3D Processing and Analysis with ImageJ

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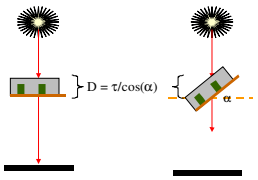
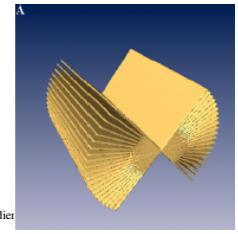
Introduction

- Rapid development of technologies yielding multidimensional data
  - LSCM / Video
  - Electron tomography
  - IRM / CT scan

Introduction

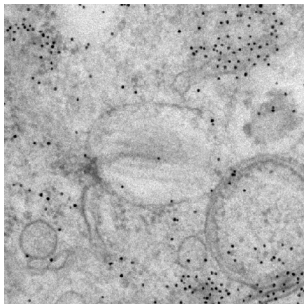
- Electron tomography
  - Series of tilted projections
  - Reconstruct original volume





Introduction

- Set of 3D projections
  - -60° to 60°
- Melanosomes
  - © Hurbain et al. Electron tomography of early melanosomes PNAS 2008.

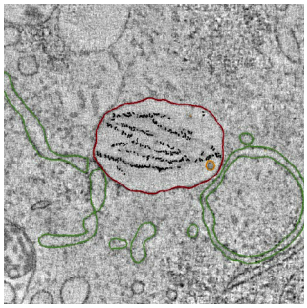


Introduction

- Reconstruction
  - 3D volume
- Melanosomes
  - © Hurbain et al. Electron tomography of early melanosomes PNAS 2008.

Introduction

- Modelisation
  - Drawing of 3D structures
- Melanosomes
  - © Hurbain et al. Electron tomography of early melanosomes PNAS 2008.



Introduction

- Steps of analysis :
  - Reading data
  - Visualization
  - Processing
    - Reduce noise
    - Enhance objects
  - Segmentation
  - Analysis

Reading data

- 3D data :
  - X-Y + Z
  - Stack of 2D images
- 4D data :
  - 3D data + time
- 5D data :
  - 3D data + time + channel

Reading data

- A set of 2D files
  - Import image sequence
- One file storing all images
  - Tiff
  - Proprietary formats
    - Stk, lif, zvi, dm3, …
- → Use LOCI Bio-Formats plugin

2D visualisation

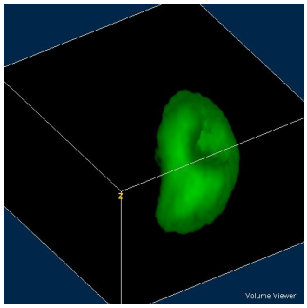
- Only one slice is displayed
  - Adjust brightness/contrast
- Normalize values for all slices
  - Thickness increase in tomography
  - Bleaching in fluo

2D visualisation

- Reslicing
  - Coronal, horizontal and sagittal sections
- Different spacing XY and Z
  - Interpolation
- Isotropic data
  - Electron tomography

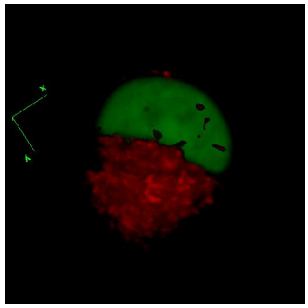
3D visualization

- Volume Viewer plugin
  - Interactive cross-sections
  - Volume rendering
- Volume Slicer
  - Macro for making animations



3D visualization

- ImageJ 3D Viewer
  - Volume and surface rendering
  - Multiple data
  - Registration
  - Transparencies
  - 4D data
  - ...



3D processing

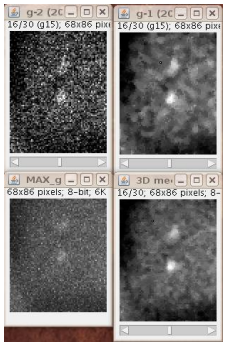
- 2D filtering slice by slice
- 3D filtering
  - A sphere of a given radius
  - Usual filters : mean, median, min, max, …
- Time-consuming
  - Use of JNI or multi-threading

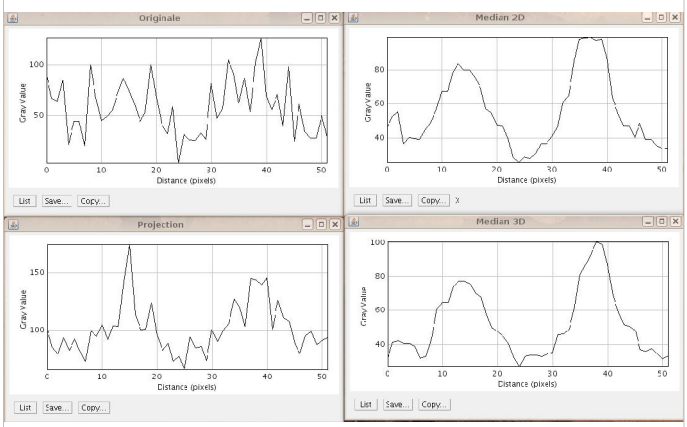
3D processing

- Noise reduction
  - Objects same location in consecutive slices
  - Not noise
- Common filters :
  - Mean, gaussian
  - Median
- « Enhanced » filters : sigma or shift
- Anisotropic filtering

3D Processing

- 2D vs 3D median processing
  - Radius = 2



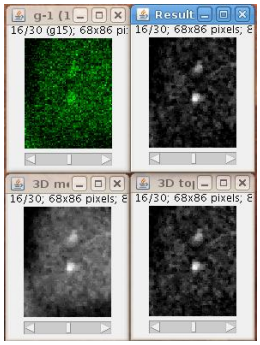


3D processing

- Enhance objects
  - « Hard » smoothing to homogeneize values inside the objects
- Bright spots detection
  - Tophat filtering
    - Minimum filtering (supress bright spots)
    - Maximum filtering (compute background)
    - Difference between original and background
    - → spots

3D processing

- 2D vs 3D top hat processing
  - Radius = 7

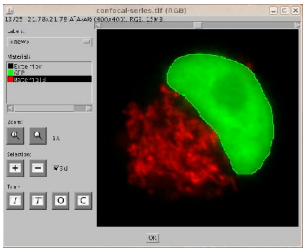


3D segmentation

- Detection of 3D objects
  - 3D objects may be quite complex (ex: golgi)
- Manual segmentation
  - Set of ROI
  - Segmentation Editor
- Manual binarization
  - Threshold each slices independently
    - Only 2D objects

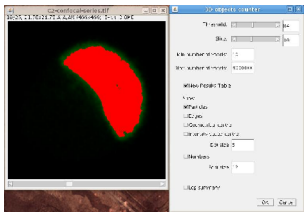
3D segmentation

- Segmentation Editor
  - Draw structure on each slice
  - Display 3D structure
- LiveWire Tool
- see TrackEM2



3D segmentation

- Manual binarization
  - Create a 3D object by connecting 2D cross-sections
- 3D Object Counter
  - One treshold for all slices



3D segmentation

- Mathematical morphology
  - Two basic operations
  - Erosion and dilatation
- Improve binarization
  - Smooth objects (close)
  - Separate objects (open, watershed)
  - Fill holes inside objects
-

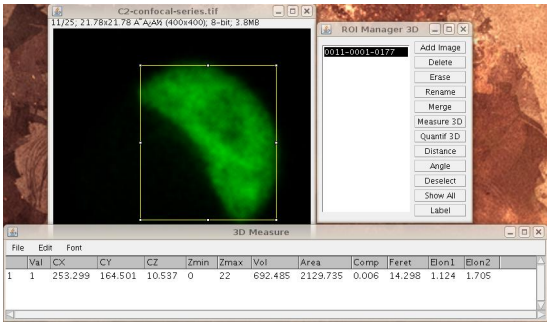
3D analysis

- Geometrical features
- Distances
- Intensity features
- Surface analysis
- Granulometry

3D analysis

- Geometrical features
  - Volume, surface, center
    - Can be computed from 2D slices
  - Feret's diameter
    - Needs 3D computation
  - Ellipsoid fitting
    - Main axes
    - Main and median elongation

3D analysis



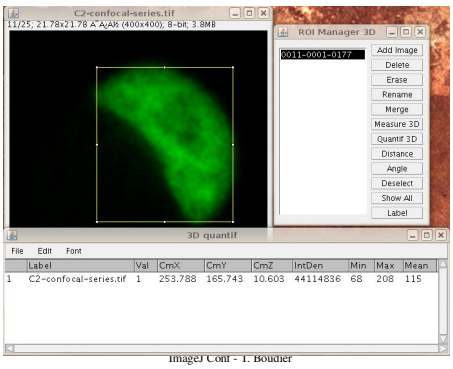
3D analysis

- 3D distances
  - Center to center
  - Center to border
  - Border to border
  - Distances along a direction

3D analysis

- Intensity features
  - Integrated density
  - Mass centers
  - Statistical values
    - Mean, variance, min, max
    - Intensity distribution

3D analysis

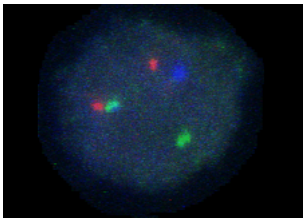


3D analysis

- Surface analysis
  - Curvatures computation
  - Complex mathematics
- Granulometry
  - Series of opening anf closing
  - Objects sizes analysis
  - Distribution of distances between objects

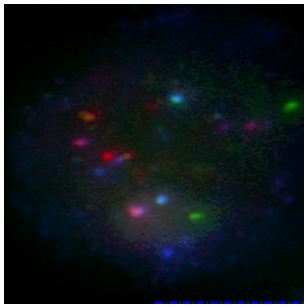
3D analysis

- Example :
  - 3D F.I.S.H
  - Intergenic distances
  - Interaction with CTs



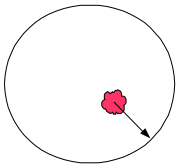
3D analysis

- Example :
  - multi F.I.S.H
  - 7 genes with colocalization



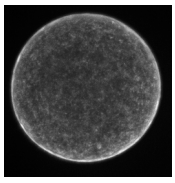
3D analysis

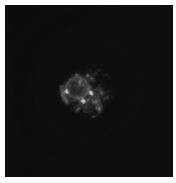
- Example :
  - Spindle positioning in ovocytes
  - Compute possible poles
  - Check if spindle moves towards closest pole



3D analysis

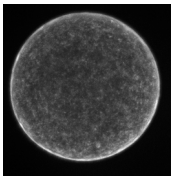
- Example : Spindle positioning in ovocytes

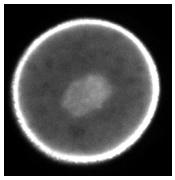




3D analysis

- Example : Spindle positioning in ovocytes





Conclusion

- Growing multidimensional data
- 3D visualization is not more the big issue
- 3D Processing is related to 2D processing
  - 2D filtering slice by slice may be an alternative
- 3D analysis may be a bit more complex than 2D analysis
  - However biology is mainly (only ?) 3D (4D ?)