Single-Cell Live Image Analysis

HTI major 2013

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Supervision

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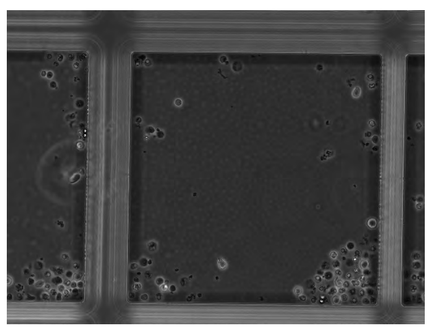
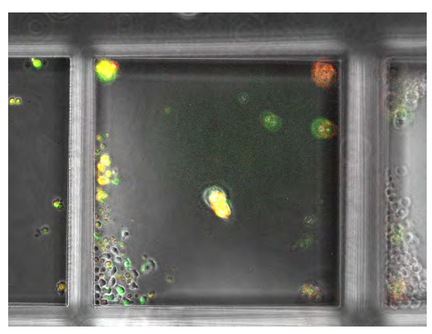
MOTIVATION & OBJECTIVES

Single-cell microscopic imaging

- Single-cell imaging in phase-contrast and fluorescence microscopy allows for monitoring *in vivo* human T cells confined in μ-trap arrays
- Analyzing time-lapse video sequences could bring new insights on the way T cells evolve into T-reg cells which govern immune tolerance
- This requires image processing methods for automated cell counting, segmentation and tracking, coupled to data processing techniques for understanding cell interactions



phase-contrast microscopy



fluorescence microscopy

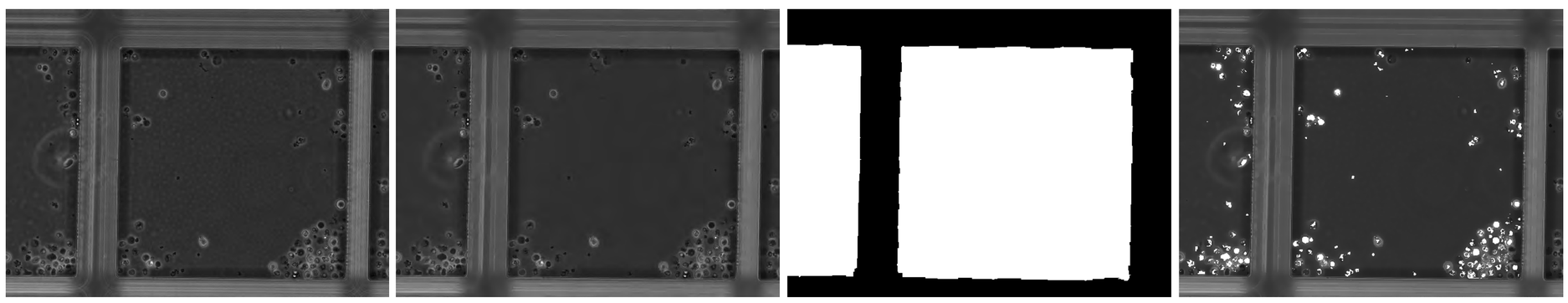
Challenges

- Detect all the objects in the field of view
- Distinguish between impurities, dead and alive cells
- Track individual cell to reconstruct trajectories

MATERIAL & METHODS

- **Setup:** Human T cell lines in μ-trap arrays with macrophages and beads connected to specific molecules
- **Database:** 12 time-lapse video sequences = phase-contrast images of μ-grid sub-regions at regular time intervals during 24 hours + fluorescence images acquired 2-3 minutes after phase-contrast data
- **μ-grid segmentation** using temporal mathematical morphology on frames groups
- **Object segmentation** in each frame:
  - > Preprocessing: μ-trap background texture homogenization via PDE filtering (anisotropic diffusion with Tukey biweight-based conduction function)
  - > Detection using geodesic morphology using μ-trap background and object dark sub-region markers

RESULTS



original frame | μ- trap texture filtering | *μ-grid-segmentation* | detected objects

- Sound performances showing high sensitivity / low specificity: all objects are detected, including beads and impurities
- Spurious over-segmentation artefacts can occur due to possibly non simply connected object markers

CONCLUSION & PERSPECTIVES

- Using temporal features would improve robustness and allow for distinguishing between dead/alive cells
- Specificity could be enhanced by relaxing the requirement to detect impurities
- Future work:
  - > implement a cell tracker based on frame-by-frame object segmentations
  - > use fluorescence data to discriminate T-reg cells

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