Identifying Weak Linear Features with the “Coalescing Shortest Path Image Transform”

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Abstract: The detection of line-like features in images finds many applications in microanalysis. Actin fibers, microtubules, neurites, pilis, DNA, and other biological structures all come up as tenuous curved lines in microscopy images. A reliable tracing method that preserves the integrity and details of these structures is particularly important for quantitative analyses. We have developed a new image transform called the “Coalescing Shortest Path Image Transform” with very encouraging properties. Our scheme efficiently combines information from an extensive collection of shortest paths in the image to delineate even very weak linear features.

Key words: shortest paths, image transform, linear feature, neurite tracing, membrane tracing, image analysis, image processing

INTRODUCTION

In addition to the biological examples outlined in the abstract, there are many other examples of line-like features, such as fine cracks in materials, grain boundaries, roads, tracks and rivers in landscapes, and hairs and wrinkles. Accordingly, a lot of attention has been devoted to the topic of automated linear feature detection (Lindeberg, 1998). In this contribution, we introduce a new image transform that shows great promise for identifying linear features displaying very low contrast.

Our new transform is based on finding minimum cost (or shortest) paths in a network, which can be done efficiently using Dijkstra’s method (Dijkstra, 1959; Mehlhorn & Näher, 1999). As an example, Figure 1A shows a simple network with a minimum cost (or shortest) path indicated in bold between nodes S and T. A raster image can naturally be represented as a network by associating a node with each pixel and by introducing edges to capture adjacency information as shown in Figure 1B. A good choice for edge costs is the product of their Euclidean length by the average image intensity of the pixels at their end points. One is then able to trace dark linear features by computing minimum cost paths between two pixels. Commonly, these distal pixels are interactively selected along linear features in a methodology that has become known as “livewire” (Poon et al., 2008).

MATERIALS AND METHODS

Graph Structure and Cost Refinement

Intuitively, the more edges that are included in the network, the more accurate the traces obtained for linear features. We

therefore have included in our network not only links to direct neighbors, but also to neighbors of neighbors as indicated by the two dashed edges in Figure 1B. Furthermore, following Meijering et al. (2004), we have augmented the intensity and distance cues embedded in the edge costs with additional information. It is well appreciated that the eigenvector associated with the largest eigenvalue of the Hessian matrix of second-order image derivatives is directed parallel to linear features. Therefore, we include as a term in the edge cost the scalar product $|\vec{v} \cdot \vec{n}|$, where $\vec{v}$ is the displacement vector corresponding to an edge, and $\vec{n}$ is the normalized Hessian eigenvector. Including this term helps steer the minimum cost path along linear features as it favors motion in their direction (see Fig. 1C, which also illustrates how second-nearest neighbors increase the angular resolution). Including this term also improves path smoothness by implicitly exploiting the underlying smoothness of the eigenvector field.

Shortest Paths Generation

In this contribution, we are specifically interested in the full automation of the tracing process even for weak linear features, and we want to avoid any manual intervention. A few software solutions exist that are able to do that (Sun & Vallotton, 2009), but they often fail to accurately trace the very weak linear features.

We observed that if one selects a pixel at random as an attractor site and one examines many shortest paths toward it, launched from arbitrary origins (in practice, we use local intensity maxima as origins) in the image, even weak linear features could be detected: shortest paths are seen “coalescing” onto the latter as they approach the attractor (see Fig. 1E; the origins correspond to the leaves of the tree rooted at the attractor). Moreover, the position of the traces tended to be quite robust against the exact location of the

Figure 1. **A:** Dijkstra's algorithm identifies shortest paths in costed networks very rapidly. Here, the shortest path from node S to node T is indicated in bold. **B:** Images can naturally be associated with costed networks. Linear features in images can be identified by finding shortest paths in such networks, where the cost is a function of Euclidean distance, image intensity, and additional geometric cues such as the alignment between candidate graph edges with the local direction $\vec{v}$ of linear features as determined using the Hessian matrix. **C:** Example of linear feature configuration where second nearest neighbours allows to better estimate the quality of traces: without the edge a-c, the edges a-b and b-c would have to be visited successively, both of which are poorly aligned with the true direction of the linear feature. Thus, the path quality would be underestimated as a result of discretisation effects. **D:** To ensure approximate translational invariance for our transform, the image is treated as a doughnut by connecting its opposite sides. **E:** Coalescence in action: a single attractor in yellow is located at the center of the image and a subset of shortest paths are seen coalescing onto linear features. We use local intensity maxima as paths origins. The essence of our method is to combine information obtained for all attractors on a grid indicated with yellow markers. **F:** The level sets of the geodesic distance from an attractor in red are overlaid on the original image. Observe that, thanks to periodic boundary conditions, level sets are continuous across the image limits shown by black lines. **G:** Neuronal cells with neurites presenting very dim contrast—not unusual for this type of preparation (courtesy Jenny Gunnersen, Howard Florey Institute, Melbourne, Australia). **H:** Our algorithm is able to highlight continuous traces even for weakly contrasted neurites (512×512 pixels). **I:** Immunostained cell membranes under fluorescent confocal microscopy (courtesy David James, Garvan Institute, Sydney, Australia). The closed contour in red was correctly traced by relying on our algorithm as a preprocessing step, followed by circular shortest path extraction (Vallotton et al., 2010). Two neighboring cells were considerably more contrasted than the membrane of interest and the contour would be pulled away from the true contour were it not for our new transform (600×600 pixels).

selected shortest path origins [“livewire” magic (Falcao et al., 2000)]. This suggests that it should be possible to obtain most linear features by sampling from a collection of shortest paths. Unfortunately, linear features that run perpendicular to the axis between a particular origin and the attractor will not be traced by shortest paths (traveling perpendicular to the direction of one’s destination is not the quickest way to get there). Therefore, multiple attractors have to be used to ensure that, on average, the transversality condition just described occurs only rarely. For example, a set of 3×3 attractors regularly arrayed as shown by the yellow markers in Figure 1E represents a suitable selection. In practice, little improvement is obtained by increasing the number of attractors beyond 3×3 , so we use this number systematically. The choice of such a regular array also avoids biasing the sensitivity of the transform toward particular linear features in the image.

Imposing Periodic Boundary Conditions

Another issue related to avoiding bias effects is that close to the image boundaries, weak features may be missed because shortest paths have no opportunities to form and coalesce prior to reaching these regions. Translational invariance is a fundamental requirement in image processing and to help guarantee it, we introduce additional edges in the network, linking nodes representing pixels at the boundary of opposite sides of the image (thus creating a doughnut, see Fig. 1D). In this topology, every pixel is equivalent, and the geodesic distance from a point close to the image boundary is correctly seen to propagate continuously across the image limits as apparent in Figure 1F. Results in Figure 1G,H demonstrate that weak linear features close to the image border can also be detected. Periodic boundary conditions may exceptionally induce spurious traces at one boundary of the image due to the presence of a strong signal at the opposite boundary. This was observed only for images where the subject extends to the very image boundaries. One can easily guard against such effects by padding the image with thin (e.g., 30 pixels width) strips obtained by reflection, prior to creating the doughnut.

Combining Information from All Shortest Paths

It remains to describe how the information contained in all the sample shortest paths is combined for all origins and all attractors. We use an accumulator matrix of the same size as the original image, and we count how many times each pixel in the image is visited by the shortest paths. Very often, paths from different origins were seen to rapidly coalesce along the linear features in a process analogous to rain pouring on a landscape and carving trenches. This had the effect of greatly amplifying the signal of even weak linear features.

For improved results, the final sections of the shortest paths (e.g., within 40 pixels from the attractors) were ignored from the accumulation phase for the reason that they depend on the particular position of the attractor. While the

respective zones for that particular attractor are excluded from the analysis, they are included by all other attractors, such that there is effectively no loss of relevant traces. Similarly, the accumulation did not include pixels further than 150 pixels from the attractors. The value of these parameters is not critical, but they should be scaled proportionally in case one is dealing with images much larger than 512×512 pixels. In that sense, our transform offers only few parameters and they are easy to tune—a very desirable property from a user point of view. The output of the coalescing transform is the accumulated image itself (see Fig. 1H).

We point out that while our approach is reminiscent of the watershed transform (Yan et al., 2006) and of the image foresting transform (Falcao et al., 2004) (and probably of several other transforms, including the Hough Transform), the computational process is clearly distinct: combining stochastic elements, geodesic concepts, and an *ad hoc* mechanism for signal amplification. We note also that the idea of aggregating information from an ensemble of nonlinear transformations applied to subsets of image data has interesting connections to the statistical theory of model averaging (Hastie et al., 2009). The method is summarized below:

Coalescing Shortest Path Image Transform

1. Setup graph G with second-order image connectivity; costs as in the text; and periodic boundary conditions. Include reflexive strips if necessary.
2. From G , select the 3×3 “attractor” nodes $n_i, i = 1:9$ on a regular grid.
3. Identify all local intensity maxima $m_k, k = 1:M$ in the image as “origins.”
4. Create accumulation image $I = 0$ of the same size as the original image: I_0 .
5. FOR $i = 1:9$
 FOR $k = 1:M$
 IF $d(m_k, n_i) < 150$: Trace shortest path $S_j, j = 1:J$
 from m_k to n_i where S_j is the j ’th pixel on the path
 FOR $j = 1:J$
 IF $d(S_j, n_i) > 40$: $I(S_j) = I(S_j) + 1$
 END j
 END k
 END i
 Return I

RESULTS AND DISCUSSION

We illustrate the benefits of the coalescing transform on two applications: neurite tracing and cell membrane detection under fluorescence confocal microscopy. In the first case, neurites were too faint to be traced using traditional solutions (neurite traces appeared broken and branched). In contrast, the coalescing transform was able to outline the main neurites even when they appeared discontinuous and displayed a very low signal-to-noise ratio (see Fig. 1G,H).

Processing time for this image (512×512 pixels) was within 10 s on an Intel Core i5 processor.

In the second application, the main challenge was to trace weak cell membrane compartments in the presence of other membranes nearby featuring better contrast and higher intensity (Fig. 1I). Without our new method, the detected contour would invariably jump on these neighboring cells, producing wrong contours. However, by using the coalescing transform as a preprocessing step, we were able to accurately recover the circular shortest path shown in red (see, e.g., Sun et al., 2009 for a description of the circular shortest path algorithm). Because the process is fully automated, this technique is suitable for a membrane protein quantification assay in a drug discovery and development setting.

SUMMARY

We have developed a new image transform capable of outlining even very weak linear features in images. Several variations and improvements on the theme suggest themselves, including iterating the transform to capture linear features in close proximity to stronger linear features, extension to three dimensions, and speeding the computations on parallel architectures. Finally, the transform has a tendency to create spurious traces in the presence of extended, featureless area. Examining and eliminating stretches from the traces obtained on the basis of the measured intensities and contrast represents a possibility. We aim to explore these directions in the future.

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