Elastic Atlas Registration of β - Autoradiograms Using Scattered Data Interpolators

J. Cabello*, A. Metaxas, A. Bailey, I. Kitchen and K. Wells

Abstract—Autoradiography is a widely extended pre-clinical nuclear imaging modality used in life sciences to investigate and localise radiolabelled biological pathways in thin *ex-vivo* tissue sections. After the tissue section has been exposed to an ionising radiation detector the resulting labelled regions are subsequently analysed. Typically, the resulting autoradiograms are analysed manually by an expert life scientists using a visual template as reference to measure the different radioligand uptake levels in the different areas of, in our case, mouse brain. This process is extremely time consuming and error prone, with the expertise of the life scientist playing a significant role. In this paper we describe a semi-automatic method to register a template brain atlas on to the brain autoradiogram making the analysis process more efficient, repeatable and independent of the expertise of the life scientist. The method first identifies those regions with high and low level of radioligand uptake by region growing segmentation. Subsequently, the counterpart regions in the corresponding atlas image are manually identified. Finally a set of control points is extracted from each region contour in the autoradiogram and the atlas image to apply a scattered data interpolator.

Index Terms—Autoradiography, Atlas-based registration, Elastic registration, Scattered data interpolators.

I. INTRODUCTION

Autoradiography (AR) is a method used to map the distribution of radiolabelled biomolecules deposited in a thin tissue specimen. After the sample has been exposed to autoradiographic film, subsequent analysis is performed on the digitised autoradiogram. This is currently undertaken by manually segmenting those regions of functional importance [1], usually showing significant high levels of radioligand density. In terms of throughput, this is considered as one of the main bottle-necks in AR that remains. A possible approach to semi-automatically warp a digitised mouse brain atlas on to the autoradiogram is presented here. The scientific literature published so far has shown some alternatives to co-register autoradiograms of the same subject to obtain a 3D brain model [2], [3] to better understand the areas where the ligand uptake has been located, but under this situation the same problem arises. The regions of interest have to be manually selected. No attempt has been found, to the best of our knowledge, to register an autoradiogram with an atlas so far.

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The algorithm we present is comprised of two steps: first those regions with significant high and low levels of ligand density are automatically segmented, by using a region grower-based approach, and secondly the atlas is elastically warped on to the autoradiogram using the previously segmented regions as landmarks. Whilst principally applied here to AR data, the method is also applicable to the registration of PET and SPECT functional image data.

II. SEGMENTATION OF FUNCTIONAL DATA

The segmentation method used here is based on region growing, as this has been demonstrated to be a robust method of segmenting data highly corrupted with statistical noise, as in the case of autoradiographic data. An initial pre-processing step is undertaken before segmentation. This is sub-divided in (1) thresholding based on gradient descent, to remove the background of the autoradiogram, and (2) anisotropic filtering [4], to reduce the amount of statistical noise but keeping the edges.

The two main parts of any region grower-based algorithm are the similarity criterion, which is the condition used to append pixels to the region grower, and the termination criteria, which represent the conditions that the region grower has to fulfil to halt the appending of pixels. This algorithm is described in more detail in [5] and briefly summarised below.

A. Initialisation

The algorithm starts by locating automatically an initial seed pixel, which corresponds to the pixel with the highest intensity value. When the region grower has segmented the first region, the algorithm segments recursively the remaining of the autoradiogram by locating automatically consecutive seed pixels following the same criterion. When a seed pixel is located, an $N \times N$ seed region, centred on the seed pixel, is placed to obtain the mode (m_o) and the lower (σ_l) and upper (σ_u) standard deviation of the pixels within the seed region. These σ_l and σ_u correspond with the resulting σ of only those pixels below and above m_o respectively. The use of two different standard deviations is based on the typical case of a textured region where the histogram of the original seed area is modelled with a Gaussian mixture [6]; thus the mode of the histogram approximately sets the middle of the mixture, one standard deviation (σ_l) represents the lower values of the mixture describing, in an approximate way, the variation of width of an assumed Gaussian-like (but contaminated) distribution, and the second (σ_u) is a similar descriptor for the higher values of the mixture (see Figure 1). If the seed region

is placed on a very homogeneous region and the histogram can be modelled with a single Gaussian, i.e. σ_l and σ_u are very similar.

Fig. 1. Model of a low Contrast-to-Noise-Ratio Gaussian mixture where two different standard deviations, σ_l for lower values and σ_u for higher values, are computed to better model the statistics of the original seed region.

It has been observed that after several iterations of the region grower, in some cases there still exist some regions with low levels of ligand uptake (low intensity values) that have not been segmented. Therefore, once the region grower has segmented all the possible regions starting with high intensity pixels, the entire process is applied to an intensity-inverted version of the same autoradiogram.

B. Similarity criterion

The similarity criterion is a condition that allows candidate pixels to be appended if their pixel intensity values are within a certain range, automatically defined in terms of region statistics by the upper and a lower threshold explained in the previous section. In this work, a dynamic similarity criterion has been used [7], capable of adaptively modifying the aforementioned thresholds as the region grower appends pixels. An example of the evolving boundary at three different positions, corresponding to three different values of one of the control parameters in the similarity criterion, is shown in Figure 2. Once the localised seed region statistics are computed, then a one pixel wide border, defined by T (equation 1), is considered, and each pixel of the border is evaluated.

$$T = \{x_{ij} \notin \bigcup_{i=1}^n R_i : N(x_{ij}) \cap \bigcap_{i=1}^n R_i \neq \emptyset\} \quad (1)$$

where $N(x_{ij})$ is the set of immediate neighbours of pixel x_{ij} . The candidate pixel intensity, x_{ij} , will be appended to the region $\{R_i\}$ if it fulfils the homogeneity criterion, i.e. if its intensity value is within the margins defined by equation 2:

$$x_{ij} \begin{cases} \in R_i & \text{if } m_o - k\sigma_l < \hat{x}_{ij} < m_o + k\sigma_u \\ \notin R_i & \text{otherwise} \end{cases} \quad (2)$$

where $\hat{x}_{ij}$ is the mean of the 3×3 ROI centred in x_{ij} , m_o , σ_l and σ_u represent the parameters shown in Figure 1, and k is a parameter that controls the marginal step size. This

parameter k gives this region grower the capability of adapting the homogeneity criterion to the different difficulties exhibited in typical autoradiographic data with low Contrast-to-Noise-Ratio, such as blurred edges and high statistical noise.

In actuality, the reference pixel x_{ij} is typically used in equation 2, instead of $\hat{x}_{ij}$, but due to the high statistical noise present in this kind of images, this represents an improvement to avoid a premature halt of the region growing. An initial value of k is set manually but this is later increased dynamically as in [7] (see Figure 2).

Fig. 2. Evolution of the boundary as the similarity criterion is modified, for different values of one of the control parameters defined as k . The initial seed region, painted in red, is indicated with an arrow.

C. Termination criteria

When the similarity criterion is modified four different termination criteria are evaluated to assess whether the region grower has to halt or continue growing. These termination criteria consider (1) the region's intensity histogram, (2) region size, (3) edge of the region being grown and (4) how frequent the region grower has updated the similarity criterion.

If any of these criteria determines that the an edge has been found or the region grower has leaked in to an adjacent region then the growing process stops.

III. ELASTIC REGISTRATION WITH SCATTERED DATA INTERPOLATORS

After the region grower has delineated those functional regions with significant high and low levels of ligand density, counterpart regions in segmented autoradiogram and the atlas image are manually defined, resulting in two sets of N regions, one corresponding to the autoradiogram and another corresponding to the atlas. Before applying the elastic registration the autoradiogram and atlas are first registered using an initial affine registration with 5 degrees of freedom: translation (2), rotation(1) and scaling(2), using the principal axes transformation method [8]. However, this initial alignment is unable to match the internal functional regions with the atlas due to the sample stretching that occurs during its preparation and sample-to-atlas variations. Thus, an elastic approach based

on scattered data interpolators is then used to improve the accuracy of the registration.

Initially, a set of equidistant control points is automatically extracted from the autoradiogram contour and the atlas contour, making both contours match the deformations caused by the cleaving of the tissue during sample preparation. Subsequently a set of control points is extracted from each of the functional regions previously segmented. Special attention is paid to the number of control points extracted from each functional region and the location of those control points: too many control points will generate unexpected behaviour in the data interpolators; too few control points will not represent accurately the region contour. Conversely, if the control points do not represent accurately each contour the registration will present significant local misalignments.

Each set of control points is post-processed to obtain an accurate representation of the contour of each region while avoiding over-crowding of control points along the contour. This is achieved by limiting the maximum distance between adjacent control points along the region contour, and also by limiting the minimum distance between control points from different functional structures. Figure 3(a) represents an exemplar region contour from the atlas. The extracted set of control points and the resulting final set of control points after post-processing are shown in Figures 3(b) and 3(c) respectively. Locations where control points have been shifted from their original positions, to better describe the region contour, are indicated in Figure 3(c) with red arrows.

Fig. 3. Contour of an atlas region (a), extracted set of 128 control points (b) and post-processed set of control points (c).

After obtaining the set of control points a scattered data interpolator is applied to both data sets. Several global support data interpolators, such as Inverse Distance Weighted [9] and Radial Basis Functions (RBFs) [10], and local support interpolators, such as B-Splines [11] and RBFs based on the Wendland function [12], have been implemented. The *locality* of the B-Splines is controlled by the distance between control

points of the underlying lattice and in the Wendland radial basis functions, by the radius of the function. For a detailed description of each interpolator see references [9], [10], [11], [12].

IV. RESULTS

Figure 4 represents an example of an autoradiogram of ^{125}I -epibatidine density binding to $\alpha_4\beta_2$ heteromeric nAChRs in a mouse brain section. This example shows the resulting segmentation of the original autoradiogram and its intensity inverted version. Observing the original autoradiogram (Figure 4(a)) it can be noted how some structures have a counterpart in the atlas image (Figure 4(b)), and some other structures do not, the reason being that atlases are created based on large data sets of images to obtain a generic atlas. This means that in practise a specific autoradiogram will show a limited level of agreement with the corresponding atlas image. The accuracy of this method is strongly dependent on this level of agreement between autoradiogram and atlas.

Observing Figures 4(a) and 4(d) it can be identified how most of the brightest structures in the autoradiogram are nonetheless correctly segmented.

Fig. 4. Original autoradiogram of $\alpha_4\beta_2$ nAChRs binding in a mouse brain section (20 μm thick) labelled with ^{125}I -epibatidine (0.1 nM), cut at the level of Bregma -3.16 mm (a), corresponding atlas section (b), resulting segmented autoradiogram (c) and original autoradiogram with those regions segmented delineated (d).

The result of applying the affine registration between the atlas (source image) and the autoradiogram (target image) is shown in Figure 5. Internal functional structures show significant mismatches between the atlas and the autoradiogram, making elastic registration necessary.

The resulting registered autoradiogram after applying a scattered data interpolator based on thin-plate splines RBFs, as representative global support interpolator, is shown in Figure 6(a). The resulting control grid and vector field are shown in Figures 6(b) and 6(c) respectively.

Fig. 5. Affine registration of the autoradiogram and the slice of the atlas corresponding to that brain section (Bregma -3.16 mm). In general poor correspondence between the internal functional regions can be observed.

Fig. 6. Autoradiogram of mouse brain section with corresponding atlas section superimposed on top after applying a scattered data interpolator based on thin-plate splines RBFs (a). The image in (b) shows the resulting control grid showing the warping function, and the image in (c) shows the displacement vectors corresponding to the warping function.

The resulting registered autoradiogram after applying a scattered data interpolator based on B-splines with a lattice interspace of 10 pixels, is shown in Figure 7(a). The resulting control grid and the vector field, are shown in Figures 7(b) and 7(c) respectively.

V. CONCLUSIONS

Autoradiographic data, an imaging modality typically represented by high levels of statistical noise and low contrast, has been successfully segmented using an approach based on region growing. This process has been observed to be highly dependant on the chemical ligand and the radioisotope used

Fig. 7. Autoradiogram of mouse brain section with corresponding atlas section superimposed on top, after applying a scattered data interpolator based on B-splines with a lattice interspace of 10 pixels (a). The image in (b) shows a control grid showing the warping function and the image in (c) shows the displacement vectors corresponding to the warping function.

as these play a significant role on the contrast to noise ratio and the spatial resolution respectively.

Several interpolators, with global and local support, have been implemented showing that these two families of interpolators do not represent significant differences in general for this particular application. However local support interpolators showed slightly better accuracy registering small structures in brain AR. The level of agreement between the autoradiogram and the corresponding atlas image has been observed to be an important parameter to obtain a successful registration.

These results will be extended for other autoradiograms using different radioligands and a quantitative comparison between interpolators will be presented in a forthcoming paper.

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