4 Discussion and Conclusions

The results of this study demonstrate that ratiometric mapping of electrical activity is possible using a single video camera. Kinsley [2] was the first to show that ratiometry could be used to minimize artifact due to heart wall motion and photobleaching in di-4-ANEPPS stained hearts. Fluorescence was excited regionally with 488nm excitation and a ratio signal formed from the emission recorded over short and long wavelength windows with two photodetectors. Our approach can be used sequentially with a single photodetector. Two widely available long pass filters are used rather than custom-made band pass filters, and improved SNR is expected because these filters covers more of the emission spectra than band pass filters.

We have shown the performance of our method is very similar to that of Knisley [2] under comparable conditions. The results presented here also demonstrate that it is possible to recover APs using a single 2D detector in the presence of substantial motion. However, signal-to-noise ratio in this case was low (SNR≈2) and motion artifacts were only partly suppressed. Difficulties include the need to maintain a high level of synchronization and accuracy when filters are switched. Also, motion signal must be reproduced throughout the recording period if it is to be removed using a sequential correction method. Finally this method cannot remove motion artifact that is due to altered registration of the heart within the 2D imaging frame.

An interesting finding is the failure of ratiometry to remove artifact with 532nm laser excitation, when this is highly effective with 473nm excitation. This suggests that the spectral response of di-4ANEPPS may be different at these two excitation wavelengths. The interesting is also possibility to use this approach in combination with image registration technique [3].

Acknowledgement

This work has been partly supported by the grant 102/07/1473 from GACR

References

[1] Asimov RI, Mikulski PV, Salaam G. Optical Imaging of the Heart, Circulation Research 2004; 95:21-33.

[2] Kinsley S.B., et al. Ratiometry of transmembrane voltage-sensitive fluorescent dye emission in hearts. American Journal of Physiology Heart and Circulatory Physiology 2000;279:1421-1433.

[3] Svrcek, M.; Rutherford, S.; Chen, A.; Provaznik, I.; Smaill, B. Characteristics of Motion Artifacts in Cardiac Optical Mapping Studies. In Proceedings of the IEEE EMBS Conference. IEEE, 2009. s. 1-4.

[4] Chen, A.; Vanholsbeeck, F.; Tai D.; Svrcek, M.; Smaill, B. Time- resolved all fiber fluorescence spectroscopy system. In BiOS 2010. San Francisco, California , USA: SPIE Photonics West, 2010. s. 1-4.

[5] Salama G., Morad, M. Merocyanine 540 as an optical probe of transmembrane electrical activity in the heart, In Science, 1976;191:485-487.