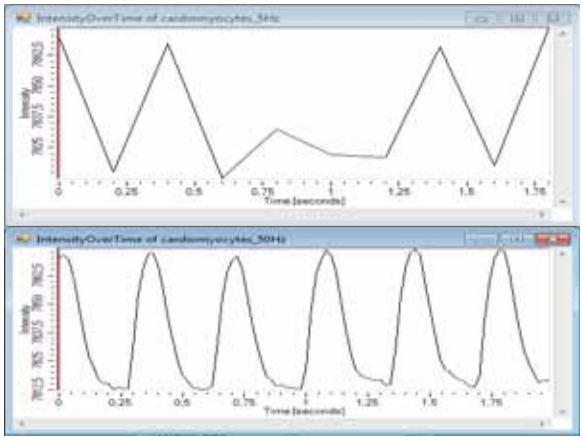
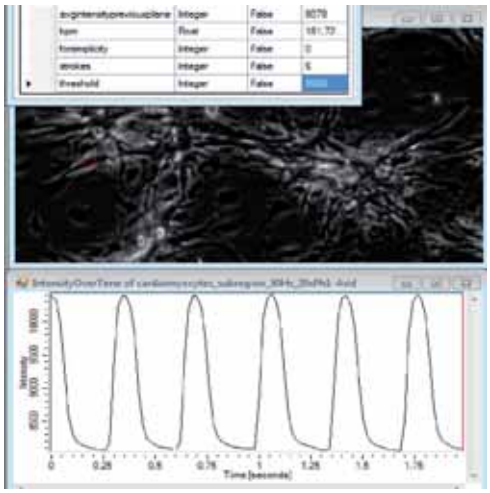


Figure 1: 3Hz contraction of cardiomyocytes acquired with 5fps and with 50fps using PCO edge. Several contraction are not visible at lower frame rate.

Software

The VisiView Software is used to control the camera and acquire images. The addition of drugs can be denoted in the experiment files using event markers. The CMM application module of VisiView easily counts the contractions and calculates the bpm on the basis of intensity changes over time. The VisiView software further allows to store all relevant data for easy reproduction of your analysis.



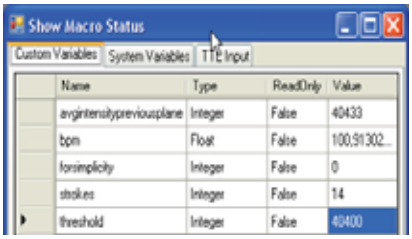
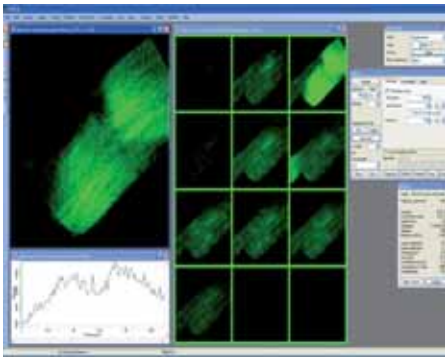


Figure 2: CMM of cardiomyocytes growing in clusters. The images were obtained with a 20x LD A-Plan Ph1 and a PCO Edge acquiring 50fps. The CMM application module automatically calculates the bpm from the image or a certain region of interest.

Fluorescence Techniques

To gain deeper insight into the ion homeostasis of cardiac myocytes certain fluorescence techniques can be applied. There are many fluorescent dyes available which selectively bind certain ions. Binding of the ion then shifts the either the excitability, excitation or emission spectra and can be measured with VisiFluor. Ca2+ concentration for example can be assayed with Fura-2, Indo-1, Rhod-3, Fluo-4 etc., Na+ can be visualized with SBFI, K+ with PBF1, H+ with BCECF. The easiest way of loading is to incubate cells for 10-60min at 37°C with about 10µM of the cell-permeant acetoxymethyl (AM) esters.



VisiView Software with Montage Display

If AM ester loading is insufficient there are alternative but more invasive methods of dye loading like electroporation, microinjection, ballistic bombardment etc . For proper concentration calculation the calibration in the same sample is very important. Usually cells are permeabilized using ionophores to equilibrate the intracellular ion concentration with the buffer.

Although VisiFluor imaging requires quite different microscope equipment compared to phase contrast imaging described before, most microscopes can be upgraded to combine VS-CMM and VisiFluor Ratio Imaging within one setup.