

# Non-Rigid Registration of 3D Microscopy Images for the Normalization of Different Cell Nuclei

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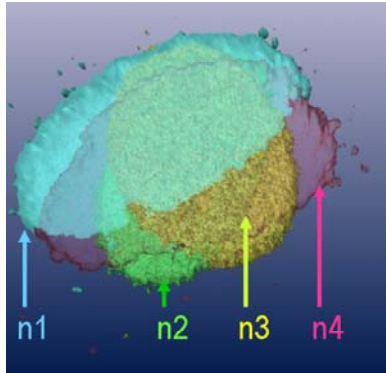
**Abstract.** We present an intensity-based approach for non-rigid registration to normalize 3D multi-channel microscopy images of different cell nuclei. A main problem with cell nuclei images is that the intensity structure of different nuclei differs very much, and thus an intensity-based scheme cannot be used directly. Instead, we first perform a segmentation of the images, smooth them by a Gaussian filter, and then apply an intensity-based algorithm. To reduce the computation time we use a multi-resolution scheme. Our approach has been successfully applied using 2D cell-like synthetic images, 3D phantom images, as well as 3D multi-channel microscopy images representing different chromosome regions and gene regions (BACs).

## 1 Introduction

For building maps of the 3D structure of chromatin fiber of the human genome in the cellular interphase, different cell nuclei need to be evaluated. The aim is to relate geometric information to genome function. Hence, one of the essential tasks is to analyze a large amount of multi-channel 3D microscopy images, which represent cell nuclei as well as genomic structures like chromosome regions and gene regions (BACs). However, since nuclei of different cells are considered it is necessary to normalize these images (see Fig. 1), i.e., we have to put them into the same coordinate system. Normalization can be achieved by image registration and generally one has to cope with both global rigid transformations as well as local non-rigid deformations.

Non-rigid registration approaches can be divided in two main categories: geometry-based and intensity-based schemes. With geometry-based approaches first geometric structures such as points or surfaces are extracted from the images and based on that a non-rigid transformation is computed. One main difficulty is the finding of correspondences which often requires some kind of user-interaction.

**Fig. 1.** Four different nuclei of HeLa cells overlaid with different colors (without normalization).



Intensity-based approaches, on the other hand, directly work on the image intensities and have the advantage that they are fully automatic. Recently, also hybrid approaches based on both geometry and intensity information have been proposed. The above-mentioned types of approaches have been widely applied in medical image analysis, however, application to biological image data is rare. According to our knowledge non-rigid registration schemes have not yet been used for 3D multi-channel microscopy images of different cell nuclei. For rigid registration of 3D images we refer to [1].

We have developed an intensity-based approach for non-rigid registration of 3D multi-channel microscopy images of different cell nuclei. Our approach is based on the demons algorithm [2], which has been successfully applied in medical image analysis. However, a main problem with 3D cell nuclei images is that the intensity structure of different nuclei differs very much, and thus an intensity-based scheme cannot be used directly. Therefore, in contrast to previous work, we first perform a segmentation of the images by global thresholding (after noise reduction using an anisotropic diffusion filter), smooth them by a Gaussian filter, and then apply an intensity-based algorithm. Since the computation time of the approach is relatively high, we use a multi-resolution scheme which improves the speed significantly. Our approach is fully automatic and has been successfully applied using 2D cell-like synthetic images, 3D phantom images, as well as 3D multi-channel microscopy images.

## 2 Method

To register 3D microscopy images of different cell nuclei we first reduce the noise in the images by applying a structure-preserving anisotropic diffusion filter [3] and then segment the images by a global thresholding scheme. We found that the selection of a threshold value is uncritical in our application. Then the 3D images are aligned globally by applying a rigid registration approach based on the mean-squared intensity error and a gradient descent optimization algorithm.

For local shape alignment we then employ a variant of the demons algorithm [2], which is driven by symmetric forces defined by the following equation:

$$\mathbf{u} = \begin{cases} \frac{2(g-f)(\nabla f + \nabla g)}{d} & d \geq \epsilon \\ 0 & d < \epsilon \end{cases} \quad (1)$$

where  $\mathbf{u}$  are the forces,  $f$  and  $g$  represent the intensity values in the target and source image,  $d = (\nabla f + \nabla g)^2 + (g - f)^2$ ,  $\nabla$  denotes the nabla operator, and  $\epsilon$  is a small positive constant. Our experimental results showed that this variant yields better results compared to the standard approach, where the forces are not symmetric. Application of a prior rigid transformation improves the registration result and also reduces the overall computation time. The standard demons approach assumes that the intensities remain constant (optic flow based scheme), which is not true in our application since different cell nuclei significantly differ in substructure shape and intensity. Direct application therefore results in unrealistic compression and dilation. In contrast, we here consider cell nuclei as a whole and apply the algorithm to the segmented images. However, our experiments showed that we obtain good results for the contour regions of the objects but not for the inner (homogeneous) parts. To overcome this drawback, we suggest to smooth the segmented images first using a Gaussian filter. The purpose of smoothing consists in producing larger non-zero gradient regions in the neighbourhood of edges. Typically we use a relatively large value of the standard deviation of the Gaussian leading to strong smoothing. The deformation field obtained in this way is more homogeneous and appears to be more correct.

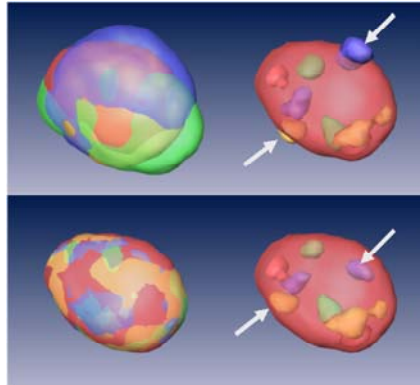
One problem still is the relatively high computation time of the algorithm, which is typical for intensity-based non-rigid registration schemes. For 3D images typically several hours are required. To reduce the computation time we have used a multi-resolution scheme, which follows the principle "from coarse to fine", so that the registration process can be accelerated significantly. Using this scheme we could reduce the computation time, e.g., for an image size of  $512 \times 512 \times 24$  voxels from 845 min to 86 min, and for an image size of  $256 \times 256 \times 33$  voxels from 44 min to 7 min.

For 3D multi-channel images the transformation is computed based on the first channel which includes the cell nuclei and then the transformation is applied to this channel as well as to the other channels which represent the different chromosome or gene regions (BACs).

### 3 Experimental Result

To systematically test our approach we have generated 2D cell-like synthetic images. Application of our non-rigid registration approach showed that symmetric forces lead to better results than asymmetric forces. We also found that a prior rigid transformation improves the registration result and also reduces the overall computation time.

**Fig. 2.** Top: Four different HeLa cell nuclei (left) with chromosome 4 regions (right) before registration. Bottom: Corresponding images after registration overlaid with a nucleus of one cell as a reference. The arrows point to corresponding chromosomes.



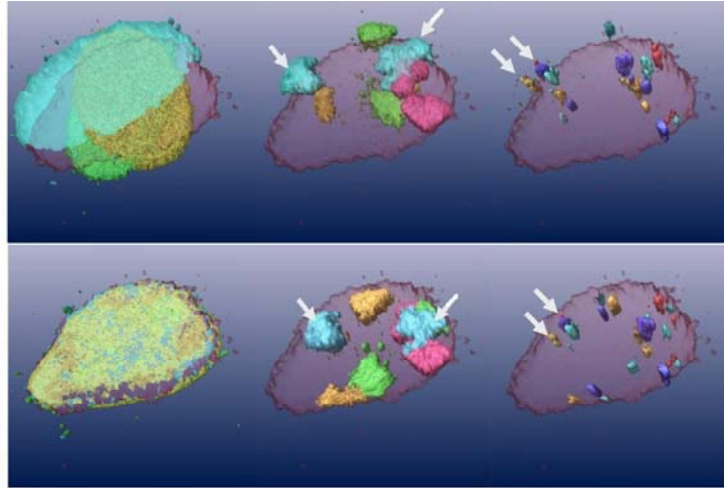
Next, we have validated our scheme using 3D images of the deformation of a silicon cube [4]. Comparing the displacements from the computed transformation with the real displacements at 343 points we obtained an acceptable correlation coefficient of  $r = 0.96$  (for 12.5% strain).

Moreover, we have successfully applied our approach to 3D multi-channel cell nucleus images representing different chromosome regions and gene regions (BACs). For example, in one experiment we used four confocal 3D images from a 3D FISH (fluorescence in situ hybridisation) experiment with HeLa cells and using DAPI counterstain of the nucleus (image size  $256 \times 256 \times 33$  voxels). We considered chromosome 4 and visualized the 3D registration result by overlaying the transformed segmented chromosome territories together with the surface of one of the nuclei as a reference. Fig. 2 illustrates that before registration some chromosomes lie outside the reference cell nucleus. After registration using our approach, all chromosomes are inside the reference cell nucleus and the chromosome structures from the different cells are located more closely together. In this way we achieve a normalization of the image data. In another experiment with 3D images (image size  $512 \times 512 \times 24$  voxels), where chromosome 2 as well as gene regions (BACs) were stained, we obtained the results in Fig. 3. Recently, we also successfully applied our approach for the registration of 3D images of a moving cell nucleus.

## 4 Conclusion

We have presented an intensity-based non-rigid registration approach for the normalization of 3D multi-channel microscopy images of cell nuclei. The approach is based on a variant of the demons algorithm, uses segmented images, and employs a multi-resolution scheme. We have successfully applied our approach to confocal 3D images from different FISH experiments with HeLa cells. The ap-

**Fig. 3.** Top: Four different HeLa cell nuclei (left) with chromosome 2 regions (middle), and gene regions (right) before registration. Bottom: Corresponding images after registration overlaid with a nucleus of one cell as a reference. The arrows point to corresponding chromosome und gene regions (BACs).



proach is quite robust and can cope with relatively large shape differences. The achieved normalization should facilitate accurate quantification and statistical analysis of cell nuclei data. Further improvement of the computation speed is expected by using a distributed computer cluster.

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## References

1. Rieger B, Mollenaar C, Dirks RW, van Vliet LJ. Alignment of the Cell Nucleus From Labeled Proteins Only for 4D In Vivo Imaging. *Microscopy Research and Technique* 2004;64:142–150.
2. Thirion JP. Image matching as a diffusion process: an analogy with Maxwell's demons. *Medical Image Analysis* 1998;2:243–260.
3. Perona P, Malik J. Scale-space and edge detection using anisotropic diffusion. *IEEE Trans on Pattern Analysis and Machine Intelligence* 1990;12:629–639.
4. Kerdok A, Cotin S, Ottensmeyer M. Truth cube: Establishing physical standards for soft tissue simulation. *Medical Image Analysis* 2003;7:283–291.
5. Ibanez L, Schroeder W, Ng L, Cates J. *The ITK Software Guide*. Kitware Inc.; 2005.