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Non-rigid multi-frame registration of cell nuclei in live cell fluorescence microscopy image data

Marco Tektonidis [a,*], Il-Han Kim [a], Yi-Chun M. Chen [b], Roland Eils [a], David L. Spector [b], Karl Rohr [a]

[a] Dept. Bioinformatics and Functional Genomics, Biomedical Computer Vision Group, University of Heidelberg, BioQuant, IPMB, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 267, 69120 Heidelberg, Germany
[b] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

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ABSTRACT

The analysis of the motion of subcellular particles in live cell microscopy images is essential for understanding biological processes within cells. For accurate quantification of the particle motion, compensation of the motion and deformation of the cell nucleus is required. We introduce a non-rigid multi-frame registration approach for live cell fluorescence microscopy image data. Compared to existing approaches using pairwise registration, our approach exploits information from multiple consecutive images simultaneously to improve the registration accuracy. We present three intensity-based variants of the multi-frame registration approach and we investigate two different temporal weighting schemes. The approach has been successfully applied to synthetic and live cell microscopy image sequences, and an experimental comparison with non-rigid pairwise registration has been carried out.

1. Introduction

Analyzing the dynamic behavior of subcellular particles based on live cell microscopy images yields important information about the underlying biological processes. For example, promyelocytic leukemia nuclear bodies have been proposed to be involved in tumor suppression, viral defense, or DNA repair, and the movement of these particles within the cell nucleus is important for understanding cell function. However, in the images both the motion and deformation of a cell as well as the motion of particles within the cell are observed (Fig. 1). To cope with this problem, the dynamic image sequences need to be registered w.r.t. a reference time point. Registration yields a mapping of homologous points of a cell along the sequence of images, which can be used to compensate cell motion and deformation. The registration task can be quite challenging, for example, for image sequences which depict cell nuclei going into mitosis (cell division), in particular, during prophase and prometaphase. During these cell phases, the shape and the intensity structure of the nucleus are changing strongly due to, for example, chromatin condensation which causes a shrinking of the nucleus, nuclear envelope breakdown which causes a disassembly of the nucleus (cf. Fig. 1) or the disappearance of internal structures such as the nucleoli.

In previous work, different registration approaches have been proposed to compensate cell motion and deformation in live cell microscopy image data. Mostly rigid and affine transformations have been used comprising translation, rotation, and scaling of cells. In Rieger et al. (2004), for example, 3D rigid registration of cell nuclei is performed based on the center of mass and the intensity distribution of labeled proteins. Correlation-based approaches have been proposed by Wilson and Theriot (2006) for rigid registration of cells and by Goobic et al. (2005) to compute 2D translation in intravital video microscopy images of rolling leukocytes. Sage et al. (2005) described a model-based approach where a least-squares fit of an ellipse to segmented 2D images of a nucleus has been used to determine a rigid transformation. Matula et al. (2006) perform rigid registration of cell images by minimizing the mean-squared differences between two sets of corresponding points which represent subcellular structures. De Vos et al. (2009) described a two-step rigid registration approach for cell nuclei which uses the correlation coefficient and the geometric center of telomeric dots. In Dzyubachyk et al. (2010), a shape-based approach using distance functions is introduced for correction of affine cell motion in fluorescence microscopy images. For attenuation measurement and motion correction in live cell FLIP (fluorescence loss in photobleaching) 2D image sequences, van de Giessen et al. (2012) proposed a rigid registration approach based on a photobleaching model. Raza et al. (2012) employed a block-matching approach to determine translations in static 2D images from multi-tag fluorescence microscopy stacks.

* Corresponding author.
E-mail addresses: marco.tektonidis@bioquant.uni-heidelberg.de (M. Tektonidis), k.rohr@dkfz.de (K. Rohr).

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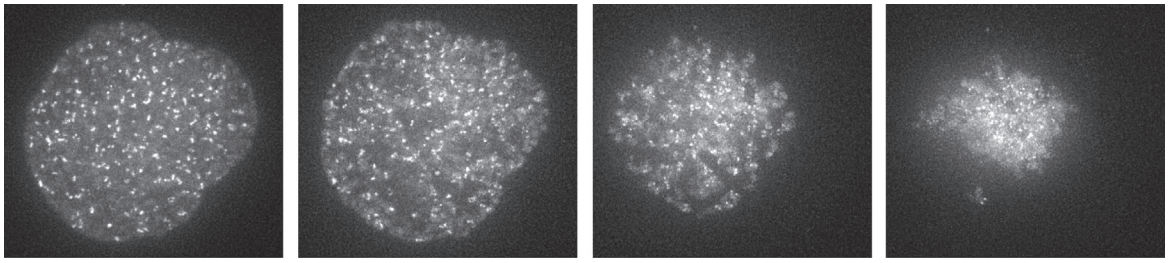

(a) $t = 1$ (b) $t = 30$ (c) $t = 60$ (d) $t = 90$

Fig. 1. Example images from a multi-channel microscopy image sequence (nucleus channel, contrast enhanced).

In previous work on *non-rigid* registration of cell nuclei in live cell microscopy image data, only few approaches have been described. Mattes et al. (2006), for example, described a landmark-based registration approach for cell nuclei using thin-plate splines. Yang et al. (2008) used an extension of the demons algorithm with symmetric forces to register static images of different cells as well as live cell microscopy images based on segmented images. Kim et al. (2011) introduced an intensity-based non-rigid registration approach for dynamic cell nuclei images which is based on an extension of the Lucas–Kanade optic flow algorithm. A contour-based non-rigid registration approach which utilizes thin-plate splines has been proposed by De Vylder et al. (2011) for registration of cell nuclei. Non-rigid registration of microscopy images has been also used in other biological applications. For 3D reconstruction of static fluorescence confocal microscopy images, Du and Wasser (2009) introduced an intensity-based non-rigid registration approach using thin-plate splines, and Hogrebe et al. (2012) introduced a landmark-based non-rigid registration approach using B-Splines. For 3D reconstruction of static electron microscopy images, Akselrod-Ballin et al. (2011) described a feature-based non-rigid registration approach and Saalfeld et al. (2012) used a block-matching non-rigid registration approach. Lorenz et al. (2012) employed intensity-based registration using B-Splines for 3D reconstruction and reduction of motion-induced artifacts in intravital images of living animals. To analyze gene expression data, Tomer et al. (2010) described an intensity-based approach using B-Splines, and Peng et al. (2011) introduced a landmark-based approach using thin-plate splines for non-rigid registration of static confocal microscopy images. For cell shape modeling, Rohde et al. (2008) used an intensity-based approach for non-rigid registration of segmented images of different cell nuclei, and Yoshizawa et al. (2010) used features from segmented nuclei and the plasma membrane for interactive non-rigid registration of microscopy images with different fluorescent markers of the same cell.

All approaches described above for non-rigid registration of dynamic cell microscopy images employ *pairwise* temporal registration. In pairwise registration, each frame of a sequence is registered either to a chosen reference frame (*reference* registration method) or to the previous frame (*consecutive* registration method). For registration of cell nuclei, the consecutive method is preferred since it can cope better with intensity variations of cell nuclei over time. Pairwise registration uses only two successive images for registration. However, to better exploit the temporal information and improve the registration result it is advantageous to use multiple images by a *multi-frame* registration approach.

In medical image analysis, *temporal groupwise* registration approaches have recently been proposed, which simultaneously take into account all images of a *dynamic* image sequence (e.g., Castillo et al., 2010; Metz et al., 2011; Yigitsoy et al., 2011; De Craene et al., 2012; Wu et al., 2013). Related approaches have been introduced for analyzing growth patterns of several subjects based on follow up image data (e.g., Durrleman et al., 2013). However, there the type of image data as well as the considered objects are very different compared to our application leading to different requirements for image analysis approaches. First, in our case we study fluorescence microscopy image sequences of cells which go into mitosis (cell division), whereas in medical image analysis *temporal groupwise* registration approaches for dynamic data have been used for MR, CT, and US images of organs (e.g., lung, liver, heart, brain). Second, another difference is that with temporal groupwise registration approaches typically periodic movements are considered, in particular, respiratory movements of the lung and liver (e.g., Castillo et al., 2010; Metz et al., 2011; Yigitsoy et al., 2011; Wu et al., 2013) or movements of the beating heart (e.g., Metz et al., 2011; De Craene et al., 2012). Periodic movement means that the considered organs periodically return to the same shape. Thus, an image sequence consists periodically of the same (or very similar) images. In contrast, in our application we consider cells that go into mitosis, where the structural differences increase with time. Thus, the images are increasingly different compared to the first time point which makes registration generally more difficult compared to periodic movement. In addition, in medical image analysis the intensity structure of the considered object is typically more homogeneous compared to our application where strong changes of the intensity structure of the cell nucleus occur since, for example, the nuclear envelope breaks down causing a disassembly of the nucleus, or internal structures such as the nucleolus can disappear. Because of the non-periodicity and the strong structural changes in our application, it is not advantageous to compute the intensity similarity measure between very distant time points (e.g., between the first and the last time point of an image sequence) as in temporal groupwise registration approaches. Instead, we propose a consecutive incremental and multi-frame non-rigid registration approach where the intensity similarity measure is determined between time points within a certain time range of an image sequence. Third, in our application due to the strong changes in intensity structure and the strong deformations, it is important to compute the deformation vectors for each pixel of an image. To this end we use a local differential registration approach. In comparison, *temporal groupwise* registration approaches typically use B-spline-based approaches and image grids that are coarser than one pixel spacing (e.g., grid spacing of $13 \times 13 \times 13$ voxel up to 20×20 pixel in Metz et al. (2011), grid size of $3 \times 3 \times 3$ up to $10 \times 10 \times 6$ control points in De Craene et al. (2012)), or consider a sparse number of image points with distinctive features (e.g., Wu et al., 2013), or take into account a subset of time points of a dynamic image sequence (e.g., Yigitsoy et al., 2011), while the information at other image points or time points is obtained by interpolation. Using a subset of the image information has the advantage that the complexity of the optimization problem is significantly reduced, however, the full image information is not exploited. Forth, in our application we consider a relatively large number of frames of a dynamic image sequence (100–200 time points). In comparison, *temporal*