# Improved Elastic Registration of Low-Contrast Fluorescent Microscopy Images Using the Behaviour of Local Similarity

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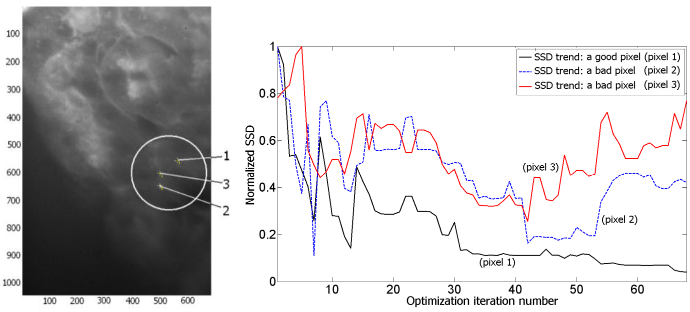
Recent confocal imaging techniques have been developed to utilise a per-pixel weighted subtraction on a pair of simultaneously acquired images. It is therefore necessary to ensure that the images are successfully registered before this processing step. Elastic image registration is commonly addressed as a parametric modelling problem based on B-splines [1, 2] combined with an optimization algorithm to maximize a similarity measure. Sum of squared differences (SSD) is a common similarity measure in use. For low contrast microscopy images, the background pixels and the low-intensity data pixels have a negative contribution to the overall similarity measure calculation. Such pixels drive some of the B-spline coefficients onto an incorrect optimization path. Therefore, there must be a dynamic mechanism for controlling the optimization process and excluding misleading pixels from the calculations. Figure 1(a) shows a sample "mouse kidney" image acquired by low light fluorescent microscopy. Three pixels are landmarked in this figure. Figure 1(b) shows the normalized value of local SSD for these pixels during image registration. Local SSD of pixel number one is minimized, whilst local SSD value for pixels two and three show fluctuations and do not converge to a minimum value.




(a) (b)

Figure 1: (a) A sample low-contrast "mouse kidney" image. Three pixels are landmarked; (b) The behaviour of local SSD for the pixels in (a).

In this paper we present a novel mechanism that monitors the value of local similarity measure during registration and excludes pixels that mislead the optimization. Our novel algorithm uses the fluctuating behaviour of Figure 1(b) to exclude such pixels. Consider two images $I_t(x,y)$ and $I_s(x,y)$, denoted target and source respectively, which are functions defined as mappings from the regions of interest $\Omega_t \subset \mathbb{Z}^2$ and $\Omega_s \subset \mathbb{Z}^2$ to the intensity domain $\mathbb{R}$. Image registration aims at finding a transformation function $\mathbf{T}(x,y): \mathbb{R}^2 \to \mathbb{R}^2$ from the coordinates of $I_t$ to those of $I_s$, which is a combination of an affine and an elastic transformation. In this paper we only consider the latter, which can be modelled using the cubic B-spline basis functions as in equation (1):

$$\mathbf{T}(x,y) = \sum_{k=0}^{3}\sum_{l=0}^{3} \begin{pmatrix} c_{1,k,l} \\ c_{2,k,l} \end{pmatrix} \beta_3\left(\frac{x}{s_x} - k\right) \beta_3\left(\frac{y}{s_y} - l\right) \tag{1}$$

In this equation, $\beta_3$ is a third-order B-spline basis function, $s_x$ and $s_y$ control the image resolution in the multiresolution registration framework, and $c_{1,k,l}$ and $c_{2,k,l}$ are the set of coefficients to be optimized. Many different implementations of the B-spline based image registration have been developed. The one we employ here is the multiresolution implementation presented in [3]. Because the optimization process is performed over time, we define the three dimensional space of SSD values $Z$. The members of $Z$ are the local SSD value for the patches $\mathcal{P}^{l}_{t,(x,y)}$ at iteration steps $\zeta$, where $\zeta = 1,2,3,...M$ represents the iteration number, with $M$ representing the final optimization iteration. The value of $M$ varies depending on the images and the algorithm in use. For each pixel $(x_t, y_t)$ and each iteration step $\zeta$ we define a stack of size $m$ of the most recent SSD values. This forms a two dimensional space $\Lambda_\zeta$ of vectors $\lambda_{\zeta,t,(x,y)}$ of length $m$, where $\lambda_{\zeta,t,(x,y)}$ is defined as in equation (2).

$$\lambda_{\zeta,t,(x,y)} = \left[ SSD_{\mathcal{P},\zeta,t}, SSD_{\mathcal{P},\zeta-1,t}, ..., SSD_{\mathcal{P},\zeta-m+1,t} \right] \tag{2}$$

The discrete time derivative of $\lambda_{\zeta,t,(x,y)}$ is equal to:

$$D\lambda_{\zeta,t,(x,y)} = \left[ d\lambda_{\zeta,t,(x,y)}, d\lambda_{\zeta-1,t,(x,y)}, ..., d\lambda_{\zeta-m+2,t,(x,y)} \right] \tag{3}$$

Where:

$$d\lambda_{\zeta-k,t,(x,y)} = SSD_{\mathcal{P},\zeta-k,t} - SSD_{\mathcal{P},\zeta-k-1,t}, \quad k \le m-1 \tag{4}$$

If the number of consecutive sign changes in $D\lambda_{\zeta,t,(x,y)}$ is more than a certain value, which we call $\mu$, the algorithm excludes the pixel $(x_t, y_t)$ from further SSD calculation. For example, assume two pixels $(x_1, y_1)$ and $(x_2, y_2)$, with $m = 6$ and:

$$\begin{cases} sign\left(D\lambda_{\zeta,(x_1,y_1)}\right) = [-,+,-,+,-] \\ sign\left(D\lambda_{\zeta,(x_2,y_2)}\right) = [+,+,-,+,-] \end{cases} \tag{5}$$

If $\mu = 3$ for example, we exclude $(x_2, y_2)$ from further SSD calculation, but include $(x_1, y_1)$. We discuss about changing the above parameters. We stop the optimization when fifty percent of the pixels are excluded. Figure 2 shows the value of overall SSD for Figure 1(a) during registration before and after masking poor pixels. As we see, the number of iteration steps is reduced down to about one-fourth of the original approach in [3]. The value of average registration error is reduced from 6.20 to 3.64 pixels in this experiment.

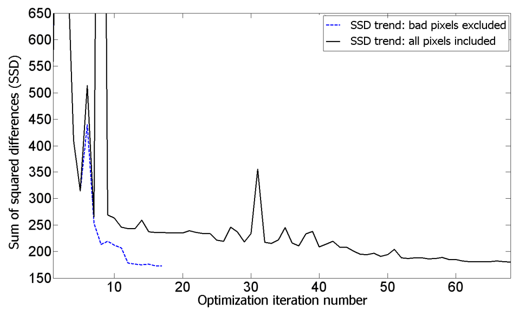



Figure 2: Overall SSD for the mouse kidney image of Figure 1 before and after excluding poor quality pixels.

We show that omitting such pixels during registration improves both accuracy and performance of image registration and produces faster convergence. In contrast to available methods, which treat the optimization as an input/output block, this approach constantly monitors what happens in each iteration step and prevents the deformation field from falling into incorrect directions which are sometimes irreversible.

[1] C. Ó. S. Sorzano, P. Thévenaz, and M. Unser. Elastic Registration of Biological Images Using Vector-Spline Regularization. *IEEE Transactions on Biomedical Engineering*, 25(4): 652-663, 2005.

[2] D. Rueckert, L. I. Sonoda, C. Hayes, D. L. G. Hill, M. O. Leach, and D. J. Hawkes. Nonrigid Registration Using Free-Form Deformations: Application to Breast MR Images. *IEEE Transactions on Medical Imaging*, 18(8): 712-721, 1999.

[3] J. Kybic, P. Thévenaz, A. Nirkko, and M. Unser. Unwarping of Unidirectionally Distorted EPI Images. *IEEE Transactions on Medical Imaging*, 19(2): 80-93, 2000.