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Applications Note

Systems biology

TiQuant: software for tissue analysis, quantification and surface reconstruction

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Abstract

Motivation: TiQuant is a modular software tool for efficient quantification of biological tissues based on volume data obtained by biomedical image modalities. It includes a number of versatile image and volume processing chains tailored to the analysis of different tissue types which have been experimentally verified. TiQuant implements a novel method for the reconstruction of three-dimensional surfaces of biological systems, data that often cannot be obtained experimentally but which is of utmost importance for tissue modelling in systems biology.
Availability and implementation: TiQuant is freely available for non-commercial use at msysbio.com/tiquant. Windows, OSX and Linux are supported.
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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

During the last decades, sophisticated techniques for imaging of cells and tissues have been established. However, the translation of this information into new knowledge is hampered by the difficulty to form consistent hypotheses on the complex interplay between components of biological systems resulting either in physiological function or a diseased state. In recent years, mathematical models became increasingly important addressing this question by formalizing the relations between and the interplay of these components in well-controlled model scenarios (Schliess *et al.*, 2014). The construction and parameterization of informative models, however, crucially depends on our ability to quantify structure and dynamic behavior of tissues by image processing and analysis techniques. Moreover, modeling often requires information that cannot be obtained by available imaging methods either because the structure of interest cannot be accessed experimentally or due to technical limitations. For example, while in most cases the two-dimensional (2D) structure of surfaces in tissues can relatively easily be obtained, the reconstruction of the full three-dimensional (3D) picture usually is much more complicated. Cell margins, e.g. can easily be obtained in 2D using a beta-catenin or phalloidin staining, but in most available 3D microscopy software an automated segmentation and quantification of the corresponding individual cells is not possible. Existing methods for the reconstruction of cell surfaces (Klauschen *et al.*, 2009) typically rely on a proper segmentation of cells. Quantification of individual cell surfaces is required in many situations. For example, in liver physiology cell shapes determine cell–cell contact areas that in turn impact metabolic transmembrane fluxes and thus liver function (Hoehme *et al.*, 2010).

Moreover, surfaces often represent a conceptual or functional boundary rather than an actual biological structure that can be explicitly stained and imaged. For example, liver is subdivided in many small functional units called lobules. Only in few species such as pig the surface of these lobules is represented explicitly by an anatomical membrane-like structure, while in most other species including human no

such structure exists. This makes the borders between the functional and anatomical units experimentally very hard to determine. However, detailed knowledge of the full 3D shape of liver lobules is essential to quantify the anatomy of key vascular systems in liver as the sinusoidal or bile networks which is of utmost importance to understand the interplay of components. In this article, we present comprehensive software for the analysis and quantification of tissue that implements inter alia a novel method for the reconstruction of 3D surfaces. The technique is applicable to well-established and widely used imaging techniques even if staining of some cellular structures is incomplete.

2 Software

The presented software TiQuant is implemented in portable object-oriented ANSI C++. The GUI is based on QT and supports real-time visualization using OpenGL. TiQuant is embedded in the tissue modelling framework CellSys and thus is tightly linked with TiSim, a versatile and efficient simulation environment for tissue models (Hoehme and Drasdo, 2010, wherein TiSim was preliminarily called CellSys). TiQuant provides an interface to the popular volume visualization tool VolView and further complements its functionality by linking to the open-source libraries ITK and VTK (itk/vtk.org) that implement a wide variety of thoroughly tested state-of-the-art image processing and visualization methods. The image/volume processing chains currently implemented in TiQuant for example include techniques to segment central and portal veins, sinusoidal and bile canaliculi networks as well as hepatic and non-hepatic nuclei from 3D confocal micrographs of liver tissue based on the Adaptive Otsu Thresholding method and a number of morphological operators as described in detail in (Hammad *et al.*, 2014) and the supplemented user guide.

3 Surface reconstruction method

Recently, a novel method for the reconstruction of 3D surfaces was added to TiQuant. First, two classes of objects are identified: one preferentially located in the interior (class *CI*) of objects and another located on their sought 3D surface (class *CS*). Naturally, the structures in class *CS* do not need to cover the entire 3D surface. We assume both *CI* and *CS* to be available as binary masks for example obtained by TiQuant's segmentation methods indicated above (see also user guide). Next, we apply a Signed Maurer Distance Transformation (Maurer *et al.*, 2003) to obtain the Euclidean distance $d_{CI,CS}(\underline{x})$ to the nearest voxel of *CI (CS)* for each point $\underline{x} = (x, y, z)$ in order to compute the gradient function $g(\underline{x})$using Eq.1:

$$g(\underline{x}) = \beta d_{CI}(\underline{x}) / \left(\beta d_{CI}(\underline{x}) + (1-\beta) d_{CS}(\underline{x})\right) \quad (1)$$

The parameter β ($0 \leq \beta \leq 1$) allows balancing the influence of the two classes on the resulting gradient magnitude profile; lower values of β reduce the influence of class *CI*. Supplementary Figure S1 in the supplement illustrates the behavior of $g(\underline{x})$ for different β.

In a final step, we apply a variant of the Beucher–Watershed algorithm termed Morphological Watershed (Beare and Lehman, 2006) to $g(\underline{x})$ that aggregates points whose gradient descent leads to the same local minimum. The resulting space partitioning yields sought surfaces in 3D.

In liver, the described method has successfully been used to obtain (i) individual cell shapes (Fig. 1A–C) and (ii) the surfaces of liver lobules (Fig. 1F). For the reconstruction of cell shapes, cell nuclei were used for class *CI* as they are typically located in the interior of cells while sinusoids and bile canaliculi which are always located at the cell surface were used for class *CS* (Fig. 1A).

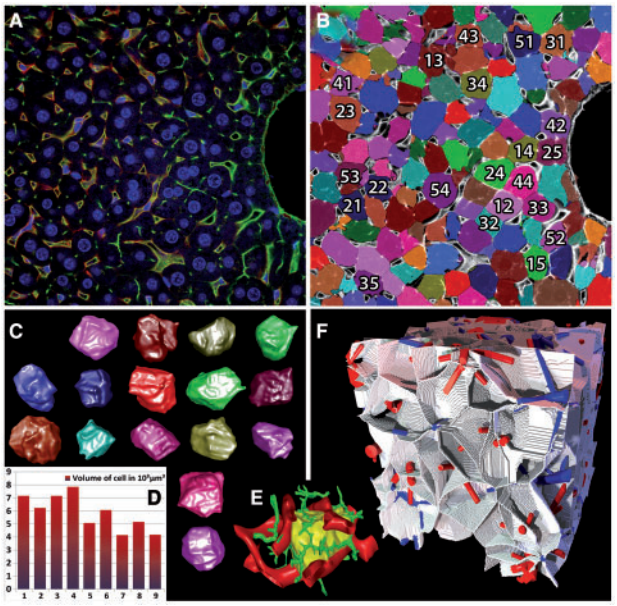

Fig. 1. (**A**) Confocal dataset: Nuclei (blue, DAPI), bile canaliculi (green, DPPIV), sinusoids (yellow, DPPIV, DMs). Resulting cell surface reconstruction in (**B**) 2D and (**C**) 3D. Marked cells are randomly selected. Numbers in (**B**) denote row (1st digit) and column (2nd digit) in (**C**). (**D**) Volume quantification of cells 1–9 (complete plot in Supplementary Fig. S4) (**E**) Single cell in tissue context (yellow: cell, green: bile, red: sinusoids). (**F**) Reconstruction of lobule surfaces in liver. See Supplementary Figure S3 for reconstruction in 2D

Since hepatocytes can have more than one nucleus and nuclei do not have to reside in the center of the cell, the influence of *CI* has been decreased to $\beta = 0.1$. The result of the surface reconstruction method is shown in Figure 1B–D. We compared the result of our method to a reconstruction based on beta catenin which allowed for precise cell membrane segmentation that we considered a reference cell shape reconstruction. The volume deviation of our method is less than 10% for half the cells and maximally 30% which represents a reasonably accurate reconstruction (see Supplementary Fig. S2).

In case of liver lobule surface reconstruction, central veins were used for class *CI* as they are per definition located in the center of lobules, while portal veins which are typically located at the border of lobules were used for class *CS*. In this example, both vascular structures were based on μCT images. Since the geometry of central and portal veins equally defines the shape of the lobules, we choose $\beta = 0.5$ (Fig. 1F). On a modern system (Intel i7 Quadcore), a complete run of the implementation of the cell surface reconstruction method in TiQuant completes in less than 30 minutes for a dataset of size $1024 \times 1024 \times 100$ (~100 m voxels). The RAM requirement for processing datasets of this size is 16 GB.

4 Summary

TiQuant provides a robust and efficient way to reconstruct, visualize and analyze different types of tissue. Additionally, the software implements a novel, widely applicable technique for the reconstruction of surfaces of biological structures based on incomplete information without using explicit staining. Open-source release of extended versions of TiQuant is planned.

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