Imaging Cardiomyocyte Beating Across Entire Microtiter Plates

Channel Islands CALIFORNIA STATE UNIVERSITY

Julian Ramirez[1], Brandon Eckert[1,2], Angelea Gephart[2], Jasmine Salgado[2], Natali Chavez[1,3], Raphael Hicks[3], Brian Rasnow, Ph.D.[3]

Dept. of Biology[1], Biochemistry[2], and Physics[3], CSUCI

Introduction

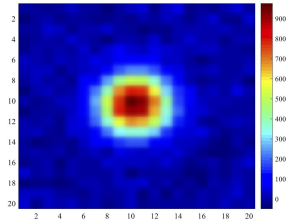
Embryonic cardiomyocytes (CMs) grown in culture spontaneously beat in synchrony with their neighboring cells. Measuring changes in the beating of heart cells is important for discovering safe and effective drugs. Conventional assay methods utilize phase-contrast microscopy to observe how drug treatments modulate CMs beating in one well at a time [1]. This method is tedious, requires expensive apparatus, and cannot provide data across multiple test conditions, concurrently. Photographing an entire microtiter plate (~15cm diameter) has inadequate resolution to detect the micron- scale beating of CMs. We demonstrate here a method using fluorescence super-resolution (SR) imaging to reconstruct the sub-pixel beating motion of CMs simultaneously in 384 well plates.

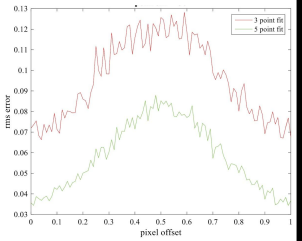
Materials and methods

~1µm polystyrene fluorescent (FITC) beads were diluted in a clear-bottom 384 well microtiter plate. The plate was illuminated in an Amgen/Etaluma FlashMini (right) and imaged with a Nikon D810A camera filtered for fluorescence. Matlab software was used for image analysis and simulation [2].

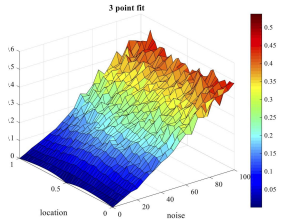
Results

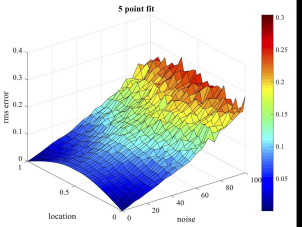
Our images of FITC beads are defocused over several pixels, similar to the simulation at right, with signal/noise = 50. As the bead moved across a pixel, two SR algorithms reconstructed the bead's location, with resulting RMS error = 0.09 & 0.06 pixels.



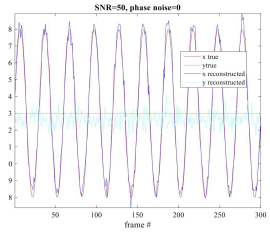


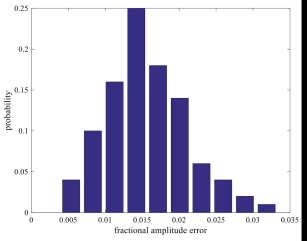
These surface plots show how the RMS error of two SR algorithms are sensitive to bead location within a pixel and the image noise level. Without noise, the algorithms locate the beads with systematic errors <1/20 pixel (bottom), and as noise increases to S/N=10, accuracy drops to 0.45 and 0.26 pixels (RMS).





Sinusoidal motion of the bead with amplitude = ½ pixel was recovered from 300 frames at S/N=50. SR was applied to each frame, and resulting positions were Fourier transformed recovering the beat amplitude = 0.508+-.003, usually within 2% of the correct value (far right). Motion phase was accurately recovered: 0.02±0.4°.





Conclusions

We have demonstrated:

1) We can image fluorescent beads concurrently in all wells of microtiter plates (not shown here).
2) Superresolution can localize beads to ~1/20 of a pixel with low noise.
3) Sub-pixel amplitude periodic motions of beads can also be reconstructed accurate to ~1/10 of a pixels.
4) Our algorithms are very robust to noise.

Future Directions

1) We are working to culture embryonic CMs [3], label them with dilute beads, & image their motions.
2) We will explore SR algorithms in the frequency domain.

References

1] Butler JP, Tolić-Nørrelykke I M, Fabry B, Fredberg JJ (2002) Traction fields, moments, and strain energy that cells exert on their surroundings. Am. J. Physiol. Cell Physiol. 282, C595-605.

[2] MATLAB - www.mathworks.com

[3] Louch WE, Sheehan KA & Wolska BM (2011) Methods in Cardiomyocyte Isolation, Culture, and Gene Transfer. J Mol Cell Cardiol. 51, 288–298.

Acknowledgments

* Society for Advancement of Chicano/Hispanics and Native Americans in Science (SACNAS) supported our multicultural, multidisciplinary team.
* Etaluma (www.etaluma.com), Amgen, and U4EA Ranch provided fluorescence imaging apparatus and reagents.