Application of Image Analysis Techniques in Environmental Biotechnology

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Opportunities for Image Analysis Applications

- Development of faster computers
- Advanced frame grabbers
- Sophisticated software

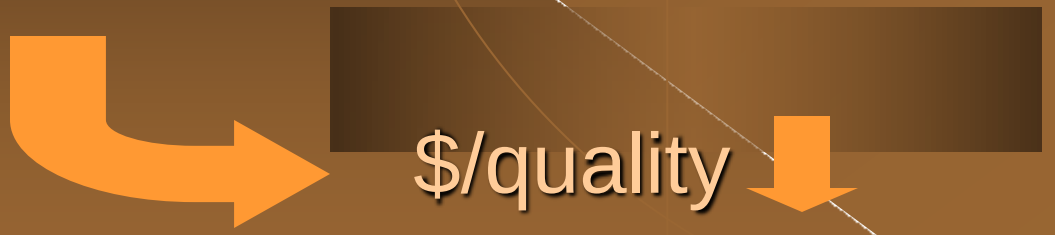
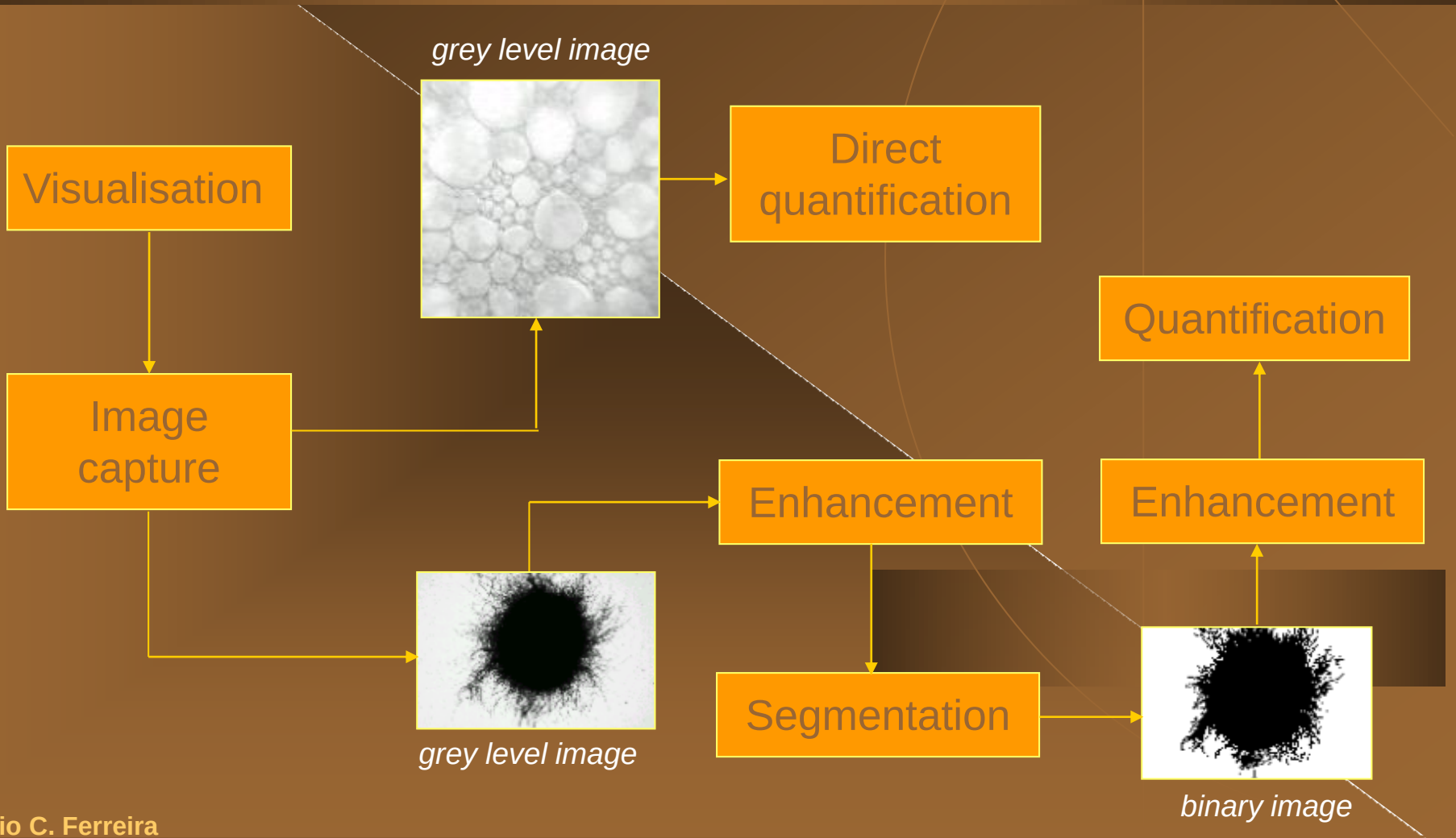


Image Analysis allows for:

- 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



Application of Image Analysis Techniques in Wastewater Treatment

- Activated Sludge
- Protozoa
- Anaerobic Digestion

- Other Applications...

IA in Wastewater Treatment – Activated Sludge

- Morphological sludge characterization a WWTP using Partial Least Squares
- Automated Monitoring of Activated Sludge using Image Analysis (correlation with settleability, SVI)
- Characterisation of activated sludge by automated image analysis: validation on full-scale plants

IA in Activated Sludge

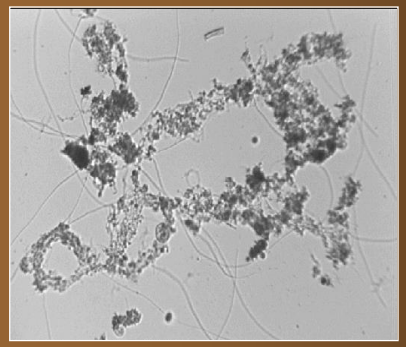
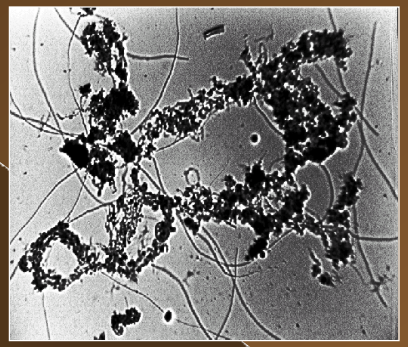


Imagem inicial



Melhoramento

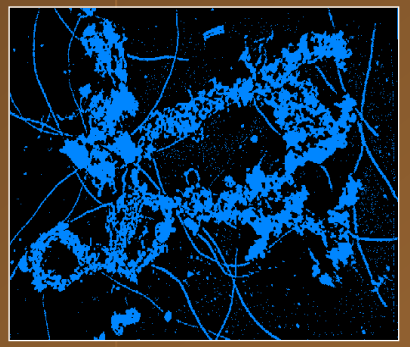
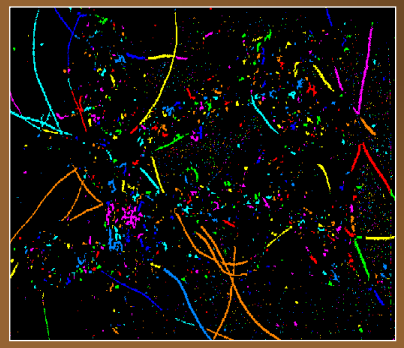
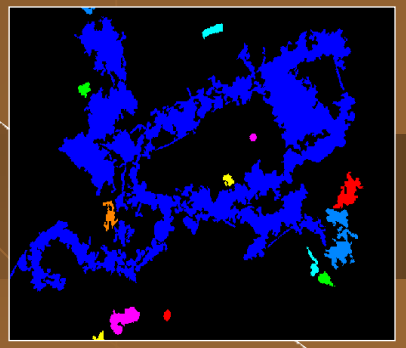


Imagem binária



Marcação

Filamentos



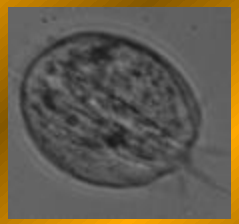
Flocos

Automatic Recognition of Protozoa by Image Analysis

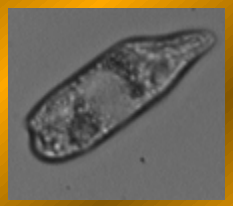
- Protozoa are commonly used as biological indicators of the performance of wastewater treatment. Their identification is not only time consuming but also demands high expertise.
- Programs were created to automatically analyse protozoa digitised images.
- A PCA and Discriminant Analyses techniques were explored for the species identification. Several protozoa species could be completely separated from the others.

Ciliate Protozoa in Wastewater Treatment Plant

Colpidium



Glaucoma



Litonotus

Tetrahymena

Trachelophyllom

Free swimming

Epistylis

Zoothamnium

Opercularia

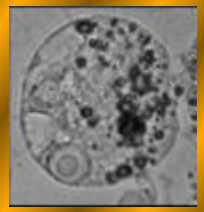
V. convallaria

V. microstoma

Sessiles

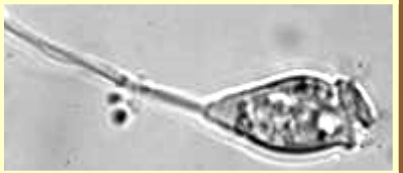
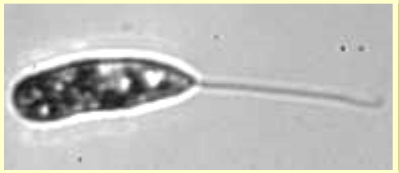
Euplotes

Crawling



Prorodon

Carnivorous

Ciliates	Carnivorous	
	Crawling	
	Stalked	
	Free Swimming	
Flagelates		
Metazoan		
Testate Amoebae		

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

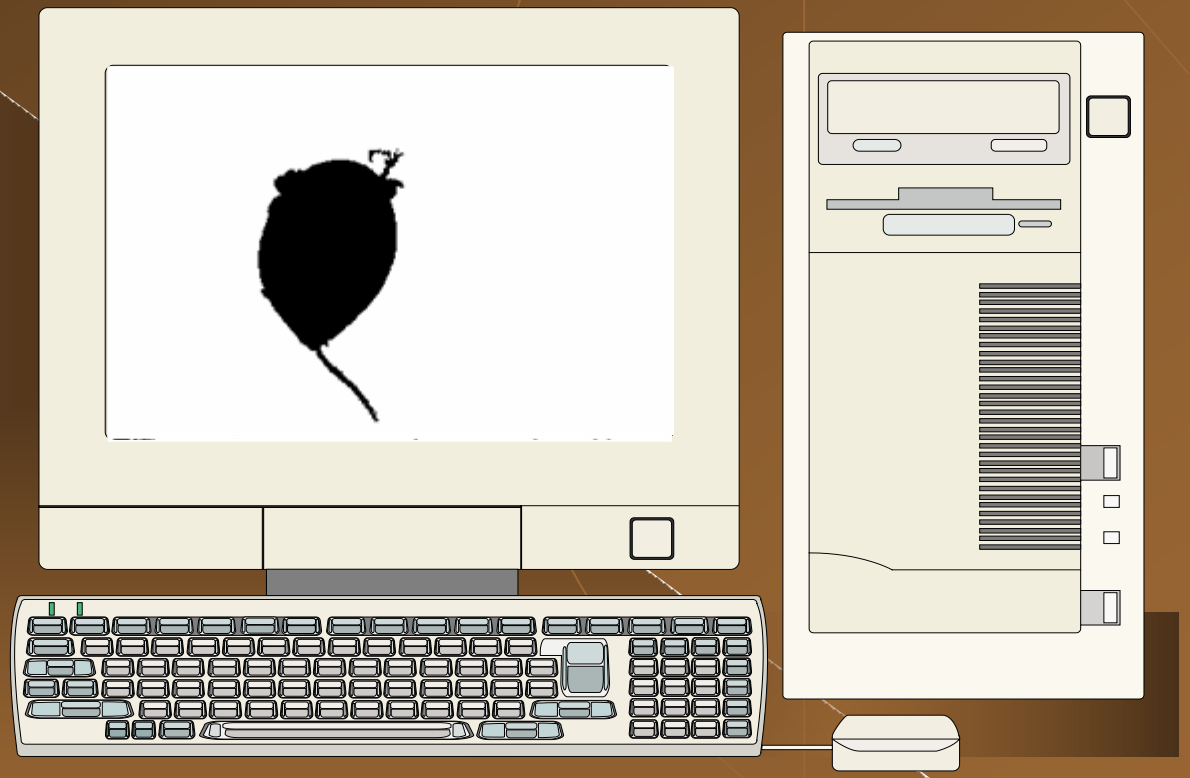
- Pequenos flagelados: revela uma má eficiência que pode ser causada por lamas pouco oxigenadas ou entrada de substâncias em vias de fermentação
- Pequenas amebas nuas e flageladas: revela uma má eficiência que pode ser causada por uma carga elevada ou de baixa degradabilidade
- Pequenos ciliados nadadores (< 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 (*V. microstoma* and *Opercularia* sp.)**
- 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

IA in Wastewater Treatment – Protozoa

- Automatic recognition of protozoa by image analysis
- Survey of a Wastewater Treatment Plant Microfauna by Image Analysis (Discriminant Analyses)
- Study of Protozoan Population in Wastewater Treatment Plants by Image Analysis (PCA)
- Determination of the movement changes of ciliates exposed to toxics

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

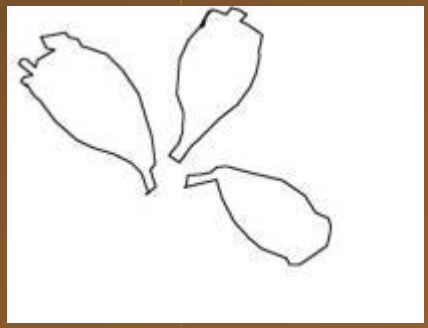
1. Initial image with a x400 magnification
2. Contour enhancement by histogram local equalization
3. Background suppression by opening and closing to remove the halo.
4. Semi-automated segmentation based on the Euclidian Distance Map.
5. When the protozoan is not in contact with the frame, part of the 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)

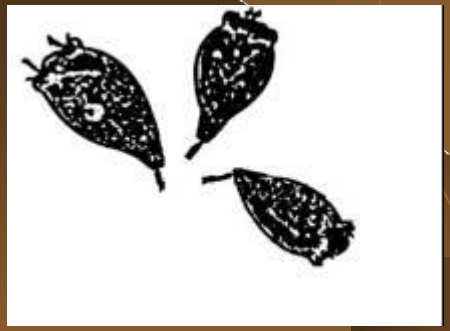
Acquired image

Pre-Treated image

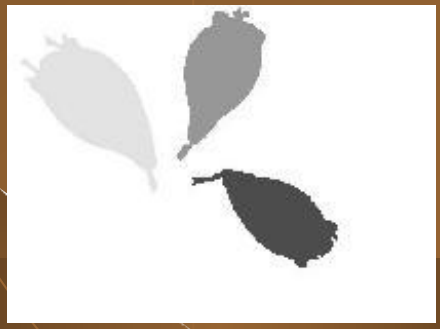


Regions of interest

Recovered protozoan



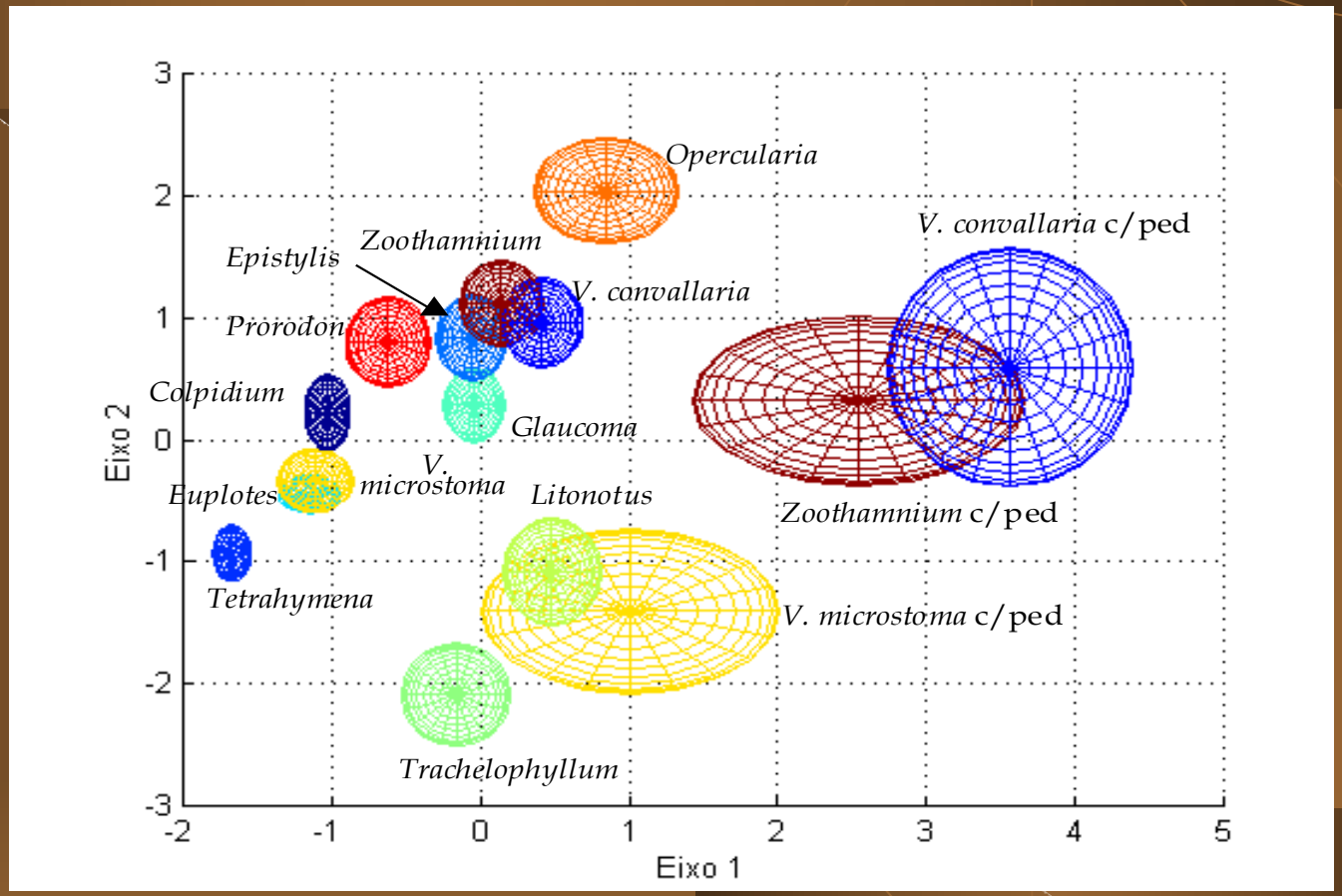
Binary image



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.

3D

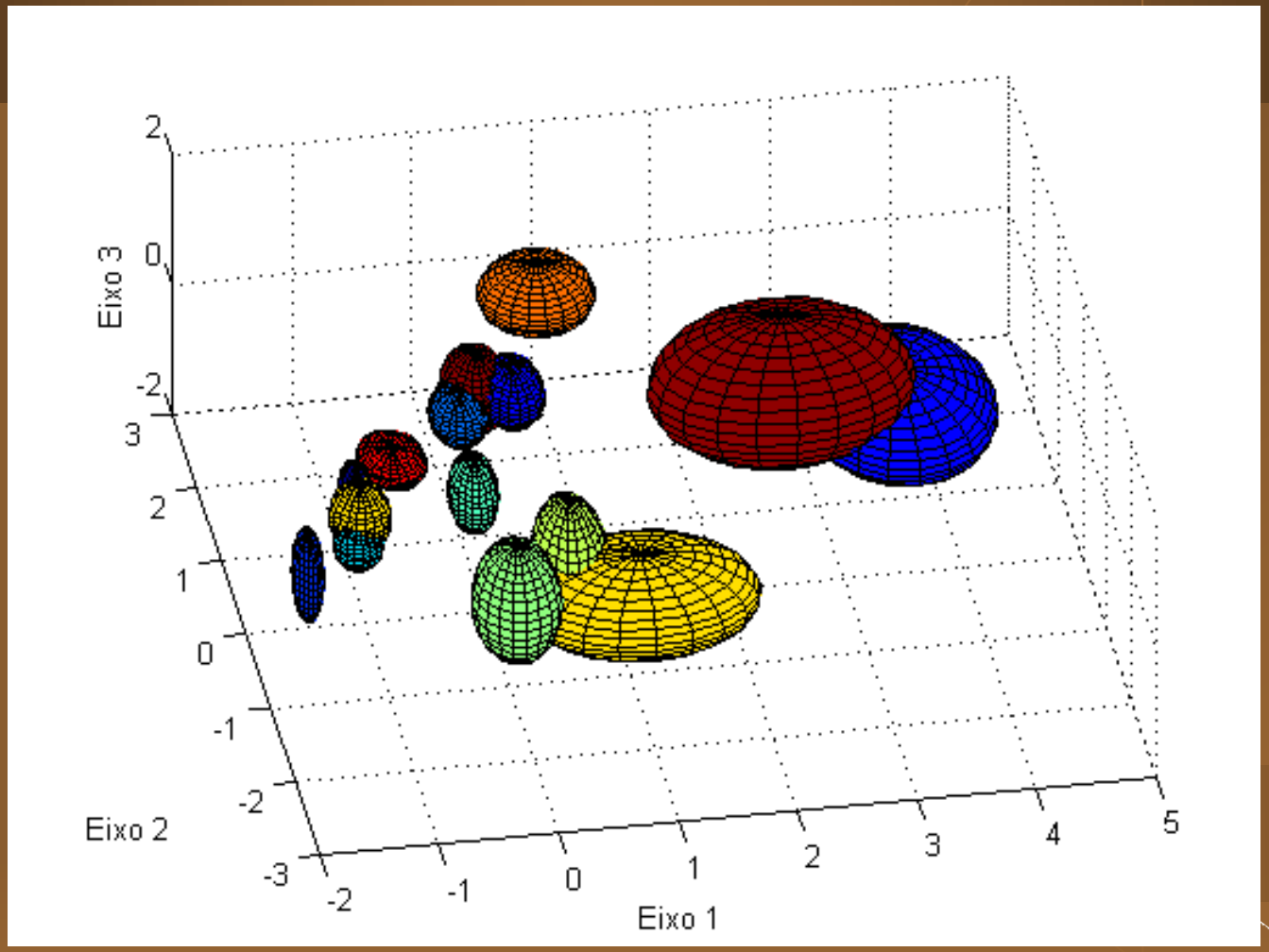


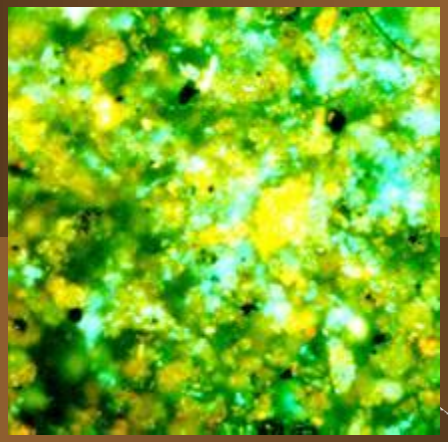
Image Analysis in Anaerobic Digestion

- Monitoring methanogenic auto-fluorescence and granulation in anaerobic digestion
- 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

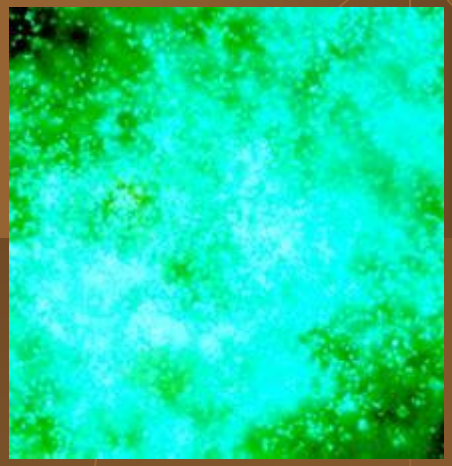
Monitoring Methanogenic Fluorescence by Image Analysis

- 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.

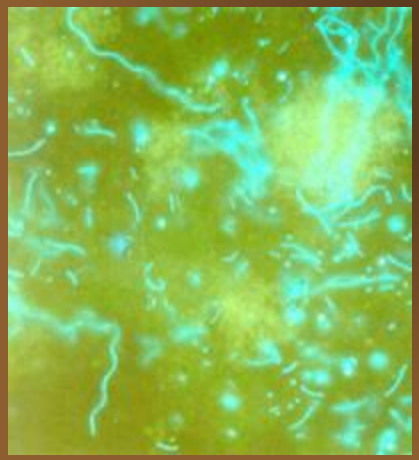
Examples of fluorescent anaerobic sludges



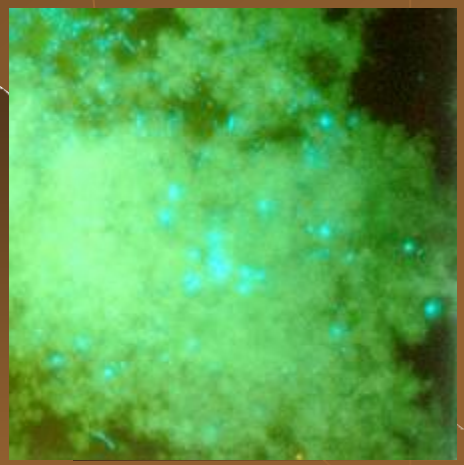
Low intensity



High intensity



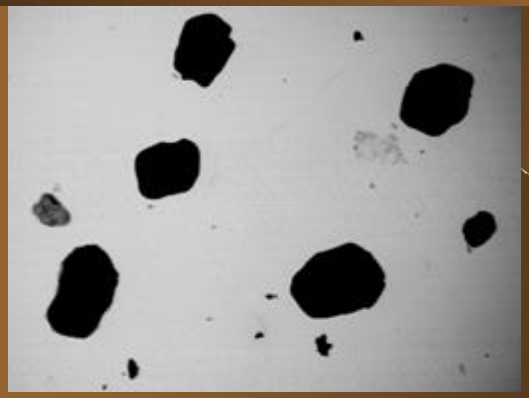
Different morphologies



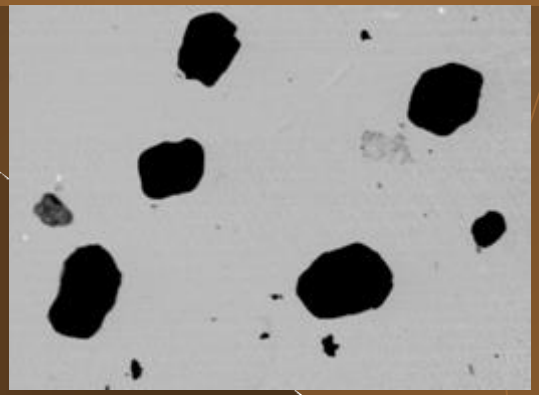
floc

Granulation in Anaerobic Digestion

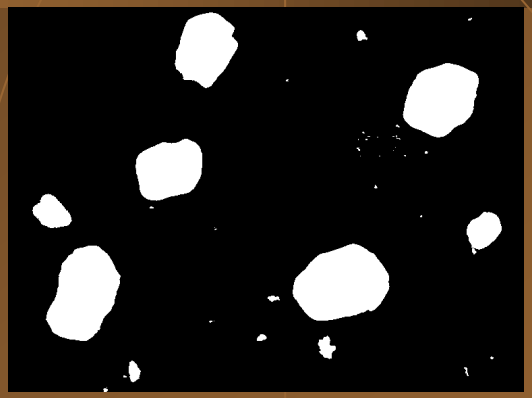
Some steps of the *Flocs* image processing



Acquired image



After background subtraction



Final image

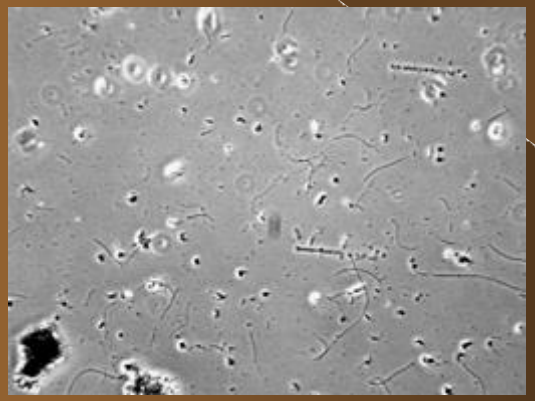
The *Flocs* program consists of three major parts:

- **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.

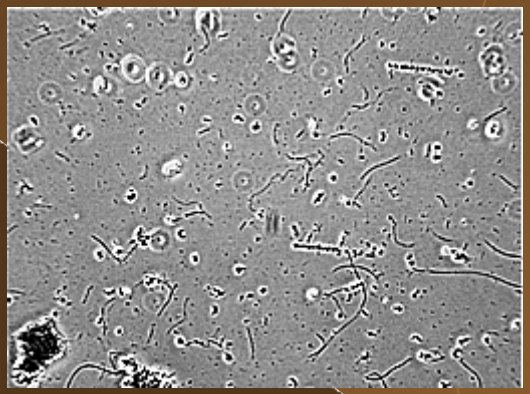
The *Filaments* program consists of three major parts:

- 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.

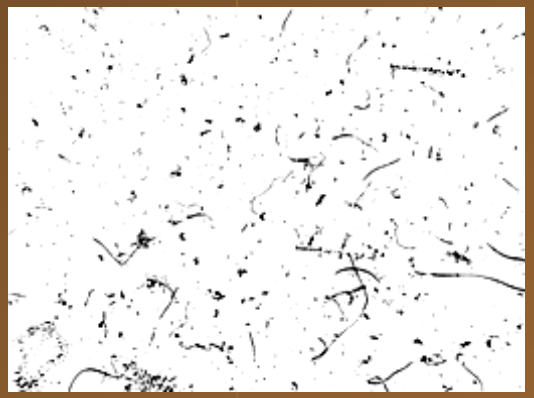
Some steps of the *Filaments* image processing



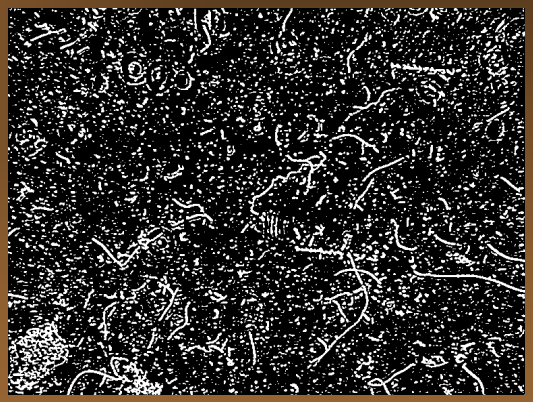
Acquired image



Mexican hat image



Homogenisation image



First binary image

Filaments image

Other Applications (Biotechnology and Food Technology)

- Classification of *Saccharomyces cerevisiae* morphology using image analysis
- Morphological Analysis of *Yarrowia lipolytica* under Stress Conditions through Image Processing
- Automatic counting of viable/non-viable yeasts by epifluorescence microscopy with acridine orange as dying agent
- Characterization of bubbles in a bubble column by image analysis
- Simultaneous monitoring of lactic acid bacteria and yeast during Vinho Verde fermentation using phase contrast microscopy coupled to image analysis

More Information about Projects and Resources may be browsed throughout the BioPSE group's web page

www.deb.uminho.pt/BioPSEg