



# Spatial Frequency Content in Optical Mapping of Cardiac Cell Monolayers

Harold Bien, Emilia Entcheva, Arkady Pertsov, Sergey Mironov and Frederick Vetter

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The following is the abstract of the article discussed in the subsequent letter:

**Mironov, Sergey F., Frederick J. Vetter, and Arkady M. Pertsov.** Fluorescence imaging of cardiac propagation: spectral properties and filtering of optical action potentials. *Am J Physiol Heart Circ Physiol* 291: H327–H335, 2006.—Fluorescence imaging using voltage-sensitive dyes is an important tool for studying electrical propagation in the heart. Yet, the low amplitude of the voltage-sensitive component in the fluorescence signal and high acquisition rates dictated by the rapid propagation of the excitation wave front make it difficult to achieve recordings with high signal-to-noise ratios. Although spatially and temporally filtering the acquired signals has become de facto one of the key elements of optical mapping, there is no consensus regarding their use. Here we characterize the spatiotemporal spectra of optically recorded action potentials and determine the distortion produced by conical filters of different sizes. On the basis of these findings, we formulate the criteria for rational selection of filter characteristics. We studied the evolution of the spatial spectra of the propagating wave front after epicardial point stimulation of the isolated, perfused right ventricular free wall of the pig heart stained with di-4-ANEPPS. We found that short-wavelength (<3 mm) spectral components represent primarily noise and surface features of the preparation (coronary vessels, fat, and connective tissue). The time domain of the optical action potential spectrum also lacks high-frequency components (>100 Hz). Both findings are consistent with the reported effect of intrinsic blurring caused by light scattering inside the myocardial wall. The absence of high-frequency spectral components allows the use of aggressive low-pass spatial and temporal filters without affecting the optical action potential morphology. We show examples where the signal-to-noise ratio increased up to 150 with <3% distortion. A generalization of our approach to the rational filter selection in various applications is discussed.

*Spatial Frequency Content in Optical Mapping of Cardiac Cell Monolayers*

*To the Editor:* An elegant study by Mironov et al. (4) used experimental data from paced activation in pig hearts to formulate practical resolution limits in optical mapping of cardiac excitation. In the examined conditions, little to no content was found >130 Hz in the temporal domain and <3 mm in space, thereby justifying the use of aggressive filters to improve signal-to-noise ratio with minimal overall distortion (<3%). The remarkably low spatial frequency content was attributed to tissue absorption and photon scattering in thick samples. The authors suggested that in purely two-dimensional systems, such as cultured cardiomyocyte monolayers, higher frequencies might still be obtained. We find that when imaging cell monolayers with very high-resolution detectors (1), the aforementioned interference factors are not present, but surprisingly, the spatial spectra remain similar to those reported for thick tissue, indicating that factors other than tissue absorption and photon scattering affect spatial resolution.

As discussed by Mironov et al. (4), the fastest component of an action potential in the time domain (the upstroke) under normal propagation conditions translates into the highest spatial frequency seen at the wave front. For typical conduction velocities of 25–50 cm/s, imaging the wave front at 250 frames/s (fps, 4 ms) or 500 fps (2 ms) would yield minimum “upstroke wavelengths” of 0.5–2 mm regardless of tissue thickness. Large-kernel spatial filters would be unlikely to influence the outcome if the original image only contained features larger than 2 mm as discovered by Mironov et al. (4).

However, finer spatial details may be seen at lower conduction velocity, such as near the tip of a spiral or around sharp

obstacles. Coarse filtering in this situation can underestimate local wave front curvature, even though the overall shape remains unaffected. The spatial frequency spectrum may gain higher-frequency components through regions with closely spaced wave fronts, including wave collisions, tight wave front curvature (wave breaks or tip of a spiral wave), and steep spatial gradients (fine obstacles or spatially discordant alternans). Such regions tend to also exhibit lower conduction velocities as per the eikonal equation, thus further increasing the spatial frequency. They are found in only a small portion of the field of view but are most affected by low-pass filtering; the abundance of lower-frequency domains can mask such distortions from overly aggressive filtering.

Because the dynamics of excitable tissue impose a limit on wave front curvature, an ideal spatial cutoff size might be one-half the critical radius of curvature. The latter has been estimated to be <200 μm, but the exact value in cardiac tissue remains unknown (2). Computer simulations have demonstrated underestimation of maximum curvature even when resolution was chosen at one-fourth the simulated critical wave front curvature and was only partially recovered after interpolation (3).

In conclusion, the filtering parameters proposed in Ref. 4 for thick tissues can be generalized for imaging normal propagation in cell monolayers as well. Only when the local dynamics in areas with high wave front curvature is of particular interest, such filtering is not applicable. High-resolution imaging of such areas in cell monolayers has the potential to provide more precise estimates of the critical wave front curvature and the curvature-velocity relationship for cardiac tissue.

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Harold Bien  
Emilia Entcheva  
*Department of Biomedical Engineering  
Stony Brook University  
HSC T18-030  
Stony Brook, New York  
e-mail: emilia.entcheva@sunysb.edu*

REPLY

*To the Editor:* Fluorescence imaging using voltage-sensitive dyes has become an important tool for studying electrical propagation in the heart. Yet, the low amplitude of the voltage-sensitive component in the fluorescence signal and high acquisition rates dictated by the rapid propagation of the excitation wave front make it difficult to achieve recordings with high signal-to-noise ratios. Whereas digital filtering of the acquired signals has become a de facto key element of optical mapping, there is no consensus regarding



its use. We are very pleased that our study (3), in which we propose a systematic approach to rational selection of filtering procedures, stirred interest in the optical mapping community. We hope that the Letter to the Editor by Harold Bien and Emilia Entcheva will provoke a broader discussion, leading to better understood caveats of the filtering methods and to gold standards for processing various types of optical mapping data.

In their Letter, Bien and Entcheva report the interesting observation that, similar to thick tissues, optical images recorded during normal propagation in cell monolayers also lack the high-frequency spatial components and thus can be safely processed using aggressive low-pass filters (3). The only exceptions in which high-frequency components played a role were the areas of high-curvature excitation fronts observed during reentrant activity. While the absence of high-frequency components in thick tissues has a rather straightforward explanation and is a consequence of blurring resulting from multiple-scattering of photons inside the tissue (1, 2), similar observations in cell monolayers are quite unexpected.

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Arkady Pertsov  
Sergey Mironov  
*Department of Pharmacology  
State University of New York  
Upstate Medical University  
Syracuse, New York  
e-mail: pertsova@upstate.edu*

Frederick Vetter  
*Department of Electrical and Computer Engineering  
University of Rhode Island  
Kingston, Rhode Island*