# Segmentation of biological 3D images

Juan Cardelino

juanc@fing.edu.uy Grupo de Tratamiento de Imágenes, IIE- UDELAR



# Summary

- ★ Introduction
- ★ Background
- ★ Feature Extraction
- ★ Our approach
- ★ Results
- ★ Concluding Remarks



### Introduction - Objective

Goal:

★ Devise a general segmentation algorithm, independent of the acquisition technique and the type of cellular tissue involved that is able structures of interest in 3D data in the most robust and accurate way possible.



#### Introduction - Motivation



(a)

(b)



(C)

(d)

Figure 1: (a), (b) Two consecutive (partial) slices of genital tissue of a Spider (IIBCE-2003). (c), (d) Manual reconstruction of the sequence.

#### Introduction - Motivation



(a)





Figure 2: Sample sequences: (a), (b) Ovaries taken from sea-seals (Fac. Vet. 03). (c), (d) Seminiferal tissue of a ram (Fac. Vet. 01).



## Introduction - The problem

- ★ Usually coming from biological tissue (great diversity).
- ★ Coming from different sources: Computed Tomography, Magnetic Resonance, Optical and Confocal, etc.
- ★ Approach: 3D image as series of 2D images (great difference between sampling in the x y and z axes).



Figure 3: Data structure

## Introduction - Objectives

- ★ Explore the capabilities of a state of the art family of algorithms and evaluate possible improvements.
- ★ Generic segmentation framework integrating as much information as possible: boundaries, texture, color, shape, movement.
- ★ Test the proposed algorithms and their improvements over very different sequences.



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### **Background - Curve evolution**

Segmentation: search for an F which goes to zero at the border of the objects.



#### Figure 4: Segmentation with a time evolving curve

$$\begin{cases} \frac{\partial \Gamma}{\partial t} = F\vec{N} \\ \Gamma(s,0) = \Gamma_0(s) \end{cases}$$

(1)



# **Background - Curve evolution**

Variational formulation: F = F(L, G, I) usually designed to minimize a cost criterion including:

- ★ L: Local properties of the curve (e.g. curvature)
- ★ G: Global properties of the curve (shape, integrals).
- ★ I: Properties dependent of the underlying image (boundary or region).

Characteristics:

- ★ PDE's: formal framework, large amount of mathematical tools.
- ★ Singularities: non-classic solutions.
- ★ Careful numeric analysis required.
- ★ Level-set implementation: topology independent.



#### **Background - Region Based Active Contours**

$$E(\Gamma) = E_{boundaries}(\Gamma) + E_{regions}(\Gamma) + E_{smooth}(\Gamma)$$
 (2)

$$E_{regions}(\Gamma) = \int_{\Omega_0} k_0(\mathbf{x}, \Omega_0) d\mathbf{x} + \int_{\Omega_1} k_1(\mathbf{x}, \Omega_1) d\mathbf{x}$$
(3)

 $k_i(\mathbf{x})$ : global region descriptor such that:

$$\begin{cases} k_i \approx 0 : if x \in \Omega_i \\ k_i >> 1 : if x \notin \Omega_i \end{cases}$$
(4)

#### Ref: [Deriche, 2002] [Vese, 2001] [Aubert, 2002]



### Background - RBAC energy

Example:  $k_0 = |I(x) - \mu_A|$  and  $k_1 = |I(x) - \mu_B|$ . Where  $\mu_A \approx 255$  and  $\mu_B \approx 0$ .



Figure 5: Data term: (a) small energy, (b) big energy.



## Background - RBAC energy

Maximum Likelihood Estimation: equivalent to take  $k_i = p(I(x)|\Omega_i)$ .

$$E(\Gamma, \theta_i) = -\int_{\Omega_0} \log[p_0(x)] dx - \int_{\Omega_1} \log[p_1(x)] dx$$

where  $p_i(x) = p(I(x)|\Omega_i)$  and  $\theta_i$  are the parameters of the conditional PDF in each region.

Requisite: define a PDF model for each region.

Ref: [Deriche, 2002]



# **Background - Energy minimization**

The gradient descent yields

$$\frac{d\vec{\Gamma}}{dt} = \log\left(\frac{p_1(\mathbf{x})}{p_0(\mathbf{x})}\right)\vec{N}_{\Gamma}$$
(5)

and if we suppose a gaussian model  $\theta_i(\mu_i, \sigma_i)$ :

$$\begin{pmatrix} \mu_i = \frac{\int_{\Omega_i} I(\mathbf{x}) d\mathbf{x}}{\int_{\Omega_i} d\mathbf{x}} \\ \sigma_i^2 = \frac{\int_{\Omega_i} (I(\mathbf{x}) - \mu_i)^2 d\mathbf{x}}{\int_{\Omega_i} d\mathbf{x}} \end{pmatrix}$$

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(6)

#### **Background - Multiple Features**

Instead of considering an image  $I(\mathbf{x})$  we will use a feature vector  $U(\mathbf{x})$ . Then

$$p_i = \prod_{i=1}^N p_i^j$$

Thus the energy in this case yields

$$E(\Gamma) = -\int_{\Omega_0} \sum_{j=1}^N w_j \log p_0^j(\mathbf{x}) d\mathbf{x} - \int_{\Omega_1} \sum_{j=1}^N w_j \log p_1^j(\mathbf{x}) d\mathbf{x}$$
(7)

and its gradient descent is

$$\frac{\partial \Gamma}{\partial t} = \left(\sum_{j=1}^{N} w_j \log\left[\frac{p_1^j(\mathbf{x})}{p_0^j(\mathbf{x})}\right]\right) \vec{N} + \lambda \kappa \vec{N}$$
(8)



# **Background - RBAC algorithm**

- 1. Compute features.
- 2. Perform some pre-processing.
- 3. Start with an arbitrary curve  $\Gamma_0$ .
- 4. For each region *i* compute  $\theta_i$  ( $\mu$  y  $\sigma$  in the Gaussian case).
- 5. Evolve the curve according to the gradient descent equation.
- 6. go to 4 until steady state is reached  $(\frac{\partial \Gamma}{\partial t} \approx 0)$ .



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## Feature extraction

#### ★ Features:

- $\triangleright$  Color: (R, G, B), (Y, Cb, Cr), (K1, K2, K3), (H, S, V), (L, a, b)
- ▷ Texture: Structure tensor, Gabor filters, Wavelet analysis.
- Motion estimation: optic flow.
- ★ PDF model: choose a suitable model for each set of features.
- ★ Pre-processing: anisotropic diffusion, histogram correction.



## Feature extraction - Color features

- ★ Separate intensity from chromaticity: (Y, Cb, Cr) or (H, S, V). For histogram correction and geometry extraction.
- ★ De-correlate feature channels (approx.)
- ★ non-linear: singularities, perceputally accurate.
- ★ Accurate texture description: color texture, linear space.

Choice:

- ★  $(K_1, K_2, K_3)$ , Karhunen-Loève approximation for natural images.
- ★ PDF model: gaussian.

Ref: [Sakai, 1980]



#### Feature extraction - Texture modeling

Approaches:

- ★ Statistical: co-occurrence matrices.
- ★ Structural: elements and placement rule.
- ★ Filtering: Roberts, Gabor, Wavelets.
- ★ Model based: Markov Random Fields.

#### Ref: [Jain, 1998] and [Brady, 2003].



### Feature extraction - Wavelet Texture Descriptors

- ★ Properties: multiscale, frequency information, spatial localization, orientation information.
- ★ Intensity independent: No approximation channel.
- ★ Color space: (K1, K2, K3) minimum correlation between channels.
- ★ PDF model: Generalized Gaussian (Mallat).





Ref: [de Wouwer; P. Scheunders; S. Livens; D. Van Dyck, 1999], [Brady, 2003]. Juan Cardelino Segmentation of 3D Biologica

### Feature Extraction - Segmentation of 2D images



Figure 6: Two regions with different textures (Brodatz)



# Feature extraction - Optic flow

Goal: Compute the velocity between two frames in each point. Approaches:

- ★ Region-based matching: cross-correlation, etc.
- ★ Energy based : peaks of modulus of the Fourier transform.
- ★ Phase-based: phase output of band-pass filters.
- ★ Grouping: tensor voting.
- $\star$  Differential:
  - Lucas-Kanade (local, first order), Horn-Schunck (global, second order)
  - Recent: Weickert, Lucas-Kanade with Anisotropic Diffusion.

#### Ref: [Weickert, 2002].



## Feature Extraction - Optic Flow Results



#### Figure 7: Optic Flow examples



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# **Approach - Extension to Tracking**

Goal: Follow objects of interest in video sequences.



- ★ Idea: use the same formulation with motion as an additional feature.
- ★ First frame: manually drawn (or segmentation of *moving* objects).
- $\star$  Initial condition: use the curve from previous frame.
- **\star** The parameters  $\theta_i$  of the PDF are fixed during each frame.

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# Approach - The algorithm

- 1. Compute features ( $(K_1, K_2, K_3)$  + Wavelets + OF).
- 2. Perform anisotropic diffusion for each frame.
- 3. Start with an arbitrary curve  $\Gamma_0$ .
- 4. For each frame k
  - (a) For each region *i* compute  $\theta_i$  from  $I_{k-1}$
  - (b) With  $\theta_i$  evolve the curve according to (5) until steady state is reached.



# Approach - Example of tracking





## **Approach - Discussion**

- $\star$  Strong dependence on the amount of diffusion.
- ★ Integrates information in a global manner but evolves locally.
- ★ Number of regions a-priori fixed.
- ★ Fixed weights for the feature channels
- ★ Strong dependence on initialization: inaccurate parameter estimation, falls into poor local minima (inability to jump across regions).



Figure 8: Initialization problems



## **Approach - Re-initialization**

- ★ Decision: (need and side)
  - Compute a Gaussian Mixture Model (Expectation Maximization).
  - Compare the PDFs: if  $d(p_0, p_1) < th$  and area > th then re-initialize.
- ★ Construction of the front: original curve fixed, additional curve evolving outwards(inwards).



## **Approach - Reinitialization example**



Figure 9: Example of the reinitialization algorithm: (c) initial front, (d) after normal segmentation, (d) after the reinitialization algorithm



#### **Approach - Reinitialization results**



Figure 10: Frames 1:3 of the *Ram* sequence. Up: without reinitialization, down: with forced reinitialization



## **Approach - Weighting**

- ★ Feature weighting: *distance* between segmented regions  $d_j = d(p_0^j, p_1^j)$ .
- ★ Weights:

$$w_j = \frac{d_j}{\sum_{i=1}^N d_i}$$

★ PDF metrics: Battacharyya, Kullback-Leibler.





# Approach - Our algorithm

- 1. Compute features ( $(K_1, K_2, K_3)$  + Wavelets + OF).
- 2. Perform anisotropic diffusion for each frame.
- 3. Start with an arbitrary curve  $\Gamma_0$ .
- 4. For each frame *k*:
  - (a) For each region *i*, compute  $\theta_i$  from  $U_{k-1}$ .
  - (b) For each feature channel j, compute  $w_j$  from  $U_{k-1}$ .
  - (c) With  $\theta_i$  and  $w_j$  evolve the curve according to the gradient descent equation, until steady state is reached.
  - (d) Detect if re-initialization is needed, and the side.
  - (e) Construct the reinitialized front.
  - (f) Evolve the new front according to the gradient descent equation until steady state.



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#### **Results - Segmentation of video sequences**



Figure 11: Segmentation of video sequences (without reinitialization)





Figure 12: *Spider*: Results of segmentation with automatic weighting (frames 1,2,6:5:31).





Figure 13: *Spider*: Results of segmentation with automatic weighting and reinitialization (frames 1,2,6:5:31)





Figure 14: Spider: evolution of the optimal weights





#### Figure 15: Spider: 3D reconstruction





Figure 16: Ram: 3D reconstruction





Figure 17: Objects detected in a sample image of our data sets. Left: without re-initialization (using the previous frame). Center: using evenly spaced spheres in each frame. Right: using the re-initialization algorithm.





Figure 18: Ram: number of objects detected



Figure 19: Ram: Iterations performed by each algorithm



## **Results - Biological sequences (Echinococcus)**



Figure 20: *Echinococcus*: Results of the complete algorithm (frames 14,15:5:45).



# **Results - Biological sequences (Echinococcus)**



Figure 21: *Echinococcus*: 3D reconstruction



## **Results - Biological sequences (Echinococcus)**



Figure 22: Echinococcus: evolution of the optimal weights



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## **Discussion - Conclusions**

- $\star$  In-depth study of a family of algorithms.
- ★ Two simple improvements over the orginial formulation: reinitialization and weighting.
- ★ Test over real sequences: good but improvable results.



# **Discussion - Improvements**

- $\star$  Weighting: extension to *M* regions.
- $\star$  Re-initialization: extension to *M* regions.
- ★ Validation: quantitative evaluation (TP,FP,TN,FN), extend database.



The end

### Thank you!





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- \* Acknowledgments



## **Results - Biological sequences (Crypt)**



#### Figure 23: *Crypt*: frames 1,2,6:5:31



## **Results - Biological sequences (Crypt)**



Figure 24: Crypt: evolution of the optimal weights



## **Results - Biological sequences (Crypt)**



Figure 25: Crypt: 3D reconstruction



# Discussion

#### Model:

- ★ Same tests with different types of PDF estimation (refinement).
- $\star$  *M*-regions: already introduced in the literature
- ★ Shape-priors: introduce shape information.
- ★ Integrate the information in a local manner: (e.g. regioncompetition on a neighborhood of the point): difficult to derive the energy.

#### Implementation:

- ★ Careful and efficient numerical analysis (Semi-implicit, highorder schemes)
- ★ Narrow band approaches: reduce dimensionality.



# Discussion

Tracking:

- ★ Introduce invariants: area, volume, shape, statistical parameters.
- ★ Smooth variation of the same measures

Features:

- $\star$  Weighting with the area of the regions.
- ★ Auto-selection of the wavelet sub-bands.
- ★ Intelligent diffusion: color restrictions, modulus and directions for optic flow.
- ★ Alternative approach: Vector Probability Diffusion.



# **Discussion - Improvements**

#### ★ Re-initialization:

- Extension to *M*-regions: distance measure to each other region (no background). Easy in the case of *M* fronts, challenging in the multiphase case
- No clear notion of outside for the re-initialization algorithm
- Relies on EM accuracy.
- User contradiction: local minima sometimes wanted (bus sequence). Features with poor separability.
- ★ Weighting:
  - Extension to N-regions: cluster separability measure instead of distance between two regions (distance matrix).
  - Exahustive comparison on PDF metrics.
  - Memoryless estimation.



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