Bubble Segmentation Based on Shape From Shading for In-Situ Microscopy

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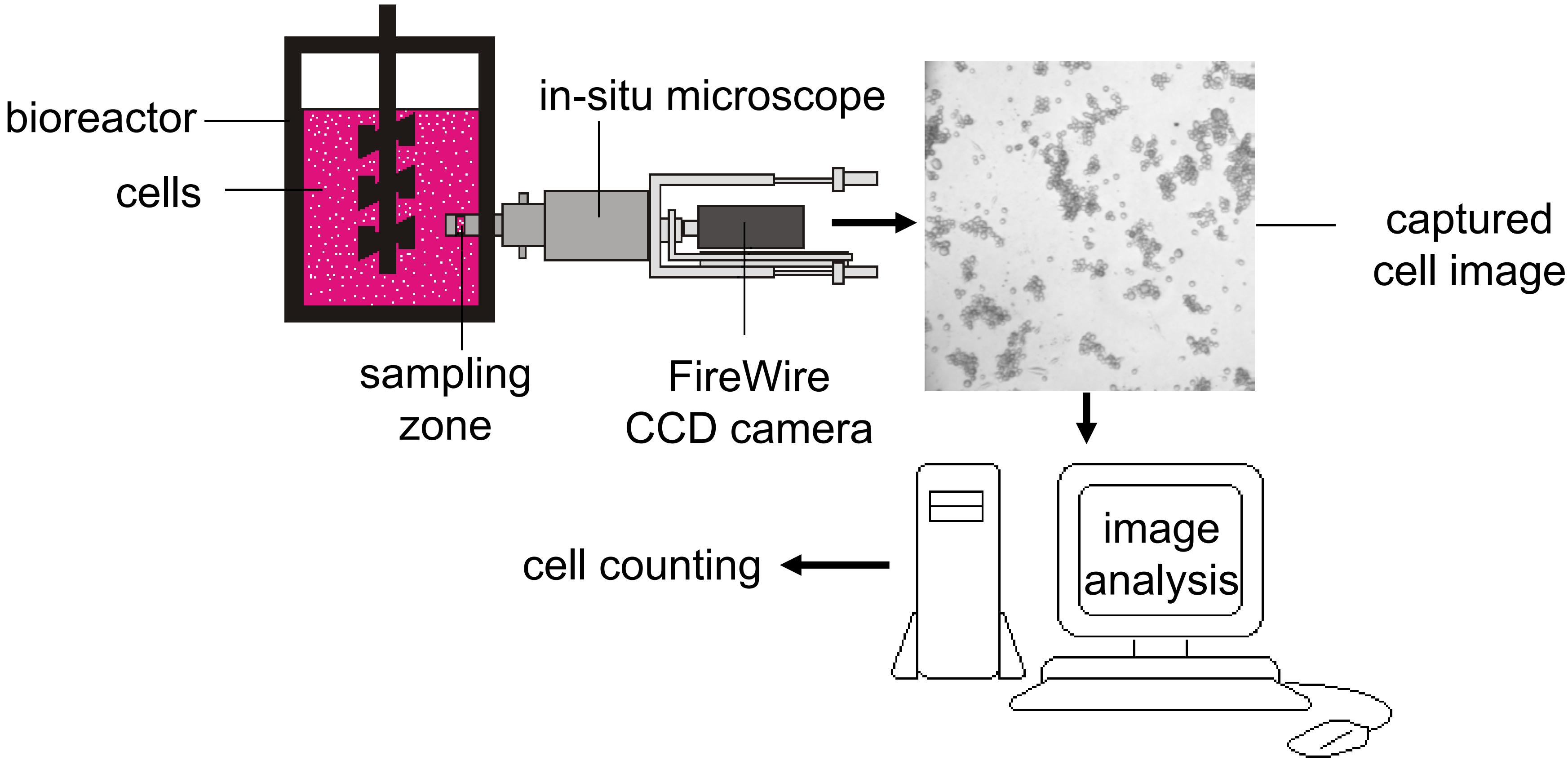
CONIELECOMP 2011, Cholula, Puebla, Mexico, March 2011

Overview

- Introduction
- Problem
- Approach
- Bubble segmentation algorithm
- Experimental results
- Summary

Introduction

On-line automatic cell counting with no risk of culture contamination

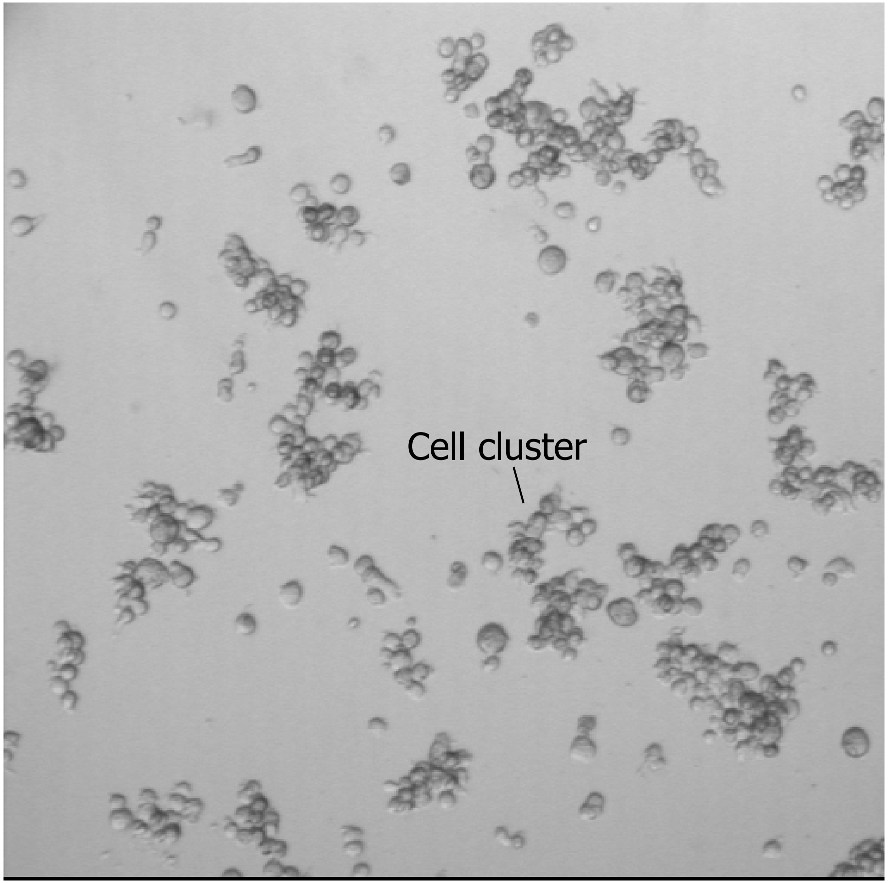


Assumptions: the cells are round and they aggregate forming symmetrical 3D clusters up to three-layers high

Introduction

3D Cell Counting

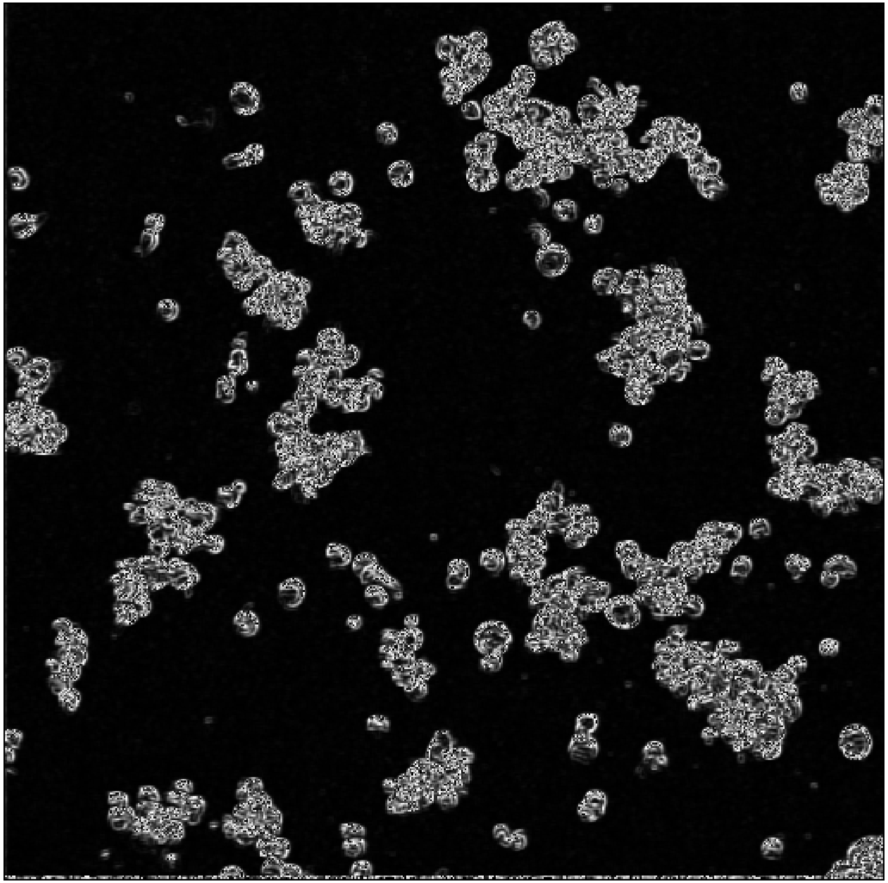
1) Capture an intensity image I



Introduction

3D Cell Counting

2) Estimate the local intensity variance at each pixel position



Variance image V

Introduction

3D Cell Counting

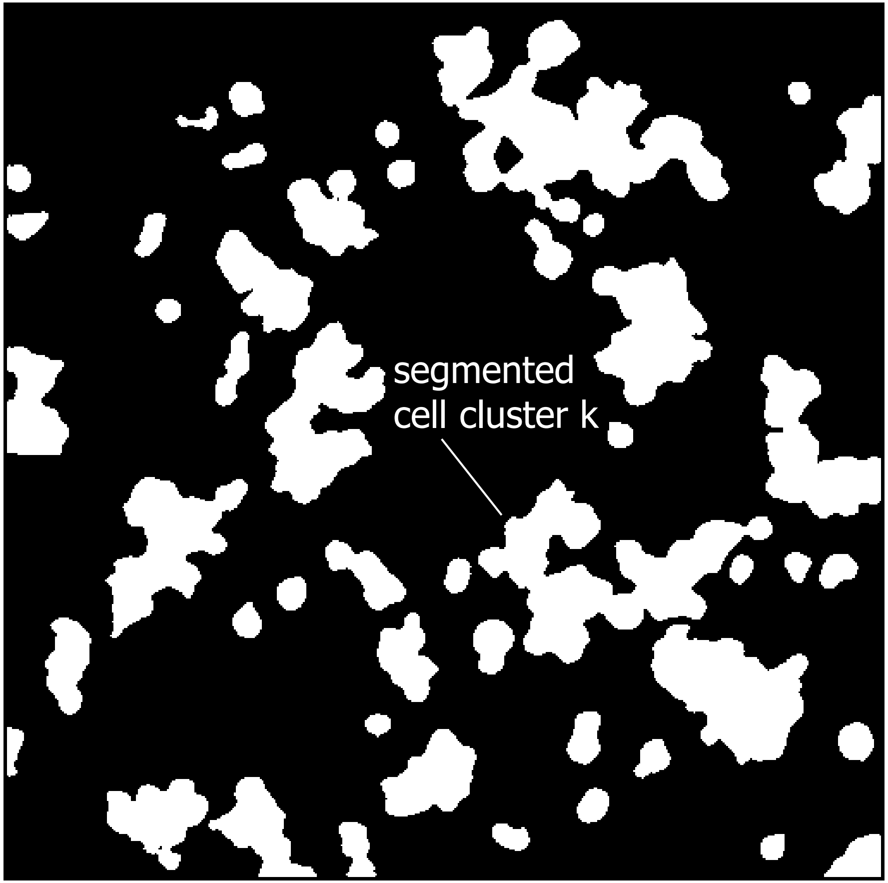
3) Classify all the pixels of the variance image V into pixels of the background and pixels of the cell clusters by using the threshold th_m

   $V_i \leq th_m$: background (black)

   $V_i > th_m$: cell cluster (white)

   Estimate th_m by maximizing a likelihood function according to Kittler

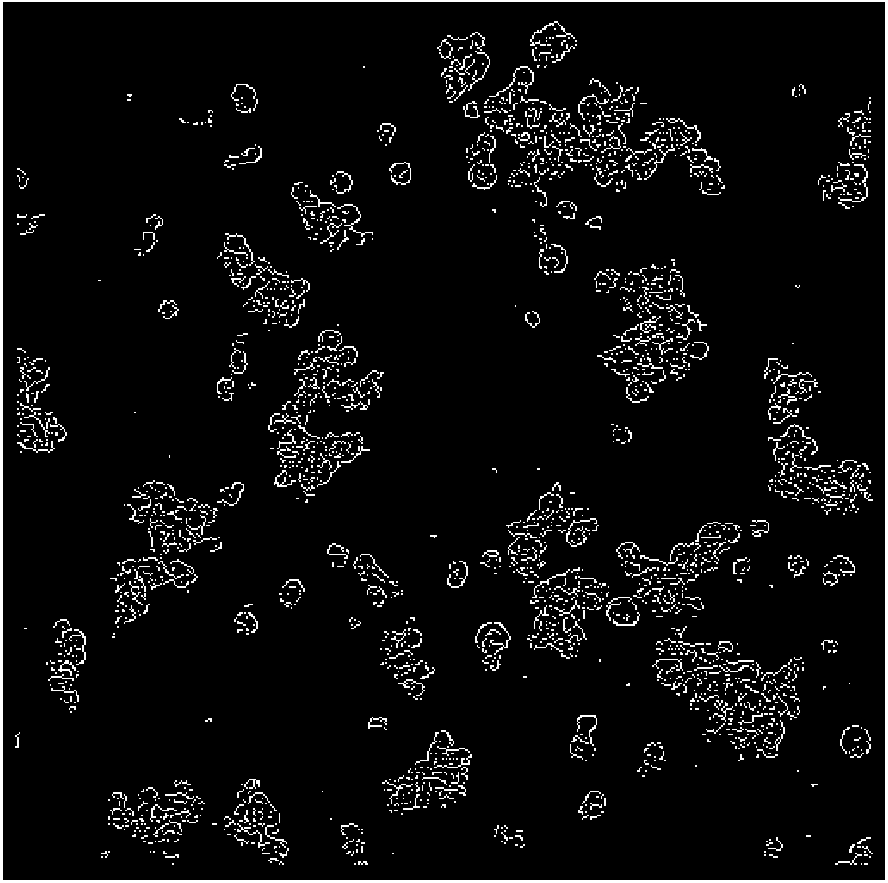
   Correct misclassifications by applying particle filtering



Introduction

3D Cell Counting

4) Extract the edges of cell clusters by applying the Smallest Univalue Segment Assimilating Nucleus algorithm (SUSAN algorithm) to the intensity image I

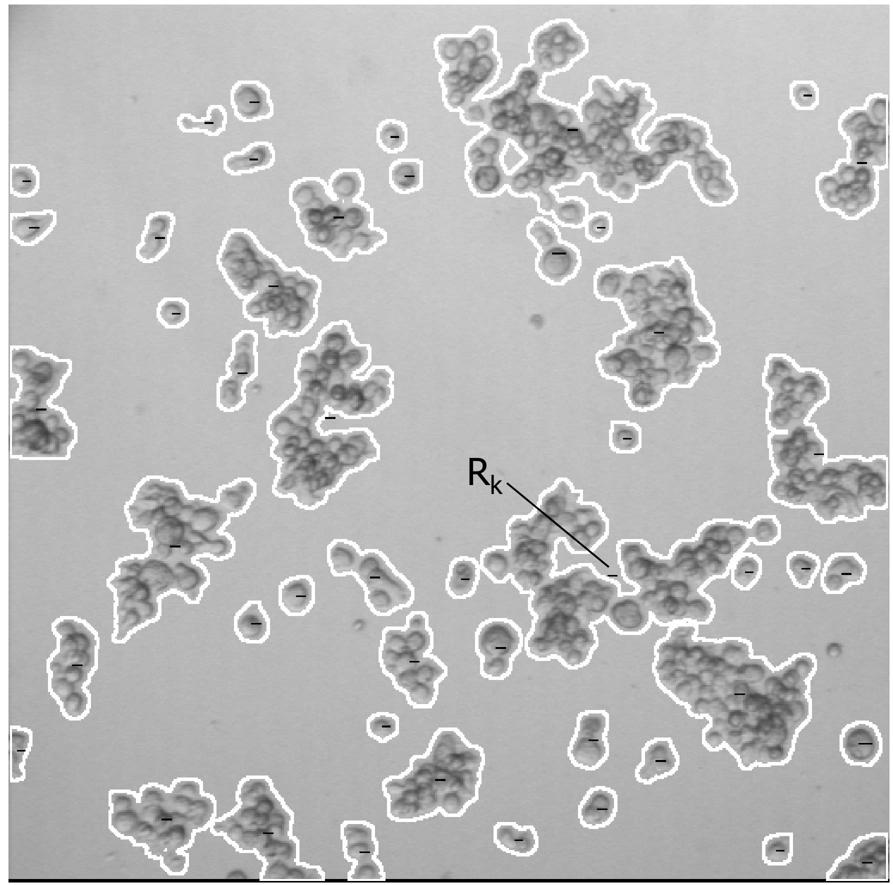


Introduction

3D Cell Counting

5) Estimate the average cell radius R_k of each segmented cell cluster k by maximizing the variance of the circular Hough transform of the edges inside the cluster

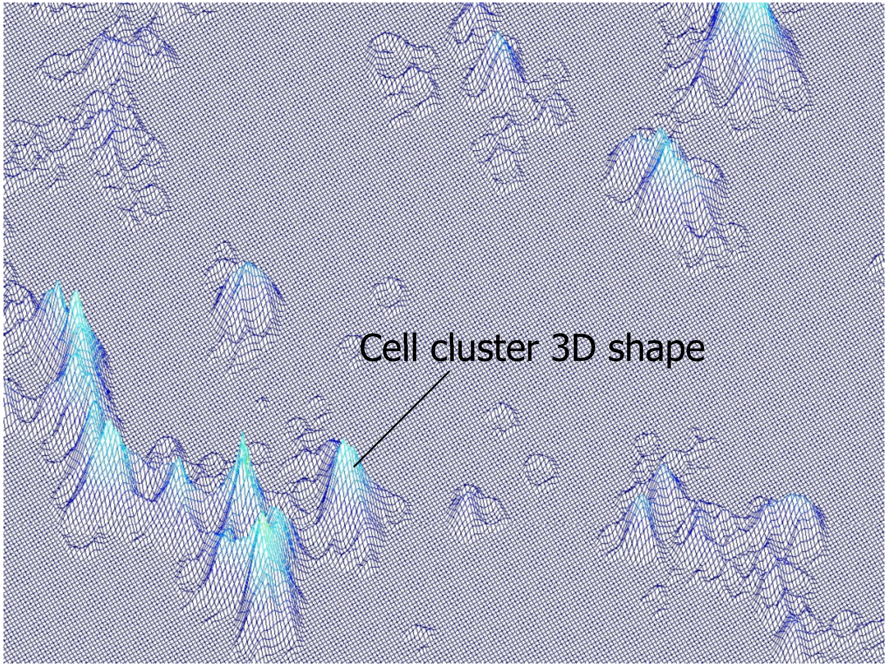
$$\sigma(H_{R_k,k})^2 \geq \sigma(H_{r,k})^2 \;\; \forall \;\; r = 1,2,3,..$$



Introduction

3D Cell Counting

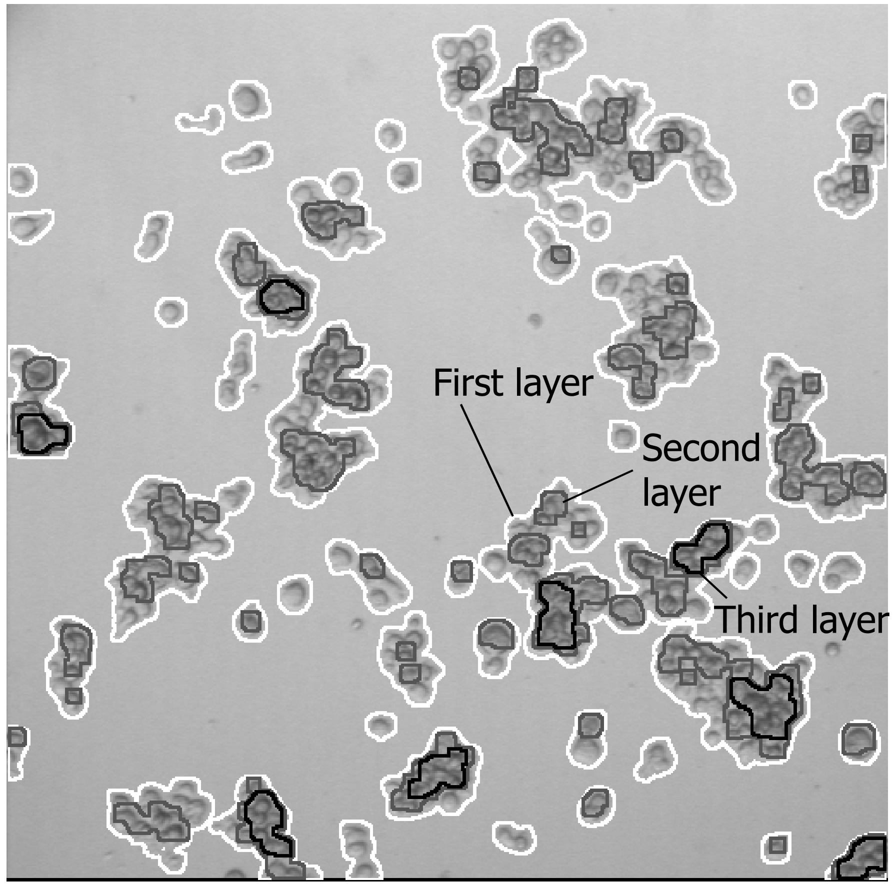
6) Estimate the 3D shape (depth map) of the cell clusters by applying the Bichsel and Pentland's Shape from Shading algorithm to the intensity image I



Introduction

3D Cell Counting

7) Quantize the estimated depth map up to 3 layers and compute the image regions of the parallel projections of the 3 layers into the image plane



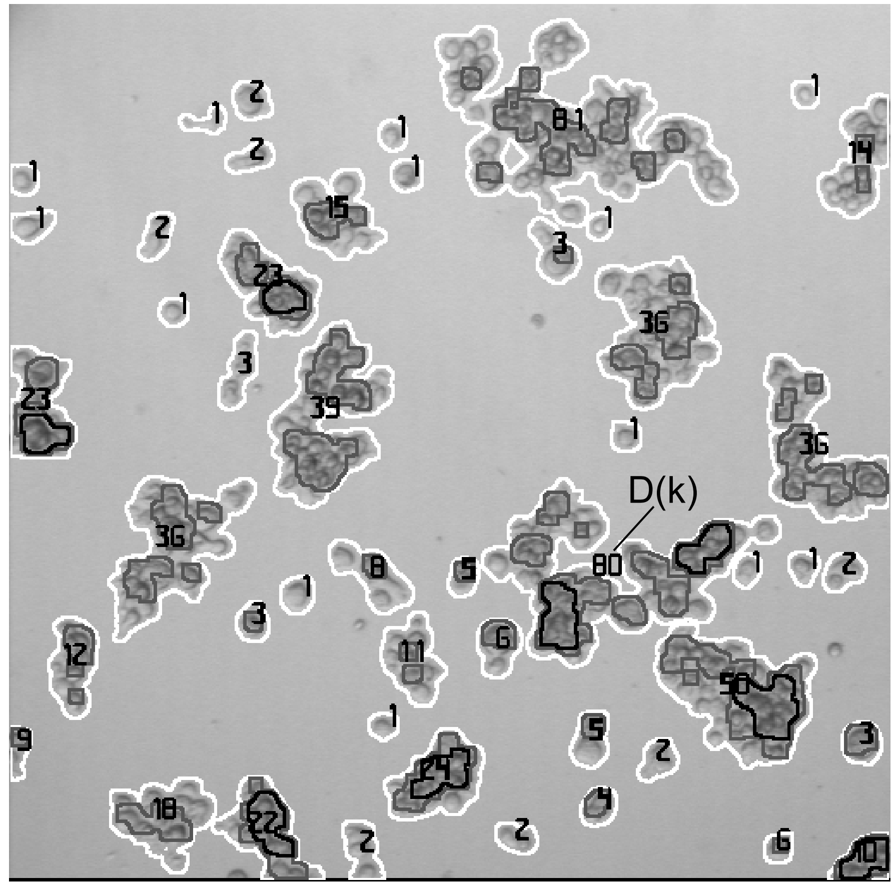
Introduction

3D Cell Counting

8) Estimate the number of cells D(k) in each segmented cell cluster k as follows:

$$D(k) = \frac{A_1(k) + 2 \cdot A_2(k) + 2 \cdot A_3(k)}{\pi \cdot R_k^2},$$

where $A_1(k)$, $A_2(k)$ and $A_3(k)$ are the areas of the image regions of the parallel projections of the first-, second- and third-layers of the cell cluster k into the image plane



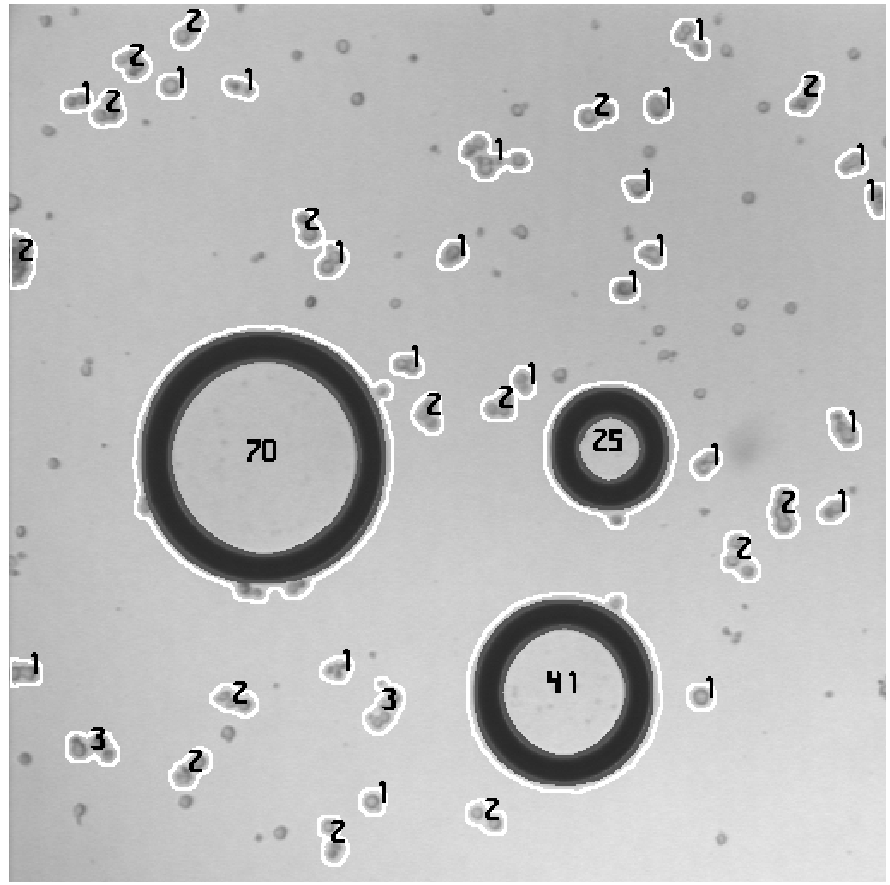
Problem

What happen when there are bubbles in the scene?

3D Cell Counting fails !

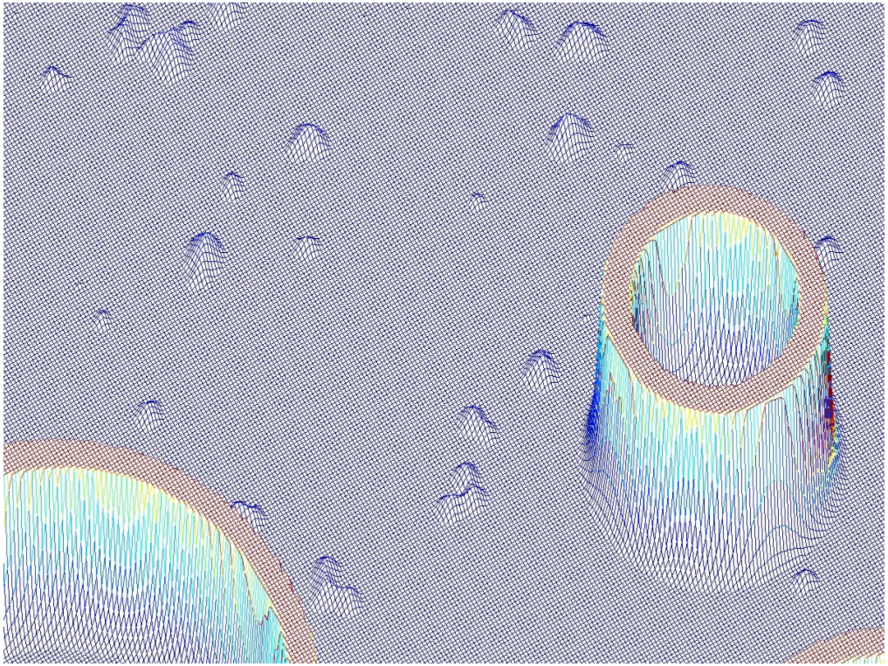
Thus, the image regions of the bubbles must be segmented and excluded from cell counting

But, how can we segment the bubble regions?



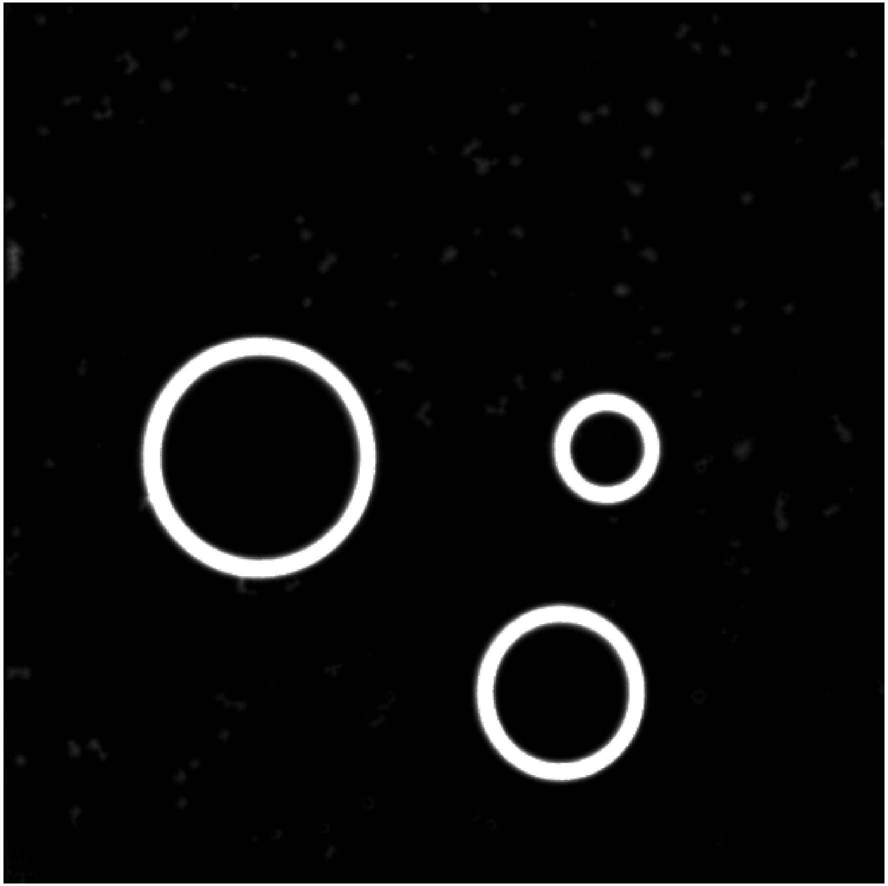
Approach

- Reuse the previously estimated depth map
- First, segment the bubble boundaries by pixel classification comparing the depth at each pixel position to a high threshold value
- Then, obtain the complete bubble regions by filling in each one of the segmented bubble boundaries



Bubble Segmentation Algorithm

1) Re-scale and quantize the estimated depth map to 256 levels



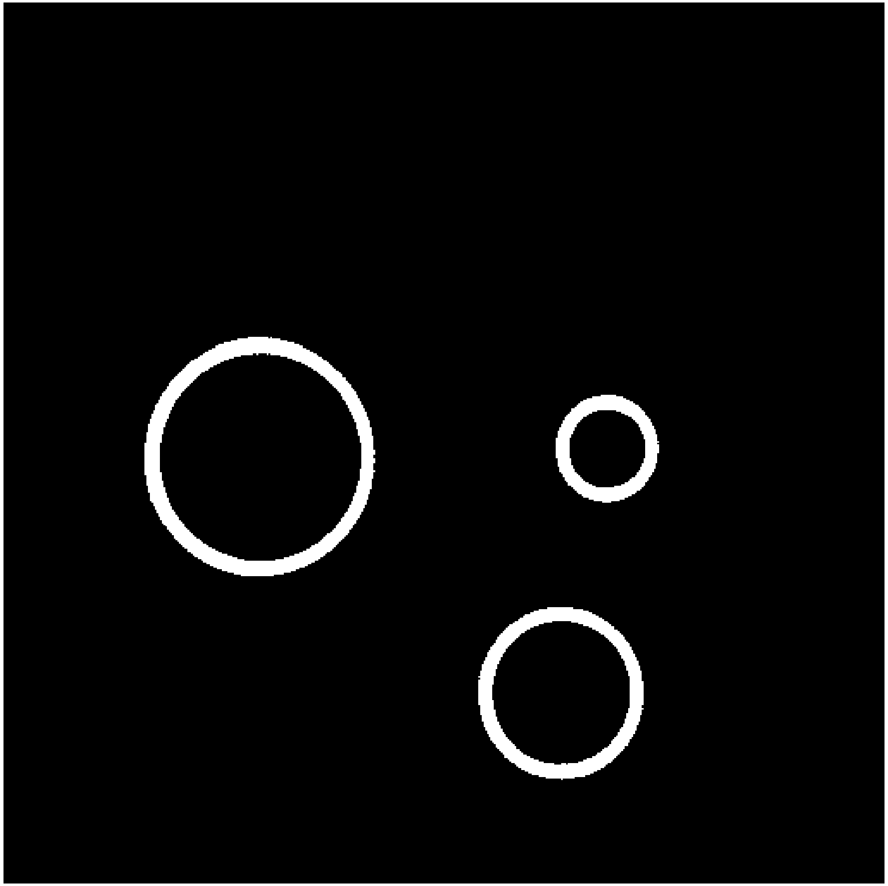
Re-scaled and quantized depth map D

Bubble Segmentation Algorithm

2) Classify all the pixels of the re-scaled and quantized depth map D into pixels of the background and pixels of the bubble boundaries by using the heuristically found high threshold value of 240

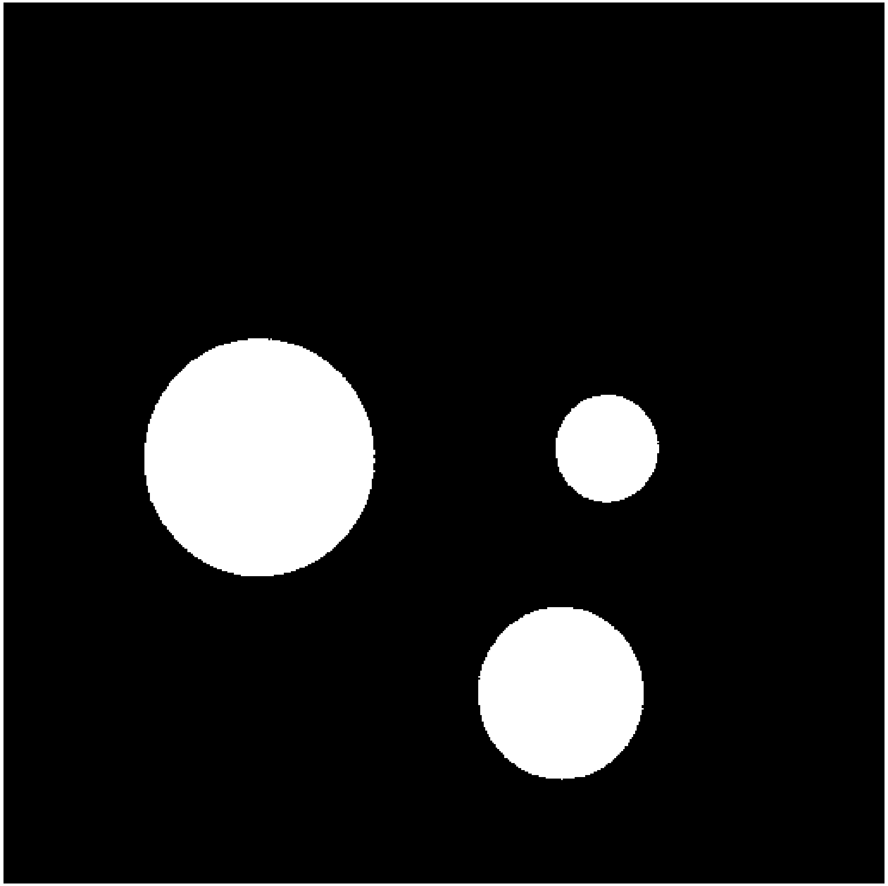
$D_i \leq 240$: background (black)

$D_i > 240$: bubble boundary (white)



Bubble Segmentation Algorithm

3) Fill in with white the black hole inside each segmented bubble boundary

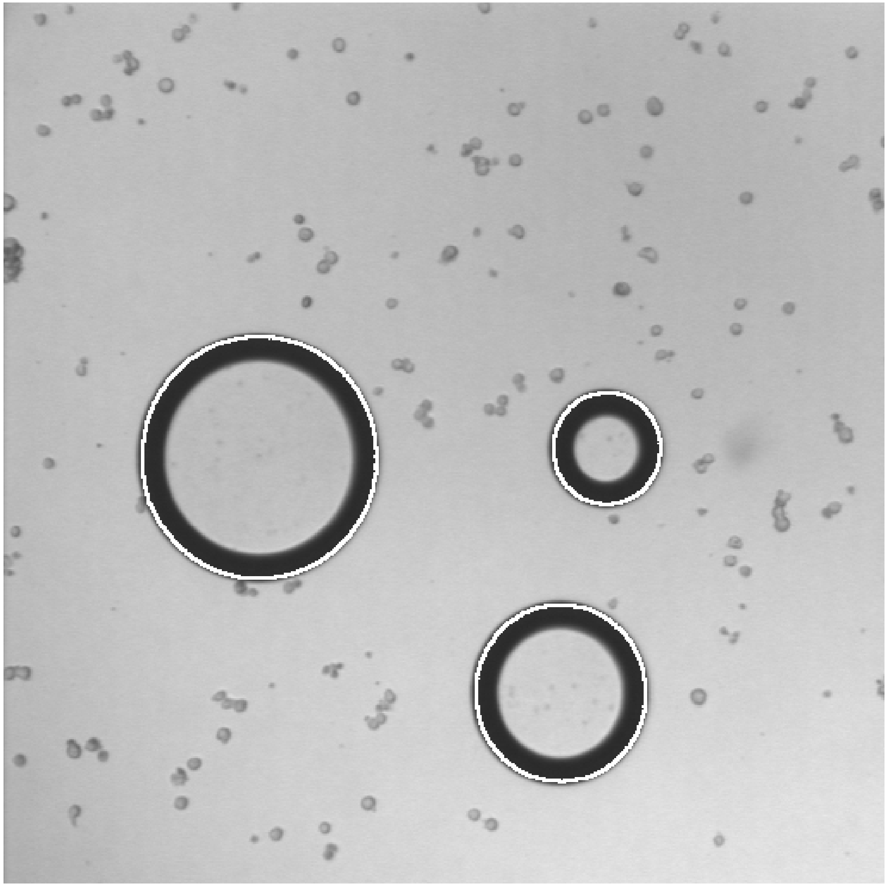


Experimental Results

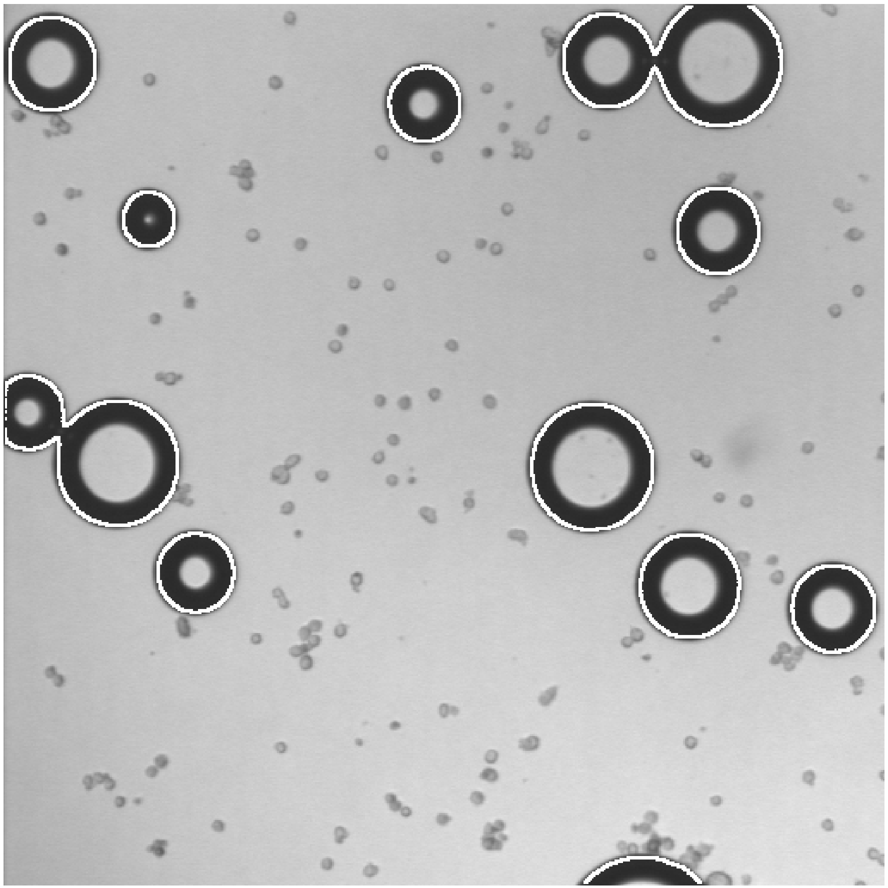
Average processing time 2.62 sec/image

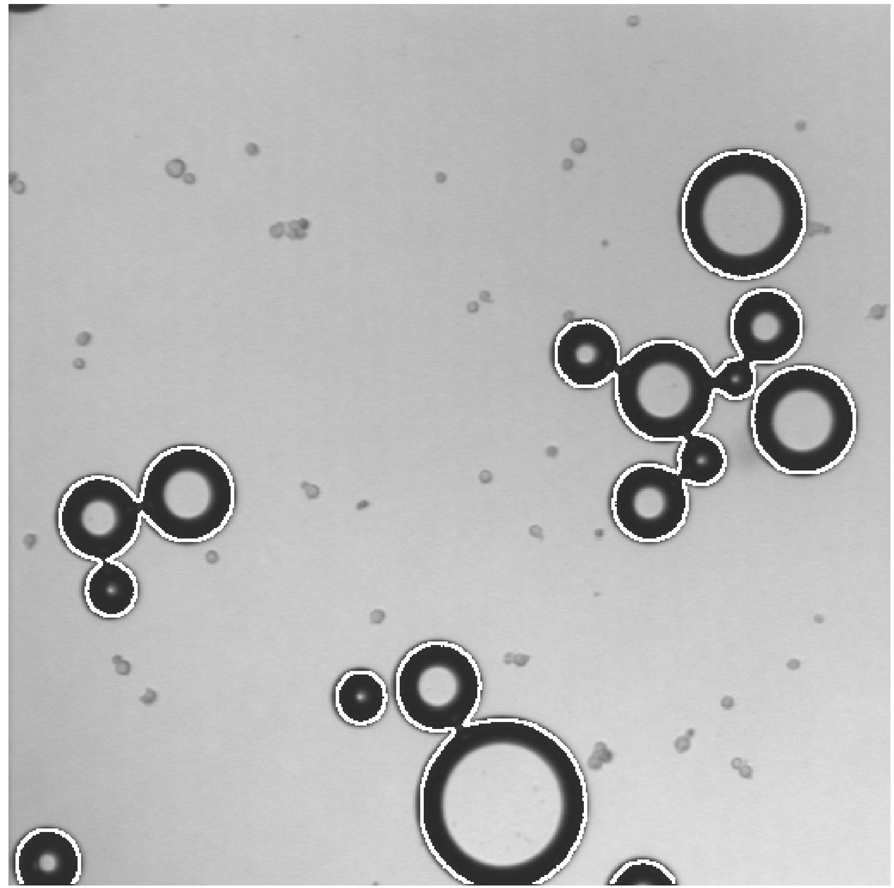
High reliability and accuracy

Contours of the segmented bubbles overlapped with the original image

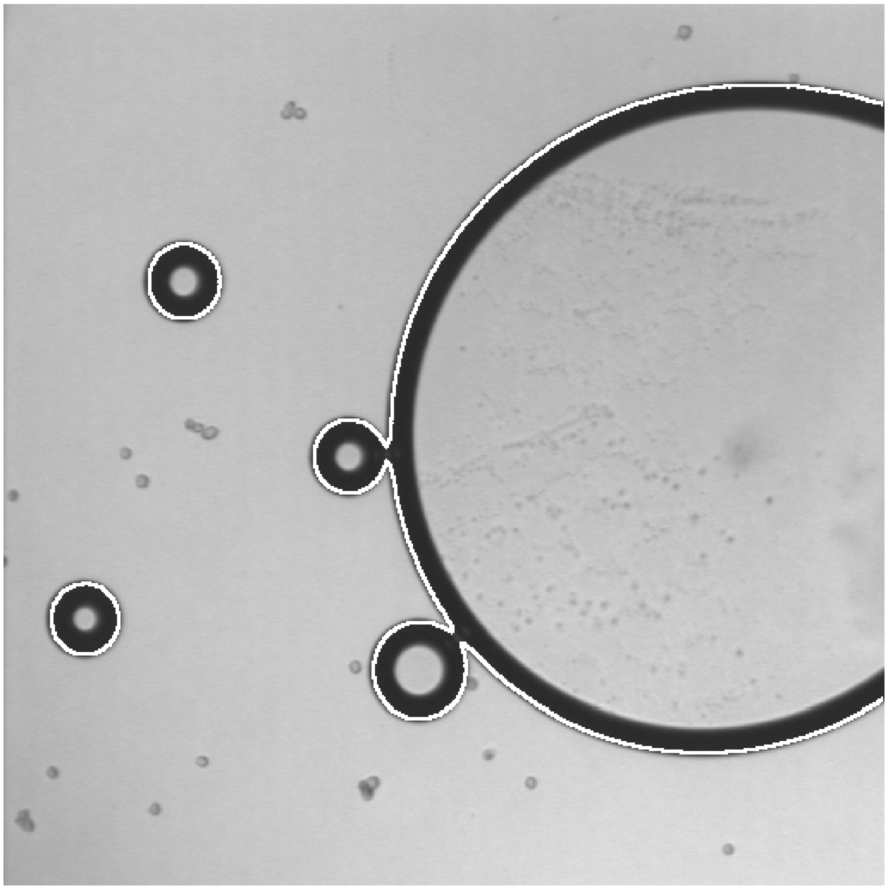


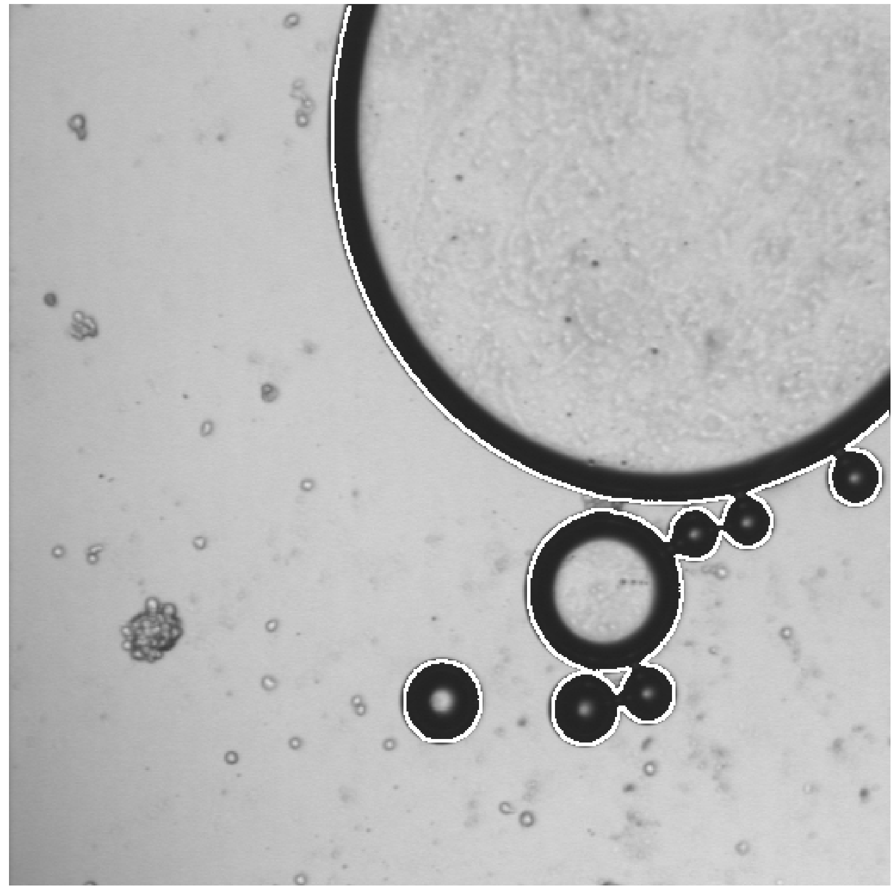
Experimental Results



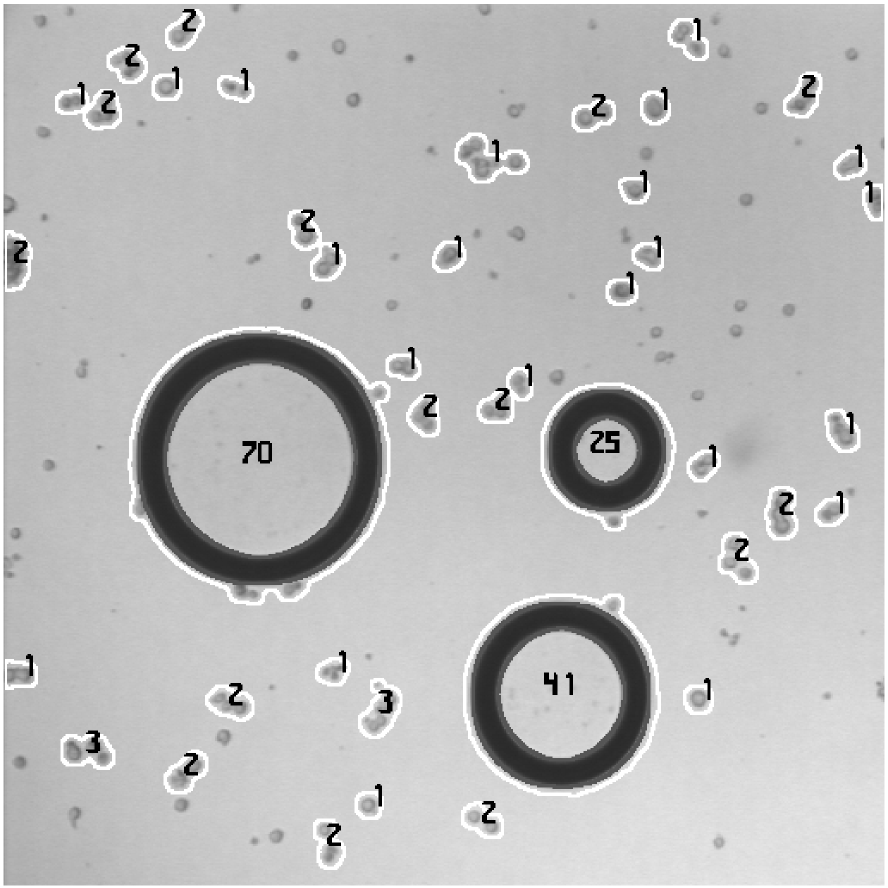


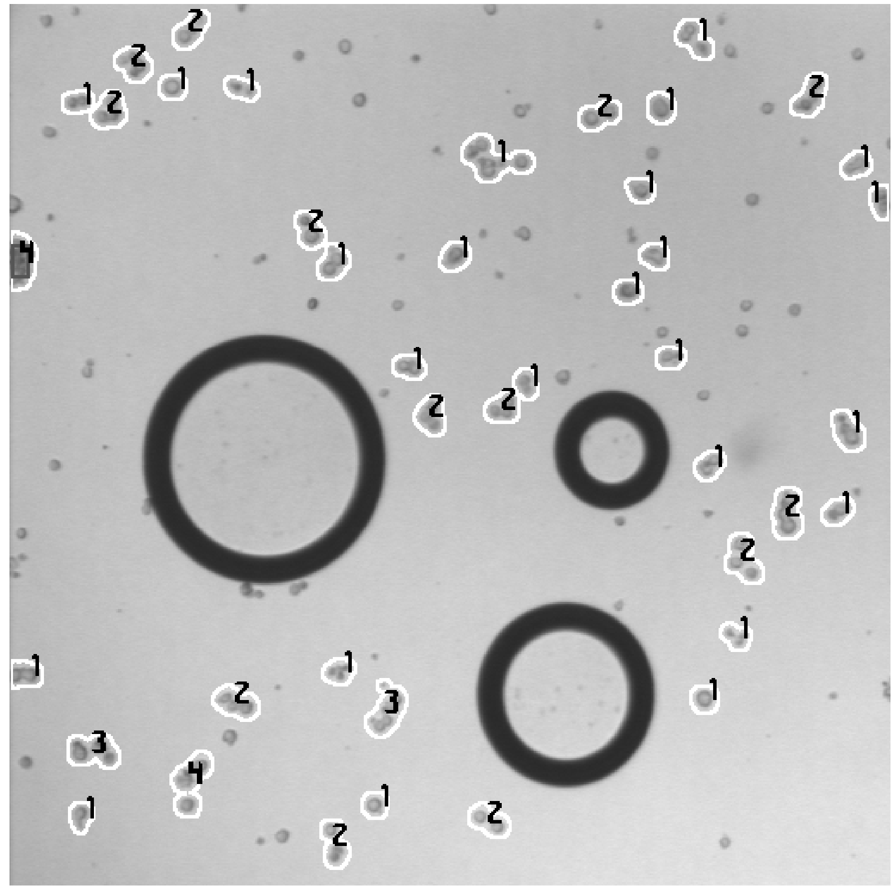
Experimental Results





Experimental Results





Bubble excluded from cell counting

Summary

- A bubble segmentation algorithm was proposed
- First, the bubble boundaries are segmented by thresholding a depth map using a high threshold value, where the depth map is estimated by applying the Bichsel and Pentland`s Shape From Shading algorithm
- Then, the segmented bubble boundaries are filled in to obtained the complete bubble regions
- The experimental results revealed an average processing time of 2.62 sec/image and very promising bubble segmentation results