Foreground Segmentation of Human Insulin Crystal Images for In-Situ Microscopy

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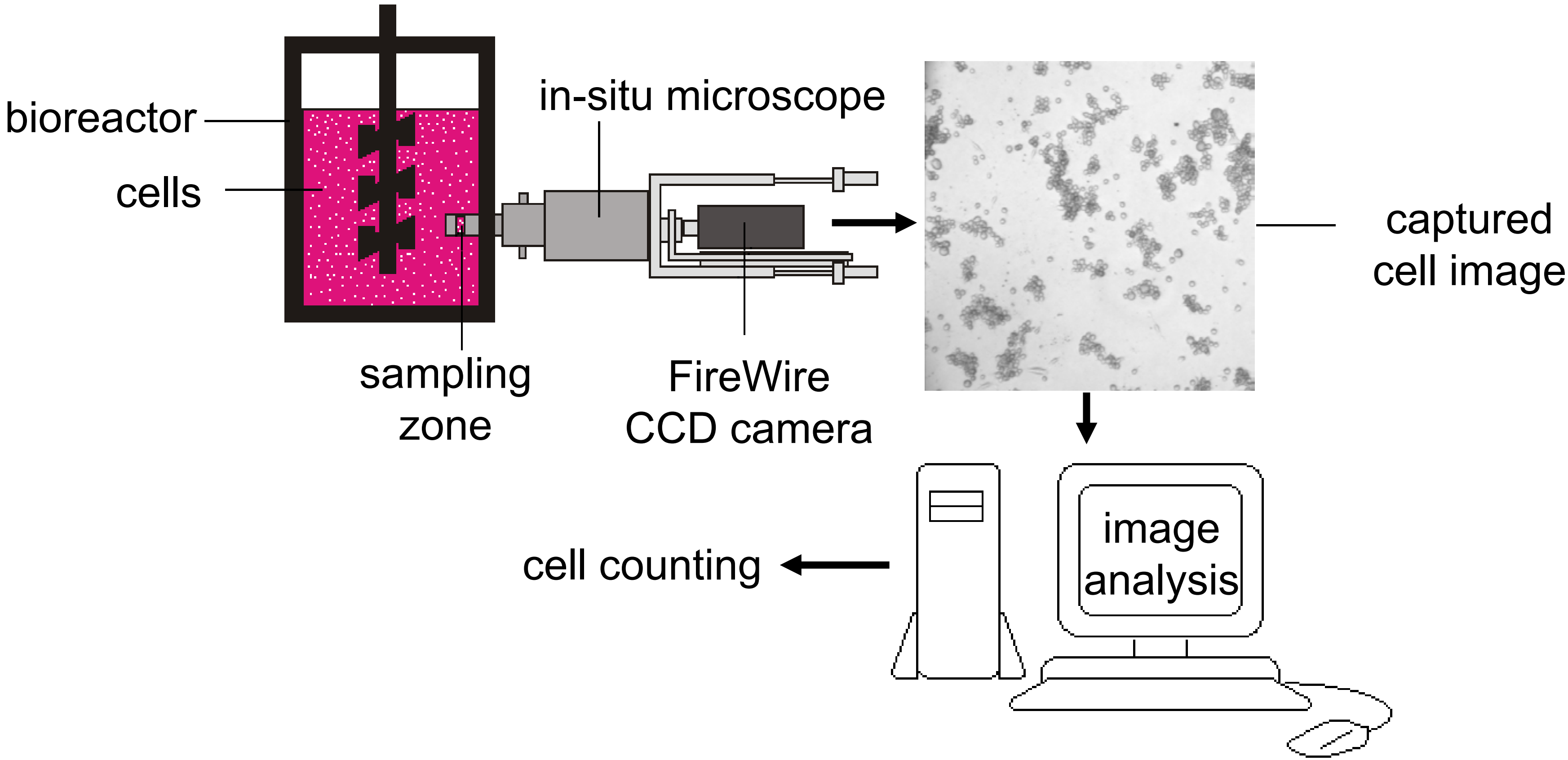
Overview

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

Introduction

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



Introduction

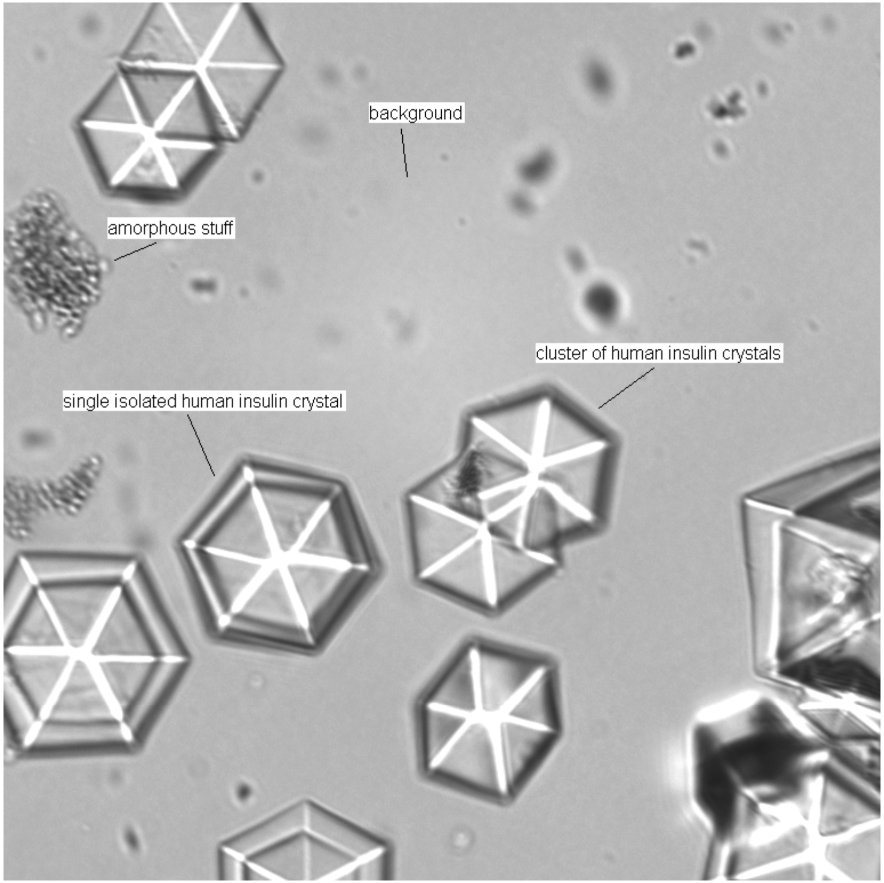
Is it possible to use an in-situ microscope to capture images of a human insulin crystal process?

Yes!

First images show:

Background: homogeneous

Foreground: single crystals, crystal clusters, amorphous stuff and mixed regions



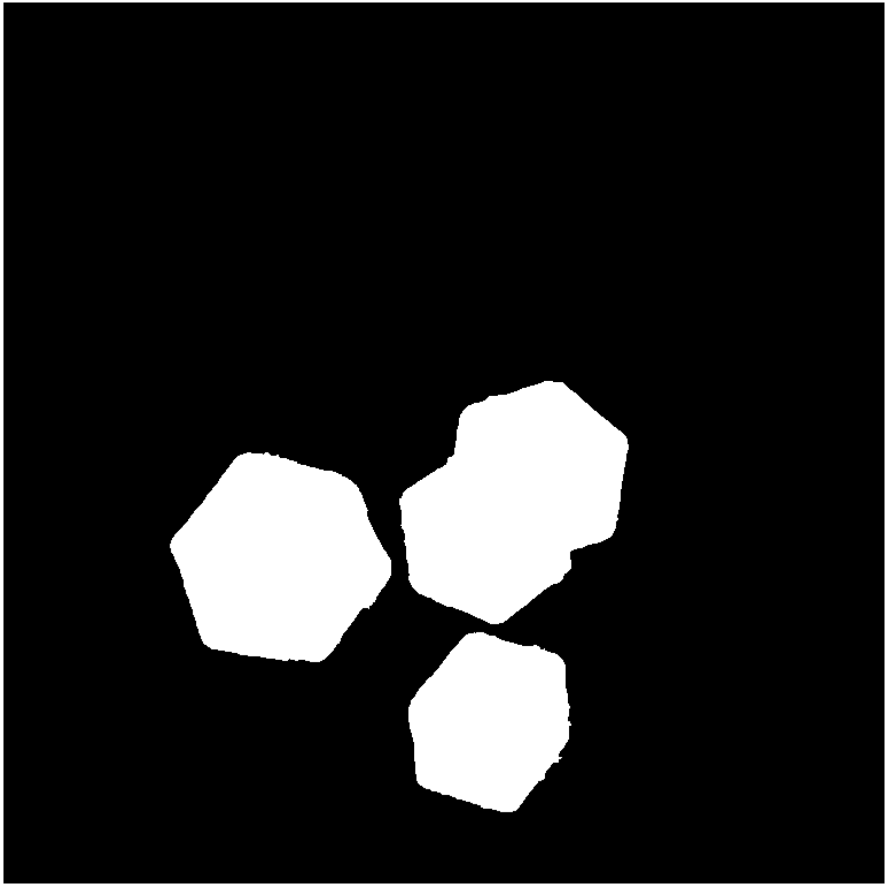
Human insulin crystal image captured by an in-situ microscope

Introduction

Is it possible to measure the size of the human insulin crystals from the captured images?

We believe yes!

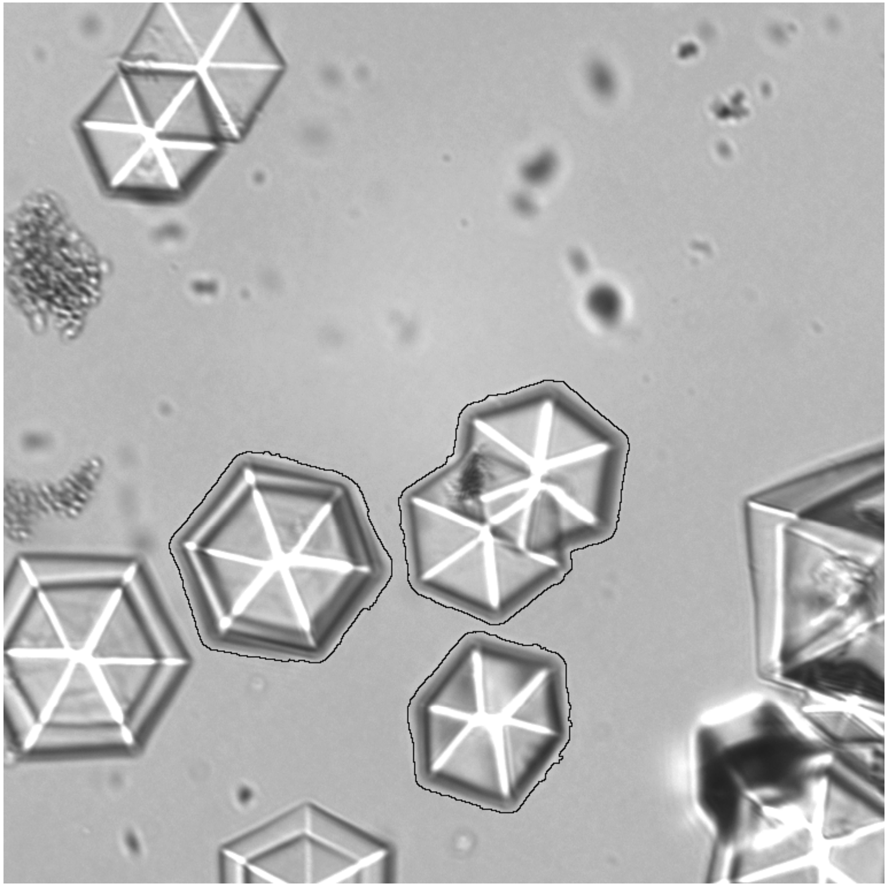
To this end, the first challenge that we have to overcome is to develop an algorithm able to automatically segment the captured images into background and foreground regions



Foreground regions

Problem

But, how can the captured images be segmented into background and foreground regions?



Approach

- Pixel classification:

  The image pixels are classified into two classes: the class of pixels belonging to the foreground regions and the class of pixels belonging to the background region

- Classification rule:

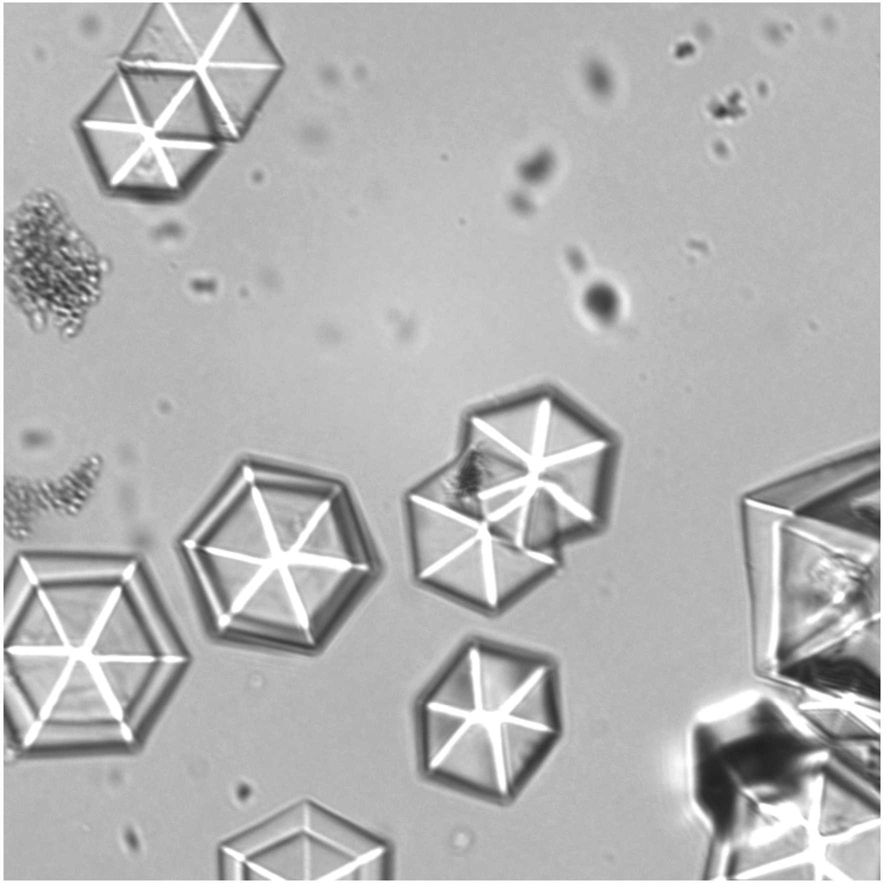
  Pixels whose local intensity variance is less than a threshold are classified as belonging to the background regions, otherwise they are classified as belonging to the foreground regions

- Threshold:

  Linear combination of the first and second order statistical characteristics of the local intensity variance values at the pixels in the background region

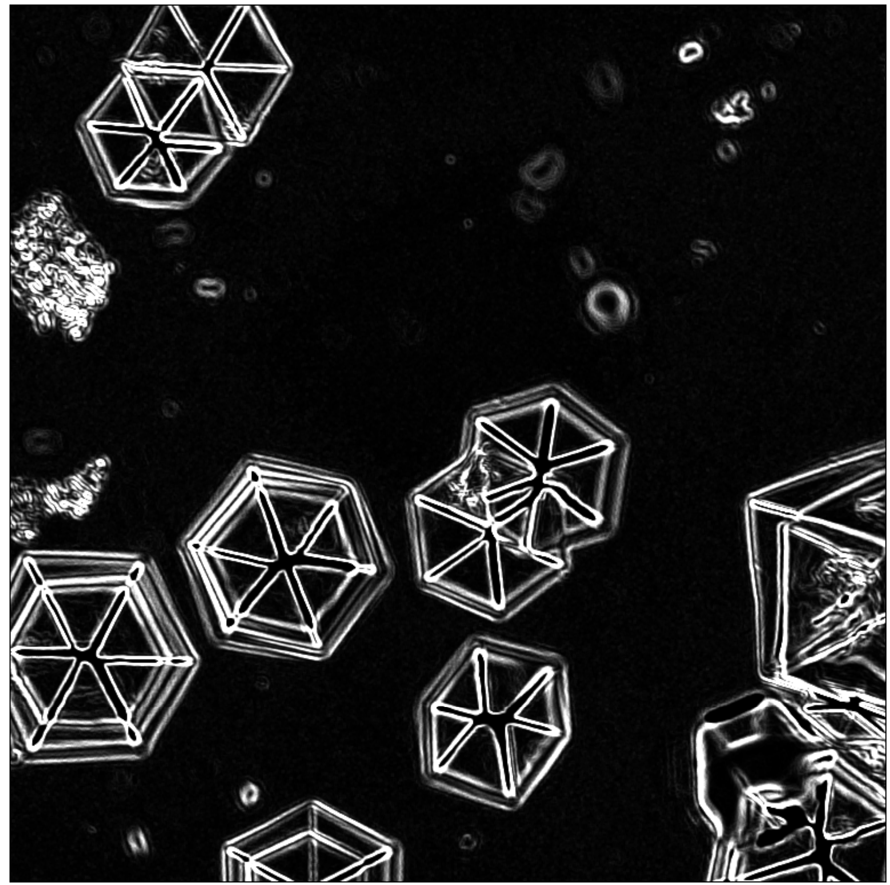
Segmentation Algorithm

1) Capture an intensity image I



Segmentation Algorithm

2) Estimate the local intensity variance at each pixel position

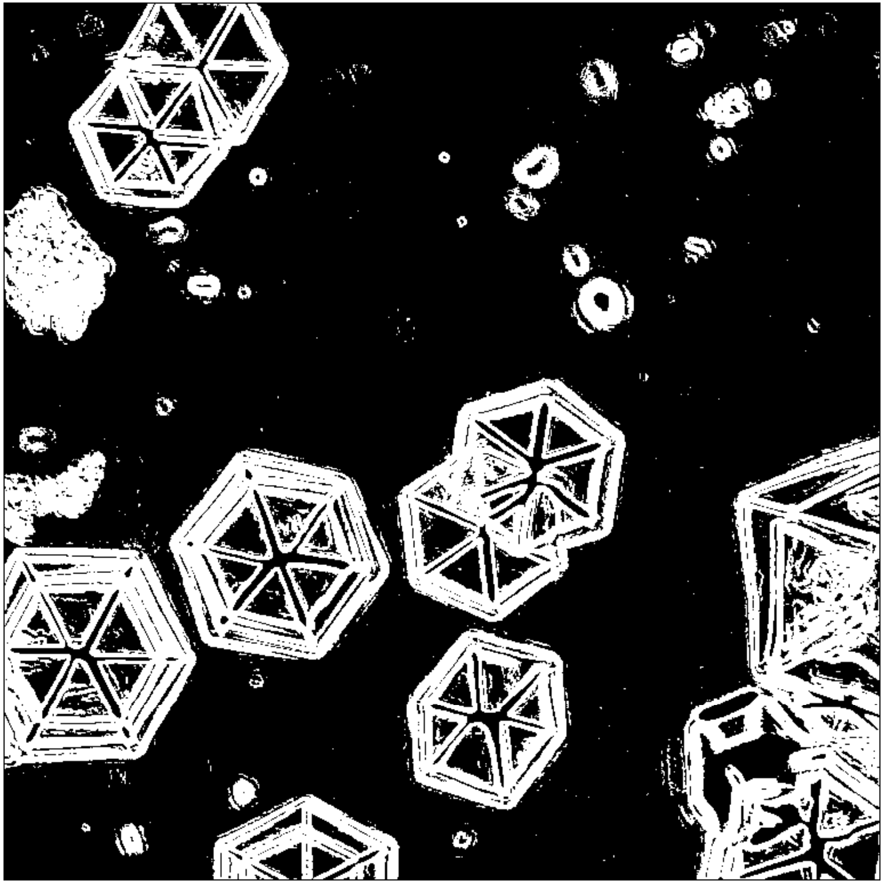


Variance image V

Segmentation Algorithm

3) Classify all the pixels of the variance image V into pixels of the crystal regions and pixels of the background by using the global threshold th_g

$V_i \leq th_g$: background (black)
$V_i > th_g$: foreground (white)



Segmentation Algorithm

$$th_g = m_1 + 4\sigma_1$$

σ_1 : standard deviation of the variance values at the background
m_1 : mean of the variance values at the background

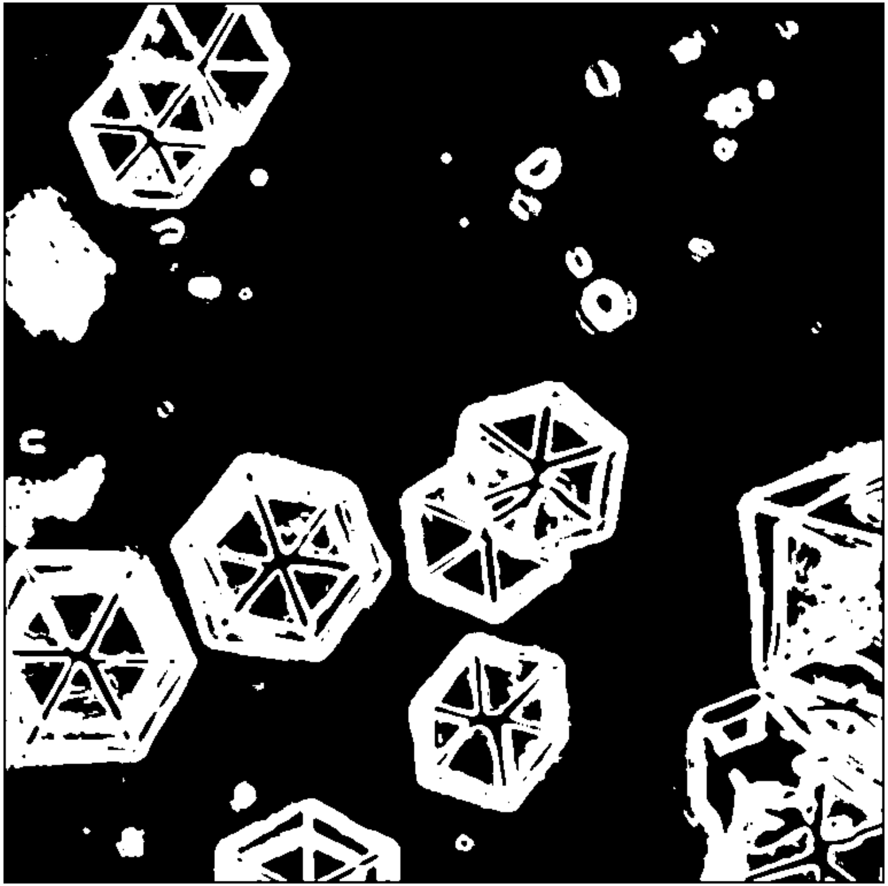
Those statistical characteristics are estimated, along with the mean and the standard deviation of the local variance values at the pixels in the foreground regions, by maximizing a likelihood function

The likelihood function is computed by modeling the probability density function of all local variance values with a population mixture model consisting of the sum of two weighted Gaussian probability densities functions given the classes to which each pixels belongs to

For maximization an Expectation Maximization (EM) algorithm is applied

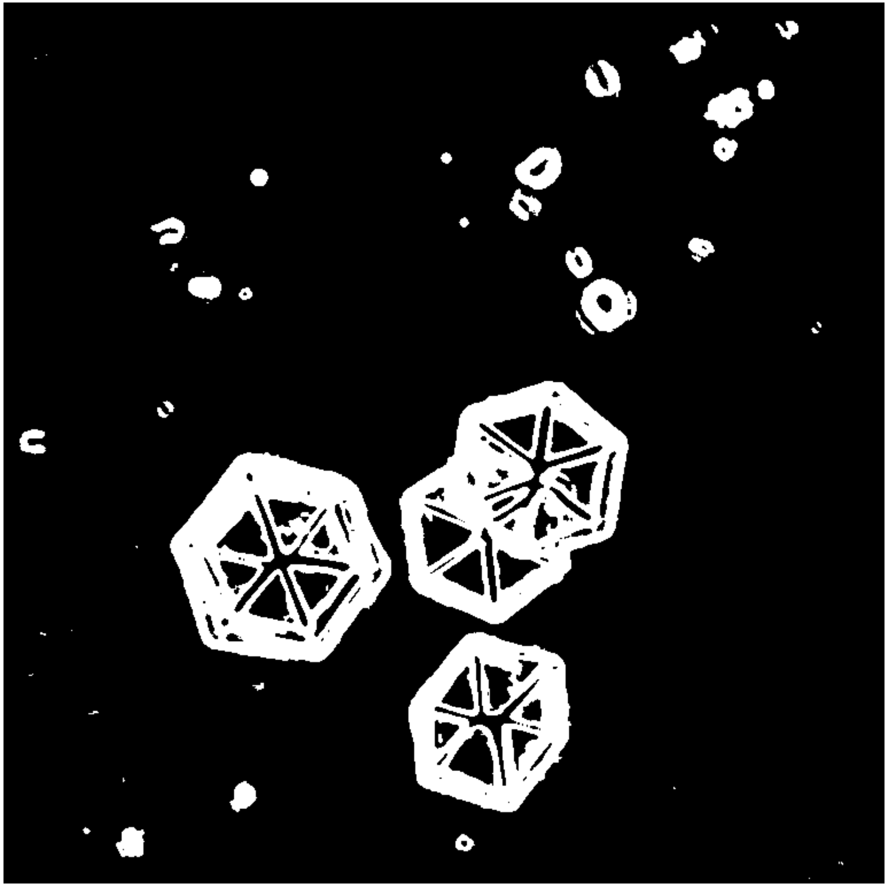
Segmentation Algorithm

4) Eliminate isolated white pixels by applying a 5x5 median filter



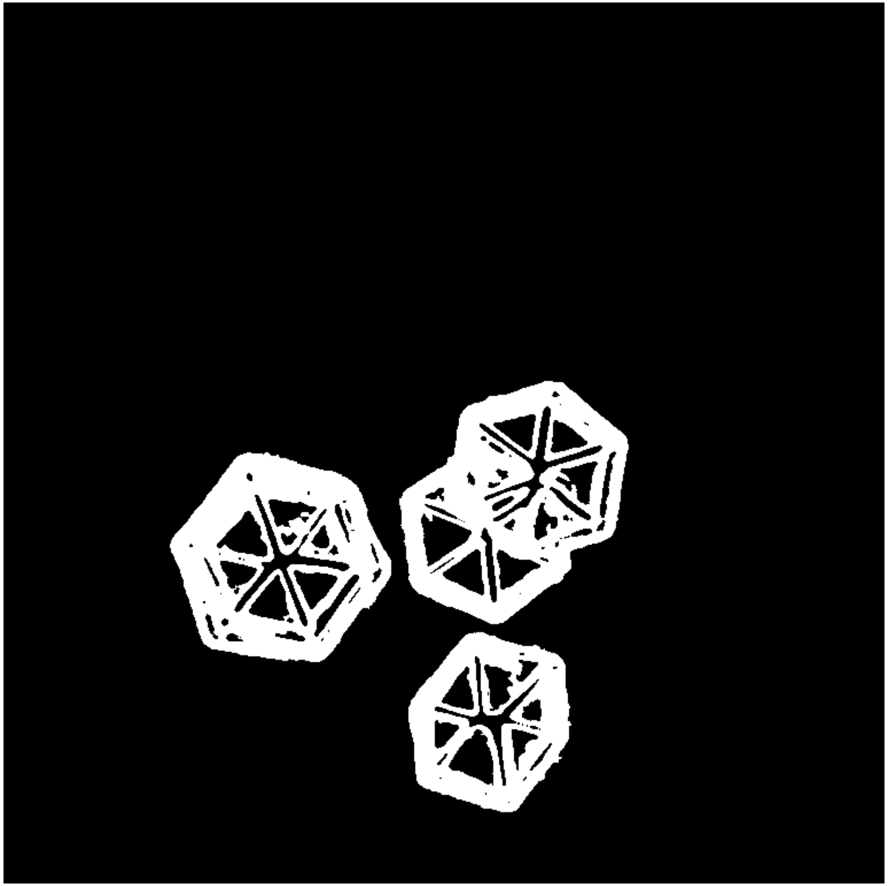
Segmentation Algorithm

5) Eliminate any white region touching any image border



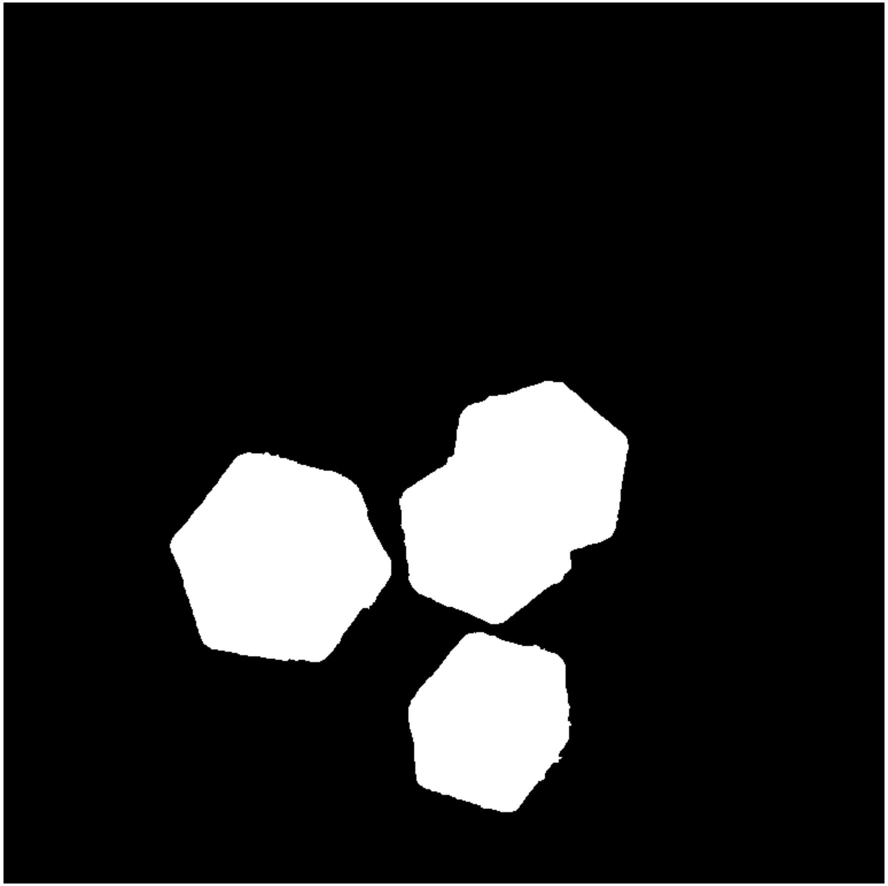
Segmentation Algorithm

6) Eliminate white regions whose image area is less than 0.09% of the total image area



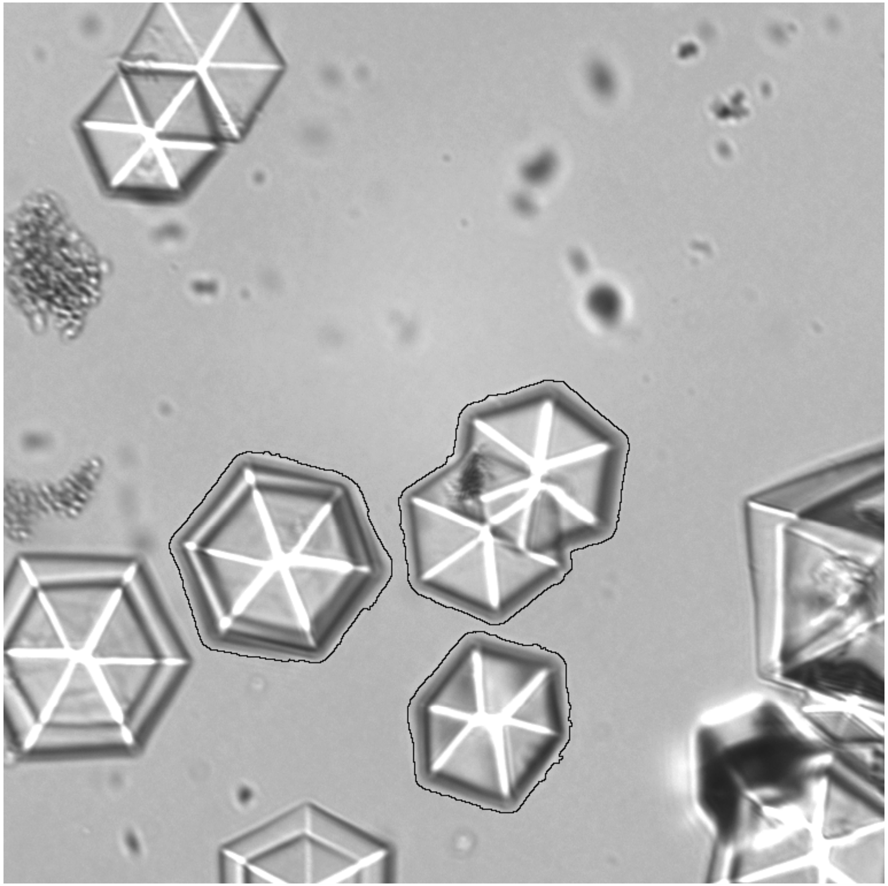
Segmentation Algorithm

7) Eliminate black holes inside white regions

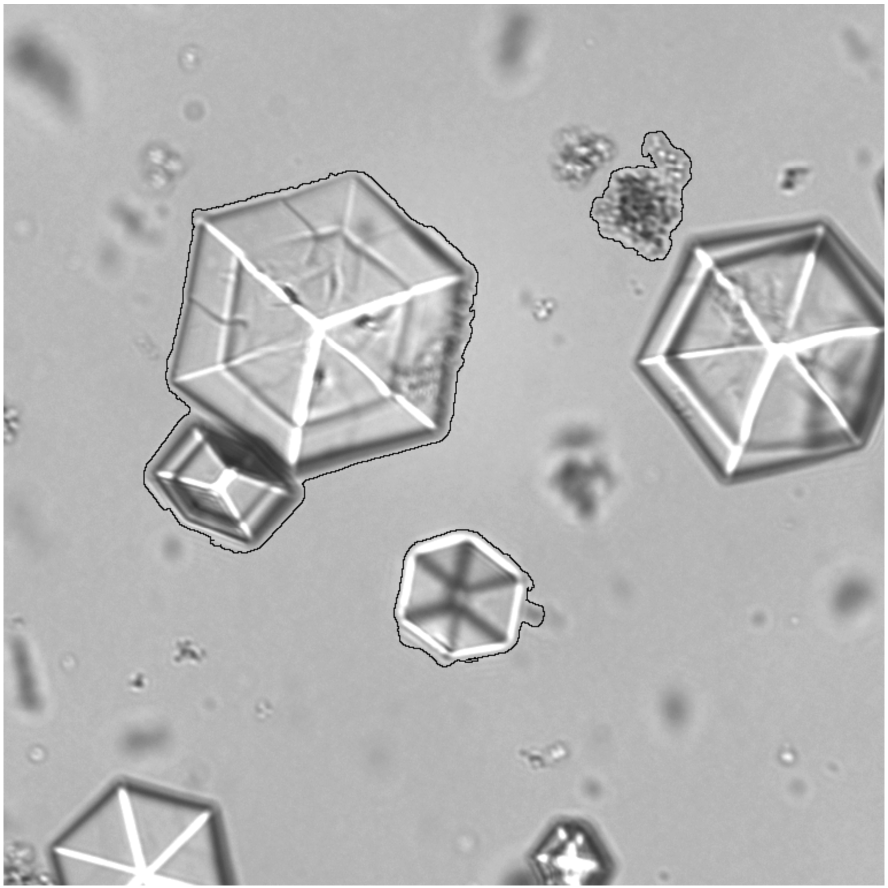


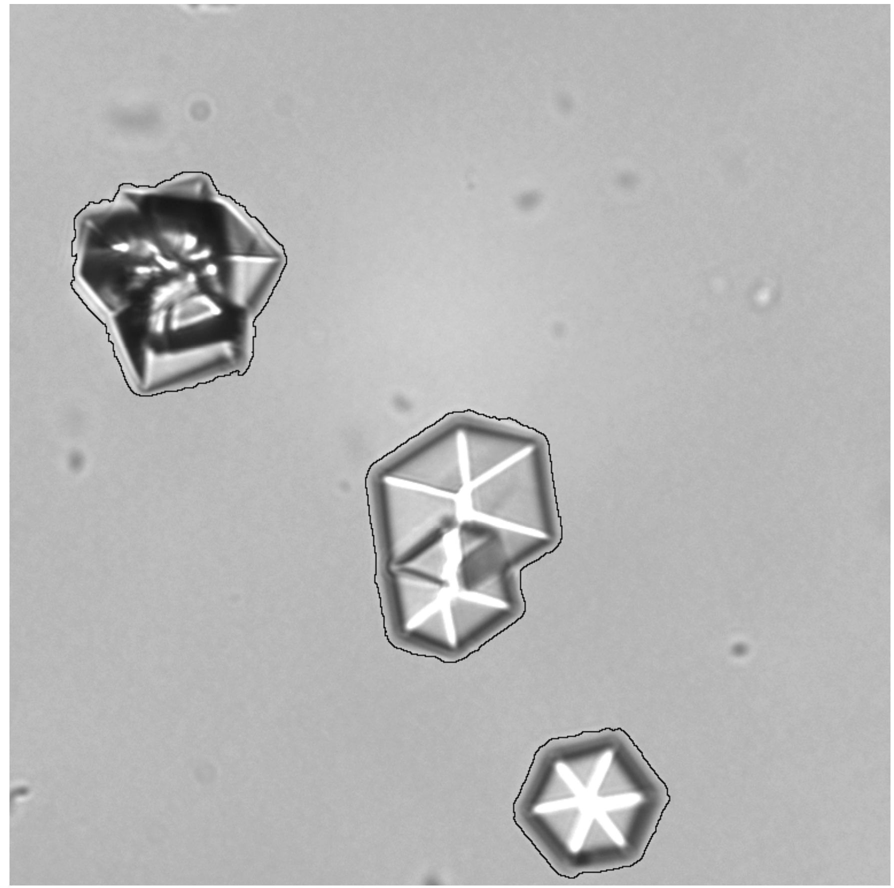
Experimental Results

- Average processing time: 12 sec/image
- High reliability
- Segmentation error of approximately 14 pixels
- Contours of the segmented foreground regions overlapped with the original image I

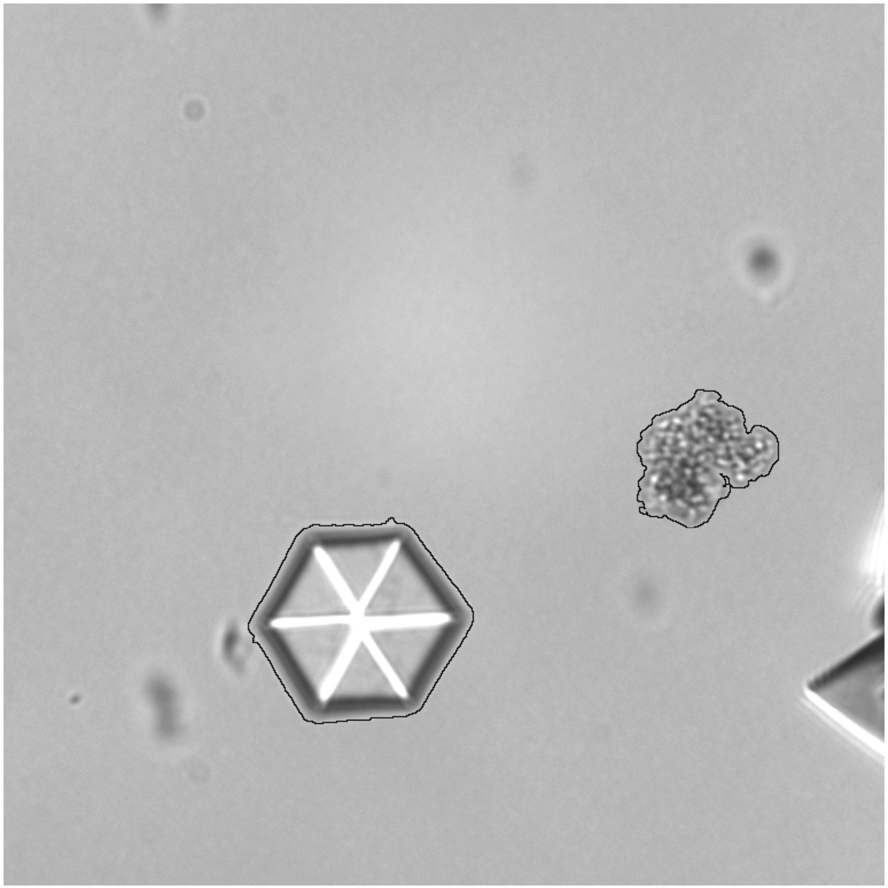


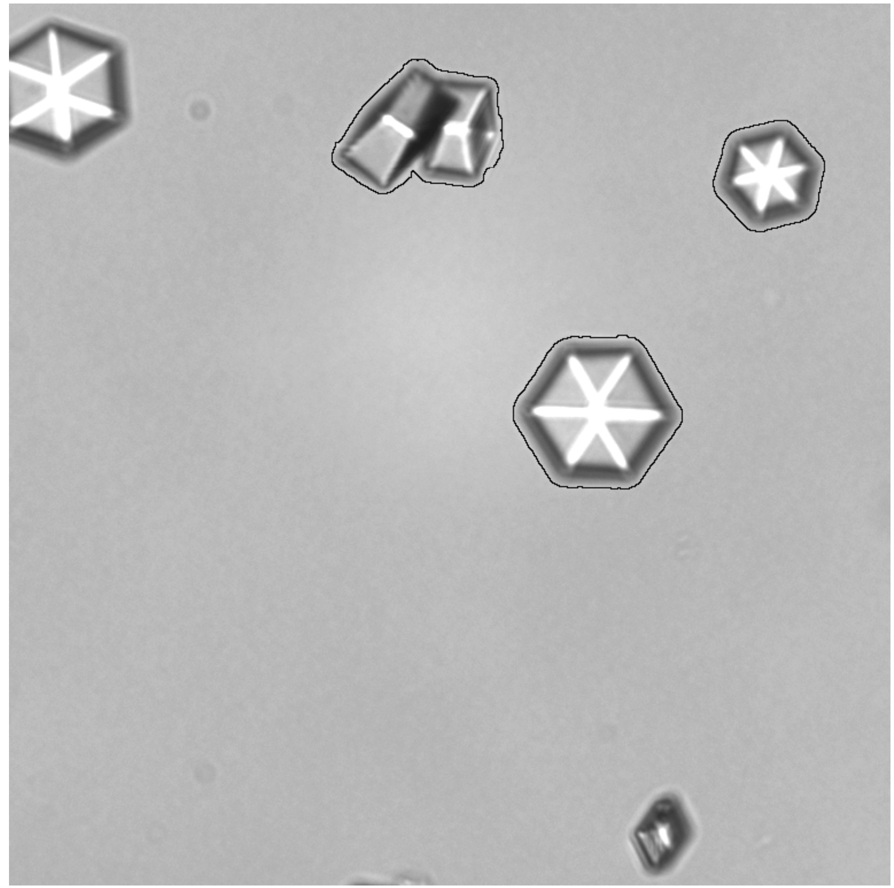
Experimental Results





Experimental Results





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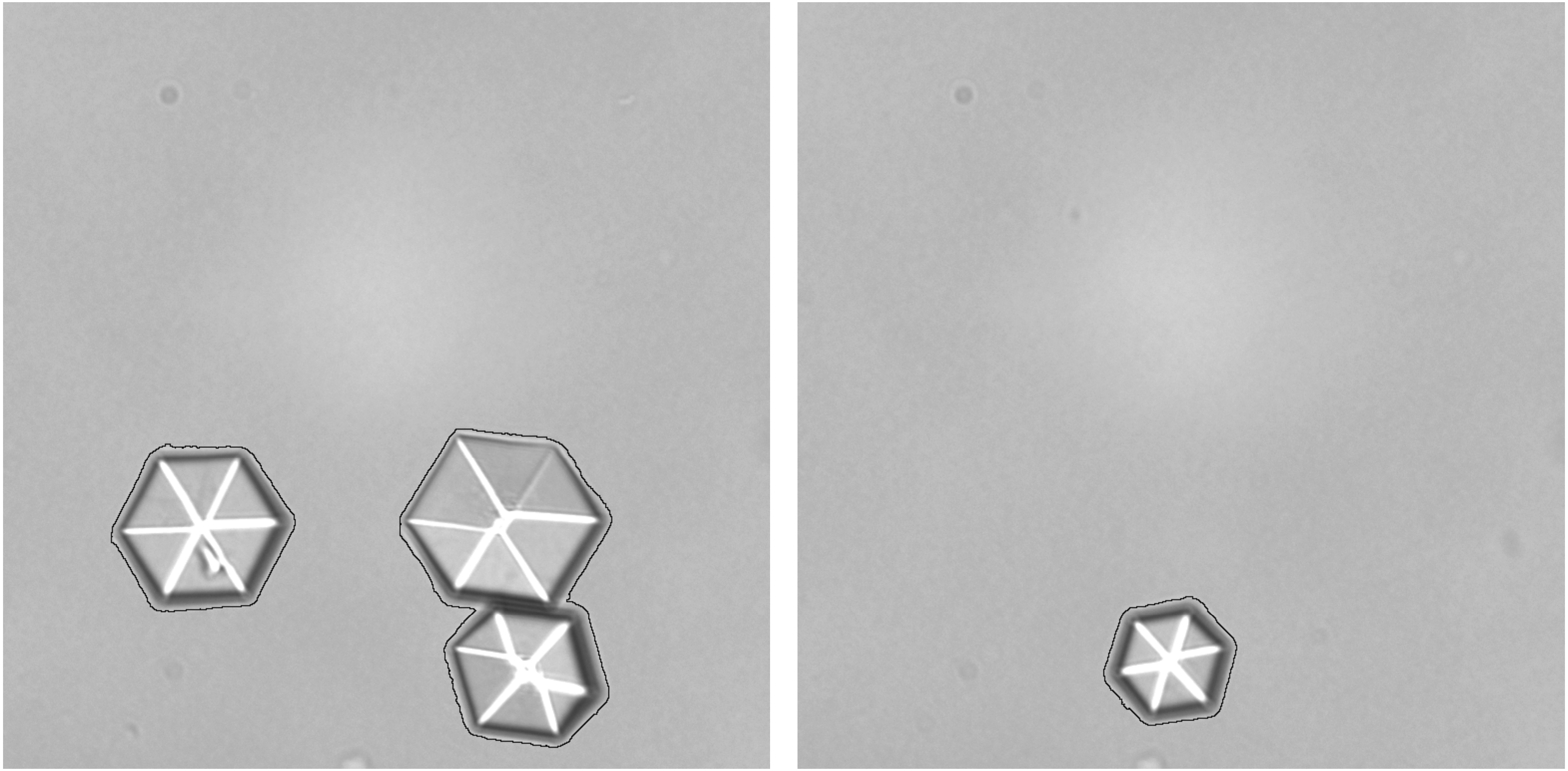
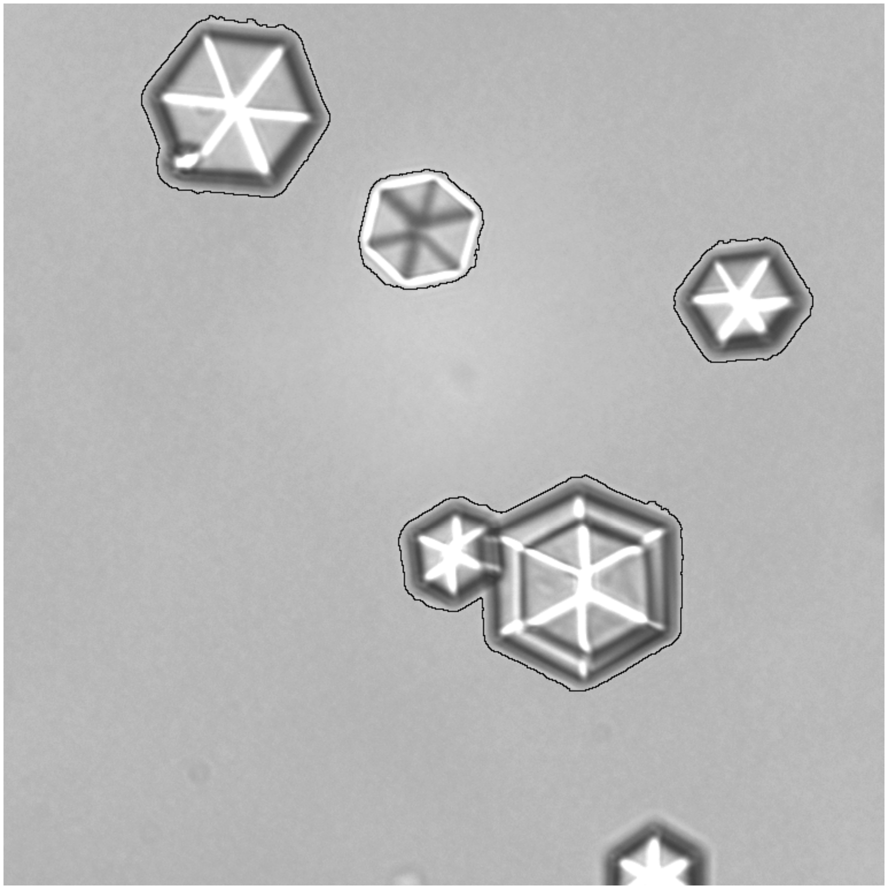
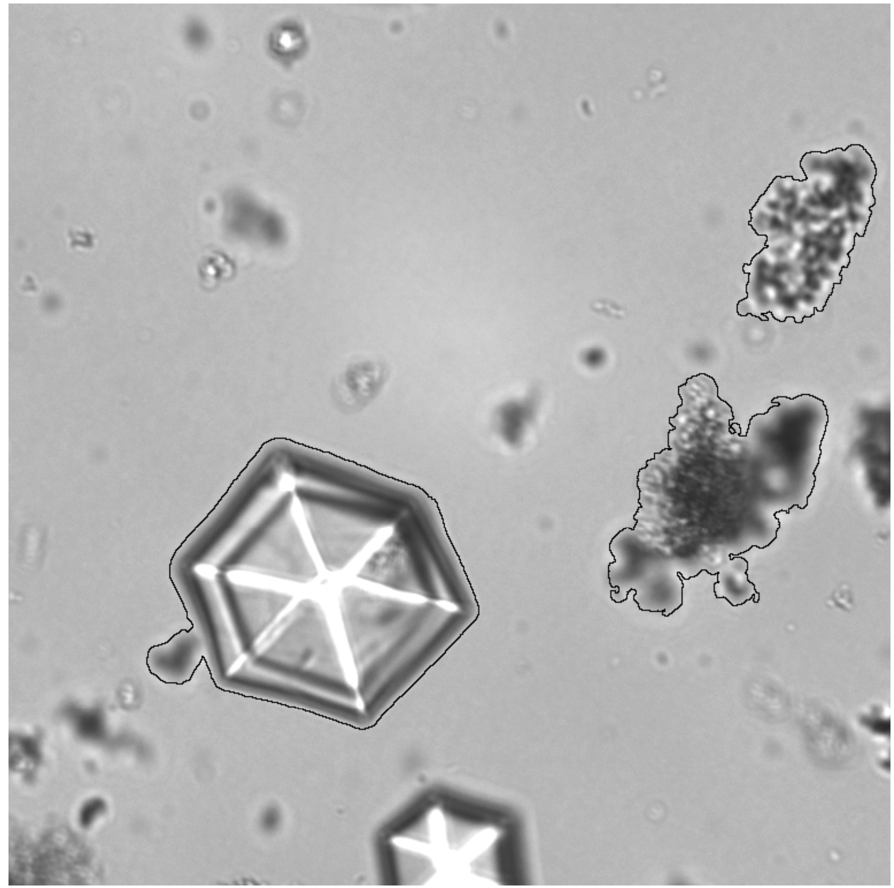


Experimental Results





Summary

- For foreground segmentation a thresholding technique is applied
- The optimal threshold was found heuristically to be the mean plus four times the standard deviation of the local intensity variance values of the pixels at the background region
- The misclassifications are corrected by particle filtering assuming that the foreground regions are bigger than 0.09% of the total image and that they do not contain holes
- Experimental results revealed an average processing time of 12 sec/image, an excellent reliability and a segmentation error of approximately 14 pixels