3D Reconstruction of Histological Sections: Application to Mammary Gland Tissue

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ABSTRACT In this article, we present a novel method for the automatic 3D reconstruction of thick tissue blocks from 2D histological sections. The algorithm completes a high-content (multiscale, multifeature) imaging system for simultaneous morphological and molecular analysis of thick tissue samples. This computer-based system integrates image acquisition, annotation, registration, and three-dimensional reconstruction. We present an experimental validation of this tool using both synthetic and real data. In particular, we present the 3D reconstruction of an entire mouse mammary gland and demonstrate the integration of high-resolution molecular data.


INTRODUCTION

The mammary gland is a ductal tree that develops during puberty. In neonate mammals, the mammary epithelium consists of a branched sprout contiguous to the nipple. During puberty, there is considerable growth of the ductal tree, which invades and eventually fills the fat pad. In the adult animal, ducts are formed by secretory, luminal epithelial cells surrounded by contractile myoepithelial cells. At each developmental stage, the phenotype of the cells and therefore the function of the entire gland is driven by their response to external factors and among those, to steroid hormones. The interrelationship between the three-dimensional structure of the mammary gland (macroarchitecture) and its cellular composition (microarchitecture) has been only described in a qualitative way in studies on humans and animal models. This is mainly due to the lack of 3D imaging methods to simultaneously capture the morphology of the gland and the phenotype of its constituent cells, defined by the morphology and the expression of specific markers within the cells.

Some studies based on whole mount preparations (Bagheri-Yarmand et al., 2003; Cleary et al., 2004; Giovann et al., 2004; Westerlind et al., 2002) provide global information on the development of the gland based on the number and spatial distribution of its different morphological structures: primary and secondary ducts, lobular units, etc. Before the use of digital image processing, whole mount preparations were imaged with conventional analog cameras and analyze manually (Russo and Russo, 1978a,b). The use of digital tools gave a significant boost to the analysis of whole mount preparations. This technology was used in some studies of mammary gland development in rats, combining digital acquisition with manual measurements and counts of epithelial areas and lobular terminal units (Ip et al., 1999). However, whole mount analysis is a low-resolution approach that works from a 2D flat projection of the entire gland and provides only limited morphological information that cannot be combined with information about cellular morphology or the expression of cellular markers.

Three-dimensional microscopy—confocal, two-photon, etc.—can provide high-resolution cell morphological and phenotypical information, at the expense of being only of local extent, disconnected from the macroscopic structure of the gland. For instance, working with high-numerical aperture lenses, one can, visualize and analyze tissue volumes of ≈200 × 200 × 40 μm³, a very limited volume given the size and thickness of the gland (Pawley, 2006).

Recently, Capek et al. (2009) approached the problem of combining macroscopic and microscopic data by physically sectioning rat embryo specimens in 40-μm sections and then acquiring confocal stacks that were stitched in the x-y plane and registered in the z-vertical—direction, thus creating a complete digital version of the specimen. However, this is only suitable for small tissue volumes because of data management and analysis issues. Another shortcoming of this method is that it relies on confocal imaging of fluorescent markers, which might be the best approach for high-resolution...
The problem of recovering the three-dimensional location or morphology, are imaged automatically grouped (Section Contour Grouping). Next, the structures of interest are segmented (Section Segmentation of Structures of Interest), and their resulting contours are automatically grouped (Section Contour Grouping). Finally, the registration is locally and/or elastically refined (Section Local Group Registration), and the structures are reconstructed in 3D based on the grouped contours (Section 3D Reconstruction). We evaluated the system using an artificial model (Section Validation of the Method Using a Phantom) and then applied it to real mammary gland tissue blocks (Section Experiments With Real Data). We show the benefit of using these integrated techniques to generate novel realistic, smooth, and compact 3D reconstructions of histological tissues. We discuss the conclusions of our approach in Discussion Section.

MATERIALS AND METHODS

Image Acquisition

Formalin-fixed, paraffin-embedded tissue blocks containing entire mouse mammary glands were fully sectioned at 5 μm thickness and every even section stained with H&E. The number of sections per block depended on the thickness of the tissue block, ranging from the—low-resolution—images of the even H&E-stained sections, which is the main topic of this article. A topologically accurate rendition of a tissue requires that all the images of the sections are correctly aligned, ensuring the continuity of structures crossing several sections. Manual registration of two sections is a relatively simple task that involves either manually rotating and translating a half-transparent image over the reference image or marking sets of corresponding points—fiducial points—on both images and calculating the affine transform that aligns the images. However, this approach becomes impractical when registering large tissue blocks, made of hundreds of sections. Moreover, manual registration adds certain error to the result, as the user can bias the registration by incorrectly selecting the fiducial points. Finally, this approach assumes that the registration between consecutive sections can be done using an affine transformation. However, there are often nonlinear effects introduced during tissue sectioning that cannot be corrected for using an affine transformation as described by Deverell et al. (1989), Schormann et al. (1995), and Brey et al. (2002). Examples of these effects are missing sections and/or nonlinear distorting effects, such as tissue folding, stretching, and tearing. Another consequence of serial sectioning is that the resolution of the images in the Z direction (i.e., the distance between sections) is considerably lower than the XY resolution. This causes substantial differences between the same structures in consecutive sections, and consequently complicates the registration process. Ourselin et al. (2001) proposed a strategy to compensate these nonlinear effects. They compute local displacements between sections using a block-matching approach. Then, a global rigid transformation is calculated from these matches as the solution of a robust regression problem. We propose instead the use of a local nonrigid registration method to compensate for nonlinear effects. Nonrigid registration methods are well suited for the task as complex transformations can be modeled by them. Moreover, they are applied locally to reduce the computational complexity and to preserve the topology of the tissue.

Several groups have developed systems to reconstruct tissues from histological sections (Capuco et al., 2002; Fiala, 2005; Haas and Fischer, 1997; Hofstadler-Deiques et al., 2005; MacKenzie-Graham et al., 2004; Streicher et al., 1997, 2000); but to the best of our knowledge, they all rely on manual and/or rigid-body registration of the images, which, as it has been argued, is not appropriate for large tissue volumes. In addition, some of those studies require using several software platforms for acquisition, analysis, registration, and reconstructions, adding compatibility issues between platforms, and complicating the entire reconstruction process.

Other authors combined molecular and 3D morphological information using episcopic methods (Weninger and Mohun, 2002; Weninger et al., 1998). These methods do not require registration because all images are acquired before sectioning. However, they are quite limited in terms of the extent reconstructed area, given that the staining that these methods allow is only superficial, the range of possible applications is very limited. In our previous work (Fernandez-Gonzalez et al., 2002), we presented a system that simplifies and semiautomates the acquisition, handling, and reconstruction of histological structures from fully sectioned tissue blocks. However, the registration used in that system was fully manual, thus only allowing affine transforms, which are not optimal for large tissue blocks.

Here, we introduce a method for the automatic reconstruction of entire tissue blocks in a way that could be later used to store cellular-level phenotypic information. This requires the method to be automatic, work at high resolution in the vertical direction, and rely on elastic registration to account for nonlinear distortions. In practice, our algorithm proceeds as follows: once the images are acquired (Section Image Acquisition), they are automatically and rigidly registered (Section Rigid Registration). Finally, the registration is locally and/or elastically refined (Section Local Group Registration), and the resulting contours are automatically grouped (Section Contour Grouping). Finally, the registration is locally and/or elastically refined (Section Local Group Registration), and the structures are reconstructed in 3D based on the grouped contours (Section 3D Reconstruction). We evaluated the system using an artificial model (Section Validation of the Method Using a Phantom) and then applied it to real mammary gland tissue blocks (Section Experiments With Real Data). We show the benefit of using these integrated techniques to generate novel realistic, smooth, and compact 3D reconstructions of histological tissues. We discuss the conclusions of our approach in Discussion Section.

MATERIALS AND METHODS

Image Acquisition

Formalin-fixed, paraffin-embedded tissue blocks containing entire mouse mammary glands were fully sectioned at 5 μm thickness and every even section stained with H&E. The number of sections per block depended on the thickness of the tissue block, ranging...
from the high tens to the low hundreds. Low-resolution (2.5× magnification) multiple-snapshot images of all the sections were acquired using a computer-assisted wide-field microscope (Fernandez-Gonzalez et al., 2002). The size of the images depended on the size of the tissue sections, but ranged typically from 3,000 × 3,000 to 5,000 × 5,000 pixels. A background correction algorithm based on a phantom (Fernandez-Gonzalez et al., 2004) was used to correct the mosaic-like effect of the images caused by uneven illumination of the field of view of the microscope. The sections were saved in either TIFF or ICS (Dean et al., 1990) format (gray scale mode) and grouped as sets of related images that we will refer to as cases.

Rigid Registration

We first apply an automated rigid-body registration algorithm to register each image to the previous image of the block. Because of the large size of the images, we implement a computationally efficient multiresolution pyramidal version of the algorithm (Borgefors, 1988). The algorithm used to calculate the optimum transformation at each pyramid level is schematically described in Figure 1. First, both target and source images are subsampled with a factor given by the pyramid level. Second, image noise is removed using a Gaussian filter, and the image gradient is calculated with a Sobel operator. Third, to determine the location of the relevant image edges and create a binary contour map, the images are binarized applying a modified Otsu threshold (Otsu, 1979; Rasband, 1997–2009). Finally, the inverse distance transform of the target image is computed.

To speed up the search for the maximum of the matching function, the number of rotations and translations that we use depend on the pyramid level. Using this multiresolution hierarchical algorithm, the best matching transformation is progressively found, by first approaching it at low resolution in large search areas and then refining it at high resolution in reduced search spaces. Consequently, the reduction of the error in every pyramid level is implicit, as lower levels use higher resolution images containing more autothresholding function. Then, a matching function is applied between the source image after a rigid-body transformation and an inverse distance transform version of the binarized target image.
image information. The efficiency of the algorithm is also guaranteed, as the size of the search space decreases as the size of the subsampled images increases.

**Segmentation of Structures of Interest**

To automatically extract the contours of the structures of interest, we use a method (Fernandez-Gonzalez et al., 2004) that combines two well-established schemes for interface propagation: the fast-marching method (Sethian, 1996) and the level-set method (Osher and Sethian, 1988). The fast marching algorithm is first run to provide a quick good approximation of the boundaries of the objects that we are trying to segment. This is just a quick estimate of the segmentation, as the fast marching approach assumes monotonic speed functions—always positive or negative. Then, the approximation provided by the fast marching method is used as initial condition for the level set method. This is a computationally expensive algorithm, which is just run for just a few steps to adjust the evolving front to the contours of the structures of interest.

**Contour Grouping**

In the segmentation step, we annotate the structures of interest of our tissue: ducts, lymph nodes, etc. We then group the contours that belong to the same structure so that they can be rendered together. We have developed an automatic grouping algorithm, which proceeds in two steps. The first step assigns the same group number to the contours of consecutive sections if their projected bounding boxes—minimum squared areas containing the shapes—overlap. If there is not overlap the contour remains ungrouped. During this process, if the bounding box of a grouped contour overlaps an already grouped contour, then both contours receive the same supergroup number. Following this idea, we iteratively create a hierarchy of groups. In the second step, all the remaining ungrouped contours are assigned the group and supergroup number of the closest grouped contour in the next section.

**Shape-Based Rigid Registration**

When we consider that is necessary to improve the result of the initial rigid registration, we apply a variation of the rigid-body registration, called shape rigid registration. This method requires the sections to be already segmented and represented as a binary set of contours corresponding to the edges of the structures of interest (see Section Segmentation of Structures of Interest). We use it after the grouping step described in Section Contour Grouping. Therefore, in the process described in Figure 1, the results of the autothreshold of the source and target images are replaced by the binary representation of the segmented contours of the images. The rest of the algorithm does not change from what has been described. Figure 2 shows the difference between the binary image used as input by the standard and the shape rigid registration method, respectively. This method has two main advantages over the previous one. First, assuming that the segmented contours correspond only to the relevant structures in the image, the matching between the images will be based only on relevant image information, thus mitigating the effect of noise or artifacts. The second advantage of this method is its increased efficiency. As the number of evaluation points is drastically reduced, as most of the pixels in the binary image are black.

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Fig. 2. Graphical example of the reduction in complexity of the rigid registration algorithm when using as input a binary image containing only the most representative contours of the structures of interest or containing all the gradient information above a modified Otsu threshold. (a) Histological section image already segmented. The contours from the structures of interest are represented in white. (b) Binary image constructed from the contours. (c) Binary image obtained with the preprocessing described in Section Rigid Registration (subsampling and Gaussian filtering, application of the Sobel operator, and modified Otsu thresholding). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Local Group Registration

As already mentioned in the introduction, manual sectioning can produce nonlinear deformations in the tissue, such as folding, stretching, and tearing. Occasionally also, because of the tissue conditions or improper maintenance of the microtome, some sections can be damaged and must be disposed. Consequently, there might be gaps in the sequence of sections. All these effects may cause heavy misalignment between areas that cannot be corrected by using a global affine transformation. Therefore, we opted for combining a local correlation-based registration method with a consistent elastic registration algorithm (Arganda-Carreras et al., 2006). As the algorithm is applied locally, the topology is preserved and accurate results are produced in a reasonable time. The selection of the structures for which a consistent elastic registration algorithm is needed is described next.

First, we calculate a common bounding box for each group of contours that were automatically grouped in the previous step. Then, we estimate a translation offset for each group by computing the cross-correlation between the content of the corresponding bounding box in consecutive sections. In particular, we use the popular phase correlation method (PCM) by Kuglin and Hines (1975). It is based on the Fourier shift theorem, which states that a shift between two images results in a linear phase difference in their Fourier transform. In consequence, in the frequency domain, the modulus of the correlation has a peak at a distance from the center that corresponds to the applied shift. A graphical description is shown in Figure 3.

Consequently, we obtained a correction vector and a correlation coefficient for each group of contours in every section. The shift vector is computed as the difference between the coordinates of the center of the subimage and the coordinates of the brightest peak in the modulus subimage. The correlation coefficient is calculated between the original target subimage and the translation corrected source subimage and does provide a measure of the accuracy of the correction vector. Correlation values lower than a minimum value—corresponding to strongly distorted areas—trigger a consistent elastic registration routine (Arganda-Carreras et al., 2006) that corrects the misalignment.

Fig. 3. Image alignment by phase correlation. (a) Selected region of a tissue section. (b) Same region of the tissue section but shifted. (c) Modulus of the autocorrelation image. Note the peak at the image center. (d) Modulus of the cross-correlation between images (a) and (b). Note how the peak is shifted. (e) Correction vector in white.
This routine simultaneously calculates the direct and inverse transformations and minimizes the similarity error between the target and source images after imposing a consistency constraint. Consistency here refers to the invertibility of the transformations, in fact, this approach provides bidirectional registration—from image A to B or from B to A—in a single computation. Image A is elastically deformed to look as similar as possible to image B, and, at the same time, the “inverse” transformation (from B to A) is also calculated so a pseudo-invertibility of the final deformation could be guaranteed. This method uses B-splines to efficiently represent both images and deformations in multiresolution pyramids. A powerful optimizer is used to iteratively converge fast to the best image alignment minimizing an energy functional computed from the grayscale pixel-to-pixel similarity between the images and the consistency of the direct and inverse deformations.

3D Reconstruction

The system reconstructs the 3D structures of interest based on the grouping of the segmented contours, which have been aligned using the rigid and elastic registration. The surfaces of the volume are created with a refined Delaunay triangulation (Boissonnat and Geiger, 1993). Some examples and validation experiments of the complete rendering process are shown in the next section. Alternatively, our system offers the option of creating a 3D binary image, containing the segmented and aligned contours in white and the background in black. This binary image can be used for further studies of the structures of interest such as generating their skeleton, counting, and measuring their branches, volumes, etc., as it is also shown in the next section.

High-Resolution Acquisition

From the 3D reconstruction of the tissue, calculated from the H&E-stained sections—the even sections of the block—we select areas of interest. These areas of interest—selected based on interesting morphological features or their location within the gland—are then imaged again with an optical wide-field microscope at high resolution—40×—in the odd sections and can be used to quantify molecular events at cellular level, as defined by specific markers. This information can then be incorporated and retrieved from the 3D reconstruction of the tissue. An example of this will be described in the Results section.

RESULTS

Validation of the Method Using a Phantom

We validated our registration and reconstruction protocol using a phantom model in which we can artificially simulate the most common problems found during the reconstruction of histological tissue. To this end, we created a phantom model similar to the type of structures we want to render. Our phantom model was mouse airway tree, segmented from an X-ray tomographic image of a mouse chest. We stored the model in a 444 × 471 × 568 3D image and calculated the isosurfaces of the structures of interest using the ImageJ 3D Viewer (Schmid, 2008), a popular open source software that extracts the volume surface from the 3D image and allows storage in virtual reality modeling language (VRML) format. Figure 4 shows the volume. We used the model to study the robustness of our 3D reconstruction system against variations in the direction of tissue sectioning, the loss of sections, and the presence of nonlinear tissue distortions.

Direction of Sectioning. We first created three sets of artificial slices from the phantom, by sectioning it in the x-, y-, and z-direction. Thus, we obtained three sets of images or cases that we used to test the accuracy with which our algorithm can reconstruct the original phantom. For these three cases, we followed the steps previously described in the Methodology: segmentation, grouping, and rendering. At this point, we did not perform any registration because the sections kept the alignment of the original 3D image.

We then compared the resulting isosurfaces with the original model surface. For this we used the Hausdorff distance (Rote, 1991), which measures how far two compact nonempty subsets of a metric space are located from each other. We used the software toolbox MESH (Aspert et al., 2002) to compute the minimum, maximum, mean, and root-mean-square errors, between the surfaces and to visualize the differences between them. Table 1 shows the mean symmetric Hausdorff distance between the model surface and the three surfac-
ces generated by our method. As can be seen from the table values, our system produces surfaces, which are approximately one pixel away from the original model. If we take into account that our segmented contours are one pixel thick, we can conclude that our system reconstructs the model accurately, regardless of the sectioning direction. Figure 5 shows one of the three reconstructions. The error is codified using a color code on top of the surface that represents the distance to the model. Blue areas represent perfect fit, whereas green and yellow areas represent larger errors.

**Missing Sections.** A common problem when manually processing histological tissue blocks is the loss of sections that may happen during sectioning. We wanted to test how robust our algorithm is against this problem that can easily lead to loss or distortion of small tissue structures. To quantify the effect of missing sections, we used one of the three cases generated previously and carried out 10 renderings. In the first rendering, all sections were used. For the $n$th rendering, only one of every $n$th section was used. Figure 6 shows the evolution of the error versus the percentage of sections used to generate the reconstruction. Using 100% of the sections, we obtained 1.27 pixels of average error (mean symmetric Hausdorff distance), but the number gradually increased up to 3.11 pixels when we used only 10% of the sections. More representative of this degradation is the maximum distance error, which increases from 8.90 when we used all the sections up to 40.92 in the 10% case. Figure 7 shows a detail of the 3D renderings of the synthetic case, superimposing the original surface and the degraded surface from the case of 100% of the sections down to using only 10%. We can see how the surface becomes gradually degraded as the distance between sections increases. As the image and accompanying table shows, our algorithm is quite robust against the loss of one section.

<table>
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<tr>
<th>Directions</th>
<th>X-</th>
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<tr>
<td>Mean</td>
<td>1.28</td>
<td>1.14</td>
<td>1.21</td>
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<tr>
<td>RMS</td>
<td>1.58</td>
<td>1.42</td>
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<tr>
<td>Max</td>
<td>15.11</td>
<td>19.79</td>
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*Fig. 5.* Visualization of Hausdorff distance. From left to right: histogram with color distance code scaled in microns (1 pixel = 6.8 µm) and our 3D reconstruction of the x-axis sectioned case colored with the distance to the model.
(\(n = 2\) case), which is the most common case, and produces acceptable results even when subsampling down to \(n = 5\). The final decision on the subsampling rate must be therefore taken as a trade-off between the acceptable error of the reconstruction and the amount of work required to process the sections.

**Rigid and Nonrigid Deformations.** Next, we tested the robustness of our algorithm against misalignment between sections. To this end, we first applied random rigid deformations to all the sections of the model. Namely, we applied \(\pm 20\) pixels maximum displacements and \(\pm 15^\circ\) maximum rotations to the sections and then registered and reconstructed the model. We obtained a reconstructed volume similar to the original model but with different orientation. To measure the surface distances, we aligned the volumes using the basic Iterative Closest Point (Zhang, 1993) method, which allowed us to match both reconstructions and calculate the Hausdorff distance between them. Even when the entire case was rigidly distorted, we obtained an acceptable mean symmetric distance of 3.48 pixels and maximum error of 26.60. This results confirms that our registration is robust against rigid distortions.

The final synthetic experiment combined rigid and nonrigid deformations. Thus, we applied the same type of random rigid transformations used in the previous experiment and added one elastic deformation (Arganda-Carreras et al., 2006) every 20 sections. To create the elastic deformations, we used SplineDeformationGenerator. The elastically deformed sections were corrected using our consistent elastic registration (Arganda-Carreras et al., 2006) and then we proceeded to reconstruct the case. Again, we first matched the resulting volume and the model calculating the symmetric distance between surfaces, which amounted to 3.57 pixels (maximum error 28.74). This result proves that our registration method is robust against nonlinear distortions, as the distance is similar to the distance of the previous experiment.

**Experiments With Real Data**

We finally used our algorithm to reconstruct real mammary gland tissue sections. An entire mouse mammary gland was fully sectioned and stained with H&E (even sections) and alternatively with an antibody against the estrogen receptor (ER), progesterone receptor (PR), or Her2-neu (odd sections). The case was made of 205 \(\mu\)m sections.

Then, we imaged all H&E sections and registered them using the algorithm described earlier. A total of 6,106 structures of interest were segmented and 821 subgroups were automatically detected and distributed under two large supergroups: the lymph node and the ductal network, which were then reconstructed in 3D. Figure 8 shows two views of the entire gland. The top image shows the result of the reconstruction before the rigid and local group registration process and the bottom image shows the gland reconstructed after registration. The effect of the registration is clear from this example, where the registered case shows much more compact and smoother volumes than the unregistered one.

![Distance error versus section loss.](image)

Fig. 6. Distance error versus section loss.

![Volume degradation.](image)

Fig. 7. Volume degradation. From top to bottom and from left to right, zoom over the 3D renderings of the synthetic case (from using 100% of the sections down to using only 10%). The different reconstructions are shown in red, while the model is shown in translucent yellow.

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Then, to increase the quality of the reconstruction, we also used the structures of interest in the odd sections of the case. We registered the even and odd sections refining the previous result using the local consistent elastic algorithm. We analyzed the 3D volume reconstruction. We generated a binary image containing the ductal network and then calculated its skeleton by applying a 3D thinning algorithm (Lee et al., 1994). Next, we analyzed the skeleton visiting its tree and measuring it. We found that this ductal network consists of 6,209 branches—regions between junctions and/or end points—averaging 191.99 μm and 2,762 junctions. Figure 9 shows an example of the volume skeleton rendered with the lymph node volume to better appreciate the real distribution of the ductal network.

Fig. 8. 3D reconstruction of a whole mouse mammary gland. Top image: Reconstruction before rigid and local group registration. Bottom image: Reconstruction after registration. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Fig. 9. Mammary gland skeleton reconstruction view. The ductal network (lines and spheres representing branches and end-points) is shown along with the lymph node volume (in the center of the skeleton). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Study of Cell Distribution

As mentioned earlier, the system can be used to visualize the distribution of cellular markers in the gland. To this end, the user can manually mark cells as being positive or negative for each marker in areas selected and imaged at high resolution in the intermediate—odd numbered—sections. As a proof of principle, we have manually selected three structures from the 3D reconstructed—one large mammary duct, one small duct, and one growing end bud—and manually marked the epithelial cells as being positive or negative for each of the markers in 12 consecutive odd sections, i.e., four images for each cellular marker. Then, we have averaged the results of the number of positive cells for each of the markers and type of structure, and color coded the surface of the corresponding 3D structures using a fire lookup table. This way, we can easily visualize differences in the percentage of positive cells for a particular structure—and between the same markers in different tissue structures (see Figure 10). In this particular case, the percentages of positive cells for ER, PR, and Her2-neu were, respectively, 46.22, 50.38, and 48.10% in the growing end bud; 72.97, 72.72, and 62.50% in the small duct; and 33.09, 40.45, and 71.82% in the large duct. This is just a proof of principle of how the system could be used to store and visualize molecular information attached to the corresponding tissue structures. This in turn can be used to study the relationship between the phenotype of the gland in different stages of its development. Another aspect of interest that can also be analyzed are the phenotypic differences between different tissue structures or gland locations within a given gland.

DISCUSSION

We have presented a method for the 3D reconstruction of mammary gland tissue blocks. The algorithm includes rigid and nonrigid image registration, automatic segmentation, contour grouping, and volume rendering. We validated the method using synthetic data, which allowed us to evaluate it in realistic situations such as different sectioning orientations, loss of sections, and rigid and nonrigid misalignments. We finally applied the system to reconstruct real data and showed renderings of an entire mammary gland tissue blocks, the corresponding ductal network skeleton, and its measurements.

This algorithm completes an integrated computer-based microscopy system for simultaneous morphological and molecular analysis of thick tissue samples. This system overcomes the penetration limitations of other microscopy modalities—confocal microscopy, two-photon excitation microscopy, etc.—while allowing single-cell resolution and novel 3D views of the mammary gland.

Many of the methods described and used in this work (elastic registration, 3D skeletonization...) have been developed as ImageJ plug-ins (Rasband, 1997–2009) and can be freely downloaded from http://bioweb.cnb.csic.es/~iarganda/software.html.
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