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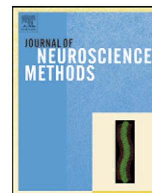


# Image analysis of time-lapse movies—A precision control guided approach to correct motion artefacts

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ABSTRACT

In long-term time-lapse studies of cell migration, it is often important to distinguish active movement of individual cells from global tissue motion caused, for instance, by morphogenetic changes, or due to artefacts. We have developed a method to define and correct global movements. This is realized by the sequential morphing of image sequences to the initial image based on the position of immobile reference objects. Technically, the approach is implemented in *ImageJ*, using the plugin *UnwarpJ*. We describe an efficient way to select parameter settings such as to optimize image correction. To this end, we implemented a strict statistical control that allows to quantify image registration quality. We document this approach using a time-lapse sequence of migrating interneurons in slice cultures of the developing cerebellum.
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## 1. Introduction

Within recent years, the development of increasingly sophisticated methods of vital staining and ever more sensible techniques of imaging have opened new approaches to study the cell and tissue dynamics underlying a wide variety of biological and pathological processes, from development, tissue turnover and regeneration to inflammation, malignant invasion and tumor spreading (e.g. Mareel and Leroy, 2003; von Andrian and Mempel, 2003; Ross and Walsh, 2001; Filla et al., 2004; for a review, see Dormann and Weijer, 2006). A common goal of such studies is to derive a quantitative description of cellular locomotion, or cell–cell and cell–extracellular matrix interaction that will either allow to infer basic molecular mechanisms of these processes, or to provide a basis for quantitatively assessing the effect of molecularly defined manipulations (e.g. treatment protocols) on cellular motility.

However, cell locomotion has to be defined relative to the surrounding matrix or tissue, which itself can perform deformations and movements, in particular over time spans typically covered in developmental studies of morphogenetic processes (Kafer et al., 2006; Palsson and Othmer, 2000; Rembold et al., 2006). The central nervous system of vertebrates, for example, develops in a series of highly dynamic morphogenetic rearrangements which transform a small plate-like structure first into a tube and later into the complex structures of the brain and spinal chord (Gilbert and Scott, 2008). These dramatic global tissue changes are caused by the finely controlled growth and migration of neural cells (Hatten, 1999) within a remodelling extracellular matrix (Rauch, 2004). Moreover, when cell locomotion in developing tissues is examined *in vitro* additional global movements as, for example, anisotrophic swelling or a time-dependent flattening of tissue slices may occur due to limitations in the physiological control of specimens.

Therefore, the assessment of the locomotion of actively migrating cells relative to such a deforming tissue or matrix requires an adequate quantification of the global, dynamic changes in tissue morphology parallel to single cell movement. Here, we have chosen image sequences recorded from slice cultures of mouse cerebellum, in which inhibitory interneuron precursors were fluorescently labelled by expression of GFP from the Pax2 locus (Pfeffer et al., 2002; Weisheit et al., 2006) to address this issue. In these cultures, global tissue motion is a common phenomenon and easily recognized (e.g. Fig. 1): the challenge, then, is to establish a reproducible protocol to quantify movement of individual cells relative to global movement.

Here, we present an approach, based on the iterative application of image unwarping to time-lapse sequences of microscopic images to solve this problem. Our approach maps each image of the

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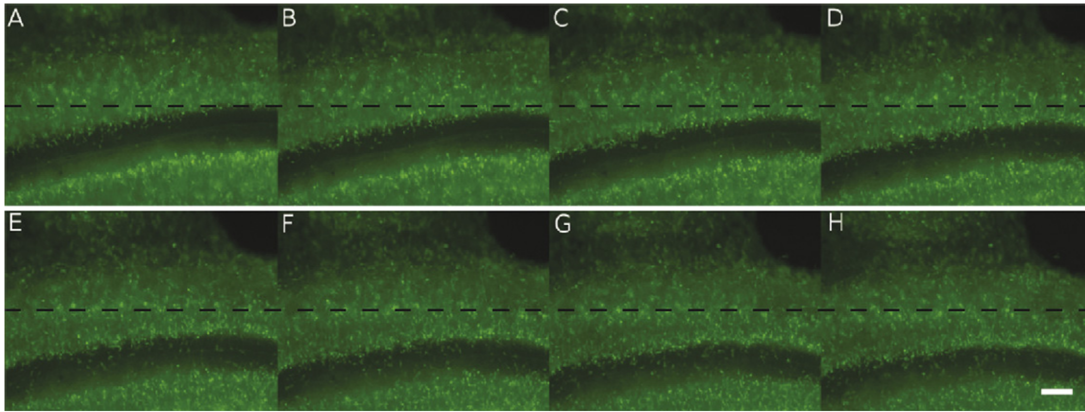


**Fig. 1.** Deformation of a slice culture prepared from an 8-day-old cerebellum over a culture period of 16 h. Shown are 8 out of 96 images. Time between subsequent images is 120 min. Images are 870 μm × 650 μm (696 × 520 pixels) in size. A fixed dashed line at the mid of the image frame is drawn to facilitate visualization of tissue distortion. Scale bar = 100 μm (full movie in supplemental material).

series under investigation onto the geometry of the initial image, which thus can be used as a fixed reference frame for the movement of single cells. The main goal of the following analysis is to document how stringent quantitative assessment of image unwarping precision may be utilized to guide image registration in order to optimally account for global tissue movement. Our data also provide an estimate of the limits of analytical precision of our method, and they show its implementation with standard computational resources.

## 2. Material and methods

### 2.1. Animals

Animal handling was done in strict adherence with local governmental and institutional regulations, and all efforts were made to minimize the numbers of animals used and their suffering. Pax2-GFP transgenic mice were kindly provided by M. Busslinger (Pfeffer et al., 2002). They were kept as heterozygotes on a C57Bl/6 background. To establish organotypic slice cultures, 8-day-old pups were killed by decapitation. Cerebella were rapidly dissected, taking care not to disrupt the meninges. Sagittal sections were obtained using a McIlwain tissue chopper (Campden Instruments Ltd.) at 250–300 μm. They were collected in neurobasal medium supplemented with B27 and 2 mM L-glutamax I (all from Invitrogen) at room temperature. Subsequently, slices were transferred, with 500 μl complete medium to 12-well cluster plates that had been coated with poly-L-lysine for 2 h at 37 °C. The slices were cultured for approximately 4 h in a standard incubator (5% $CO_2$/air, water-saturated, at 37 °C) to allow them to attach to the wells. Thereafter, the cluster plates were transferred to a heated (37 °C), gassed (5% $CO_2$/air), and humidified chamber fitted onto an inverted microscope (Leica DM IRE2 HC Fluo) with a motorized cross-stage (Märzhäuser) and a HBO 100 lamp. Images were recorded every 10 min, using a 10× objective (10×/0.22 LMC −/−7.8 objective, Leica) and a GFP Filter set (BP470/40; RKP500; BP525/50). Illumination was minimized by use of a shutter synchronized with the camera.

Images were recorded with a digital camera (Leica DFC350FX) and stored with the FW4000 database software (Leica). All further image processing was done using *ImageJ* software (NIH; http://rsb.info.nih.gov/ij/). In addition to the basic *ImageJ* configuration, we used the following plugins: *ManualTracking* (http://rsb.info.nih.gov/ij/plugins/track/track.html), *Mtrack2* (http://valelab.ucsf.edu/~nico/IJplugins/MTrack2.html), *MtrackJ* (http://www.imagescience.org/meijering/software/mtrackj/), and *UnwarpJ* (Sorzano et al., 2005).

### 2.2. UnwarpJ

As the procedures implemented in the *ImageJ* tool *UnwarpJ* are of central importance for the approach presented here, a brief description seems appropriate. For further detail see Thevenaz et al. (2000) and Sorzano et al. (2005).

The method of registration used by *UnwarpJ* to morph two images $I_1$ and $I_2$ by constructing a suitable map $R_{1,2}: I_1 \rightarrow I_2$ is based on a numerical procedure for minimizing the energy functional:

$$\begin{aligned} E &= E_{w_{\mathrm{im}},w_{\mathrm{div}},w_{\mathrm{curl}}}(R_{1,2}) \\ &= w_{\mathrm{im}} \times E_{\mathrm{im}}(R_{1,2}) + w_{\mathrm{div}} \times E_{\mathrm{div}}(R_{1,2}) + w_{\mathrm{curl}} \times E_{\mathrm{curl}}(R_{1,2}). \end{aligned} \quad (1)$$

This is an affine linear sum of three quadratic functionals, each multiplied by a positive weight factor $w_i$. The first integral $E_{\mathrm{im}}$ simply measures the mean quadratic deviation between the image grey scale values $I_2$ and $R_{1,2}(I_1)$, whereas the other two are implemented as penalty functionals measuring the mean quadratic divergence ($E_{\mathrm{div}}$) and curl ($E_{\mathrm{curl}}$) of the probed transformation $R_{1,2}$. In consequence, the larger the factors $w_{\mathrm{div}}$ or $w_{\mathrm{curl}}$ are chosen, the more does the minimization procedure reduce the mean values of $\mathrm{div}(R_{1,2})^2$ or $\mathrm{curl}(R_{1,2})^2$, respectively, for the obtained “optimal” morphing map $R_{1,2}$. Thus, if one knows beforehand that the tissue movement between $I_1$ and $I_2$ shows a clear component of dilatational volume expansion or compression, then the optimal map $R_{1,2}$ should have a significant divergence and the weight $w_{\mathrm{div}}$ has to be chosen small relative to the image weight $w_{\mathrm{im}}$. Moreover, if the tissue reveals minor (or almost no) rotational components, then the curl weight $w_{\mathrm{curl}}$ should be chosen large (or very large) compared to $w_{\mathrm{im}}$.

### 2.3. Strategy for a precision-controlled approach to sequential image registration

Our unwarping process follows the schemes sketched in Figs. 2 and 3 and includes a rigorous statistical quality control (see below). For any pair of successive images, $(i-1)$ and $i$, in a given stack, we use *UnwarpJ* to determine the change from image $(i-1)$ to image $i$ ($R_{(i-1),i}$: registration) and its inverse transformation ($U_{(i-1),i}$: unwarping). This procedure is sequentially applied to successive pairs of image frames, from $i = 2$ up to the last image in the stack ($i = n$). Thus, a chain of $(n-1)$ transformations ($U_{(i-1),i}$) is calculated.

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