**MARS Software package**

The software Mars\_v2 will run on a 64-bit PC (not Mac) in the absence of Matlab access.

It evaluates muscle defects from SHG (or from immunofluorescence) periodic patterns and calculates both R and S values at the same time. To understand the meaning of these values and how the program calculates them, please go to our paper “Quantitative evaluation of skeletal muscle defects in second-harmonic generation images” W. Liu, N. Raben, E. Ralston *Journal of Biomedical Optics****.*** 2013 Feb 1: 18(2). A pdf is in the present package. Please cite the paper in your references if you publish data obtained with it.

We recommend that you create a desktop folder in which you place the images you want to analyze in .tif format. The images can be black and white or in color. In the latter case the software will analyze the green channel. Do not try to use images that have more than 3 (RGB) channels.

When you start the program it asks you to select the first image to analyze. Navigate to the folder and select the image.

You will then be asked to confirm the period and orientation of the muscle fibers. The period is in pixels; the orientation is the angle between the fiber axis and a horizontal line. This step is useful for muscle fibers in really bad condition when the detection method may fail. Click yes if the two values are close to your estimate (does not need to be very accurate). Click no if the values are way off and then type in your estimates in the following pop-up window.

The program will then build the offsets and calculate the texture correlation for each pair by using the Fourier transform, The frequency plot is made and the ratio of the first fundamental frequency over the intensity of the origin indicates the regularity of the sarcomere arrangement. The program will also use the curve fitting and the texture correlation to calculate the S value. All the results will be saved on FtRatio and strRatio and will be passed to the next step.

The result figures for the Fourier Transform and fitting will be saved to a subfolder "results" that the software creates inside your image folder. The final results and scores will be saved in an Excel file. The “R score” and “S score” columns store R and S score. The rest of the columns can be useful if you are interested in other results as well.

At the end of the operations on one image you will be asked if you want to analyze another image. If your answer is yes, make sure that the results Excel file is not open. If it is open you will get an error message because the software will not be able to add data to the file.

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