Scanning Gradient Fourier Transform
V0.2.17 (6/2/2016)
Quick-Start Manual

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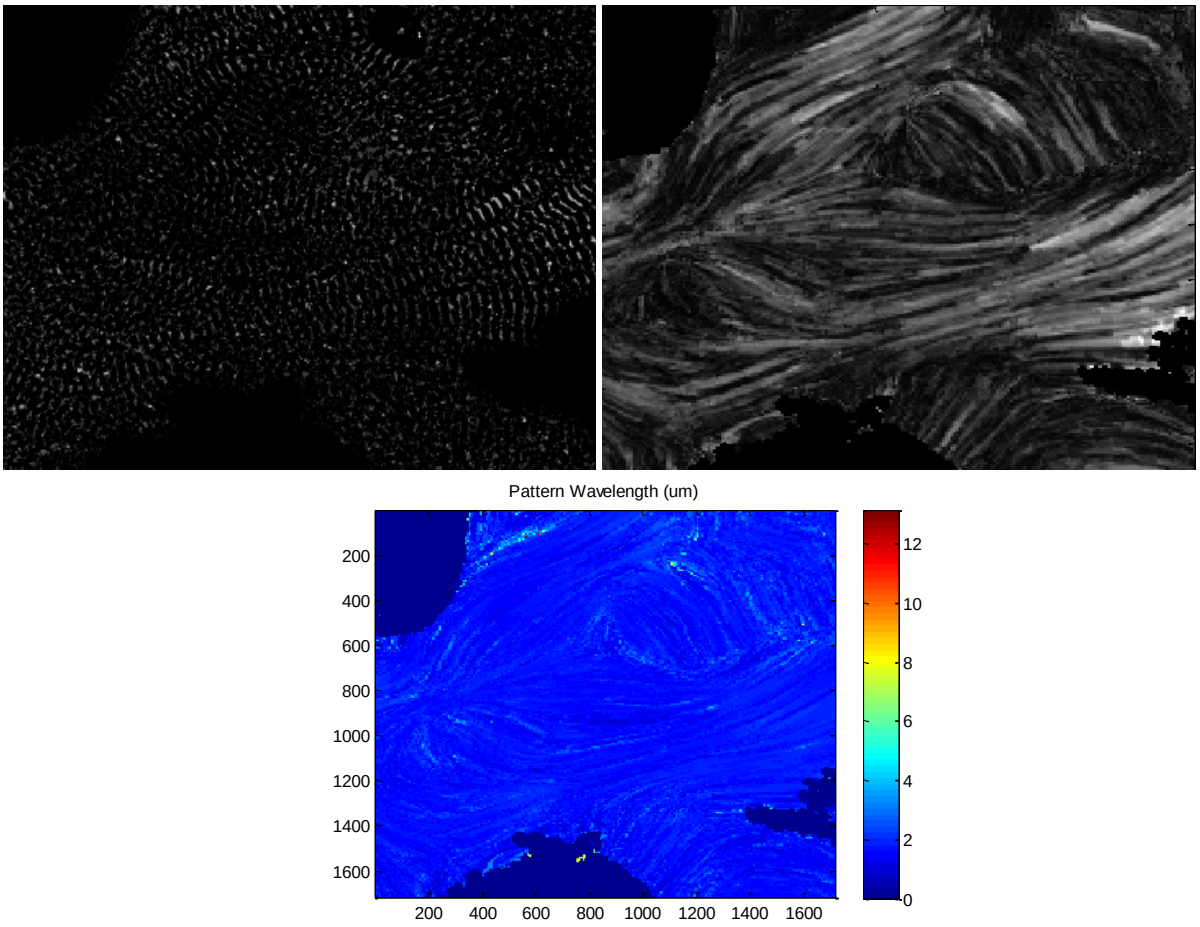
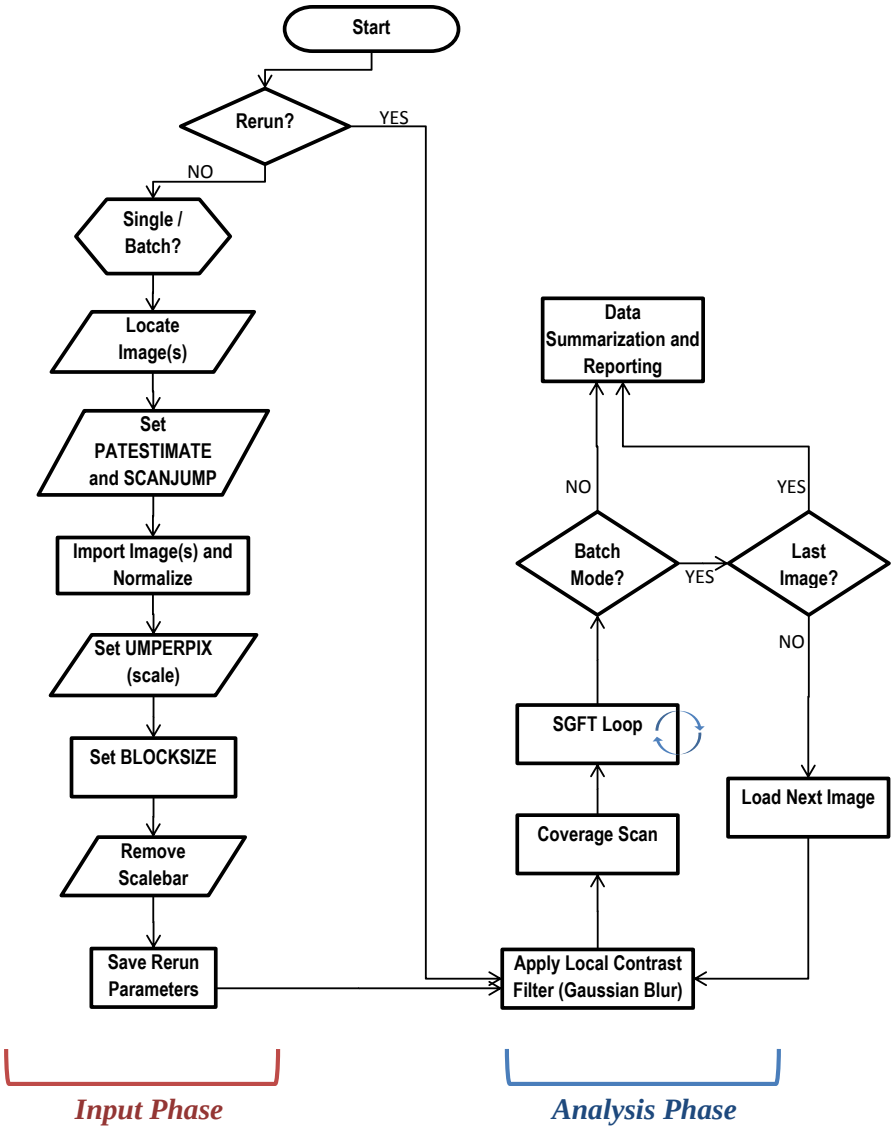


Thank you for downloading the SGFT program for MATLAB! The Scanning Gradient Fourier Transform (SGFT) program allows for quantification of striated patterns, such as those formed by sarcomere structures in myocytes. The software has been designed to automatically determine striation direction, wavelength (i.e. distance between stripes or sarcomere length), and uniformity in 2D images. The code has been designed to analyze either single images or large batches for a more high-throughput approach. Exported data includes an Excel file with the analyzed pattern strengths, alignments, and wavelengths, as well as parameters and a summary of the image's patterning. The complete, analyzed dataset is also output as a MATLAB data file.

A few things to note:

- This program requires both MATLAB and the MATLAB Image Analysis Toolbox to run. We've done our best to ensure that it functions properly on all versions of MATLAB, though cannot guarantee that obsolete functions will work properly on the latest releases.
- Since this code involves a large number of Fourier transforms to determine pattern strength, it can be computationally demanding. A modern desktop or laptop computer can handle most standard (~2000 X 2000) images; however, a faster computer or cluster may be recommended for large datasets. Reducing the SCANJUMP will also speed up analysis, at the cost of output resolution.
- Since several *in vitro* myocyte models have unaligned cells, the code does not depend on any prior assumption of directionality; however, outputs such as 'lanewidth' do assume vertical orientation of any lanes for micropattern-based myocyte models.



Getting Started:

1) Unzip all files into the same directory.
2) Open MATLAB and open the "sft_main_v17.m" file. This is the main calling function for the program.
3) Run the program with F5 and follow the prompts in the console. The following will describe each prompt:
    a. **Is this a [RERUN]?**
        This allows the user to re-run the last image that was analyzed, using the same parameters. This is useful for troubleshooting or if there was an interruption during the last analysis run.
    b. **Is this being run on a [SINGLE IMAGE] or a [BATCH OF IMAGES]?**
        For most beginning users, single images will first be analyzed. If the code is to be used for screening or other high-throughput purposes, batch-mode can be selected. This allows multiple images to be loaded at once, which will then be sequentially analyzed using the same parameters. Note: For batch analysis, images should be taken at the same magnification.
    c. **Please locate image. Image must be in TIF format.**
    d. **Please provide estimate of [PATTERN SIZE].**
        The size of each wavelength must be estimated in order to optimize the size of the scanning window. A ballpark estimate (within 60%-140% of actual size) will suffice. For example, in the case of sarcomere striation, the standard length in relaxed human heart tissue is ~1.9 microns. Thus, the user would type in 1.9 at this prompt.
    e. **Please provide a [SCANNING RESOLUTION].**
        The software 'skips' a number of pixels between each scan to hasten the analysis. For the most detailed analysis, a scanning resolution of "1" be used (but this is not recommended). At first, multiple values may be attempted, depending on the image size, number of images, computation speed, and time available for analysis. Once the analysis begins, you will get time predictions that will help you determine whether this value was properly set.
    f. **Scale must be set…**
        1 – Use scalebar in image to calibrate.
            You will be given the option to identify a scalebar in the image, then type in the value of the scalebar in order to calibrate the software.
        2 – Direct input of pixel/um ratio
            Some microscopes include pixel/um ratios in their metadata. If this value is known, it can be directly input by the user.
        3 – Set no scale
            For troubleshooting purposes only. Wavelength or pattern width outputs will be unitless.
        4 – Use last-used pixel/um ratio
            If the same microscope settings were used in all images, it is recommended to use this option.
    g. **The scalebar must next be removed from the image**
        Due to the shape of scalebars, they often provide a false positive region in images. This blurs out the scalebar so it does not affect analysis. To do this, outline the scalebar with multiple points. Next, right click on the points and select "FILL AREA." If there is no scalebar in the image, simply outline a small region that does not contain any sample. Not that in BATCH analysis mode, scalebars will not be removed. It is recommended that the user use images without scalebars if they are to be used in batch analysis.
4) The code will now scan through the image, quantifying the patterning of the entire image based on the given parameters.

5) Once completed, the data will be exported as an .xls file and .mat file, each containing the calculated pattern values for each scanned X and Y position, as well as parameters and data summaries for the analysis run. These files will be placed in the same folder as the original image file.

The user may also explore the analyzed data contained in the **`results`** variable, which contains **`results.params`**, **`results.summary`**, **`results.plots`**. The following is a description of each cell:

`results.data`	A large data matrix, with each row containing the analysis results for each individual scan. Each column represents the following, in this order: 1) X coordinate 2) Y coordinate 3) distance-from-edge(um) 4) pattern strength 5) pattern direction 6) pattern wavelength 7) specimen width 8) organization index(OI) 9) alignment index(AI) 10) combined myofibril index(CMI) *Note: this data is all contained in the 'data' page in the exported excel file*
`results.params.filename`	Original image file name
`results.params.pathname`	Folder location for original image
`results.params.umperpix`	Microns-per-pixel conversion
`results.params.scanjump`	Number of pixels jumped between individual scans
`results.params.blocksize`	Size of the subset matrix used for each individual scan
`results.summary.OI`	Organization index
`results.summary.AI`	Alignment index
`results.summary.CMI`	Combined myofibril index
`results.summary.superiorang`	The angle that the most high-strength patterns are pointed toward
`results.summary.sarcarea`	Percentage of the image that contains patterns that meet a set strength threshold
`results.summary.sl_ave`	Average pattern strength
`results.summary.sl_std`	Standard deviation of pattern strength
`results.summary.p20`	Percentage of detected patterns that are within 20 degrees of the superior angle (higher value indicates uniform directionality)
`results.summary.p15`	Percentage of detected patterns that are within 15 degrees of the superior angle (higher value indicates uniform directionality)
`results.summary.p10`	Percentage of detected patterns that are within 10 degrees of the superior angle (higher value indicates uniform directionality)
`results.plots.im`	Sample image
`results.plots.str`	Pattern strength matrix
`results.plots.dir`	Pattern direction matrix
`results.plots.quiv`	Pattern direction quiver plot
`results.plots.sl`	Pattern wavelength matrix
`results.plots.align`	Weighted histogram of pattern directions

Thank you for using this software, and good luck on all of your pattern analyses! For questions and feedback, please contact **maxsalick@gmail.com**.