Application of the linear scale space in segmentation of cells in microscopic images

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Introduction

Segmentation of touching cells, or cells having branching structures presents a major challenge for *intensity threshold*-based techniques. The situation can be further complicated in histological sections, where various extracellular structures overlap with the cells or structures of interest. In contrast, *regional approaches* benefit from existing spatial correlations of the data dictated by the underlying anatomical structures.

Multiscale approaches appear to be very promising in a variety of applications. *Linear scale space* is spanned by increasing convolutions of Gaussian kernels with the image under study. In the English speaking literature this approach was pioneered by Marr and Hildreth [4], who introduced a preprocessing Gaussian convolution step. While the approach has been conceptualized earlier in Japan in the context of scale space theory by Iijima [2] and his disciples.

Signal Processing

Isotropic smoothing

Interactions of the Gaussian kernels with the image can be viewed as realizations of a diffusion process and can be described well by the diffusion equation with an initial condition the original image $L_0(x) = I(x, y)$.

$$L_s(\mathbf{x}) = G_s * I = \int I(x)\, G_s(x - z)\, dz \tag{1}$$

The special properties of the Gaussian kernel rely on the fact that it is a generic solution of the diffusion equation where the scale of the kernel is viewed as ”time”

$$\frac{\partial G(x, y)}{\partial s} - \frac{1}{2}\nabla^2 G(x, y) = 0 \tag{2}$$

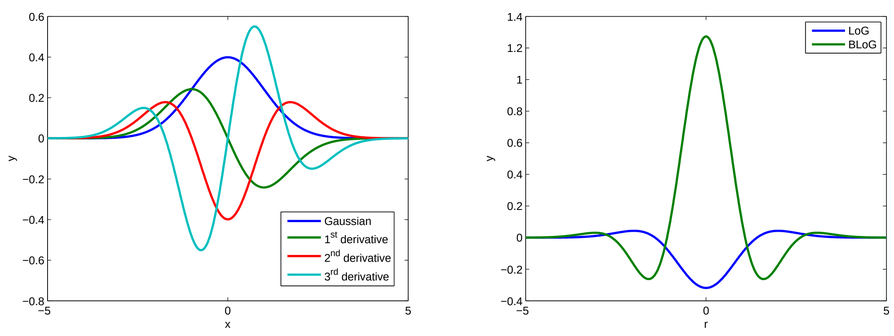

Figure 1: Gaussian derivative (GD) kernels. Laplacean of Gaussian (LoG) and Bi-Laplacean of Gaussian (BLoG) kernels

GD kernels can be combined isotropically in the Laplacian of Gaussian (LoG) or in a power of LoG (PLoG). The first resulting kernel is also known as the Mexican hat filter, while the second is denoted as Bi-Laplacean of Gaussian (BLoG):

$$\mathrm{LoG}_s(r) = -\frac{\left(2s - r^2\right) e^{-\frac{r^2}{2s}}}{2\pi s^3} \tag{3}$$

$$\mathrm{BLoG}_s(r) = \frac{\left(8s^2 - 8r^2 s + r^4\right) e^{-\frac{r^2}{2s}}}{2\pi s^5} \tag{4}$$

where r conventionally denotes the distance from the origin and s represents the scale. Edges in this way correspond to sign alternation in the filter response (zero-crossing event; see Fig. 1).

Isotropic edge-detection

Edges are characterized with a step increase in the image brightness. This fact can be modeled as estimation of a directional derivative. In order to stabilize the filter response against additive noise the derivation step can be combined with smoothing, which will result in convolving the original image with a derivative of the Gaussian kernel (GD).

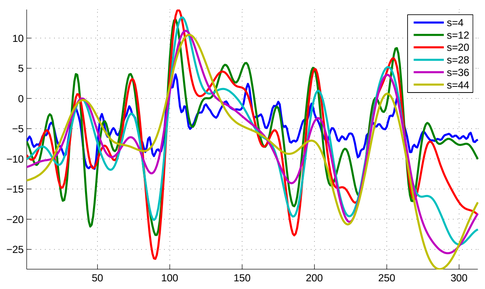

Figure 2: Response of of the LoG filter for several different scales. Scale space projection of Giemsa stained granulocyte (seen next two figures. Left: LoG operator zero set: Right: BLoG operator zero set. Note the possibility to discern nuclear features. Scales, s=4, 12, 20, 28, 36, 44 pixels. X – sample number; Y - arbitrary intensity units.

Implementation

In the concrete work-flow we use a combination of the Laplacean of Gaussian or Bi-Laplacen of Gaussian, detection of zero crossings and 4/8-connected component labeling. The algorithms are implemented as a set of independent ImageJ plugins.

Results

Segmentation of nuclear lobes

Blood smears have been freshly prepared and stained according to the standard Romanovsky-Giemsa protocol and observed under an inverted Zeiss Axiovert 40 microscope using a 20x objective. Images were taken using a 5 MPix Sony DSC W30 camera in RGB mode. Further processing is demonstrated on the green channel.

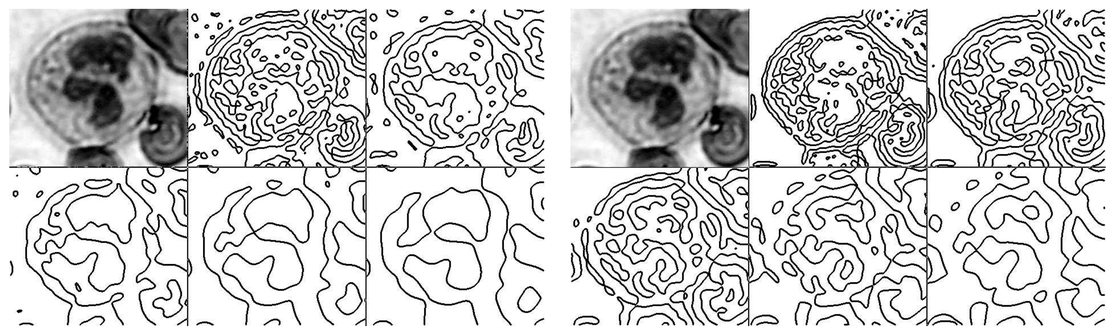

Figure 3: Scale space projection of Giemsa stained granulocyte. Left: LoG operator zero set: Right: BLoG operator zero set. Scales, s=4, 12, 20, 28, 36, 44 pixels.

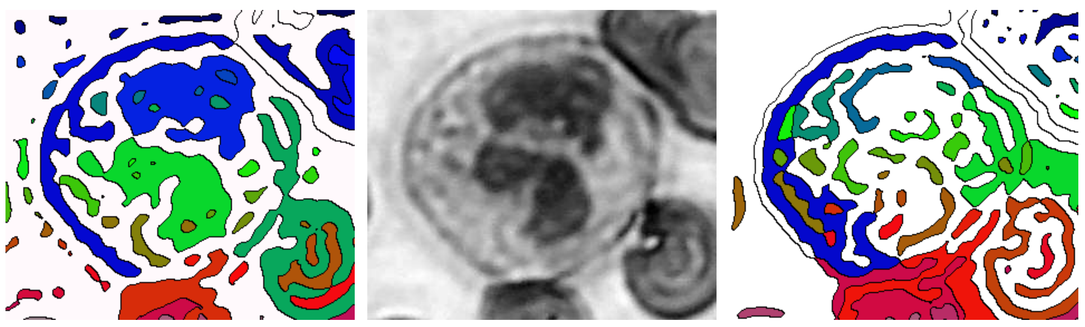

Figure 4: Blob segmentation based on median projections. LoG (left) and BLoG (right) operators were computed in the scales from 6 to 26 pixels. Blobs, representing distinct regions, were constructed from the 4-connected components of the complement to the zero-space. The original image is displayed in the middle for appreciation. Note the possibility to discern nuclear features.

Segmentation of astrocytes

Frozen cryostate sections (20 μm) of mouse brain were immunostained for GFAP using the following protocol: pre-incubation - 4h in 10 % normal goat serum, containing 0.1 % Triton-X 100; incubation - rabbit GFAP antibody (1:500) overnight at room temperature. Following secondary incubation for 4h with anti-rabbit Alexa-568 (1:500) in blocking buffer, the sections were cover-slipped with Vectashield-DAPI mounting medium. Confocal images were acquired on Zeiss 700 confocal microscope (Carl Zeiss Microimaging GmbH, Germany) using Plan Apochromat 10x objective at 16 bit dynamic range.

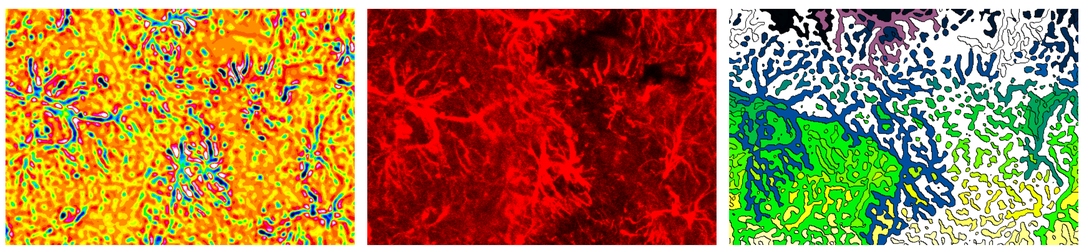

Figure 5: Blob segmentation based on median projections. LoG (Left) was applied in the scales from 5 to 14 pixels. Blobs (Right), representing distinct regions, were constructed from the 4-connected components of the complement to the zero-space. The original image is displayed in the middle for appreciation. Note the astrocyte depicted in navy blue for better appreciation.

Summary and Outlook

- Linear scale spaces operators demonstrate robustness against additive and impulse image noise and represent an alternative to threshold-based an mrophological techniques [3].
- Median projections across the image scale space are promising dimension reduction approach and can present an alternative to region growing methods [1]

Spaces spanned by the powers of the Laplacen operators represent a connection between scale space and wavelet theory. Indeed, when the convolution step is combined with a derivation, the resulting kernel fulfills the admissible conditions for a wavelet. In such way, the resulting transform becomes sensitive to a variety of local features of the image, which can be used for classification.

References

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