SarcLen Algorithm

Sarcomere Spacing

Mean sarcomere length of a muscle can be recorded using the IonOptix tool SarcLen. This tool runs within IonWizard and allows online visualization of the muscle specimen and the recorded mean sarcomere length within a user-defined region of interest.

We provide this documentation to explain the numerical methodology used in determining the mean sarcomere length within SarcLen. In short, we employ a fast Fourier transform to determine the mean frequency of sarcomere spacing that is defined by the A- and I-bands of the muscle.

Sarcomere Defined

A sarcomere is defined as the space between neighboring Z-lines within a muscle. Unfortunately, Z-lines are very difficult to resolve optically. However, the dark and light bands, i.e., the A- and I- bands, of the sarcomere are easily resolved and can act as markers of sarcomere spacing.

Region of Interest Defined and Line Optical Density

With the longitudinal axis of the muscle aligned horizontally, the user must define an area in the muscle image for which sarcomere spacing is to be determined. This area is called the region of interest (ROI). The image intensity across the longitudinal axis of the ROI is termed the Line Optical Density (LOD) and holds the image information from which mean sarcomere length is ultimately determined. Figure 1 illustrates an example muscle image, ROI (magenta), and LOD (black line).

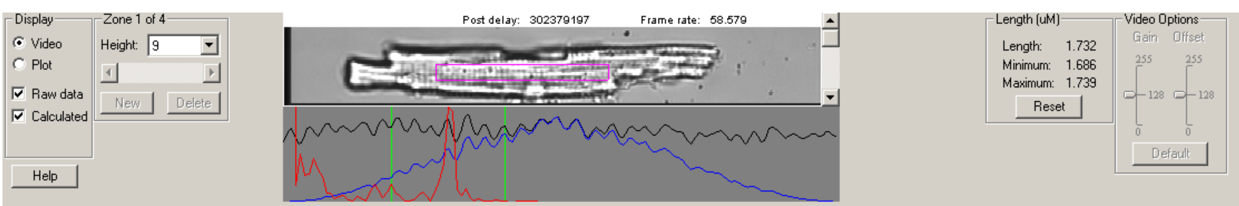

Figure 1. Example image of muscle with its longitudinal axis aligned horizontally, a selected ROI, and the corresponding LOD, as seen when using SarcLen. Note that the LOD depicts the A- and I-bands of the muscle as low and high intensity values, respectively. It is the resolved spacing between these low and high intensities that allows for calculation of the mean sarcomere spacing.

Line optical Density Conditioned by a Window

We plan to perform a Fourier transform to the LOD. But before doing so, it is important to condition the LOD such that the results are not influenced by large discontinuities in the LOD that often occur at its boundaries. Therefore, we condition the LOD by first subtracting the mean value for the LOD and then multiplying the LOD by a weighting function, w(n), called a window. The window we use is a Hanning window, which is used extensively for this purpose in signal analysis, and is define mathematically as follows:

$$w(n) = 0.5 \times (1 + \cos(2\pi n/n\_lod)), \qquad (1)$$

where n = a specific pixel number across the LOD, and n_lod = the total number of pixels across the LOD. The application of this window assures that the discontinuities at the boundaries of the LOD are not of significance consequence and that the center of the LOD is weighted most heavily in the calculation of mean sarcomere spacing.

Fast Fourier Transform

The fast Fourier transform (FFT) is the name given to an optimized method used for determining the Fourier transform numerically. This method, however, requires that the data destined for FFT contain a number of points that is a power of 2, such as 256, 512, 1024, etc. The length of the LOD, however, is usually not one of these convenient lengths, so we add values of zero to the LOD so as to pad the LOD until the length is a power of 2. This technique, called zero padding, is also a standard technique used extensively for this purpose in signal analysis.

The FFT is then performed on the zero padded LOD (ZLOD) and the results are squared to produce a magnitude of the FFT in the frequency space. The magnitude of the FFT at a given frequency corresponds to the amplitude of that frequency being discernible in the original LOD. Figure 2 illustrates a windowed and padded LOD and the corresponding magnitude of the FFT. Note that the high peak in the FFT around the index 21, which corresponds to 0.5 µm^{-1} spatial frequency and therefore refers to the high incidence of the ~2 um spacing within the LOD. This 2 µm corresponds to the sarcomere spacing.

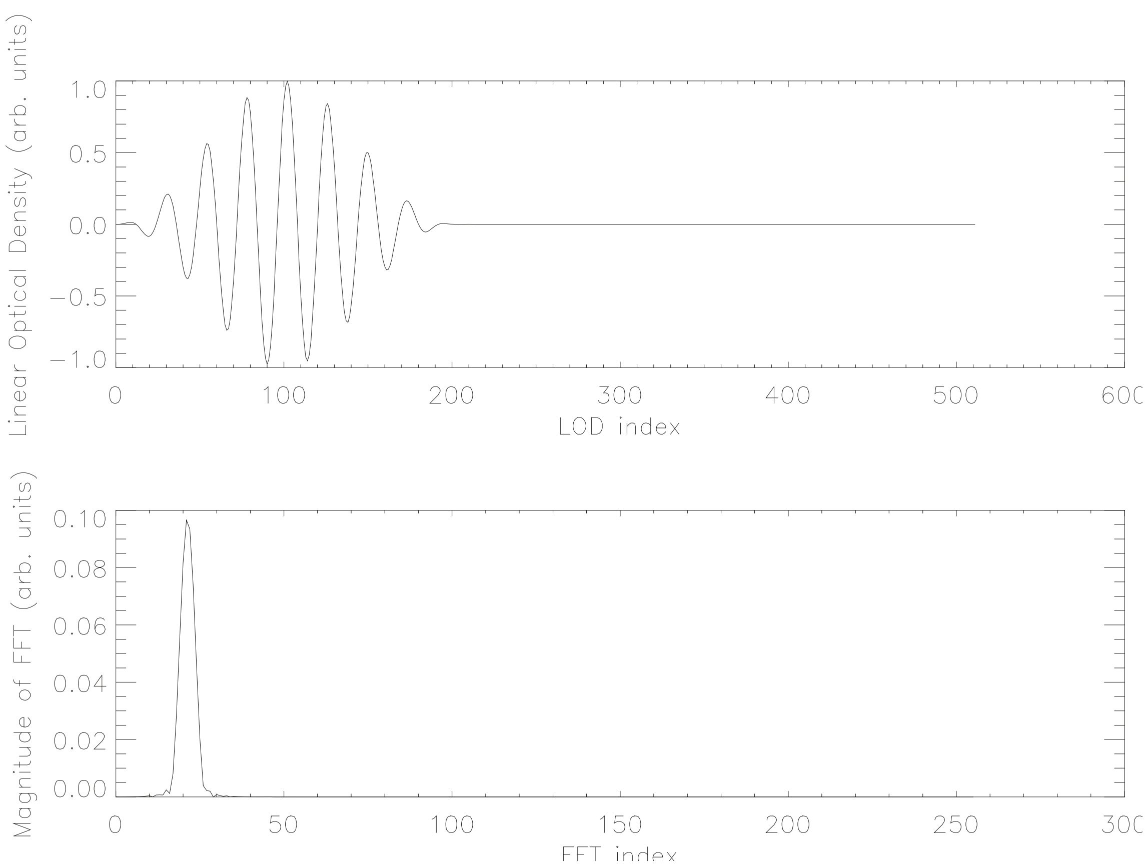

Figure 2. Conditioned LOD and the corresponding magnitude of the FFT. Note that the peak of the FFT falls near index 21 (0.5 um^{-1}), which corresponds to sarcomere spacing of ~2 µm.

Finding Mean Sarcomere Spacing

The problem of finding the mean sarcomere spacing involves three steps: (1) find the range within which we expect to find the peak frequency of sarcomere spacing in the FFT, (2) approximate the position of the peak magnitude of the FFT, and (3) determine the corresponding mean sarcomere spacing.

The range of sarcomere lengths we expect is between 1 µm and 6 µm. The corresponding spatial frequencies are 1 μm^{-1} and 0.167 μm^{-1}. We actually determine the indices of the FFT to which these values best correspond. For example, index corresponding to 1 μm^{-1} is (n_zlod x cal x 1) and the index corresponding to 0.167 μm^{-1} is (n_zlod x cal x 0.167), where n_zlod = number of points in the ZLOD, and cal = the calibration relationship of microns per data point.

Within the range defined above, we then simply find the peak value and its index. Because the spatial frequency axis of the FFT is discrete, i.e., there is a finite number of frequencies represented, it is imperative that the discrete results be analyzed for the non-discrete possibility. The two neighboring points very close to the peak value are therefore included to help approximate where the actual peak would lie within the neighborhood. This approximation is performed as determining the position at which a second-order power series would be a maximum. Specifically:

$$\text{Position relative to peak} = 0.5 \times (f_{-1}-f_1) / (f_{-1}-2f_0+f_1), \qquad (2)$$

Where f_{-1} = value at neighbor prior to peak, f_0 = value at peak, f_1 = value of neighbor after peak. Figure 3 shows an example of approximating the mean frequency of sarcomere spacing from the magnitude of the FFT.

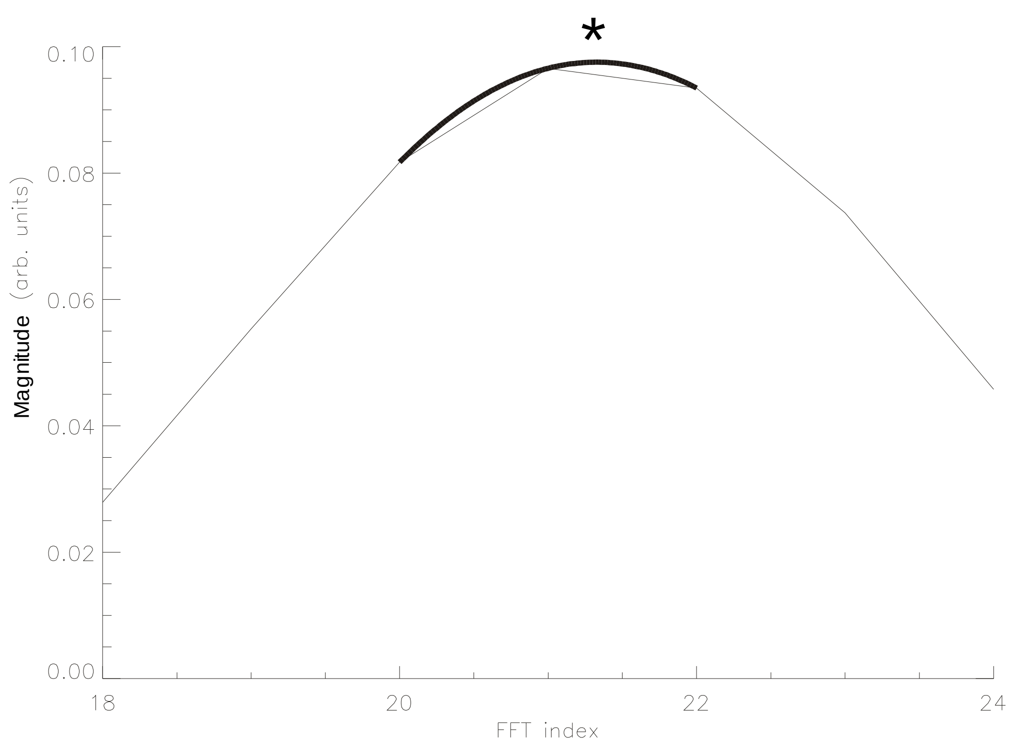

Figure 3. Example of peak magnitude of the FFT, the second-order power series approximation of the neighborhood, and the approximate position (*) of the peak.

The mean sarcomere spacing is simply calculated from the position of the approximated peak magnitude of the FFT. For example, mean sarcomere spacing = n_zlod x cal / index of approximated peak. In the example shown in figure 3, index of the approximated peak was 21.32, n_zlod = 512, and cal = 0.083 microns / pixel, and the mean sarcomere spacing was calculated to be 2.001 um.

Limitations of the Method

The method for determining the mean sarcomere spacing has some limitations. We have found that the bias associated with the above method is less than 1 % (in fact usually less than 0.2%) when the mean sarcomere length is between 1-4 um and the number of sarcomeres in the ROI is greater than 6. The bias, however, can fluctuate between + bias and – bias as the sarcomere length increases, depending on the phase of the sarcomeres relative to the window. So, be careful in interpreting any oscillations observed in the sarcomere length as it increases, it may simply be a slight oscillation in this bias. You can minimize this bias and its oscillation by incorporating more than 6 sarcomeres in the ROI.

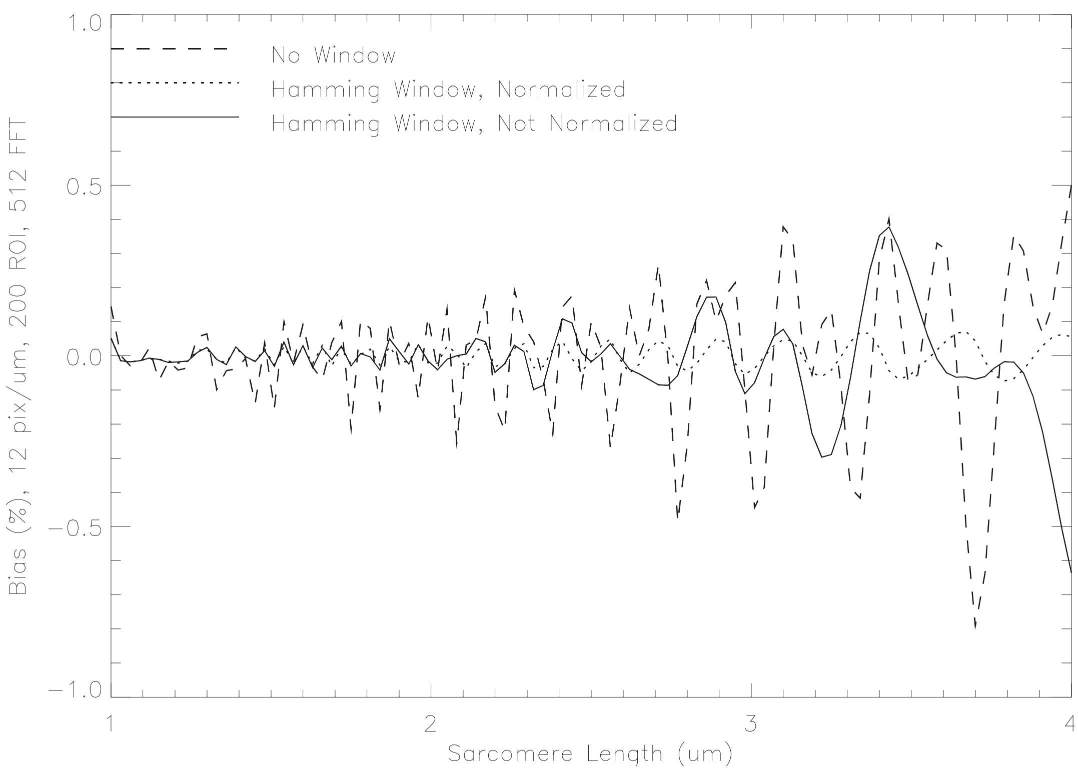

Figure 4. Example of calculated bias over simulated sarcomere lengths between 1-4 um. The number of points used in the FFT was 512, the number points in the ROI was 200. In this example, resolution was 12 pixels/um and the number of sarcomeres in the ROI ranged from 16-4 as the sarcomere length increased from 1-4 um.

Acknowledgements

The SarcLen PMT software has benefited from the input of all of our users. Specifically IonOptix would like to thank the following:

Dr. Henk Granzier for sparking our interest and providing the initial FFT technique.

Dr. Brad Palmer for help on developing the FFT algorithm.