Recognition of Linear Stress Fibers Based on Hough Transform

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OBJECTIVE: To present an algorithm based on Hough transform for recognition and extraction of linear stress fibers formed on exposure to lysophosphatidic acid (LPA).

STUDY DESIGN: A ridge set of head points with lower shoulders is calculated, followed by a thinning process shrinking long, narrow regions to regions of single pixel thickness, then converted into a rectangular map whose value is the number of regional points in the path of a straight line at the angle and intercept determined by two coordinates. The location of the maximum in the map is sought, and the corresponding line with an unlimited length is constructed from the paired coordinates. We removed the line before repeating the process for the next longest straight line, continuing until all lines with reasonable lengths are extracted.

RESULTS: Application of the algorithm to the stress fiber images of DOV13 cells stained with Texas red–phalloidin on LPA and AG1478 demonstrates close matches between stress fibers in the original images and linear lines.

CONCLUSION: An algorithm for recognition of linear stress fibers formed on exposure to LPA is described and applications to stress fiber images using DOV13 cells with Texas red–phalloidin staining are demonstrated. (Anal Quant Cytol Histol 2011;33:121–131)

Keywords: cell invasion, cell migration, image segmentation and recognition, peripheral actin microspike, stress fiber.

Stress fibers are a specific cytoskeletal organization of actin monomers. These fibers are involved in cell shape and structural functions of the cell. Actin monomers form long polymers that attach to the plasma membrane at focal adhesions. Formation of stress fibers and focal adhesion complexes are a key regulatory event in cell growth and movement, including migration and invasion. Contraction of actin is an important part of controlling morphogenesis fibers by allowing the cells to exert tension on the substratum. One traditional way for quantifying stress fiber formation is the examination of the percentage of cells with stress fiber formation in a given field. This method is fine when stress fiber change is dramatic, such as from none to apparent or from apparent to none in cells, although it requires cell counting and arbitrarily defining cells to...
be with or without stress fiber formation. However, when the cells undergo subtle changes in the extent of stress fiber formation, only representative pictures can show qualitative differences. Generally, the perpendicular orientation of stress fibers relieves the increase in tension generated by stretch, resulting in a more stable configuration for the actin cytoskeleton. Petroll et al applied Fourier transform, which produced an elliptical pattern in the magnitude spectrum with the major axis at 90 degrees to the long axis of the wound in the original image. Sato et al used an ellipse to fit to the binary cell outline and calculated the angle between the major axis of the ellipse and the direction of strain. Unlike the scarring fibrosis that may have variant width and curves, stress fibers appear linear with relatively uniform thickness. In this paper, we describe an algorithm based on Hough transform to extract the individual stress fibers so that measurement can be performed on individual fiber basis, which may provide better understanding in fiber formation, such as the length, angle, and distributions of the stress fibers.

Materials and Methods

For immunostaining of stress fibers, DOV13 cells were cultured on glass coverslips and fixed in 4% paraformaldehyde in 20 mM of HEPES (pH 7.4) and 150 mM of NaCl for 20 minutes, permeabilized in 0.1% Triton X-100 in phosphate-buffered saline for 10 minutes, blocked with 1% bovine serum albumin/phosphate-buffered saline for 1 hour, and then incubated at room temperature for 1 hour with Texas red–phalloidin (Molecular Probes; Invitrogen Corporation, Carlsbad, California, U.S.A.) at a 1:100 dilution in blocking solution. Images were acquired on a Nikon Eclipse TE2000-U microscope (Nikon Corporation, Tokyo, Japan) using the SPOT digital camera (SPOT Imaging Solutions, Sterling Heights, Michigan, U.S.A.) and MetaMorph 5.0 software (Molecular Devices, Inc., Sunnyvale, California, U.S.A.).

The stress fiber has a periodic sarcomeric structure similar to that of muscle fibers, with myosin motor proteins exerting contractile force by pulling on actin filaments. On stimulation, the actin filament lined up parallel to the direction of cell movement. Thus the number of lines (F-actin bundles) may represent the extent of stress fiber formation. The stress fibers appear as straight lines with relatively uniform thickness. Therefore a single fiber can be represented and determined by the two end points of the line. Although an ideal line has an almost zero thickness in continuous signal, we assume the line thickness as 1 pixel width in the discrete images.

Figure 1A is a typical image of DOV13 cells, an ovarian cancer cell line, immunostained with phalloidin and showing the stress fibers. Let the stress...
fiber image of size $N_1 \times N_2$ be represented by a 2-D discrete sequence $x(n_1,n_2)$, for $n_1 = 0,1,\cdots,N_1 -1$, and $n_2 = 0,1,\cdots,N_2 -1$. To reduce the effect of image noise, we use a low-pass filter with the finite-impulse response given by $h_w(n_1,n_2) = (4 - (n_1 + n_2))/W$ for $-2 \leq n_1 \leq 2$ and $-2 \leq n_2 \leq 2$, 0 otherwise, where the normalization factor $W = \sum_{n_1=-2}^{2} \sum_{n_2=-2}^{2} h_w(n_1,n_2)$. The filtered image, $x_w(n_1,n_2) = h_w(n_1,n_2) * x(n_1,n_2)$, for $n_1 = 0,1,\cdots,N_1 -1$ and $n_2 = 0,1,\cdots,N_2 -1$, is smoother than the original image.

We call the point at $(n_1,n_2)$ a horizontal head point in $x_w(n_1,n_2)$, if $x_w(n_1,n_2)$ is higher than its two shoulders or immediate neighbors on both sides at $(n_1-1,n_2)$ and $(n_1+1,n_2)$. The set of horizontal head points is $G_h = \{(n_1,n_2) | x_w(n_1,n_2) > x_w(n_1-1,n_2) \text{ and } x_w(n_1+1,n_2) \}$. The sets of the head points in the other three directions are $G_v = \{(n_1,n_2) | x_w(n_1,n_2) > x_w(n_1,n_2-1) \text{ and } x_w(n_1,n_2+1) \}$, $G_{h-v} = \{(n_1,n_2) | x_w(n_1,n_2) > x_w(n_1-1,n_2-1) \text{ and } x_w(n_1-1,n_2+1) \}$, and $G_{v-h} = \{(n_1,n_2) | x_w(n_1,n_2) > x_w(n_1+1,n_2-1) \text{ and } x_w(n_1+1,n_2+1) \}$. Because the lines are generally brighter than their immediate surroundings, intensities below a significant low level are excluded. We define $G_T = \{(n_1,n_2) | x_w(n_1,n_2) \geq T_g \}$, where $T_g$ is a low-intensity value such as $\leq 10$. We selected $T_g$ as 10 so that those pixels in the smoothed image with intensities $\leq 10$ are preexcluded for faster computation. The set of the head points can be written as $G = (G_h \cup G_v \cup G_{h-v} \cup G_{v-h}) \cap G_T$.

**Figure 1** (A) Original stress fiber image of DOV13 cells with Texas red–phalloidin staining, $256 \times 256$ pixels, metric size $58.086 \times 58.086$ μm$^2$. (B) Thresholding at intensity of 100. (C) $G$ set. (D) Ridge set, $E$. (E) Skeletonized and spur-removed. (F) Linear stress fiber lines, white for bright fibers and gray for dimmer ones.
homogenous brightness and existence of gray value thresholds.\(^{14,15}\) The original image in Figure 1A shows a high variation in background intensity. It has significantly lower average intensities at the lower, right area of the image than in the rest. In the thresholding method, the intensity variation in the image background is equivalent to the brightness inconsistency. Comparison of the two images in Figure 1A and B demonstrates the different effects of the inconsistent image brightness in the two algorithms. Set \(G\) in Figure 1C shows robust segmentation regardless of the variations in background brightness. The poor result shown in Figure 1B is from the thresholding method, which has missed most of the dimmer lines and oversegmented the brighter background regions.

It is expected that set \(G\) should include the line pixels because the pixels along the center of a line are usually higher in intensity than their neighbors in lines perpendicular to the stress fiber line. Assuming a thickness of 1 pixel size, further refining is needed to shrink set \(G\) to reduce the width of lines. Let the difference between the head and shoulders in horizontal direction be measured by \(e_h(n_1,n_2)=\frac{1}{2}((x_{w,h}(n_1,n_2)-x_{w,h}(n_1-2,n_2))+ (x_{w,h}(n_1,n_2)-x_{w,h}(n_1+2,n_2)))\),

where \(x_{w,h}(n_1,n_2)=\frac{1}{2}\sum_{k=2}^{5} x_{w}(n_1,k,n_2)\) and \((n_1,n_2) \in G\).

Similarly, \(e_v(n_1,n_2)=\frac{1}{2}((x_{w,v}(n_1,n_2)-x_{w,v}(n_1,n_2-2))+ (x_{w,v}(n_1,n_2)-x_{w,v}(n_1,n_2+2)))\),

where \(x_{w,v}(n_1,n_2)=\frac{1}{2}\sum_{k=2}^{5} x_{w}(n_1,n_2,k)\) and \((n_1,n_2) \in G\). The differences of head and shoulders in the other two directions are measured by \(e_{h,v}(n_1,n_2)=\frac{1}{2}((x_{w,h,v}(n_1,n_2)-x_{w,h,v}(n_1-1,n_2-1))+(x_{w,h,v}(n_1,n_2)-x_{w,h,v}(n_1+1,n_2+1)))\) and \(e_{v,h}(n_1,n_2)=\frac{1}{2}((x_{w,v,h}(n_1,n_2)-x_{w,v,h}(n_1-1,n_2+1))+(x_{w,v,h}(n_1,n_2)-x_{w,v,h}(n_1+1,n_2-1)))\),

where \(x_{w,h,v}(n_1,n_2)=\frac{1}{2}\sum_{k=2}^{5} x_{w}(n_1,k,n_2)\) and \((n_1,n_2) \in G\). If the current point at the location \((n_1,n_2)\) is on a bright line, we expect one of the differences of the head and shoulders to be significantly higher than the rest. We defined the head difference at \((n_1,n_2)\) to be the largest of the four measurements in the four directions such as \(e_{h}(n_1,n_2)=\max(e_h(n_1,n_2), e_v(n_1,n_2), e_{h,v}(n_1,n_2), e_{v,h}(n_1,n_2))\). If \(e_{h}(n_1,n_2)\) is large enough, we name it as a ridge point because the point belongs to a raised and narrow strip resembling to a segment of ridge. The accumulation of all the ridge points forms a set of the ridge points. Thus we define a ridge set \(E\) as \(E = \{(n_1,n_2) | e(n_1,n_2) \geq T_e\}, \quad \{n_1,n_2) \in G\}\), where \(T_e\) is a given constant. Figure 1D is the corresponding ridge set extracted from the original image in Figure 1A with \(T_e = 1.5\). The ridge set \(E\) contains the stress fiber points, and its regions appear long and narrow, similar to lines. However, the narrow and long regions in \(E\) may still be much wider than the single point width of discrete lines. To reduce the width of the narrow regions, we use the morphologic procedures to shrink the line regions. A morphologic skeletonization procedure is used and followed by removal of the remaining spurs. In this study, the algorithm is implemented in the Matlab (The MathWorks, Inc., Natick, Massachusetts, U.S.A.) environment. The skeletonization is implemented by the procedure of \textit{bwultramorph} (*skel*,Inf), which removes repetitively the pixels on the boundaries of objects without allowing objects to break apart. The spur removal is implemented by the procedure of \textit{bwultramorph}(*spur*,3), which repeats three times the removal of the line end points. Figure 1E shows the ridge lines after the thinning process. Note that lines are thinned too close to 1 pixel width except at some joint locations.

Let \(R\) be the set of pixels in the ridge lines thinned from the ridge set \(E\). We define the corresponding binary ridge line image as \(r(n_1,n_2)=\begin{cases} 1, & (n_1,n_2) \in R; \\ 0, & \text{otherwise.} \end{cases}\)

Suppose that the set \(R\) has a total of \(L\) separate regions or objects where each region is an 8-connected object that is 8-disconnected from any other objects in the set. If we process one object at a time, a loop of \(L\) iterations will complete the whole image. Let \(r_{l}(n_1,n_2)\) be the image of the \(l\)-th object such as \(r_{l}(n_1,n_2) = r(n_1,n_2)\) if the point \((n_1,n_2)\) belongs to the \(l\)-th object and otherwise \(r_{l}(n_1,n_2) = 0\). For example, Figure 2A shows one such object where the object pixels are in black and the rest are in white. The reversed displays in intensity in Figure 2 enhance clarity in the tiny area of the object regions.

There is only a limited number of nonzero points in \(r_{l}(n_1,n_2)\). We can find the smallest rectangle of size \(M_1 \times M_2\) that can contain the whole object by finding the outermost points in the four directions. In other words, there is at least one nonzero point in each of the rectangle borders and no nonzero points are located outside the rectangle. For easier computations in the subsequent processing, we purposely add a narrow strip, for example, a width of 2 pixels,
of zeros to each border of the rectangle. The new, larger rectangle will be of size $M_1 \times M_2 = (M_1'+4) \times (M_2'+4)$, as shown in Figure 2A, where the strips are the small white gaps between the object and the image borders.

The $l$-th object in the rectangle of size $M_1 \times M_2$ may contain multiple straight lines in contact because of the vicinities between any two neighboring

\[ R_k, S_k, \text{ and } A_k \text{ are in the } k\text{-th row, from left to right, respectively, for } k = 1, 2, \ldots, 5. \]

After each iteration, one line $S_k$ shown in the middle, is removed from the set $R_k$ on the left and is added to the set $A_k$ on the right.
lines. Our procedure is to extract one line at a time. Thus a subloop procedure is needed. Because the lines may have different lengths, the order is to extract the longest line from the remains of the object in each loop.

The binary \( r_l(n_1,n_2) \) is nonzero only inside a smaller rectangle; we use an axis translation so that the new image is nonzero only in the rectangle where \( 0 \leq n_1 < M_1 \) and \( 0 \leq n_2 < M_2 \), and without loss of clarification, we still use \( r_l(n_1,n_2) \) to represent the translated image. We use the Hough transform\(^{11-13}\) to map the image into the parameter space of angle and intercept of lines. The purpose is to find the long straight lines in the image; therefore we create an image map as in Hough transform, \( y \), as the discrete image of gray levels whose intensity is the number of nonzero points in \( r_l(n_1,n_2) \), for \( 0 \leq n_1 < M_1 \) and \( 0 \leq n_2 < M_2 \), in the path along a straight line of unlimited length in particular angles.

Let \( \Delta \alpha \) be a small angle increment. We used 1 degree for the angle increment or \( \Delta \alpha = \frac{\pi}{180} \). In a rectangle of size \( M_1 \times M_2 \), any straight line cannot exceed \( \sqrt{M_1^2 + M_2^2} + 1 \) in length. Thus we use a matrix of size \( L_1 \times L_2 = 180 \times (\sqrt{M_1^2 + M_2^2} + 1) \) to contain the map image, \( y \), that is computed as the following:

\[
\text{for } l_1 = 0 \text{ to } L_1-1 \text{ do }
\{ \\
\quad \text{for } n_1 = 0 \text{ to } M_1-1 \text{ do } \\
\quad \quad \text{for } n_2 = 0 \text{ to } M_2-1 \text{ do } \\
\quad \quad \quad \text{if } r_l(n_1,n_2) \neq 0 \\
\quad \quad \quad \quad \quad l_2 = \text{round} \left( c_2 + (n_1-C_1) \sin(l_1 \Delta \alpha) + (n_2-C_2) \cos(l_1 \Delta \alpha) \right); \\
\quad \quad \quad \quad \quad y(l_1,l_2) = y(l_1,l_2) + 1; \\
\quad \quad \}
\]

where \( C_1 = \frac{M_1}{2}, C_2 = \frac{M_2}{2}, c_2 = \frac{L_2}{2} \), the function \( \text{round}(\bullet) \) rounds the variable to its closest integer and pixels in \( y \) are at first initialized to zeros as \( y(l_1,l_2) = 0 \) for

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**Figure 3**  Top Row: the normalized map images in the first 5 iterations, \( y_k(l_1,l_2) \) for \( k = 1,2,\ldots,5, l_1 = 0,1 \ldots, L_1-1, \) and \( l_2 = 0,1,\ldots, L_2-1, \) displayed inversely in intensity, derived from the 5 binary images in the left column in Figure 2, respectively. The weighted row index, \( l_1 \Delta \alpha \) or \( \frac{l_1 \pi}{180} \) (\( l_1 = 0 \) for top row and \( l_1 = -1 \) for bottom row), represents the line orientation and the column index, \( l_2 \), determines the axis intercept. 'Bottom Row: the same as the images immediately above them, except the added dark and gray dots showing the locations of the high local maxima of the images. In the \( k \)-th iteration, the single dark dot, corresponding to the location of the global maximum in the map image, determines a straight line overlapping the longest straight-line segment in \( R_k \).
The atlas is in left of the upper row in Figure 3A shows the normalized map image, \( y(l_1,l_2) \) for \( 0 \leq l_1 < L_1 \) and \( 0 \leq l_2 < L_2 \), where the axis \( l_1 \) points downward from top to bottom and the other axis \( l_2 \) points to the right. The intensity is normalized with the highest intensity of 255 and the lowest 0 for the best contrast without distortions. As seen in Figure 2, the image intensities are displayed inversely for better representing that darker intensity means higher image value.

Because the value of \( y(l_1,l_2) \) corresponds to the number of pixels in the path of a particular line, larger value may mean a longer line. If the image has a local peak at the location of \( (l_1,l_2) \), that means \( y(l_1,l_2) \) is the highest in a small neighborhood around the location of \( (l_1,l_2) \), and we expect that \( r_1(n_1,n_2) \) has a line of length of \( y(l_1,l_2) \) in the orientation and intercept determined by the indices \( l_1 \) and \( l_2 \), respectively. If \( y(l_1,l_2) \) is a local maximum and is large enough, we determine that a line exists. Frequently, \( y(l_1,l_2) \) may contain multiple locations with high local maxima as indicated by the black and gray dots in the map images in the lower row in Figure 3. Among the multiple peaks, we seek the highest corresponding to the longest line. If \( (l_1,l_2) \) is the location at the highest peak, the distance of the location in the image of \( r_1(n_1,n_2) \) to the line, determined by \( l_1 \) and \( l_2 \), is \( \rho(n_1,n_2) = (n_1 - c_1) \sin(l_1 \Delta \alpha) + (n_2 - c_2) \cos(l_1 \Delta \alpha) - (l_2 - c_2) \). The line length is \( \Xi_1 = \{ (n_1,n_2) \ | \ \rho(n_1,n_2) < 0.5 \} \), a set of pixels that are half-pixel width or less in distance to the line determined by \( l_1 \) and \( l_2 \). The line segment is obtained as \( S_1 = \text{dilate}(\Xi_1, B) \cap R_k \), where \( R_k = \{ (n_1,n_2) \ | \ r_1(n_1,n_2) = 1 \} \) and \( B \) is a small structure element such as a square of size 5. Set \( S_1 \) may have multiple isolated 8-connected regions. Retain the largest 8-connected region as \( S_1 \) and discard the rest. The image in the middle of the top row in Figure 2 shows the line segment \( S_1 \) from the location of the highest peak indicated by the dark dot in map in the left of the lower row in Figure 3 and the ridge line set \( R_1 \) to its left in Figure 2. After the longest line is removed, the remaining portion of the ridge object becomes \( R_k = \text{xor}(R_{k-1}, S_1) \), as shown in second row in Figure 2. In the \((k)\)-th loop, we update

1. \( R_k = \text{xor}(R_{k-1}, S_1) \);
2. \( y_2(l_1,l_2) = | \{ (n_1,n_2) \ | \ (l_1, \text{round}(c_2 + (n_1 - c_1) \sin(l_1 \Delta \alpha) + (n_2 - c_2) \cos(l_1 \Delta \alpha))) \in R_k \} |, \) for \( l_1 = 0,1,2,\cdots, (L_1 - 1) \) and \( l_2 = 0,1,2,\cdots, (L_2 - 1) \);
3. \( (l_1,l_2) = \max_{(m_1,m_2)} y_2(m_1,m_2) \) \( 0 \leq m_1 < L_1 \) and \( 0 \leq m_2 < L_2 \);
4. \( \Xi_k = \{ (n_1,n_2) \ | \ (n_1 - c_1) \sin(l_1 \Delta \alpha) + (n_2 - c_2) \cos(l_1 \Delta \alpha) - (l_2 - c_2) \leq 0.5 \} \);
5. \( \tilde{S}_k = \text{dilate}(\Xi_k, B) \cap R_k \);
6. \( S_k \subseteq \tilde{S}_k \), the largest object in \( \tilde{S}_k \);
7. Measure \( d_{k,v} \), the length of the line segment, computed as the distance between the two end points of the object and \( \theta_{k,v} \) the orientation of the line valued by \( l_1 \Delta \alpha \);
8. \( A_k = A_{k-1} \cap S_k \);

where \( A_k \) is the accumulation of all previously extracted line segments, the initial states \( A_0 = \phi \), an empty set, and \( R_k = \{ (n_1,n_2) \ | \ r_1(n_1,n_2) = 1 \} \).

Figure 2 shows the sets \( R_{k,v} \), \( S_{k,v} \), and \( A_{k,v} \) for \( k = 1,2,\cdots,K \) in the first five iterations for a single isolated region of \( R_1 = \{ (n_1,n_2) \ | \ r_1(n_1,n_2) = 1 \} \). The sets of \( R_{k,v} \), \( S_{k,v} \), and \( A_k \) are displayed in the \( k \)-th row, from left to right, respectively. After each iteration, one line, \( S_{k,v} \), shown in the middle, is removed from the set \( R_k \) on the left, and then added to the set \( A_k \) on the right.

The \( k \)-th image in the upper row in Figure 3 shows the normalized map in which the value of \( l_1 \Delta \alpha \), the weighted vertical axis displayed inversely from top to bottom, stands for the angle of the line, the other axis \( l_2 \) for the axis-intercept, and the intensity, \( y_1(1,l_2) \), at \( (l_1,l_2) \) is inversely proportional to the length of the line determined by \( l_1 \Delta \alpha \) and intercept \( l_2 \). The images on the second row in Figure 3 show the maps with dark dots for the locations of the high local maxima of the images. When the highest value of \( y_2(m_1,m_2) \) \( 0 \leq m_1 < L_1 \) and \( 0 \leq m_2 < L_2 \), with the increasing number of iteration \( k \), becomes smaller than a given number such as 10, the iterations can then be terminated.

The procedure yields the final set, \( A_K \), the accumulated set of the line segments, after a total of \( K \) iterations. Information obtained about the individual lines also includes the length of each line, \( d_{k,v} \) for \( k = 1,2,\cdots,K \) and the angle of each line, \( \theta_{k,v} \) for \( k = 1,2,\cdots,K \).

The procedure extracts \( K \) line segments from the \( l \)-th object, one of the \( L \) objects in the set of the ridge lines. Repetitive applications of the above procedure on all the \( L \) objects will produce the set of all extracted line segments along with their orientations and lengths. Figure 1F shows the complete set of line segments that are grouped into two categories: white lines for the bright fibers and gray ones for the others.
Results

Figure 1A shows one stress fiber image from vehicle-treated DOV13 cells with Texas red–phalloidin staining. The digital image is of 256 × 256 pixels or 58.086 × 58.086 μm² in actual size. Figure 1F shows the recognized linear stress fibers; the white straight lines represent bright stress fibers and gray lines indicate dimmer ones. Ideal lines should be almost zero in thickness. To display the lines in discrete images, we let the lines be 1 pixel thick so that the lines, in minimal width, are not visually broken. Each line, determined by its two end points, gives

Figure 4  (A) Image of DOV13 cells stained with phalloidin on LPA and AG1478 treatment, discrete size 512 × 512 pixels and metric size 116.173 × 116.173 μm². (B) Ridge set. (C) Ridge set morphologically skeletonized and spur-removed. (D) Extracted linear stress fiber lines, white for bright fibers and gray for dimmer ones.
information about its length, its orientation, and the average intensity of the stress fiber.

Compared with the region or thresholding-based methods, the proposed algorithm has two major advantages. One advantage is its high robustness to the uneven brightness in the original images. Whereas the intensity-sensitive thresholding-based method produces the poor segmentation in Figure 1B, the proposed algorithm can yield the highly correlated and desired segmentation in Figure 1C. Another advantage of the algorithm is the straightforward interpretation of the results that provide the

Figure 5  (a) Stress fiber image of DOV13 cells stained with phalloidin on LPA and AG1478, size 512 × 512 pixels, metric size 116.173 × 116.173 μm². (b) Stress fiber image from vehicle-treated DOV13 cells with Texas red-phalloidin staining, the same size as that in (a), (a’) extracted linear stress fiber lines from image in (a), (b’) extracted linear stress fiber lines from image in (b).
Figure 4 shows an application of the proposed algorithm to a discrete image of DOV13 cells stained with palloidin on lysophosphatidic acid (LPA) and AG1478 (an EGF receptor inhibitor) treatment. The discrete size is $512 \times 512$ pixels and actual size is $116.173 \times 116.173 \, \mu m^2$. Figure 4B and C show the ridge set and the ridge lines after being morphologically skeletonized and having the spurs removed, respectively. Figure 4D shows the extracted linear stress fibers; the bright fibers are indicated by white lines while the dimmer ones by the gray lines. Comparing the stress fibers in Figure 4A and the extracted fiber lines in 4B, we can observe the close matches between the fibers and their representative lines.

Figure 5 shows the applications applied to two additional original images: (A) stress fiber image of stained DOV13 cells treated with LPA and AG1478 and (B) stress fiber image of DOV13 cells treated with vehicle control. Both discrete images are $512 \times 512$ pixels and metric size $116.173 \times 116.173 \, \mu m^2$. Figure 5a’ and b’ show the results of extracted fiber lines by the proposed algorithm. Both results show the matches between the fibers in the original images and the lines extracted.

Figure 6 shows the histograms of the lengths vs. the line angles for both bright and dim fibers in the four sample images. The left column is for the bright fibers, and the right column is for dim fibers. The four rows, from top to bottom, show histograms of fibers in Figures 1A, 4A, 5a, and 5b, respectively. In each chart, the horizontal axis is for the line angle, ranging from 0 to $\pi$. The horizontal domain of $(0, \pi)$ is sampled into 40 points with a sampling period of $\frac{\pi}{40}$. The vertical axis is for the accumulated line length that is calculated as the sum of the lengths of all lines whose angles fall into the same sampling period.

We present an algorithm for recognition of linear stress fibers formed on exposure to LPA. An initial procedure gathers the ridge elements that have lower immediate neighboring intensities on both sides along the respective four evenly spaced directions to form a ridge set that is subsequently thinned into single pixel depth by the binary morphologic operations. For each isolated region in the thinned ridge set, a rectangular map is generated as
the number of pixels in the path of the straight lines whose angle and intercept are determined by the two coordinates in the map. The highest value in the map corresponds to the longest straight line in the region. We search for the location of the maximum in the map and construct the line from the paired coordinates of the location. The longest straight line is found by intersecting the constructed line and the dilated region. After the longest line is extracted and removed from the set, the same procedure is repeated for the next longest line in the set. This process continues until all the lines longer than a given length are exhausted. After each region undergoes the same process, we obtain all of the extracted linear lines. This algorithm has been applied to a number of stress fiber images of DOV13 cells with Texas red–phalloidin staining and also those treated with LPA and AG1478. Experimental results show the close matches between the fibers in the original images and the resulting linear lines.

References