We resize the input image to 512×512 pixels. Then, the standard Canny edge detection algorithm [1] is employed to outline the edges of stress fibers. Technically speaking, a Gaussian filter is applied to smoothing the image whilst reducing noise. Afterwards, image gradients are computed so that potential edges can be identified.

The detected edges are then processed using a Non-Maximum Suppression (NMS) technique [2] to eliminate false detections, followed by edge screening via a dual-threshold approach of Canny edge detection [1]. The dual thresholds are determined, based on statistical characteristics of image pixels. To be specific, the standard Sobel operator [3] is used to calculate horizontal and vertical gradients ( and ). The gradient magnitude G at each pixel, representing gradient intensity, is computed as:

$$G = \sqrt{G\_{x}^{2} + G\_{y}^{2}}$$

The mean (M) and standard deviation (S) of the gradient magnitudes are then calculated. The upper threshold () and the lower threshold () are determined as follows, where 𝒯 denotes the reduction ratio:

$$T\_{\max} = M + 2 \times S$$

$$T\_{\min} = \frac{T\_{\max}}{\mathcal{T}}$$

Next, the resized image is converted to the counterpart in the HSV color space. The center point of the blue region is identified based on a predefined HSV range. For each stress fiber edge, two parameters are computed:

1. The distance () from the center of the blue region to the edge.

2. The angle () between the edge and the horizontal axis (0° < Aⁱ < 180°).

For the images containing multiple blue regions, the average distance () from each stress fiber edge to all the blue centers is calculated. During this process, small blue regions and short stress fiber edges are filtered out.

Finally, spatial dispersion (\*dsp\*) is used to measure the degree of disorder. Each pair (, ) is treated as a point in the 2D space. The space is divided into \*n\* subspaces, and the probability \*p\* of points falling into each subspace is computed as the ratio of points in that specific subspace against the total number of the points. The entropy (\*etp\*) and normalized spatial dispersion (\*dsp\*) are derived as follows:

where etp is used to measure the uncertainty of the point distribution within spatial sub-regions, where p represents the proportion of the points in each sub-region relative to the total number of the points. n denotes the total number of the sub-regions. By dividing ETP by the maximum entropy (log₂n), the result is normalized to a scale of [0,1], facilitating the comparisons across different regions.

$$etp = \sum \left( -p \times \log(p) \right)$$

$$dsp = \frac{etp}{- \log\left(\frac{1}{n}\right)}$$

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This code is available at https://github.com/zhaoaite/ActinDetection.