

Automation of quantitative muscle cell analysis by image segmentation

Zdenka Uhríková

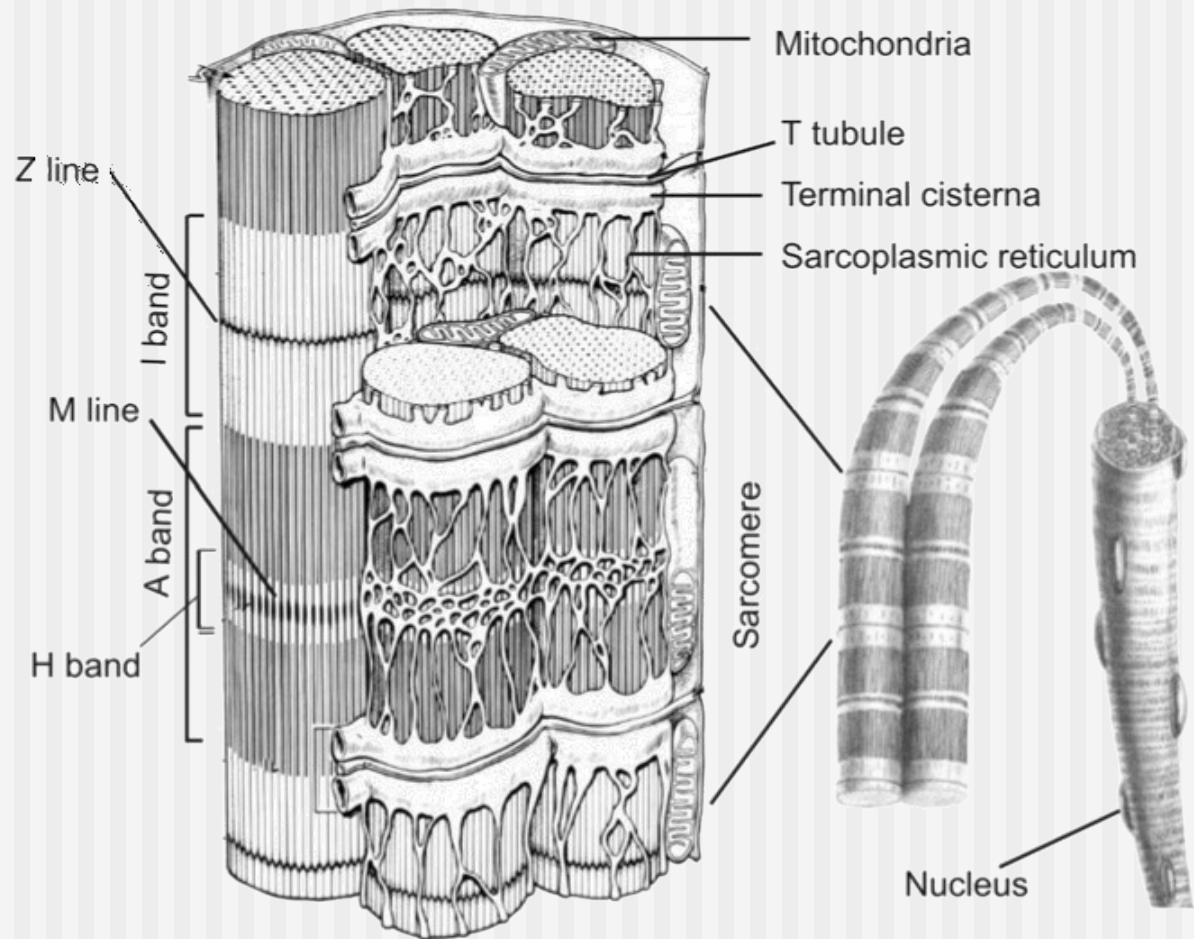
Supervisor: Mgr. Július Parulek

Overview

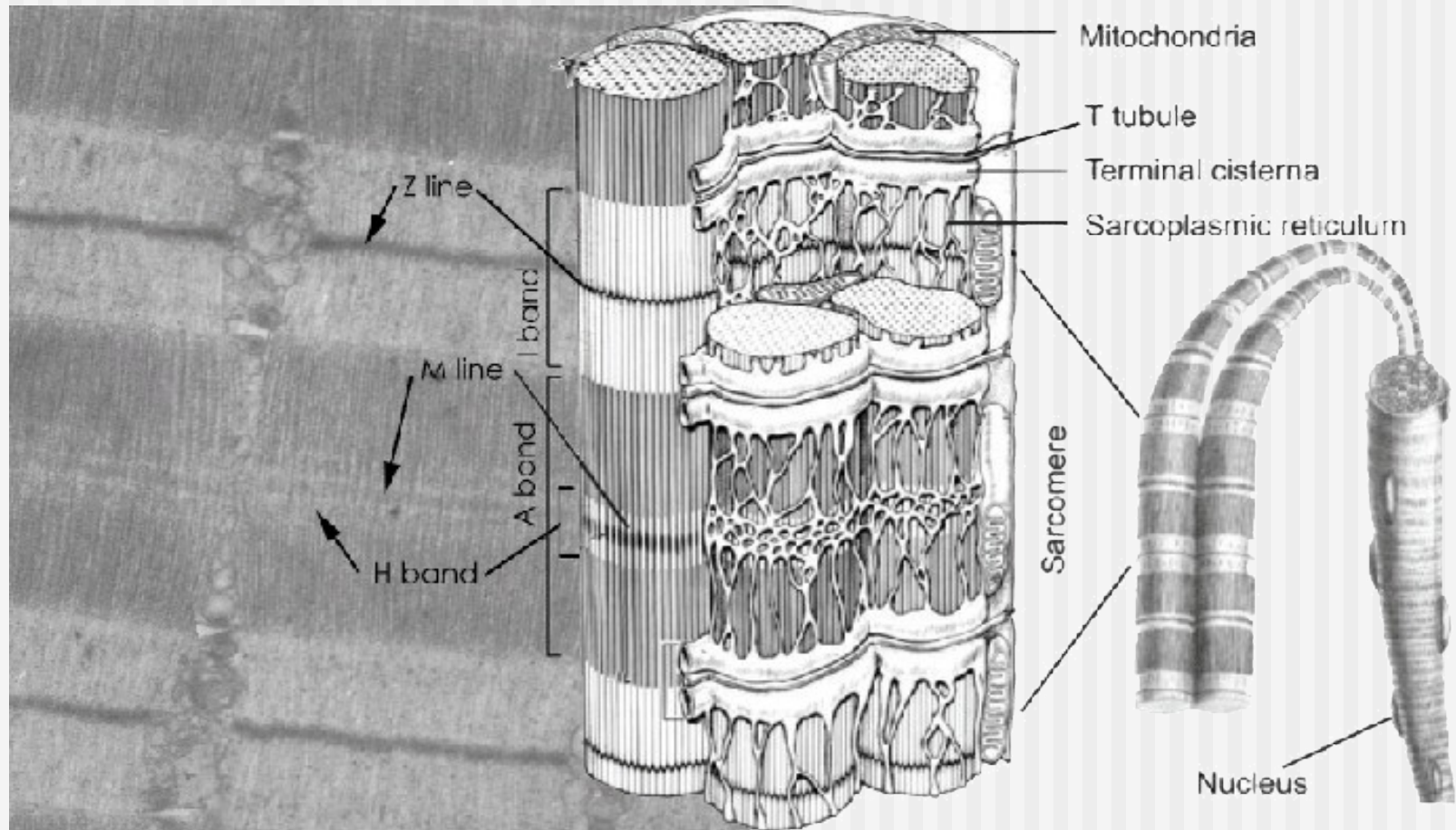
- Introduction
 - Muscle fiber structure
 - Classic quantitative analyze
- Automatic quantitative analyze
 - Fiber direction
 - Mask based thresholding
 - Boundaries detection
 - Length measuring
- Results and Conclusion

Muscle fiber structure

- Z line
- A band
- I band
- Mitochondria
- T tubules

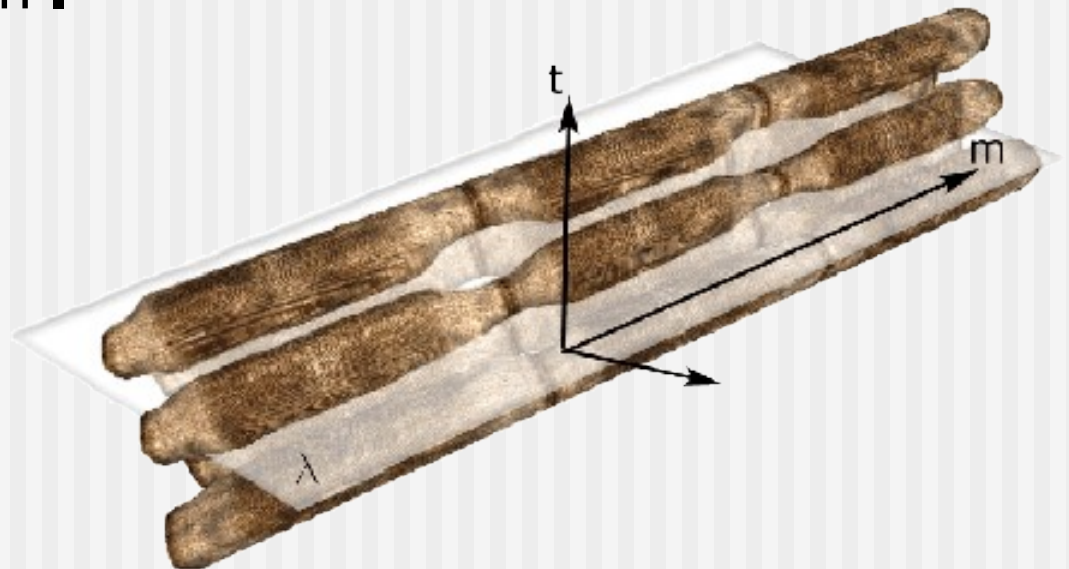


Muscle fiber structure



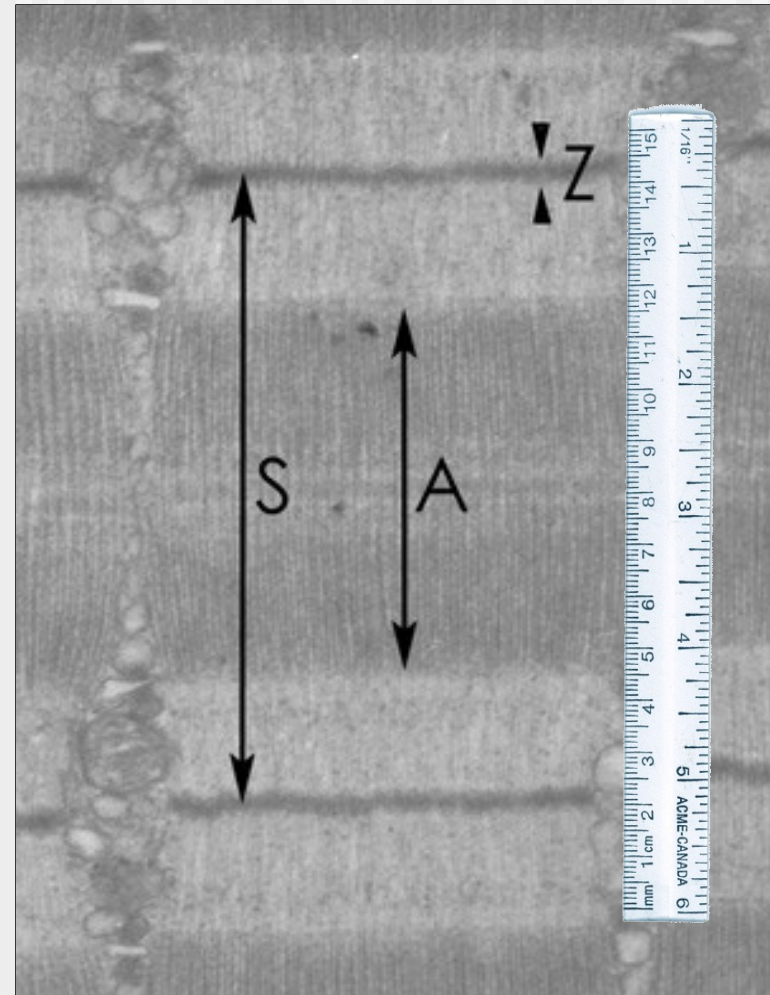
Fiber direction

- Longitudinal direction is defined by the main fiber axis m
- Transversal direction t
- Longitudinal section \parallel



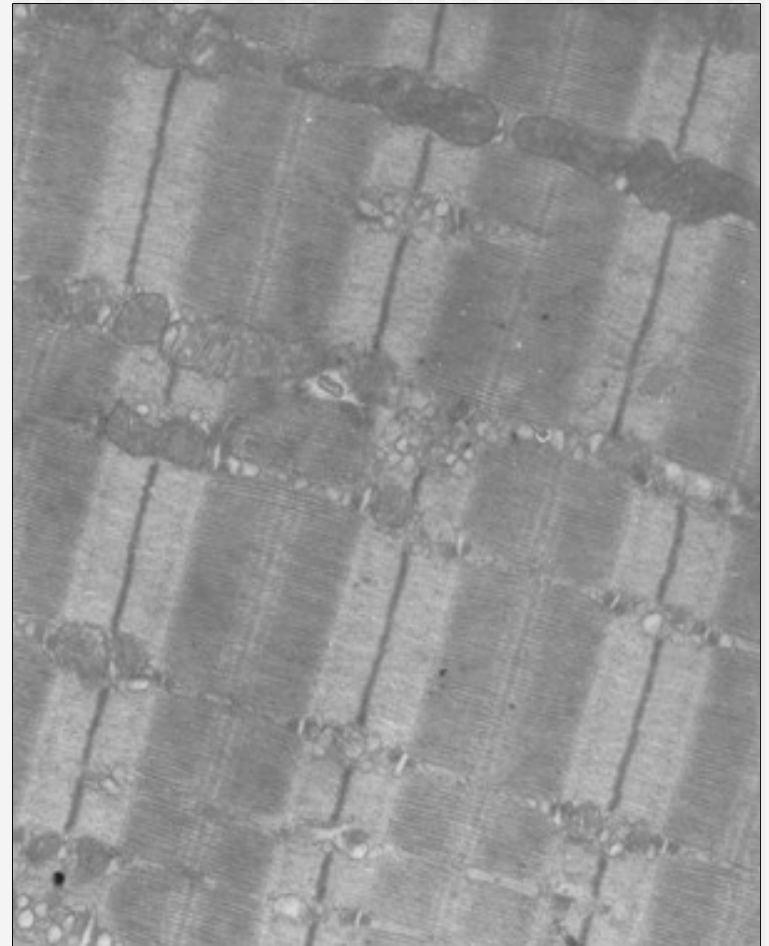
Classic quantitative analyze

- Morphometric data:
 - Sarcomere length
 - A band length
 - Z line length
- All lengths are computed in longitudinal direction



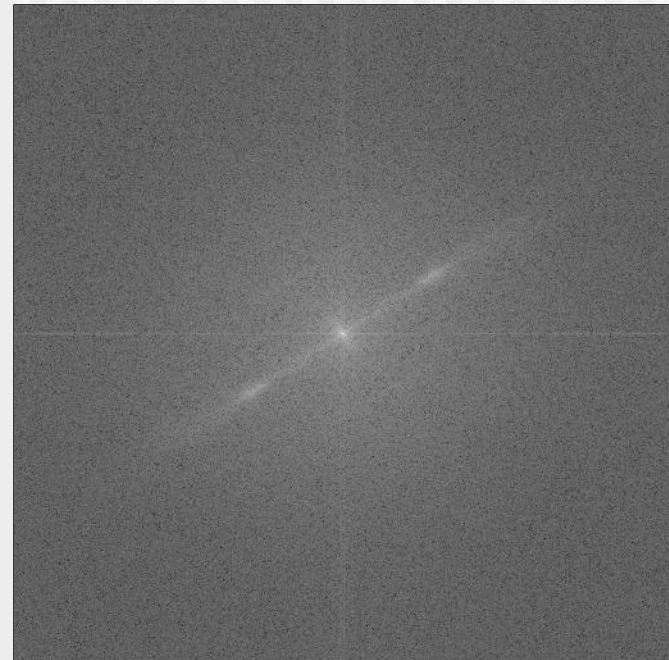
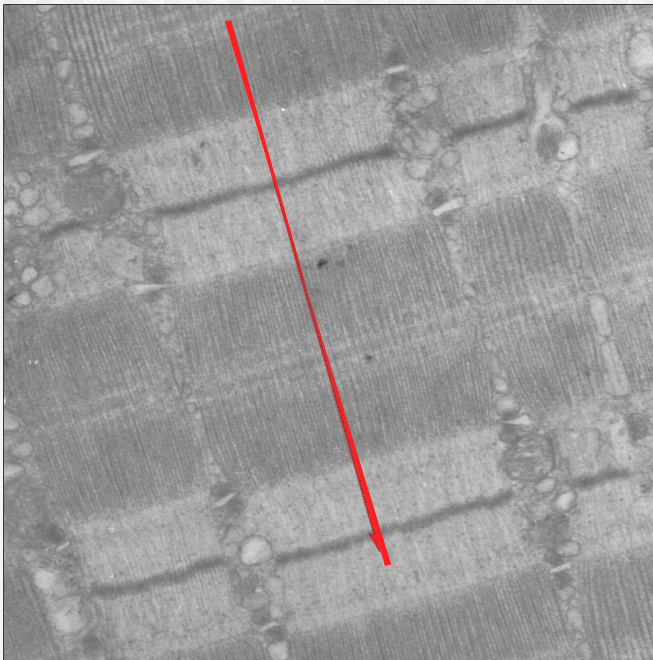
Automatic quantitative analyze

- Automate the manual processing of images
- Defining fiber constituents
- Provide morphometric measuring



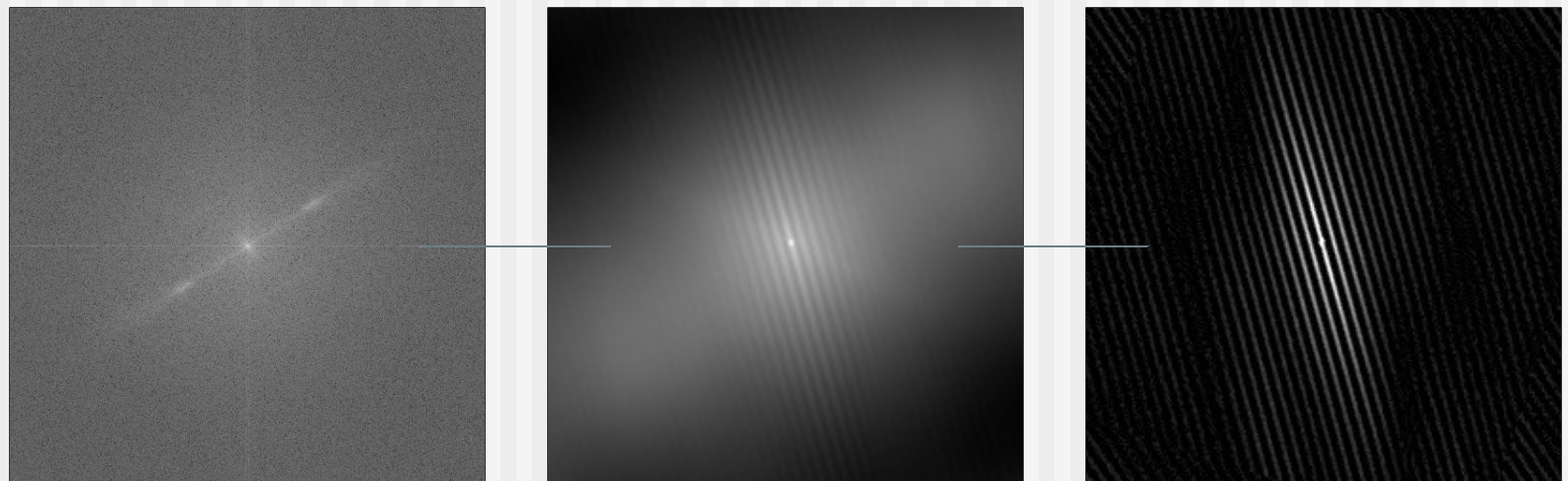
Fiber direction detection

- Using image periodicity, we apply Fourier transform to detect the direction



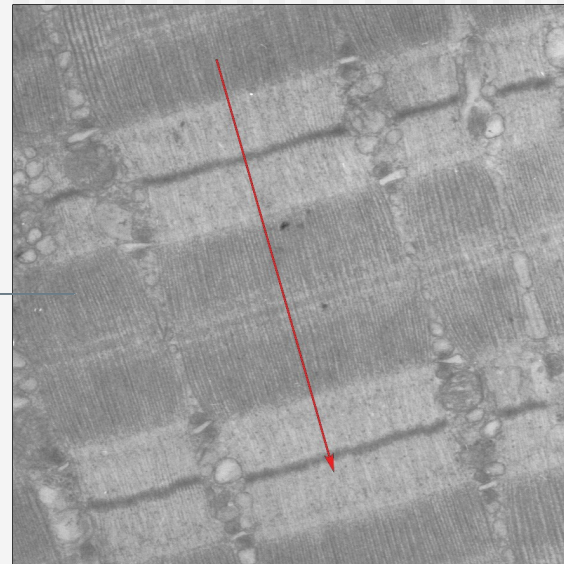
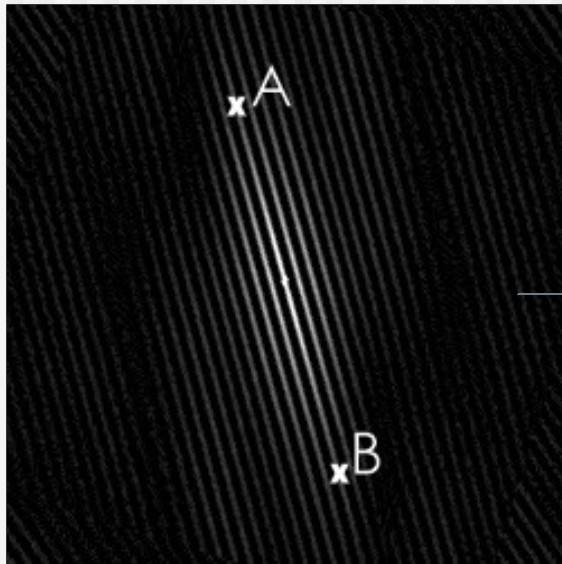
Fiber direction detection

- Sharpening by conjugate multiply of frequency domain image
- Applying inverse Fourier transform
- Convolve mask to make image clearer



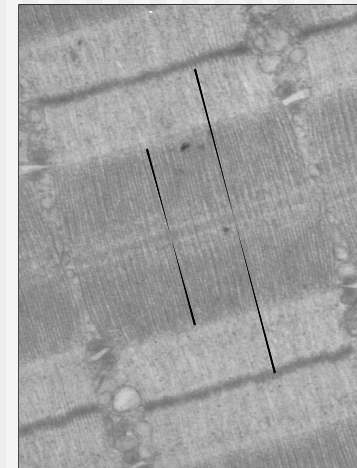
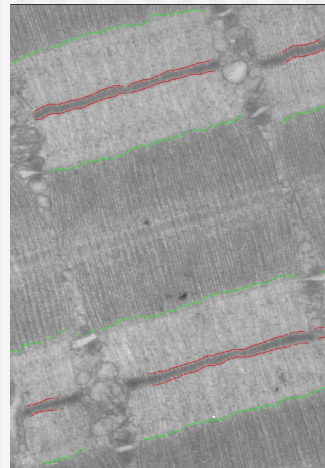
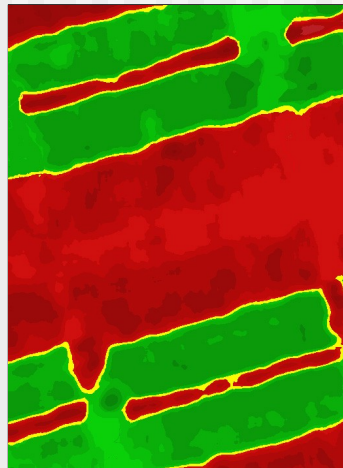
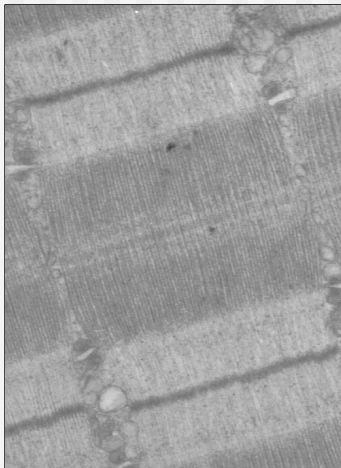
Fiber direction detection

- Direction: Line passing through center is defined by two points A and B
- Finding A and B by following high intensity points



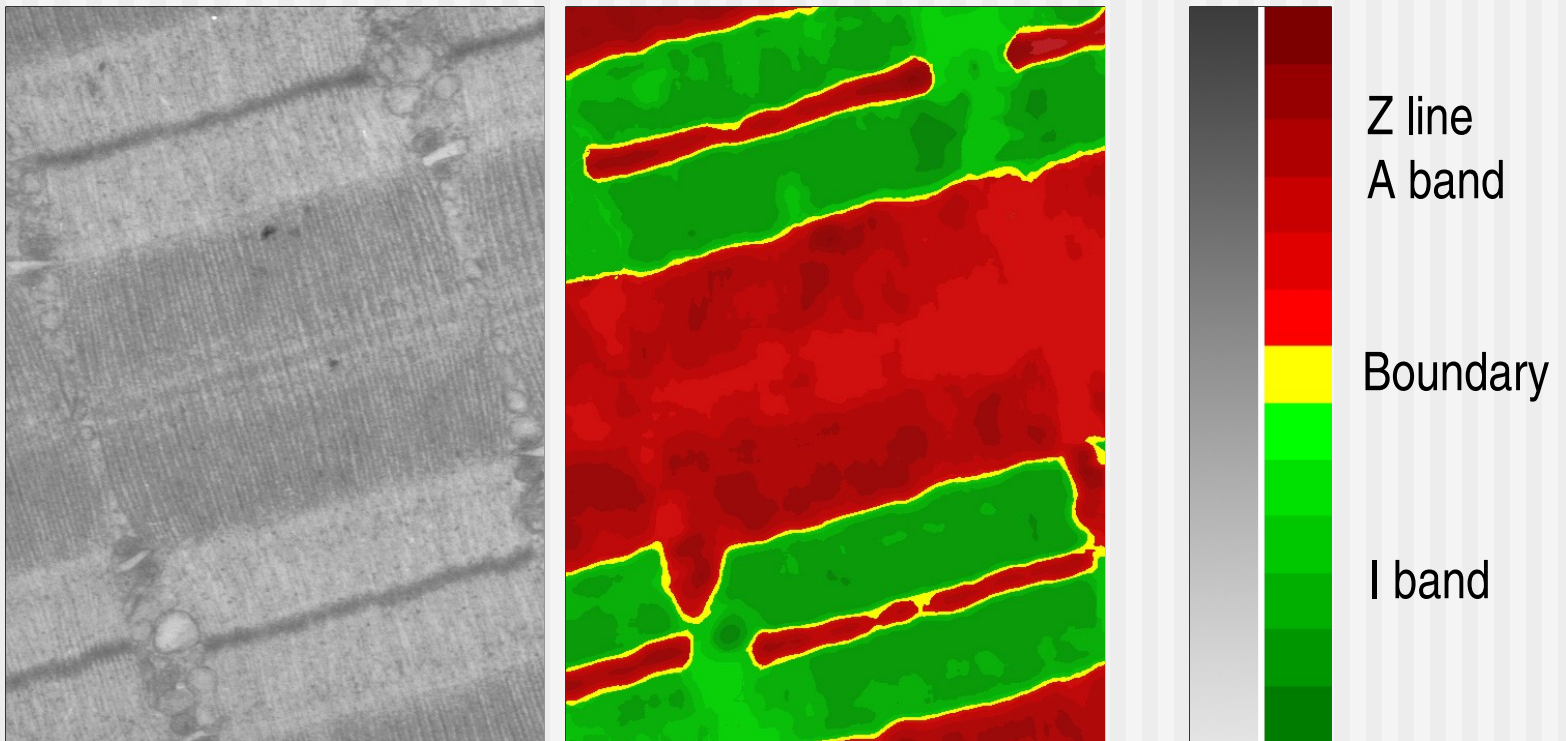
Segmentation and quantification

- Image decomposition - 3 areas
- Longitudinal direction and areas are used to detect boundaries
- Boundaries provide measurement of lengths



Mask based thresholding

- Image is divided into three color zones



Mask based thresholding

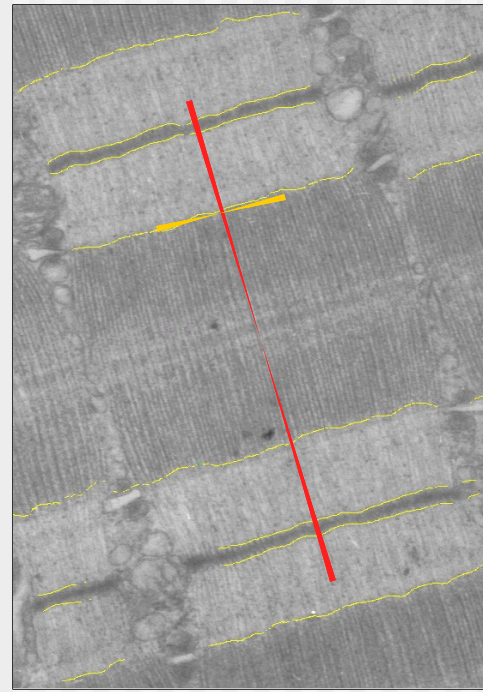
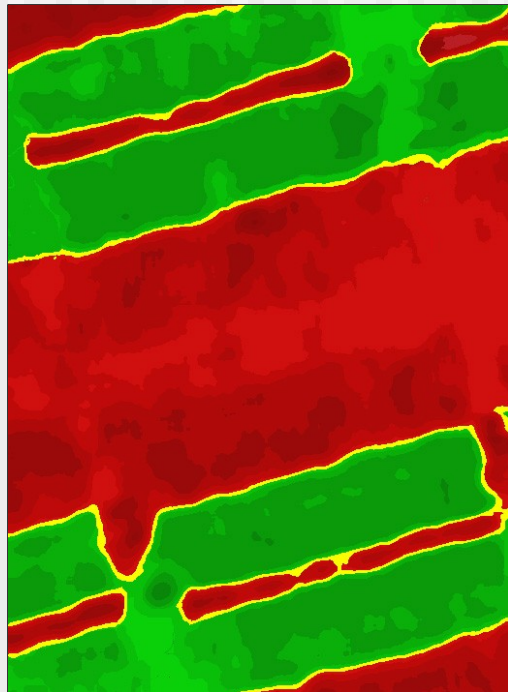
- Each point T belongs to a single zone, which depends on its 5 values (m^k , where $k=1..5$) obtained from 5 different masks

$$T_{ij} = \begin{cases} yellow & \text{if } \exists k : m_{ij}^k = \bar{m} \\ red & \text{else if at least 3 } m_{ij}^k \text{ are less than } \bar{m} \\ green & \text{else if at least 3 } m_{ij}^k \text{ are more than } \bar{m} \end{cases}$$

- Threshold value m is mean of all point intensities

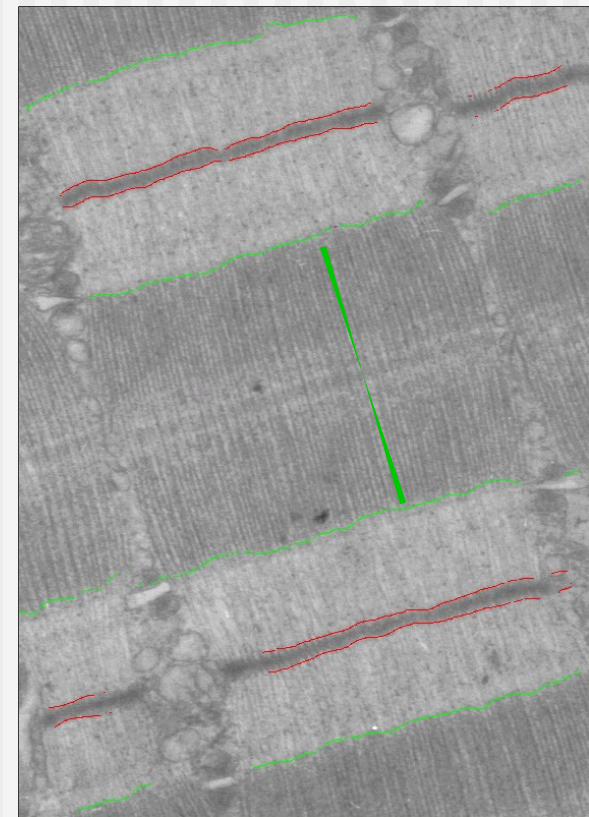
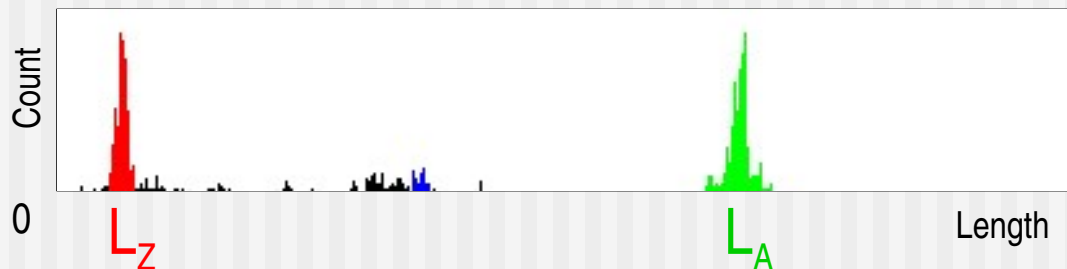
Boundaries

- Boundaries are extracted from yellow area
 - They must be perpendicular to the longitudinal direction



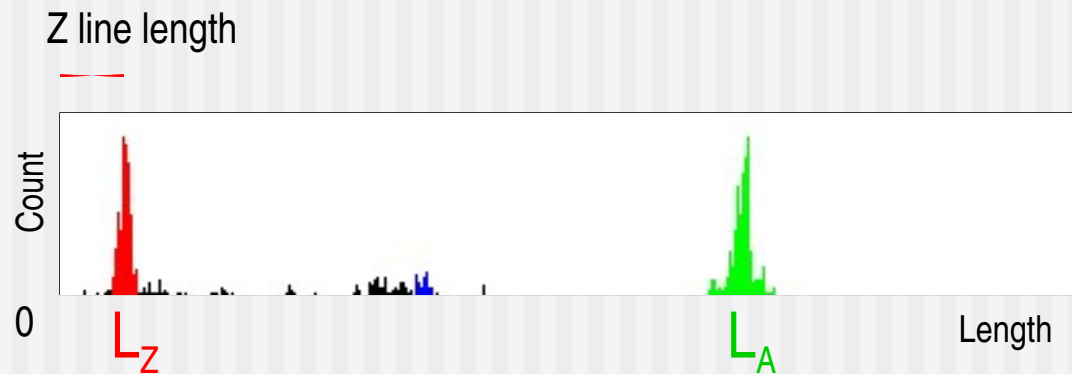
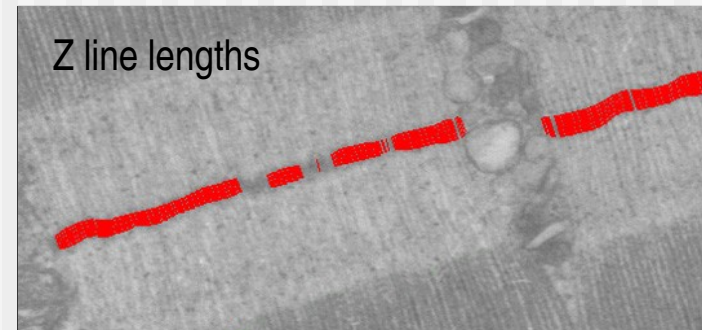
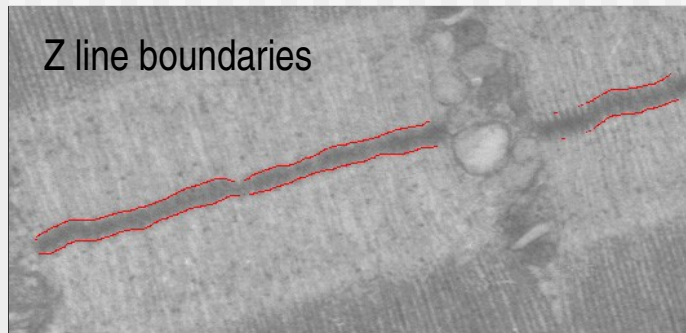
Estimation of distances

- Distance of two neighboring boundaries in longitudinal direction
- These distances create bimodal histogram
 - First peak determines length of Z line (red) - L_Z
 - Second peak determines length of A band (green) - L_A



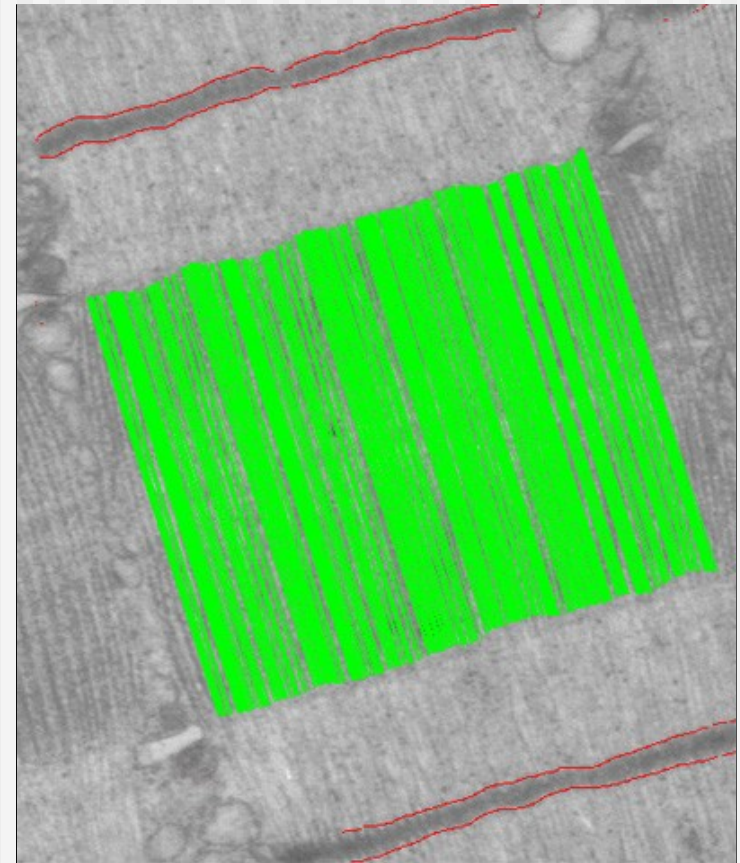
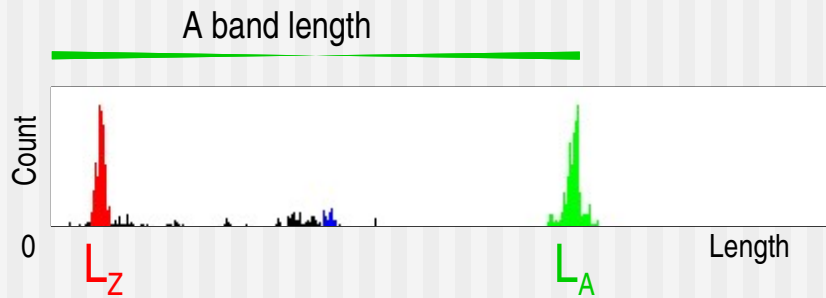
Z line length

- The value L_z determines length of Z lines



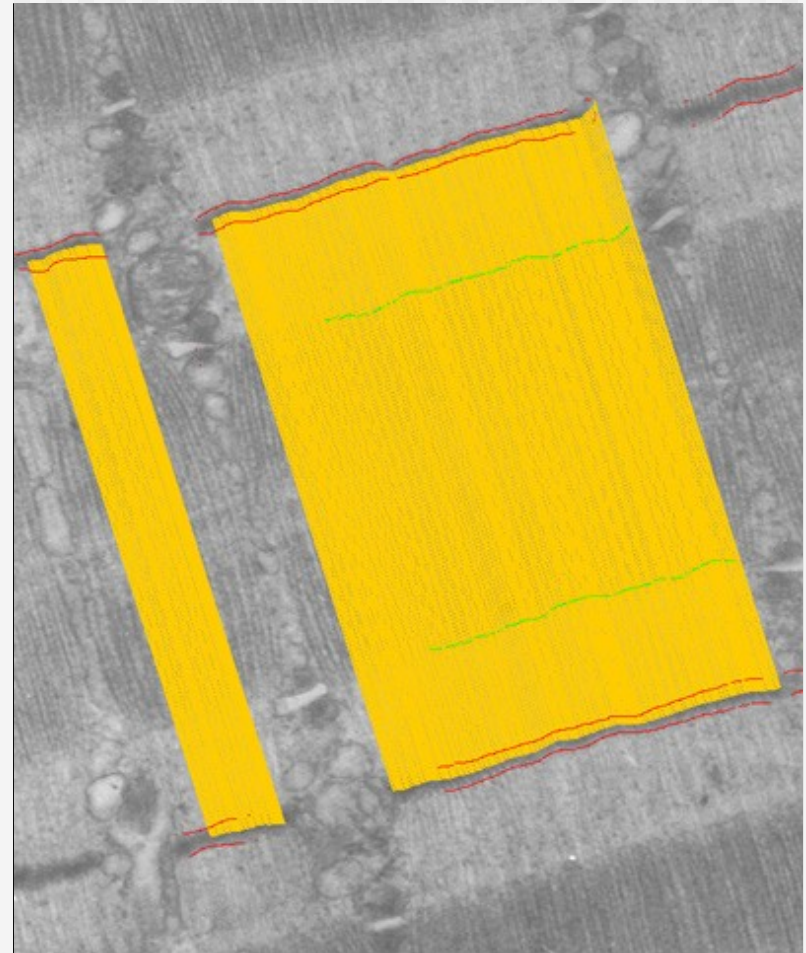
A band length

- The value L_A determines length of A band



Sarcomere length

- Distance between two Z lines
- Measured from first Z line center to second Z line center



Results

- Working with two sets of images
 - Totally 15 images
 - Two different types of muscle cells
- Results compared with manually obtained measurements of
 - Z line
 - A band
 - Sarcomere

Results

#1	L_m	L_a
S	1580	1578
A	992	989
Z	39	39

#2	L_m	L_a
S	3049	3050
A	1222	1223
Z	84	84

Results are in nanometers, 1px=3,5nm.

Difference between manual and automatic measuring is less than 1 pixel.

Over 500 measuring of one value was taken.

L_m : manually measured length

L_a : automatically measured length

S: length of Sarcomere, A: length of A band, Z: length of Z line

Conclusion

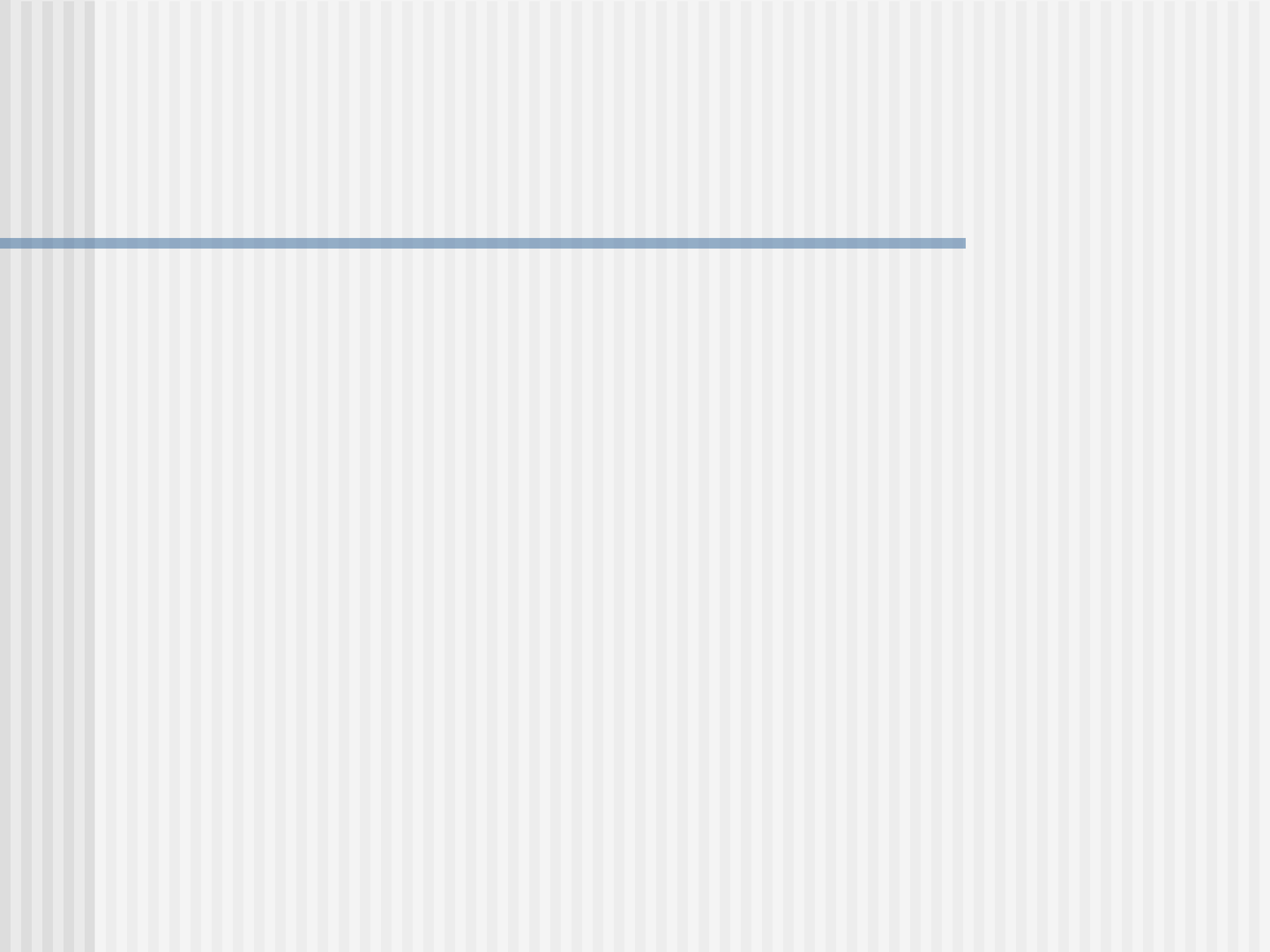
- We introduced algorithm for automatic measuring
 - Fiber direction, Segmentation, Boundaries
 - Lengths of A band, Z line and sarcomere were measured
- Results are very close to those obtained manually
 - T-test – our results are not significantly different from manually obtained results

Future work

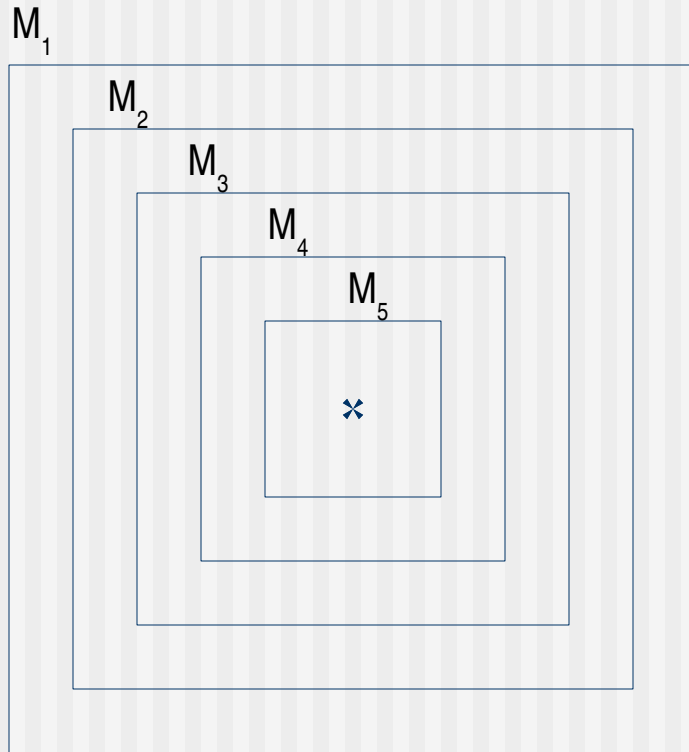
- Optimization of segmentation
 - A band
 - Mitochondria
- Provide stereological measuring
 - Volume and surface ration of observed organelles

Thank You.

zdenka.uhrikova@gmail.com

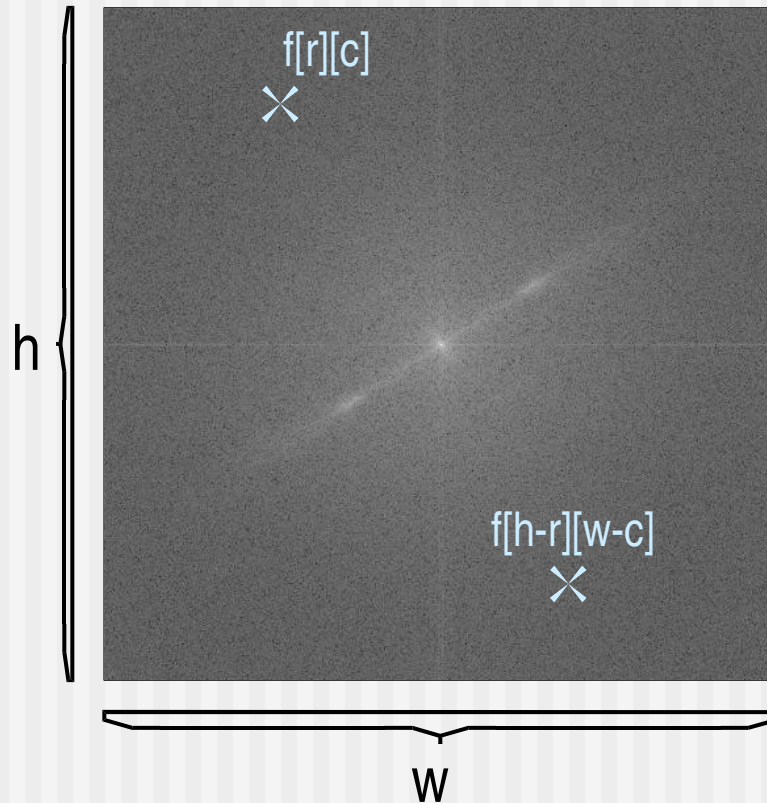


Masks



Conjugate multiplication

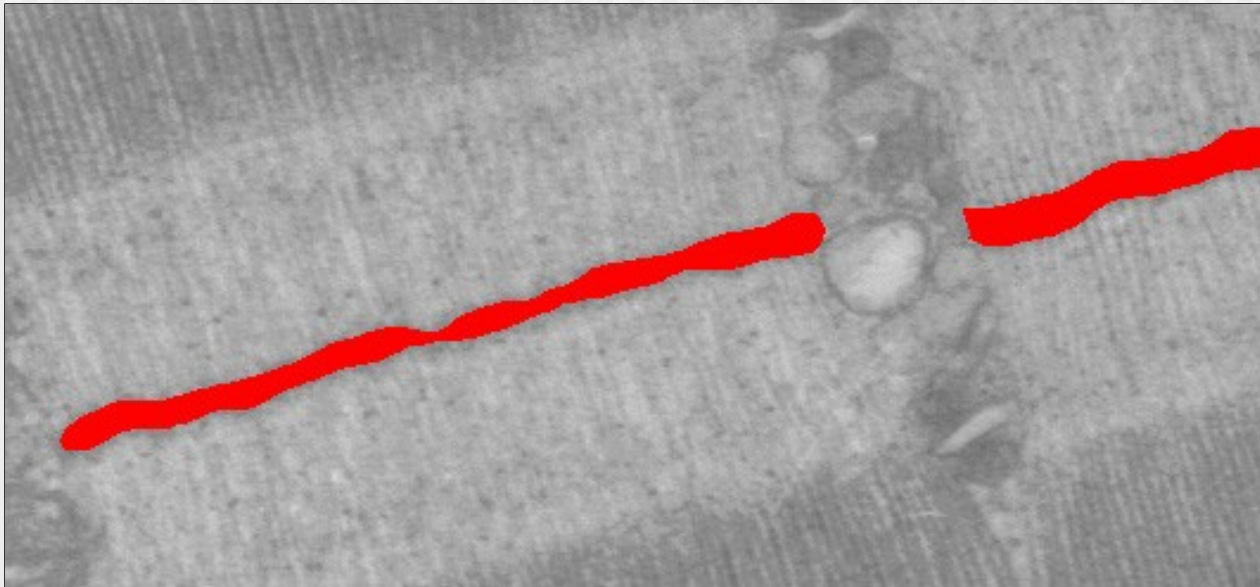
- $F[r][c] = \frac{1}{2} * f[r][c]^2 + \frac{1}{2} * f[h-r][w-c]^2$



Students T-test

- Widely used in biological sphere
- Allows to compare two sets
 - Paired T-test
- Two sets are significantly different
 - Depending on resulting probability P and defined threshold probability
 - $P > 0.05$

-
- Z line detection using thresholded image



Histogram

