

# Tracking cell dynamics from time-lapse LSM imagery

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## Introduction

In many organs, including the mammary gland, the spherical acinus forms one of the structural units required for tissue homeostasis, but the cell behaviors that specify acinar geometry in adult tissue are insufficiently understood. Using 3D culture models of human mammary epithelial cells [Bissell 01], we investigate both acinar morphogenesis and tumor formation using 4D live confocal fluorescence imaging to elucidate the mechanism of this architectural specification. We demonstrate that non-malignant cells maintain strong cell-cell adhesion while generating actomyosin-regulated centripetal force, resulting in coherent angular motion (CAMo) during acinar morphogenesis. That this motion is instrumental to the formation of the architecture is demonstrated by the disorganization that results when either polarity genes or cell-cell adhesion molecules are disrupted. The volume of the data is a couple of gigabytes and often challenging to analyze quantitatively. While we have quantified CAMo using simple trajectory models of the dynamics, further developments/tools are necessary to derive geometrical descriptors and track the evolution of acinus formation.

## From CLSM to ROI

Modern microscopes as the confocal laser scanning microscope (CLSM) LSM710 can reduce distortion and optical aberrations, however no optical system is free of artifacts that may compromise the SNR. We minimize signal corruptions by running 3D bilateral filtering. This anisotropic filter combines geometric closeness (domain) and photometric similarity (range) to recalculate a pixel value. The algorithm is an attractive alternative to iterative diffusion methods due to its efficiency, particularly when dealing with large images as those considered in this research.

We noticed that the inclusion of the frequency distribution of the intensity values in the calculation of the new voxel has proven to mitigate spurious fluctuations by compressing the histogram to a narrower vicinity. Our approach uses Gaussian kernels to attenuate the influence of pixels in the vicinity of a target pixel, and they depend on geometrical closeness ( $\sigma_d$ ) and pixel depth ( $\sigma_r$ ) difference. A possible way to estimate these parameters is to use image acquisition metadata. This gray level remapping supports the next processing step, the statistical region growing, by restricting the variation according to distance while preserving the borders of the cell structures.

Statistical region merging (SRM) is a region growing segmentation algorithm based on an adaptive statistical merging predicate on intensity levels. Particular advantages of using this algorithm for dealing with large images are that it dispenses dynamical maintenance of a region adjacency graph (RAG), allows defining a hierarchy of partitions and it runs in linear-time by using bucket sorting algorithm while transversing the RAG [Nock 05].

The key numerical schemes are summarized below:

- Fringe removal using non-linear bilateral filtering:

$$h(x) = k^{-1}(x) \int_{-\infty}^{+\infty} f(\xi) c(\xi, x) s(f(\xi), f(x)) d\xi$$

$$k(x) = \int_{-\infty}^{+\infty} c(\xi, x) s(f(\xi), f(x)) d\xi$$

*(Note: 'geometric' is associated with c(ξ, x) and 'photometric' with s(f(ξ), f(x)))*

- Particle segmentation using statistical region merging:

$$b(R) = g \sqrt{\frac{1}{2Q|R|} \left( \frac{\ln S_{|R|}}{\delta} \right)}$$

$$P(R_i, R_j) = \begin{cases} 1 & \text{if } |R_i, R_j| \leq \sqrt{b^2 R_i + b^2 R_j} \\ 0, & \text{otherwise} \end{cases}$$

*(Note: 'merging predicate' is associated with the entire expression)*

- Standard geometrical descriptors: surface area, volume, surface-area-to-volume ratio.

## Structural changes over time

The geometry-sensing mechanisms in single-celled organisms help them to control both the decision to enter into cell division and the physical orientation of the chromosome segregation machinery [Moseley 10].

Recent research in cell development showed that culture monitoring from time-lapse videos allowed identification of subsets of healthy human embryos [Pera 10] using computer vision techniques. Their investigation identified parameters to track time lags between mitosis and synchronicity among cells.

One of our goals is to look into cell descriptors that can indicate HMEC mitosis, and identify if these descriptors correlate with certain windows of time. Our hypothesis is that variation in cell-division schedules are possibly related to variation in gene expression profiles, and it may be connected to the structural formation of the acini.

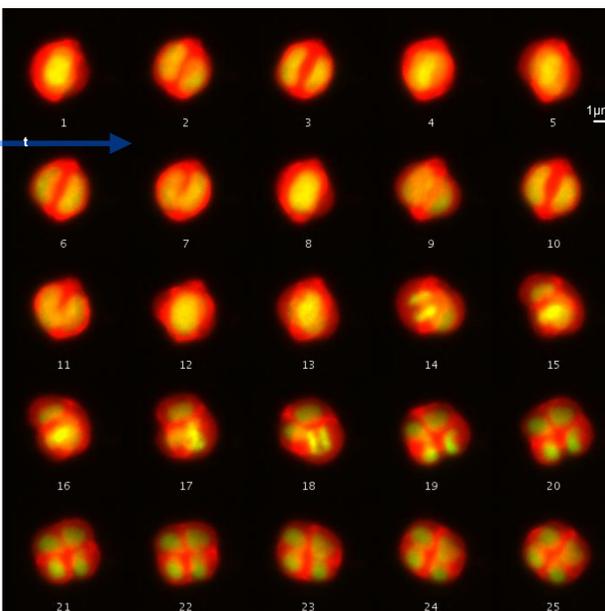


Figure 1. Four dimensional live confocal fluorescence imaging of S1 acinar morphogenesis time steps using, showing DNA (green) and F-actin (red), marked with H2B-GFP and mCherry LifeAct respectively: HMEC dynamics is not evident from projections.

Visual inspection of the dynamics of 3D culture models have supported the hypothesis of cell spiraling movement – our goal is to continue developing tools to derive geometrical descriptors and track the evolution of such features in time.

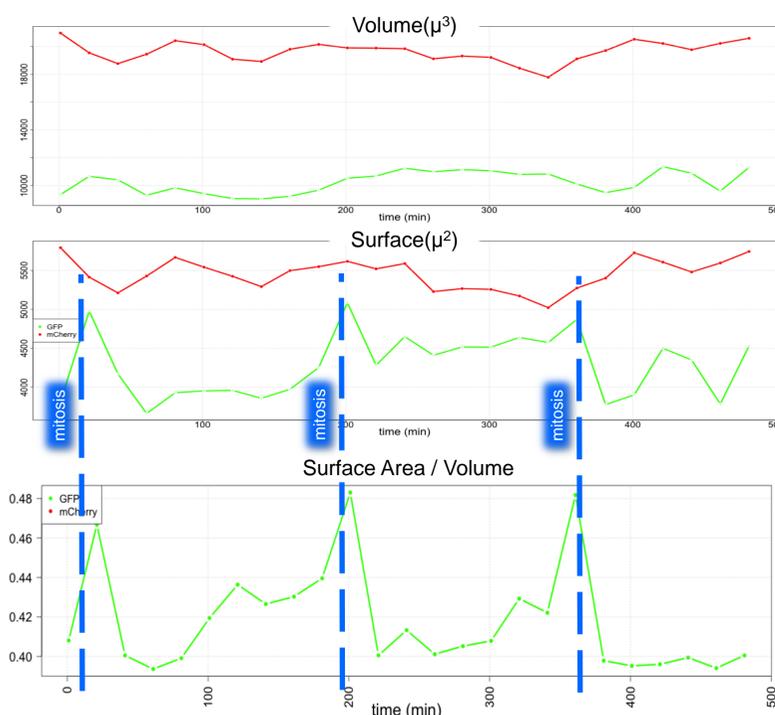
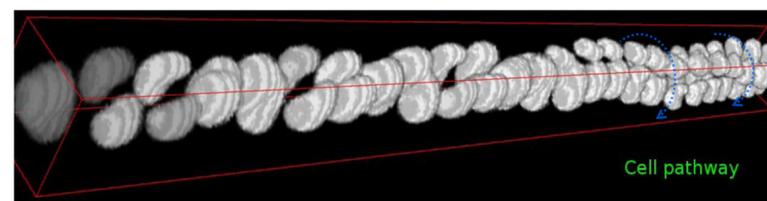


Figure 2. Standard descriptors of nucleus and cytoplasm of cell culture, as in Figure 1, illustrate potential regular time-schedules and correlation between mitosis and increased surface area of nucleus.

## Analysis pipeline

We designed a quantitative image analysis pipeline to process HMEC from time-lapse microscopy data. This process includes three main steps: (i) identify protein fluorescence associated to cell structure as F-actin and DNA and eliminate regions corresponding to non-specific binding; (ii) estimate the position of the structure boundaries at each time frame; (iii) calculate structural changes over time. We focus on sub-cellular protein localization and have designed computer algorithms that include artifact minimization using bilateral filtering and segmentation of regions of interest using statistical region merging. Regions are associated to nucleus and cytoplasm structures, and characterized in terms of cell surface and volume.

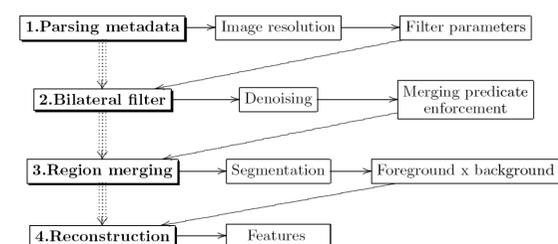


Figure 3. Quantitative image analysis pipeline to track cells under division.

## Results

Preliminary results illustrate the quantification of structural features from time-lapse movies of the S1 acinar formation, and describe patterns in terms of simple geometrical descriptors as volume, surface area and their ratio. We noticed that variations of these parameters are correlated to time schedules in which mitosis are occurring. We are developing better methods for analyzing cell trajectories, as well as assembling a database, and will soon be able to determine the circumstances in which proteins likely colocalize. New investigation of the underlying mechanisms will include tracking of surface parameters and image restoration and deblurring to deal with inherent image degradation due to optical sectioning.

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