# Algorithm for Detection of Single Isolated Human Insulin Crystals for In-Situ Microscopy

G. Martinez<sup>1</sup>, P. Lindner<sup>2</sup>, A. Bluma<sup>2</sup>, and T. Scheper<sup>2</sup>

<sup>1</sup>Image Processing and Computer Vision Research Laboratory (IPCV-LAB), Universidad de Costa Rica

<sup>2</sup>Institute of Technical Chemie (TCI), Leibniz Universität Hannover, Hannover, Germany

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### Overview

- Introduction
- Problem
- Approach
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- Experimental results
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#### Introduction

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



### Introduction

As an alternative, we are using the insitu microscope for on-line analysis 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 stuff  $(C_2)$  and mixed regions  $(C_3)$ 



Human insulin crystal image captured by an in-situ microscope

### Problem

We are interested in the regions of the single crystals!

but how can them be automatically found in the captured images?



### **Two-Staged Approach**

First, all the foreground regions are segmented



 Then, the single crystals are detected among the segmented foreground regions





# **Two-Staged Approach**

Foreground Region Segmentation

- Pixel classification based on the evaluation of the local intensity variance:
  - pixels whose local intensity variance is less than a threshold th<sub>g</sub> are classified as belonging to the background regions, otherwise they are classified as belonging to the foreground regions

Single Crystal Detection

- Single nearest shape prototype detection:
  - a foreground region is detected as a single crystal if in the Euclidian space its shape is much closer to the shape prototype of the single crystals than to the shape prototypes of the other classes of foreground regions

1) Capture an intensity image I



2) Estimate the local intensity variance at each pixel position



- 3) Classify all the pixels of the variance image V into pixels belonging to the foreground regions and pixels belonging to the background region by using the global threshold  $th_g$   $V_i \le th_g$ : background (black)  $V_i > th_g$ : foreground (white) where  $th_g=m_1+4\sigma_1$   $\sigma_1$ : standard deviation of the
  - variance values at the background
  - m<sub>1</sub>: mean of the variance values at the background



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



5) Eliminate any white region touching any image border



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



7) Eliminate black holes inside white regions

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



F<sub>s</sub>, s=0, 1, 2

8) 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



9) 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

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

where

$$\|C_{s} - P_{n}\| = \sqrt{\sum_{k=0}^{6} (P_{n}(k) - C_{s}(k))^{2}}$$

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



- Implemented in C under Windows XP
- Intel Core Duo CPU at 2.2 GHz and 2 GB RAM
- Tested with 289 real images
- Average processing time of 0.15 sec/image
- Detection reliability (F score) of 95%





Segmented foreground regions

Detected single crystal regions



Segmented foreground regions

Detected single crystal regions



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Segmented foreground regions

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Segmented foreground regions

Detected single crystal regions



Segmented foreground regions

Detected single crystal regions

## Summary

- First, the foreground regions are segmented by using a global threshold technique. Then, an arbitrary segmented foreground region is detected as the region of a single crystal if in the Euclidian space its shape vector is much closer to the shape vector prototype of the class of regions of single crystals
- A shape vector is a 7-dimensional vector of rotation, translation and scale invariant shape characteristics
- The Euclidian distance is used to compute the closeness between shape vectors
- Tested with 289 real images captured by the in-situ microscope
- Average processing time of 0.15 sec/image
- Detection reliability of 95%

### **Contact Information**

Prof. Geovanni Martínez, PhD. Image Processing and Computer Vision Research Lab. (IPCV-LAB) Escuela de Ingeniería Eléctrica Código postal 2060 Universidad de Costa Rica San José, Costa Rica

Tel: +506 2511 3864 Fax: +506 2511 3920 E-mail: gmartin@eie.ucr.ac.cr www: http://ipcv-lab.eie.ucr.ac.cr