

Correcting Distorted Histology Slices for 3D Reconstruction

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Abstract. Histology acquisition often introduces tissue distortions that make 3D reconstruction difficult. We present a framework to detect distorted slices, distorted structures and eventually correct the distortions by a reasonable estimation. The first detection specifies distorted slices by comparing similarity measures in a group of neighbouring slices. The distorted structures detection attempts to predict the type, size and position of the distortions in the absence of a priori information about expected distortions. The last algorithm uses undistorted tissue information in corresponding regions in neighbouring slices to correct distorted areas, facilitating pair-wise slice registration for 3D reconstruction.

1 Introduction

Building and studying 3D representations of anatomical organs, such as the brain, plays an important role in modern biology and medical science. While 3D imaging methods such as MRI and CT provide accurate 3D structural information, 2D imaging methods such as histology and optical microscopy typically generate images with much higher resolution and better specific contrast. When studying the mouse brain, it is best to combine the advantages of both 3D and 2D imaging technologies. The classical approach is to reconstruct a 3D mouse brain volume from a series of images of histology slices, which provide more tissue detail than MR images [1][2][3]. However, histology acquisition generally induces a lot of artifacts (holes, folding, tearing, sketching, etc.). Detecting and correcting the artifacts becomes a central issue when reconstructing a 3D volume from a series of histology slices. Indeed, they can make the distorted regions significantly different from the corresponding regions in adjacent slices. In a typical pair-wise registration approach, registration errors tend to propagate to adjacent slices and prevent a smooth 3D brain volume from being reconstructed. Therefore, post-acquisition histology distortion detection and correction are preferable [1] [2].

Researchers have proposed approaches to detect and remove distorted slices by evaluating the quality of image registration between slices. [4][5] have chosen weighted registration error as the metric to eliminate badly distorted slices. Those methods need to register all the slices pair by pair in order to construct a weighted graph of registration errors. The methods are based on the idea of eliminating the distorted slices rather than keeping and correcting them where possible. Quite often, most of histology slices in a series have different types of distortions in different regions. If they are all classified as distorted and then removed totally, there will be too little or no information left from which to reconstruct a 3D volume.

Consequently, in addition to detecting distorted slices in a series of histology slices, the work reported here also aims to detect distorted structures within those slices. This will give us the ability not only to identify which slices are distorted, but also to predict how they might have been distorted, allowing distortion correction to be more precisely targeted and guided. Several approaches to distortion detection have been proposed, i.e. pre-segment [6][7] holes or detect tears, or exploit prior knowledge of position, shape, size and type of distorted tissues [8][9]. However, those approaches need significant amounts of a priori information about distortions in order to model them.

In this paper, we introduce a new similarity-measure-based method of detecting distorted slices and distorted structures. Without knowledge of the identity of the original object, only the following assumption is made: the shape of an anatomical structure varies slowly with respect to section thickness. In other words, neighbouring slices look similar to each other and corresponding points on adjacent slices are likely to be located close together. After distorted slices and structures are identified, we apply a novel method of distortion correction which takes undistorted tissue information in corresponding regions of a group of neighbouring slices into account. The information generated by this process is artificial, but it can be used to facilitate local affine registration between slices, so that the histology slices can be warped together more precisely. This method serves as an efficient and effective pre-processing tool for traditional pair-wise slice registration, and leads to an improved 3D volume reconstruction.

2 Method

2.1 Distorted Slice Detection

We firstly align all the slices globally using automated rigid-body registration [10] to eliminate the effect of slice misalignment. Then, we compute and compare the similarity measures (correlation coefficient in our case) between slices in a consecutive group. When slice thickness is reasonably small ($<50 \mu\text{m}$), undistorted consecutive slices have similar (almost identical) structures because of the little anatomical transition across section.. Therefore, the similarity measures computed on pairs of neighbouring slices should not be significantly different. However, when a distortion occurs, similarity measures of consecutive slices usually differ significantly. Based on this, we make the assumption that if the similarity between the two neighbours of the slice under detection test is larger than the mean similarity measure between the testing slice and each of its neighbours, the testing slice has likely to have been distorted. This is based on the previous slice having been acknowledged as undistorted. If we run the detection in the forward direction (first slice to last), the quality of the previous slice is a standard of the comparison. If it was detected as distorted, and the similarity measure between the two neighbours of the testing slice is smaller than the mean similarity measure of the testing slice and each neighbour, the testing slice is probably distorted. Since we also want to consider the quality of following slices, the algorithm runs in two directions. When running backward, the quality of the next slice becomes the condition of comparison. A third detection pass runs from the slices detected by both the first two tests. It also runs in two directions to improve the precision of the results. The final result only counts the slices specified by all of the three detection steps. As this approach is based on quality of previous and following slices, the first and the last slices must be manually evaluated before running the algorithm.

Distorted Slice Detection

1. Globally align the slices using automated rigid-body registration.
2. Compute similarity measure (CC: Correlation Coefficient) between the each slice to be tested and its neighbours, e.g. detecting at slice 3, compute CC (2, 3), CC (3, 4) and CC (2, 4).
3. Test the slices in forward direction.

Pseudo code:

```
for slice = 2 to (last -1)
  if (slice-1) is good
    if  $CC(\text{slice} - 1, \text{slice} + 1) > \frac{CC(\text{slice} - 1, \text{slice}) + CC(\text{slice}, \text{slice} + 1)}{2}$ 
      then slice is distorted
    else mark slice as good
  endif
elseif (slice-1) is bad
  if  $CC(\text{slice} - 1, \text{slice} + 1) < \frac{CC(\text{slice} - 1, \text{slice}) + CC(\text{slice}, \text{slice} + 1)}{2}$ 
    then slice is distorted
  else mark slice as good
endif
endif
```

Table 1. Principle of forward distorted slices detection

4. Test the slices in backward direction. (The rule is the same as the forward test but begins at the last image and moves towards the first.)
5. Run forward and backward detections outwards from the detected distorted slices
6. Combine the results from above detections, mark the distorted slices based on weighted statistical analysis of the three tests. Weights are forward: 0.4, backward: 0.4, and the third detection phrase: 0.2. If final possibility is larger than or equal to 50% (0.5), the detected slice is marked as distorted.

2.2 Distorted Structure Detection

This method provides a prediction of the shapes and locations of the distortions in the damaged histology sections detected by the algorithm described above. The distorted slices found by our detection method are first globally aligned with their undistorted adjacent neighbour slices.

In our case, we want to avoid comparing cellular details, and rather focus on macro structure information, i.e. slice artifacts. Therefore, a bilateral filter [11] was used to blur details of most areas of images while still preserving boundaries between different tissues sufficiently for distortion detection.

In order to distinguish the possible distorted areas, we apply a statistical test to classify regions that significantly differ from their correspondences in adjacent undistorted slices. Initially, two consecutive images are divided into corresponding blocks. After computing similarity measure of each of the block pairs (excluding background), the Inter-quartile Outlier Test [12] identifies outliers among those similarity measures. We define an outlier as a similarity measure more than 1.5 times the IQR (inter-quartile range, $Q_3 - Q_1$) above or below the first quartile (Q_1 , cut-off of the lowest 25% of similarity measure data) and third quartile (Q_3 , cut-off of the highest 25%).

$$\text{Outlier} < Q_1 - 1.5 \cdot \text{IQR} \text{ or } \text{Outlier} > Q_3 + 1.5 \cdot \text{IQR}$$

This coefficient 1.5 has been widely used in finding mild outliers, while a coefficient of 3 can be used to find extreme outliers [12]. The coefficient 1.5 has proved sufficient to find out the outlier regions in the current application.

We mark the areas of outliers in white, normal areas in grey and background in black to distinguish them and make a map of distorted structures. However, we do not want to take inter-section anatomical differences into account. Therefore, we need to apply this method to a group of slices, find out which parts of the detected distortions are anatomically correct and should not be changed. The choice of the block size is made empirically. Normally, we go from 10x10 pixels down to 2x2 to optimize the final output.

2.3. Distortion Correction

The goal of the work reported here is to correct for tissue loss caused by distortions such as holes and folding and also to correct for tissue stretching. In undistorted histology slices, corresponding regions across slices are normally at the same or similar coordinates, provided that slice thickness is reasonably small (which implies anatomical translation is also small). Based on this hypothesis, we examine a group of consecutive slices around the slice identified as suffering tissue loss. In our case, two slices on each side are considered according to the slice thickness ($25 \mu\text{m} \times 2$). We also make sure all those slices are globally aligned by rigid body transformation. Regions that have been lost are labelled by masking the detected distortion map. Then, for every pixel in the lost region, we look for areas within a certain radius range at the same coordinate in the neighbouring slices. If there is no other distortion except tissue lost, grey levels of pixels at the same position across slices should display similar values. We average the grey levels in those areas within a radius (2 pixels) to take small anatomical translation into account. After that, we average the mean gray levels again to estimate the grey level of that pixel in the lost region. The estimate is then used to fill in the hole.

Folding is viewed as a combination of two types of distortion: tissue loss (parts have been folded over) and overlapping (dark parts). For tissue loss, we can apply the same solution as for holes. For overlapping, because of it is not in the colour of background (as tissue lost), instead of filling we replace the dark pixels with the estimated ones.

Tissue stretching is also treated as tissue loss. This is because most part of stretching has normally been compressed, original tissue information cannot be recovered by warping back. An estimation of tissue at least smoothes the damaged part and hence is good for reconstruction. Therefore, stretching is fixed in the same way as tissue loss which means our method is generic without knowing the type of distortions.

Since there is no way to restore lost tissue 100% precisely, without access to a model of the original tissue, our approach is designed only to add more information to improve the accuracy of slice registration during 3D reconstruction.

3 Experimental results

3.1 Distorted Slice Detection

In this experiment, we applied our algorithm to a set of 8 consecutive slices from a stack of 350 ($25 \mu\text{m}$ thick) Nissl-stained images acquired by cryo-sectioning coronally a single frozen C57BL/6J adult mouse brain from LONI

Research Lab at UCLA, as shown in Figure 1. Slice 2 has a big bubble on the top right corner, slice 4 has been torn, forming a horizontal gap on the top, and slice 6 has an obvious intensity inhomogeneity.

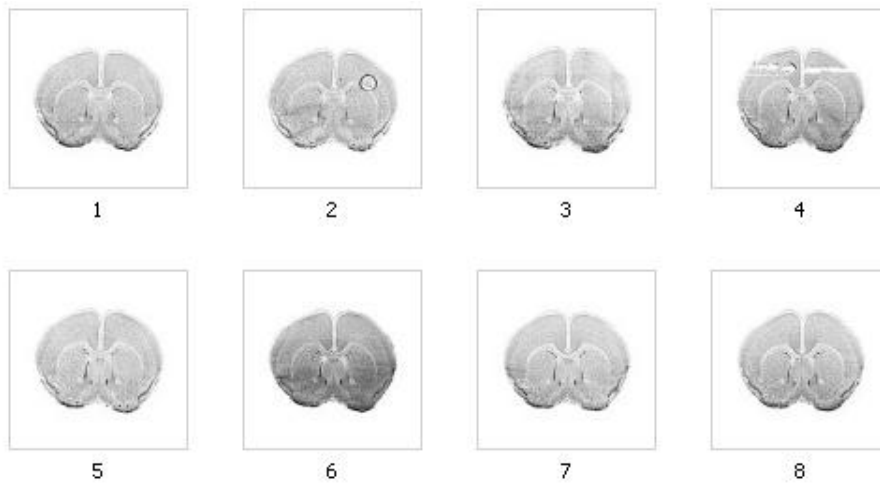


Figure 1. A series of histology slices

In the forward detection phase, slices 2, 4, 6 and 7 were detected as distorted. In the backward detection, only slice 2, 4 and 6 were highlighted. Taking the intersection of those two sets of results, the testing step is run starting from slices 2, 4 and 6. Because the last slice (8) is manually checked and marked as good and slice 6 is labelled as distorted in the forward detection, which give the two direction detections of slice 7 conflicting priori information. This leads to two different sets of results, and indicates the necessity of running the algorithm in both directions. To reduce the risk of a distorted slice affecting 3D reconstruction, we act conservatively and chose to mark slice 7 as distorted.

As a result, slices 2, 4, 6 and 7 were detected as distorted slices. Those four slices are the ones which must be corrected for a smooth volume to be reconstructed.

3.2. Distorted Structure Detection

To validate the method of Distorted Structure Detection, we tested a distorted slice (slice 4 in Figure 1) with a tearing gap on the top with its undistorted neighbour (slice 5). Similarity measures (Correlation Coefficient) are computed in the corresponding blocks of the two slices.

Figure 2 shows the detected distorted structure in 2x2 block size. The detected structure matches the original distortion. The white circle around the outer circumference is very likely an inter-section anatomical difference. It implies the next slice is slightly bigger which is an anatomical transition in this part of mouse brain and will not be corrected.

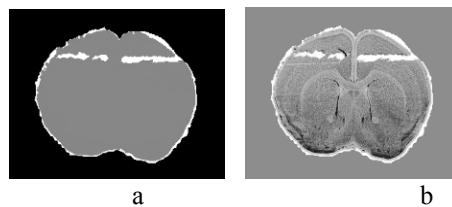


Figure 2. a) Detected distorted structure; b) result on distorted slice

3.3 Distortion Correction

The grey level information from slices 2, 3, 5, and 6 (in Figure 1) is used to estimate the pixel values in the tearing gap of slice 4. For every pixel site, we compute the mean grey level in a region with radius of 2 pixels. Figure 3 shows the result, with the gap filled.

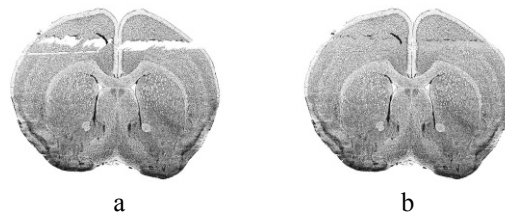


Figure 3. a) Before; b) after correction

In Figure 4, we also show some results for folding and stretching distortions (not shown in slice series Figure 1.). The corrected slices have more information for a more precise local affine registration.

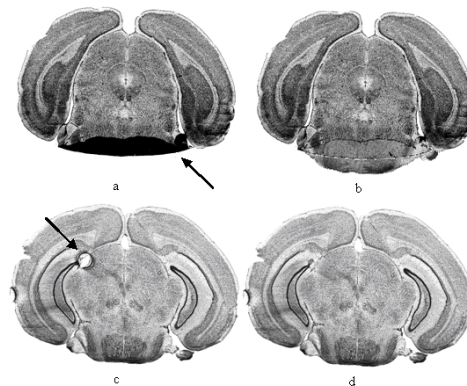


Figure 4. a) Folding before; b) after fix; c) stretching before; d) after fix

4 Conclusion

We have presented a novel method for fixing histology artifacts. It consists of three parts: Distorted Slice Detection, Distorted Structure Detection and Distortion Correction. Similarity-measure-based detections can not only find damaged slices among a series of slices, but also predict distorted structure without a priori information. Both detection methods are demonstrated as straightforward and reliable pre-processing for distortion correction. Eventually, our correction algorithm fixes those slice artefacts, removing obstacles which might prevent reconstruction of a smooth 3D volume. The proposed methods can be easily applied to different histology datasets, since no priori information is needed. In the future we will compare the performance of our method to other methods proposed to incorporate more spatial information into distortion detection and correction.

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