Fluorescent imaging of optogenetic pacemaker cells for the heart

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Optogenetics uses light to stimulate genetically modified cells. Researchers at Stony Brook University plan to use these cells as optical cardiac pacemakers. A dedicated high-speed camera is being employed to monitor the work of the cells, providing relevant insight into the propagation patterns of the activation signal.

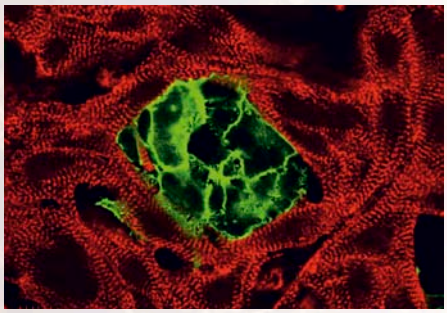

Figure 1: Genetically modified, optically excitable cardiac tissue: light-sensitive cells (green) integrated within heart cells (red)

Each year, around 65,000 patients in Germany are given a cardiac pacemaker. Since the first fully implantable pacemaker was used in 1958, there has been no change in the underlying operating principle: if the regular heartbeat stops, the electronic system delivers electrical impulses in lieu of the the sinus node (the natural pacemaker in the heart), thereby triggering contractions in the heart muscles. Although electronic pacemakers have already benefited millions of patients, these devices come with drawbacks: the batteries need to be changed every five years or so, and the electronic circuitry is highly sensitive to electromagnetic radiation.

Researchers at New York's Stony Brook University are now experimenting with a completely new concept. The basic principle is not to deliver electrical pulses rather optical ones, using a small population of genetically modified, photosensitive cells (**figure 1**). These cells act as a kind of biological relay system: a light impulse excites the cells, and when coupled to the surrounding tissue, transmit electrical impulses to the rest of the heart muscles. The contraction of the heart would then be triggered optically by a fibre-optic cable. Considering improvements in solid-state light sources, this could yield energy saving benefits and could mean a tenfold increase in the battery's service life, extending it to as much as 50 years. Fibre-optic cables may also be more biocompatible and less prone to breaks than electrical leads.

1 Manipulations with light

The new research field of optogenetics is where biomedical specialists have achieved important breakthroughs. Optogenetics, a combination of genetic engineering and optical methods, is regarded as one of the most exciting research fields in biomedicine over the last few years, and was chosen as Method of the Year in 2010 by the renowned research journal, *Nature Methods*. In optogenetic applications, cells are genetically modified to respond to light impulses. Biomedical specialists have succeeded in manipulating brain cells using light, and subsequently have advanced research influencing the behavior of animals [1]. The researchers, led by bioengineering project manager Professor Emilia Entcheva, have coupled optogenetic modified cells with heart-muscle cells for the first time, establishing a basis for developing optical cardiac pacemakers [2,3].

The "tandem unit" concept, which is a coupled system of light-sensitive donor cells and native heart cells, is the core idea. Light-sensitive switch molecules - genetically modified Channel rhodopsin proteins (ChR2) from algae - were introduced into the donor cells. Blue light pulses (wavelength of 470 nm) are used to open the protein ion channels, which then produce an electrical signal, that is instantly transmitted to the second type of cells in the tandem unit, the heart-muscle cells. This cell-to-cell signal transmission thereby causes the synchronous heart-muscle contraction.

One advantage of this approach is that the heart-muscle cells are not genetically manipulated, nor are they directly excited by an electrical lead. As a result, future therapy would need only to inject a small population of genetically modified cells, such as stem cells from the patient, into the heart-muscle cells to function as the pacemaker. The team estimates that using just half a million of the genetically modified cells as a relay (less than a few square millimeters of tissue) would be enough to trigger an electrical current of sufficient strength for the entire heart to contract.

2 Monitoring tandem cells

The tandem cell unit idea was validated in cell pairs and in cardiac tissue by optically

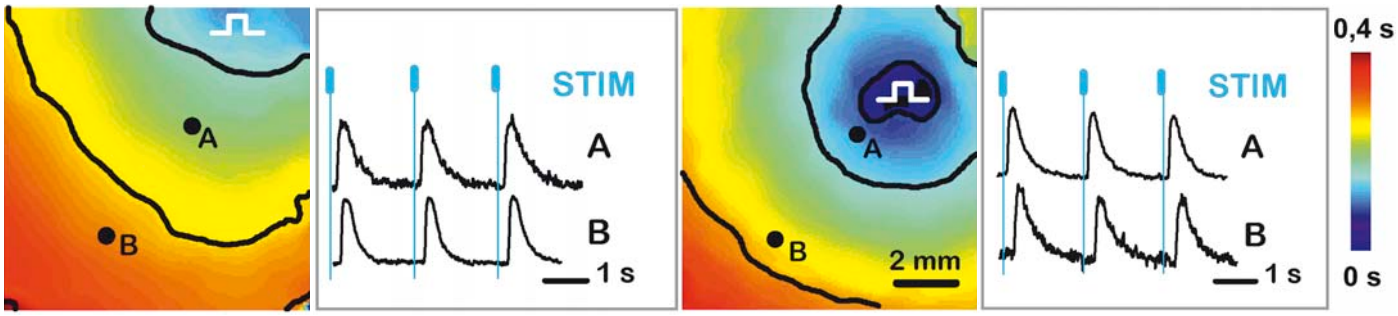

Figure 2: Optically-recorded propagation maps and single-site records in response to electrical (left) and optical stimulation (right) of cardiac tissue

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tracking the light-triggered waves of excitation. An ingenious measurement system was used to obtain detailed, high-speed and high-resolution images that record the propagation of the electrical waves in the cell clusters and engineered cardiac tissue (**figure 2**). This is important, because the electrical impulses must be propagated quickly and synchronously for the proper pumping activity of the heart. Results in cell samples in the laboratory have been encouraging: the heart-muscle cells behave identically, irrespective of whether they are excited by optically stimulated neighbouring cells, or directly by an electrical impulse, as used in a standard cardiac pacemaker.

But how can the function of the entire cell relay be measured – from the start of the optical light impulse to the contraction of the docked heart-muscle cells? And are measurements possible over a longer series of contractions? For answers, the team used a second light source that projects green 535 nm light into the cell cluster from the side without exciting the light-sensitive ion channels. Instead, the light serves to excite a fast reporter dye in the cells. With each electrical impulse triggered in the cardiac tissue, the dye changes its fluorescence on a millisecond time scale, emitting red light at a wavelength of 630 nm. This fluorescent light is recorded by a light-sensitive camera (**figure 3**) and processed into a series of images that map the exact propagation of the electrical activity in the cells over time.

3 Pushing the limits of camera technology

The camera, which has to meet extremely stringent demands, plays a crucial role in the experiment set-up. The role is important because, in addition to the high-speed and high-spatial resolution requirements, the fluorescent signal from the cells is extremely weak, and requires high-sensitivity detection. EMCCD (electron multiplying charge-coupled device) cameras have been specially designed for weak light signals of this kind, and they intensify the signals captured on the sensor. However, they fail to meet the second requirement – the ability to deliver a rapid sequence of images in excess of 200 images per second over a million of pixels. The trade off is, on the other hand, that extremely fast cameras offer a lower image resolution. "We tried out a lot of different cameras, but to begin with, none of them managed to meet our stringent requirements," Emilia Entcheva recalls. In 2005, at the end of an EMCCD selection process, Professor Entcheva finally decided on a pco.1200 hs high-speed camera from the company PCO. This camera has a high frame rate of 636 images per second, at a full resolution of 1280 x 1024 pixels, and a dynamic range of ten bits (**figure 4**). One drawback is that the CMOS image sensor does not provide sufficient sensitivity for the weak fluorescent signals from the cells. However, this can be overcome by using an appropriate high-speed image intensifier.

4 Data transmission

One bottleneck in the measuring equipment is the data streaming, because the high frame rate also means a high data volume. With a capacity of four gigabytes, the storage unit in the camera can buffer only shorter image sequences, but at full speed, the camera is recording one gigabyte per second. For that reason, the scientists connected a fast RAID storage unit with several hard disks to the camera to handle monitoring periods of several minutes. This solved the problem of data acquisition, and meant that the CMOS camera could make its contribution to the success of the experiments.

EMCCD cameras have of course also made great strides over the last six years, but their performance cannot yet match the requirements, particularly in terms of combined spatio-temporal resolution.

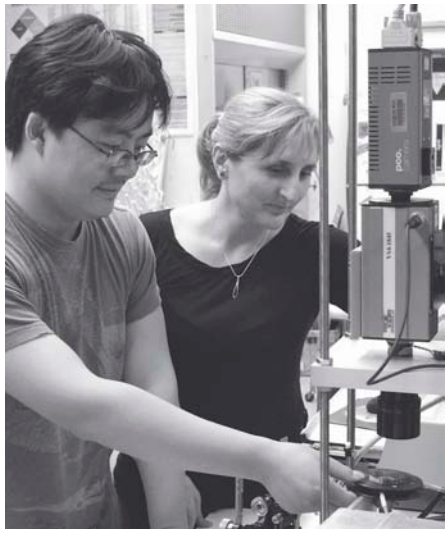

Figure 4: Prof. Emilia Entcheva and her student Zhiheng Jia along with the pco.1200 hs camera system

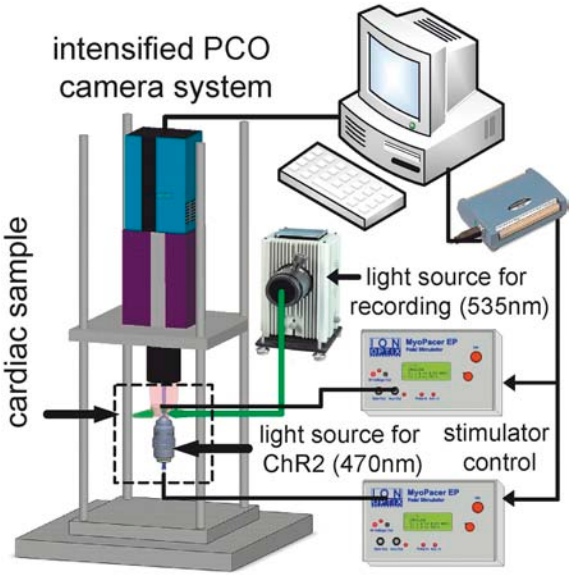

Figure 3: Optical mapping system for monitoring the cell-relay operation. Genetically modified cells within the tissue sample, being illuminated from below with blue light (470 nm), are stimulated to emit electrical pulses. A migrating pulse generates weak fluorescence emission at 630 nm from the natural cells that are being additionally illuminated at 535 nm. The 630 nm emission is monitored by the fast, high-resolution camera

5 Outlook

Light-sensitive cells could possibly serve as a "relay" for future pacemakers. Within the next few years, biomedical specialist Emilia Entcheva intends to further improve the efficiency of the measurement set-up, with help from PCO and with new, more sophisticated camera systems, such as the fast, sensitive scientific CMOS camera system, pco.edge.

Literature:

[1] K. Deisseroth, *Controlling the brain with light*. Sci Am. Nov. 2010, 303(5), p.48-55. *www.stanford.edu/group/dlab/papers/deisserothsciam2010.pdf*

[2] E. Entcheva et al., *Stimulating Cardiac Muscle by Light: Cardiac Optogenetics by Cell Delivery*, Circulation: Arrhythmia & Electrophysiology, 4(5):753-60, 2011, www.ncbi.nlm.nih.gov/pubmed/21828312

[3] E. Entcheva, H. Bien, *Macroscopic optical mapping of excitation in cardiac cell networks with ultra-high spatiotemporal resolution*, Progress in Biophysics & Molecular Biology 2006

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