# A GENERAL APPROACH FOR SEGMENTING ELONGATED AND STUBBY BIOLOGICAL OBJECTS: EXTENDING A CHORD LENGTH TRANSFORM WITH THE RADON TRANSFORM

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## ABSTRACT

Automatic, high-throughput, quantification of the precise position and orientation of biological objects is essential for studying living, biomedically relevant processes from time-lapse microscopy images. These measurements frequently include precise estimates for the center-of-mass as well as the location of the true object boundaries e.g. the membranes of cells. This paper describes a region-oriented segmentation approach applied to the detection of both insects at the mm length scale as well as bacteria at the µm length scale. Despite the differences in length scale, images of both objects have similar aspect ratio, and it is common to have overlapping objects in images of both. This thus presents a challenge for any segmentation algorithm. Our approach performs all orientation detection through a chord length transform, so the task of separating overlapping objects in a two-dimensional image is reformulated as a voxel-labeling problem within a three-dimensional volume. It then utilizes the directional information from the Radon transformed image. Experimental results in simulation show that our method is effective in separating clustered elongated but stubby objects with aspect ratios not far from 1. The applications in detecting insects and *Escherichia coli* bacteria demonstrate the value of our approach.

***Index Terms***— high-throughput, in vivo microscopy, image analysis, chord length transform, Radon transform

## 1. INTRODUCTION

Quantifying individual features of large numbers of cells or animals simultaneously is a problem confronted in a number of disciplines. It is common for the number of objects requiring detection and segmentation to vary from hundreds to many thousands. Manual analysis is likely to show inconsistencies and be excessively time-consuming. Here we are especially interested in the location of each object and its orientation. The two particular biological problems this work helps address are as follows. 1) We want to track the dynamics of fluorescently labeled components inside single bacteria when we are simultaneously imaging large numbers of cells. To establish a co-ordinate system for the motion of subcellular components relative to the cell we first need to have good tools for segmenting the cells (a typical image is Figure 1b.1). 2) We want to find the alignment and position of large numbers of insects crowded into a domain. This allows us to investigate how collective behavior of insects depends on their densities (a typical image is Figure 1a.1). Note that the images have elongated organisms that frequently overlap.

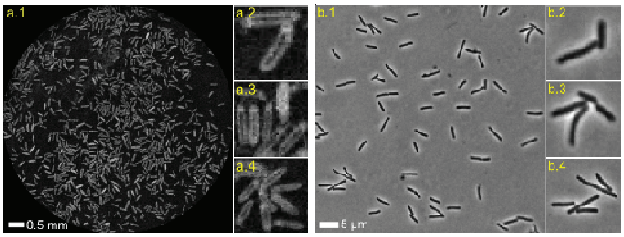


Figure.1 Example images containing elongated biological objects. (1) *Collembola* insects of 0.2 to 0.25 mm length in a circular enclosure (a.1) and clusters in magnified view (a.2-a.4). (b.1) A bright-field image of *E. coli* bacteria of length 1 µm and clusters in magnified view (b.2-b.4).

High noise, contrast fluctuations and the presence of overlapping structures have serious detrimental effects on overlapping object segmentation techniques. Pixel-oriented segmentation techniques [1] include threshold techniques, edge methods and "growing-region" approaches, which are often ineffective for segmentation with complicated image conditions because these methods depend only on intensity or gradient information and neglect geometrical characteristics. The watershed algorithm [2] and its variants [3] are enhanced pixel-oriented methods used in many applications. However, they are limited by noise and intensity fluctuations, which generally result in over-segmentation and need appropriate modification in post-processing to enhance the accuracy to an acceptable level. Level set methods [4, 5] combined with prior shape information can provide accurate results but can be computationally demanding for large-scale datasets, thus necessitating time-consuming and subjective manual user-intervention. To recognize partially occluded objects, some researchers use varied features, e.g. matching geometrical features by ellipse fitting [6], using convex hull attributes

[7], or by classification driven segmentation [8], directional morphological filters [9], and extracting directional characteristics by transforms [10, 11].

Here, we propose an improved region-oriented segmentation approach, which builds on the Chord Length Transform (CLT) proposed in Ref. [10] by combining it with the Radon transform [11]. This is particularly useful for the stubby elongated objects we consider (which have maximum aspect ratios near 1:3).

## 2. ALGORITHMS APPLICATION

We prepare our image by first using a complex filter derived from Ref. [12] to suppress background noise. This provides a more uniform object which is then mapped into multiscale space after Ref. [13] to distinguish the details of boundaries. The objects are then classified into two sets by a simple intensity segmentation using a local adaptive threshold method. We then have either clusters of overlapping objects or isolated objects depending on the foreground intensity. We can compute the attributes of isolated objects directly, so the bulk of the methods presented here are focused on obtaining the location and orientation of each individual object from overlapping clusters, which is normally a challenging question for segmentation approaches.

### 2.1 Region-oriented chord length transforms

We now briefly describe how a Chord Length Transform (CLT) can be used to map the 2D image into a 3D space to allow easy segmentation. The method we use is closely modeled on the CLT described in Ref. [10].

The input to the CLT is a binary image, E, containing a cluster of objects obtained from the aforementioned preprocessing (e.g. Figure 2a). Each point inside the cluster (i, j) is assigned an orientation θ and the CLT of E, C (i, j, θ), is an integer valued 3D image with elements which are the length of the chord of orientation θ passing through (i, j). An example of the 3D image C can be found in Figure 2b. A full range of θ is not considered; instead we consider a smaller, evenly spaced, sample of the full set of discrete orientations. In order to define chords and chord lengths in this discrete case the Bresenham line construction is used.

### 2.2 Radon transforms and connected component labeling

Having obtained an, integer-valued, 3D image, C, from the 2D binary image of the cluster we next enhance this image by a morphological filter using Bresenham line as a directional kernel. The filtered image is then thresholded using its mean; this generates a binary image C' with reduced background noise. Connected components of C' are identified by the following procedure. If C'(i, j, θ) is non-zero, it is placed in the same component as the component of the 26 nearest neighbors of the point (i, j, θ) in the three dimensional volume that are also non-zero. One can thus expect this procedure to generate multiple, distinct, connected components. Each distinct component is given a different label. We now project these labels onto, E, the original 2D thresholded image of the cluster. Each point E(i, j) in the 2D image can have multiple labels as it can participate in multiple components of the 3D binary image C'. We perform a small amount of size filtering and delete connected components which either have a very small 2D projection (<<organism size) or a large one (>2×organism size). We call the lower threshold $l_{min}$ and the upper $l_{max}$. We are now in a position to use information derived from the Radon transform.

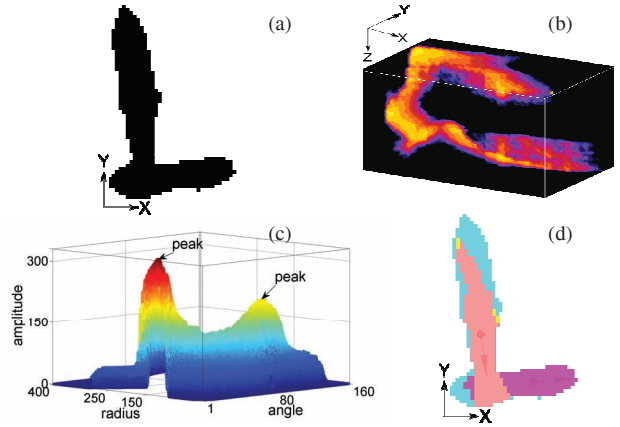


Figure 2. Segmentation and detection for overlapping clusters of stubby elongated biological objects. (a) Smooth filtered image to be transformed. (b) 3D volume which is the CLT of the image in a. (c) Component alignment estimation from the Radon transform (d) Detection result for center-of-mass and orientation in an overlapping cluster.

The Radon transform [11] of a binary object E(**x**) returns the distance, E'(r, **θ**), travelled, through the object, by a particle following a straight-line trajectory which has a closest approach distance r to the origin and orientation **θ**. We take the binary image of the cluster, E and find its Radon transform E'(r, θ) (an example is Figure 2c). The maxima of E'(r, θ), of height greater than a threshold, $t_R$, are found and the corresponding θ values are identified. The hope is that these angles will be those that are aligned with the principal axes of the organisms contained within the clusters. The final step is to find the alignment angles of each component of C' when they are projected into the (i, j) plane. The extracted components are labeled and shown in Figure 2d with different colors overlaid on original blue objects, which have an alignment angle close to the principal angles identified by the Radon transform, and are then the candidate individual organisms. Given the centers of each organism a watershed algorithm is used to find the boundaries and then the best alignment of the organism can be found.

## 3. EXPERIMENTS AND RESULTS

We first tested our approach on simulated data. We generated fixed-size rod-like objects with random position

and alignment and varied their densities. We picked an aspect ratio to mirror that of the bacteria and insects. Different parameter settings were tested, and the one with the best performance was then used for the segmentation of all the subsequent experimental images. In our specific applications, the following parameters were selected and provided satisfactory results: $\theta$ was considered at intervals of 5 degrees with $l_{max}$=32 pixels, $l_{min}$=8 pixels and Radon threshold $t_R$ =0.8. The results for extracting location and orientation for each elongated object corresponding to its segmentation are shown in Figure 3. It can be seen that our proposed algorithm approaches that actual number of rods in the simulated image. It is evident that our segmentation method combining the Radon transform outperforms the original CLT. The unmodified CLT algorithm has a tendency to oversegment and is better for more extreme aspect ratios; our method is particularly suited to elongated objects which are stubby rather than strip like. This is clarified in Figure 4.

In our application we find that the intervals between the θ values in CLT are important. When the intervals are large, much orientation information will be skipped to produce under-segmentation. On the contrary, if the intervals are too small, too much detail will be obtained from the interior of the binary bodies we would like to identify as objects; this tends to oversegment the clusters. Here we find that 5° is a good compromise.

We applied our method to movies of real experimental elongated biological objects using the optimized parameter settings based on our simulated data. Figure 5 illustrates insect image patches of two tightly connected objects from low density to high density, and *E. coli* microcopy in bright field is shown in Figure 6. The quantitative performance of our new method was compared to the method embedded in the open source software CellProfiler [3], which provides an enhanced watershed method combining distance information and intensity gradients from the objects. Typically, the boundaries of objects generated by CellProfiler were divided at the regions in the image with strong pixel intensity gradients, so that over-segmentation phenomena were serious. The direct results from the level set approach show that under-segmentation often occurs because it is difficult to divide a connected object without sufficient edge information. Overlapping especially makes the area of occluding part large and there are no obvious gradient changes to map the contour to the exact boundary. Our method is able to get significantly better detection for both location and orientation of elongated biological objects. It was evident that our method offered distinct advantage in adding directionality into the segmentation algorithm via the transformation into an oriented domain. By counting the number of segmented objects compared with manual results from several typical frames labeled with yellow color, Figure 7 also indicates that our proposed method can provide more accurate results.

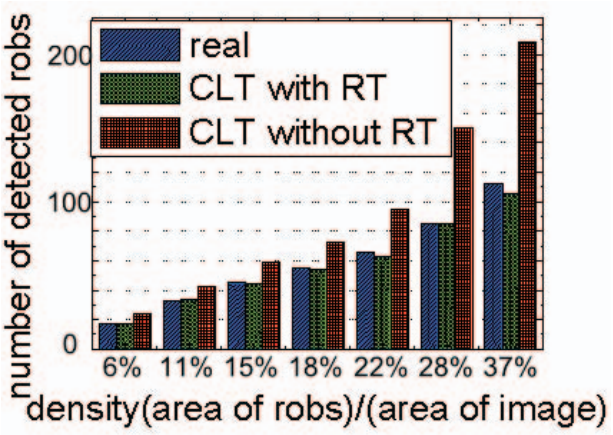


Figure 3. Accuracy comparison for actual value of rods, CLT with RT and CTL without RT

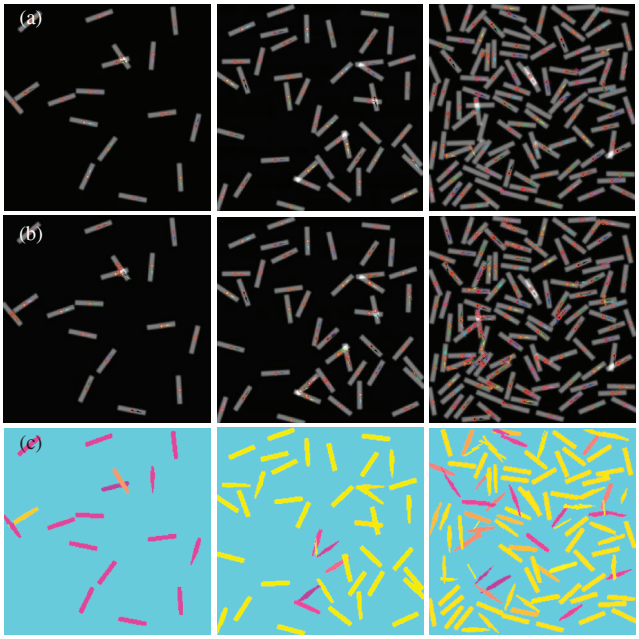


Figure 4. Location and orientation detection (row a) based on its segmentation (row b) by CLT+RT for simulated data for low density (1st column), middle density (2nd column) and high density (3rd column). Row c is obtained by CLT without RT to the same image as a row.

## 4. CONCLUSION

We described a scheme for segmenting elongated biological objects and extracted the corresponding information e.g. center-of-mass and orientation. The proposed method starts by using a Chord Length Transform to convert the 2D image into a 3D volume. The Radon transform is then applied to help segment this volume. This result may prove applicable to many types of biological/biomedical microscopy image analysis, and we have used this algorithm in both quantifying the dynamics of components inside single bacteria and in tracking the dynamics of large populations of insects.

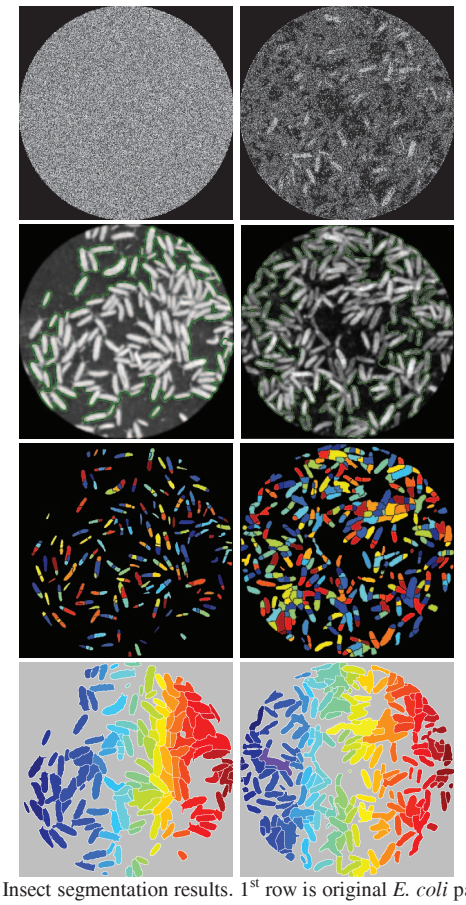

Figure 5. Insect segmentation results. 1st row is original *E. coli* patches; 2nd row is obtained from the level set method; 3rd row is the result from CellProfiler and the 4th row comes from our novel detecting method.

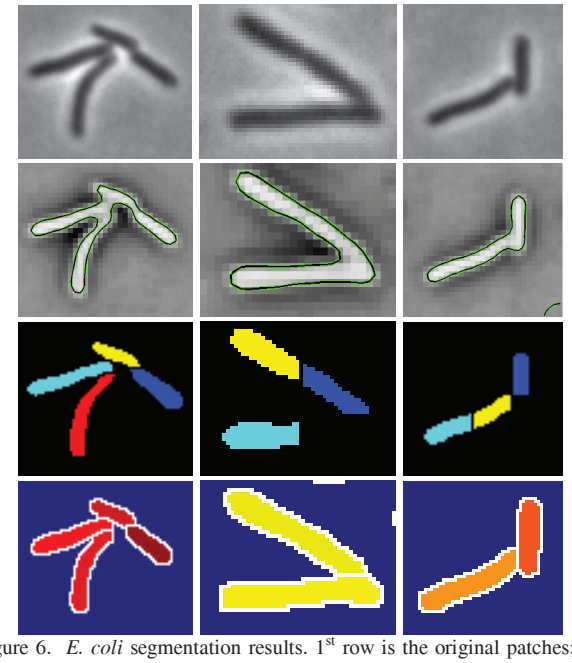

Figure 6. *E. coli* segmentation results. 1st row is the original patches; 2nd row is obtained from the level set method; 3rd row is the results from CellProfiler and the 4th row comes from our novel detecting method.

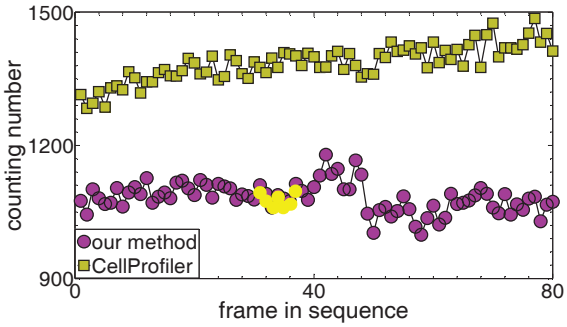


Figure 7. Comparing our method with CellProfiler for segmented insect numbers in frames.

## 5. AKNOWLEDGEMENTS

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