Automated length measurement based on the Snake Model applied to nanoscience and nanotechnology

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Abstract: *This work describes the development of an automated method for metrological purposes to measure the length of filament-shaped objects. The proposed methodology can determine the length of the object(s) of interest using image segmentation, where the Snake Model (SM) was applied. The measurement procedure starts from the segmentation step, where a mathematical morphology technique called erosion is applied, resulting in a thin curve representation with only two endpoints (skeleton). A set of 3x3 kernels was defined for the erosion procedure. The piecewise linear approximation concept was also employed to measure the total length of an object, based on the dimension of the pixel (unitary length) that composes the skeleton. The accuracy of the algorithm was evaluated using a sort of DNA filaments from Atomic Force Microscopy (AFM) images.*

1. Introduction

The motivation for the development of a reliable algorithm capable to measure the length of objects automatically began with the DNA images obtained from the Atomic Force Microscopy (AFM) technique [1], having in view the importance of their nanometrological characteristics. Several efforts have been done so far in automating a method to determine DNA filament lengths. *Ficarra et al* [2] developed an automated algorithm to determine the DNA fragment size from AFM images and extract the molecular profiles. *Spisz* and co-workers [3] presented an automated sizing DNA program for segmentation using a threshold selection method, thinning and neighborhood pixel processing. Different software packages have been described and applied to DNA fragment length determination. One of them is the ImageJ® software (National Institutes of Health – NIH, USA) [4], that offers a manual procedure to trace segmented lines over the image in order to approximate the correct length of the object.

This work proposes a technique that comprises image segmentation and length estimation of the objects of interest in an automatic way. The main idea is to segment the image using Parametric Deformable Models, especially the Snake Model (SM) [5], to better represent the edges of the object(s). The erosion procedure was used to skeletonize the selected objects, and a linear piecewise approach was employed to estimate their lengths. The original proposal of the SM has been successfully applied in a variety of problems for computer vision and image analysis, such as motion tracking and segmentation. The SM consists basically on an elastic curve that can dynamically fits to the real object shape due to the action of internal (elastic) and external (derived from the image) forces. The simplest external force is a gradient of the image function, but another approach called Gradient Vector Flow (GVF) [6] allows the SM to push the curves into boundary cavities, converging in a more accurate representation of the border. The GVF deformable model is a version of the SM that uses the GVF as an external force (GVF-SM). The GVF-SM was used to improve the precision of the segmentation, which is a determinant feature in the nanoscale.

2. The Snake Model (SM)

The SM is a 2-D parametric deformable model that is defined as a curve $x(s)=[x(s),y(s)]$, $s\in[0,1]$ - here assumed to be closed - that moves through the spatial domain of an image to minimize the energy functional

$$P=\int_0^1 \frac{1}{2}\left(\alpha\left|x'(s)\right|^2+\beta\left|x''(s)\right|^2\right)+ \\ +E_{ext}^i\left(x(s)\right)ds \tag{1}$$

where α and β are weighting parameters that control the Snake's tension and rigidity, respectively. The terms $x'(s)$ and $x''(s)$ denote the first and second derivatives of $x(s)$ with respect to s.

The external potential function E_{ext}^i is derived from the image so that it takes on its smaller values at the features of interest, such as boundaries. Given a grey-level image $I(x,y)$, viewed as a function of continuous position variables (*x,y*), typical external potential functions (designed to lead a deformable contour toward step edges) are:

$$E_{ext}^a\left(x,y\right)=-\left|\nabla I\left(x,y\right)\right|^2 \\ E_{ext}^b\left(x,y\right)=-\left|\nabla G_\sigma\left(x,y\right)*I\left(x,y\right)\right|^2 \tag{2}$$

where $G_\sigma\left(x,y\right)$ is a two-dimensional Gaussian and ∇ is the gradient operator. A deformable contour that minimizes P must satisfy the Euler equation**:**

$$\alpha x''(s)-\beta x''''(s)-\nabla E_{ext}=0 \tag{3}$$

The external potential force pulls the deformable contour toward the desired image edges, while the intrinsic forces minimize stretching and bending. Defining F_{int} as $\alpha x''(s)-\beta x''''(s)$ and F_{ext} as ∇E_{ext}, equation 3 can be rewritten as:

$$F_{\text{int}}-F_{ext}=0 \tag{4}$$

representing a force balance equation. When the forces are balanced by means of equation 4, the state solution is achieved.

A numerical solution can be found by discretizing equation 3 and solving the discrete system iteratively. The partial derivative of *x* with respect to *t* is then set equal to the left side of equation 3:

$$x_t(s,t)=\alpha x''(s,t)-\beta x''''(s,y)-\nabla E_{ext} \tag{5}$$

Figure 1.a illustrates a low signal to noise ratio image containing the object “U” with no well defined edges. Figure 1.b shows the vector field $-\left|\nabla I\left(x,y\right)\right|^2$ and figure 1.c shows the steps of the SM evolution.

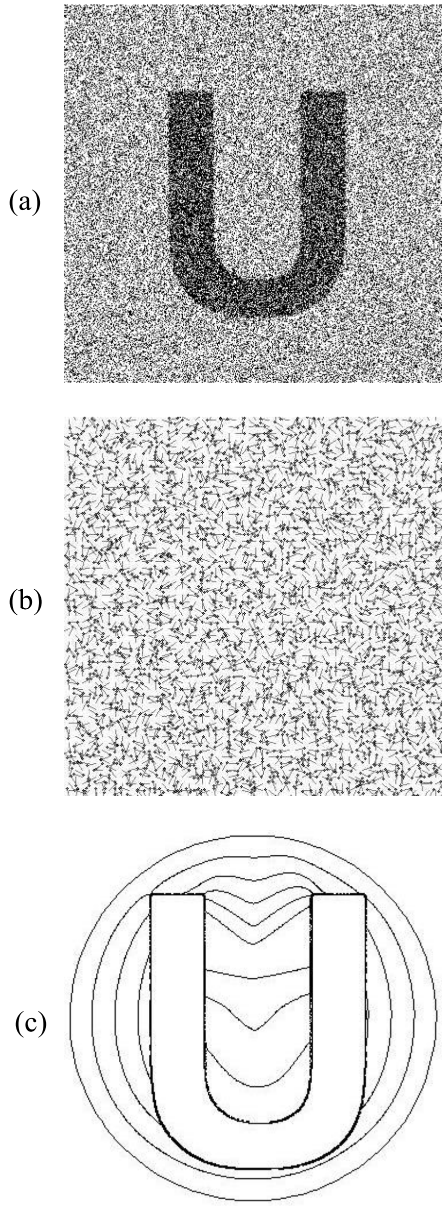


Figure 1 – (a) The original image to be processed, (b) the correspondent external vector field $-\left|\nabla I(x,y)\right|^2$ and (c) the steps of the SM evolution.

3. The Gradient Vector Flow (GVF)

The GVF is another external potential function introduced by *Xu et al* [6] that can be used to improve the solution. The GVF is a vector diffusion method that can be defined through the following diffusion-reaction equation:

$$\begin{aligned} v_t &= g\left(\left|\nabla E_{ext}\right|\right)\nabla^2 v - h\left(\left|\nabla E_{ext}\right|\right)\left(v - \nabla E_{ext}\right) \\ v_0 &= \nabla E_{ext} \end{aligned} \tag{6}$$

where $g(\bullet)$ and $h(\bullet)$ are nonnegative weighting functions, defined on the image domain, or can be set as constants. The relationship between weight parameters was observed by *Marturelli et al* [7] with respect to the quality of the generated field. Particular advantages over a SM are: i) insensitivity to initialization and ii) the ability to move into boundary concavities. The GVF-SM uses GVF as an external force:

$$x_t(s,t) = \alpha x''(s,t) - \beta x''''(s,y) + GVF \tag{7}$$

Figure 2 shows the vector field computed by GVF and the result of the GVF-SM.

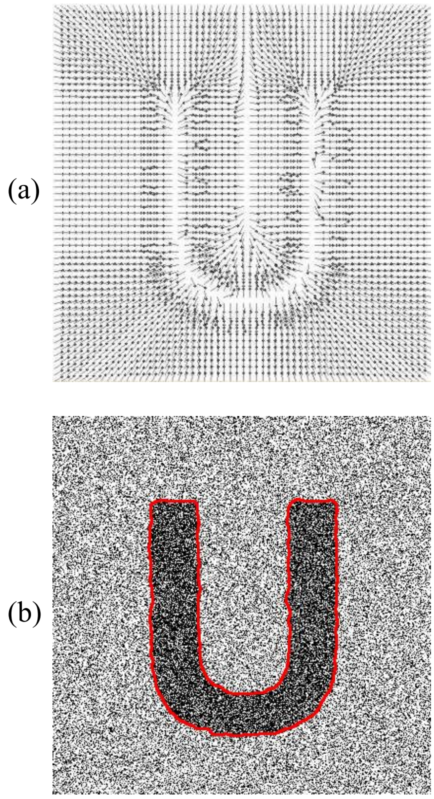
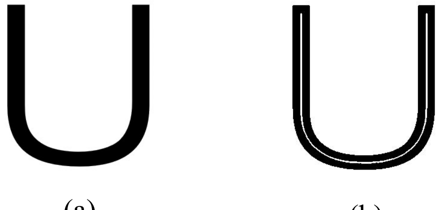


Figure 2 - (a) Vector field computed by the GVF model, g = 2 and h = 0.02, and (b) the GVF-SM solution (red contour).

4. Skeletonization

In this work, the skeleton is defined as an open curve that fits to a median line of a polygon. In order to compute the skeleton, the mathematical morphology erosion operator is applied [8]. First, a binary (black & white) image from the result of the GVF-SM is computed (figure 3.a). From this image, the segmented object is eroded in order to wiredraw it, resulting in the skeleton representation showed in the figure 3.b. This procedure works repeatedly analyzing each pixel belonging to the border of the object. To implement this procedure a comparison between the analyzed pixel with a list of pre-defined 3x3 kernels was defined, resulting in forty different templates (figure 4), following a simple rule: *The analyzed pixel will be removed if there exist two or more self connected neighbours. On the other hand, the pixel can not be removed having in view that a segment fault may occur, resulting in disconnected curves for the same object.*



(a) (b)

Figure 3 - "U" object (a) its shape, and (b) the representation of the skeleton (white line).

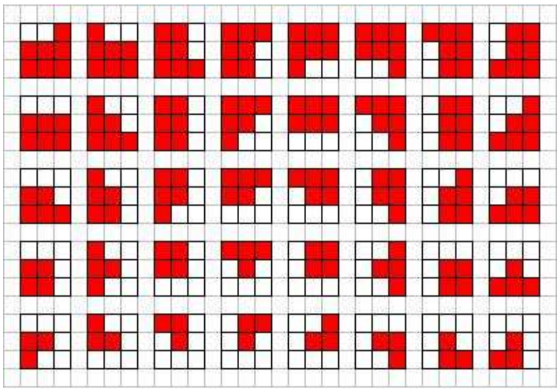


Figure 4 – Predefined kernels (templates) used to erode the object.

5. Length Measurement

From the skeletons a piecewise linear approximation was used to measure the length of the object(s). The main idea is to compute the length unit of each pixel that constitutes the skeleton, using 1 for vertical and horizontal directions and $\sqrt{2}$ for the diagonal. The sum of values results in a dimensionless length measurement that must be scaled to the original image dimension by means of multiplying the skeleton's length by a scale factor defined by the user.

6. Measurements Results

This section shows the results of two measurement experiments using AFM images of DNA filaments, in which an Intel® Core™2 CPU 6420 @ 2.13 GHz computer with 2 GB of RAM was used.

In the first experiment, an AFM image containing plasmid DNA filaments was used (figure 5.a). When prepared, the sample was supposed to have tiny filaments around 896 base pairs, which should represent separated filaments with 264 nm. A simple visual inspection shows that differences appear, due to, probably, different conformational arrangements after the sample preparation for AFM imaging. Table 1 shows some coherent results revealing such differences. Figure 5.b illustrate the skeletons drawn over the original image. In this case the method took 0.9 sec to process the measurements. The image was obtained from a Nanowizard AFM (JPK Instruments) in tapping mode, in air.

Table 1 - Data from DNA filaments of figure 5.b.

Filament	Length (nm)
1	264
2	250
3	316
4	202
5	263
6	230

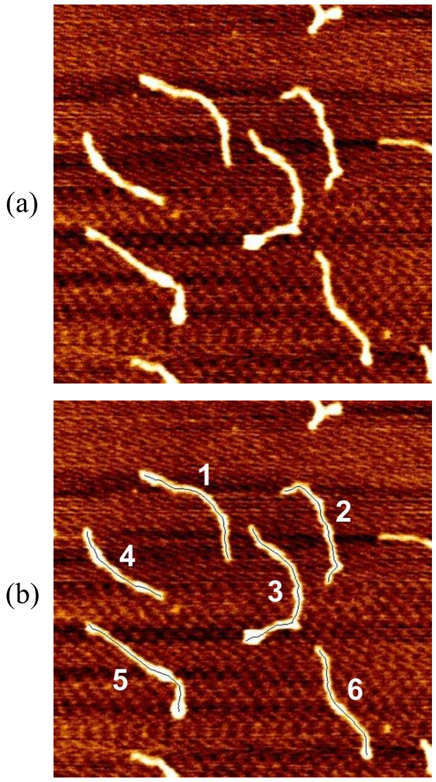


Figure 5 - (a) 512 x 512 AFM image of DNA filaments (700 nm x 700 nm) with 896 base pairs and (b) the skeletons after erosion (black lines).

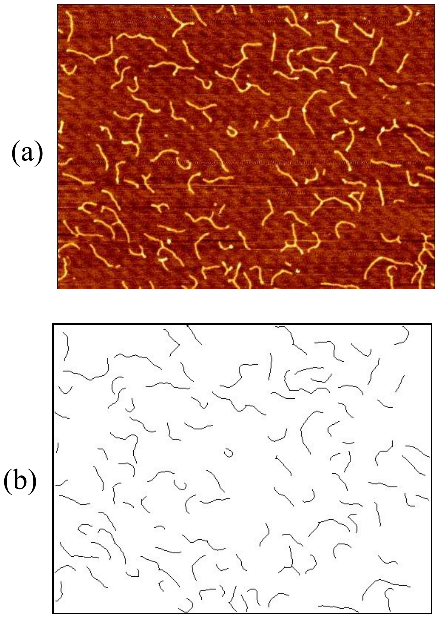


Figure 6 - (a) 512 x 387 AFM image of DNA filaments (3 µm x 2.27 µm) and (b) the skeletonization results.

The second experiment, considers a huge amount of objects (approx. 100 DNA filaments) in the same image (figure 6). In this case, tiny objects (compared to the average) were rejected, as well as the superimposed ones. The algorithm took 2 sec to process them.

7. Conclusions

The proposed technique is not intended to replace any other methodology, but to show the efficiency of the GVF-SM approach over poor signal-to-noise ratio images. The main advantage of the GVF-SM is to obtain more precision in the segmentation stage. The erosion procedure depends strongly on the initial shape of the object, resulting in a more accurate skeleton representation.

For future work, the Topological Snake (T-Snake) [9] will be used to make the initialization easier, minimizing the inhomogeneous illumination problems.

8. Acknowledgments

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9. References

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