Comparison of two detection algorithms for spot tracking in fluorescence microscopy images

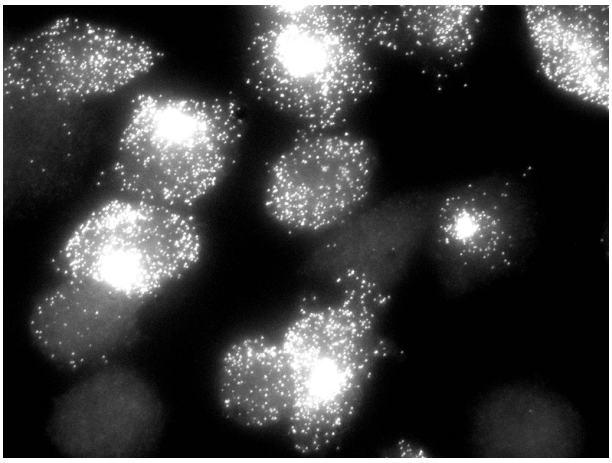
Matsilele Mabaso*, Daniel Withey‡, Bhekisipho Twala†
* ‡MDS(MIAS)
Council for Scientific and Industrial Research
Pretoria, South Africa,
Email: *MMabaso@csir.co.za
†Department of Electrical Engineering
University of Johannesburg
Auckland Park, South Africa

***Abstract*—Research in biological image analysis plays an important role in understanding the underlying mechanisms of cellular processes, which may lead to better knowledge of certain aspects of the cell function. The primary analysis of biological images requires the detection and tracking of hundreds of spots. In this paper we presents an approach for the tracking of spots in microscopy images based on the modification of the algorithm presented by Feng et al. The improved algorithm consists of replacing the original detection algorithm, Feature Point Detection(FPD) with Isotropic Undecimated Wavelet Transform (IUWT). The tracking algorithm based on Interacting Multiple Model(IMM) remains fixed. The performance of the presented method, IMM-IUWT along with two others, MHT, based on Multiple Hypothesis Tracking (MHT) and IMM-FPD, were validated on numerous challenging realistic synthetic image sequences, and their performance was evaluated using root mean square error (RMSE) metrics. The results indicate that the presented method outperforms the original method. At high level of signal to noise ratio (SNR), it is noted that the performance of the modified method, IMM-IUWT, is similar to that of MHT method. The quantitative comparative results demonstrated the importance of spot detection in tracking contexts.**

I. Introduction

In recent years, the field of fluorescence microscopy has been improved and automated, and a large volume of image data are being generated in biology and biomedical fields. The issue of how to accurately process and analyze the data becomes one of the major issues in the field because manual analysis is not practical anymore [1]. This issue has drawn much attention to systems for bioimage analysis based on computer vision and image processing. Biological images obtained through fluorescence microscopy contain a wealth of objects appearing in the images as bright spots. These spots exhibit a Gaussian-like appearance with high mean intensity compared to their background, as shown in Figure 1. The analysis of these images involves the detection and tracking of these bright spots. Tracking of these spots is becoming a requisite for describing biological processes and the working mechanisms in the living cells.

The key idea of spot tracking is to establish correspondence between spots in a sequence of images in order to construct their trajectories throughout the time-lapsed sequence. However, there are several challenges which hinder the construction of a robust spot tracking methods. These challenges include high levels of noise, inhomogeneity in microscopy images, temporary high spot density, spot disappearance, spot merging, spot splitting and complex motion patterns. Several tracking methods have been proposed to overcome these challenges but often fail to yield satisfactory results in cases of the above mentioned problems [3].



(a)

Fig. 1. (a) Example of a real fluorescence image containing multiple bright spots [2]
.

In this paper, we introduce a modified tracking algorithm based on modifying the original tracking method developed by Feng et al. [4]. The algorithm presented in this paper, replaces the detection algorithm with the Isotropic Undecimated Wavelet Transform (IUWT) [5]. Based on our previous work [6], it is noted that the IUWT performed well compared to other methods. The new approach has been applied to realistic, synthetic image sequences containing multiple spots. The results are then compared with tracking methods proposed in Feng et al. [4] and Chenouard et al. [7].

The layout of the paper is as follows: Section II discusses various studies on tracking. Section III, IV and V describe the various algorithms used in the experiments. Section VI presents the performance measures and in section VII experiments are discussed. Section VIII discusses the experimental results, and finally, the conclusions are given in section IX.

II. Related Work

Several studies have investigated the tracking of spots in microscope images and a number of methods have been proposed to resolve the tracking issues but often fail to yield satisfactory results in cases of high level of noise, complex motion patterns and high spot density [3]. One approach for spot tracking is based on the nearest neighbor method. However, this approach is most useful for spots which are far apart and with low density, which is not the case in fluorescence microscopy images. In cases of high spot density the search strategy employed by nearest neighbor becomes ambiguous since several correspondences are possible. Recently, several methods for particle tracking have been proposed [8]–[10], which can help to overcome the above mentioned problems.

Sbalzarini et al. [8] presented a 2-D feature tracking method for the automatic detection and analysis of particle trajectories in video imaging. The tracking algorithm requires no assumption about the motion model, it is self initialized, discriminates spurious detections and can handle temporary occlusion as well as particle appearance and disappearance. The algorithm, as presented in [8], starts by finding the particle locations using feature point detection [8] then employs a motion correspondence method for data association based on a graph theoretical approach to solving the transportation problem [11].

Genovesio et al. [9] presented a method for tracking of multiple moving spot-like particles showing different kinds of dynamics. The method uses the 3-D undecimated wavelet transform to detect spots and the prediction of spot future states is accomplished by the Interacting Multiple Model (IMM) algorithm [12]. Several models corresponding to different biologically realistic movement types are used. Then, association is performed to establish the trajectories based on the maximum likelihood of the innovation among the IMM filters. The last step consists of updating the filters to compute the final estimate.

Similar to Genovesio et al. [9], Feng et al. [4] used an IMM filter to predict and maintain the particle state integrated with the feature point detection algorithm presented in [36]. A data association method based on multidimensional assignment was applied to establish tracks. They combine IMM filter, Feature Point Detection [8], multidimensional assignment, particle occlusion handling, and merge-split events in a single software package.

Chenouard et al. [13] studied the movement of spots in biological images using a Multiple Hypothesis Algorithm. The proposed algorithm, first detects spots in images using Isotropic Undecimated Wavelet Transform (IUWT) [5].

An investigation which was done by Cheezum et al. [14] focused on evaluating some popular tracking methods in microscopy images. The authors evaluate the performance of four non-probabilistic tracking algorithms using only synthetic images and only for particle localization. It was found that the performance of the tracking methods deteriorates as the signal to noise ratio of the images decreases. However, the study imposed certain limitations. First they were interested in the localization error while the correspondence findings were ignored, no real images were used and only non-probabilistic methods were considered.

The recent study by Chenouard et al. [15] investigated the performance of 14 tracking methods using synthetic image sequences. This study included most of the tracking methods, however, it did not include the algorithm presented in Feng et al. [4]. The evaluation of the methods was based on synthetic images of different scenarios. It was found that at present no single method performs best in all scenarios.

III. Detection Methods

A. Feature Point Detection (FPD)

The method of feature point detection was proposed in [16] and used for the detection of bright particles in [8].

The algorithm consists of four steps:

1) Image restoration step: this step corrects imperfections in the image by using a box-car average estimation and simultaneously enhances spot-like structures by convolving with a Gaussian kernel. The convolution kernel is given by:

$$K^w = \frac{1}{K_0^w}\left[\frac{1}{B}exp(-\frac{i^2+j^2}{4\lambda_n^2}) - \frac{1}{(2w+1)^2}\right], \quad (1)$$

where K_0^w and B are normalization factors, λ_n defines the kernel width and w is the user tunable constant. Thus the image after restoration is given by:

$$I_f(x,y) = \sum_{i=w}^{w}\sum_{j=w}^{w} I(x-i, y-j)K^w(i,j), \quad (2)$$

where(x,y) and (i,j) are pixel coordinates in the image and kernel respectively.

2) Estimating the particle location: this is done by locating local intensity maxima in the filtered image, $I_f(x,y)$. A local maximum is considered to be a spot if it has the highest intensity within a local window and the intensity is in the r^{th} highest percentile.

3) Refining the particle location: this step reduces the standard deviation of the position measurement. It is based on the assumption that the local intensity maximum of point P at $(\hat{x}_p, \hat{y}_p)$ is near the true geometric center (x_p, y_p) of the spot. The offset is approximated by the distance to the gray-level centroid in the filtered image $I_f(x,y)$:

$$\begin{bmatrix} \varepsilon_x(p) \\ \varepsilon_y(p) \end{bmatrix} = \frac{1}{m_0(p)} \sum_{i^2+j^2 \le w^2} \begin{bmatrix} i \\ j \end{bmatrix} I_f(\hat{x}_p+i, \hat{y}_p+j)$$

Factor $m_0(p)$ is the sum of all pixel values over a feature point P given as:

$$m_o(p) = \sum_{i^2+j^2 \le w^2} I_f(\hat{x}_p + i, \hat{y}_p + j) \quad (3)$$

Then the refined location estimate is determined as:

$$(\tilde{x}_p, \tilde{y}_p) = (\hat{x}_p + \varepsilon_x(p), \hat{y}_p + \varepsilon_y(p)) \quad (4)$$

4) Non-particle discrimination: this step rejects false identifications such as auto fluorescence signals and

dust. This step is based on the intensity moments of order 0 and 2, and identifies true spots as those within a cluster in the m_0, m_1 plane. A detailed description of the discrimination step can be found in [8].

B. Isotropic Undecimated Wavelet Transform (IUWT)

The method of IUWT was proposed in [5] for the detection of spots in biological images. 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.

The algorithm starts by convolving the image $I(x, y)$ row by row and column by column with a symmetric low pass filter $h = [1, 4, 6, 4, 1]/16$, resulting in a smoothed image $I_1(x, y)$. The process is repeated for J scale levels, augmenting the filter with $2^{i-1} - 1$ zeros between taps in each case. The corresponding wavelet coefficients $W_i(x, y)$ are given as:

$$W_i(x, y) = I(x, y) - I_1(x, y) \tag{5}$$

Then a hard thresholding is applied to reduce the effect of noisy wavelet coefficients with $t_i = k\sigma_i$, where σ_i is the standard deviation of the noisy wavelet coefficients at scale i and $k = 3$.

$$t_{hard}(W_i, t_i) = \begin{cases} W_i(x, y), & W_i(x, y) \geq t_i, \quad (6) \\ 0, & W_i(x, y) < t_i \quad (7) \end{cases}$$

Thus after hard thresholding, a multiscale product of each wavelet coefficient is computed to get a correlation image $P_J(x, y)$

$$P_J(x, y) = \prod_{i=1}^{J} W_i(x, y) \tag{8}$$

All the values in the correlation image are compared to predetermined detection level l_d to discriminate between particle and background, and get a binary image of particles. A spot is accepted only at positions where the correlation is above l_d,

$$P_J(x, y) = \begin{cases} 255, & |P_J(x, y)| \geq l_d, \quad (9) \\ 0, & Otherwise \quad (10) \end{cases}$$

IV. Tracking Methods

A. Interacting Multiple Models (IMM)

The IMM filter is a state estimation algorithm for systems with multiple motion models. Internal model changing is based on a finite state Markovian switching coefficient. The algorithm was first developed for radar tracking systems [12], and introduced to biological applications in [4], [9]. The generic IMM filter using the Kalman filter as proposed in [9] contains several models of linear systems:

$$x_k = A_k^j x_{k-1} + w_{k-1}^j \tag{11}$$

$$z_k = H_k^j x_k + v_k^j \tag{12}$$

Where x_k is the system state at time k, j denotes the index of models, A_k^j is the state transition matrix for model L_k^j at time k and $w_k^j \sim N(0, Q_k)$ is the process noise. z_k is the measurement state, H_k^j is observation matrix for model L_k^j at time k, and $v_k^j \sim N(0, V_k)$ is the measurement noise. The n models of the IMM filter form a discrete set denoted as: $L = \{L^1 ... L^n\}$ and the probability of switching from L_{k-1}^i to L_k^j given as: $\pi_{ij} = P\{L_k^j | L_{k-1}^i\}$.

B. Multiple Hypothesis Tracking

The Multiple Hypothesis Tracking method was previously used in radar tracking and video surveillance [17]–[19] and proposed for biological particle tracking by Chenouard et al. [13]. The algorithm is based on Bayesian tracking principles. The main idea of this algorithm is to build a set tracks that maximizes the likelihood, $\mathcal{L}(B^m)$, of the associations between tracks, B^m, and measurements, Z^m, from the sequence of m images:

$$B^i \triangleq \arg \max_{B^m \epsilon \Omega^m} \mathcal{L}(B^m) \tag{13}$$

Where, Ω^m is the set of hypothesis tracks and, $\mathcal{L}(B^m)$, the likelihood of the tracks is defined as:

$$\mathcal{L}(B^m) = P\{B^m | Z^m\} \tag{14}$$

A detailed description of the algorithm can be found in [7], [20].

V. Detection and Tracking

A. IMM-FPD

The algorithm presented by Feng et al. [4], first uses the Feature Point Detection (FPD) [8] method to detect spots in time lapse image sequence. Then, the Interacting Multiple Model (IMM) method is used to model the movements of spots and generate cost values for particle linkage. The data association based on multidimensional assignment [4] is used to establish tracks.

B. IMM-IUWT

The proposed method in this paper is based on the original tracking algorithm presented by Feng et al. [4] but we replaced the original spot detection, Feature point Detection (FPD), with, Isotropic Undecimated Wavelet Transform (IUWT) [5]. The IUWT method is applied to image sequences to provide spot locations in each frame, then these spot locations are linked to form tracks based on the tracking algorithm presented by Feng et al. [4] using the Interacting Multiple Model (IMM) filter.

C. MHT

The Multiple Hypothesis Tracking method presented by Chenouard et al. [7] uses the detections provided by Isotropic Undecimated Wavelet Transform(IUWT) [5] detector, then these detections are then linked to form tracks based on a Bayesian framework. The MHT algorithm aims at building iteratively the set of tracks that maximizes the likelihood in equation (14).

VI. Evaluation

The particle tracking benchmark generator plugin [20], [21] in the ICY software [22], [23] from Institut Pasteur in France, was used to create the synthetic image time sequences. The plugin creates realistic $2D/3D+$ time fluorescent particles. By adjusting parameters in the plugin configuration text file, different characteristics of particles can be achieved, for example, particle motion type, number of particles, background noise, particle intensity, and dimension and length of sequences.

In order to test the performance of the different algorithms, the Root Mean Square Error (RMSE) was used. This measure indicates the overall accuracy of matching points in the paired tracks and was successfully used in Chenouard et al. [15].

$$RMSE = \sqrt{\frac{\sum(y_i - \hat{y}_i)^2}{n}} \tag{15}$$

Where y_i is the ground truth value for i^{th} observations and $\hat{y}_i$ is the predicted value (value reported by the algorithm) and n is the number of observation. This measure is implemented in an Icy [22] plugin called tracking performance measures [24].

VII. Experimental set-up

A. Experiments with synthetic data

The performance of the proposed tracking methods was evaluated using synthetic (with ground truth) image sequences, as shown in Figure 2.

Six types of synthetic image sequences, Seq A, Seq B, Seq C and Seq D, Seq E, Seq F, were created using the synthetic data benchmark generator [21]. These synthetic sequences simulated different imaging conditions and different spot movement. Each synthetic sequence was a two dimension plus time $(2D+t)$ image with size of 512×512 pixels containing multiple spots in random locations. The length of the time sequences was fixed at 50.

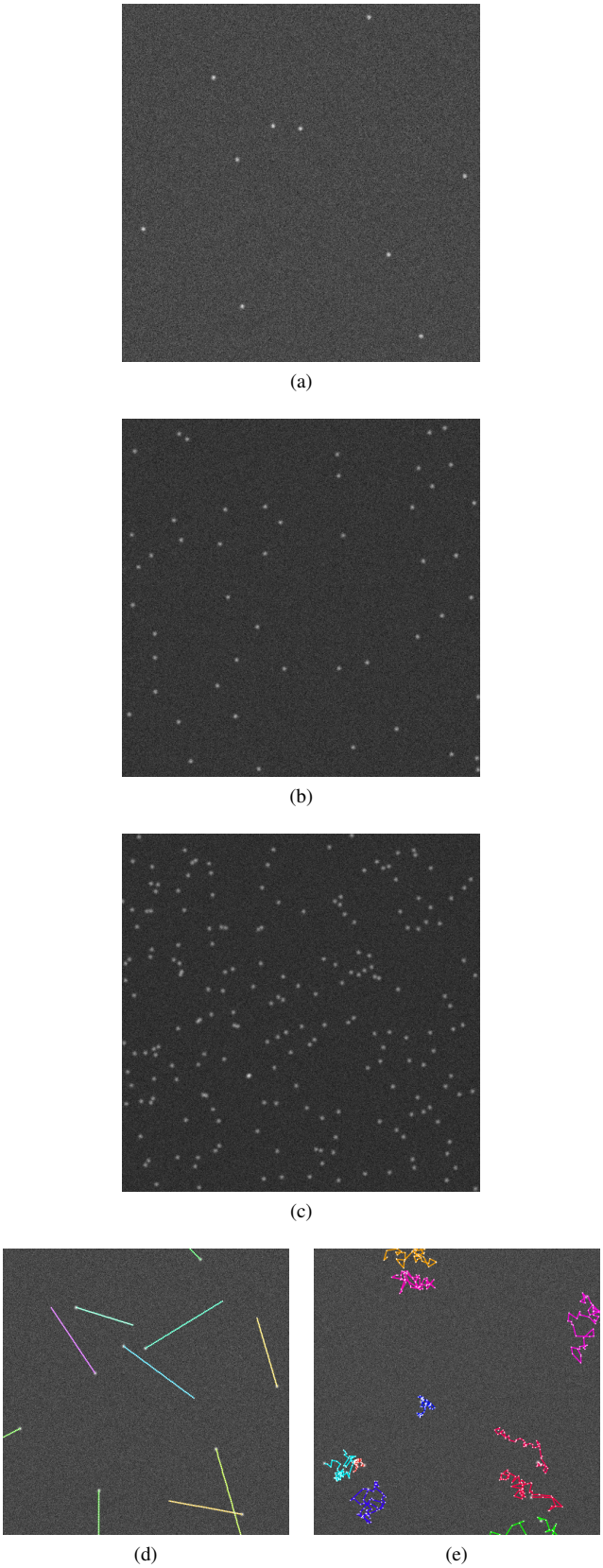
The first three sequences (Seq A, Seq B and Seq C) contained 10, 50 and 200 spots respectively, moving in linear motion with random directions, and with randomly varying velocity between $v_{min} = 2$ and $v_{max} = 4$. Gaussian noise was then added to each sequence resulting in noisy synthetic sequences with signal to noise ratio (SNR) values of $\{10, 7, 5, 4, 3, 2, 1\}$. SNR in our experiments was defined as the ratio of spot intensity, I_{max}, divided by the noise standard deviation, σ_{noise}:

$$SNR = \frac{I_{max}}{\sigma_{noise}} \tag{16}$$

The last three sequences (Seq D, Seq E, Seq F) contained 10, 50 and 200 spots moving with Brownian motion and with the standard deviation of the motion fixed at $sigma_bMax = 4$ and $sigma_bMin = 2$. Gaussian noise was added to produce SNR values of $\{10, 7, 5, 4, 3, 2, 1\}$.

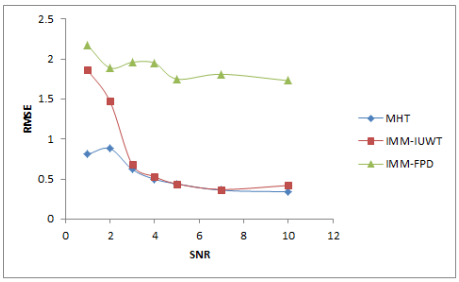
VIII. Experimental results and discussion

In this section, the performance of the proposed tracking methods is evaluated using synthetic images with ground truth by computing the root mean square error.

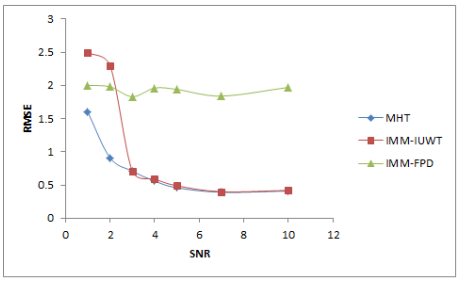


(a) (b) (c) (d) (e)

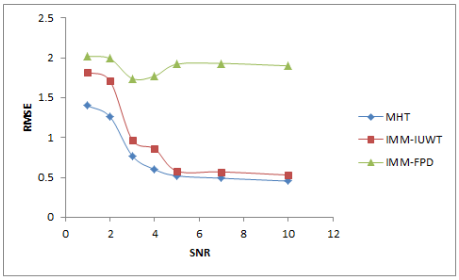
Fig. 2. Examples of synthetic images used in the experiments. (a, b, c) shows the images with increasing number of spots, 10, 50 and 200. (d and e) shows the types of motions considered in our study, linear motion (d) and Brownian motion (e).



(a)

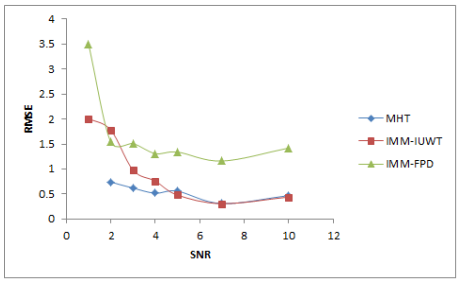


(b)

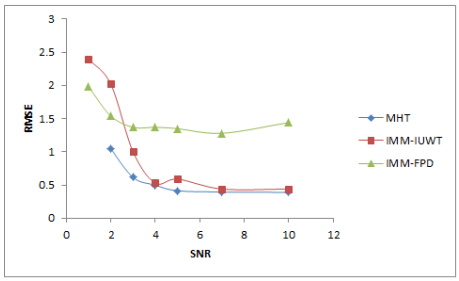


(c)

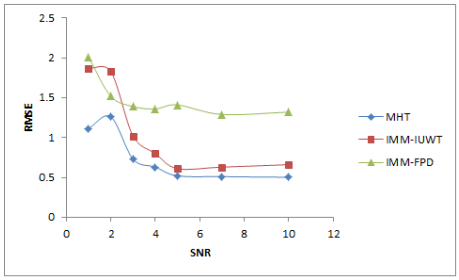
Fig. 3. Results for tracking spots moving in linear motion at different SNR. (a) Seq A (10 spots) (b) Seq B (50 spots) (c) Seq C (200 spots).



(a)



(b)



(c)

Fig. 4. Results for tracking spots moving in Brownian motion at different SNR (a) Seq D (10 spots)(b) Seq E (50 spots)(c) Seq F (200 spots).

Figures 3 and 4 show the results of three tracking methods using synthetic images. The results from Figure 3 indicate that the MHT method performs well compared to IMM-IUWT and IMM-FPD at low SNR (1 to 2). However, as the SNR increases from 3 to 10 the difference in performance between MHT and IMM-IUWT appears to be less significant. The IMM-FPD method produces results with higher error compared to all other methods even at high SNR.

Results from Figure 4 also indicate that the MHT method performs well at low SNR compared to other methods. However, at SNR $= 1$, in Figure 4(a, b) it is noted that the MHT failed to find tracks. As SNR increases, it is noted that the IMM-IUWT performance increased. The improvement in performance of the IMM-IUWT over IMM-FPD was the result of replacing the detection algorithm, FPD with IUWT.

IX. Conclusion

This paper introduced a new method, IMM-IUWT, for particle tracking and compared its performance with two other tracking methods, MHT and IMM-FPD based on synthetic images. The proposed method is a modification of the algorithm presented in [4], here named IMM-FPD. The results from experiments on synthetic image sequences indicate that the proposed method generally outperforms the original method, IMM-FPD especially at higher SNR levels. At low SNR, no single method perform best. It is also noted that at high level of SNR, the performance of IMM-IUWT and MHT is relatively the same. This results show that the performance of the detection algorithm is critical for best tracking performance. As part of future work, we are planning to investigate the performance of IMM-IUWT on real microscopy image sequences.

Acknowledgment

This work was carried out with the financial support of the Council for Scientific and Industrial Research (CSIR) and the Electrical and Electronic Engineering Department at the University of Johannesburg. We also thank the Feng et al. [4] research group for providing their tracking software and Institut Pasteur Image Analysis Group for their Icy [22] and benchmark generator [21] software which are freely available [22].

References

[1] H. Peng, "Bioimage informatics: a new area of engineering biology," *Bioinformatics*, vol. 24, no. 17, pp. 1827–1836, 2008.

[2] A. Raj, "Raj laboratory for system biology[online available]." http://rajlab.seas.upenn.edu/. Accessed September 9, 2014.

[3] I. Smal, E. Meijering, K. Draegestein, I. Grigoriev, A. Akhmanova, M. E. van Royen, A. Houtsmuller, and W. Niessen, "Multiple object tracking in molecular bioimaging by rao-blackwellized marginal particle filtering," *Medical Image Analysis*, vol. 12, no. 6, pp. 764–777, 2008.

[4] L. Feng, Y. Xu, Y. Yang, and X. Zheng, "Multiple dense particle tracking in fluorescence microscopy images based on multidimentional assignment," *Journal of structural biology*, vol. 173, pp. 219–228, 2011.

[5] J.-C. Olivo-Marin, "Extraction of spots in biological images using multiscale products," *Pattern Recognition*, vol. 35, no. 9, pp. 1989–1996, 2002.

[6] M. Mabaso, D. Withey, N. Govender, and B. Twala, "Performance evaluation of spot detection algorithms in fluoresence microscopy images," *in Proceedings of the Twenty-Third Annual Symposium of the Pattern Recognition Association of South Africa*, pp. 89–93, 2012.

[7] N. Chenouard, I. Bloch, and J.-C. Olivo-Marin, "Multiple hypothesis tracking for cluttered biological image sequences," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 35, no. 11, pp. 2736–3750, 2013.

[8] I. Sbazarini and P. Koumoutsakos, "Feature point tracking and trajectory anaysis for video imaging in cell biology," *Journal of Structural Biology*, vol. 151, no. 2, pp. 182–195, 2005.

[9] A. Genovesio, T. Liendl, V. Emiliana, W. Parak, M. Coppey-Moisan, and J.-C. Olivo-Marin, "Multiple particle tracking in 3-d+t microscopy: Method and application to the tracking of endocytosed quantum dots," *IEEE Transactions on Image Processing*, vol. 15, no. 5, pp. 1062–1070, 2006.

[10] W. Godinez, M. Lampe, S. Worz, B. Muller, and K. Rohr, "Deterministic and probabilistic approaches for tracking virus particles in time-lapse fluorescence microscopy image sequences," *Medical Image Analysis*, vol. 13, no. 1, pp. 325–342, 2009.

[11] F. Hitchcock, "The distribution of a product from several sources to numerous localities," *J. Math.Phsy*, vol. 20, pp. 224–230, 1941.

[12] H. Blom and Y. Bar-shalom, "The interacting multiple model algorithm for systems with with Markovian switching coefficients," *IEEE Trans. Autom. Control*, vol. 33, no. 8, pp. 780–783, 1988.

[13] N. Chenouard, I. Bloch, and J.-C. Olivo-Marin, "Multiple hypothesis tracking in microscopy images," in *IEEE International Symposium on BioImaging: From Nano to Macro*, pp. 1346–1349, 2009.

[14] M. Cheezum, W. Walker, and W. Guilford, "Quantitative comparison of algorithms for tracking single fluorescent particles," *Biophys.J*, vol. 81, no. 4, pp. 2378–2388, 2001.

[15] N. Chenouard, I. Smal, F. de Chaumontand M. Maka, I. F. Sbalzarini, Y. Gong, J. Cardinale, C. Carthel, S. Coraluppi, M. Winter, A. R. Cohen, W. J. Godinez, K. Rohr, Y. Kalaidzidis, L. Liang, J. Duncan, H. Shen, Y. Xu, K. E. G. Magnusson, J. Jaldn, H. M. Blau, P. Paul-Gilloteaux, P. Roudot, C. Kervrann, F. Waharte, J. Y. Tinevez, S. L. Shorte, J. Willemse, K. Celler, G. P. van Wezel, H. W. Dan, Y. S. Tsai, C. O. de Solrzano, J. C. Olivo-Marin, and E. Meijering, "Objective comparison of particle tracking methods," *Nature Methods*, vol. 11, no. 3, pp. 281–290, 2014.

[16] J. Crocker and D. Grier, "Methods of digital video microscopy for colloidal studies," *Journal of Colloid and Interface Science*, vol. 179, 1996.

[17] D. Reid, "An algorithm for tracking multiple targets," *IEEE Transactions on Automatic Control*, vol. AC-24, no. 6, pp. 843–854, 1979.

[18] I. Cox and S. Hingorani, "An efficient implementation of reid's multiple hypothesis tracking algorithm and its evaluation for the purpose of visual tracking," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 18, no. 2, pp. 138–150, 1996.

[19] S. Blackman, "Multiple hypothesis tracking for multiple target tracking," *IEEE Aerospace and Electronic Systems Magazine*, vol. 19, no. 1, pp. 5–18, 2004.

[20] N. Chenouard, "Advanced probabilistic particle tracking for biological imaging," *Phd dissertation, Institut Pasteur-TELECOM Paris Tech*, 2010.

[21] N. Chenouard, "Particle tracking benchmark generator[online available]." http://icy.bioimageanalysis.org/plugin/Particle_tracking_benchmark_generator. Accessed May 9, 2013.

[22] F. de Chaumont, S. Dallongeville, N. Chenouard, H. Herve, S. Pop, T. Provoost, V. Meas-Yedid, P. Pankajakshan, T. Lecomte, Y. L. Montagner, T. Lagache, A. Dufour, and J. Olivo-Marin, "Icy: an open community platform for bioimage informatics [online available]." http://icy.bioimageanalysis.org. Accessed June 26, 2013.

[23] F. de Chaumont, S. Dallongeville, N. Chenouard, H. Herve, S. Pop, T. Provoost, V. Meas-Yedid, P. Pankajakshan, T. Lecomte, Y. L. Montagner, T. Lagache, A. Dufour, and J. Olivo-Marin, "Icy: an open bioimaging informatics platform for extended reproducible research," *Nature Methods*, vol. 9, no. 7, pp. 690–696, 2012.

[24] N. Chenouard, "Tracking performance measures[online available]." http://icy.bioimageanalysis.org/plugin/Tracking_Performance_Measures. Accessed September 9, 2014.