Performance evaluation of spot detection algorithms in fluorescence microscopy images

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ABSTRACT
Detection of messenger Ribonucleic Acid (mRNA) spots in fluorescence microscopy images is of great importance for biologists seeking better understanding of cell functionality. Fluorescence microscopy and specific staining methods make biological molecules appear as bright spots in image data. Manual analysis of such data is both time-consuming and laborious, and can lead to errors.

In this study, we compare two computer-based detection methods used in fluorescence microscopy images for the detection of spots. The algorithms compared are the Isotropic Undecimated Wavelet Transform (IUWT) and the Feature Particle Detection (FPD). The performance of these algorithms is validated on synthetic images. Our study finds a major difference in the performance of the two algorithms. IUWT performs better than FPD.

INTRODUCTION
In recent years, advances in molecular and cell biology have triggered the development of a highly sophisticated imaging tool known as fluorescence microscopy. This is used to visualise and study intracellular processes. The use of fluorescence microscopy and a specific staining method make biological molecules appear as bright particles (spots) on image data (see **Figure 1**).

Spot detection is a fundamental step for biologists to better understand intracellular processes. Although manual analysis of spots can yield the most accurate results, it is very time consuming, and impractical for use on large data sets. Therefore, it is useful to use computer-based algorithms to automate this process.

In this study, we compare the performance of two computer-based detection methods used for the detection of bright particles in fluorescence microscopy.

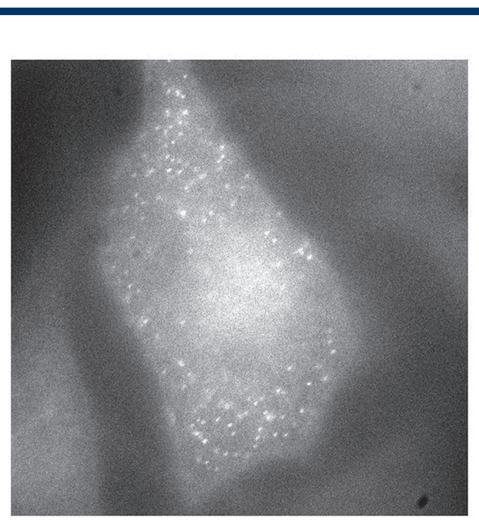


Figure 1: Sample image of Hela Cells obtained using fluorescence microscopy

DETECTION METHODS
Feature particle detection (FPD)
The method of feature particle detection was proposed by Sbalzarini and Koumoutsakos [1], for detection of gold colloids in micrographs.

The algorithm consists of four steps:

1. Image restoration step: this step corrects imperfection in the image by using a box-car average estimation and simultaneously enhances spot-like structures by convolving it with a Gaussian kernel.
2. Estimating the particle location: this is done by locating local intensity maxima in the filtered image. A point is considered to be local maxima if it has the highest intensity within a local window.
3. Refining the particle location: this step reduces the standard deviation of the position measurement to less than 0.1 per pixel.
4. Non-particle discrimination: this step rejects false identifications such as auto fluorescence signals, dust and fluorescent vessels. This step is based on the intensity moments of order 0 and 2. A detailed description of the discrimination step can be found in Sbalzarini and Koumoutsakos [1].

Isotropic Undecimated Wavelet Coefficient (IUWT)
The method of IUWT was first used in astronomical applications [2] to detect isotropic objects, and was then introduced to biological applications [3]. Olivio-Marin[3] approached the problem of feature extraction based on undecimated wavelet representation of the image, and on the selective filtering of wavelet coefficients. The algorithm is based on the assumption that spots will be present at each scale of wavelet decomposition and thus will appear in the multiscale product. A detailed algorithm can be found in Olivio-Marin[3].

EXPERIMENTS
Experiments with synthetic images
One of the fundamental problems in evaluating the performance of algorithms for spots detection in fluorescence microscopy images is that one is never certain about the number of spots in images. Therefore, when evaluating and analysing different detection methods applied to fluorescence images, synthetic images were created with a known number of spots on them (**Figure 2**).

Each synthetic image consists of 512×512 pixels containing 10×4 2D Gaussian intensity spots with decreasing intensity across the rows and decreasing in radius across the columns. A Gaussian noise (σ ranging from 2 to 10) was added to each image resulting in noisy images (**Figure 2**).

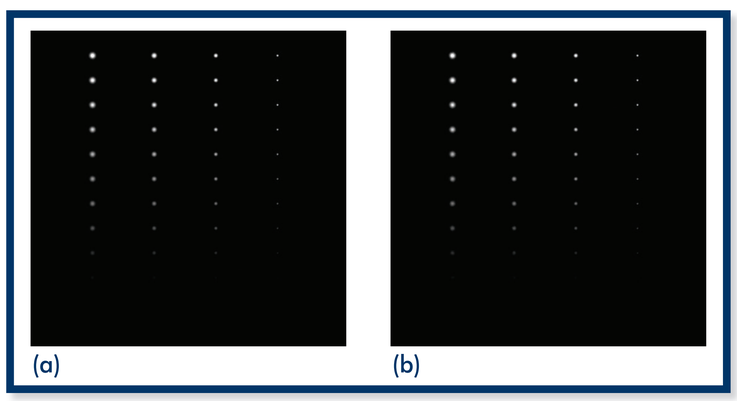


Figure 2: Examples of synthetic images with different noise levels (σ) (a) σ = 2, (b) σ = 8

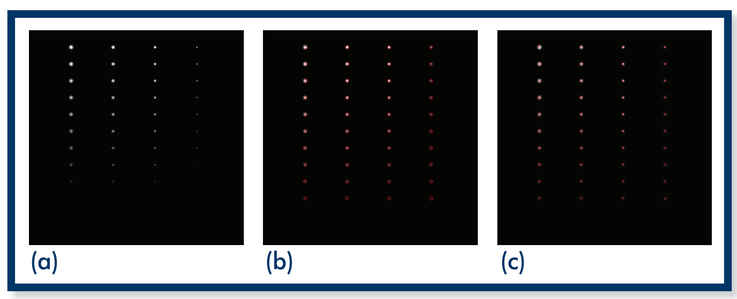
RESULTS


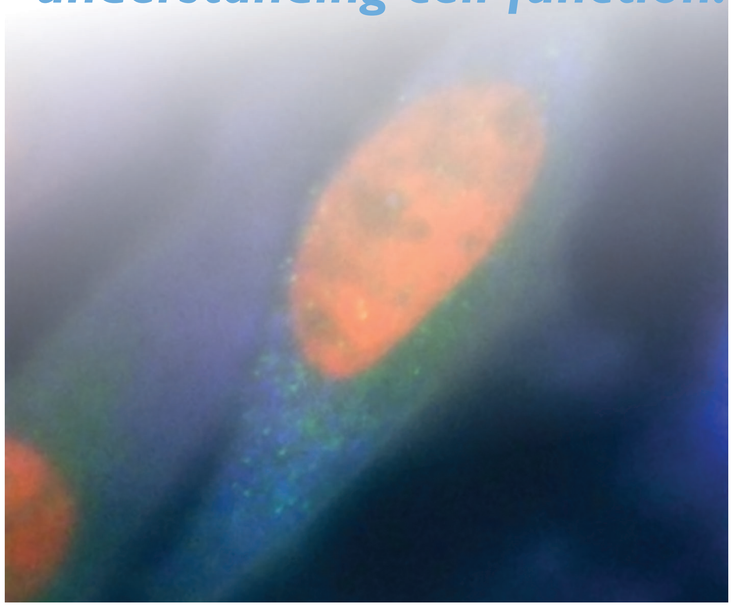
Figure 3: Spot detected in a noisy synthetic image (a) Synthetic noisy image with additive Gaussian noise (σ = 6), (b) Detected spots in red using IUWT, (c) Detected spots with FPD

For quantitative comparison, we evaluated the performance of the two methods by computing the true positive rate (TPR) and false positive rate (FPR). We define N_{TP} as the number of true positives and N_{FP} as the number of false positives. Then $TPR = N_{TP}/((N_{TP}+N_{FN}))$, and $FPR = N_{FP}/((N_{TP}+N_{FN}))$

Table 1: The table shows the ratio of true positives and false positive rates

Test datasets		*IUWT*	*FPD*
1	TPR	0.977	0.975
	FPR	0	0
2	TPR	0.975	0.975
	FPR	0.025	0.025
3	TPR	0.951	0.941
	FPR	0.075	0.252
4	TPR	0.905	0.730
	FPR	0.191	0.269
5	TPR	0.892	0.62
	FPR	0.275	0.33

We are using algorithms for image analysis and computer vision to automatically detect the position of molecules within fluorescence microscopy cell images. This information is important to biologists who are interested in understanding cell function.



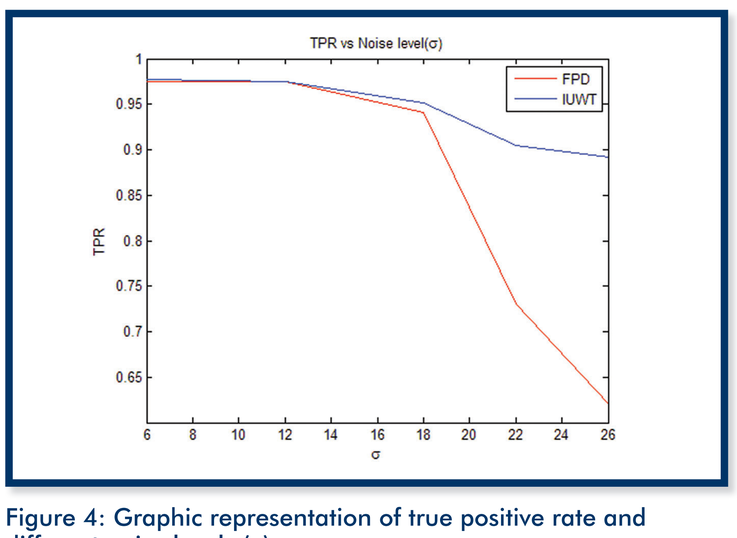


Figure 4: Graphic representation of true positive rate and different noise levels (σ)

CONCLUSION
In this paper, we introduced two methods for particle detection and compared their performances using synthetic images. The results from experiments indicated that IUWT performs better than FPD, and is less sensitive to noise than FPD.

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