

# Segmentation and analysis of notochord cells in 3D microscope data

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## Motivation

The notochord is an embryonic structure that undergoes dramatic cell shape changes as it itself changes shape from a roughly isodiametric primordium of mesenchymal cells to a long extended rod. Ascidians (sea squirts) have small, compact embryos with a notochord consisting of only 40 cells, allowing comprehensive 3D confocal imaging that would be challenging in other chordate model organisms (Fig. 1).

Here we show a semiautomated method (Fig. 2) for the 3D segmentation of individual notochord cells, and a method for quantifying notochord cell-cell contacts.

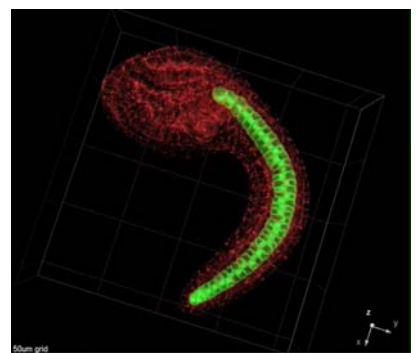


Fig. 1. Volume rendering of an early tailbud stage ascidian embryo. Red: phalloidin staining labels cell peripheries. Green: notochord specific Green Fluorescent Protein transgene.

## Algorithm – Image Analysis

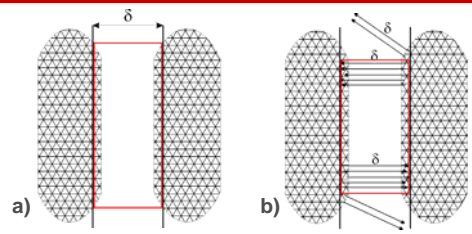


Fig. 3. Contact surface analysis approach: a) Tassy et al.: Current Biology 16, 1–14, 2006, b) Our approach.

Cell surface area is estimated by an algorithm, which calculates the sum of the surfaces of the triangles composing the object's surface with the Heron formula.

Notochord cell to notochord cell contact area is established by first computing the normal vector for each triangle and then searching in this direction for the points that are closer than a user-specified threshold from the other object. Once a point is selected, the triangles it belongs to in the mesh are kept in memory and used to display, on each object, the surface of contact in 3D (Fig. 3).

## Algorithm – Image Segmentation

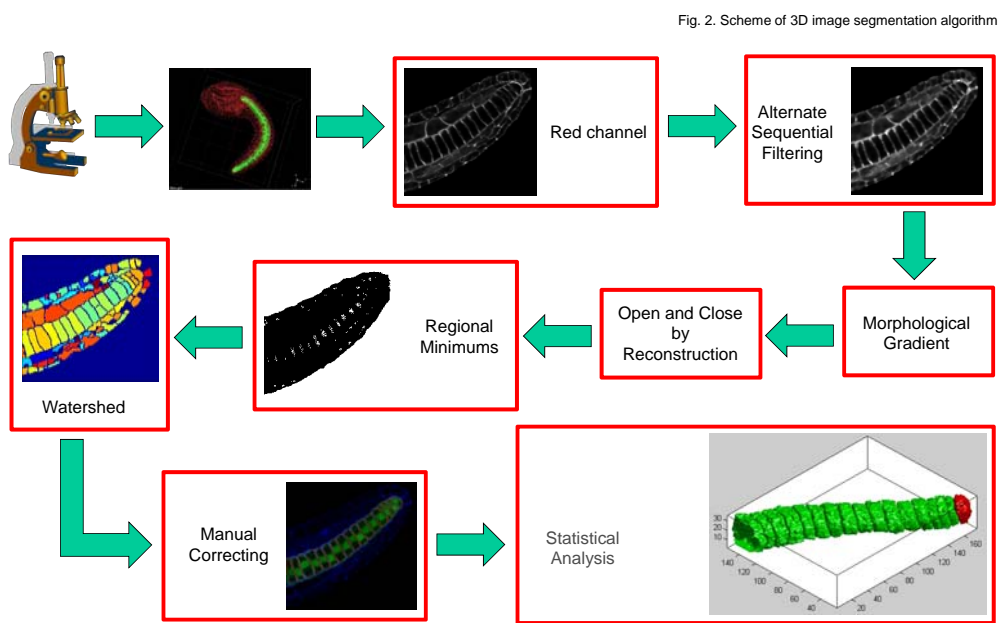


Fig. 2. Scheme of 3D image segmentation algorithm

## Result and Implementation

This image analysis approach is able to reconstruct topological images and provide precise statistical data about the notochord cells (Fig. 4). It can also be easily extended to any other tissues stained in a similar way. Those tools have been implemented in Matlab together with a graphical user interface (Fig. 5).

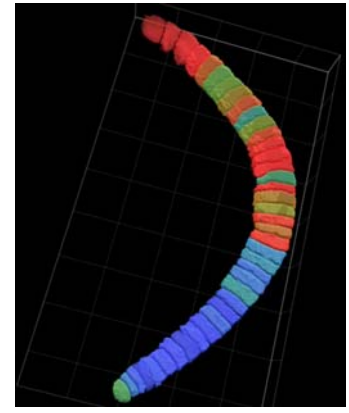


Fig. 4. 3D segmented confocal microscope image of ascidian

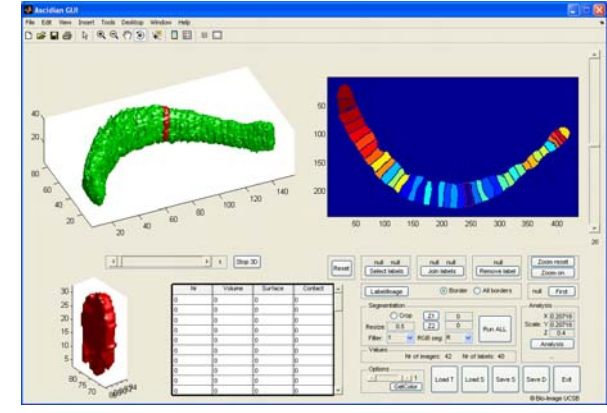


Fig. 5. Ascidian 3D - image analysis and processing tool.

## Conclusion and Future Work

- ❑ 3D shape measurements will suggest biological mechanisms – Are surface area or volume conserved?
- ❑ 3D models derived from high quality confocal images of fixed and cleared specimens will be useful in segmenting datasets using other imaging modalities (such as DIC timelapse).