Supplemental Methods of TRACTS

Normalized error measure

The difference between the section found by TRACTS and the position of the expected section is measured by the normalized fit error *Fe* , which takes into account both the angle between the planes of the sections and the average distance of the centres of mass of each section to the other section.
Let *S* and *T* be two sections and let *V* be the plane through *S* and *W* the plane through *T*. Then we define the first component of the fit error as follows.

(1) $D_{\angle}(S,T) = 1 - |\cos(\alpha)|$

where α is the angle between planes *V* (trough section *S*) and *W* (trough section *T*).

For the second component, we use the fact that the distance of a point **x** to a plane *V* can be computed by $|\mathbf{n_V} \cdot (\mathbf{x} - \mathbf{a_V})|$, where $\mathbf{n_V}$ is the unit normal of the plane *V* and $\mathbf{a_V}$ is an arbitrary point on the plane *V*. (See Weisstein, E.W. "Point-Plane Distance." From MathWorld--A Wolfram Web Resource. http://mathworld.wolfram.com/Point-PlaneDistance.html)

(2) $D_p(S,T) = \frac{1}{2}(|\mathbf{n_V} \cdot (\mathbf{m_T} - \mathbf{a_V})| + |\mathbf{n_W} \cdot (\mathbf{m_S} - \mathbf{a_W})|)$

where $\mathbf{n_V}$ is the unit normal of the plane *V*, $\mathbf{m_T}$ is the centre of mass of section *T*, $\mathbf{a_V}$ is arbitrary point on the plane *V*, and $\mathbf{n_W}$, $\mathbf{m_S}$ and $\mathbf{a_W}$ are defined analogously.

To make $D_p(S,T)$ equal in weight to $D_{\angle}(S,T)$, which ranges from 0 (at 0 degrees) to 1 (at an angle of 90 degrees), $D_p(S,T)$ is divided by the size of the reference model (3) before both components are combined into the fit error (4).

(3) $Size(M) = \sqrt{A_x^2 + A_y^2 + A_z^2}$

where A_x is the length of the x-axis of reference model M, and A_y and A_z are defined analogously.

(4) $Fe(S,T) = (D_{\angle}(S,T) + \frac{D_p(S,T)}{Size(M)}) \cdot 50$.

$Fe(S,T)$ is normalized to be 100 at maximum

Pre-processing of input section

Before an input section can be compared to the reference model, pre-processing of this image has to take place. After input into TRACTS a bounding box is fitted around the segmented heart and the image is cropped. The resulting binary image is rotated in 32 steps and mirrored at each position. The resulting 64 images are processed like the pre-computed reference sections (reference model images): determine its size, resize to 128x128 pixels, determine features, compute contour, and distance transform. These images are then used for fitting to the virtual sections of the reference model.

Similarity metric

The similarity between the input image and the cross section from the reference model (reference section) is given by a similarity measure that is based on the mean Euclidean distance between the inner and outer contours of the heart in the input section to the contours in reference section, and vice versa. The contour is considered to be a good representation of the shape of the heart. To compute this similarity measure the input section and the reference sections are all resized to 128x128 pixels from which the contour is derived. From these contour images a distance transformed image is generated. The pixel values in these distance transformed images represent the Euclidean distance to the heart contour. To find the similarity between the two contours, the distance transformed image of one contour image is masked by the contour of the other image and vice-versa. The mean distance is determined from the remaining distance values in the contour masks. By using the sum of both average distance values the resulting similarity metric is symmetric, that is, the resulting value would be the same when comparing a reference section to an input section and the other way around.

Reference model images

The database of cross sections of the reference model (Fig. 1A) contains sections that are generated at every second voxel over the three main axes of the reference model. At every position 64 cross-sections are computed with a different combination of tilting angle and tilting direction. The tilting angle ranges from 0 to 40 degrees (in steps of 10 degrees) and the number of tilting directions increases with each increment of the tilting angle. The number of tilting directions is determined in such a way that the normal vectors of the resulting sections are distributed approximately uniformly in 3D space. The small, nearly cubic, voxels of an episopic reconstruction are such that cross sections in all directions can be made without loss of morphology. All model sections are converted to a pixel size equal to the original x and y resolution of the model. Only those sections containing over 20 pixels of myocardium are used. Every section is cropped to the bounding box around the heart. The size of this bounding box is determined and stored. Then the model images are resized to a standard size of 128x128 pixels. The contours of the heart in those resized images are determined and their distance transformed images are computed. Both the contour and the distance transformed images are stored. For the E11.5 reference model this results in 32813 image pairs stored in the database. The positional and size information are stored in an index table.

Selection of images on features

To reduce computation time, not all sections from the reference model are compared to the input section. Initially only those that are similar in size are selected. The size difference between input and reference section may not exceed 20%, which is based on expert knowledge that embryonic hearts never differ over 20% in size at the same developmental stage. In an attempt to exclude the misplaced sections additional features were implemented. Since selection based on these features results in a lower number of distance transform-based comparisons, it has the added benefit of making the program faster. The decision which features to use is based on an analysis in which experts did a visual assessment of input sections and the recovered reference sections (de Boer et al., 2007). Features that exclude the unsatisfactory fit results without affecting the well fitted images were preferred. Several potential features were tested, and three of these were selected based on their individual performance. These features were (1) centre of mass, (2) overall tissue density and (3) regional tissue density. Thresholds for each of these features where determined after some experimentation and in consultation with experts. All these features are based on the myocardial area of a cross section.

1. The centre of mass feature is defined as the average position of the myocardial tissue of a cross section. This feature was used to avoid that images with a very different tissue distribution are matched.
2. The overall tissue density is measured by counting the number of pixels containing tissue in the resized images. The density feature was applied because the basic program regularly fitted sections with a large amount of tissue, for instance a section containing a thick ventricular wall, to sections with a small number of tissue pixels, for instance a section containing only a thin atrial wall. Such a difference in wall thickness is reflected by the part of the section containing tissue.
3. The last feature uses a regional tissue density measure. For this feature the density in the four corners of every section is measured. To compensate for both technical and biological deformations of the tissue, the density measurement of the input sections is done by moving the measurement areas 6 pixels around each quarter of the image and selecting the minimum and maximum tissue area of each quarter.

Reference

de Boer,B.A., Ruijter,J.M., and Voorbaak,F.P.J.M. (2007). Towards the automatic registration of histological sections into a 3D reference model. In *BNAIC'07. Proceedings of the 19th Belgian-Dutch Conference on Artificial Intelligence, Utrecht: 5-6 November 2007*. (ed. Dastani,M.M. and de Jong,E.), pp. 41-48.