Tracking Virus Particles in Fluorescence Microscopy Images Using Multi-Scale Detection and Multi-Frame Association

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Abstract—Automatic fluorescent particle tracking is an essential task to study the dynamics of a large number of biological structures at a sub-cellular level. We have developed a probabilistic particle tracking approach based on multi-scale detection and two-step multi-frame association. The multi-scale detection scheme allows coping with particles in close proximity. For finding associations, we have developed a two-step multi-frame algorithm, which is based on a temporally semiglobal formulation as well as spatially local and global optimization. In the first step, reliable associations are determined for each particle individually in local neighborhoods. In the second step, the global spatial information over multiple frames is exploited jointly to determine optimal associations. The multi-scale detection scheme and the multi-frame association finding algorithm have been combined with a probabilistic tracking approach based on the Kalman filter. We have successfully applied our probabilistic tracking approach to synthetic as well as real microscopy image sequences of virus particles and quantified the performance. We found that the proposed approach outperforms previous approaches.

Index Terms—Virus particle tracking, multi-frame association, multi-scale particle detection, Kalman filter, tracking algorithms.

I. INTRODUCTION

Autom atic tracking of subcellular structures in fluorescence microscopy images is important to study the behavior of virus particles under different experimental conditions. Virus particle tracking is challenging due to the similar appearance of different virus particles, the large number of spurious objects, the appearance and disappearance of particles, as well as their unpredictable motion. Moreover, frequent clustering and unclustering of the virus particles, and out of focus movement of the virus particles are additional challenges for automatic tracking. Main steps of particle tracking approaches are particle detection and association finding. Reliable and accurate particle detection is important because detection errors generally propagate to the subsequent tracking steps and degrade the tracking performance. Particle detection is difficult because of the small size of the objects and the generally high level of image noise. Other challenges are particles in close proximity and particles that go out of focus. Image noise generally induces false detections that may lead to erroneous trajectories (e.g., false positive trajectories). Particles in close proximity and particles going out of focus generally lead to missed detections causing association errors and broken trajectories.

Previous work on particle tracking can be subdivided into deterministic and probabilistic approaches. Deterministic approaches consist of particle detection and association finding (e.g., [1], [2]). While being computationally efficient, deterministic approaches have difficulties in dealing with spurious objects and detection errors [3]. In comparison, probabilistic approaches perform spatial-temporal filtering to robustly estimate the position of particles under noisy conditions (e.g., [3]–[11]). However, a disadvantage of certain probabilistic approaches is that for multiple particle tracking only two frames are taken into account for association finding (e.g., [5]). Probabilistic approaches which use multiple frames (e.g., [6], [7]) may not include locally best associations in the globally optimal solution in which associations are likely to be influenced by neighboring spurious particles. Other approaches have difficulties in scenarios with several crossing objects (e.g., [8]).

Particle detection approaches can be categorized as single-scale and multi-scale approaches. Single-scale approaches
use information from only one scale for particle detection (e.g., [12]–[14]). In [12] the spot-enhancing filter (SEF) with a single scale was proposed to detect spots in fluorescence microscopy images. Compared to other single-scale spot detection approaches, SEF has been found to be one of the best detection approaches for images displaying different types of objects (e.g., round and elongated objects) [15]. However, the detection result of SEF depends on the standard deviation of the filter which needs to be adapted to the size of the particles [12]. A relatively small filter size leads to a high number of false positive detections and a relatively large filter size leads to missed detections for particles in close proximity. Some approaches have been presented to specifically deal with the problem of particles in close proximity. The approaches in [13] and [16] fit a mixture of Gaussian models for detecting close and overlapping spots. To cope with the problem of particles in close proximity, an approach based on an adaptive h-dome transform has been described in [17]. However, all approaches described above use only the information from a single scale. Multi-scale approaches have been widely used for object detection in computer vision (e.g., [18]–[20]) and medical image analysis (e.g., [21]–[24]). For detection of biological particles in microscopy images, a wavelet-based approach was introduced which uses information from multiple scales to increase the robustness to noise [25], [26]. The approach can well detect separated particles but has problems with clustered particles (particles in close proximity and overlapping particles). In [27], a related approach using a normalization step was described.

Approaches for association finding can be characterized on the basis of their temporal and spatial scope. Regarding the temporal scope, approaches for association finding can be categorized as two-frame (e.g., [3], [5]) or multi-frame (e.g., [2], [6], [28], [29]) approaches. Two-frame approaches have problems with difficult tracking tasks (e.g., appearance and disappearance of particles) for which information from a larger temporal context is important. In comparison, multi-frame approaches exploit more temporal information by using multiple frames. Particle tracking approaches based on multi-frame and spatially global optimization schemes are likely to perform better than approaches using two-frame and spatially local nearest-neighbor schemes [30]. However, the computational costs increase with the number of time points. The efficient deterministic approach in [1] achieves multi-frame association by multiple two-frame associations. Thus, the approach is in fact a two-frame approach. The method in [31] is based on a temporally global optimization scheme but uses a nearest neighbor approach for the initialization of trajectories. Deterministic association finding approaches that uncouple particle linking from the detection of clustering and unclustering events (e.g., [28]) may yield incorrect associations because they do not fully exploit the available information. Deterministic [2] and probabilistic [29] multi-frame association finding algorithms perform particle linking and detection of clustering and unclustering jointly. However, these approaches are spatially global and consider all particles jointly at a time point. This may lead to incorrect associations in the presence of a large number of spurious objects because locally best associations may not be included in the globally optimal solution.

In this work, we introduce a particle tracking approach based on a multi-scale detection scheme as well as a multi-frame association approach and the Kalman filter. For particle detection, we use a multi-scale approach which extends the single-scale spot-enhancing filter (SEF) [12]. This allows coping with particles that are in close proximity. For association finding, we have developed a two-step multi-frame association finding algorithm which is based on a temporally semi-global formulation as well as spatially local and global optimization. The multi-scale detection approach and the multi-frame association finding algorithm have been integrated with a probabilistic tracking approach based on the Kalman filter. Compared to spatially global approaches, our association finding algorithm can better cope with spurious objects by selecting locally best associations in the first step and using multi-frame optimization for the unmatched particles in the second step. Key properties of our probabilistic tracking approach are multi-scale detection, multi-frame optimization, verification of associations with past and subsequent positions of the particles, correction of erroneous associations, and robust estimation of the position of particles. Compared to our previous work [32], the new approach utilizes multiple scales for particle detection. In addition, we performed a quantitative evaluation based on synthetic data and we significantly extended the evaluation for real microscopy image data. Compared to [1], our approach simultaneously exploits the information from several consecutive time points. Compared to [2] and [29], we generate reliable associations prior to multi-frame optimization and use a multi-scale detection scheme. Unlike [31], we initialize trajectories using multiple frames. Unlike [28], our approach performs particle linking and handling of clustering and unclustering jointly. Compared to [17], we use multiple scales to detect particles in close proximity. We have quantitatively evaluated the performance of our approach using synthetic image sequences as a function of the signal-to-noise (SNR) ratio, the number of out of focus particles, as well as the object density (number of particles). We have also applied our approach to real fluorescence microscopy image sequences displaying avian leukosis virus (ALV) particles and performed a quantitative comparison with previous approaches.

This paper is organized as follows. In Section II, we present the multi-scale detection scheme. Our multi-frame tracking approach is described in Section III. Experimental results for synthetic and real image sequences are presented in Section IV. Conclusions are given in Section V.

II. PARTICLE DETECTION

In this section, we present the multi-scale particle detection approach used in our probabilistic tracking approach. We first describe a previous single-scale detection approach and then extend it to multiple scales.

A. Single-Scale Spot-Enhancing Filter (SEF)

In fluorescence microscopy images, biological particles typically appear as bright spots. Often, the images have a
high level of noise and include other biological structures. For particle detection in such images, a single-scale approach based on the spot-enhancing filter (SEF) has been proposed in [12]. The SEF enhances the particles while suppressing background structures and removes noise efficiently. Particle detection using the SEF approach has been found to be among the best performing approaches [15] and has been applied for spot detection in several biological applications including detection of centrosomes [33] and single molecules [34]. The first step of the SEF consists in convolving an original image with the Laplacian-of-Gaussian (LoG) kernel:

\[ f(x, y) = \text{LoG}(x, y, \sigma) \ast g(x, y) \]  

where \( g(x, y) \) is a given image, \( f(x, y) \) is the filtered image, and \( \ast \) denotes the convolution operator. The LoG kernel is given by:

\[ \text{LoG}(x, y, \sigma) = \frac{x^2 + y^2 - 2\sigma^2}{2\pi\sigma^6} e^{-\frac{x^2 + y^2}{2\sigma^2}} \]  

where \( \sigma \) is the standard deviation of the filter and should be adapted to the size of the observed particles. After convolution with the LoG the next steps for particle detection are intensity thresholding of the filtered image (see Section II-B below for more details) and connected components labeling for particle localization.

\[ \text{B. Multi-Scale Spot-Enhancing Filter (MSSEF)} \]

For the single-scale SEF approach described above, the detection result strongly depends on the standard deviation \( \sigma \) of the filter which needs to be adapted to the size of observed particles [12]. Since in fluorescence microscopy images, biological particles appear as relatively small spots and multiple particles are often located in close proximity, using only one scale for noise filtering and particle detection has several limitations. Particle detection at a coarse scale typically yields missed detections for multiple close particles. On the other hand, particle detection at a fine scale often results in false positive detections due to image noise. By exploiting multiple scales, missed detections and false positive detections can be avoided. Below, we describe an approach for multi-scale particle detection which takes advantage of effective noise filtering at a coarse scale and accurate detection of particles in close proximity at a fine scale.

In our approach, a filtered image \( f(x, y, \sigma^{(k)}) \) at a scale \( \sigma^{(k)} \) is obtained after \( k \) recursion steps by convolution of the LoG kernel \( \text{LoG}(x, y, \sigma^{(k)}) \) with the original image \( g(x, y) \) masked with a binary image \( b(x, y, \sigma^{(k-1)}) \) obtained at a previous (coarser) scale \( \sigma^{(k-1)} \):

\[ f(x, y, \sigma^{(k)}) = \text{LoG}(x, y, \sigma^{(k)}) \ast (b(x, y, \sigma^{(k-1)}) \ast g(x, y)) \]  

(3)

The binary mask \( b(x, y, \sigma^{(k-1)}) \) is obtained by applying a threshold \( T^{(k-1)} \) to the image \( f(x, y, \sigma^{(k-1)}) \):

\[ b(x, y, \sigma^{(k-1)}) = \begin{cases} 
0 & f(x, y, \sigma^{(k-1)}) < T^{(k-1)} \\
1 & f(x, y, \sigma^{(k-1)}) \geq T^{(k-1)} 
\end{cases} \]  

(4)

The threshold \( T^{(k-1)} \) is computed for the image at each scale as:

\[ T^{(k-1)} = \mu_{\text{intens}}^{(k-1)} + c \sigma_{\text{intens}}^{(k-1)} \]  

(5)

where \( c \) is a user-defined factor, \( \mu_{\text{intens}}^{(k-1)} \) is the mean intensity of the image \( f(x, y, \sigma^{(k-1)}) \), and \( \sigma_{\text{intens}}^{(k-1)} \) is the standard deviation (for other thresholding schemes we refer to [35] and [36]). The binary mask is used to remove noisy background pixels of an image. By multiplying an image with a binary mask, the intensities of noisy background pixels are set to zero while the intensities of foreground pixels remain unchanged. At the beginning of the recursion \( k = 1 \), we define the binary mask as \( b(x, y, \sigma^{(0)}) = 1 \). The scale \( \sigma^{(k)} \) at step \( k \) is given by \( \sigma^{(k)} = \sigma_{\max} - (k - 1) \Delta \sigma \), where \( \sigma_{\max} \) is the coarsest scale. After \( N \) steps, our coarse-to-fine approach reaches the finest scale \( \sigma_{\min} \), where \( \Delta \sigma = (\sigma_{\max} - \sigma_{\min})/(N - 1) \). Connected components of the binary image \( b(x, y, \sigma^{(N)}) \) are identified as particles by connected component labeling (e.g., [37]) and a particle’s position is associated with the intensity-weighted center of mass.

Fig. 1 shows an original image in (a) and binary images with detection results using SEF in (b), (c) and MSSEF in (d). As the marked ROI in (b) indicates, SEF with a large \( \sigma \) (coarse scale, \( \sigma = \sigma_{\max} = 1.5 \)) results in erroneous detections because close particles are merged resulting in a single detection response. On the other hand, as the ROIs in (c) show, SEF
with a small $\sigma$ (fine scale, $\sigma = \sigma_{\text{min}} = 0.75$) results in several false positive detections. In comparison, the result of MSSEF shows that by exploiting multiple scales we can detect particles in close proximity as well as avoid false positive detections.

**III. MULTI-FRAME PARTICLE TRACKING APPROACH**

In this section, we describe our multi-frame probabilistic particle tracking approach. We first present our two-step multi-frame deterministic association algorithm and then combine it with the Kalman filter.

**A. Two-Step Multi-Frame Association (TSA)**

We model the association finding problem using a $w$-partite graph for a temporal window of size $w$. Vertices of the graph represent particles, and edges of the graph denote possible associations. Non-consecutive edges are used to handle clustering and unclustering events. The edges are assigned weights that correspond to the likelihood of the association. Partite sets of the graph correspond to the set of measured (detected) or predicted positions of the particles at time points within the window. A set of measured positions of the particles at time point $t$ is denoted by $P_t = \{p_{t}^1, p_{t}^2, ..., p_{t}^N\}$, where $N_t$ is the number of measurements (detected particles) at time point $t$. For a particle $i$ at time point $t$, and a particle $j$, at time point $t + r$, with position $p_{t+r}^j$, where $r = \{1, 2, ..., w - 1\}$, the likelihood that the two particles correspond to the same object over time is given by the following gain function:

$$s_{\text{gain}}(p_{t+r}^i, p_{t+r}^j) = 1 - \frac{\sqrt{(p_{t+r}^i - p_{t+r}^j)^T S_{t+r}^{-1} (p_{t+r}^i - p_{t+r}^j)}}{\sqrt{s_x^2 + s_y^2}}$$

(6)

where $p_{t+r}^i$ is the predicted position at time point $t + r$ and $S_{t+r}$ is a covariance matrix (e.g., from a Kalman filter). In our case we used the identity matrix for $S_{t+r}$. $s_x$ and $s_y$ are the image dimensions in $x$- and $y$-direction, respectively, which are used for normalization. In the deterministic approach, the prediction $p_{t+r}^j$ is computed using a motion model (e.g., constant velocity model). In the probabilistic approach (see Section III-B below), the prediction at time point $t$ is computed by the Kalman filter, and for the other time points the prediction is computed using the motion model.

We have developed a two-step multi-frame association algorithm (TSA) which combines a spatially local and a spatially global approach. First, reliable associations are determined by considering each particle individually. This step is called reliable association generation (RAG). Second, a multi-frame association approach (MFA) is applied to unassociated particles from the first step. Since the neighborhood of RAG is relatively small, a large number of particles remain unassociated in the first step and are considered in the second step. MFA performs spatially global and temporally semi-global optimization. For the two steps, we use two different circular neighborhoods of particles represented by two circles with different radii $r_{\text{RAG}}^i$, $r_{\text{MFA}}^i$ centered at a particle. The first neighborhood $\Omega_{\text{RAG}}^i$ corresponds to the region of the smaller circle and the second neighborhood $\Omega_{\text{MFA}}^i$ corresponds to the region between the two circles. An illustration of the two neighborhoods and three sample association cases are provided in Fig. 2. As can be seen, a particle may have feasible associations within $\Omega_{\text{RAG}}^i$ or $\Omega_{\text{MFA}}^i$ or both neighborhoods. Note that if the RAG step cannot assign the red particle to the green particle within $\Omega_{\text{RAG}}^i$, then the red particle will be considered by the MFA step for association with the other green particles.

By using pairs of consecutive frames, RAG generates highly likely associations on the basis of an application dependent criterion (e.g., displacement, intensity). We use RAG for establishing associations corresponding to particles exhibiting restricted motion (displacement within $\Omega_{\text{RAG}}^i$). Therefore, we set the radius $r_{\text{RAG}}^i$ of $\Omega_{\text{RAG}}^i$ equal to the average size of the particles and generate all feasible associations. For each particle $p_{t+r-1}^j$, we use a local nearest neighbor approach to find an association for which the gain is maximal:

$$j_{\text{max}} = \arg \max_j (s_{\text{gain}}(p_{t+r-1}^j, p_{t+r}^j))$$

(7)

The MFA step searches for associations within $\Omega_{\text{MFA}}^i$. We define the radius $r_{\text{MFA}}^i$ of $\Omega_{\text{MFA}}^i$ on the basis of the maximum possible displacement of the particles. This discards very unlikely (physically impossible) associations. MFA establishes associations for all unassociated particles within the temporal window. The window of MFA is initialized by two frames and progressively enlarged. With every iteration, the window is enlarged by one frame until it reaches its maximum size (i.e. $w$ frames). The MFA step generates soft associations within a growing temporal window. Soft associations can be
changed to hard associations (which are final) at a subsequent iteration of MFA. At every iteration, a split graph is constructed using all frames within a growing temporal window of MFA [2]. The optimal solution corresponds to the maximum matching of the split graph. The optimization objective has been defined as finding a hypothesis \( \mathbf{M}_{\text{max}} \) with the maximum total gain:

\[
\mathbf{M}_{\text{max}} = \arg \max_{\mathbf{M}} \sum_{k=1}^{N_{\text{total}}} \sum_{l=1}^{N_{\text{total}}} M_{k,l} G_{k,l}
\]

subject to \( \sum_{k=1}^{N_{\text{total}}} M_{k,l} = 1, \sum_{l=1}^{N_{\text{total}}} M_{k,l} = 1, \quad (8) \)

where \( N_{\text{total}} \) is the total number of measurements (detected particles) and predictions within a temporal window. A hypothesis \( \mathbf{M} \) is a feasible solution represented by an association matrix consisting of \( N_{\text{total}} \times N_{\text{total}} \) of 0 and 1, where 1 indicates presence of the association and 0 indicates absence of the association in the solution. The constraints ensure a one-to-one association. \( \mathbf{G} \) is the \( N_{\text{total}} \times N_{\text{total}} \) gain matrix consisting of gains \( G_{k,l} \) for all feasible associations, where \( k, l = \{1, 2, ..., N_{\text{total}}\} \). We solve the optimization problem (8) using the Hungarian algorithm [38].

The input for the deterministic TSA approach consists of \( P_t, P_{t+1}, ..., P_{t+w-1} \) for a temporal window of size \( w \). The two steps of TSA (RAG and MFA) are performed alternatingly. RAG iteratively generates reliable associations for all \( w \) frames. These associations are provided as hard associations to the MFA step. MFA progressively processes all \( w \) frames. Temporal windows consisting of indices of frames for RAG and MFA are denoted as \( W_{\text{RAG}} \) and \( W_{\text{MFA}} \), respectively. The size of \( W_{\text{RAG}} \) is 2 and the maximum size of \( W_{\text{MFA}} \) is \( w \). For an iteration \( r = \{1, 2, ..., w-1\} \) of the TSA algorithm, windows for RAG and MFA are defined as \( W_{\text{RAG}}^r = \{r + r - 1, t + r\} \) and \( W_{\text{MFA}}^r = \{r, t + 1, ..., t + r\} \). Our association algorithm applies RAG and MFA on their respective windows at every iteration \( r \). Fig. 3 illustrates the processing of frames by RAG and MFA, and corresponding association results. Temporal windows of RAG and MFA are represented by yellow and green rectangles, respectively (\( t = 1, w = 3 \)). Feasible associations, hard associations, and soft associations are represented by thin black, thick black, and thick blue arrows, respectively. As can be seen in Fig. 3 (a), RAG generates hard associations while keeping associations generated by the RAG step unchanged. As shown in Fig. 3 (d), MFA finds associations using all three frames \( W_{\text{MFA}}^2 = \{1, 2, 3\} \). All hard associations generated by RAG remain unchanged, a soft association generated by MFA is changed, and soft associations originating from the time point \( t = 1 \) are turned into hard associations.

The TSA approach is denoted spatially global because all physically possible associations for all detected objects at a time point are considered simultaneously in the optimization. The approach is temporally semi-global because several frames within a window are exploited to generate final associations.

### B. Probabilistic Tracking Approach Using Multiple Frames

Our multi-frame probabilistic tracking approach combines the deterministic two-step multi-frame association (TSA) algorithm described above with a Kalman filter (KF) [39],
and is denoted by TSAKF (see Fig. 4). The appearance of particles is represented by a 2D Gaussian function. Hence, the state vector for a particle is defined as \((x, y, I_{\text{max}}, \sigma_x, \sigma_y)\), where \((x, y)\) is the position in the image, \(I_{\text{max}}\) is the peak intensity and \(\sigma_{x,y}\) is the standard deviation of the 2D Gaussian function. A set of predicted positions of the particles at a time point \(t\) is denoted by \(\hat{P}_t\) and a set of measured (detected) positions of the particles at time point \(t\) is denoted by \(P_t\). Trajectories are initialized using detected positions corresponding to new particles at all time points. Note that at the first time point \(t = 1\) all detected positions correspond to new particles. TSA uses as input \(\hat{P}_t\) (computed by the Kalman filter using a random walk dynamical model) and the measured positions of the particles at time points \(t, t+1, \ldots, t+w-2\), namely \(P_t, P_{t+1}, \ldots, P_{t+w-2}\) for a temporal window of size \(w\). Thus, the input for TSA within the probabilistic TSAKF approach consists of \(\hat{P}_t, P_t, P_{t+1}, \ldots, P_{t+w-2}\). The last time point of the window is \(t + w - 2\) to meet the size requirement of the window of TSA. The goal of TSA is to find associations between \(\hat{P}_t\) and \(P_t\) by exploiting the information in all frames within the window. Based on the association results of TSA, the Kalman filter determines an estimate of the positions of the particles at the current time point.

IV. EXPERIMENTAL RESULTS

A. Experimental Setup

We have evaluated the performance of the developed multi-scale multi-frame probabilistic tracking approach using synthetic image data and real microscopy image sequences displaying avian leukosis virus (ALV) particles. First, we investigated the detection performance of the proposed multi-scale approach (MSSEF) and performed an experimental comparison with the single-scale detection approach (SEF) [12]. Then, we studied the performance of the proposed multi-scale multi-frame probabilistic tracking approach (MSSEF-TSAKF) and performed an experimental comparison with six different tracking approaches: deterministic two-frame association approach (TFA) [1], deterministic multi-frame association approach (MFA) [2], our deterministic two-step multi-frame association approach (TSA), probabilistic Kalman filter (KF) based approach which uses TFA for motion correspondence [5], probabilistic approach that combines MFA with KF (MFAKF), and probabilistic approach based on TSA and KF (TSAKF) [32]. In total, we have evaluated two different detection approaches and seven different tracking approaches.

To quantify the performance of the detection approaches we computed the detection error \(E_{\text{detect}}\) which is defined based on the number of the correct detections \(n_{\text{detect,correct}}\) and the total number of the true detections \(n_{\text{detect,total}}\):

\[
E_{\text{detect}} = 1 - \frac{n_{\text{detect,correct}}}{n_{\text{detect,total}}}
\]

A detection is considered to be correct if its distance to a true detection is within 2 pixels. To quantitatively evaluate the performance of the tracking approaches we computed the track error \(E_{\text{track}}\), which is defined based on the number of the correct trajectories \(n_{\text{track,correct}}\) and the total number of the true trajectories \(n_{\text{track,total}}\):

\[
E_{\text{track}} = 1 - \frac{n_{\text{track,correct}}}{n_{\text{track,total}}}
\]

A trajectory is considered to be correct if it contains more than 75% correct positions (positions within a tolerance of 2 pixels). For all image sequences we have used fixed parameter values for the tracking approaches. For particle detection, we employed either SEF with \(\sigma = 1.5\) and \(c = 5\) or MSSEF with \(\sigma_{\text{min}} = 0.75, \sigma_{\text{max}} = 1.5, N = 4\), and \(c = 3\). For SEF we used a higher value for \(c\) since otherwise a higher number of missed detections would be obtained for close particles. Since the virus particles exhibit random motion, we set the predicted position of a particle to its previous position.

\(\Omega_{\text{RAG}}\) or \(\Omega_{\text{MFA}}\) have been set to \(r_{\text{RAG}} = 3\) pixels and \(r_{\text{MFA}} = 10\) pixels, respectively, in accordance with the image data. We have used a temporal window of size \(w = 5\).

B. Synthetic Image Sequences

We have evaluated single-scale and multi-scale detection schemes as well as deterministic and probabilistic tracking approaches for various synthetic image sequences. We have studied the sensitivity of the developed approaches as a function of the signal-to-noise ratio (SNR), the object density (number of particles), and the percentage of out of focus particles. The synthetic images simulate real fluorescence microscopy images under different conditions. The synthetic image sequences show multiple particles with different motion patterns. We generated synthetic image sequences for 7 different SNR levels, 10 different levels of object densities reflected by the number of particles, and 5 different percentages of out of focus particles. A particle is assumed to have a Gaussian intensity profile and the image intensities are distorted by additive Poisson noise (e.g., [1], [30], [40]). The SNR is calculated as the ratio of the difference between the peak intensity of a particle \(I_{\text{max}}\) and the intensity of the background \(I_b\), and the noise level \(\sigma_n\) [1], [30]:

\[
\text{SNR} = \frac{I_{\text{max}} - I_b}{\sigma_n}
\]

We generated 20 synthetic image sequences for each of the 7 different SNR levels (1.99, 2.48, 3.49, 4.55, 6.51, 8.83, 11.63), for each of the 10 different levels of object...
density (25, 50, 75, 100, 125, 150, 175, 200, 225, 250), and for each of the 5 different percentages of out of focus particles (5.0, 7.5, 10.0, 12.5, 15.0) and computed the mean track error $\bar{E}_{\text{track}}$ (see Figs. 5 to 12). In total, we used 440 image sequences each consisting of 100 frames. The motion of the particles was simulated using different motion models (random motion, directed motion, and confined motion). Particles randomly switch between the motion models and may overlap with other particles. New particles enter the field of view throughout the image sequences. Particles may also move out of focus for a few time points (simulated by different values of the standard deviation of the Gaussian function and the image contrast) or disappear permanently. In addition, spurious particles have been added. The minimum life time of a particle has been set to 5 frames for all image sequences.

For the SNR experiments, we simulated 100 particles with a disappearance probability of 0.05 within images of size 100×100 pixels. The average number of true measurements per frame is 34.5 and the average number of out of focus particles per frame is 1.73. The results for the single-scale (SEF) and multi-scale (MSSEF) detection schemes are shown in Fig. 5. It can be seen that MSSEF yields a lower detection error compared to SEF for all SNR levels. The difference in performance between the two schemes is particularly evident for low SNRs (e.g., SNR=1.99 or 2.48). We have also computed the Jaccard similarity coefficient (JSC) to measure the detection performance using the ICY software [41]. The results as a function of the SNR level are shown in Fig. 6. It can be seen that the performance of the MSSEF approach is better compared to the SEF approach.

The results for the tracking approaches used in combination with the detection schemes (single-scale or multi-scale) are shown in Fig. 7. Regarding the deterministic approaches based on SEF, the best performance is obtained by TSA followed by MFA and TFA. For the probabilistic approaches using SEF, TSAKF yields the best performance followed by MFAKF and KF. Overall, the lowest tracking error is obtained by the multi-scale multi-frame probabilistic approach MSSEF-TSAKF which outperforms all other deterministic and probabilistic approaches.

For the experiments using different levels of object density (number of particles), we used an SNR level of 4.55, a disappearance probability of 0.05, and an image size of 150×150 pixels. We also included spurious objects. The average number of true measurements per frame, the number of out of focus particles, and the number of spurious objects varies with the object density (see Table I). As an example, two images from synthetic image sequences displaying 100 and 150 particles are shown in Fig. 8. Fig. 9 shows the results for the different detection schemes. It can be seen that for MSSEF the detection error is lower compared to SEF for all levels of the object density. The difference in performance is significant for difficult cases with a high number of particles (150 and above) because many particles are located in close proximity and MSSEF can cope better with such situations.

Fig. 5. Detection results for synthetic image sequences as a function of the SNR level (mean track error $\bar{E}_{\text{detect}}$ for 20 image sequences and standard deviation).

Fig. 6. Detection results for synthetic image sequences as a function of the SNR level (mean Jaccard similarity coefficient (JSC) for 20 image sequences and standard deviation).

Fig. 7. Tracking results for synthetic image sequences as a function of the SNR level (mean track error $\bar{E}_{\text{track}}$ for 20 image sequences and standard deviation).

### Table I

<table>
<thead>
<tr>
<th>Object density</th>
<th>True meas.</th>
<th>Out of focus particles</th>
<th>Spurious objects</th>
</tr>
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<tbody>
<tr>
<td>25</td>
<td>9.7</td>
<td>0.48</td>
<td>2.5</td>
</tr>
<tr>
<td>150</td>
<td>62.98</td>
<td>3.15</td>
<td>15</td>
</tr>
<tr>
<td>250</td>
<td>101.7</td>
<td>5.09</td>
<td>25</td>
</tr>
</tbody>
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For the experiments using different levels of object density, we used an SNR level of 4.55,
Fig. 8. Example images from synthetic image sequences displaying (a) 100 and (b) 150 particles.

Fig. 9. Detection results for synthetic image sequences as a function of the number of particles (mean detection error $\bar{E}_{\text{detect}}(\%)$ for 20 image sequences and standard deviation).

Fig. 10. Tracking results for synthetic image sequences as a function of the number of particles (mean track error $\bar{E}_{\text{track}}(\%)$ for 20 image sequences and standard deviation).

The tracking error of TFA is highest because it uses only two frames and often yields broken trajectories due to a relatively large number of missed detections. The multi-frame deterministic and probabilistic approaches, due to their larger temporal scope, yield lower tracking errors. The tracking error of the probabilistic approaches using SEF (KF, MFAKF, TSAKF) is lower compared to the corresponding deterministic approaches (TFA, MFA, TSA). TFA is outperformed by MFA, which is outperformed by TSA. Analogously, KF is outperformed by MFAKF, which is outperformed by TSAKF. Overall, the probabilistic multi-scale approach MSSEF-TSAKF yields the lowest tracking error.

Fig. 11. Detection results for synthetic image sequences as a function of the percentage of out of focus particles (mean detection error $\bar{E}_{\text{detect}}(\%)$ for 20 image sequences and standard deviation).

Fig. 12. Tracking results for synthetic image sequences as a function of the percentage of out of focus particles (mean track error $\bar{E}_{\text{track}}(\%)$ for 20 image sequences and standard deviation).

Fig. 13. FROC curve for the multi-scale spot-enhancing filter (MSSEF) approach for synthetic images displaying round objects at SNR = 2 and varying threshold factors $c$.

For the out of focus experiments we used an SNR level of 4.55 and an image size of $100 \times 100$ pixels. Each image sequence displays 100 moving particles and the average number of true measurements per frame is 34.09. The results for the detection and tracking approaches are shown in Figs. 11 and 12, respectively. Also for these images, MSSEF yields a lower detection error compared to SEF. Among the tracking approaches using SEF, the probabilistic tracking approaches (KF, MFAKF, TSAKF) yield a lower
TABLE II

<table>
<thead>
<tr>
<th>Density</th>
<th>Method</th>
<th>SNR = 2</th>
<th>SNR = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α</td>
<td>β</td>
<td>JSC compartments</td>
</tr>
<tr>
<td>Low</td>
<td>Method 5</td>
<td>0.615</td>
<td>0.409</td>
</tr>
<tr>
<td></td>
<td>Method 1</td>
<td>0.230</td>
<td>0.189</td>
</tr>
<tr>
<td></td>
<td>Method 2</td>
<td>0.546</td>
<td>0.470</td>
</tr>
<tr>
<td></td>
<td>MSSEF-TSAKF</td>
<td>0.541</td>
<td>0.463</td>
</tr>
<tr>
<td>Medium</td>
<td>Method 5</td>
<td>0.414</td>
<td>0.347</td>
</tr>
<tr>
<td></td>
<td>Method 1</td>
<td>0.210</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>Method 2</td>
<td>0.439</td>
<td>0.375</td>
</tr>
<tr>
<td></td>
<td>MSSEF-TSAKF</td>
<td>0.430</td>
<td>0.337</td>
</tr>
</tbody>
</table>

Fig. 14. Detection results of spot-enhancing filter (SEF) and multi-scale SEF (MSSEF) for selected ROIs from real image sequences and comparison with ground truth.

error compared to the corresponding deterministic approaches (TFA, MFA, TSA). The probabilistic tracking approach based on multi-scale detection (MSSEF-TSAKF) yields the best result.

We have also applied the MSSEF approach to an image sequence from [15] displaying round objects on a uniform background. The image sequence consists of 16 images each with a size of 512×512 pixels, a background intensity level of 10.0, SNR of 2, object size of 2 pixels, and a total number of true objects of 4096. To quantify the performance, we computed the true-positive ratio (TPR) and the false-positive ratio (FPR) as described in [15]. The FROC curve [42] for the MSSEF approach for varying threshold factors $c$ (see (5)) is shown in Fig. 13. It can be seen that FPR can be reduced to less than 0.001 while maintaining a TPR of more than 0.99. For FPR=0.01 the MSSEF approach yields $\text{TPR} = 0.999$. In comparison, the multi-scale variance-stabilizing transform (MSVST) approach [15] and the maximum possible height-dome (MPHD) [17] approach yielded $\text{TPR} = 0.99$ (see [17, Table 1]). Thus our approach yields comparable results.

In addition, we have applied our tracking approach to image sequences from the particle tracking challenge at ISBI 2012 [30]. We used image sequences from the receptor...
Fig. 15. Tracking results of the TSAKF approach (two-step multi-frame association and Kalman filter) using either SEF or MSSEF for the ROIs shown in Fig. 14 and comparison with ground truth.

C. Real Image Sequences

We have also applied our multi-scale multi-frame probabilistic tracking approach (MSSEF-TSAKF) to 10 real microscopy image sequences displaying avian leukosis virus (ALV) particles. The images were acquired using a confocal microscope with a frame rate of 4 frames per minute [45]. The image sizes vary between 512×512 pixels and 1024×1024 pixels, the number of time points ranges from 34 to 180, and the number of particles varies from 10 to 380. The image sequences differ in the level of the image noise, the object density, the motion pattern of the scenario with particle density levels low and medium, and SNR levels of 2 and 4. The image sequences consist of 100 images each with a size of 512×512 pixels. Particles randomly switch between Brownian motion and directed motion. To quantify the performance, we computed five performance measures (α, β, JSCθ, JSC, and RMSE) as in [30, Table 3] using the “Tracking Performance Measures” plugin in ICY [41]. The results of the MSSEF-TSAKF approach and the overall top-three methods (Method 5, Method 1, and Method 2) from [30] are shown in Table II (Method 5: Kalman filtering and probabilistic data association [5], [11], Method 1: Combinatorial optimization [1], Method 2: Multiple hypothesis tracking [43], [44]). It can be seen that the performance of the MSSEF-TSAKF approach is comparable to the other methods.

### Table III

DETECTION RESULTS $E_{detect}$(%) OF THE SINGLE SCALE (SEF) AND MULTI-SCALE (MSSEF) APPROACHES FOR 10 REAL MICROSCOPY IMAGE SEQUENCES AS WELL AS MEAN DETECTION ERROR $\bar{E}_{detect}$(%) AND STANDARD DEVIATION

<table>
<thead>
<tr>
<th>Seq.</th>
<th>No. of particles</th>
<th>SEF</th>
<th>MSSEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seq. 1</td>
<td>29</td>
<td>4.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Seq. 2</td>
<td>38</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Seq. 3</td>
<td>10</td>
<td>6.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Seq. 4</td>
<td>27</td>
<td>7.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Seq. 5</td>
<td>380</td>
<td>16.6</td>
<td>22.1</td>
</tr>
<tr>
<td>Seq. 6</td>
<td>31</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Seq. 7</td>
<td>341</td>
<td>4.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Seq. 8</td>
<td>197</td>
<td>3.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Seq. 9</td>
<td>14</td>
<td>3.6</td>
<td>2.4</td>
</tr>
<tr>
<td>Seq. 10</td>
<td>25</td>
<td>1.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

$E_{detect}$ | 5.3 | 4.6 |
Std. dev. | 4.2 | 6.0 |
TABLE IV

<table>
<thead>
<tr>
<th></th>
<th>TFA</th>
<th>MFA</th>
<th>TSA</th>
<th>KF</th>
<th>MPAKF</th>
<th>MHT</th>
<th>TSAKF</th>
<th>MSSEF - TSAKF</th>
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</thead>
<tbody>
<tr>
<td>Seq. 1</td>
<td>20.7</td>
<td>6.9</td>
<td>3.4</td>
<td>17.2</td>
<td>6.9</td>
<td>20.7</td>
<td>0.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Seq. 2</td>
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<td>12.0</td>
<td>23.7</td>
<td>15.4</td>
<td>26.3</td>
<td>13.7</td>
<td>13.2</td>
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<tr>
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<td>50.0</td>
<td>50.0</td>
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<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
<td>16.7</td>
</tr>
<tr>
<td>Seq. 4</td>
<td>55.6</td>
<td>48.1</td>
<td>33.3</td>
<td>37.0</td>
<td>44.4</td>
<td>25.9</td>
<td>22.2</td>
<td>18.5</td>
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<tr>
<td>Seq. 5</td>
<td>49.3</td>
<td>50.1</td>
<td>42.2</td>
<td>45.1</td>
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<td>41.2</td>
<td>39.6</td>
<td>49.6</td>
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<tr>
<td>Seq. 6</td>
<td>25.8</td>
<td>32.3</td>
<td>32.3</td>
<td>38.7</td>
<td>38.7</td>
<td>35.5</td>
<td>35.5</td>
<td>32.3</td>
</tr>
<tr>
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<td>15.5</td>
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<td>19.4</td>
<td>13.2</td>
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<tr>
<td>Seq. 8</td>
<td>53.3</td>
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<td>48.7</td>
<td>49.7</td>
<td>41.1</td>
<td>26.4</td>
<td>42.6</td>
<td>37.1</td>
</tr>
<tr>
<td>Seq. 9</td>
<td>16.7</td>
<td>25.0</td>
<td>25.0</td>
<td>33.3</td>
<td>41.7</td>
<td>42.9</td>
<td>33.3</td>
<td>25.0</td>
</tr>
<tr>
<td>Seq. 10</td>
<td>12.0</td>
<td>8.0</td>
<td>4.0</td>
<td>20.0</td>
<td>8.0</td>
<td>16.0</td>
<td>16.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

$E_{\text{track}}$ (%)

| Std. dev.  | 17.2| 18.2| 17.5| 12.2| 15.1  | 9.2 | 14.0  | 14.3          |

Fig. 16. (Top) Tracking results of MSSEF-TSAKF (multi-scale spot-enhancing filter combined with two-step multi-frame association and Kalman filter) for a real image sequence. (Bottom) For the selected ROI, tracking results of different approaches as well as the ground truth are shown.

particles, and the number of spurious objects. The SNR ranges from about 2 to 14 for particles in different image sequences. Ground truth for the real images has been determined by manual tracking using the ImageJ plugin MTrackJ [46]. We have evaluated the two detection approaches SEF and MSSEF for all 10 real image sequences. As an example, in Fig. 14 we show the ground truth detections along with the detection results of SEF and MSSEF for four ROIs.
from a real image sequence. From the ROIs 1, 2 and 3 it can be seen that for multiple particles in close proximity a single detection is obtained using the single scale approach (SEF). The out of focus particle in ROI 4 is also missed by SEF. In comparison, our multi-scale approach (MSSEF) correctly detects the particles in close proximity and the out of focus particle. In order to assess the effect of detection errors on the tracking accuracy, we show the tracking results of the TSAKF approach using either SEF or MSSEF for all four ROIs in Fig. 15. It can be seen that the detection errors by SEF lead to several tracking errors including missed trajectories (e.g., ROIs 1, 2, 3, 4), partial trajectories (e.g., ROIs 2, 3), localization errors (e.g., ROIs 1, 2, 3), and incorrect associations (e.g., ROIs 2, 3). In comparison, the tracking approach using MSSEF yields correct tracking results for all four ROIs. Quantitative detection results in terms of $E_{\text{detect}}(\%)$ for all 10 real image sequences as well as the mean detection error $\bar{E}_{\text{detect}}(\%)$ and the standard deviation over all 10 image sequences are shown in Table III. It can be seen that for most of the image sequences, MSSEF yields a lower detection error $E_{\text{detect}}(\%)$ compared to SEF. For Seq. 5, MSSEF results in a higher value of $E_{\text{detect}}(\%)$ because along with the noise relatively small spots are also discarded. In terms of the mean detection error $\bar{E}_{\text{detect}}(\%)$, MSSEF outperforms SEF.

We have applied all seven tracking approaches as described in Section IV-A above to all 10 real microscopy image sequences. Additionally, we performed a comparison with the multiple-hypothesis tracking (MHT) approach [6] as implemented in ICY [41]. The MHT approach uses a wavelet-based multi-scale approach for particle detection [25]. We used scale 2 with sensitivity 110 and the minimum particle size was set to 3 pixels. For tracking, both diffusive and directed motion models were used with automatic estimation of the parameters, and the inertia of the motion model switch was set to 0.2. Otherwise default values were used.

As an example, Fig. 16 shows for one image sequence tracking results of MSSEF-TSAKF and a selected ROI with tracking results of some of the other evaluated approaches. The ROI displays real particles which exhibit different motion patterns and which cluster and uncluster. Spurious objects appear for one time point and disappear. As can be seen from Fig. 16, MFA generates incorrect associations and KF generates broken trajectories because of missed detections due to particles in close proximity. The MHT approach yields a missed trajectory due to detection errors for particles in close proximity. The out of focus particle in ROI 4 is also missed by SEF. In comparison, our multi-scale approach (MSSEF) correctly detects the particles in close proximity and the out of focus particle.
proximity. TSAKF performs relatively better because of the association finding approach, however, due to detection errors this approach yields an identity switch between the particles shown in red and green color. In comparison, MSSEF-TSAKF yields correct trajectories by virtue of the multi-scale detection scheme. In Fig. 17, tracking results of MSSEF-TSAKF for another real image sequence are shown. For the ROI, we also show tracking results of some of the evaluated approaches. Because of the close proximity of the particles, the approach based on the SEF scheme fails to detect some particles and localization errors are also induced. Consequently, all tracking approaches based on the SEF scheme cannot determine the trajectory of certain particles (missed trajectory) and likewise the estimated positions include a localization error. The MHT approach also yields a missed trajectory. In comparison, by virtue of the multi-scale detection scheme, the MSSEF-TSAKF approach accurately determines the trajectories of particles in close proximity. Quantitative tracking results in terms of $E_{\text{track}}(%)$ for the different approaches for all 10 real microscopy image sequences as well as the mean track error $E_{\text{track}}(\%)$ and the standard deviation are shown in Table IV. Generally, MSSEF-TSAKF yields the best result. For Seq. 5, MSSEF-TSAKF yields a higher value of $E_{\text{track}}(\%)$ compared to TSAKF due to higher detection error (cf. Table III). In terms of the mean track error $E_{\text{track}}(\%)$, among the approaches based on SEF, TSA outperforms MFA which is better than TFA. Similarly, TSAKF outperforms MFAKF which is better than KF. The probabilistic approaches (KF, MFAKF, TSAKF) perform better than the corresponding deterministic approaches (TFA, MFA, TSA). MHT performs better than some deterministic and probabilistic tracking approaches (TFA, MFA, KF, MFAKF). Overall, MSSEF-TSAKF outperforms all other tracking approaches.

We have implemented our tracking approaches in Java. The TSAKF approach takes 20-50 seconds to track 100 particles in an image sequence with 100 time points on a laptop with Intel(R) Core(TM) i5-2410M CPU with 2.30GHz speed, 2 GB RAM and 32-bit Windows 7 operating system.

V. Conclusion

In this paper, we have proposed a new probabilistic approach for automatic tracking of particles in fluorescence microscopy image sequences. The approach is based on multi-scale detection and two-step multi-frame association. A quantitative performance evaluation and a comparison with previous approaches based on synthetic image data and real microscopy image sequences of virus particles has been performed. We studied the particle detection performance and found that multi-scale detection yields better results compared to single scale detection due to accurate detection of particles in close proximity at a fine scale as well as effective noise reduction at a coarse scale. We also studied the association finding performance. Using a two-frame association approach yielded relatively often broken trajectories. The reasons are missed detections because of temporary particle disappearance due to out of focus movement of virus particles as well as clustering and unclustering. Additional challenges are the similar shape and intensity of different virus particles as well as their unpredictable motion. To address these challenges, we have exploited information from multiple frames. From the experimental evaluation we found that the multi-frame association approach outperforms the two-frame association approach. However, the previous multi-frame association approach cannot cope well with a large number of spurious particles. A better result is obtained by a deterministic two-step multi-frame association approach which combines spatially local and global optimization, and which yielded the best result among the deterministic association approaches. The result can be further improved by probabilistic approaches due to additional spatial-temporal filtering for robust estimation under noisy conditions. Based on our experiments we found that probabilistic approaches outperform corresponding deterministic approaches. Overall, compared to these single-scale approaches, we found that our multi-scale multi-frame probabilistic tracking approach (MSSEF-TSAKF) yields the best tracking performance. The robustness and accuracy of this approach relies on multi-scale detection, reliable association generation, multi-frame optimization, handling of clustering and unclustering of particles, and position estimation by a Kalman filter. Based on our quantitative evaluation using various synthetic and real image sequences, we conclude that our approach is well suited for tracking of viruses in microscopy image sequences.

A limitation of the proposed multi-scale detection approach is that the range of the standard deviations for the SEF filter was determined empirically. In future work, the range could be determined automatically and adapted to the size of the particles in the image data. In addition, the size of the temporal window for multi-frame association could be determined automatically. The performance of the proposed probabilistic tracking approach could be further improved by explicitly identifying overlapping particles. Integrating information on particle emergence is also the subject of future work. The proposed approach will be applied for tracking of particles in different biological applications.

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References


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Maik Jörg Lehmann received the Ph.D. degree in molecular biology from the University of Heidelberg, Germany, in 2002. He studied chemistry and biochemistry with the University of Marburg, Germany, and the University of Oxford, U.K. From 1998 to 2002, he was with the Department of Applied Tumor Virology, German Cancer Research Center on Antiviral Enzyme and the Cellular Uptake of DNA. Funded by the German Academy of Sciences Leopoldina, he performed his post-doctoral studies on the visualization of virus-cell interactions with the School of Medicine, Yale University, from 2002 to 2005. In 2005, he returned to Heidelberg and started working on the pathogenesis of retroviruses with the BioQuant Center for Systems Biology. In 2010, he became a Group Leader with Humboldt-University Berlin. He is appointed as a professorship for biochemistry and cell biology with the University of Applied Sciences Bingen, Germany. His main research interests focus on the identification of host cell factors involved in the pathogenicity of viruses and parasites as well as on studying pathogen-cell interactions by using live cell imaging and electron microscopy techniques. He received the Klaus-Georg and Sigrid Hengstberger prize for outstanding young scientists at the University of Heidelberg in 2008.

Karl Rohr received the Dipl.-Ing. degree in electrical engineering from the University of Karlsruhe, Germany, and the Ph.D. and Habilitation degrees in computer science from the University of Hamburg, Germany, in 1994 and 1999, respectively. He is currently an Associate Professor and the Head of the Biomedical Computer Vision Group with the University of Heidelberg and the German Cancer Research Center. He was an Associate Professor with the School of Information Technology, International University in Germany, Bruchsal, Germany, from 2000 to 2004. In Summer 1999, he spent a research stay with the Surgical Planning Laboratory, Harvard Medical School, Boston, MA, USA. From 2007 to 2010, he was a Guest Professor with the International University in Germany. Since 2007, he has been a member of the Excellence Cluster CellNetworks with the University of Heidelberg. He has written one book entitled Landmark-Based Image Analysis (Kluwer Academic Publishers, 2001) covering landmark localization and elastic registration. He has authored over 250 peer-reviewed scientific articles. His research interests are in biological image analysis and medical image analysis with a focus on cell and particle tracking, nonrigid image registration, vessel segmentation, and landmark localization. He was an Associate Editor of the IEEE TRANSACTIONS ON BIOMEDICAL ENGINEERING (2007–2012) and served on the editorial board of the Pattern Recognition journal (2000–2006). He has also been a Program and Review Committee Member of a number of international conferences and workshops. He was a Co-Organizer of the International Workshop on Microscopic Image Analysis with Applications in Biology in 2008, 2009, and 2011. He will be the Program Chair of the IEEE International Symposium on Biomedical Imaging in 2016. For his research work, he received several prizes.