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Applications Note

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SpotCaliper: fast wavelet-based spot detection with accurate size estimation

Zsuzsanna Püspöki, Daniel Sage, John Paul Ward† and Michael Unser

Biomedical Imaging Group, École polytechnique fédérale de Lausanne (EPFL), Lausanne CH-1015, Switzerland

†Present address: Department of mathematics, University of Central Florida, Orlando, FL 32816, USA

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Abstract

Motivation: SpotCaliper is a novel wavelet-based image-analysis software providing a fast automatic detection scheme for circular patterns (spots), combined with the precise estimation of their size. It is implemented as an ImageJ plugin with a friendly user interface. The user is allowed to edit the results by modifying the measurements (in a semi-automated way), extract data for further analysis. The fine tuning of the detections includes the possibility of adjusting or removing the original detections, as well as adding further spots.
Results: The main advantage of the software is its ability to capture the size of spots in a fast and accurate way.
Availability and implementation: http://bigwww.epfl.ch/algorithms/spotcaliper/
Contact: zsuzsanna.puspoki@epfl.ch
Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

The focus of our article is the analysis of circular objects (spots), since their detection and size estimation is fundamental in the image processing of micrographs. Our method/software can be applied to a broad class of images; it is not linked to one specific biological problem. However, we note two particular applications, where the precise detection of the spots and their radius estimation is of high interest. First, in antimicrobial susceptibility testing of significant bacterial isolates, the size of the zones of the inhibition areas are measured and used as an indicator of antibiotics resistance (susceptible, intermediate or resistant) (Jorgensen, 2009). Second, detection and size measurements are also important for understanding ELISpot images (Lehmann, 2005).

2 Features and methods

There are several existing methods for the detection of circular objects and measuring their radii. Some typical examples cover morphological operators, the Circle Hough Transform, the Laplacian of Gaussian (LoG) and its multiscale extension (Lindeberg, 1998), the determinant of Hessian (DoH) and wavelet-based techniques (Olivo-Marin, 2002). Dyadic wavelet schemes are considered a powerful tool and they are widely used for feature extraction. They provide a multiscale decomposition scheme and allow a high level abstraction from the data. However, the dyadic hierarchy of the typical wavelet schemes introduces difficulties in the precise estimation of the size of the objects, since the between-scale details are diffused among the wavelet coefficients.

In the presented software, we use a novel wavelet scheme that we have developed. Our wavelets approximate intermediate scales by using several patterns (channels) that can be linearly combined with adjustable coefficients, to create disk patterns with continuously varying sizes in between two sequential (discrete) wavelet scales.

The starting point for our algorithm is a dyadic tight wavelet frame that provides a redundant multi-scale decomposition of an image (in particular, we use a Meyer-type tight wavelet frame (Daubechies, 1992). We generate our scalable wavelet frame using scalable filters. A filter is *scalable* if it and all of its scaled versions

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can be expressed in a finite basis. We apply a particular basis of scalable filters to our dyadic tight wavelet frame to create wavelet channels, and the resulting wavelet frame is arbitrarily scalable.

The wavelet coefficients of an image f at location x and dyadic scale a are computed as $g(x) = \langle f, \Psi_a(\cdot - x)\rangle$, where

$$\Psi_a(\cdot - x) = \sum_n q_n(a)\Psi_n(\cdot - x), \quad (1)$$

with Ψ denoting the scalable wavelet, n the channel, and q_n the wavelet coefficients with respect to the channels. The '$\cdot$' symbol represents the implicit (hidden) variable. Thus, we obtain

$$g(x) = \sum_n q_n(a)\langle f, \Psi_n(\cdot - x)\rangle = \sum q_n(a)g_n. \quad (2)$$

Since our wavelets, generated by such a method, are scaled continuously, we estimate the scale of objects much more precisely than what is afforded by traditional dyadic wavelet schemes. It is enough to perform the filtering operations once, the response for any specific scale being retrieved by the linear combination of a few precomputed components. Due to the scaling property of the detector wavelet, the detections are obtained in a fast and computationally effective way. The in-between-scale details are not lost, thanks to the scalable design. We note that our algorithm is able to handle overlapping spots as well.

For a thorough description on the mathematical background of our algorithm, we refer to (Püspöki *et al.* 2015).

3 The spot detection and size estimation algorithm

The detection algorithm comprises the following steps:

1. **Wavelet analysis with the proposed wavelet scheme**: We compute the wavelet coefficients for each pixel.
2. **Local maxima detection**: We apply a local non-maximum suppression on the magnitude of the wavelet coefficients in order to prevent multiple detections of the same spot.
3. **Fine estimation of the radius**: Based on the set of candidate points selected in step (2.), we provide an estimation of scale at those locations. Since our wavelets are scaled on a quasi-continuum, we obtain precise results; the local scale is chosen to maximize the detector response of the proposed wavelet.
4. **Selection**: We choose the best N results based on an image-adapted measure (e.g. highest detector response).
5. **Visualization of the detections**: The user is able to edit the results and their visualization in several ways. For further information, we refer to the supplementary material.

4 Results and validation

To evaluate the performance of the algorithm, we tested it on a variety of synthetic images in the presence of background signal, and finally on real microscope images. We generated a series of test images of size (512 × 512). The location of the spots and their radii was chosen in a way that the ground truth data contained 50 disks, with radii varying between 8 and 40 pixels. Overlap was allowed between neighboring objects, by at most 10 pixels. The results are summarized in Figure 1. For a thorough description of the evaluation and the corresponding experiments we refer to our supplementary material called Experiments.

Based on the graphs (Fig. 1A and B) we can confirm that our method performs better than other competing methods with respect to computational time, accuracy, and robustness against background with varying intensity. The quality of the detections are illustrated on synthetic data (Fig. 1C) and biological micrographs (Fig. 1D and E). The original test images and the corresponding parameter settings are presented in the Experiments supplementary material.

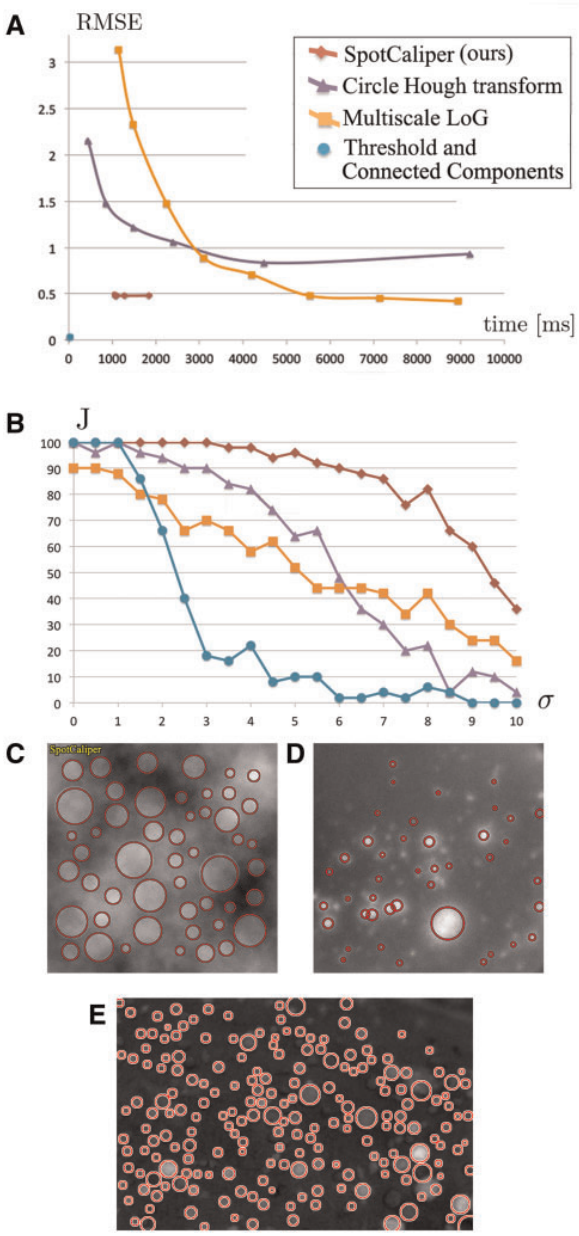

Fig. 1. (**A**) RMS error of radius estimation as a function of running time; noise free case. (**B**) Rate of detections estimated by the Jaccard index J under isotropic background signal, as a function of the standard deviation σ of the background fluorescence. (**C**) Detections using our wavelet-based method on an image with isotropic Brownian motion (mean 0, SD 4). (**D**) Semi-automated detection of spots on an ELISpot image. (**E**) Automatic detection and scale estimation of fluorescence cells on a microscopy image

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Conflict of Interest: none declared.

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