Abstract - Fluorescence imaging provides a comprehensive view of the electrical behavior of excitable cells, the relative nature of the optical signals however requires additional means for calibration. The determination of the actual electrical field strength is subject to uncertainty even when using concurrent electrode measurements. A combined approach of electrical field measurement and optical mapping of the spatially distributed polarizations provides a satisfying calibration of the optical signals in our field-stimulation experiments.

Keywords - Optical potential-mapping, fluorescent dye, action potential, electric field-stimulation

I. INTRODUCTION

Using fluorescence imaging for optical potential mapping has been established as a powerful tool to gain thorough insight into the complex behavior of cardiac activation. It is particularly suited to observe the excitation process of cardiomyocytes exposed to electrical fields.

Still there remain a number of problems to be addressed, when using this experimental method to authentically record the actual electrical membrane behavior. In the absence of additional electrode measurements from the cell or accurate estimates of the effective electric field in the bath, the observed light signals only provide information about relative potential changes. To measure the applied field strength simultaneously with the optical recording, additional electrodes sensing the actual voltages in the bath would be necessary. However, in many experimental situations this is not feasible. Moreover measuring the electric field strength separately immediately before or after the actual stimulation experiment is also subject to uncertainty. Our effort was to develop an approach using the combined information from simultaneous as well as consecutive recordings to properly calibrate optical signals and to get acceptable estimates for the potential gradients when no auxiliary measurements from separate field sensing electrodes are available.

II. METHODOLOGY

A. Problems

In our experiments single ventricular cardiomyocytes are stained with the voltage sensitive dye di-4-ANEPPS. The response to electrical stimulation is recorded by a 10x10 photodiode-array allowing for a spatial resolution of 15 µm ([1]). In field-stimulation experiments stimulus pulses of varying strength, duration and orientation are applied using a controlled current source. Because of inevitable fluctuations of the bath-height, the stimulus current can only be used as a rough estimate for the actually applied field strength.

The signals generally cumulate noise and deterministic artifacts from the light source, the optical detectors, the amplifier, and the experimental environment. Due to high light intensities of about 100 W/cm² dye bleaching occurs. Local variations of dye uptake into the membrane and inhomogeneous distribution of the excitation light result in considerable dispersion of the effective voltage-sensitivity (the percentage change of the fluorescent light intensity related to a membrane potential change).

As the observed signals only provide information about relative potential changes, proper scaling to membrane potentials is usually based on the observed change of the membrane potential from resting state to plateau state or the average action potential amplitude of cardiomyocytes recorded with patch-electrodes ([1], [2]).

In this work, the experimental records cover a period of about 10 ms after field stimulus application. Attempts to calibrate a series of consecutive records from the same cell with different field strengths support the assumption that not only the time-course of the evolving action potential but also the action potential amplitude depend on the applied field-stimulus strength. Action potential amplitude can therefore not be used as a general measure for calibrating the optical signals.

B. Approach

First, the optical signals are low pass filtered using a zero-phase finite impulse response filter ([3]).

Estimates for local voltage-sensitivities and correcting functions to account for dye bleaching were determined for every recording, assuming a common resting potential for all measuring spots within a cell before stimulus-application and also assuming that the cell exhibits a common potential again after a sufficiently long period subsequent to the stimulus application for all measuring spots within a particular cell.

Appropriate scaling of a sequence of recordings from a particular cell then comprises a two-step procedure. From the

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sequence of recordings we first select a "reference recording" with stimulation close to excitation threshold, as under this condition the cell response most closely resembles a “natural” elicited action potential. We then calibrate the optical signals of this particular experiment assuming an action potential amplitude of 128 mV. The optical signals of the remaining recordings from the particular cell can then scaled based on their relative fluorescent light intensity $\Delta F/F$. This allows an unbiased investigation of the effect of stimulus height and duration on the stimulus response.

To validate our approach we finally conducted experiments where we also measured bath impedance immediately after each stimulus using two auxiliary electrodes. This allowed getting bath-level-corrected estimates for the applied electrical field strength from the commanded stimulus current values used to generate the electrical field in the bath.

III. RESULTS

Figure 1 shows the strong linear correlation between the estimated gradient for the membrane potential based on the local polarizations and the bath-level-corrected stimulus amplitudes ($r=0.997$) for a total of 12 stimulation experiments with the same cell. Common scaling is based on a reference-recording with a 4 ms stimulus of 4.2 V/cm for that particular cell.

IV. DISCUSSION

Light intensity response may not linearly depend on the membrane potential. Linearity in the dye-response to voltage changes has previously been verified for physiological rage of potentials ([1]) and has been confirmed to be a valid assumption at least in the range -280 to +140 mV ([2]). While the membrane potential at the distant regions of a cell may be polarized beyond the range of linearity for high field strengths, in our experiments we can make use of measuring spots closer to the center of the cell, where the membrane potential is definitely in the region of verified linear dye-response.

While it would be possible to reduce bath level fluctuations by an overall increase of the bath level, this is not desirable for other practical reasons.

V. CONCLUSIONS

With our approach we were able to demonstrate that it is possible to calibrate fluorescence light intensities to membrane potentials for a sequence of recordings from the same cell using a reference recording with close-to-threshold stimulus. The responses to stimuli with increased amplitude can then be properly scaled with respect to the averaged fluorescence light intensities.

The problem of possible nonlinearities in the voltage response of the dye can be overcome for fieldstrength-estimation by using close located measuring spots.

REFERENCES

