Detection of DNA Filaments in Fluorescent Microscopy Using Feature-adapted Beamlet Transform

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Abstract

This paper presents a new method for computing the Feature-adapted Beamlet transforms [1] in a fast and accurate way. This transform can be used for detecting features running along lines or piecewise constant curves. The main contribution of this paper is to unify the Fast Slant Stack method, introduced in [2], with linear filtering technique in order to implement the Feature-adapted Beamlet transform. If the desired feature detector is chosen to belong to the class of steerable filters, our method can be achieved in $O(N \log(N))$, where $N = n^2$ is the number of pixels. This new method leads to an efficient implementation of Feature-adapted Beamlet transforms, that outperforms our previous works [1] both in terms of accuracy and speed. Our method has been developed in the context of biological imaging to detect DNA filaments in fluorescent microscopy.

Index Terms

Beamlet transform, Fast slant stack, steerable filters, feature, DNA filament detection

I. Introduction

High throughput DNA molecules screening is a technique intensely used in many applications such as drug discovery or diagnostic test development. For some of these techniques, such as microarray or Molecular Combing [3], automatic image analysis tools are mandatory to be able to process data yielded by such techniques. For example, Molecular Combing is a powerful tool that stretches DNA molecules on a slide, and produces after fluorescent microscopy acquisition, a significant amount of images that have to be analyzed (see Fig 3.a).

The problem of detecting curvilinear objects in images arises in various areas of image processing and computer vision, since such kind of objects occur in every natural and synthetic images, like roads and streams in remote sensing images, human vasculature in medical imaging or DNA filaments in biological microscopy. Edges can be also percieved as curvilinear objects since they describe discontinuities along curves [4]. Therefore, detection of such features is probably one of the most important step toward automatic object recognition and shape analysis.

Commonly, curvilinear objects are considered as 1-dimensional manifolds that have a specific profile running along a smooth curve. The shape of this profile may be an edge- or a ridge-like feature. It can also be represented by more complex designed feature. In our context of DNA filament analysis in fluorescent microscopy, the diameter of such molecule is about 25-30 $\mathring{A}$, so it is acceptable to consider the transverse dimension of a filament to be small relative to the point spread function (PSF) width of the microscope. Hence, a 2-dimensional image model for a DNA filament can be designed as follows:

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$$f(x,y) = \mathbb{1}_\beta * \rho(x,y) \quad \text{with } \mathbb{1}_\beta(x,y) = \begin{cases} \beta & \text{if } (x,y) \text{ is on the filament,} \\ 0 & \text{otherwise,} \end{cases} \tag{1}$$

and where β is the mean intensity of the filament (we suppose our filaments being relativaly free of intensity oscillations) and ρ is the PSF of the microscope. Approximation of PSF for different type of microscope can be found in [5].

One way to detect curvilinear objects is to track locally the feature of the curve-profile; linear filtering or template matched filtering are well-known techniques for doing so. Classical Canny edge detector [6] is based on such linear filtering techniques. It involves the computation of inner-products with shifted and/or rotated versions of the feature template at every point in the image. High response at a given position in the image means that the considered area has a similarity with the feature template. Filtering is usually followed by a non-maxima suppression and a thresholding step in order to extract the objects. This approach has a major drawbacks that comes from the fact that linear filtering is based on local operators: it is highly sensitive to noise but not sensitive to the underlying smoothness of the curve, which is a typical non-local property of curvilinear objects.

Alternativaly, the Radon transform is a powerful tool which may be used for line detection. Also known as the Hough transform in the case of discrete binary images, it performs a mapping from the image space into a line parameter space by computing line integrals. Formally, given an image f defined on a sub-space of $\mathbb{R}^2$, for every line parameter (t, θ), with $t \in \mathbb{R}$ and $\theta \in [0, \pi)$, the continuous Radon transform is defined by

$$R[f](t,\theta) = \int\int f(x,y)\delta(t - x\cos(\theta) + y\sin(\theta))dxdy. \tag{2}$$

Peaks in the parameter space reveals potential lines of interest. This is a very reliable method for detecting lines in noisy images. However, there are severals limitations. First, direct extension of that method to detect more complex curves is in pratice unfeasible for it increases the complexity exponentially by adding one dimension to the parameter space. In addition, Radon transform computes line integrals on lines that pass through the whole image domain and doesn't provide information on small line segments.

Given an image of $N = n \times n$ pixels, the number of possible line segments defined is about $O(N^2)$. Direct evaluation of line integrals upon the whole set of segments is practically infeasible due to the computational burden. One of the methodologies proposed to address this problem is the Beamlet transform [7]. It defines a set of dyadically organized line segments (beamlets) occupying a range of dyadic locations and scales, and spanning a full range of orientations. The collection of beamlets has a $O(N\log(N))$ cardinality. The underlying idea of the Beamlet transform is to compute line integrals only on this smaller set, which is an efficient substitute of the entire set of segments for it can approximate any segment by a finite chain of beamlets. Beamlet chaining technique also provides an easy way to approximate piecewise constant curves. The beamlet transform can be viewed as a multiscale Radon transform so it can be computed as follows: $i)$ define the set of all dyadic squares obtained by recursive partitionning of the image domain and $ii)$ on each square S, compute $R[f_S]$ thanks to equation (2), where f_S is the portion of the image induced by S.

The main objective of this article is as follows: instead of equation (2), we want to compute in a fast and and accurate way the Beamlet Transform using the following equation:

$$R[f * h^{\theta}](t,\theta) = \int\int f * h^{\theta}(x,y)\delta(t - x\cos(\theta) + y\sin(\theta))dxdy, \tag{3}$$

where h is a feature detector designed to filter specifically a 2-dimensional line profile, be it, a ridge or a more complex image feature. Our main result is that thanks to the Fast Slant Stack technique presented above, for a certain class of filters h, our objective can be fulfilled efficiently in $O(N\log(N))$. We call this transform the Feature-adapted Beamlet transform. Section 2 gives some key elements to understand the underlying mechanisms of the Fast Slant Stack methodology. Section 3 presents our contribution while section 4 presents a detection algorithm using the Feature-Adapted Beamlet transform for the detection of DNA filaments in fluorescent microscopy.

II. Fast Slant Stack

Much attention has been given over the last twenty years to adapt equation (2) to digital arrays, *i.e.* when f is represented by a discrete lattice $I = I(u,v) : -n/2 \leq u,v < n/2$. There are two distinct approaches to compute equation (2) efficiently on digital arrays. The first one is the multiscale approach presented in [8]. This approach splits the image domain by recursive partitioning into dyadic squares. Radon coefficients are computed by recombining those which have been already computed on smaller squares. The major drawback of this approach in the discrete case is the recombination process on digital grids that introduces approximations. The second kind of approach is the Fourier-based approach. It exploits the projection-slice theorem which says that the 1-dimensional constant θ-slice of the Radon transform $(R[f](t,\theta) : -\infty < t < \infty)$ and the 1-dimensional radial slice of the Fourier transform make a 1-dimensional Fourier transform pair.

Recently, a novel Fourier-based approach has been proposed, the Fast Slant Stack methodology [2]. It avoids the drawbacks mentioned above by introducing a discrete definition of equation (2) and by carefully choosing a set of *grid-friendly* lines. It yields to a fast, geometrically and algebraically exact method to compute the Radon transform in $O(N\log(N))$, where $N = n^2$ is the number of pixels. As our methodology being based on this approach, we recall here some of its key elements and refer the reader to [2] for more details.

A *basically horizontal* line is a line of the form $y = \tan(\theta)x + t$, where the slope $|\tan(\theta)| \leq 1$. Notice that the methodology described in the sequel of this paper could be applied directly to the complementary set of *basically vertical* lines of form $x = \tan(\theta)y + t$. Defining a discrete version of equation (2), the Radon transform associated with the set of horizontal lines is

$$R[I](t,\theta) = \sum_u \tilde{I}(u, \tan(\theta)u + t), \tag{4}$$

where $\tilde{I}(u,y)$ is an interpolant that takes discrete values in the first argument and continuous values in the second argument. The 1-dimensional interpolation is realized thanks to a Dirichlet kernel (see [2] for complete details). The parametrization of the Radon space is chosen as follows: one considers only the lines having an intercept $-n \leq t < n$ and the set of angles $\theta = \arctan(2r/n), -n/2 \leq r < n/2$. According to this set of angles, the fundamental property of equation (4) is driven by the following result:

Theorem 1: (Projection-Slice Theorem) Define the 2-dimensional Fourier transform of the array I via:

$$\hat{I}(k_1,k_2) = \sum_{u,v} I(u,v)\exp\{-i\frac{\pi}{n}(uk_1 + vk_2)\},$$

where $-n \leq k_1, k_2 < n$. Then, for each fixed $\theta = \arctan(2r/n)$, $-n/2 \leq r < n/2$, the 2n numbers

$$R[I](t, \theta), \qquad -n \leq t < n,$$

are a 1-dimensional discrete Fourier transform pair with the 2n numbers

$$\hat{I}(\pi \frac{k}{n} \tan(\theta), \pi \frac{k}{n}), \qquad -n \leq k < n.$$

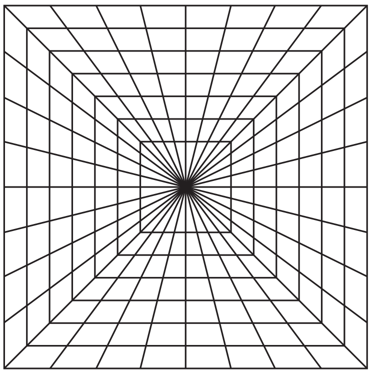


Fig. 1. The Pseudopolar Grid for $n = 8$

The key point of the Fast Slant Stack method is the special nature of the angles θ chosen above. Using theorem (1), one has a connection between Radon values and a set of spatial frequencies $\xi_{r,k} = (\pi \frac{k}{n} \frac{2r}{n}, \pi \frac{k}{n})$ with $-n \leq k < n$ and $-n/2 \leq r < n/2$. This is a special non-Cartesian pointset in frequency domain which has been known as the Pseudopolar grid, and is illustrated in Fig 1. This pointset can be efficiently computed according to the Pseudo-Polar Fast Fourier transform [2], noted $\mathcal{P}$. $\mathcal{P}[I]$ is an array of $2n$ rows by n columns where the r^{th} column refers to the values $\hat{I}(\pi \frac{k}{n} \frac{2r}{n}, \pi \frac{k}{n})$, $-n \leq k < n$. Hence, thanks to theorem (1), the Discrete Radon transform of equation (4) is reduced to $R = \mathcal{F}_{-1} \circ \mathcal{P}$, where $\mathcal{F}_{-1}$ denotes the 1-dimensional inverse Fourier transform performed on each column of $\mathcal{P}[I]$.

In the next section, we will use the Fast Slant Stack methodology in order to compute a discrete version of equation 1(3), by defining what we call the Feature-adapted Fast Slant Stack.

III. Feature-adapted Fast Slant Stack

A. *Definition*

Consider a feature detector h designed to filter specifically a 2-dimensional line profile. Let h^θ be a rotated version of h in the direction θ:

$$h^\theta(x, y) = h(\mathbf{R}_\theta(\mathbf{x}, \mathbf{y})), \tag{5}$$

where $\mathbf{R}_\theta$ is the 2-dimensional rotation matrix of angle θ. In a first step, we filter the image I with h^θ before computing equation (4). For a fixed θ, we have

$$R[I * h^\theta](t, \theta) = \sum_u \widetilde{I * h^\theta}(u, \tan(\theta)u + t). \tag{6}$$

A high coefficient means that the local feature runs significantly along the line $y = \tan(\theta)x + t$. For a fixed θ, equation (6) can be obtained by theorem (1). Unfortunately, the computation of all these coefficients for every angles is not achievable in a reasonable time, since it requires to convolve the image and to perform Pseudo-Polar Fourier transform as many times as the number of θ's, *i.e.* $2n$ times. To overcome this limitation, we propose to study the special case where h is selected to be within the class of steerable filters [9]. We can write h^θ as a linear combination of basis filters:

$$h^\theta(x,y) = \sum_{j=1}^{M} \phi_j(\theta) h^{\theta_j}(x,y), \tag{7}$$

where ϕ_j's are interpolation functions that only depend on θ and the basis filters h^{θ_j}'s are independent of θ. A convolution of an image with a steerable filter of arbitrary orientation is then equal to a finite weighted sum of convolution of the same image with the basis filters. As a result, we state the following result:

Proposition 1: Given a feature template h, for each $\theta = \arctan(2r/n)$, $-n/2 \leq r < n/2$, the 2n numbers

$$R[I * h^\theta](t,\theta), \qquad -n \leq t < n,$$

are a 1-dimensional discrete Fourier transform pair with the 2n numbers

$$\sum_{j=1}^{M} \phi_j(\theta) \widehat{I * h^{\theta_j}}(\pi \frac{k}{n} \tan(\theta), \pi \frac{k}{n}), \qquad -n \leq k < n.$$

The proof is given in [10]. We call this transform the Feature-adapted Fast Slant Stack. Thanks to this result, we compute equation (6) for every angle, as follows: first we convolve the image as many times as the number of basis filters composing our filter h. This number is typically very small. On each filtered image, we then compute the Pseudo-Polar Fourier transform and for each angle $\theta = \arctan(2r/n)$, we extract the r^{th} column of each transform and combine them thanks to Proposition 1. Finally, we perform 1-dimensional inverse Fourier transforms on each resulting series. All these steps can be performed in $O(N\log(N))$. A graphical representation of the implementation is given in Fig 2.

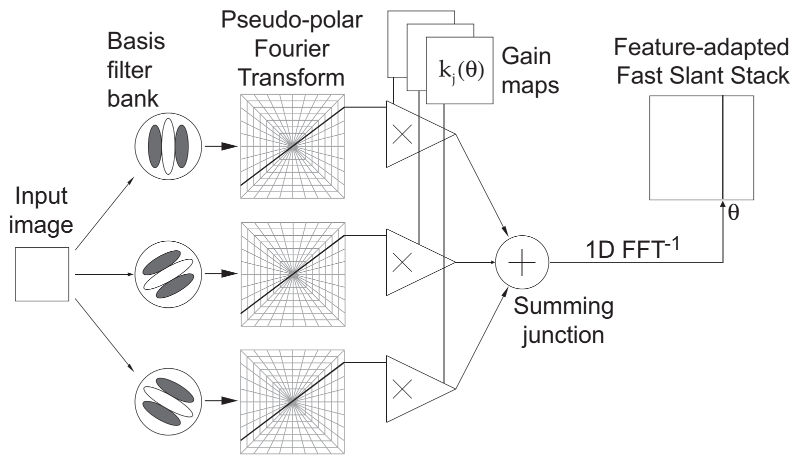


Fig. 2. Feature-adapted Fast Slant Stack diagram.

Feature-adapted Fast Slant Stack provides a fast and accurate way to compute integral of line carrying a specific line-profile (Feature-adapted Radon transform). This methodology is very general since it only requires that the feature detector designed to filter the line-profile has been selected within the class of steerable filters. In the next paragraph, we discuss about the choice of such filters.

B. Choice of filter h

Recently, detectors designed in [11] out-performed classical feature detectors like Canny edge detector or 2^{nd} derivative of gaussian-based filter for ridge detection. Authors proposed a general approach for the design of 2D feature detectors from a class of steerable filters [9]. The authors consider M^{th} order detectors of the form

$$h(x,y) = \sum_{k=1}^{M} \sum_{i=0}^{k} \alpha_{k,i} \frac{\partial^{k-i}}{\partial x^{k-i}} \frac{\partial^{i}}{\partial y^{i}} g(x,y), \tag{8}$$

where $g(x,y)$ is a gaussian function. The $\alpha_{k,i}$'s are obtained by optimization of a Canny-like criterion. Due to the steerable ability of such filters, they derive the filter's orientation that gives the highest response, by solving a M^{th} order polynomial equation at every point in the image. The choice of such filters yields to a significant performance improvement over classical linear filtering approaches.

We incorporate this class of feature detectors into our Feature-adapt Beamlet transform, and for filament detection specifically, we choose even order detectors ($M = 2, 4$, *etc.*).

C. Extension to Feature-adapted Beamlet transform

In [1], we used a technique that embedded a feature detector chosen as a steerable filter into the Beamlet transform. A two-scale recursion technique [8] was used in order to compute beamlet coefficients, where beamlet coefficients at a given scale can be obtained by the combination of those computed at smaller s cales. This strategy was quite fast at the expense, however, of a significant memory load and numerical approximations. Here we suggest that the Feature-adapted Beamlet transform can be computed thanks to the method presented in section III. The Feature-adapted Fast Slant Stack can be applied on every dyadic square that partitions the image domain to compute the Feature-adapted Beamlet transform. This completes our main objective. The next section presents how to use this tool for detection application.

IV. Application: detection using Feature-adapted Beamlet transform

In this section, we present a detection method using the Feature-adpated Beamlet transform. This method provides a list of beamlets that best represent curvilinear objects carrying a specific line-profile in an image. This method is based on a multiscale coefficient thresholding technique directly taken from [12] so we refer the reader to this paper for more details.

A. Algorithm

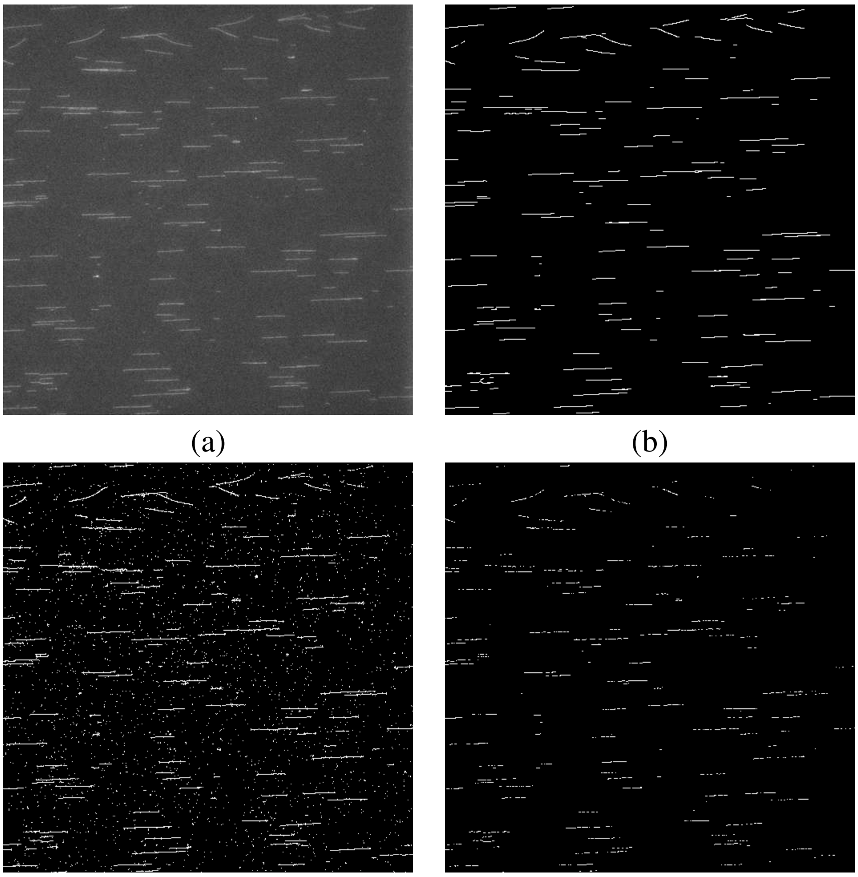
A Recursive Dyadic Partition (RDP) of the image domain is any partition, starting from the whole image domain, obtained by recursively choosing between replacing any square of the partition by its decomposition into four dyadic squares or leaving it unsplit. This concept is very similar to the *quadtree* decomposition technique. A beamlet-decorated RDP (BD-RDP) is a RDP in which terminal nodes of the partition are associated with at most one beamlet. By construction, BD-RDP provides a list of non-overlapping beamlets. In order to select the list of beamlets that best represent curvilinear objects in the image, we maximize over all beamlet-decorated recursive dyadic partitions $P = \{S_1, S_2, ..., S_n\}$ the following complexity penalized residual sum of square

$$E(P) = \sum_{S \in P} C_S^2 - \lambda^2 \# P, \qquad \text{where } C_S = \max_{L \in S} \tilde{R}[f * h^{\theta}](L) \tag{9}$$

measures the energy required to model the region S of the image f by the beamlet L and λ controls the complexity of the model as a MDL-like criteria. $\tilde{R}[f * h^{\theta}](L)$ are the normalized beamlet coefficients (see appendix). A high value of λ yields to a coarse representation of curvilinear structures; a small value leads to a quite complex model with potentially a significant number of false alarms. Notice that equation (9) can be solved very efficiently by a recursive tree-pruning algorithm due to additivity of the cost function. See [12] for complete details.

B. Experiments & Results

We evaluate the performance of the Feature-adapted Beamlet transform with respect to the pixel-wise method described in [11] on an image of multiple DNA filaments obtained by fluorescent microscopy. These filaments have a ridge-like profile. For the choice of h, we choose a 2^{nd} order detector defined in [11]. Fig 3 presents the results. We notice that the proposed method (b) outperforms the results achieved by the pixel-wise method, for a representative choice of either low or high threshold values.



(a) (b) (c) (d)

Fig. 3. Ridge detection: (a) image of DNA filaments obtaining by fluorescent microscopy. (b) detection using Feature-adapted Beamlet transform carrying a 2^{nd} order filter. (c)-(d) detection using pixel-wise ridge detector [11] with a low and high threshold values respectively.

V. CONCLUSION

In this paper, we have presented a method for computing the Feature-adapted Beamlet transform in a fast and accurate way. This transform can be used for detecting features running along lines or piecewise constant curves. Our contribution unifies the Fast Slant Stack method with the steerable filtering technique. It leads to an original and efficient implementation of the Feature-adapted Beamlet transform. This method is very general for representing curves carrying any kind of features designed by *a priori* knowledge, under the hypothesis that this feature is selected within the class of steerable filters. This work is a first step towards a more in-depth investigation of the method. We point out that precise statistical analysis of the coefficients can be easily performed due to the accuracy of the Fast Slant Stack method. This is a crucial advantage since it makes it possible to control the number of false alarms.

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Appendix
Normalization of coefficients

To simplify calculations, we change the parameterization (t, θ) of line integrals. Given a line $L = (x_c, y_c, l, \theta)$, centered at position (x_c, y_c), with length l and orientation θ, equation (3) can be rewritten, without any loss of generality, as

$$R[f * h^\theta](L) = \int_{-l/2}^{l/2} f * h^\theta(x_c + \gamma\cos(\theta), y_c + \gamma\sin(\theta))d\gamma. \tag{10}$$

Using the transitivity of convolution operator, equation (10) can be rewritten as $R[f * h^\theta](L) = f * H_b(x_c, y_c)$, where H_L is the filter detector of the whole line L defined as

$$H_L(x, y) = \int_{-l/2}^{l/2} h^\theta(x - \gamma\cos(\theta), y - \gamma\sin(\theta))d\gamma. \tag{11}$$

If we suppose now a noise model of the image f where pixels are i.i.d. normal variables $X_i \sim \mathcal{N}(0, \sigma^2)$, we have $R[f * h^\theta](b) \sim N(0, \sigma^2||H_b||_2^2)$. Then, the normalized coefficients are

$$\tilde{R}[f * h^\theta](L) = \frac{R[f * h^\theta](L)}{\sigma||H_L||_2}. \tag{12}$$

In general, equation (11) does not provide a closed form for every filter h. We choose a special class of filters defined in [11] and recalled in equation (8). These steerable filters are designed as a linear combination of derivatives of Gaussians and thus allow us to compute explicitly equation (11). To do so, since $L2$-norm is rotation-invariant, we compute equation (11) with $\theta = 0$; as a result, $||H_L||_2$ only depends on l and h and hence, can be easily precomputed.

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