# TWISTER SEGMENT MORPHOLOGICAL FILTERING.
# A NEW METHOD FOR LIVE ZEBRAFISH EMBRYOS CONFOCAL IMAGES PROCESSING.

*M.A.Luengo-Oroz*[§], *E.Faure*[‡], *B.Lombardot*[‡], *R.Sance*[§],
*P.Bourgine*[‡], *N.Peyriéras*[♮] *and A. Santos*[§]

[§]Biomedical Image Technologies lab., ETSI Telecomunicación, Universidad Politécnica de Madrid, Spain
[‡]Centre de Recherche en Epistémologie Appliquée, CNRS - École Polytechnique, Paris, France
[♮]DEPSN, CNRS - Institut de Neurobiologie Alfred Fessard, Gif-sur-Yvette, France

## ABSTRACT

We propose an extension of the classical morphological filtering based on openings by line segment structuring elements. It consists in filtering a 3D+time image with the opening by all the possible rotations of a segment in the 4D space, given an initial segment and a rotation angle. The method has been applied to remove noise from confocal laser scanning microscopy images of live zebrafish embryos engineered to fluorescently label all their cell membranes.

***Index Terms***— Biomedical image processing, image restoration, morphological operations, microscopy.

## 1. INTRODUCTION

Reconstructing the morphodynamics of cell division and pattern formation throughout embryonic development in live animals is of great interest for bio-medical research[1][2]. Among other applications, it would be the basis for a new generation of pre-clinical drug testing with automated systems for investigating drug effects at the cellular level *in vivo*. The reconstruction of cell morphodynamics greatly challenges *in vivo* imaging techniques as well as computer vision algorithms. The Embryomics project aims at fully reconstructing in space and time the cell lineage of the zebrafish (*Danio rerio*) embryo from the one cell stage throughout early steps of embryogenesis. This is achieved through time-lapse laser scanning microscopy that produces $z$ stacks of $xy$ images at every time step $t$ from live embryos engineered to highlight their cell structures. To segment cell structures and track cells requires very powerful methods of image treatment starting with image filtering. A large number of filtering methods have been described literature, nevertheless their suitability can be questioned in terms of computational cost, parametric optimization and use of contextual information. We show here that an alternative filtering method based on membranes geometry which is an extension of a classical morphological filter, removes noise from live zebrafish embryo 4D images in a very efficient way, with a predictable computational cost.

This work is supported by the spanish grant FPI-CAM(0362/2005) and the european projects Embryomics (NEST 012916) and BioEmergences (NEST 028892). We thank all the members of these projects for our fruitful multidisciplinary interaction and the anonymous reviewer suggestions.

## 2. TWISTER SEGMENT FILTERING

### 2.1. Preliminaries

A *grey-tone image* can be represented by a function $f : D_f \rightarrow T$, where $D_f$ is a subset of $\mathbf{Z}^2$ and $T = \{t_{min}, ..., t_{max}\}$ is an ordered set of grey-levels. Let $B$ be a subset of $\mathbf{Z}^2$ and $s \in \mathbf{N}$ a scaling factor. $sB$ is called *structuring element* $B$ of size $s$. The basic morphological operators are dilation $[\delta_B(f(x)) = \sup_{y \in B}\{f(x-y)\}]$ and erosion $[\varepsilon_B(f(x)) = \inf_{-y \in B}\{f(x-y)\}]$. These two elementary operations can be composed together in openings $[\gamma_B(f) = \delta_B[\varepsilon_B(f)]]$ and closings $[\varphi_B(f) = \varepsilon_B[\delta_B(f)]]$. The morphological opening (closing) filter out light (dark) structures from the images according to the predefined size and shape criterion of the structuring element. An algebraic opening $\gamma$ is increasing, idempotent and anti-extensive. One important property of openings is that any supremum of openings is still an opening. This property provides the possibility of extracting specific geometrical structures of different classes in the same filtering processing (i.e. openings and closings with line segments have been widely used in image processing to extract lines in an image [3][4]). In this paper we use the same notation that has been used in [5], so an opening by a structuring element $B$ is denoted $\gamma_B$. It is denoted also by $l_n^1, l_n^2, l_n^3, l_n^4$ the line segments of length $n$ and respective orientation $0^0, 45^0, 90^0, 135^0$. The operation $\Gamma_{n,d}^l = \vee_{i \in [1,d]} \gamma_{l_n^i}$ is an opening by line segments (see Fig.1). The two main drawbacks of this filtering is the computational cost (several independent openings and supremum calculation) and the selection of the size value $n$ (a priori knowledge of the analyzed image may provide useful information for the $n$ choice). The classical morphological alternatives to this approach are *openings by reconstruction* and *area openings*. Nevertheless both filters have their own drawbacks and the filtering by line segments is more easily parallelizable.

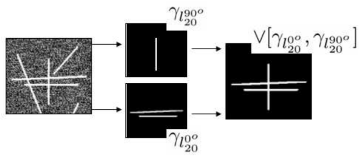


**Fig. 1**. The supremum of openings is still an opening.

## 2.2. Opening by twister segment structuring element

The proposed idea is to extend the classical filtering by line segment structuring elements to n-dimensional images. For that purpose, all the possible rotations of a line segment in a nD space will be found given an initial segment and a rotation angle (this angle is limited by the sampling rate). We found in [6][7] very interesting studies about morphological operators with discrete segments. For the sake of simplicity in the examples presented above we will allow a rotation angle of $45^o$, so the number of possible orientations in a $nD$ space is the number of boundary pixels in a $3^n$ box divided by 2. In a $2D$ image, if we note the initial segment $l_n^1$ with an orientation of $0^0$ and we permit a rotation angle of $45^o$, there exists $4$ possible segments $l_n^1, l_n^2, l_n^3, l_n^4$, where $l_n^i$ is rotated $45^o$ from $l_n^{(i+1)|4}$ so $\sharp l(dim = 2) = 4$. In these conditions, the opening by twister line segment is $\Gamma_{n,4}^l = \vee_{i\in[1,4]}\gamma_{l_n^i}$. In a $3D$ image, the interpretation remains the same as in the plane: we may think over the extraction of all the lines with specific directions embedded in the 3d space. A rotation angle of $45^o$ provides 13 possible orientations $\sharp l(dim = 3) = 13$. It should be noted that this opening allows also to extract the planes of an object which can be decomposed in lines of a given direction. We now address the spatio-temporal case. A 1D segment in the 4D space can be thought as a point which moves straight ahead in a given direction (or remains still) through time (see Fig.2). This forward movement may be seen as a differential of displacement, if the time sampling is high enough (images in two consecutive time steps are similar). The *twister segment structuring element* includes all these possible segments given an initial one and the rotation angle (note that the rotations in the 3D space are also included in this set). A rotation angle of $45^o$ provides 40 segments $\sharp l(dim = 4) = 40$.

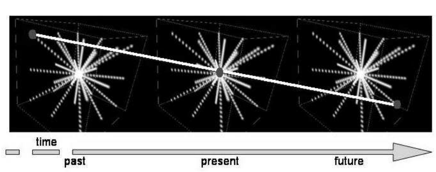



**Fig. 2**. A 1D segment structuring element in a 4D space passes through the origin(0,0,0,0) and can represent a point that moves in a straight direction over time, a fixed point over time or a segment in the 3D space in the present time.

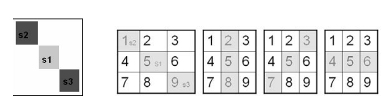
## 2.3. Algorithms

We propose an algorithm able to produce all the line segments of size $n$ which are embedded in the 4D space. Each segment is shifted $45^o$ from the previous one. The construction of each line segment is divided in two steps: finding the correct orientation and size. We define the unit structuring element of a direction $d$ as a 3 pixel line with a central pixel $S1$ and two symmetric pixels $S2$ and $S3$ (see Fig.3a). The unit structuring element is inside a n-dimensional cube of size 3. We denote the position in the $n$-dimensional cube with coordinates $(i_1, i_2, i_3, i_4, ..., i_n)$ as the position $(i_1 + i_2 * 3^1 + i_3 * 3^2 + i_4 * 3^3 + ... + i_n * 3^{n-1})$(see Fig.4). In this notation, by symmetry of the elements $S2$ and $S3$, we obtain the relation between positions $S2 + S3 = 2S1$. For instance in the 2D case $S1 = 5$, all the possible orientations are given by the combinations that make $S2 + S3 = 10$ (see Fig.3b). We proceed by analogy in the 3D case, $S1 = 14$ and $S2 + S3 = 28$, there exists 13 orientations(see Fig.4a). Finally in the 4D case, $S1 = 41$ and $S2 + S3 = 82$, there are $40$ orientations(see Fig.4b). In order to attain the length $n$ in a given orientation, a segment is dilated by the unit version of itself. As a result of several dilations, every segment of even size may be constructed $[\delta_{l_3^i}(l_n^i) = l_{n+2}^i]$. The described strategy leads to the following algorithm to implement the opening by twister line segment structuring element for 3D+time images with a rotation shift of $45^0$.

≫ **pseudocode twister segment filter (image in, length m) ⟶ (image out)**

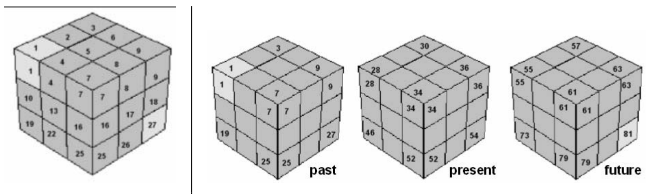
```
str.element se(2m+1,2m+1,2m+1,2m+1); .[a1]
str.element su(3,3,3,3);  ...........[b1]
for f =(1:40) {
    su(f)=1; su(82-f)=1; su(41)=1;  ..[b2]
    se(m+1,m+1,m+1,m+1)=1;  ..........[a2]
    for r = (1:m){se=δsu(se);}  .........[c]
    out=max(out,γse(in));  .............[d]
    reset su,se;
}
```

The unit structuring element $su$ of size $3^4$ is declared in [b1] and defined in [b2]. The structuring element $se$ is declared in [a1] and initializated in [a2]; $se$ is built by several dilations with $su$ in [c]. The filtered image $out$ is the maximum of all the openings by line segment in the 4D space[d].



(a) (b)

**Fig. 3**. (a) Unit structuring element. (b) Four possible directions in the 2D space with a shift of $45^0$ ($\sharp l(dim = 2) = 4$).




(a) (b)

**Fig. 4**. (a) Example of line structuring element embedded in the 3D space, formed by the points $(1, 14, 27)$. (b) Example of line structuring element embedded in the 4D space, formed by the points $(1, 41, 81)$.

## 3. CELL MEMBRANE FILTERING IN 4D IMAGES OF A LIVE ZEBRAFISH EMBRYO

We show an illustrative example of the use of the opening by a twister structuring element in 4D confocal images taken from a live zebrafish embryo with fluorescently labelled cell membranes. These images have been taken with a Leica SP2 laser scanning confocal microscope with a 40x/0,8NA water immersion objective. Image size is 512x512 pixels and voxel size is (0,58x0,58x1)$\mu m$. $Z$ stacks of $30\mu m$ have been taken every $5$ minutes for several hours starting at $3, 5$ hours of development at $28^o$C. Embryos are fluorescently stained through the expression of membrane bound mcherry red fluorescent protein. The validation of the filtering method is subjective since it should be coupled with segmentation methods. Nevertheless, it can be seen by eye that the filtering improves strongly the resolution of membranes and that the quality of the filtering is, at least, comparable to an anisotropic diffusion filter. In the presented example, the size of the line segment has been adjusted manually to seven pixels. The structuring element has been cropped in the time axis because of the low temporal sampling rate, so the line segments of the twister structuring element are embedded in a 4D box of size $(7, 7, 7, 3)$. In order to avoid abrupt jumps of intensity, the output is smoothed by a spherical averaging filter of two pixels radius. Cross-sections of original and filtered images are shown in Fig.5 and Fig.6. The method has also been tested with images in more advanced stages of embryonic development with very effective results. As embryo develops, cells differentiate and begin to build interesting structures by aligning their membranes and moving persistently in given directions. These behaviors make the twister segment filtering even more suitable for those cases than for early embryogenesis.

## 4. DISCUSSION

We have presented an extension of the classical morphological filtering based on opening by line segments. Our method performs the opening by line segments in the spatiotemporal space. This technique is suitable for images formed by polygonal shapes, huge quantity of data and uncorrelated noise over time. These characteristics are indeed those of confocal images taken from fluorescently labeled zebrafish embryos. The twister segment morphological filtering has been proved to be a very effective alternative to denoise cell membrane images. The proposed algorithm is a basic idea and further developments should be done, especially concerning the rotation shift. With a similar reasoning, we might perform morphological operations with the possible rotation of 2D planar structuring elements in the 4D space or curved line segments structuring elements that represent curved trajectories over time. The twister segment structuring element can serve also to perform other morphological operators for instance alternative sequential filters or multiscale granulometric analysis. Concerning real applications in the Embryomics project, the presented filtering methodology is currently being combined with a viscous watershed segmentation[8] producing very promising segmentation results (see Fig.7). The *oil-model* viscous flooding follows accurately the filtered membranes and prevents leakage when there are incomplete or missing boundaries in low contrast regions.

## 5. REFERENCES


[1] S.G.Megason and S.E.Fraser, "Digitizing life at the level of the cell: high-performance laser-scanning microscopy and image analysis for in toto imaging of development," *Mech.Dev.*, vol. 120, pp. 1407–1420, 2003.

[2] L.A.D'Amico M.S.Cooper and C.A.Henry, "Confocal microscopic analysis of morphogenetic movements," *Methods Cell Biol*, vol. 59, pp. 179–204, 1999.

[3] J.Serra, "Image analysis and mathematical morphology," *Academic Press, London*, vol. I,II., 1982,1988.

[4] J. Angulo and S. Matou, "Automatic quantification of in vitro endothelial cell networks using mathematical morphology," in *Visualization, Imaging, and Image Processing*. IAESTED, 2005, pp. 51–56.

[5] L. Vincent, "Morphological area opening and closing for grayscale images," in *Shape in Picture Workshop*. NATO, Springer-Verlag, 1992, pp. 197–208.

[6] P. Soille and H.Talbot, "Directional morphological filtering," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 23, pp. 1313–1329, Nov 2001.

[7] C.L.L.Hendriks and L.J.van Vliet, "Using line segments as structuring elements for sampling-invariant measurements," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 27, pp. 1826–1831, Nov 2005.

[8] C.Vachier and F.Meyer, "The viscous watershed transform," *J. Math. Imaging Vis.*, vol. 22, no. 2-3, pp. 251–267, 2005.

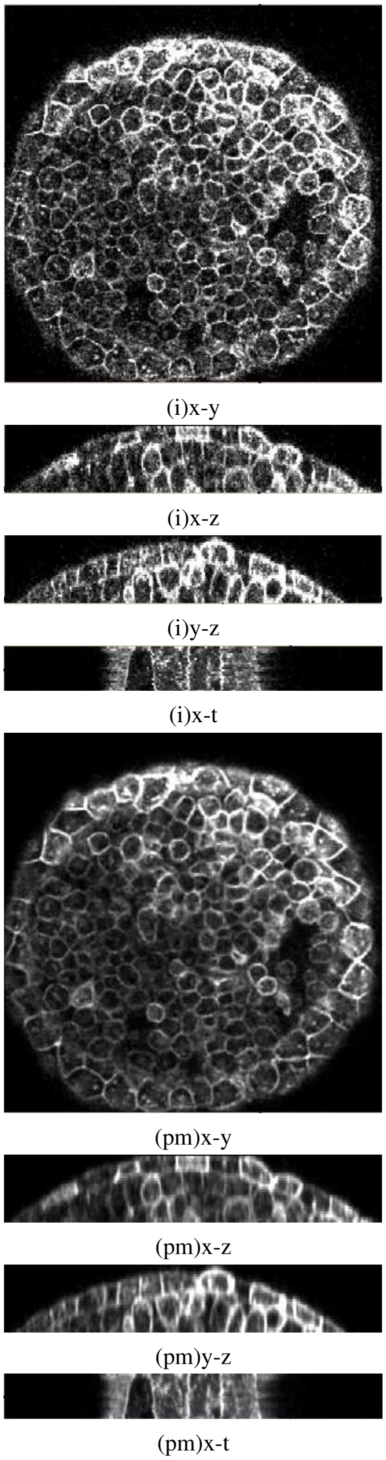



**Fig. 5**. (i) Cross sections of the original image. (pm) Cross sections of the image filtered by 4D anisotropic diffusion filter (Perona-Malik filter). The *ITK* method *[itk::GradientAnisotropicDiffusionImageFilter]* with parameters $iter = 5$, $t = 0{,}625$ and $k = 100$ has been used.

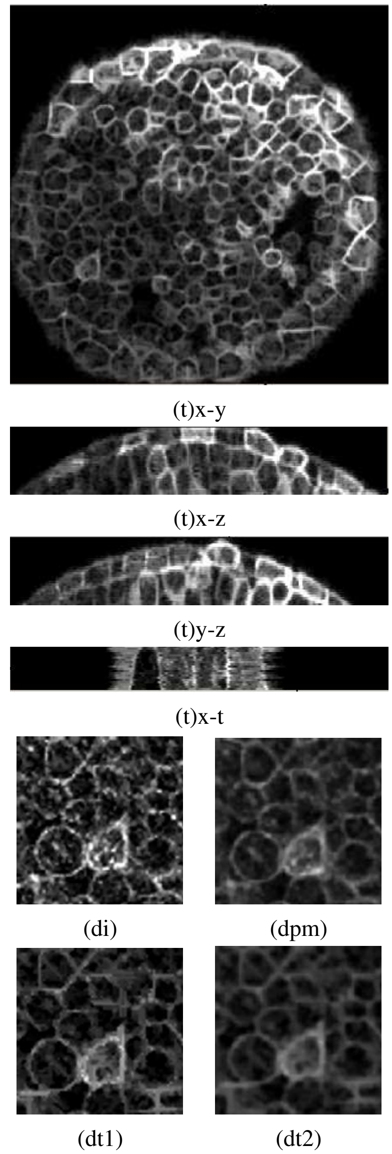



**Fig. 6**. (t) Cross-sections of the image filtered by the twister segment opening and regularized with a circular averaging. (di) Detail of the x-y cross section of the original image. (dpm) Detail of the image filtered by the Perona-Malik filter. (t1) Detail of the image opened with the twister structuring element. (t2) Detail of (t1) after circular averaging filter.

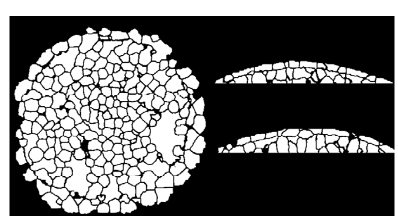


**Fig. 7**. Segmentation example produced by a viscous watershed flooding in the filtered image *t*. Contours align with cell membranes in (t)x-y, (t)x-z and (t)y-z.