SAT-0067

Development of Software for Automated Morphology Analysis (SAMA) to analyze morphogenic effects of mammotrophic hormones *in vitro*

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Summary

In vitro 3D simulations of biological systems are critical for understanding morphogenesis and patterns of normal and abnormal development in tissues.

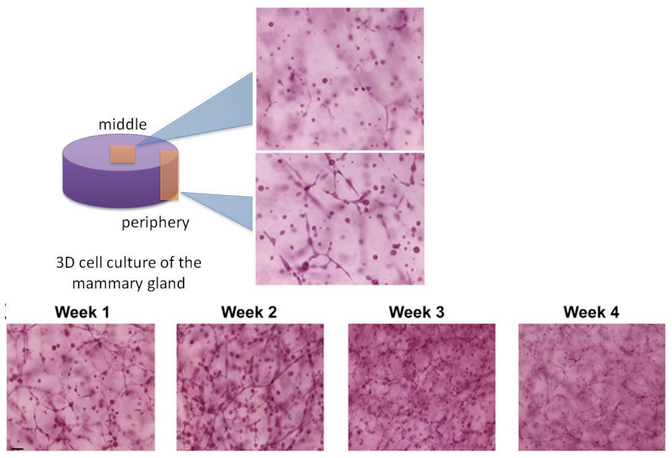
Here we present SAMA, a novel method through which epithelial structures grown in 3D cultures can be imaged, reconstructed in 3D and analyzed with minimum human intervention and, therefore, bias.

SAMA gives us an accurate picture of the epithelial structures and hence, more information compared to 2D morphometric analysis. In addition, this automated method is less time consuming than regular 2D morphometrics.

Why is SAMA needed?

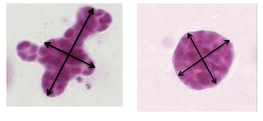
Cells in 3D culture form structures that vary in shape, size, and presence of lumen. Moreover, their distribution in the gel is heterogeneous and changes with time.

Heterogeneity in space and time



Top: MCF10A structures in the middle and peripheral area at the 10th day in culture. *Bottom:* time-course of epithelial morphogenesis; scale bar 50 µm. Adapted from Dhimolea et al, 2010.

A classic 2D morphometric approach would only provide the size and shape of a structure in 2 axes:



References

Speroni L, Whitt GS , Xylas J, Quinn KP, Jondeau-Cabaton A, Georgakoudi I, Sonnenschein C, Soto AM. *Hormonal regulation of epithelial organization in a 3D breast tissue culture model.* Tissue Eng Part C Methods. 2014 Jan, 20(1):42-51.
Dhimolea E, Maffini MV, Soto AM, Sonnenschein C. *The role of collagen reorganization on mammary epithelial morphogenesis in a 3D culture model.* Biomaterials. 2010 May;31(13):3622-30.

What is SAMA?

SAMA is a user-friendly set of customized macros that utilizes plugins operated via FIJI (http://fiji.sc/Fiji), an open-source image analysis platform in combination with a set of functions in R (http://www.r-project.org/), an open-source software for statistical analysis.

SAMA enables a fast and exhaustive analysis of the shape of all structures in a 3D image.

Advantages of SAMA over manual 2D analysis

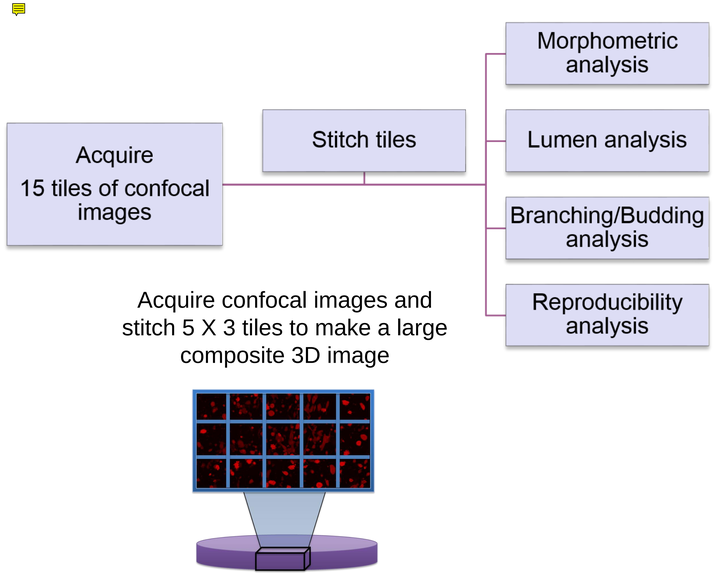
1) It is less biased as it requires minimum intervention by the user.

2) Some unique parameters can be assessed: elongation, sphericity and branching in 3 axes and presence of lumen.

3) The analysis is less time-consuming.

4) It enables the analysis of experimental reproducibility, thus providing a tool to assess and validate the robustness of the 3D culture systems in use.

How does it work?



Acknowledgments

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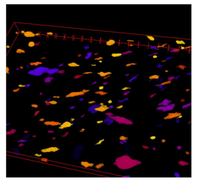
Data analysis

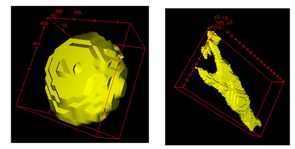
SAMA was validated using a hormone responsive 3D culture model of the breast epithelium (Speroni et al, 2014). In this model, estradiol, progestagen and prolactin induce T47D breast epithelial cells to form structures similar to those observed *in vivo*.

Morphometric analysis

SAMA processed image of a 3D gel



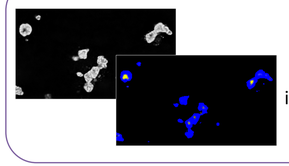
Structures are selected and rotated for a detailed analysis



Morphometric parameters:

- **Sphericity**, indicates how close is the structure to a perfect sphere.
- **Elongation** (small, middle and long axis), indicates how flat and long is a structure.

Lumen analysis

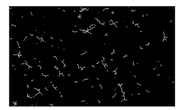


Lumen formation is analyzed by using differences in pixel intensity in the structures.

Branching/Budding analysis

Estradiol

Estradiol + Prolactin



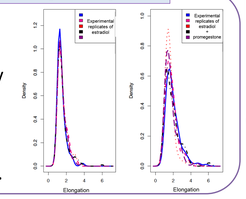
Morphometric parameters:

- **Complexity**, the sum of the length of branches.

- **Ratio volume ellipsoid**, Ratio of the volume of the structure to the volume of an ellipsoid fitted around the structure.

Reproducibility analysis

The robustness of the 3D model can be assessed automatically in terms of experimental consistency between replicates and repeats.



Results

- SAMA gave consistent results with 2D analysis by Speroni et al, 2014.
- It also provided new information, namely: 1) prolactin + estradiol generate more elongated structures among the structures that have lumen (p=0.026), and more complex structures in terms of geometrical branches (budding) (p=0.048) than estradiol alone; 2) promegestone + estradiol generate structures that are more elongated (p=0.0057), and the surface of structures is more complex than in estradiol alone (p=0.041).

Other applications of SAMA

- SAMA will be applicable to 3D culture models of a wide range of organs where a morphological analysis is needed.
- We also foresee a translational application. For example, cancer cells in 3D could be explored to monitor therapeutic effects based on quality of the epithelial structures (tumor vs. normal: size and shape of structures, luminal filling vs. clear lumen).