Non-Rigid Temporal Alignment of 2D and 3D Multi-Channel Microscopy Image Sequences of Human Cells

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Abstract. The analysis of fluroescence tagged proteins in live cells from multi-channel microscopy image sequences requires a registration to a reference frame to decouple the movement and deformation of cells from the movement of proteins. We have developed an intensity-based approach for the registration of 2D and 3D multi-channel microscopy image sequences. This approach can be directly applied to the intensity images or to the segmented images. Also, we have performed a comparison using a direct registration scheme to the reference frame and an incremental scheme taking into account results from preceding time steps. We have evaluated our approach based on 3D synthetic images of a simulated spherical cell with known deformation which has been calculated based on an analytic solution of the Navier equation given certain boundary conditions. We have also successfully applied our approach to 2D and 3D real microscopy image sequences.

1 Introduction

Current time-lapse microscopy technology allows to analyze the location and movement of fluorescent tagged structures (proteins) within a living cell (see Fig. 1). Such an analysis is important to study subcellular processes. However, live cells change in position and shape over time. Therefore, when observing the tagged structures then only a superposition of the movement of proteins and that of the cell can be seen. To determine the real motion of proteins one has to compensate the movement and deformation of the cell. To this end it is necessary to register dynamic cell microscopy images w.r.t. a reference frame.

In previous work on the registration of microscopy images of moving cells mainly rigid registration approaches recovering translation and rotation have been used (e.g., [1]). However, rigid registration cannot cope with deformations of live cells. The assumption that the shape does not change only holds for certain applications. In applications where there are considerable shape changes, non-rigid registration schemes are required to decouple the global movement of

cells from the movement of fluorescent tagged protein particles. So far, only few approaches for non-rigid registration of cell microscopy images have been described in the literature [2, 3]. The work in [2] is based on semi-automatic extraction of point landmarks and uses a thin-plate spline transformation model. 2D images of fixed cells are analyzed and the deformation between two images is due to different stainings. In [3] segmented images and an optic flow-based registration scheme are used to register 3D static images of different cells. A disadvantage of this approach is that a segmentation of the images is required.

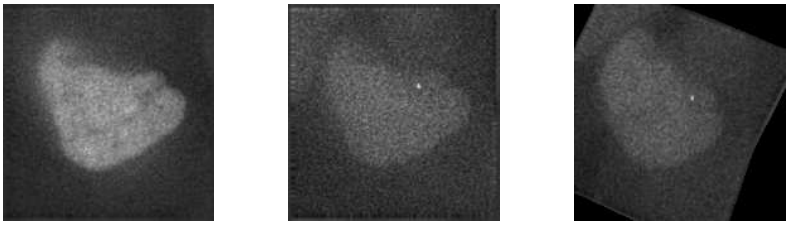
In comparison to [2, 3], we here introduce an approach for the registration of multi-channel cell microscopy image sequences which directly uses the original intensities to utilize the full image information. Our approach is fully automatic and is applied for the registration of 2D and 3D temporal image sequences of moving cells. We have compared the results of our approach with results based on segmented images. In addition, we have studied two different schemes for registering the images of an image sequence to a reference frame. In the first scheme, each image is directly registered to the first frame of the sequence (the reference frame). In the second scheme, subsequent images are registered and concatenated to obtain a registration to the first frame. We have evaluated our approach based on 3D synthetic images of a simulated spherical cell with known deformation which has been calculated based on an analytic solution of the Navier equation. We have also successfully applied our approach to 2D and 3D real microscopy image sequences.

2 Non-rigid temporal registration of cell images

For the registration of 2D and 3D multi-channel microscopy image sequences we have developed an intensity-based approach combining rigid and non-rigid transformations. The implemented scheme for registering two successive images consists of three steps: Preprocessing of the images by Gaussian filtering, rigid registration, and non-rigid registration. For rigid registration of 3D images we use a quaternion-based registration scheme which minimizes the mean-squared intensity error by a gradient descent optimizer. For non-rigid registration we use a variant of the demons algorithm [4], which is driven by symmetric forces and also incorporates a multi-resolution scheme.

We have also implemented two different schemes for the registration of the images to a reference frame. In the first approach, each image of a sequence is directly registered to the first image, without considering results from preceding time steps. Instead, in the second approach, information from previous time steps is exploited by an incremental scheme. To this end two subsequent images g_1 and g_2 at time steps 1 and 2 are registered, which results in a rigid transformation $\mathbf{R}(g_2, g_1)$ and a non-rigid transformation $\mathbf{U}$. The combination of both transformations then gives $\mathbf{u}(g_2, g_1) = \mathbf{U}(\mathbf{R}(g_2, g_1), g_1)$. For the registration of the subsequent image at time step 3 we concatenate this result with the transformation $\mathbf{u}(g_3, g_2)$ between time steps 3 and 2 to obtain the registration to the reference frame $\mathbf{u}(g_3, g_1) = \mathbf{u}(g_3, g_2) \circ \mathbf{u}(g_2, g_1)$. Generally, an image at time

Fig. 1. Example of one slice of a 3D cell microscopy image at a certain time step: (Left) Channel 1 with labelled cell, (Middle) Channel 0 visualizing the tagged protein in z-slice 25, (Right) Registered channel 0 with the tagged protein in z-slice 35



step k can be registered to the reference frame by concatenating previously calculated transformations by $\mathbf{u}(g_k, g_1) = \mathbf{u}(g_k, g_{k-1}) \circ \mathbf{u}(g_{k-1}, g_{k-2}) \circ \cdots \circ \mathbf{u}(g_2, g_1)$. To regularize the resulting deformation fields over time, we use a Gaussian filter on the final concatenated field. Our approach is directly applied to the intensity images. For a comparison we have also applied the algorithm to segmented images of the original image sequences.

3 Experimental results

To evaluate the developed approach we have generated 3D synthetic image sequences simulating the compression of a spherical cell. By solving the Navier equation $\mu \Delta \mathbf{u}(\mathbf{x}) + (\mu + \lambda)\nabla(\nabla \mathbf{u}(\mathbf{x})) = \mathbf{0}$, where $\mathbf{u}(\mathbf{x})$ denotes the deformation $\mathbf{u}$ at $\mathbf{x}$, and μ as well as λ are the Lamé coefficients, the deformation has been calculated analytically. The solution has been obtained by defining displacements at the border of the sphere, which act as boundary conditions.

The generated image sequences consist of 15 images with a resolution of 128×128×128 pixels. The spherical cell consists of an inner sphere (radius of 20 pixels) with uniform intensity of 150 and an outer sphere (radius 20 to 40) with an intensity level of 200. The compression of the simulated cell is obtained by defining displacements of 1 pixel per time step at the border of the sphere. To evaluate the effects of the registration on a moving subcellular structure a second image sequence was generated, which acts as second channel. This second sequence shows a spherical spot with a radius of 5 pixels, which moves by 2 pixels per time step from the center to the border of the sphere.

Given these 3D image sequences the performance of our approach was determined by calculating the errors in angle and magnitude of the deformation vectors w.r.t. the correct vectors as well as calculating the error in the location of the spot. The angular error and the normalized magnitude error at each pixel $\mathbf{x}$ of the computed vector $(\mathbf{u}(\mathbf{x}), 1)$ w.r.t. the correct vector $(\mathbf{u}_{true}(\mathbf{x}), 1)$ using a temporal distance of 1 are given by:

$$\bar{e}_{\text{angle}} = \arccos\left[\frac{(\mathbf{u}(\mathbf{x}), 1) \cdot (\mathbf{u}_{\text{true}}(\mathbf{x}), 1)}{\sqrt{1 + |\mathbf{u}(\mathbf{x})|}\sqrt{1 + |\mathbf{u}_{\text{true}}(\mathbf{x})|}}\right], \quad \bar{e}_{\text{magn}} = \frac{(|\mathbf{u}(\mathbf{x})| - |\mathbf{u}_{\text{true}}(\mathbf{x})|)^2}{|\mathbf{u}(\mathbf{x})|^2 + |\mathbf{u}_{\text{true}}(\mathbf{x})|^2}$$

Table 1. Averaged angular and magnitude error of the deformation field as well as spot location error for segmentation- and intensity-based registration of the synthetic image sequences

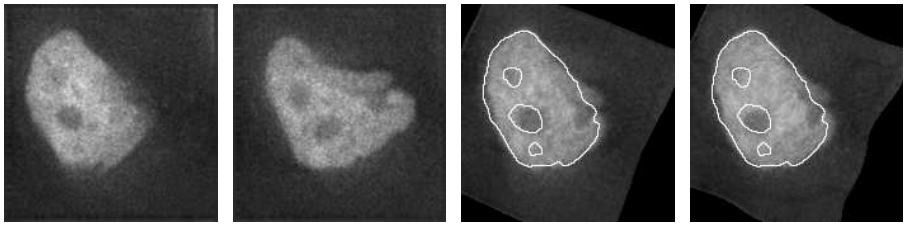
	$\bar{e}_{\text{angle}}$ [°]	$\bar{e}_{\text{magn}}$	$\bar{e}_{\text{spot}}$ [pixel]
Segmentation, incremental	7.16	0.08	2.40
Segmentation, direct	6.25	0.10	2.04
Intensities, incremental	7.87	0.08	1.59
Intensities, direct	5.90	0.09	1.31

The direct registration scheme as well as the incremental scheme have been applied to the original intensity images as well as to the segmented images. The errors have been averaged over all 14 images that have been registered to the first image of the sequence. The error in the location of the spot $\bar{e}_{spot}$ is determined by calculating the Euclidian distance w.r.t. the ground truth position. For the non-registered images, the location error of the spot is up to 13.5 pixels for the last image of the sequence. In Table 1 the calculated errors have been listed. It can be seen that registration using the original intensities gives significantly better results for the location of the spot (right column). The angular and magnitude errors (averaged over the whole images) are comparable for intensity-based and segmentation-based registration. It can also be seen that direct registration to the first image gives slightly better results than the incremental approach. The reason is probably that the assumed uniform deformation over time allows a relatively good direct registration from each image of the sequence to the first image. Instead, with the incremental approach small errors between two subsequent images accumulate.

We have also applied our approach to 2D and 3D multi-channel microscopy image sequences of live cells. The 2D image sequences depict two cells over 150 images at a resolution of 512×512 pixels. The cells show relatively small deformations between subsequent time steps but high total deformations over the whole sequence as well as considerable motion of subcellular structures. The internal structure of the cells exhibits high intensity variations. Registration based on the original intensity images yields a more accurate result for the internal structures of the cells compared to using the segmented images. In the latter case a smoother deformation field is obtained.

The 3D image sequences consist of up to 15 images per sequence with xy-resolutions between 160×152 and 196×196 pixels and z-depths between 31 and 41, showing a single cell nucleus and, in a second channel, a tagged protein, see Fig. 1. The cell nucleus exhibits relatively low intensity variations, moderate deformations between subsequent time steps, but large rigid motion. Since the sequence consists of 15 images, the overall deformation for the whole sequence is relatively small compared to the 2D image sequences consisting of 150 images. Registration based on the original intensities gave similar results as using the segmented images (see Fig. 2 and Fig. 1 (right)). The reason is probably the comparably low intensity variation inside the cell. Since here the overall de-

Fig. 2. Example of one slice of the 3D cell microscopy images: Original reference image at time step 1 (left) and step 8 (middle left), Registered image based on segmentation with edge overlay of the reference image (middle right) and using intensities with edge overlay of the reference image (right)



formations are moderate the direct registration scheme yielded more accurate results compared to the incremental scheme.

4 Discussion

We have presented an approach for the registration of 2D and 3D multi-channel temporal microscopy image sequences which utilizes the full image information. The approach has been applied to 3D synthetic image sequences as well as 2D and 3D real microscopy image sequences. It turned out that using directly the intensities gives more accurate registration results (compared to using segmented images) if the internal structure of the observed cells exhibits high intensity variations. We have also implemented and compared two different schemes for registering the images to a reference image. Here it turned out that the incremental scheme yields better results in case of large overall and non-uniform deformations. Future work will focus on analyzing a larger spectrum of 2D and 3D temporal microscopy image sequences in different applications.

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