Algorithm to Extract the Shortest Linear Edge and the Longest Diagonal of Single Isolated Human Insulin Crystals for In-Situ Microscopy

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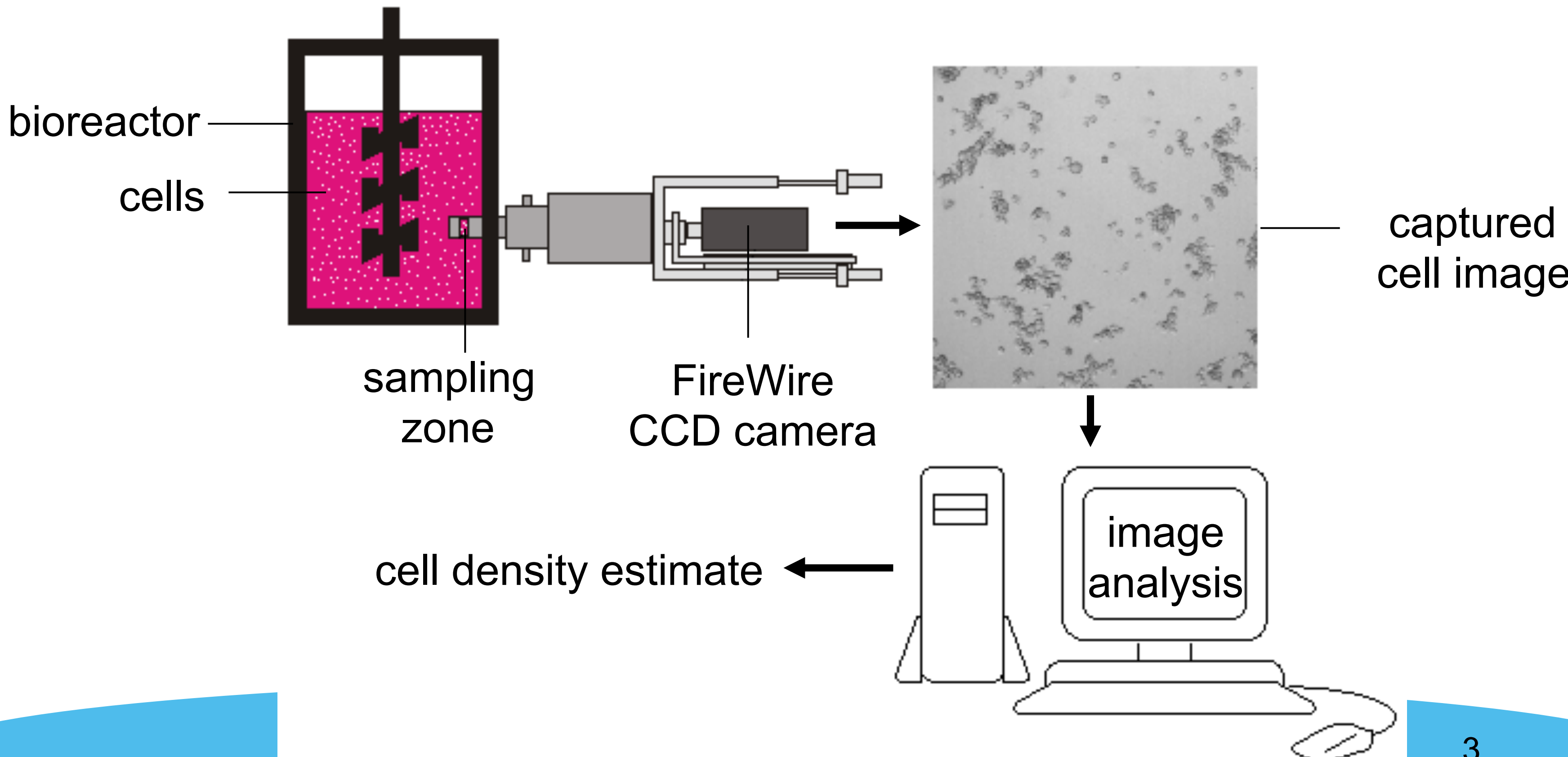
Overview

- Introduction
- Algorithm
- Experimental results
- Summary

Introduction

In-situ microscope

On-line automatic cell density estimation with no risk of culture contamination

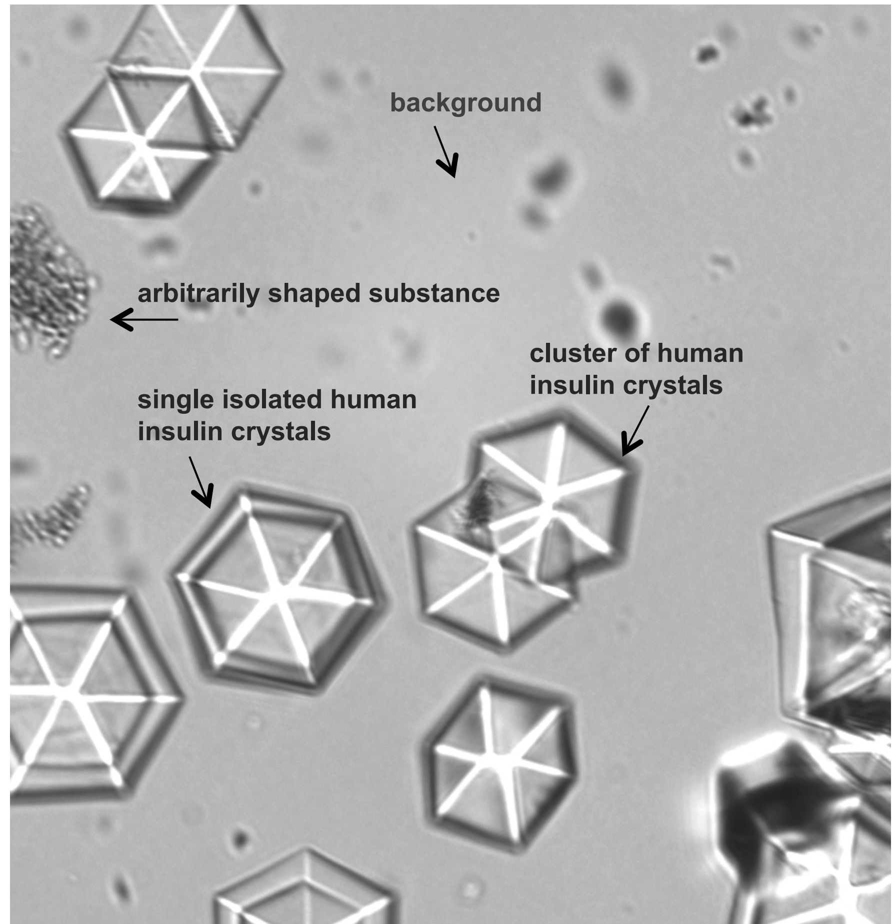


Introduction

Use of the in-situ microscope for on-line monitoring of human insulin crystallization processes

Images of first experiments show:

- Homogeneous background
- 4 different classes of foreground regions C_n, n=0,…3: single crystals (C_0), crystal clusters (C_1), amorphous substance (C_2) and mixed regions (C_3)



Human insulin crystal image

Introduction

Problem statement:

Knowledge of the crystal size distribution at any time during a crystallization process is very useful to control the crystal growth, which in turn is very important, for example, to maximize the effectiveness of the insulin diabetes treatment

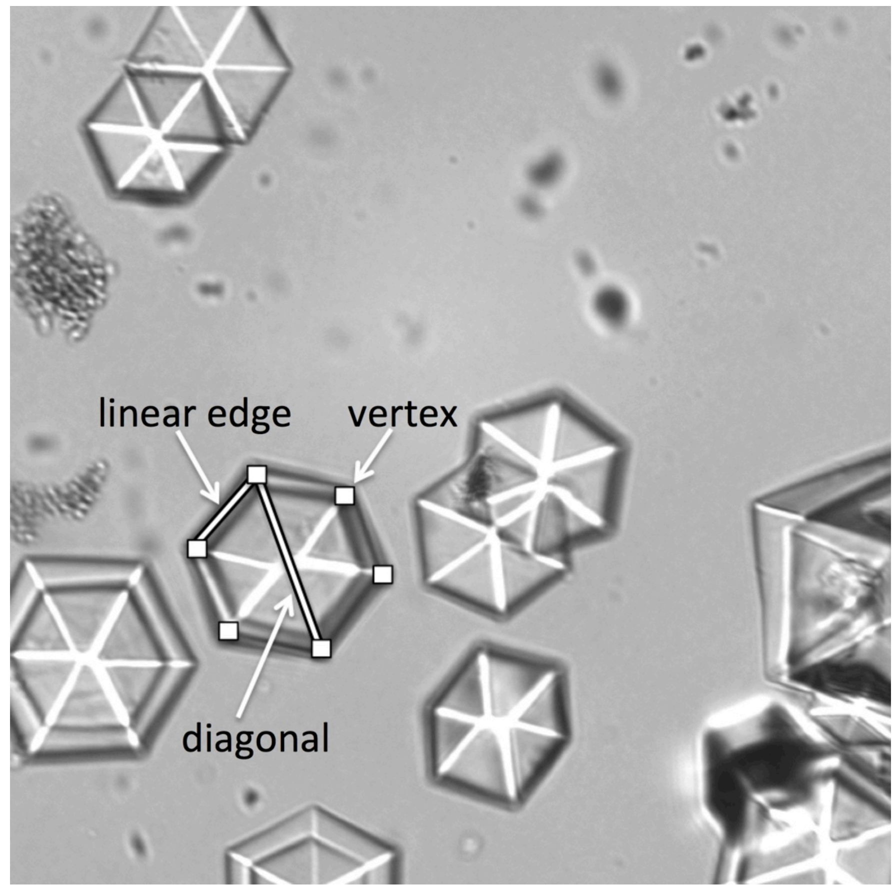
Challenge:

Development of an algorithm to measure the crystal size distribution from the images capture by an in-situ microscope

Introduction

Approach

1. Development of an algorithm to measure the shortest linear edge and the longest diagonal of the single crystals
2. Use the developed algorithm to monitor these lengths during the crystallization process
3. Inference of the crystal size distribution from the length readings

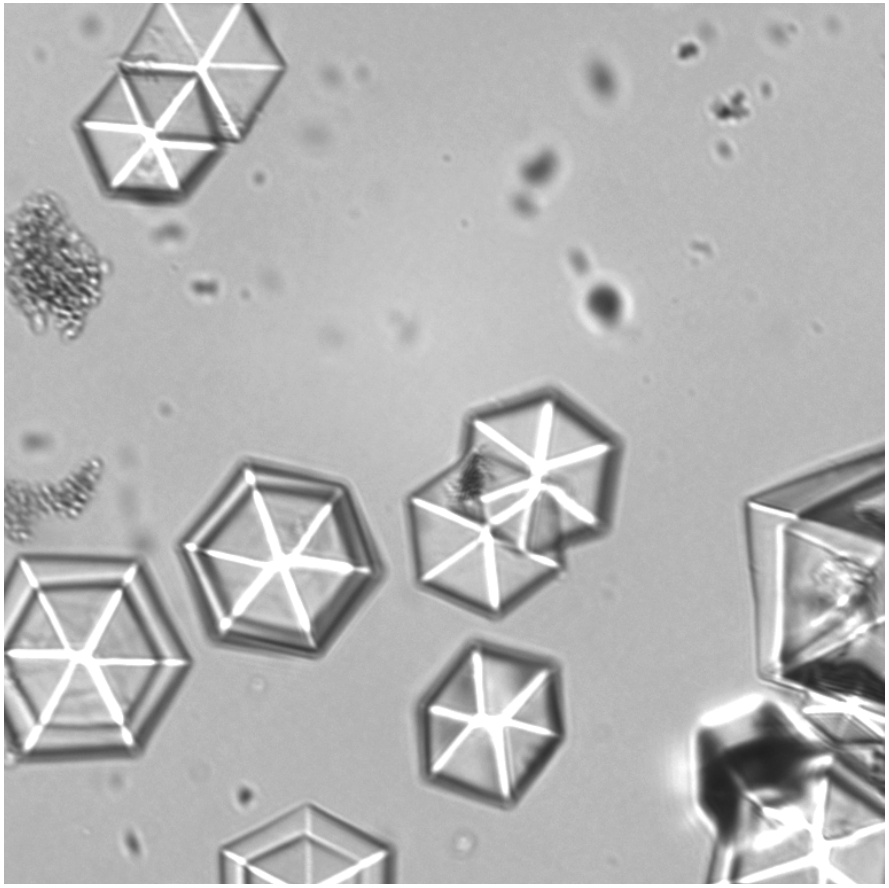


Here the algorithm to measure the above mention lengths is presented only (it consists of seven modules)

Algorithm/1st Module

Foreground segmentation

1. Capture an intensity image I

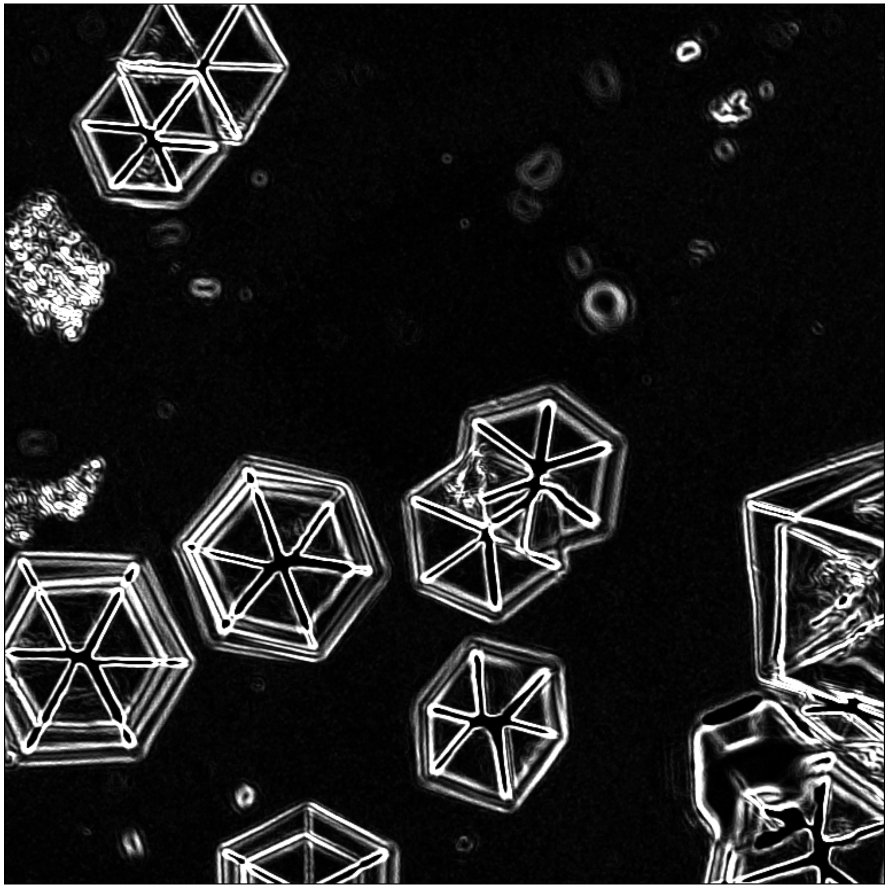


Intensity image I

Algorithm/1st Module

Foreground segmentation

2) Estimate the local intensity variance at each pixel position



Variance image V

Algorithm/1st Module

Foreground segmentation

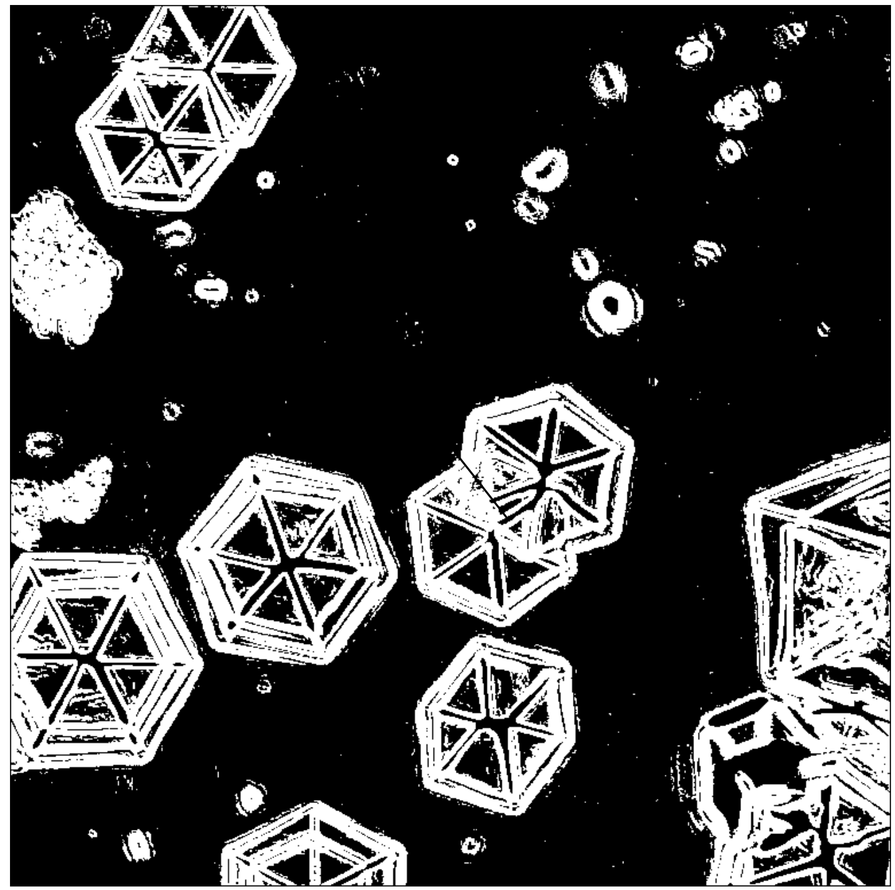
3) Threshold the variance image V

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

where $th = m_1 + 4\sigma_1$

σ_1: standard deviation of the variance values at the background

m_1: mean of the variance values at the background

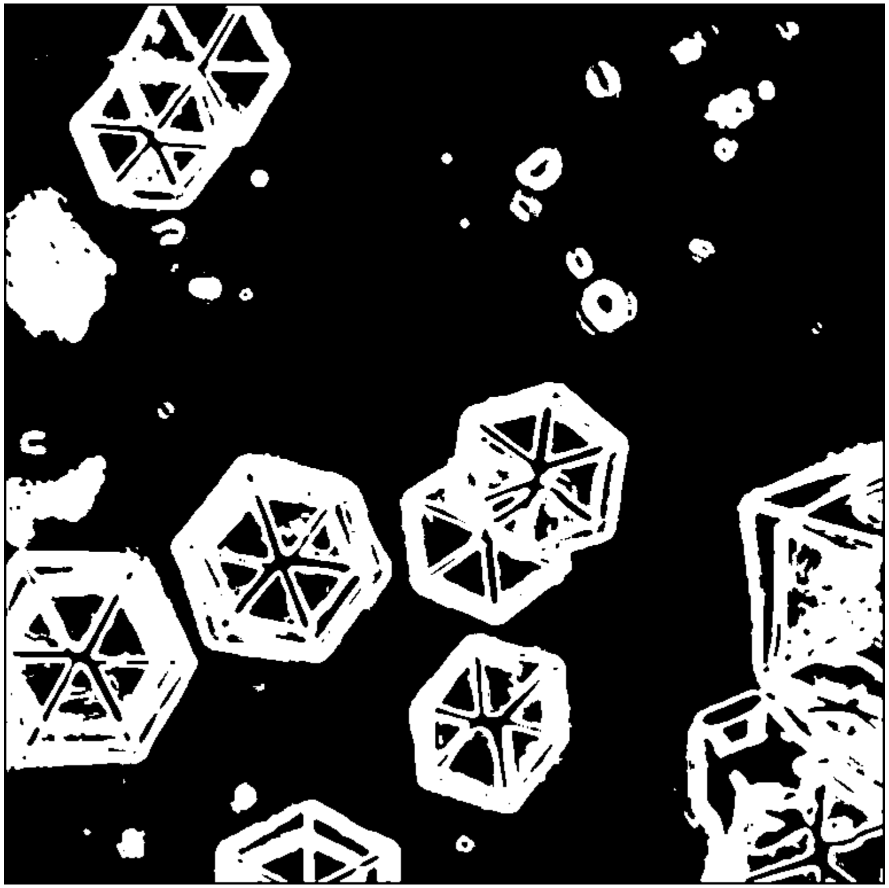


Thresholded image V_{th}

Algorithm/1st Module

Foreground segmentation

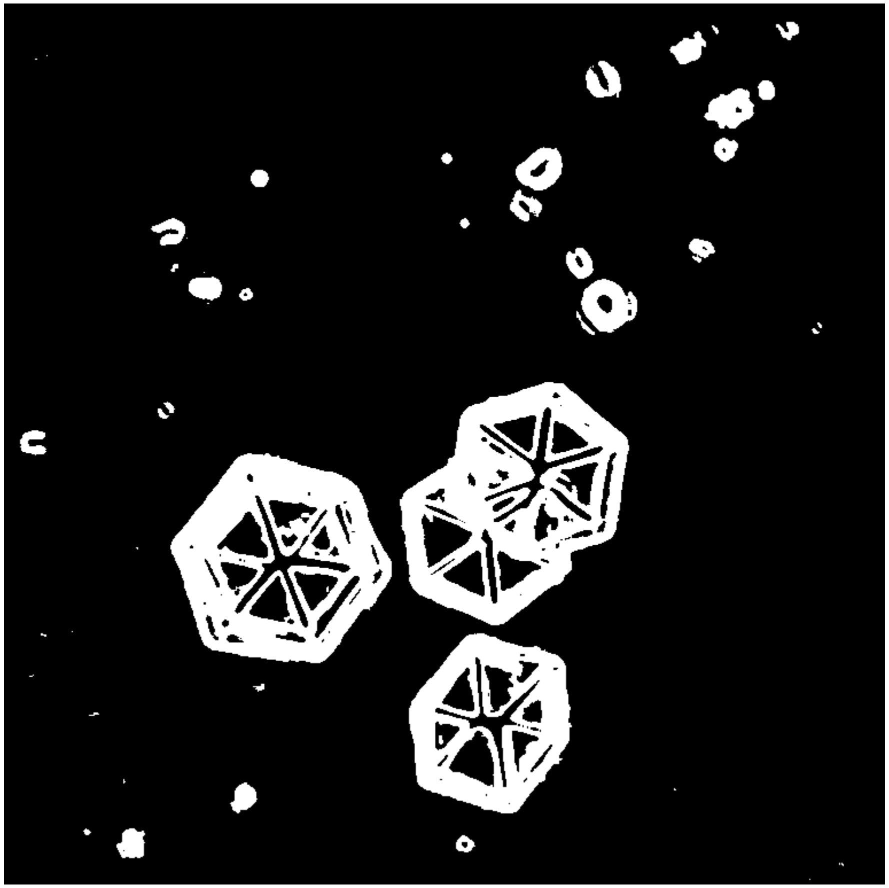
4) Eliminate isolated white pixels by applying a 5x5 median filter



Algorithm/1st Module

Foreground segmentation

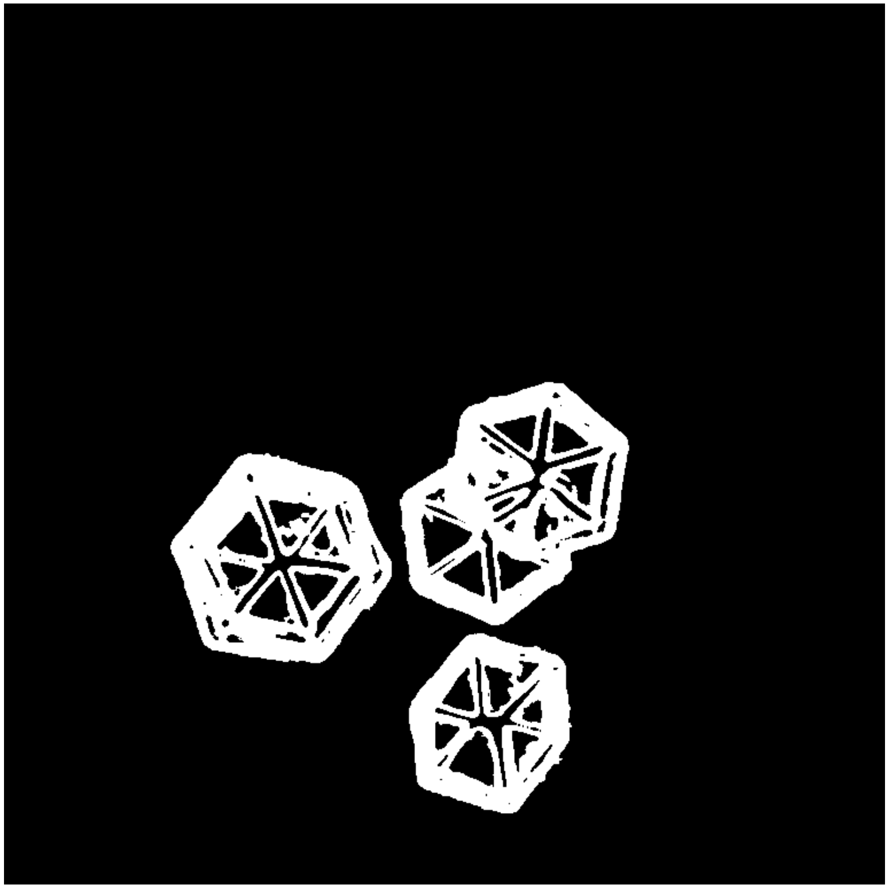
5) Eliminate any white region touching any image border



Algorithm/1st Module

Foreground segmentation

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

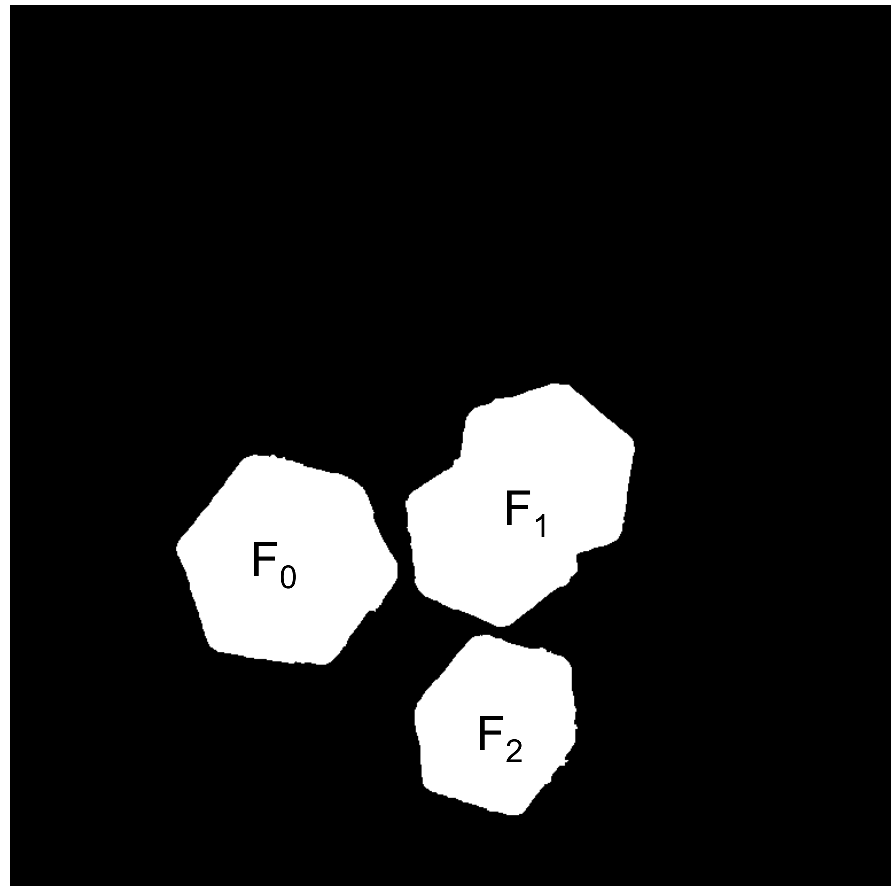


Algorithm/1st Module

Foreground segmentation

7) Eliminate black holes inside white regions

   The remaining white regions represent the segmented foreground regions F_s, s=0, …,S-1

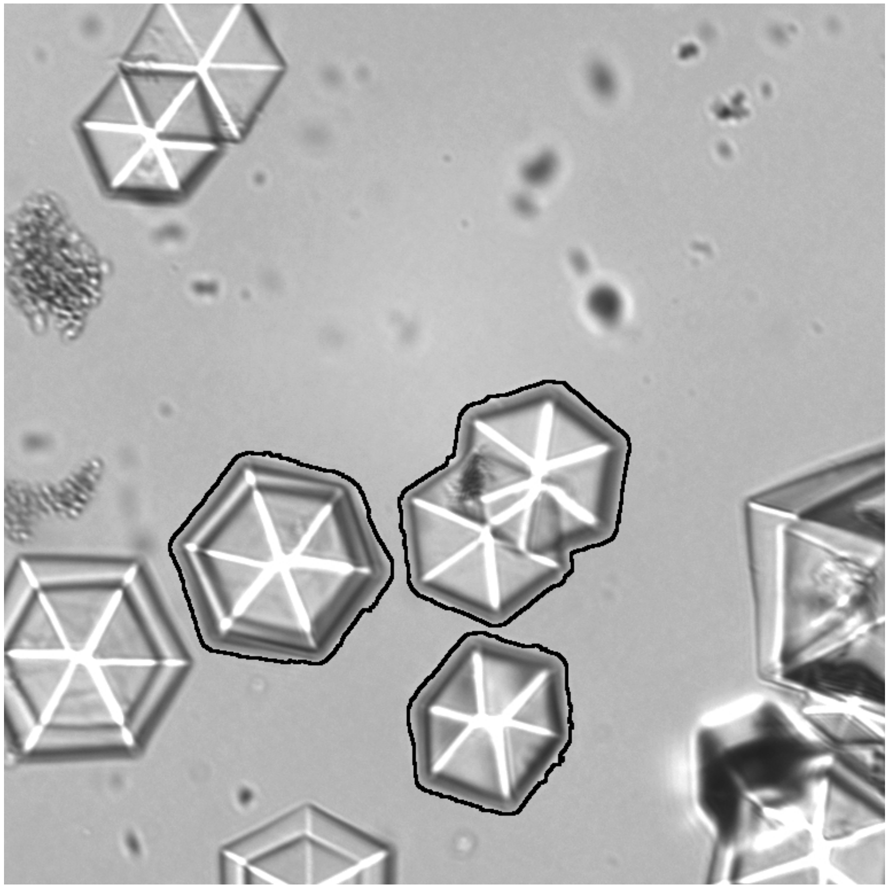


Segmented foreground regions F_s, s=0, 1, 2

Algorithm/2nd Module

Detection of single crystal regions

1) Compute for each segmented foreground region F_s a 7-dimensional vector of rotation, translation and scale invariant shape characteristics $C_s(k)$, k=0, …, 6, according to M.-K. Hu



Algorithm/2nd Module

Detection of single crystal regions

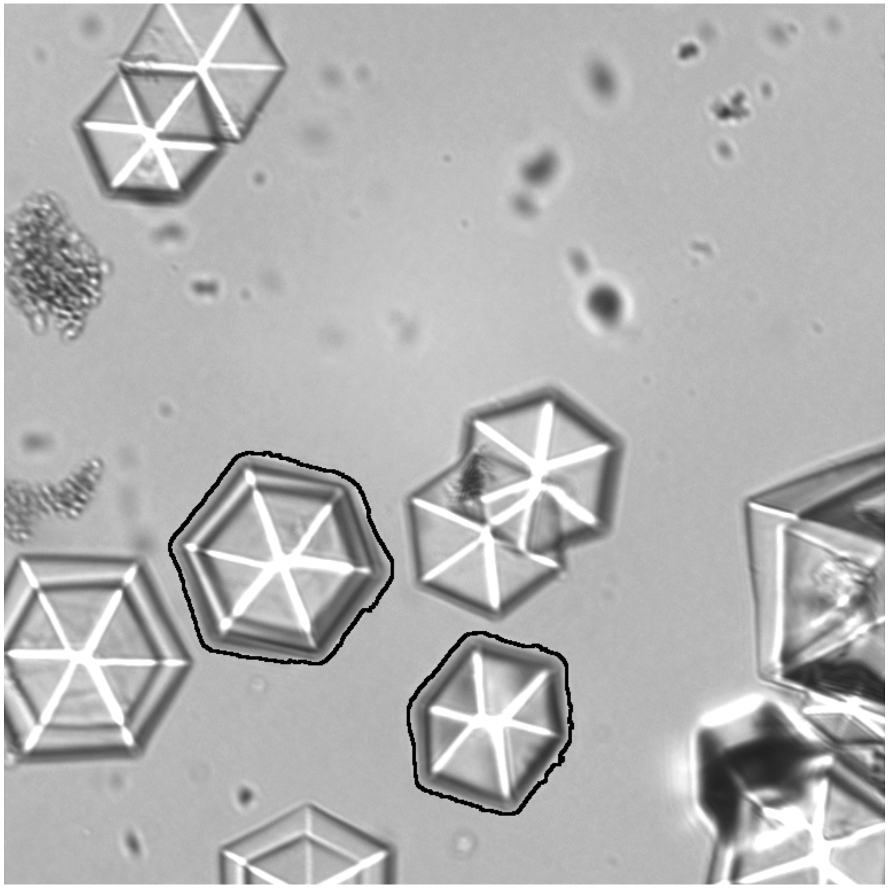
2) Detect an arbitrary segmented foreground region F_s as the region of a single crystal if its shape vector C_s is much closer to the shape vector prototype P_0 of the single crystals than to the shape vector prototypes P_1, P_2 and P_3 of the other classes of foreground regions

$$\left\| C_s - P_0 \right\| \le \left\| C_s - P_n \right\|, \ \forall \ n = 0,..,3$$

where

$$\left\| C_s - P_n \right\| = \sqrt{\sum_{k=0}^{6} \left(P_n(k) - C_s(k) \right)^2}$$

The shape vector prototypes are computed a priori from a training set of images

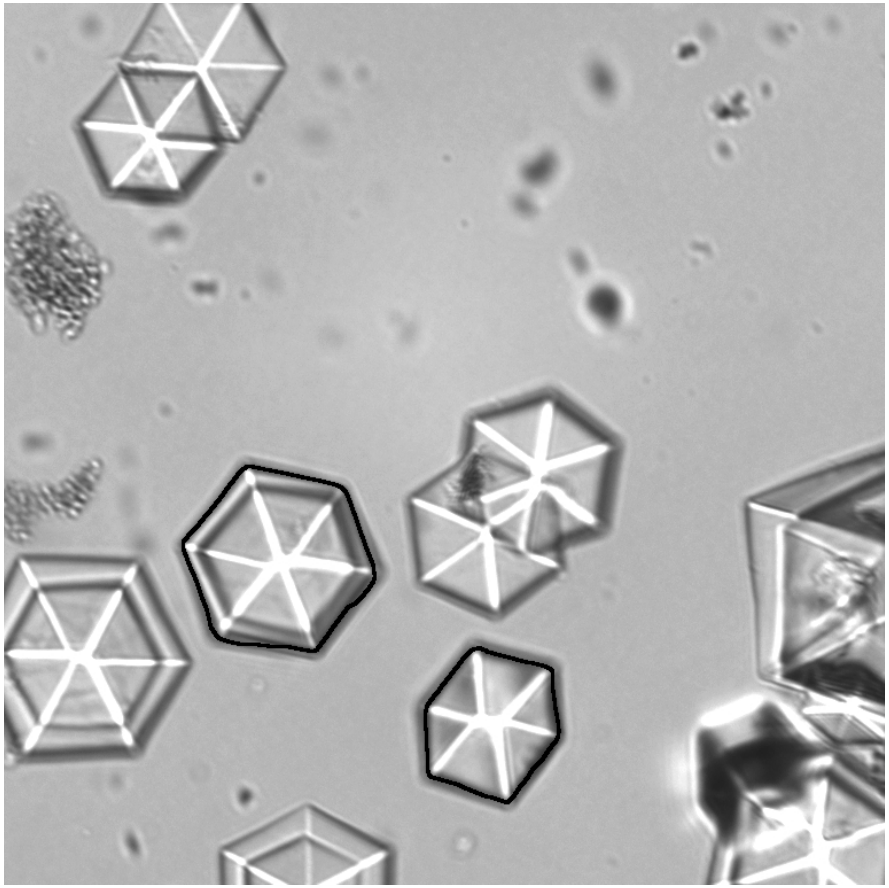


Detected single crystals

Algorithm/3rd Module

Improvement of contour accuracy

1) Move each region contour point along the line segment, which goes from the region contour point to the region center of gravity, to that image position α, where the weighted sum of the intensity value and the values of the first and the second region contour derivatives becomes minimal



Detected single crystals with improved contour accuracy

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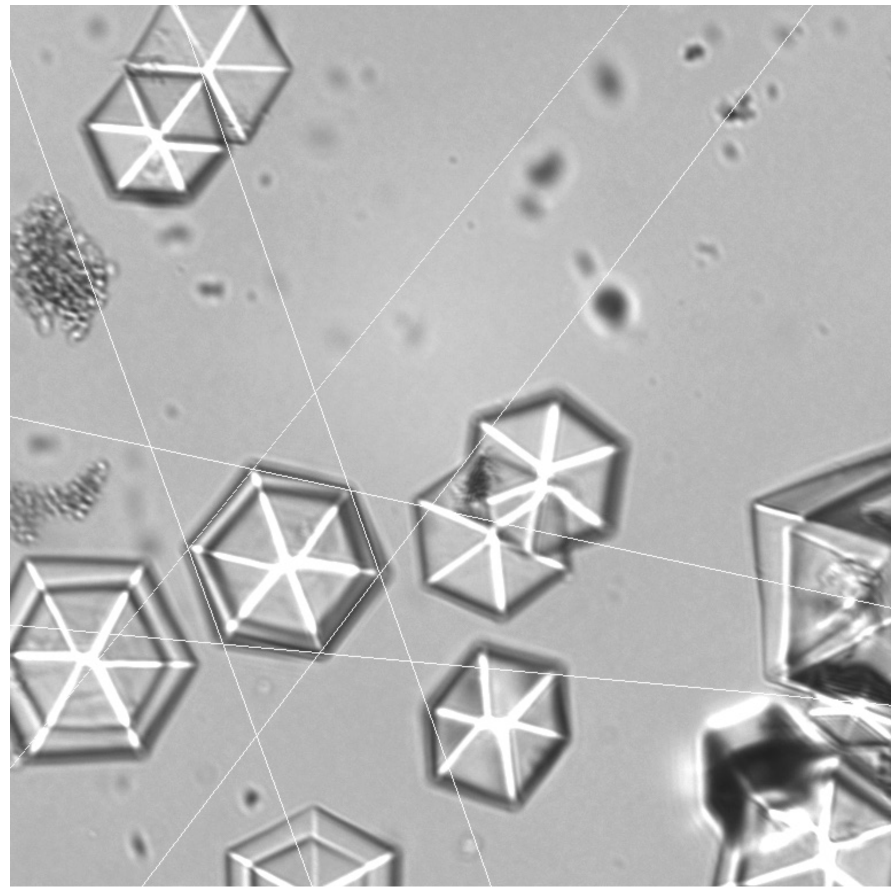
Algorithm/4th Module

Extraction of crystal lines

1) Extract the 6 lines that go through the 6 linear edges of each segmented single isolated crystal region by applying the Hough transformation to the region contour



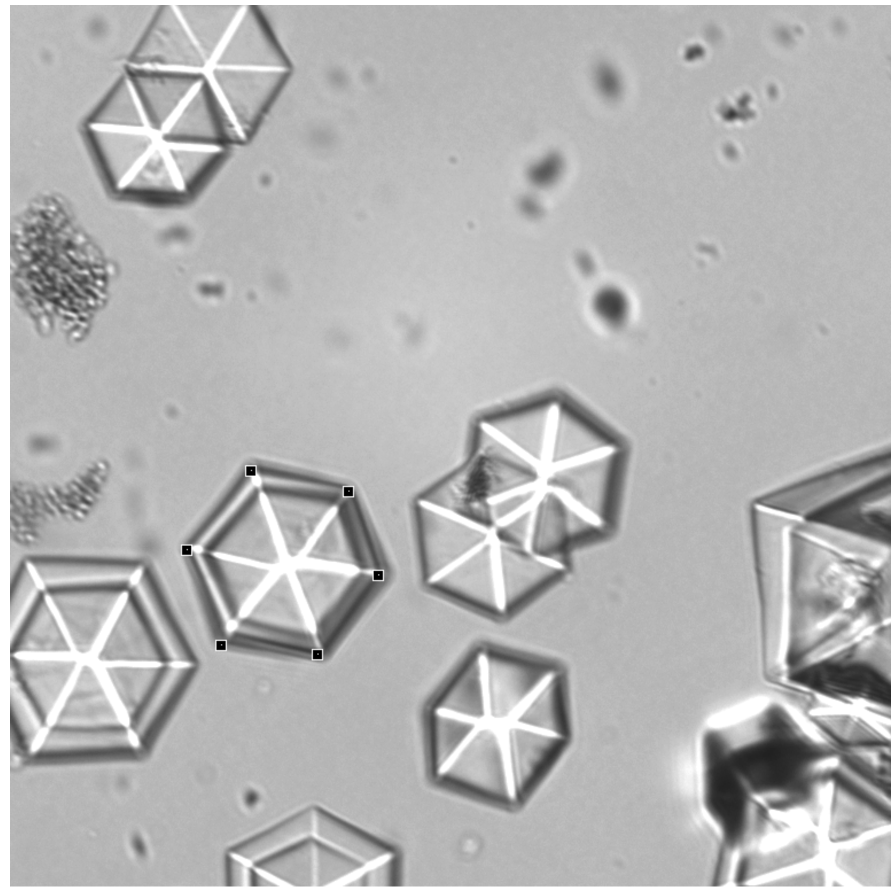
Extracted 6 lines (in white)

Algorithm/5th Module

Extraction of crystal vertices

1) Compute all the intersection points between all previously found 6 lines for each segmented single isolated crystal

2) Select those intersection points which lie inside the segmented single isolated crystal region as the 6 crystal vertices

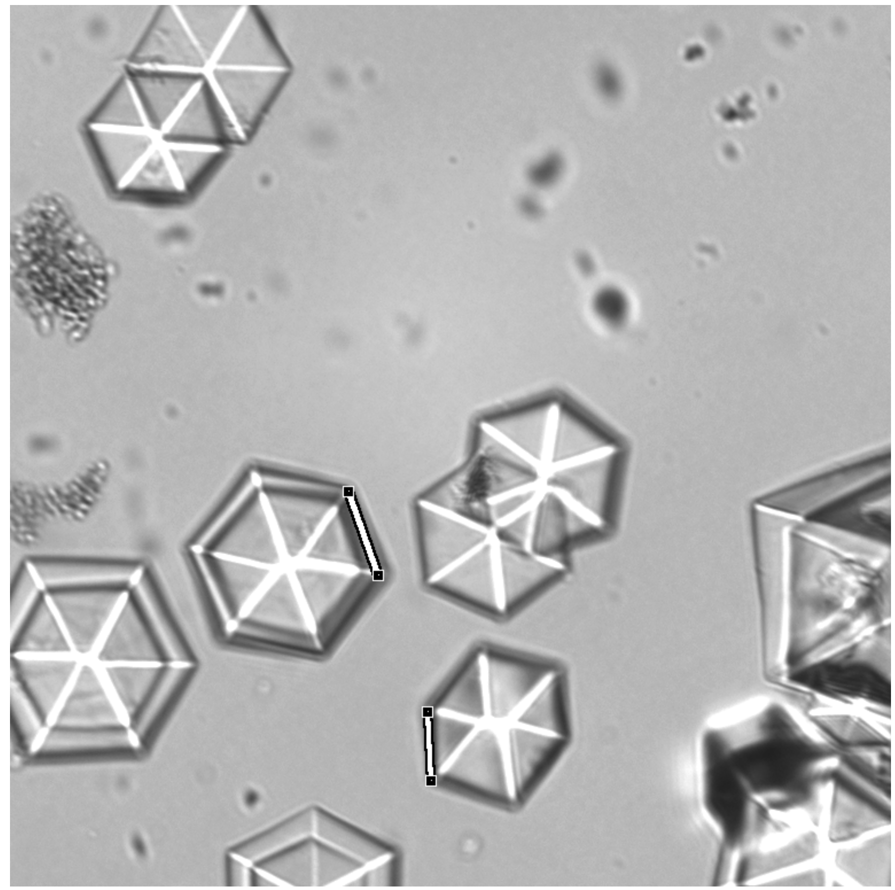


Found crystal vertices (in black)

Algorithm/6th Module

Finding the shortest linear edge

1) Find the shortest crystal linear edge of each segmented single isolated crystal region as the segment between the found vertex positions whose length represents the minimal relative distance between all found vertices

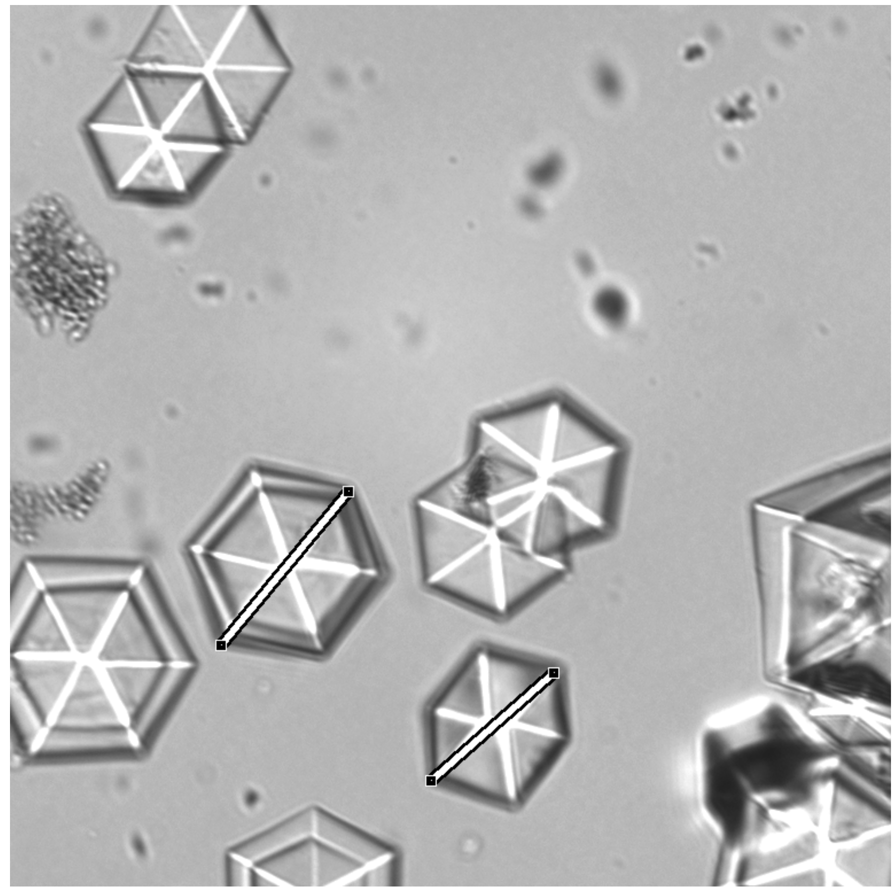


Found shortest crystal linear edges (in white)

Algorithm/7th Module

Finding the longest crystal diagonal

1) Select as the longest crystal diagonal of each segmented single isolated crystal region that line segment between the found vertex positions with maximal relative distance between all found vertices

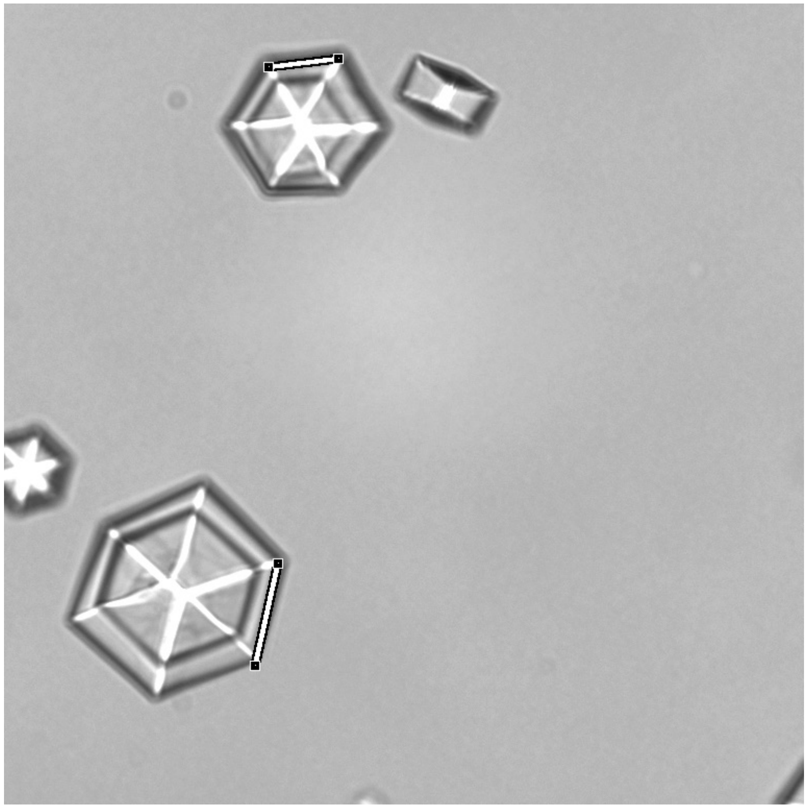


Found longest diagonals (in white)

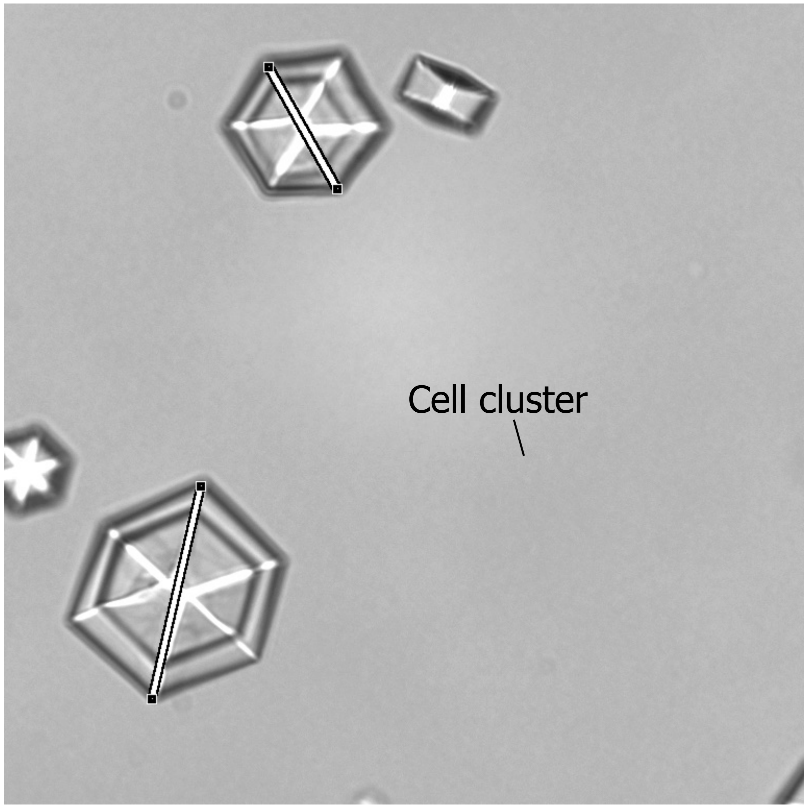
Experimental Results

- Implemented in C under Windows XP
- Tested with 60 real images
- Intel Core Duo CPU at 2.2 GHz and 2 GB RAM
- Average processing time of 1 sec/image
- Absolut length error in the extracted shortest linear edges was in average 4.18%
- Absolut length error in the extracted longest diagonals was in average 2.32%

Experimental Results

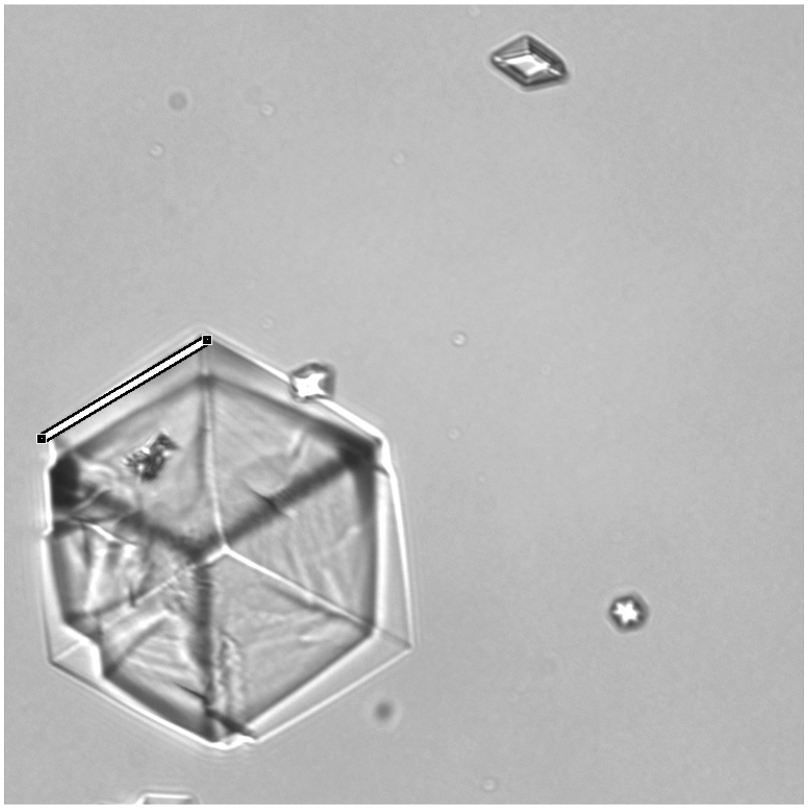


Extracted shortest linear edge
for test image 35

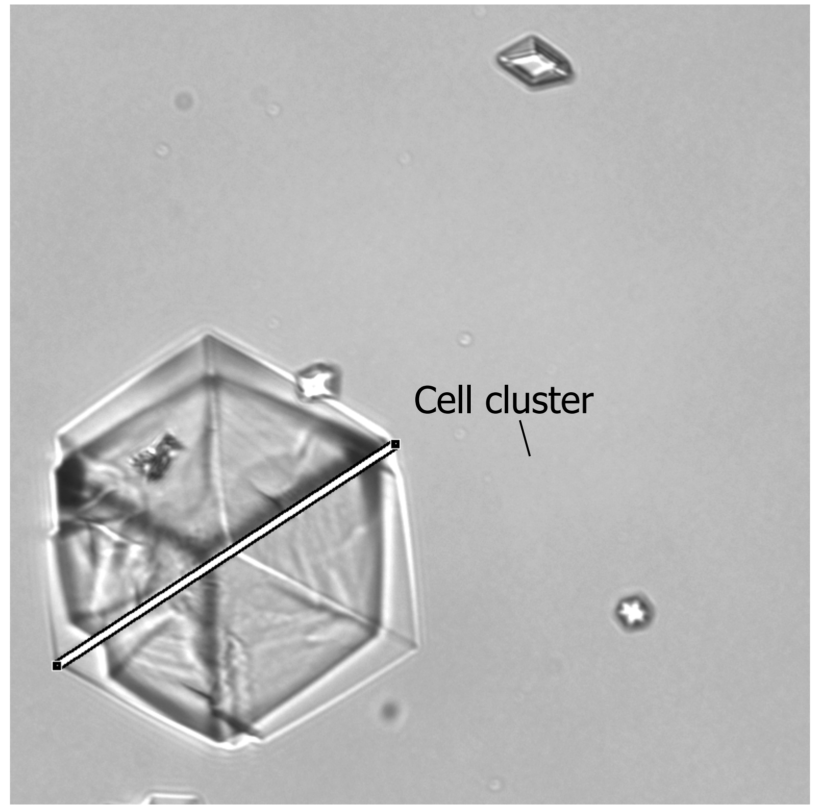


Extracted longest crystal diagonal
for test image 35

Experimental Results



Extracted shortest linear edge
for test image 44



Extracted longest crystal diagonal
for test image 44

Summary

- An algorithm was presented which is able to extract the shortest linear edge and the longest diagonal of each single crystal found in an image captured by an in-situ microscope

- In average, the lengths are delivered 1 second after the image is captured with an accuracy of 4.18% and 2.32%, respectively

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Thank you!

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Thank you again!

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