

Development of methods for automatic object recognition in microscope images of biomedical specimens

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Chair for Biomedical Technical Systems

- Scientific research
 - Biomedical image processing and pattern recognition
 - Microscope images processing
 - Other images processing
 - Pattern recognition
 - Biometrics
 - Optical methods for medical diagnostics
 - Electronic devices for medical diagnostics
 - Biomechanics

The tasks of microscopic examination of biomedical specimens

- the number of objects in a microscope image calculation,
- morphometry of objects evaluation (sizes, shapes, etc.),
- color and textural features of objects analysis.

visual analysis
(lab. assistant + microscope)



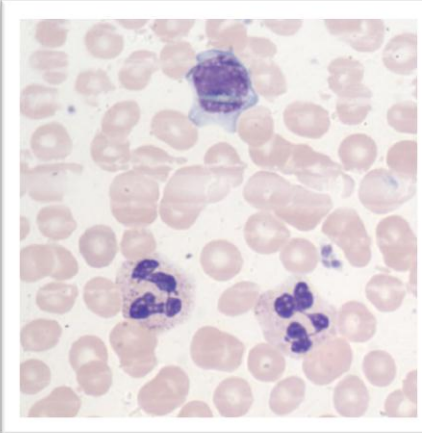
1. Significant differences in test results between different laboratories.
2. Small amount of analyzed objects.
3. Subjectivity.

automated microscopic systems (microscope
+ camera + spec. software)

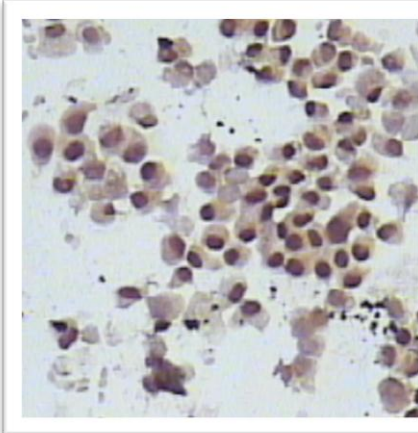


1. Repeatability and reliability of the results.
2. Ability to evaluate complex characteristics.
3. Objectivity of the analysis.
4. Reducing the complexity for the laboratory assistant.

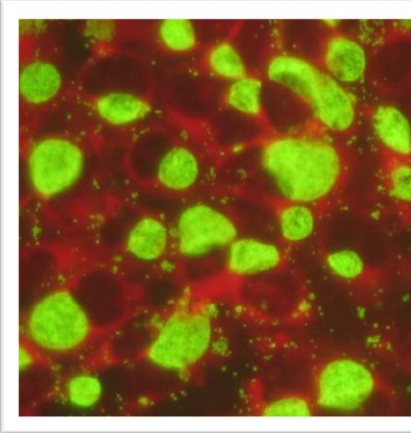
Applications of biomedical specimens microscopic analysis



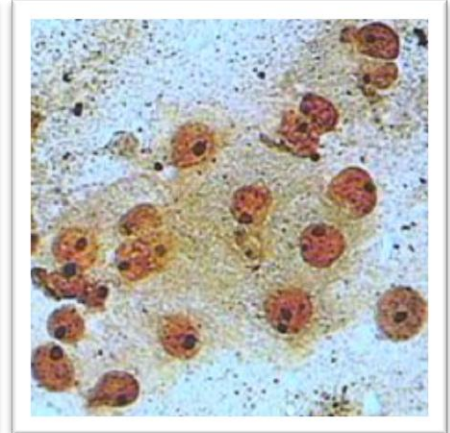
hematology



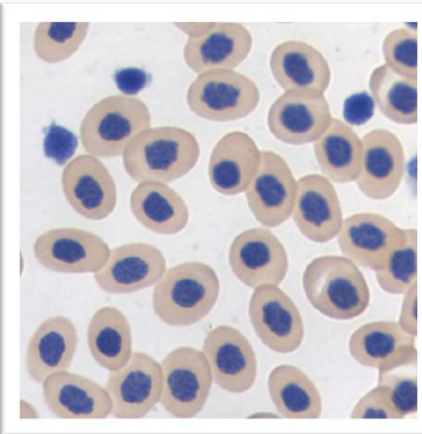
cytology (oncology)



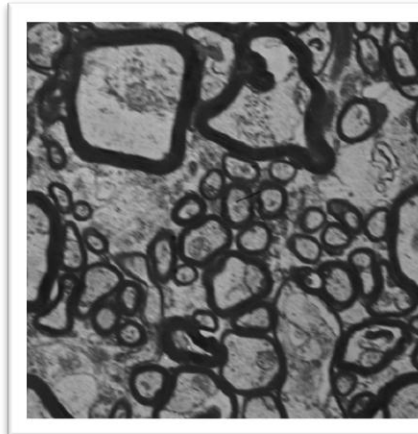
microbiology



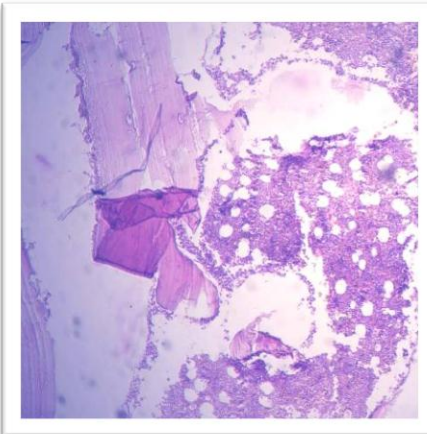
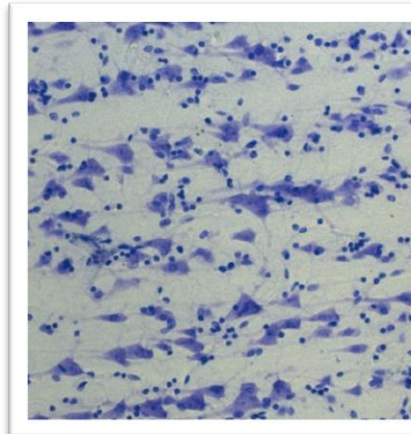
cytology (oncology)



ecomonitoring



clinical neuromorphology



forensic medicine

Our research directions

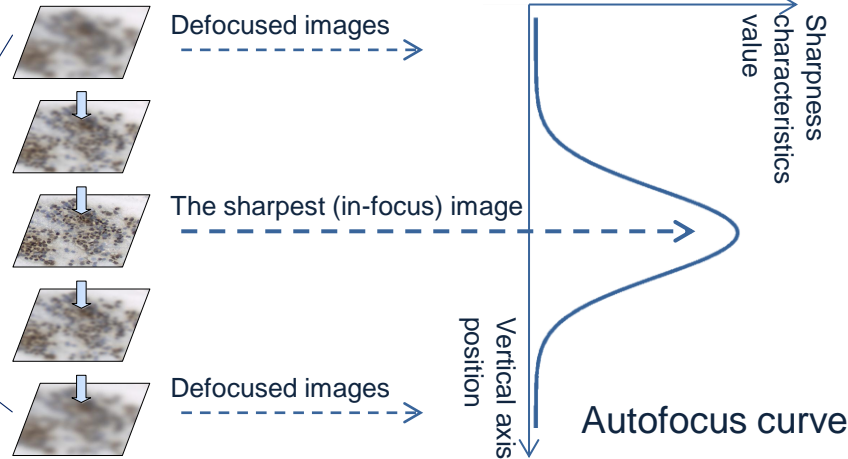
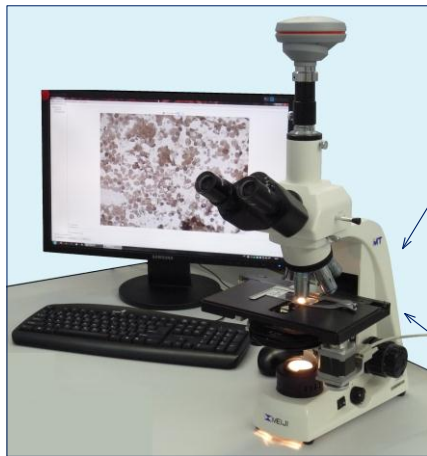
- Hardware and software design for biomedical specimens automated analysis.
- Algorithms design for specimen image processing for medical diagnostics.
- Algorithms design for specimen image processing for biomedical research.

Hardware and software complex for biomedical specimens automated analysis

- Units and components:
 - trinocular optical microscope with automated object table, autofocus unit and digital camera;
 - guiding electrical module;
 - special software for microscope guidance and image analysis.

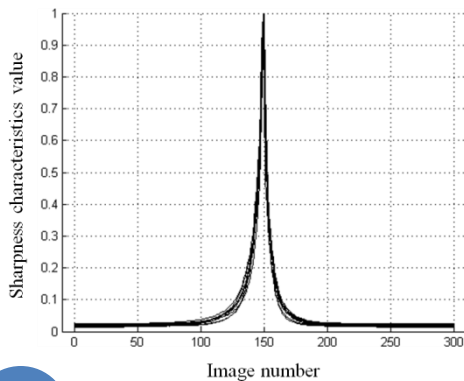


Autofocusing



Sum of squared Gaussian derivatives

$$F_{sum_Gaus_deri} = \frac{1}{H \cdot W} \sum_{Height} \sum_{Width} [f(x, y) \otimes G_x(x, y, \sigma)]^2 + [f(x, y) \otimes G_y(x, y, \sigma)]^2$$



$G_x(x, y, \sigma), G_y(x, y, \sigma)$ –
first-order Gaussian
derivatives in horizontal and
vertical directions respectively

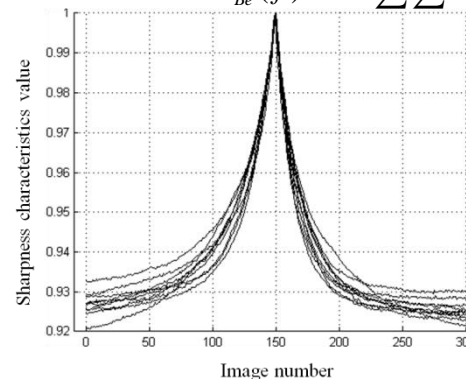
$$\sigma = \frac{d}{2\sqrt{3}}$$

d – the size of the
smallest feature in
the image

(good accuracy, sharp peak, low noise level, no local maxima, insensitivity to artifacts in the specimen)

Bayes-spectral-entropy-based focus measure

$$F_{Be}(f) = 1 - \frac{\sum_{u=0}^{M-1} \sum_{v=0}^{N-1} |F_C(u, v)|^2}{\left(\sum_{u=0}^{M-1} \sum_{v=0}^{N-1} |F_C(u, v)| \right)^2}$$



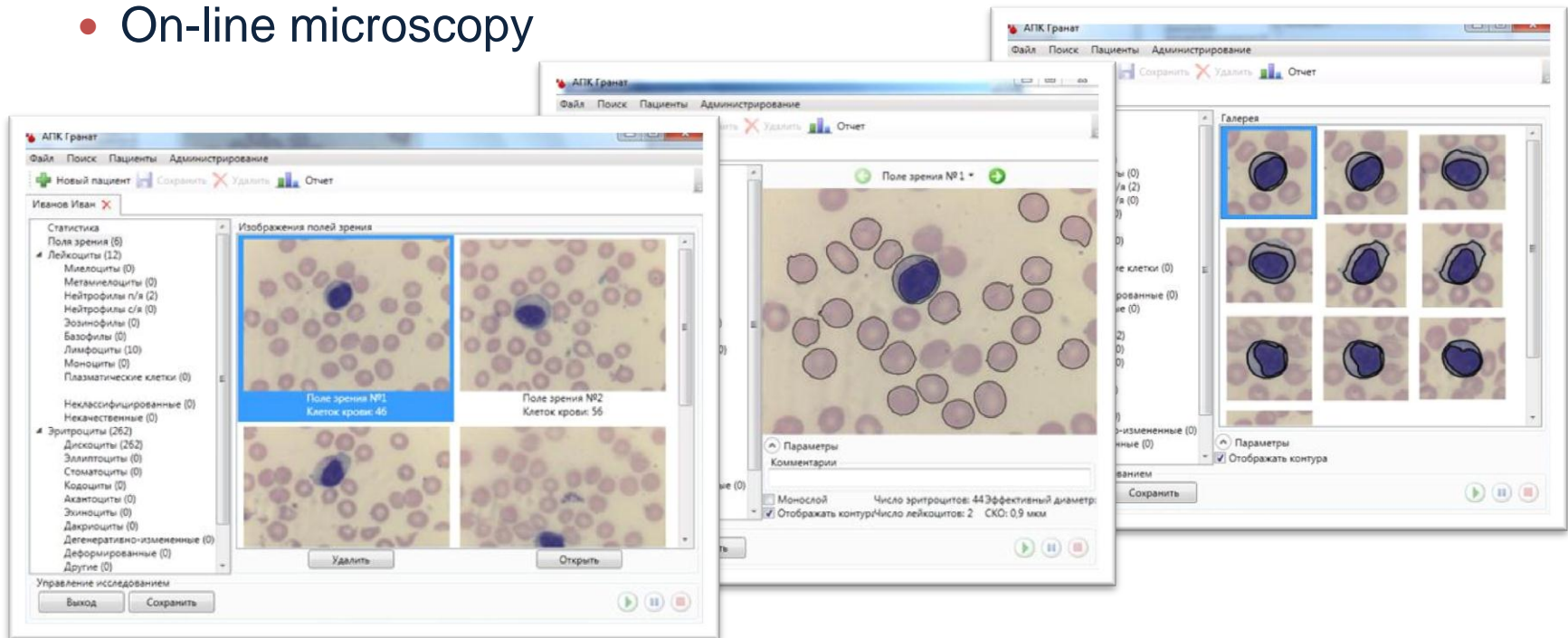
$$\tilde{F}_C[f](u, v) = \frac{|F_C(u, v)|}{\sum_{u, v \in D} |F_C(u, v)|}$$

$F_C(u, v)$ – discrete cosine
transform of the
image

(the best accuracy and wide range, enabling to find an in-focus position from totally defocused image)

Software for blood smears analysis

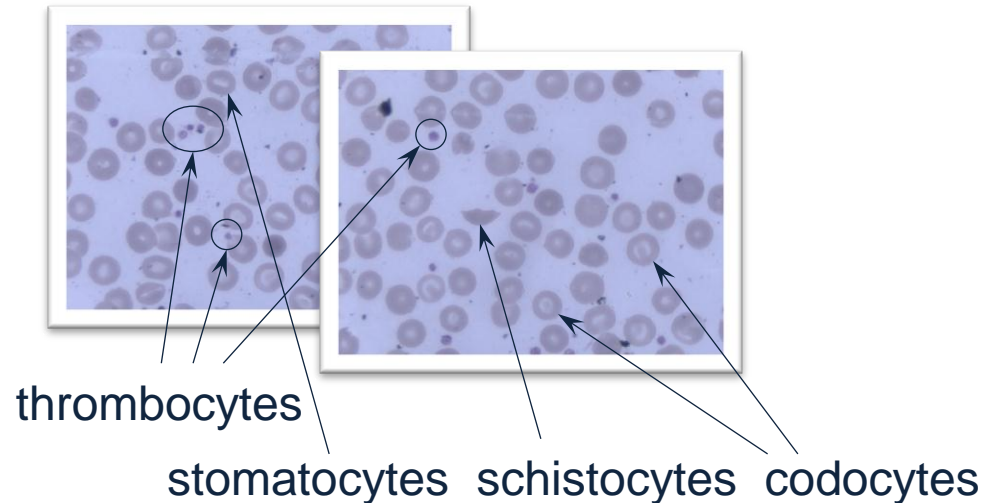
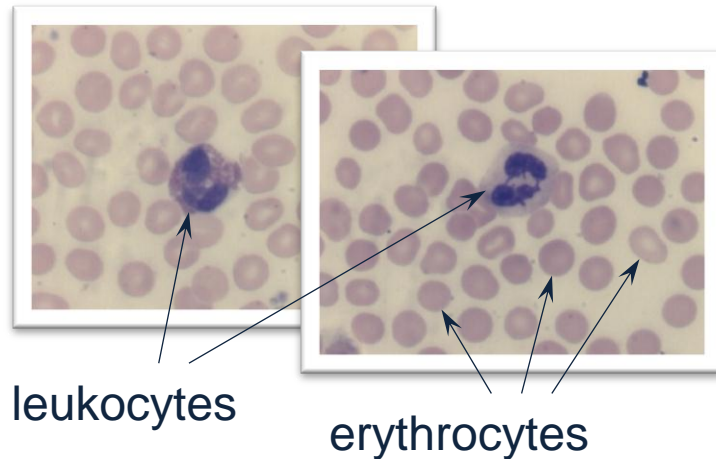
- Software features:
 - Automated and manual guidance of microscope
 - Displaying galleries of fields of view and of cells detected
 - Convenient GUI for checking of the results of automated image processing and for the correction of them if needed
 - Database management, creating reports, etc.
 - On-line microscopy



Algorithms design for specimen image processing for medical diagnostics

- Blood smears analysis
- Immunocytochemical analysis of breast cancer specimens
- Analysis of Chlamydia in cell culture

Blood smears analysis



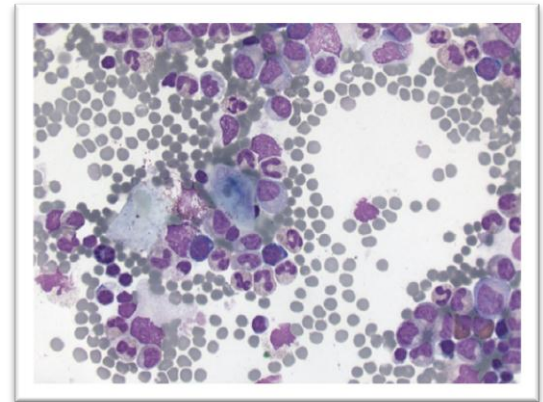
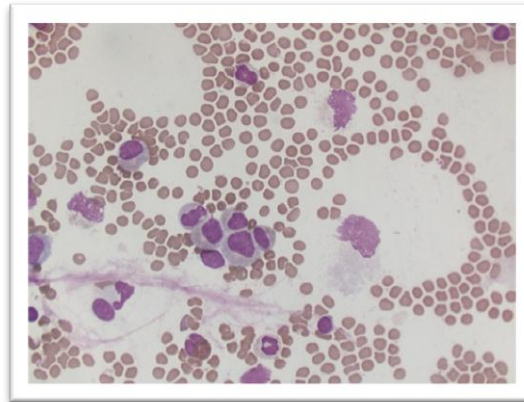
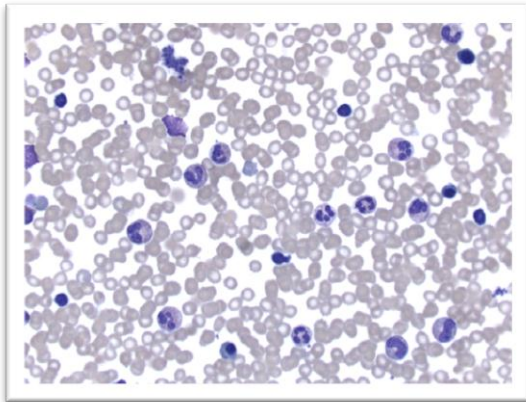
- Medical tasks:
 - screening of anemia (before clinical manifestation)
 - leukocyte examination in the case of their changes (when flow cytometers can't provide clear result)
- Medical partners: Scientific Centre of Children Health under the Russian Academy of Sciences; BMSTU polyclinic
- More than 200 smears, each having more than 100 images (image capture – in our lab)

Technical aspects

- Erythrocytes:
 - Area of analysis detection (monolayer – stationary region)
 - Segmentation
 - Morphology estimation (size, form, staining profile)
 - Classification
- Leukocytes:
 - Detection (thresholding, AdaBoost, ...)
 - Segmentation (intensity, texture, color)
 - Classification (CART, LDA, multiclass AdaBoost, kNN, Bayes, ...)

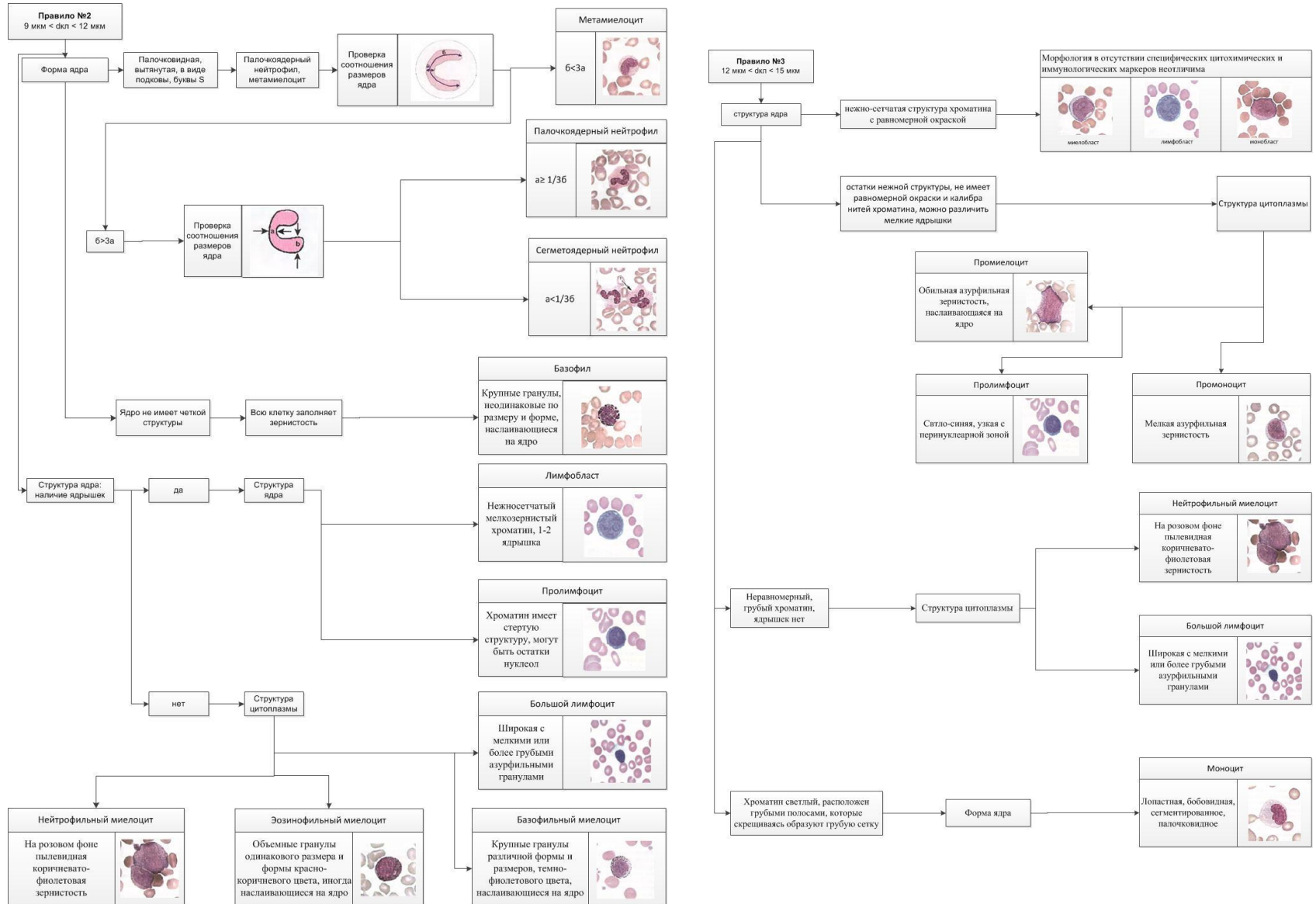
Main challenge

- Classification of young leukocytes
- Bone marrow cytological smears (~10 smears, each having more than 300 images; image capture – in our lab):

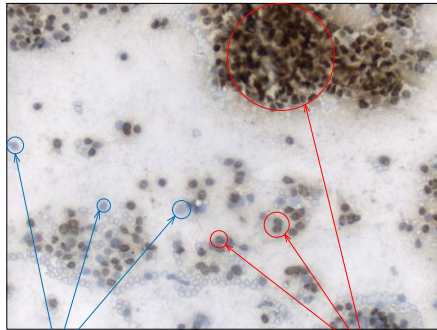


- Main problem: ground truth for classes of young leukocytes

Ground truth for classes of young leukocytes



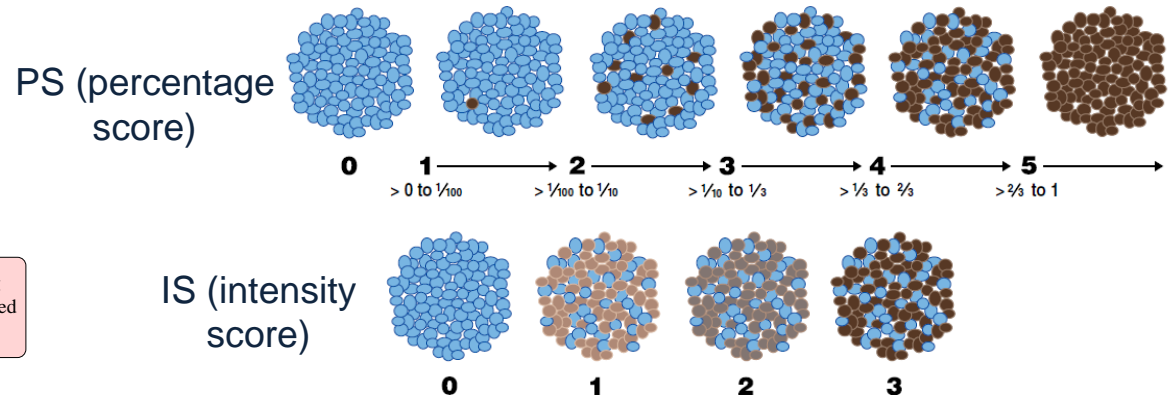
Immunocytochemical analysis of breast cancer specimens



nucleus of tumor cells stained with hematoxylin

nucleus of tumor cells containing receptors to steroid hormones stained with chromogen DAB

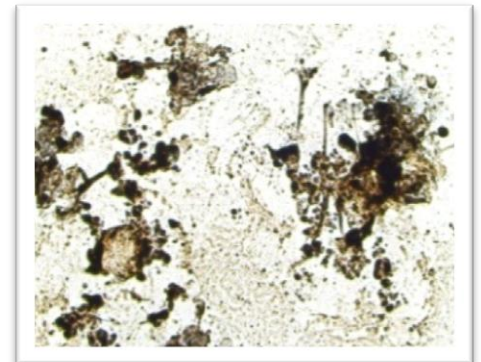
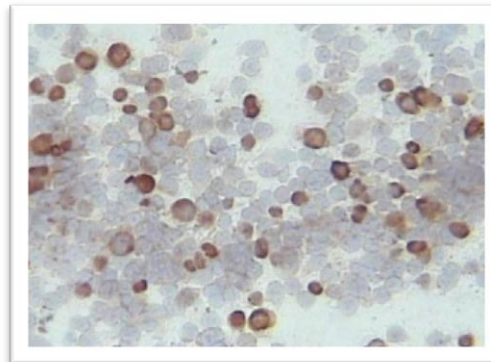
Allred scoring system:



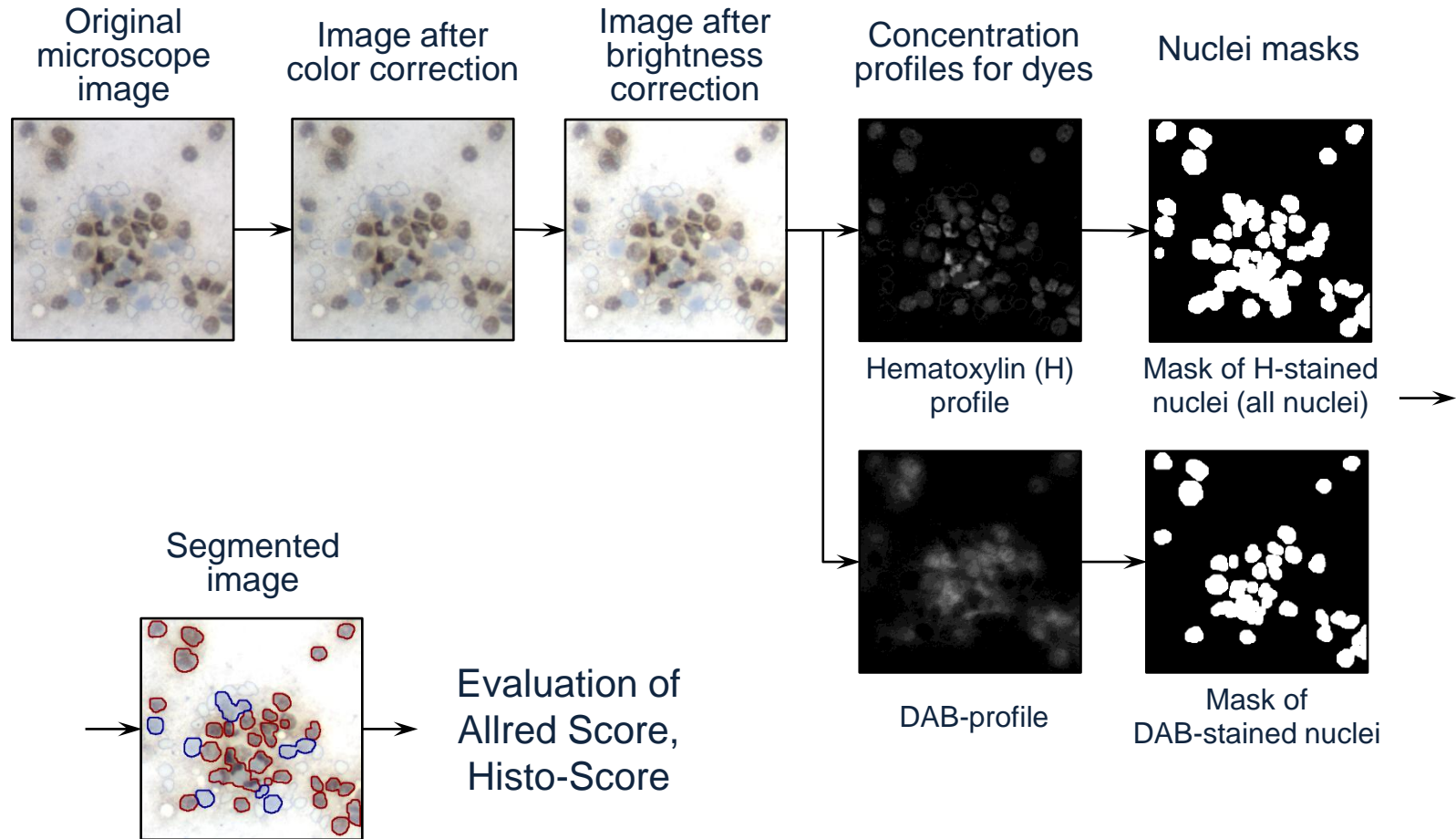
- Medical task:
 - Hormonal ER/PR status of breast cancer evaluation (influences on treatment procedures)
 - Proliferation (Ki-67) and other factors evaluation
- Medical partner: Moscow Research and Development Oncological Institute named after P.A. Gertsen
- More than 200 specimens, each having more than 100 images (image capture – in our lab).

Technical aspects

- Color and brightness correction
- Regions of cells with and without DAB staining segmentation (considering preparing artifacts, erythrocytes, etc.)
- Dyes separation (color deconvolution)
- Color intensity estimation (for DAB)
- Diagnostic scores calculation
- Main problems:
 - Quality of specimens
 - Color calibration, stable hardware (digital camera)



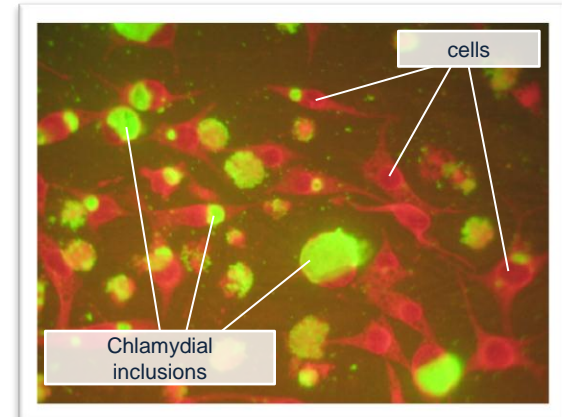
Quantification the degree of receptors expression to steroid hormones



Analysis of Chlamydia in cell culture



Fluorescence microscopy image of pure cell culture



Fluorescence microscopy image of cell culture infected with Chlamydia

- Medical task:
 - Treatment results proof ('Gold standard')
 - Estimation of new antibacterial drugs effectiveness
- Medical partner: Gamaleya Research Institute of Epidemiology and Microbiology
- More than 50 specimens, each having 10-15 images (fluorescence microscopy).

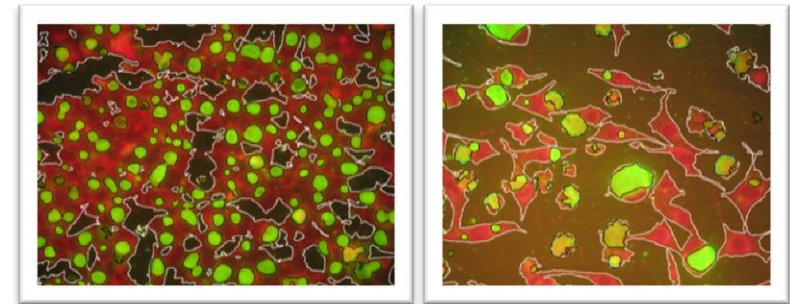
Technical aspects

- Detection of Chlamydial inclusions presence
- Segmentation of regions of cells with and without Chlamydial inclusions :
 - Fluorophore separation (adapted color deconvolution)
 - Adaptive thresholding (fast Niblack algorithm)
- Evaluation of Chlamydial inclusions geometric parameters

Estimation of new antibacterial drugs effectiveness

- Steps:
 - automated image segmentation,
 - evaluation of morphological parameters of Chlamydial inclusions,
 - quantitative assessment of antibacterial drugs effectiveness.

Examples of segmented images



An example of the results of antibacterial agent assessment (images and metadata were provided by Gamaleya Institute)

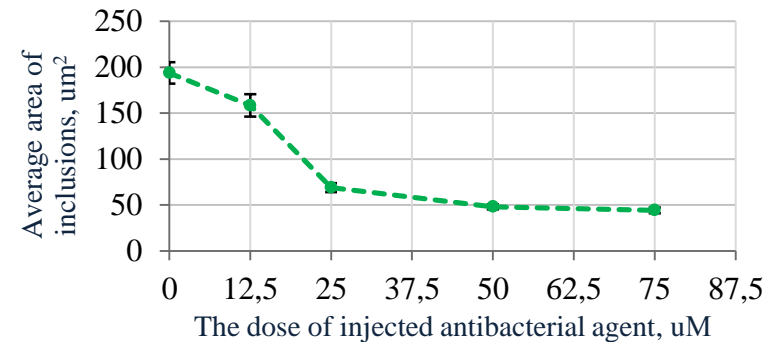
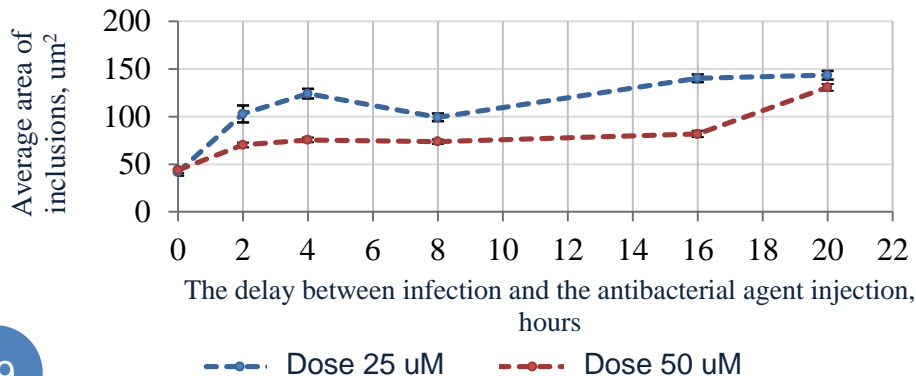
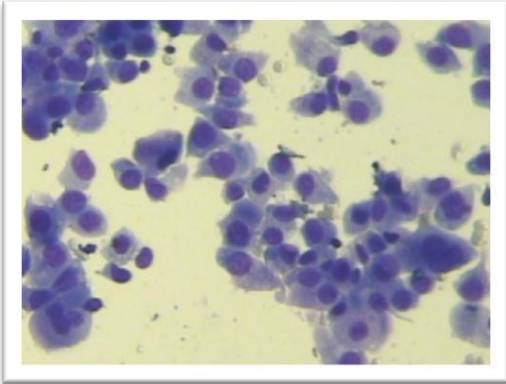


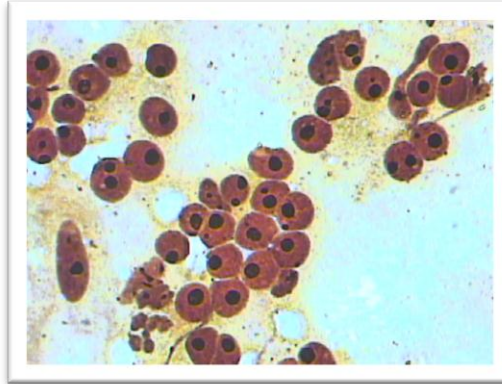
Image processing algorithms design for biomedical research

- Types of specimens:
 - Cytological specimens with different staining techniques (new cytological diagnostic criteria in oncology elaboration)
 - Grey and white matters of the brain (clinical neuromorphology)
 - Blood smears of fishes and animals (ecological monitoring and veterinary)
 - Histological images for forensic medical examination
 - Crystallograms of biological liquids

Cytological specimens with AgNOR staining analysis



Standard-stained specimen



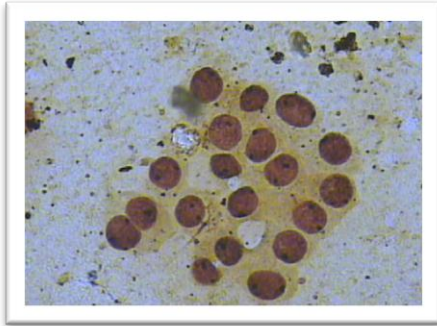
AgNOR-stained specimen

Silver nitrate staining allows to visualize the internal structure of cells nuclei

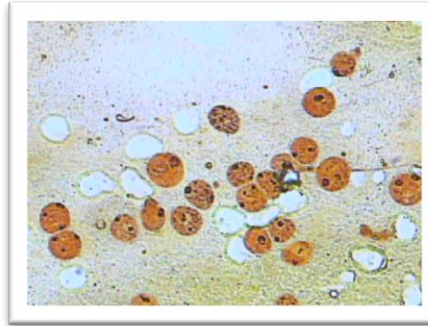
- Medical task:
 - Estimation of the malignance degree (breast cancer)
 - Morphological proof of malignancy (renal cancer)
- Medical partner: Moscow Research and Development Oncological Institute named after P.A. Gertsen
- More than 150 smears, each having approximately 30 images (image capture – in our lab)

Cytological specimens with AgNOR staining

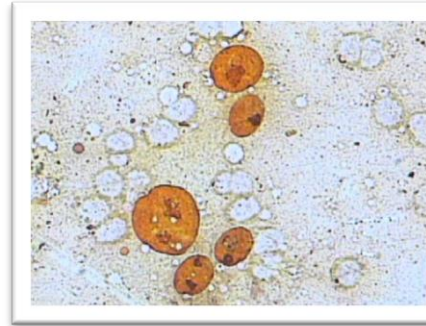
Kidney



Norm



Reactive changes

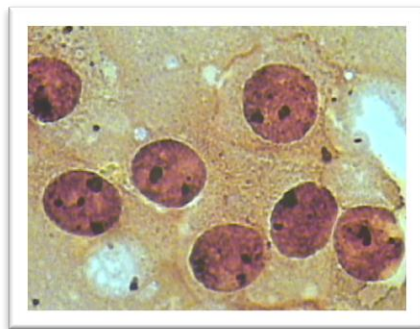


Angiomyolipoma

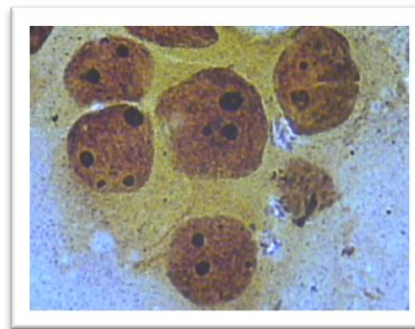


Highly differentiated renal cell carcinoma

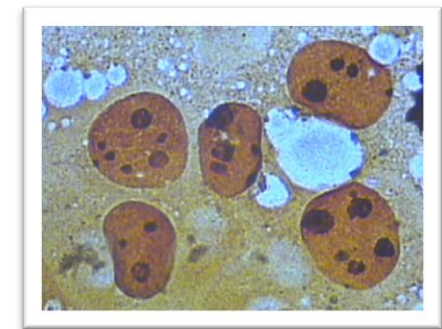
Breast



Well-differentiated
invasive ductal carcinoma



Moderately differentiated
invasive ductal carcinoma

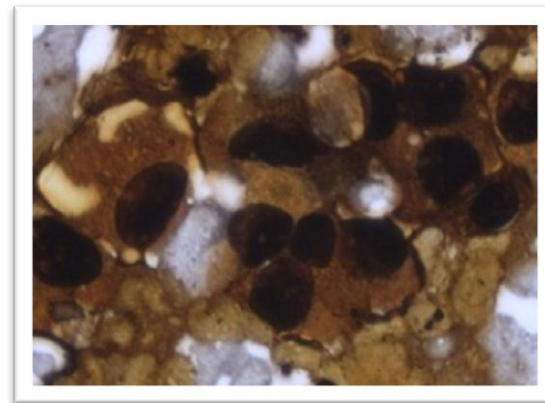
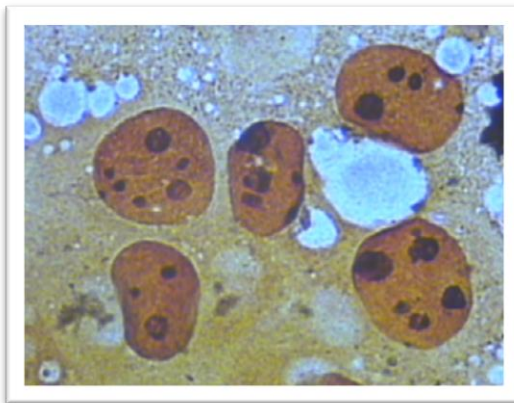


Poorly differentiated
invasive ductal carcinoma

Technical aspects

- Segmentation of cells' nuclei
- Morphology estimation (size, form, texture)
- Classification of nuclei (classes extraction at first...)
- Classification of specimen

- Problems:
 - Specimen quality, expensive dyes

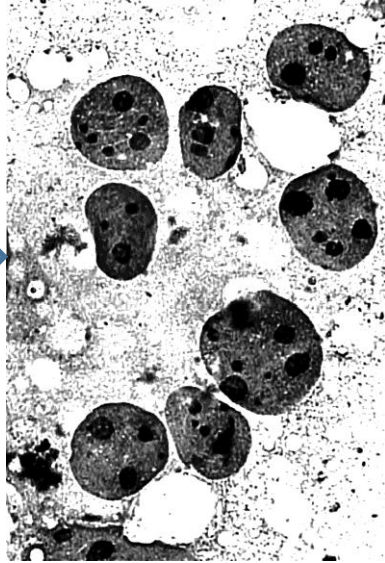


Estimation of breast cancer malignance degree

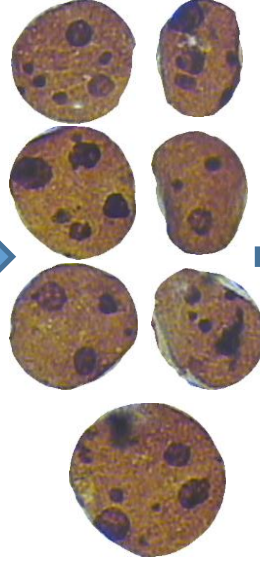
Image capture



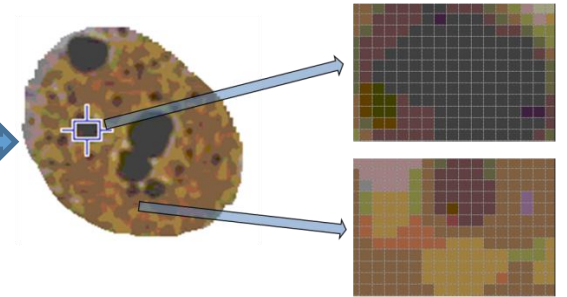
Image preprocessing



Cell nuclei segmentation



Nuclei features extraction



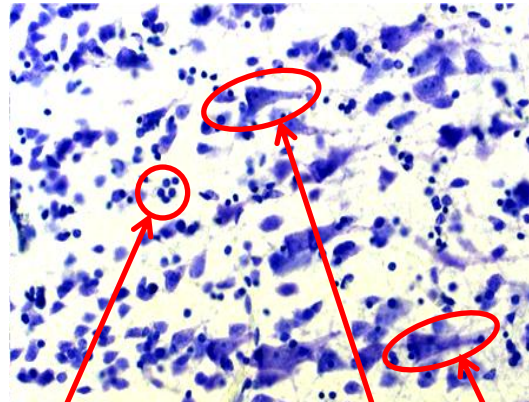
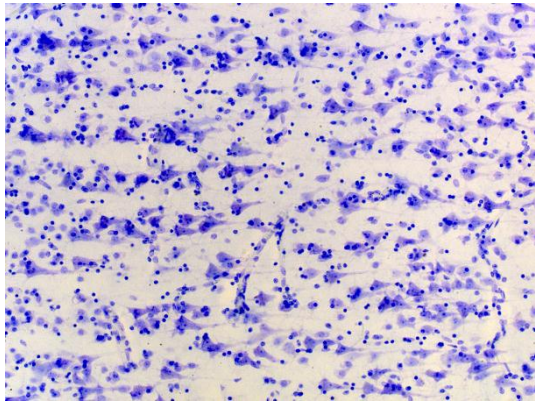
Cluster analysis:
the number of
clusters and their
characteristics

Nuclei
classification

Posterior probabilities
of cytological diagnosis
calculation

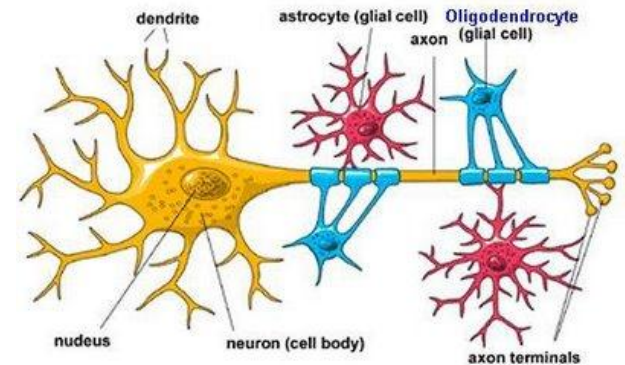
Final
decision

Histological specimens of gray matter of the brain analysis



Oligodendrocytes

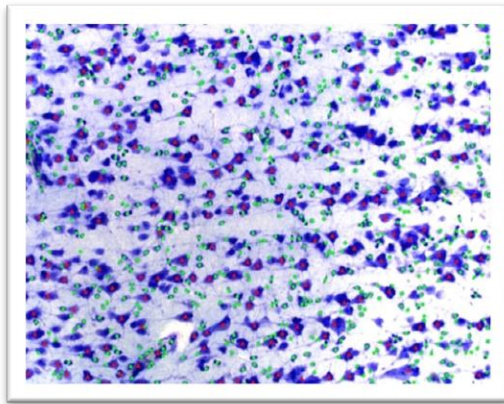
Pyramid neurons



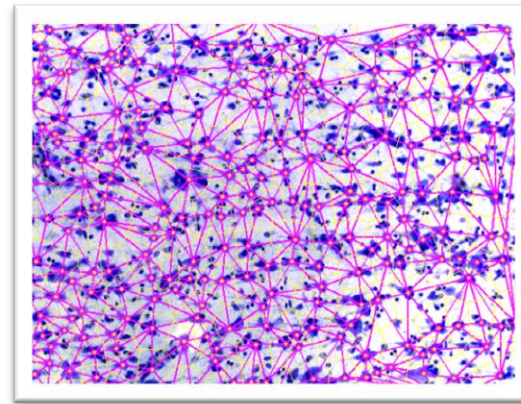
- Medical task:
 - Evaluation of spatial composition of cells (pyramid neurons and oligodendrocytes)
 - Finding differences in brain structure in the cases of mental deceases and norm
- Medical partner: Research Center for Psychological Health, RAS
- 20 tissue specimens, each having 10 images.

Technical aspects

- Cells detection (local adaptive thresholding, mathematical morphology)
- Spatial distribution features estimation (general and inside minicolumns):
 - Distance features
 - Features, derived from Voronoi polygons and Delaunay triangulation results

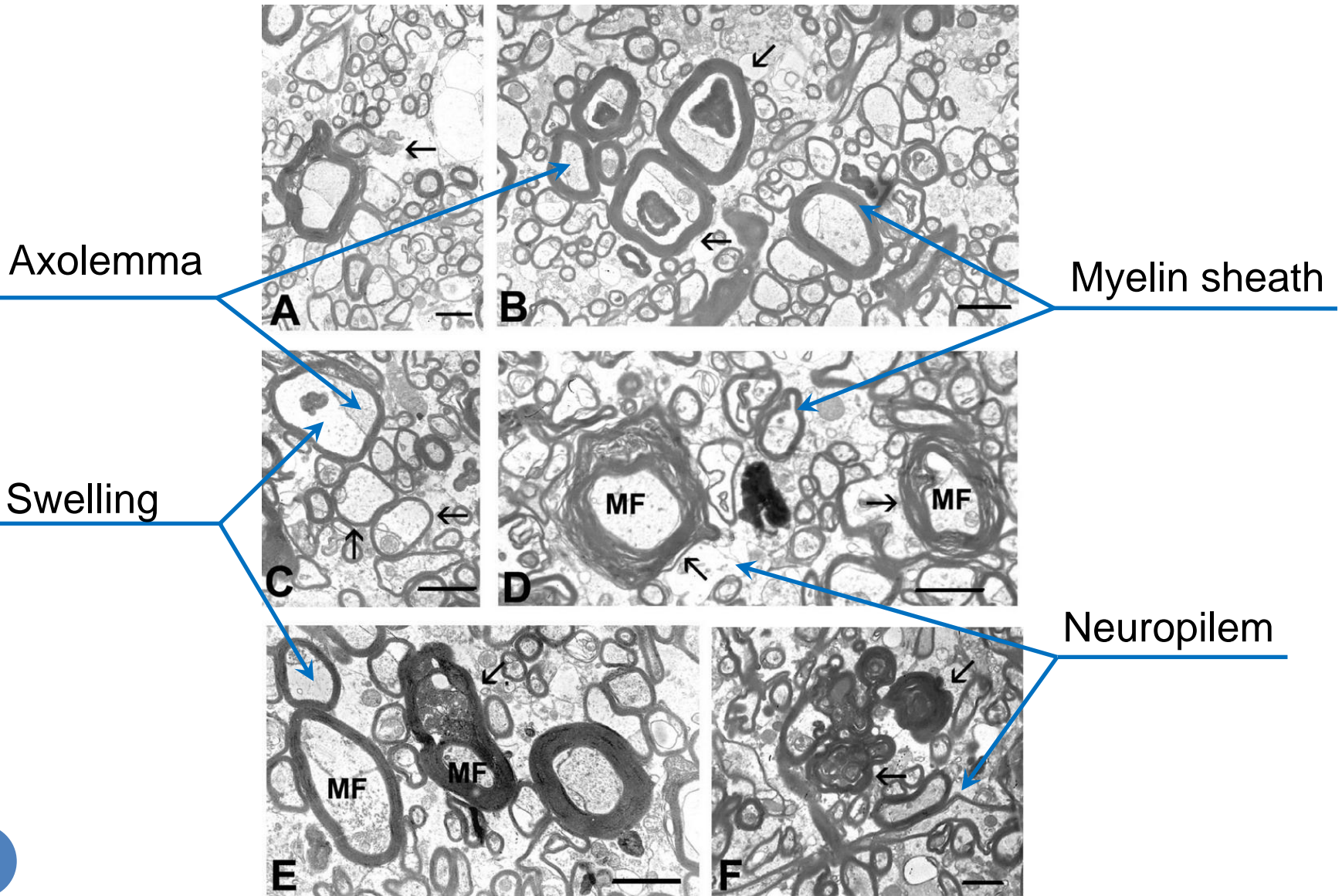


Example of detection results



Example of Voronoi polygons and Delaunay triangulation results

White matter of the brain ultrastructure analysis

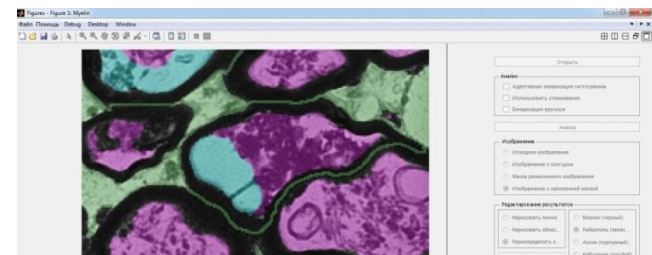
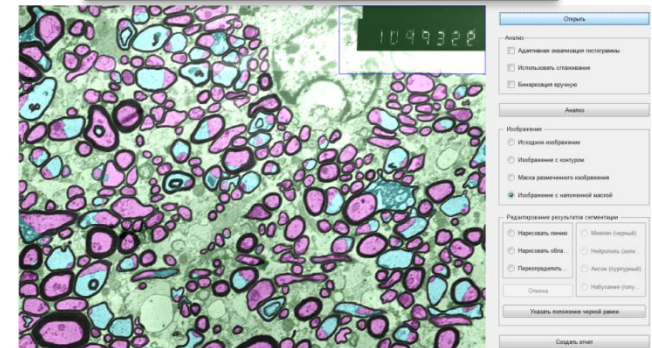
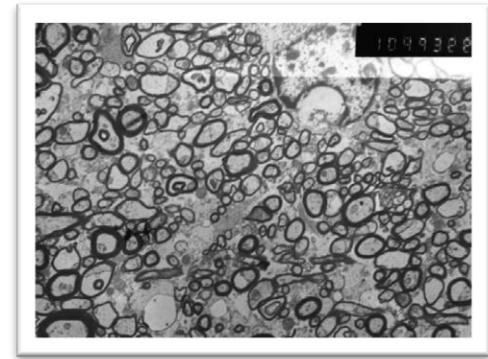


White matter of the brain ultrastructure analysis

- Medical task:
 - Evaluation of axon ultrastructure
 - Finding differences in norm and schizophrenia
- Medical partner: Research Center for Psychological Health, RAS
- 40 tissue specimens, each having 20-30 images (electron microscopy).

Technical aspects

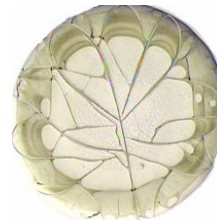
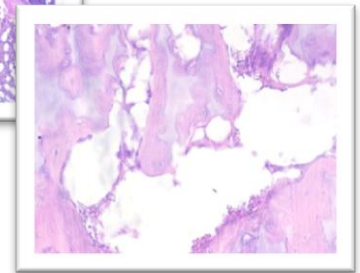
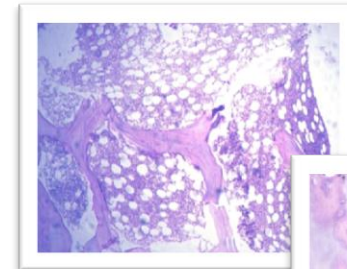
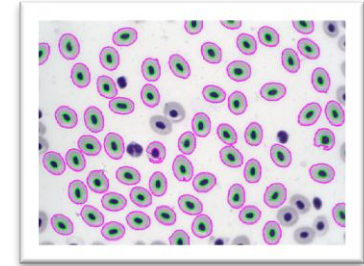
- Segmentation of myelin cover of axons
- Separation of myelin cover of neighboring axons consider their thickness
- Segmentation of inner matter of axons
- Calculation of the parameters of axons ultrastructure
- Problems:
 - Long time of ground truth evaluating



Software for image automatic and manual segmentation

Other image processing algorithms research

- Fish blood smears analysis
- Histological image analysis
 - Bone marrow: myeloid and fat tissues
 - Trabecula of bone: bone and cartilaginous tissues
- Crystallograms of biological liquids examination (blood serum, lacrimal fluid)



Our research directions in the field of microscope image processing: summary

- Hardware and software design for biomedical specimens automated analysis
- Algorithms design for specimen image processing for medical diagnostics
 - Blood smear analysis
 - Immunocytochemical analysis of breast cancer specimens
 - Analysis of Chlamydia in cell culture
- Algorithms design for specimen image processing for biomedical research
 - Cytological preparations with AgNOR staining
 - Histological specimens of gray matter of the brain analysis
 - White matter of the brain ultrastructure analysis
 - Fish blood smears analysis for ecological monitoring of basins
 - Histological image analysis for forensic medical examination
 - Cristallograms of biological liquids examination

Research directions in the field of decision-making in medicine

- Computer-aided medical reasoning
 - Differential diagnostics of early stages of arthritis
 - Estimation of the period of spleen damage
- General classification algorithms
 - Fuzzy boosting of weak classifiers: classification in the case of nonlinear decision boundaries

FuzzyBoost

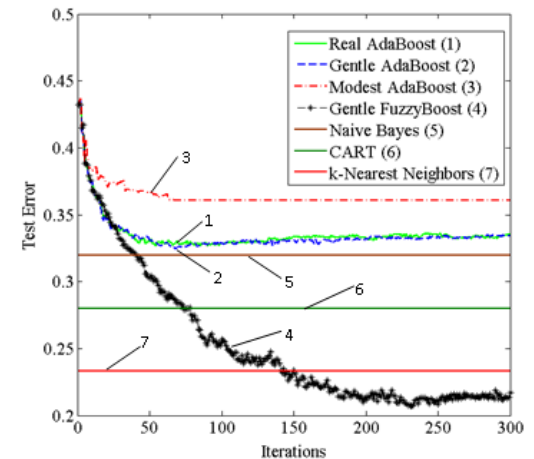
- Main ideas:
 - Generalization of AdaBoost by introducing nonlinear weak classifiers aggregation rule into boosting
 - Combining weak classifiers by Choquet integral with respect to 2-additive fuzzy measures (FM)

$$F_M^{\mu^\pm}(x) = \sum_{m=1}^M \phi_m^\pm f_m^\pm(x) - \frac{1}{2} \sum_{m \neq l} I_{ml}^\pm |f_m^\pm(x) - f_l^\pm(x)|$$

m -th weak classifier response

Shapley value (measure of weak classifier importance)

Interaction index (measure of weak classifier pairwise dependence)



Other directions of research at the Chair for biomedical technical systems

- Other types of images
 - Face (recognition, anthropometry research, emotion recognition)
 - Fingerprint (medical and genetic consultation, sports medicine)
 - Iris of an eye (medical screening)
 - Footstep (orthopedics, sports medicine)
 - Skin dermal neoplasms (medical screening)
- Electronic devices engineering
 - Automated complex for dose-correcting feedback for aeroionotherapy
 - Electrodermal activity express analyzer
- Optical systems engineering for health monitoring and dosimetry
 - Two-channel photo-plethysmograph
 - Express-spectrophotometer
 - Optical and other methods for cavitation parameters assessment
- Biomechanical systems engineering
 - Vascular microrobots
 - Biocompatible materials research



**Thank you
for your attention!**