Project Proposal

- Image analysis methods are important to extract relevant information from biomedical image data
  - ↳ typical tasks: image preprocessing, feature extraction, segmentation, object recognition, tracking and image registration

Topic 01 Team 04

1. implement and evaluate a method for segmentation of cell nuclei
   - ↳ Otsu Thresholding
2. implement evaluation measure and test using synthetically generated images
   - ↳ Dice Score
3. apply Otsu Thresholding to cell nuclei images and compute the average Dice Score
4. extend the implemented segmentation method by local thresholding using a sliding window scheme
5. individual creative ideas
   - ↳ cell nuclei counting, extend Otsu method to two level Otsu's thresholding

Structure Project Proposal

- $\frac{1}{3}$ Data analysis and Discription: Data set, Data types, differences ground truth vs. normal image, challenges (Reflections...), pixels, intensity values, explain source
  → standard data
  ↳ how to deal with challenges, problems...
  ↳ show images...

- $\frac{1}{3}$ Algorithm implementation: explain algorithm, explain Dice score, explain sliding window scheme, procedure
  → show ideas of programming (f. ex. tests, ideas...)
  ↳ use flow diagrams
  ↳ procedure, steps

- $\frac{1}{3}$ Project Management: milestones, timeling, exercise division, use function „Project, issues", implement pictures from GitHub → professional layout!

Dataset discription and analysis → 3 different datasets

→ all datasets are publicly available to enable others to use it as a benchmark for newly proposed algorithms

↳ standard data, which is provided to evaluate nuclear segmentation algorithms

① N2DH-GOWT1 cells:

- 6 images, size 1024 x 1024 pixels
- the dataset of N2DH-GOWT1 of the cell tracking challenge contains images of GFP transfected GOWT1 mouse embryonic stem cells
- captured using timelapse confocal microscopy (Leica TCS SP5 microscope)
- intersity values:
- 10-20 cell nuclei per image
- varying brightness of cells makes it difficult to distinguish the cells from the background
- low contrast and noise challauge the segmentation algrithm

GFP-GOWT1 mouse stem cells

Dr. E. Bártová, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Training dataset: http://data.celltrackingchallenge.net/training-datasets/Fluo-N2DH-GOWT1.zip* (53 MB)

Challenge dataset: http://data.celltrackingchallenge.net/challenge-datasets/Fluo-N2DH-GOWT1.zip (46 MB)

Less details

Microscope: Leica TCS SP5

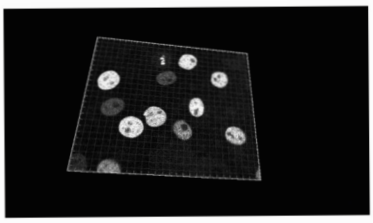
Objective lens: Plan-Apochromat 63x/1.4 (oil)

Pixel size (microns): 0.240 x 0.240

Time step (min): 5

Additional information: PLoS ONE, 2011

Mouse (mus musculus)
Embryonic stem cells
GFP = Oct4



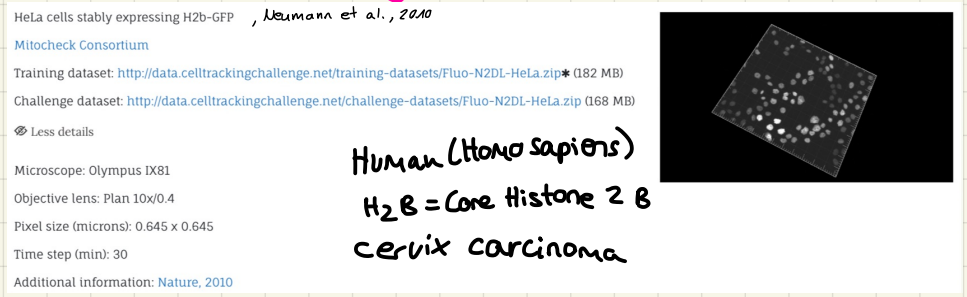
② NIH3T-cells:

- 18 images, size 1344 x 1024 pixels
- the dataset NIH3T3 contains images of mouse embryonic fibroblast cells, stained with Hoechst
- captured using fluorescence microscopy
- intensity values:
- 60 cell nuclei per image
- certain bright spots (reflections) challenge the segmentation (reduce noise with median filter and remove small non-nuclear objects by filtering out objects smaller than 2500 pixels)

(mouse (mus musculus)
Embryo fibroblast
EGFP = CD-tagged-Protein)

③ N2HL-HeLa cells:

- 4 images, size 1100 x 700 pixels
- the dataset N2DL-HeLa of the cell tracking challenge contains images of human epithelial cells of cervical cancer and shows HeLa cells stably expressing H2b-GFP
- captured with an Olympus IX81 microscope used for live imaging of fluorescently labelled chromosomes
- intensity values:
- 30-50 cell nuclei per image
- variable brightness of the image challenges the segmentation algorithm



⟹ the 3 different datasets show different features like variable brightness, reflections, low contrast and noise that challenge the segmentation algorithm (Otsu thresholding)

↳ <u>solution</u>: use different preprocessing methods (Median filter, Gaussian filter, Histogram stretching) for certain groups of data to optimize the segmentation results

→ find out which preprocessing method is the best for each dataset

Abstract → cell tracking

In developmental biology, knowledge of cell structure and their (morpho) dynamic behavior, leads to a comprehensive understanding of their conducts and the mechanisms in which they participate. This knowledge is a decisive factor in biological research and also in all drug development steps, medicinal or preventive therapies. Experimental cell analysis is hard, expensive, and time-consuming. To overcome these difficulties, in recent years, several computational object tracking methods, software system and packages have been developed in cell sciences that bring together different disciplines and branches of technologies.

Object tracking is the process of locating and monitoring specific object and its behavior in sequential images. In this paper, a comprehensive review on object tracking stages and computational methods that are utilized in terms of cell tracking has been organized. Besides, the available software packages and toolkits, challenges, and their solution in time lapse microscopy images in this scope were reviewed. The aim of describing computational cell tracking methods and tools is that biologist and cell scientists might take advantage of these computational techniques to find another method to gain complementary information for their question of interest.

Data type - Ground truth

- ground truth images show the "true and accurate" segmentation
  ↳ data is assumed to be correct
- ground truth images are used as reference images to quantify how good our final automated segmentation is

⇒ evaluate the accuracy of our obtained segmented image by using metrics (Dice Score, MSD, Hausdorff Distance) which compare the obtained segmented image to a reference image (ground truth)

Algorithm implementation - Otsu thresholding

① first step

Input (load data) → Otsu thresholding → compare obtained image to ground truth by using the dice score

↓

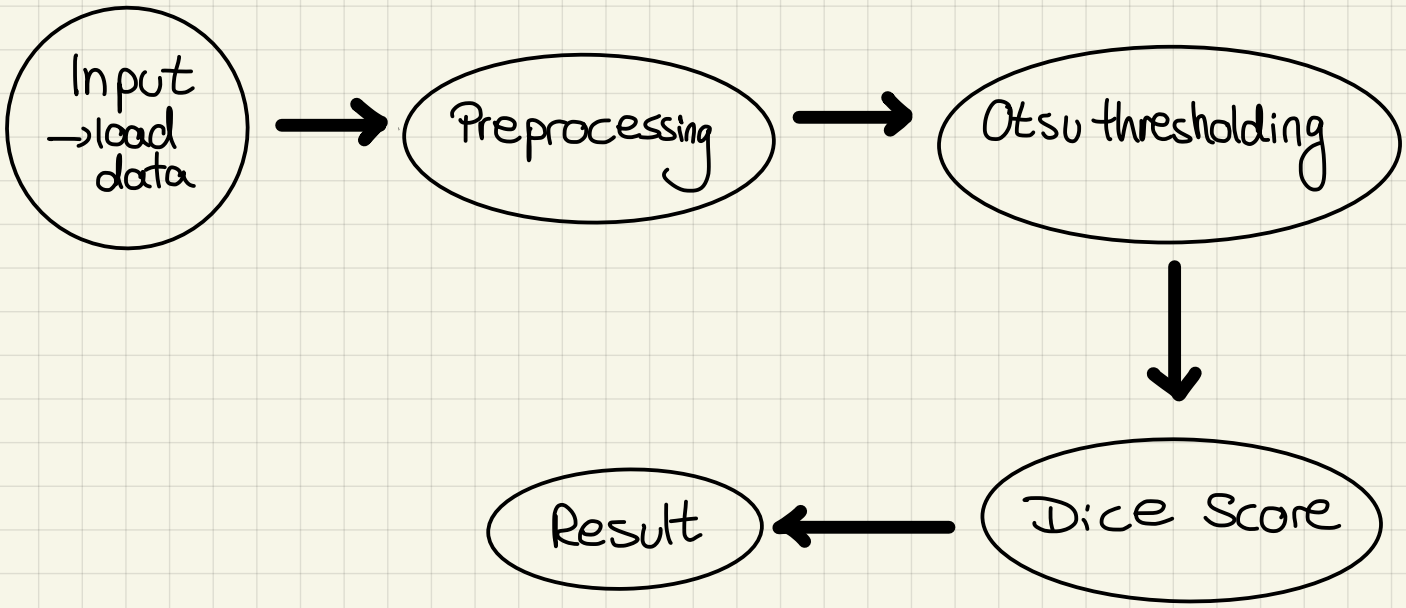
Result

② second step

find preprocessing methods to reduce factors like noice, brightness and low cotrast, which influence the accuracy of the implemented segmentation algorithm → apply tests for each dataset and evaluate each dice score

③ third step

↳ basic Procedure:



④ fourth step

→ extend Otsu thresholding to two-level thresholding

→ extend Otsuthresholding to local thresholding using a sliding window scheme

→ implement cell counting

⇒ explain Otsu thresholding

⇒ explain Dice score and evaluation methods

⇒ explain sliding window scheme

⇒ programming ideas, flow diagramm

Project Management

Milestones:

1. Biological background, input of images
2. visualization of data → analyse data and identify problems or challenges (reflections, low contrast, brightness...)
3. Implementation of Otsu thresholding
4. Implementation of Dice Score and other evaluation methods
5. apply Otsu's method on each dataset and compare the obtained segmented image to the ground truth by using Dice Score and other evaluation methods
6. apply different preprocessing methods on each dataset and evaluate theire efficiency
7. implement local thresholding → sliding window scheme
8. implement two-level thresholding and test its efficiency on each dataset
9. Results...
10. implement cell counting