Análise de Imagem e Biotecnologia

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www.deb.uminho.pt/BioPSEg

Opportunities for Image Analysis Applications

☞ Development of faster computers
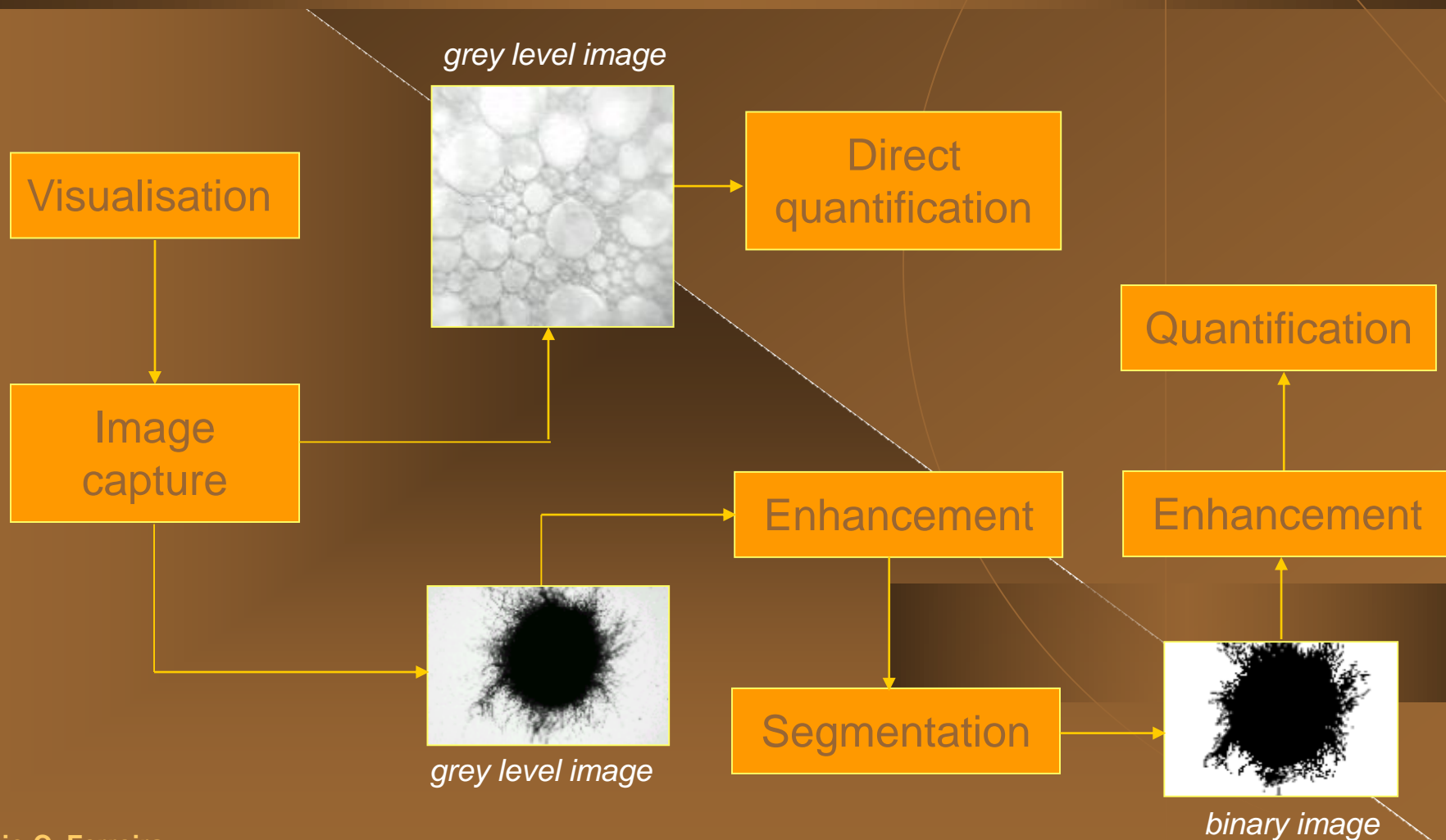
☞ Advanced frame grabbers

☞ Sophisticated software

$/quality

Image Analysis allows for:

◆ Enhancement of pictures

◆ Automatic identification and isolation of particles

◆ Fast means of getting morphologic information,
thus saving tremendous effort and time.

Principles of Image Processing

grey level image

Visualisation

Direct quantification

Image capture

Quantification

Enhancement

Enhancement

grey level image

Segmentation

binary image

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In-house software currently in use and development include:

◆ Automatic <u>recognition of protozoa</u> by image analysis

◆ Determination of the movement changes of <u>ciliates exposed to toxics</u>

◆ Automatic counting of <u>viable/non-viable</u> yeasts by epifluorescence microscopy with acridine orange as dying agent

◆ Monitoring <u>methanogenic auto-fluorescence and granulation</u> in anaerobic digestion
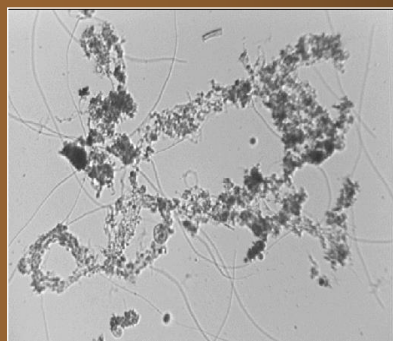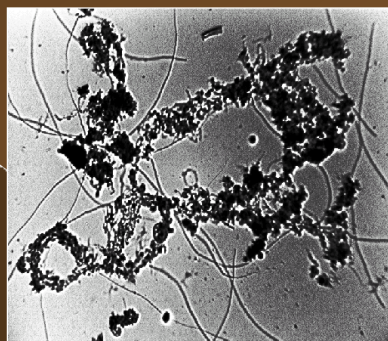
IA in Activated Sludge
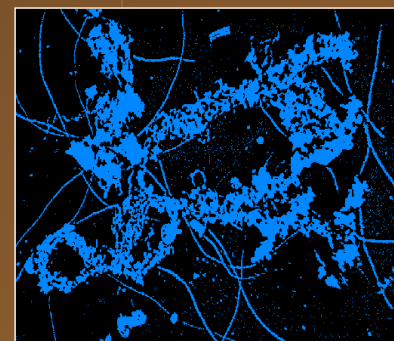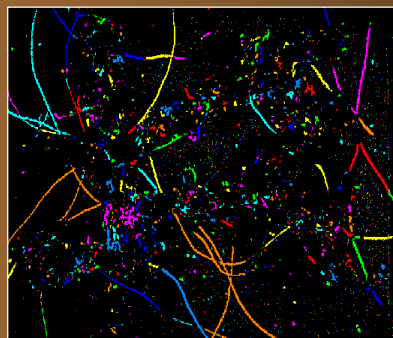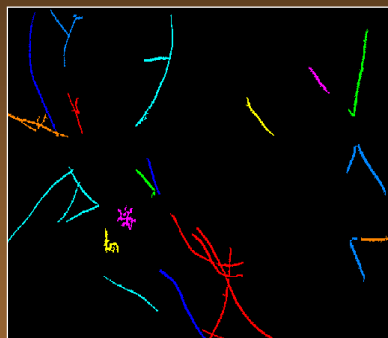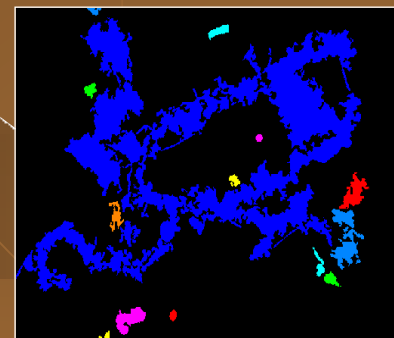

Imagem inicial

Melhoramento

Imagem binária

Marcação

Filamentos

Flocos

IA in Activated Sludge

- **<u>Morphological sludge characterization a WWTP</u>**

- <u>Characterisation of activated sludge by automated image analysis: validation on full-scale plants</u>
- <u>Automated Monitoring of Activated Sludge using Image Analysis</u>

Image Analysis in Fermentation Processes

◆ Classification of *Saccharomyces cerevisiae* morphology using image analysis

◆ Morphological Analysis of *Yarrowia lipolytica* under Stress Conditions through Image Processing

Other applications

◆ Simultaneous monitoring of <u>lactic acid bacteria and yeast during Vinho Verde fermentation</u> using phase contrast microscopy coupled to image analysis

◆ Characterization of bubbles in a bubble column by image analysis

More Information about Projects and Resources may be browsed throughout the Laboratory of Image Analysis web page :

www.deb.uminho.pt/lab_imagem

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Automatic Determination of Yeast Cells Viability by Image Analysis

◆ Epifluorescence microscopy with acridine orange as dying agent, is used for determination of viable and non-viable yeast cells

◆ The cells are viewed thereafter in an ultra violet microscope and the images can be rapidly photographed and digitized

◆ enhancement: multiplying, adding and subtracting of the channel (RGB) images to increase the contrast between viable (red/orange) and non-viable (green) cells

◆ Image analysis performed in the Global Lab Image: area determination and particles (cells) numbers

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Digitized photo from microscope observation
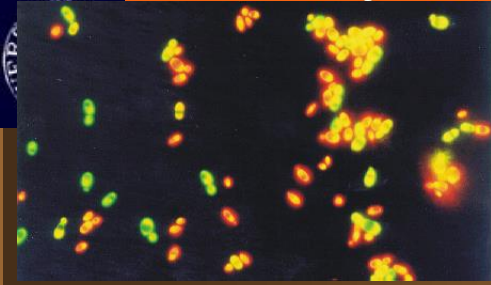
Total cells (grey scale) resulted from IA treatment
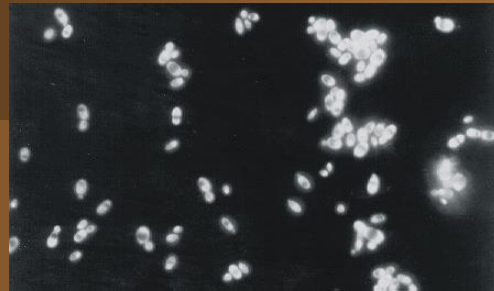
Photography of non-viable cells (grey scale) resulted from IA treatment.

Image analysis of non-viable cells. Selected cells are limited by a green outline.
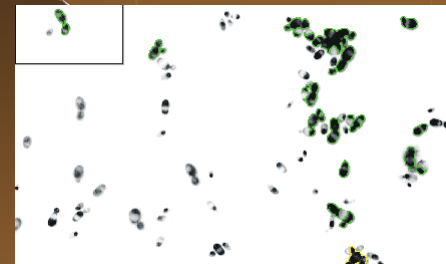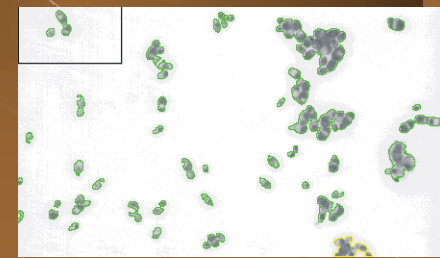
Image analysis of total cells. Selected cells are limited by a green outline.

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Monitoring Methanogenic Fluorescence by Image Analysis

◆ The co-factor F_{420} gives to the methanogenic bacteria the specific ability of auto-fluorescence when excited at a wavelenght of 420 nm. The Blue-Green (B-G) autofluorescence allows to differentiate between methanogenic and non-methanogenic bacteria.

◆ IA was used to quantify the B-G light intensity developed during the start-up of a CSTR fed with a VFA based synthetic substrate and during the S.S. operation of an anaerobic filter fed with a synthetic dairy waste

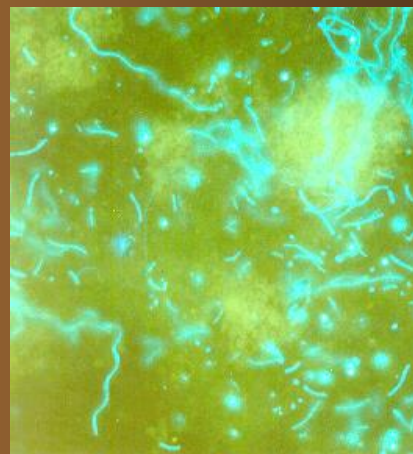◆ A program was written to calculate the number of bacterial cells and its fluorescence intensity.
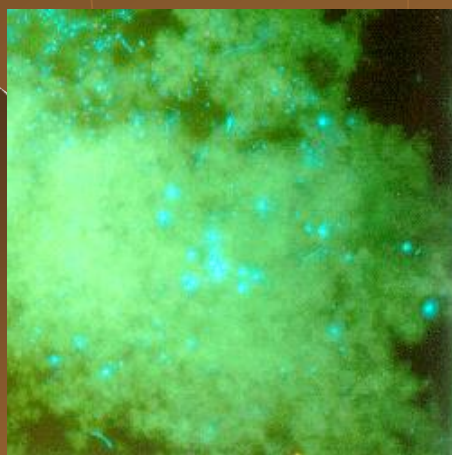
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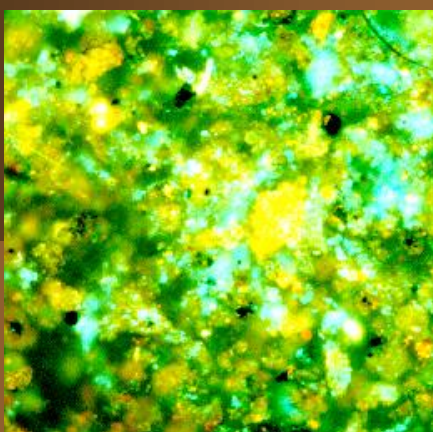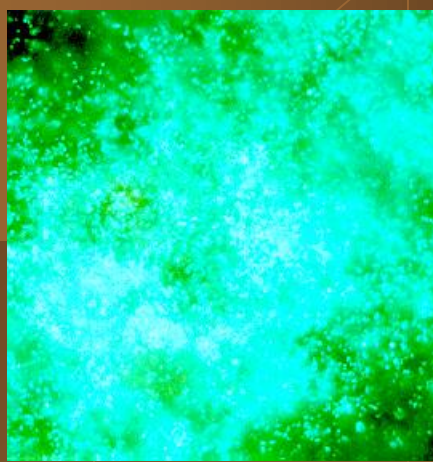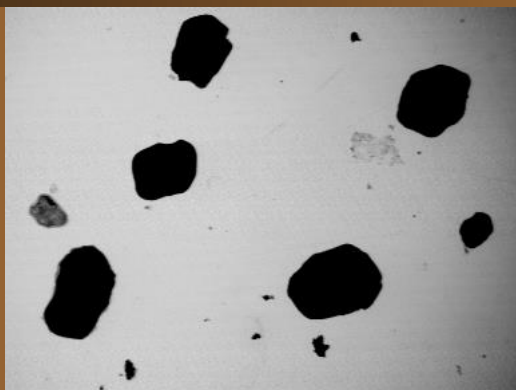
Low intensity

High intensity
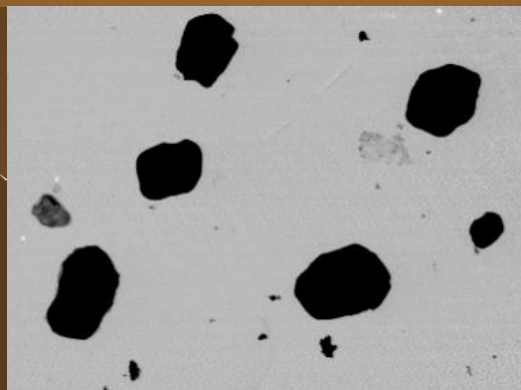

Different
morphologies
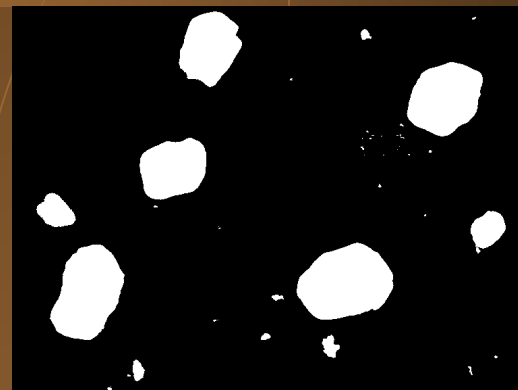

floc

Granulation in Anaerobic Digestion

Some steps of the *Flocs* image processing

Acquired image

After background subtraction

Final image

The *Flocs* program consists of three major parts:

- **Image improvement and thresholding**: subtraction of background image and thresholding by a defined threshold.

- **Floc identification**: elimination of the objects (debris) smaller than 5x5 pixels; border-kill and labelling of the remaining flocs.

- **Floc characterisation**: determination of the morphological parameters area, equivalent diameter, breadth (minimum Feret diameter), and roundness.

The *Filaments* program consists of three major parts:

- **Image improvement and thresholding**: Mexican-hat filter; background homogenisation, Wiener filtering and histogram equalization. Subsequently, the image is thresholded by a defined threshold.

- **Filament identification**: skeletonisation; end-points removal (10 pixels length); reconstruct and labelling of the remaining filaments.

- **Filament characterisation**: determination of the parameters number of filaments and average filament length.
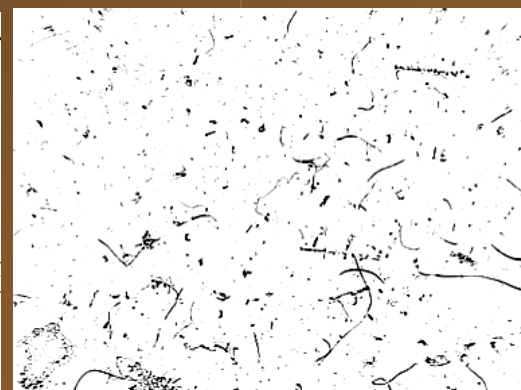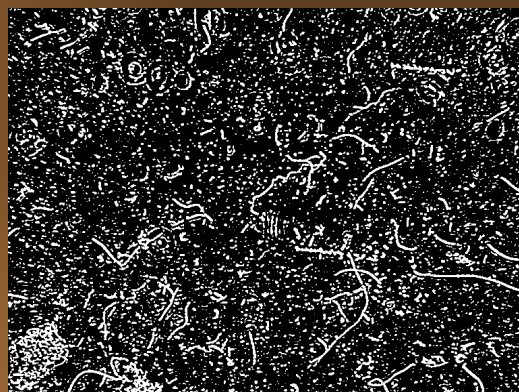
Some steps of the *Filaments* image processing
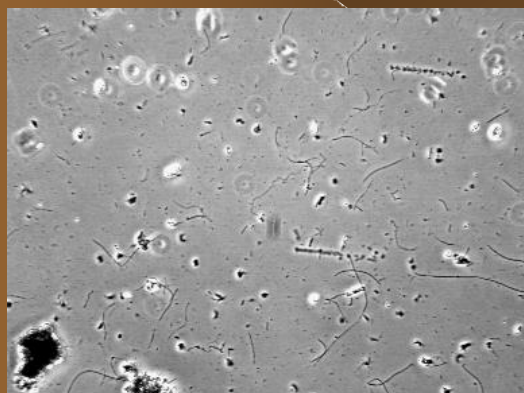
Acquired image
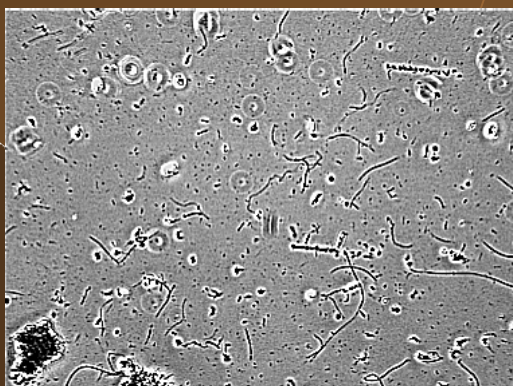
Mexican hat image

Homogenisation image

First binary image

Filaments image
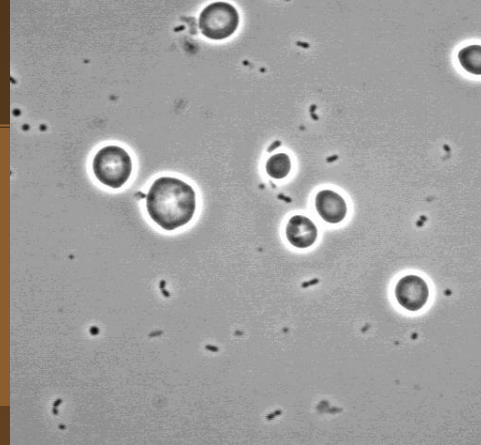
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Other developments in IA in AD

◆ Characterisation by Image Analysis of Anaerobic Sludge from Two EGSB Reactors Treating Oleic Acid: Automatic Detection of Granules Disintegration

◆ Image analysis as a tool to recognize anaerobic granulation time

◆ Image analysis, methanogenic activity measurements and molecular biological techniques to monitor granular sludge from an EGSB reactor fed with oleic acid

◆ Characterization by Image Analysis of Anaerobic Microbial Sludge under Shock Conditions

Phase Contrast Microscopy Coupled to Image Analysis as a Rapid Method to Monitor Wine Flora

The purpose of the present work, was to build a computer programme able of counting and distinguishing yeast and bacteria in a single solution

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In order to find the pixels that constitute objects, a method was built to calculate upper and lower thresholds between which the desired pixels are considered.

A sequential method along vertical and horizontal lines searches for an exact order of pixels that represents yeast

The mean value of successive small sub-matrixes is calculated and compared with an interval formed by two other calculated limits
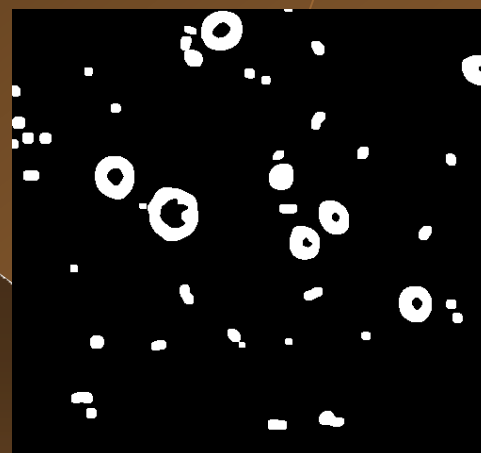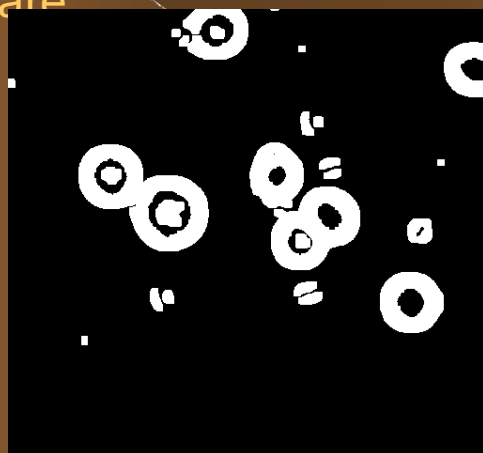
The preceding method found all the objects including bacteria, now all the objects who are not bacteria are removed from the matrix and the remaining are counted as bacteria
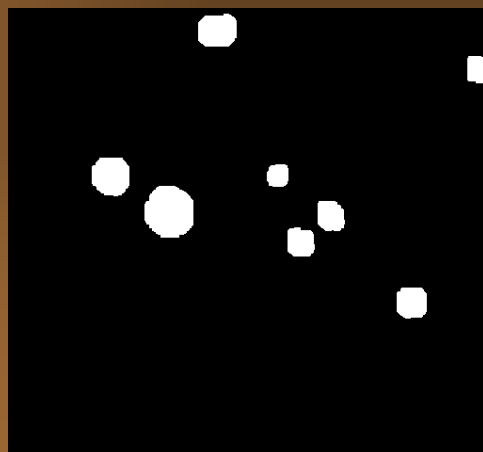
# Motility assessment of the ciliated *Tetrahymena pyriformis* after exposition to toxic compounds using image analysis

In order to determine the influence of some known toxic componds to the ciliated protozoa Tetrahymena pyriformis, a program was developed in MATLAB that allows the assessment of their motility. This program calculates both the velocity and the angle of the movement of the protozoa.

3 toxic compounds were used: cycloheximide, copper and Triton X-100.

The main objectives of this work were then:

- ◆ to identify each protozoa in a given image and calculate its position
- ◆ to recognize and isolate the same protozoa in the subsequent image
- ◆ to calculate its speed and angle of movement

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Example of movies
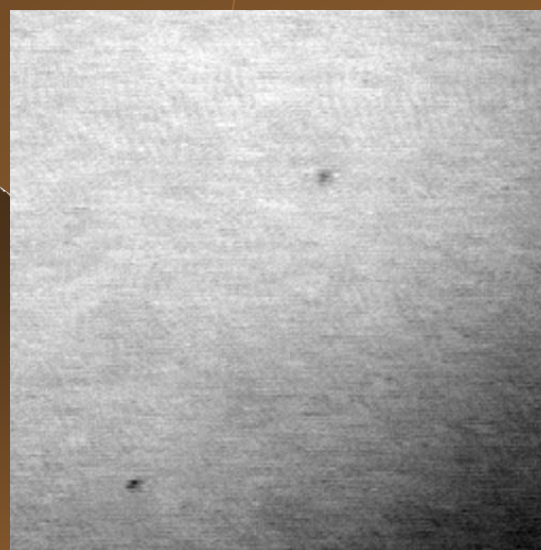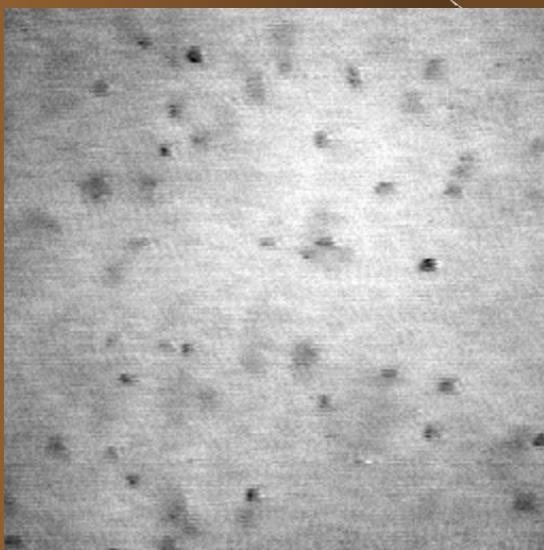
Automatic Recognition of Protozoa by Image Analysis

◆ Protozoa are commonly used as <u>biological indicators</u> of the performance of wastewater treatment. Their identification is not only time consuming but also demands high expertise.

◆ Programs were created to automatically analyse protozoa digitised images.

◆ A PCA and Discriminant Analyses techniques were explored for the species identification. Several protozoa species could be completely separated from the others.
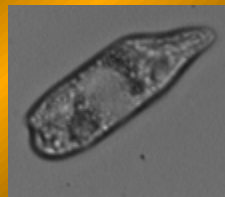
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Ciliate Protozoa in Wastewater Treatment Plant
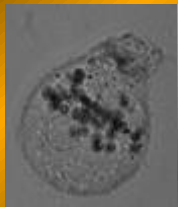
Colpidium **Glaucoma** **Litonotus**

Tetrahymena **Trachelophyllom**
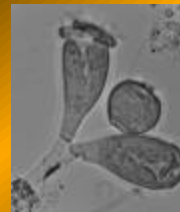
Epistylis **Zoothamnium** **Opercularia**
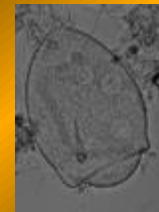
V. convallaria **V. microstoma**

Free swimming

Sessiles

Euplotes

Prorodon

Crawling

Carnivorous

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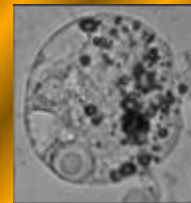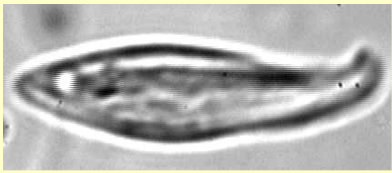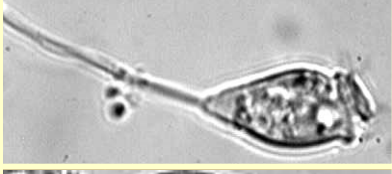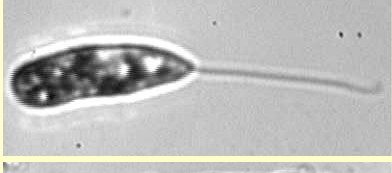
Ciliates	**Carnivorous**	
	Crawling	
	Stalked	
	Free Swimming	
Flagelates		
Metazoan		
Testate Amoebae		

Some steps of the image processing programme (v. 1)

1. Initial image with a x400 magnification
2. Contour enhancement by histogram local equalization
3. Background suppression by opening and closing to remove the halo.
4. Semi-automated segmentation based on the Euclidian Distance Map.
5. When the protozoan is not in contact with the frame, part of the flocs are eliminated by a border-killing routine. The protozoan contour is closed by openings
6. Hole-filling of the silhouette and semi-automated segmentation based on the Euclidian Distance Map.
7. Elimination of flocs by a series of erosion and reconstruction of the protozoa silhouette. If flocs are larger than protozoa, they are isolated and discarded by a logical subtraction.
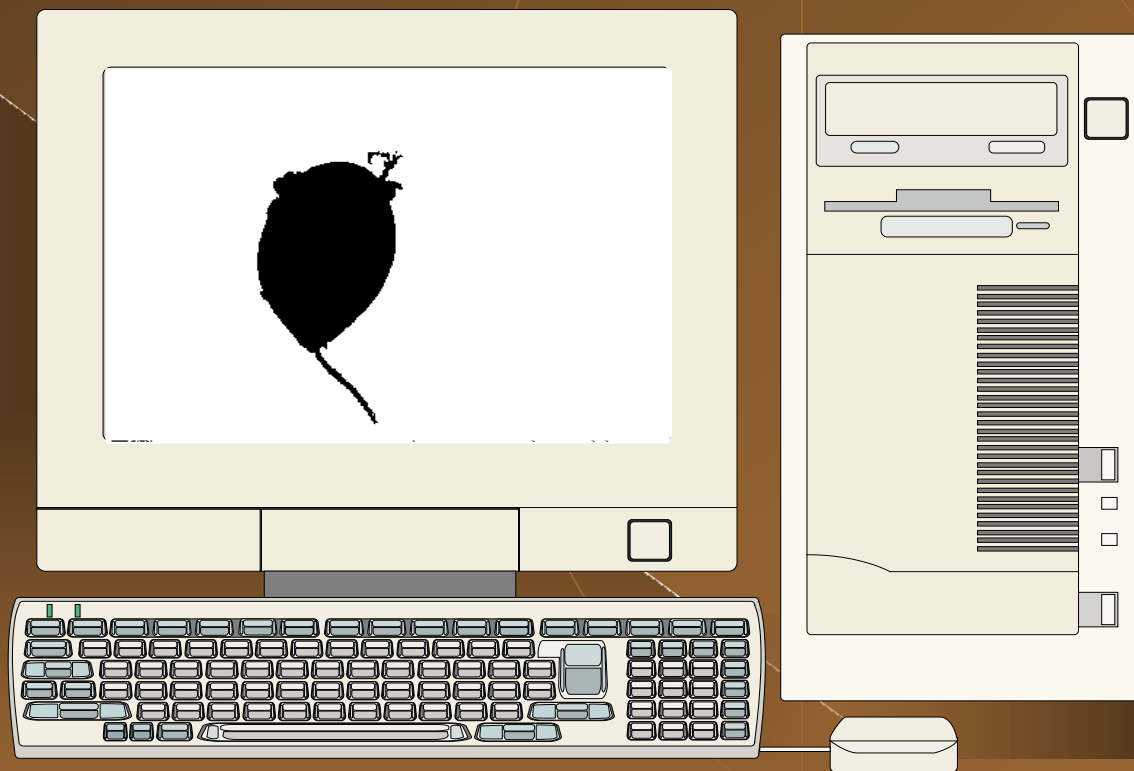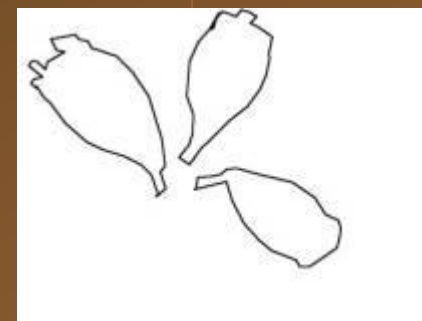
Some steps of the image processing programme (v. 2)
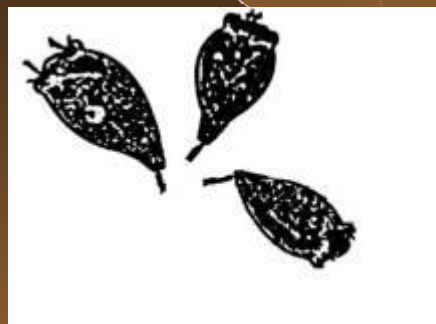

Acquired image

Pre-Treated image

Regions of interest

Recovered protozoan
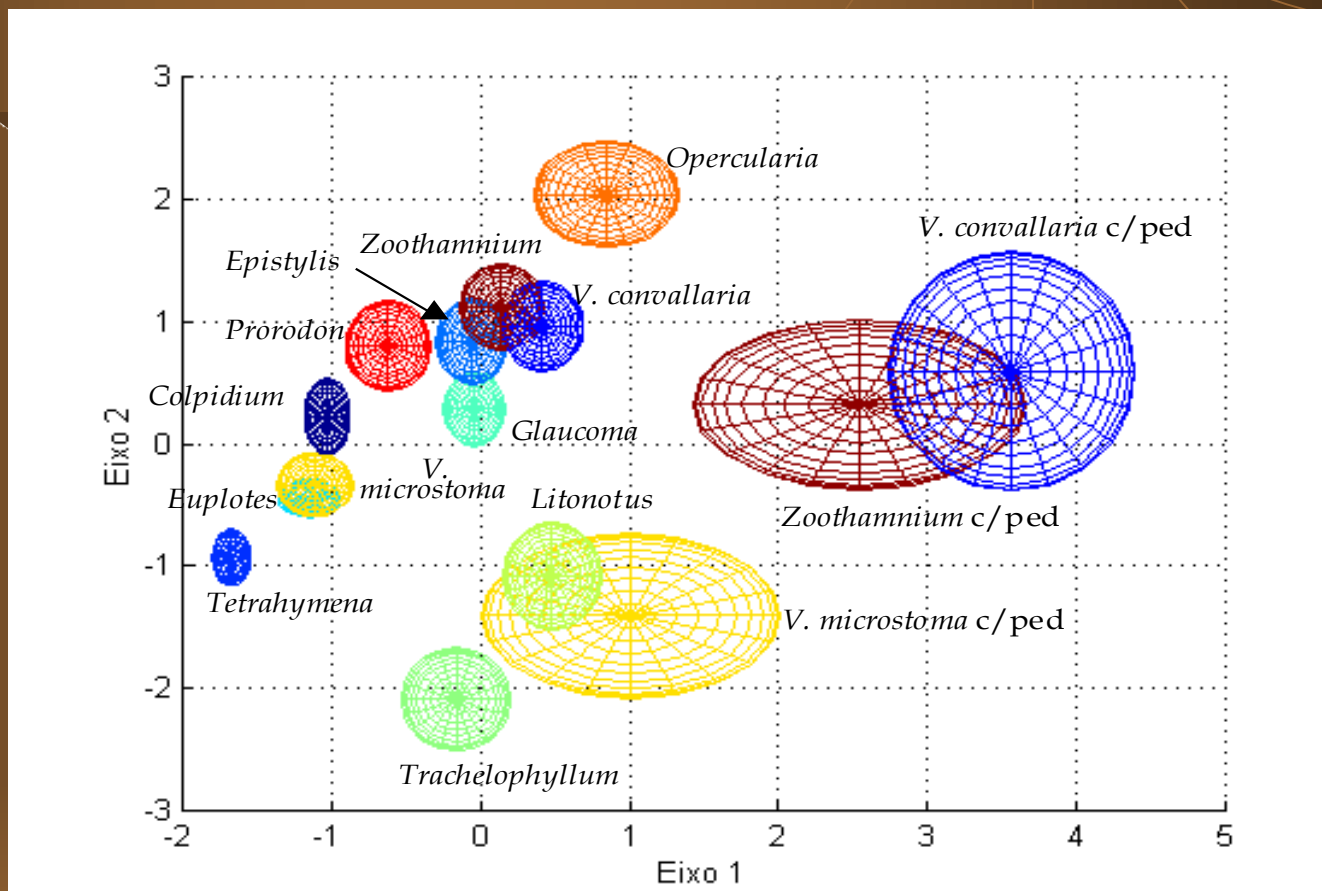
Binary image
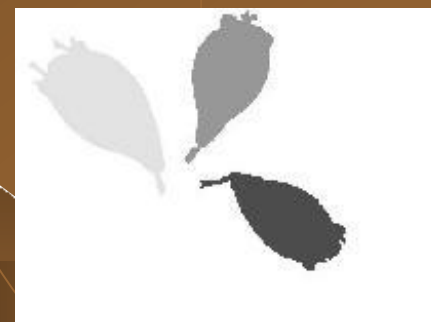
Final labeled image

Principal Component Analysis

Axis:
Linear combination of
A/P Shape,
Feret shape,
Eccentricity,
Area,
Length

V. microstoma and *Opercularia* sp., indicators of a poor efficiency of a wastewater treatment, are quite well isolated, thus allowing the determination of possible anomalies in the performance of the plant.

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3D
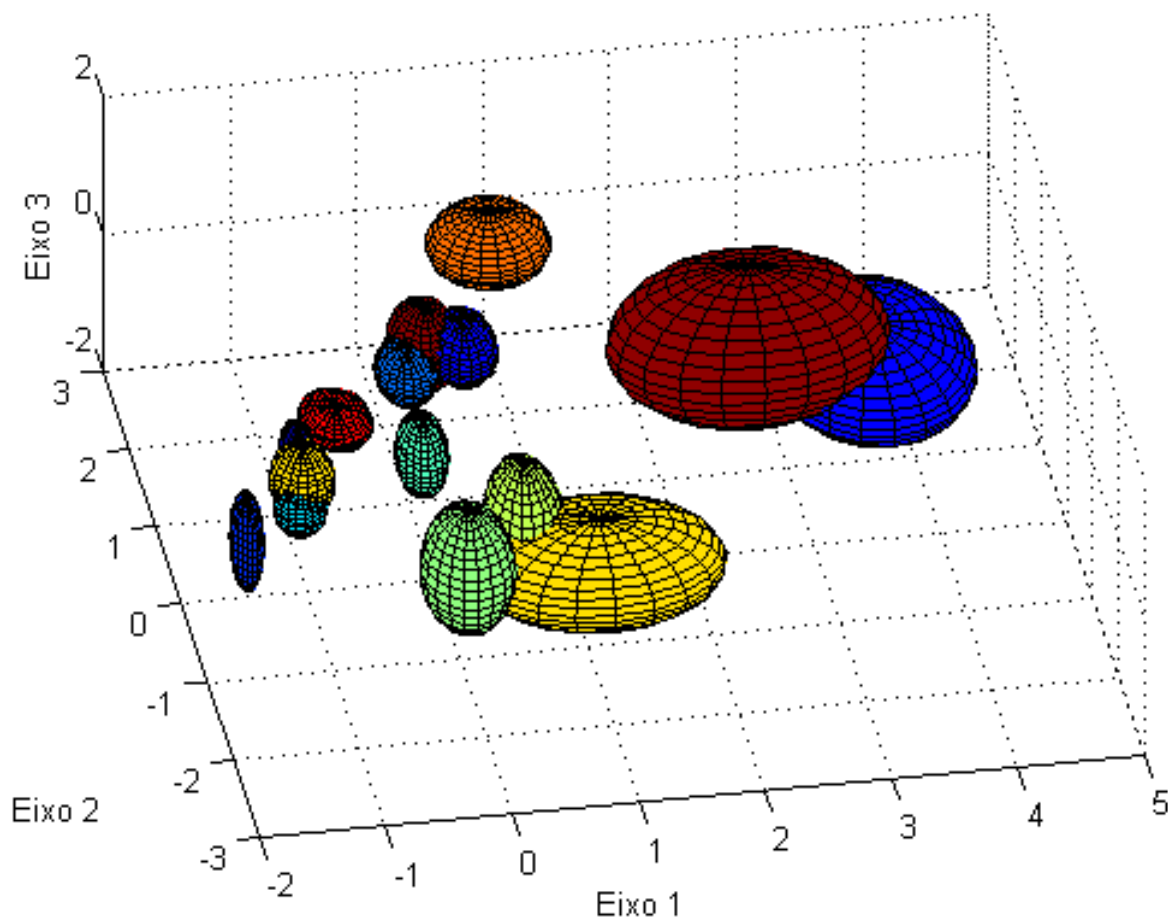
Other IA applications with protozoa

- [Survey of a Wastewater Treatment Plant Microfauna by Image Analysis](#) (Discriminant Analyses)

- [Study of Protozoan Population in Wastewater Treatment Plants by Image Analysis](#) (PCA)

O predomínio de algumas espécies pode fornecer valiosas informações sobre o estado de funcionamento de uma ETAR:

- ◆ **Pequenos flagelados**: revela uma má eficiência que pode ser causada por lamas pouco oxigenadas ou entrada de substâncias em vias de fermentação

- ◆ **Pequenas amebas nuas e flageladas**: revela uma má eficiência que pode ser causada por uma carga elevada ou de baixa degradabilidade

- ◆ **Pequenos ciliados nadadores** (< 50 mm): revela uma eficiência medíocre que pode ser causada por um tempo de residência demasiado curto ou lamas pouco oxigenadas

- ◆ **Grandes ciliados nadadores** (> 50 mm): revela uma eficiência medíocre que pode ser causada por uma carga demasiado elevada

- ◆ **Ciliados sésseis**: revela uma baixa eficiência que pode ser causada por fenómenos transitórios

- ◆ **Ciliados móveis de fundo**: revela uma boa eficiência

- ◆ **Ciliados sésseis em conjunção com móveis de fundo**: revela uma boa eficiência

- ◆ **Amebas com teca**: boa eficiência indicando estar-se perante uma carga baixa e/ou diluída e uma boa nitrificação