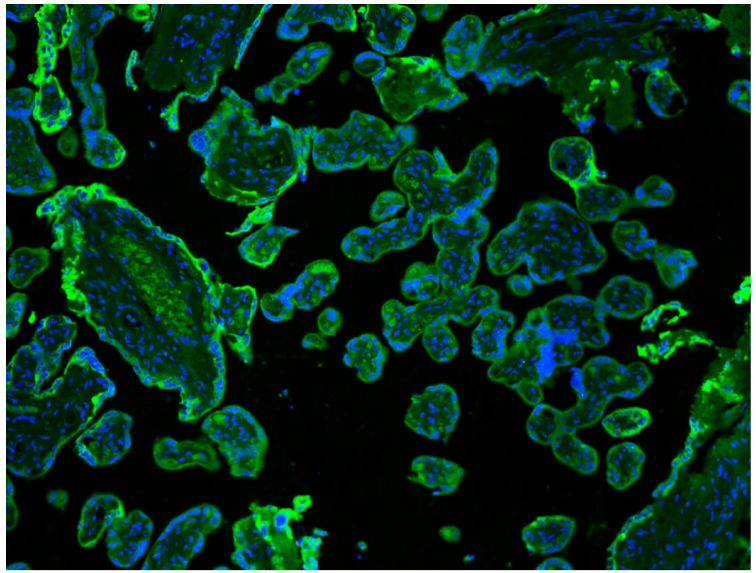
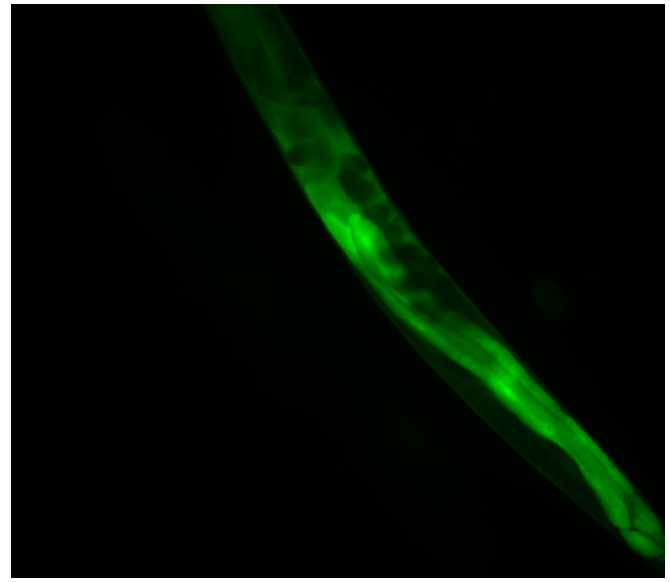
Image Data Analysis in H. Sapiens and C. Elegans





Dr. Alexander K. Seewald

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(Our) Relevant Collaborations for Biological Image Data Analysis

2009-2012
Med.Univ.Vienna, Austria
Tissue Gnostics GmbH
Funded by FFG Bridge










2007-2010
Univ. Colorado@Boulder, USA
Self-funded




2011-
IMP Vienna


2012-
FHCR/Brent Lab@Seattle, USA



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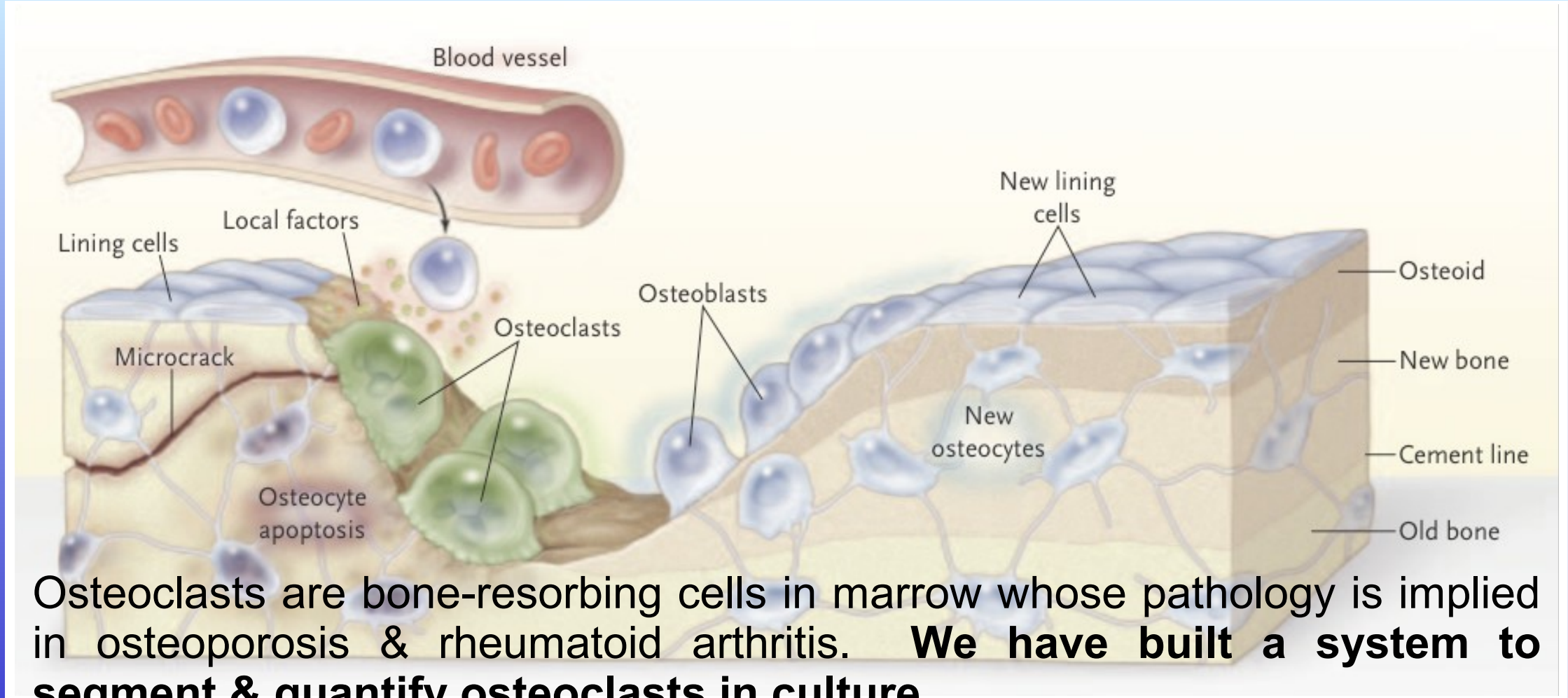
Osteoclast Characterization (1)



Osteoclasts are bone-resorbing cells in marrow whose pathology is implied in osteoporosis & rheumatoid arthritis. **We have built a system to segment & quantify osteoclasts in culture.**

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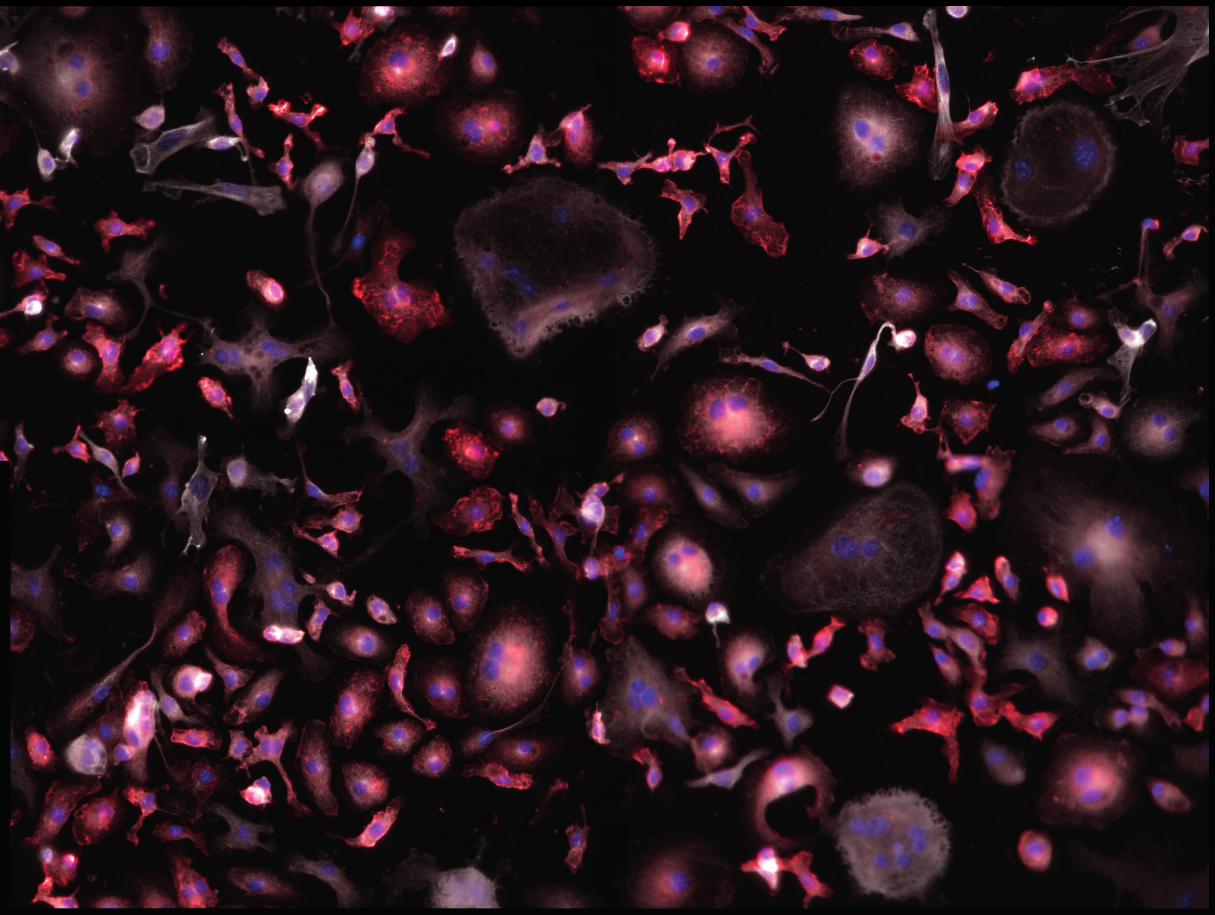
Osteoclast Characterization (2) - Sample



Triple Staining: white = cells, blue = nuclei, red = precursor / non-osteoclast

Detection works by counting nuclei (>=3) and computing red average area

Osteoclast Characterization (3) - Future Work

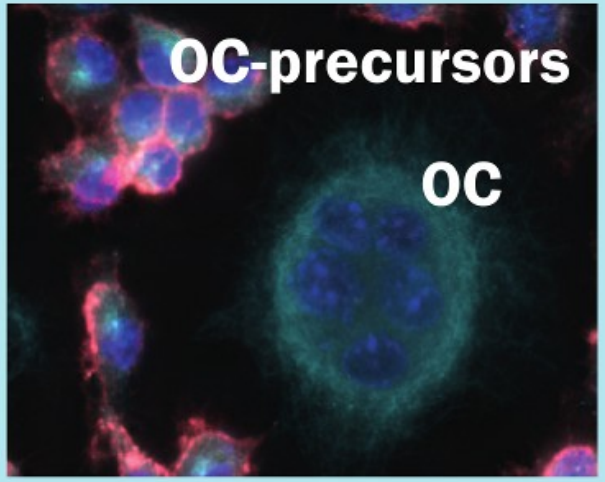


Figure 5

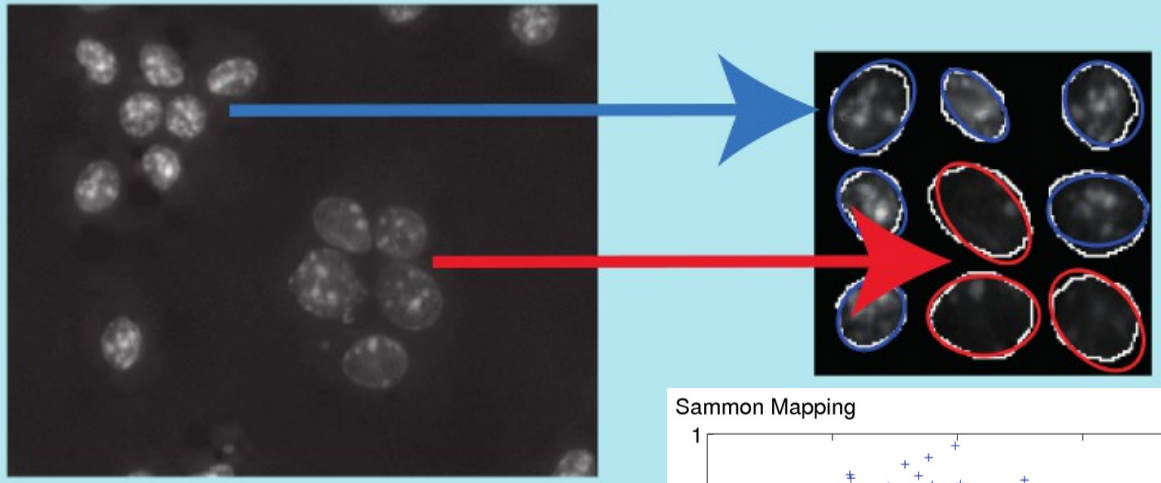
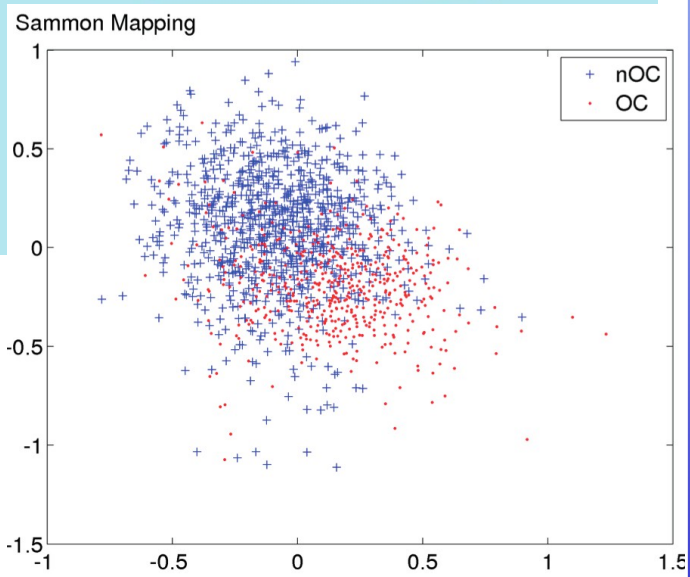


Figure 6



Shape and texture features computed from nuclei of osteoclasts (OC) and non-osteoclasts (nOC) allow to determine class with some confidence. May be combined with current system for additional precision.

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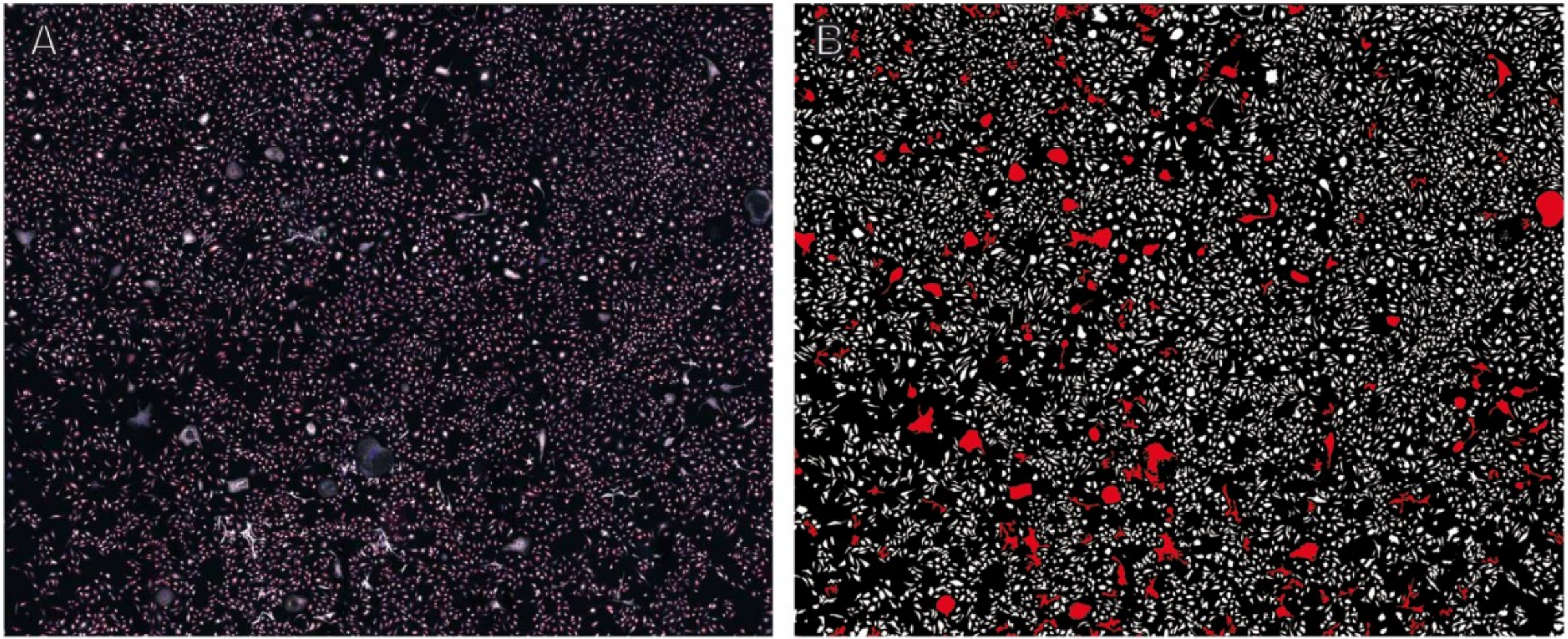
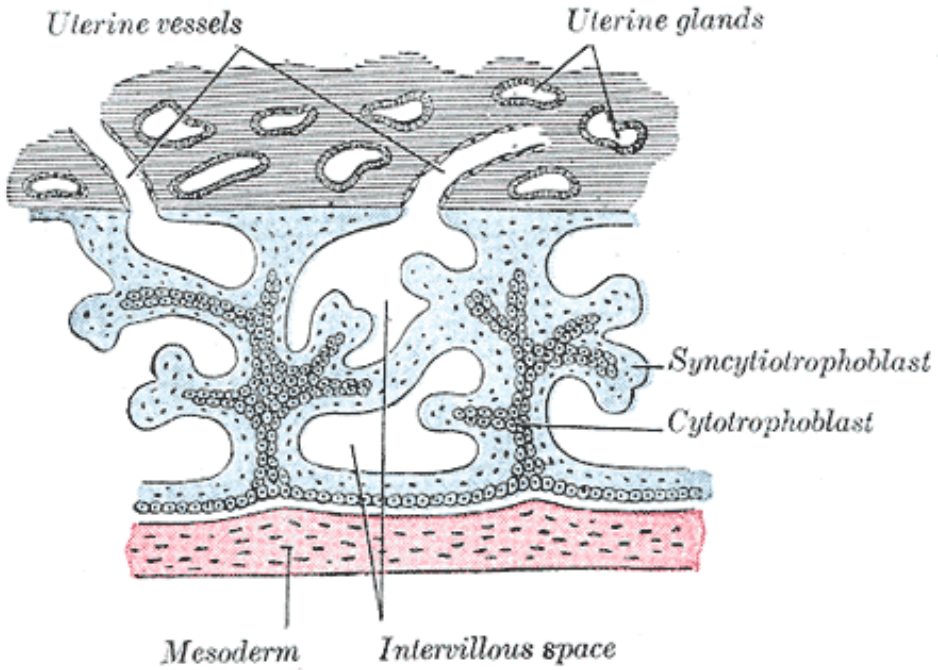


Figure 4: Image analysis of a whole sample-region consisting of 100 (10 x 10) FOVs. A: Immunofluorescence image. B: Result of cell segemntation and analysis: non-OC are marked in white, OC are marked in red.

Syncytiotrophoblast (1)

Primary chorionic villi



Syncytiotrophoblasts are multinucleated cells within the placenta of embryos at the surface of chorionic villi. Chorionic villi are part of the border between maternal and fetal blood during pregnancy. **We have built a system to segment villi & syncytiotrophoblast and applied it to protein quantification of Receptor for Advanced Glycated End products(RAGE)**

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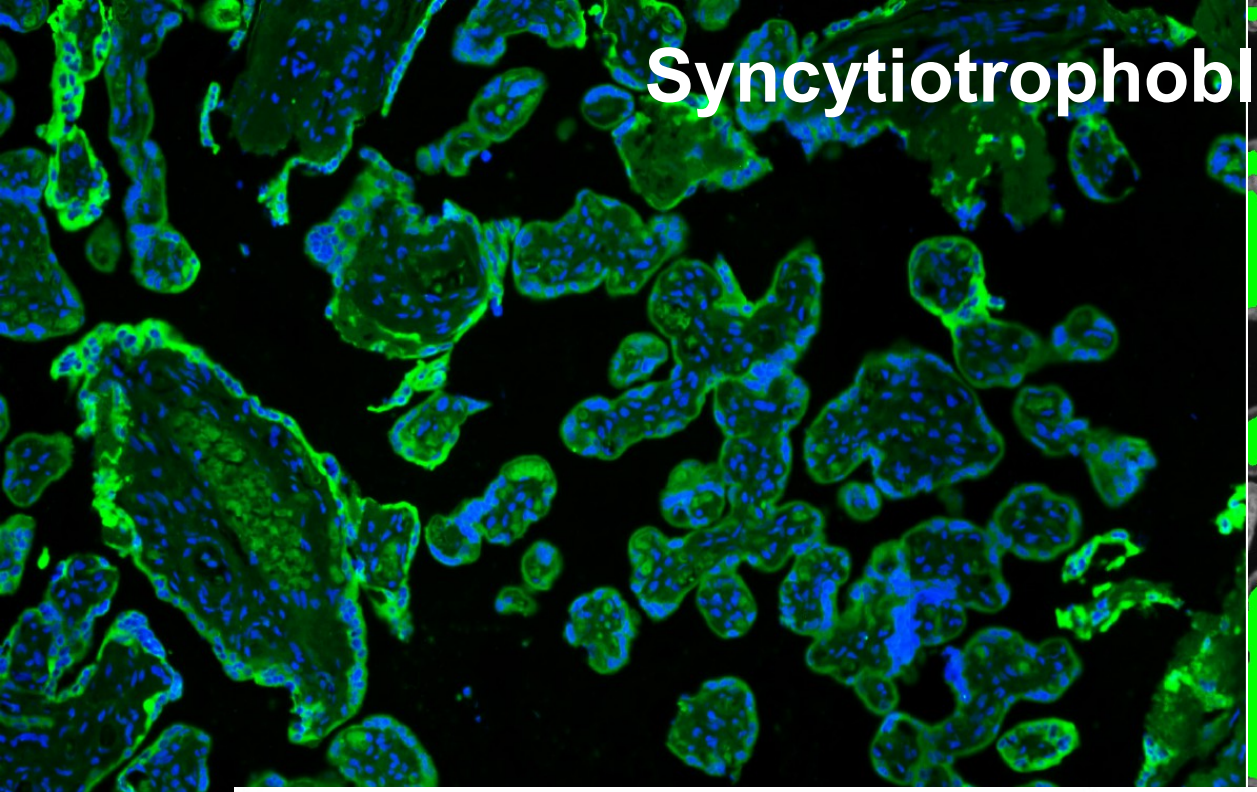
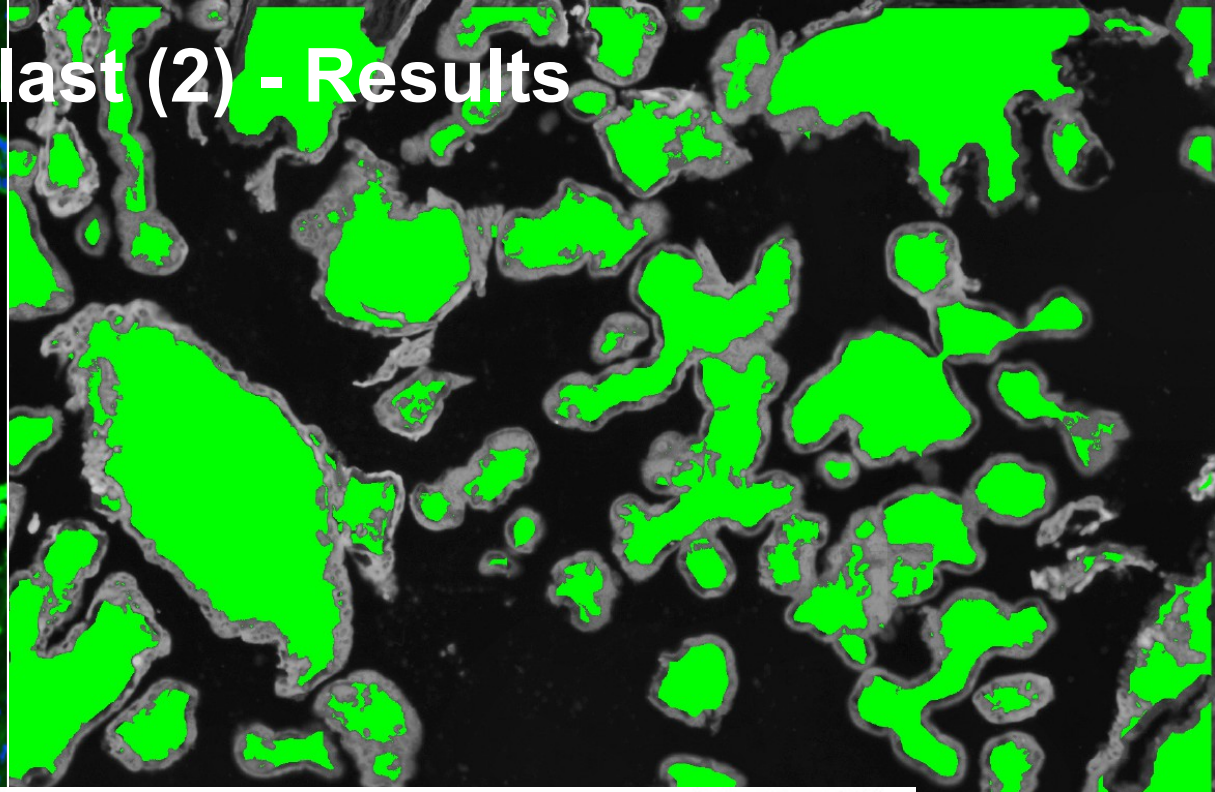
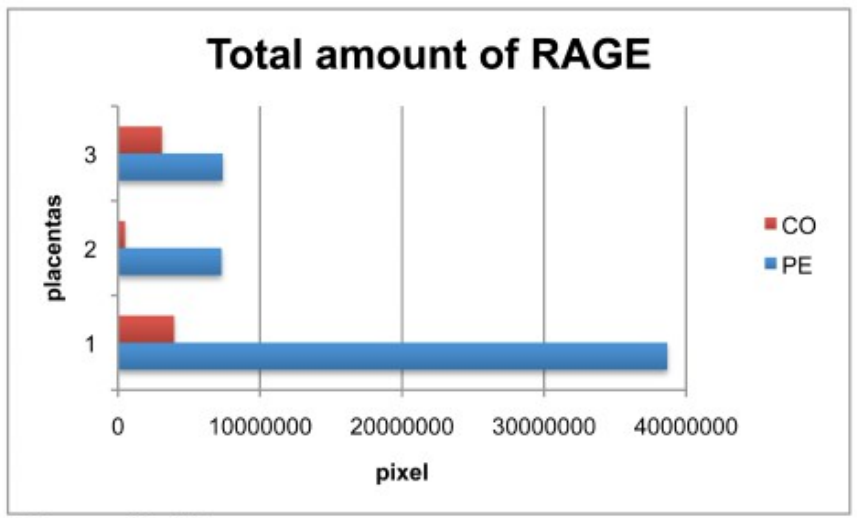
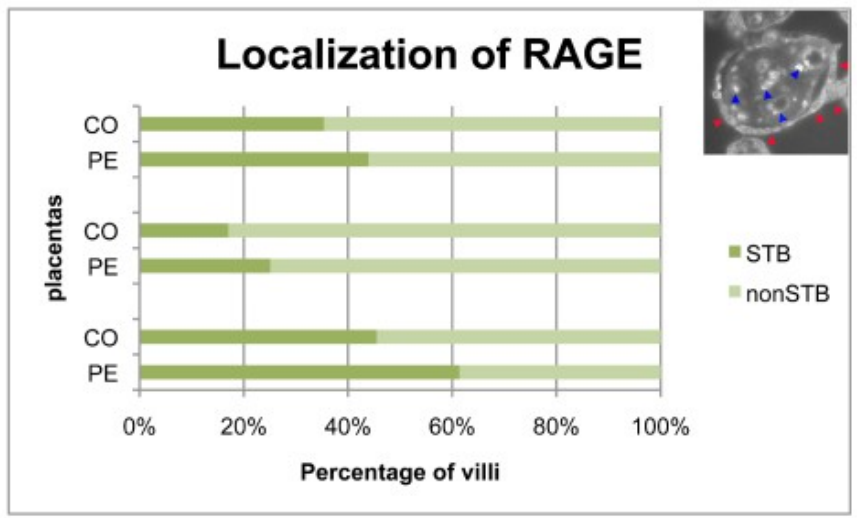
Syncytiotrophoblast (2) - Results







8



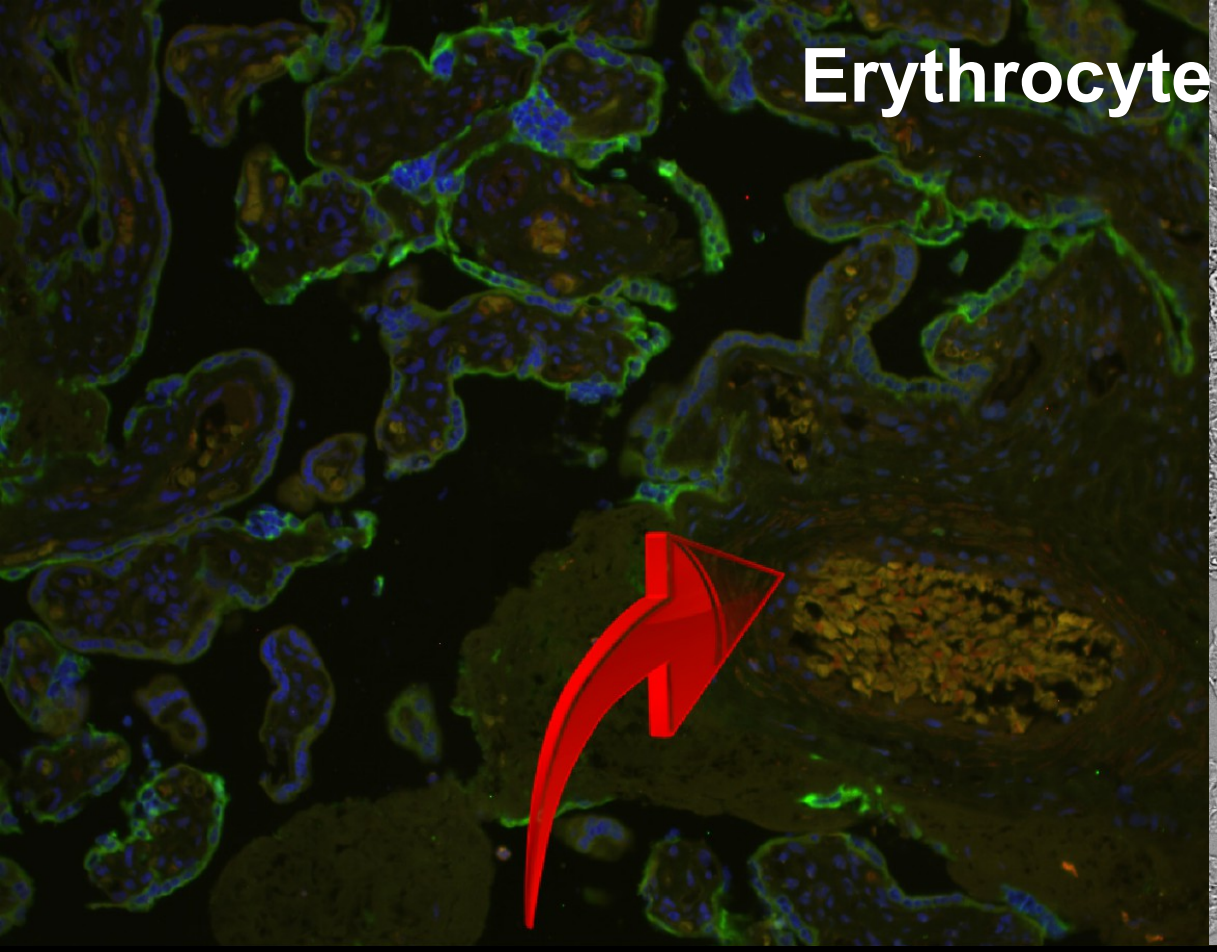
9

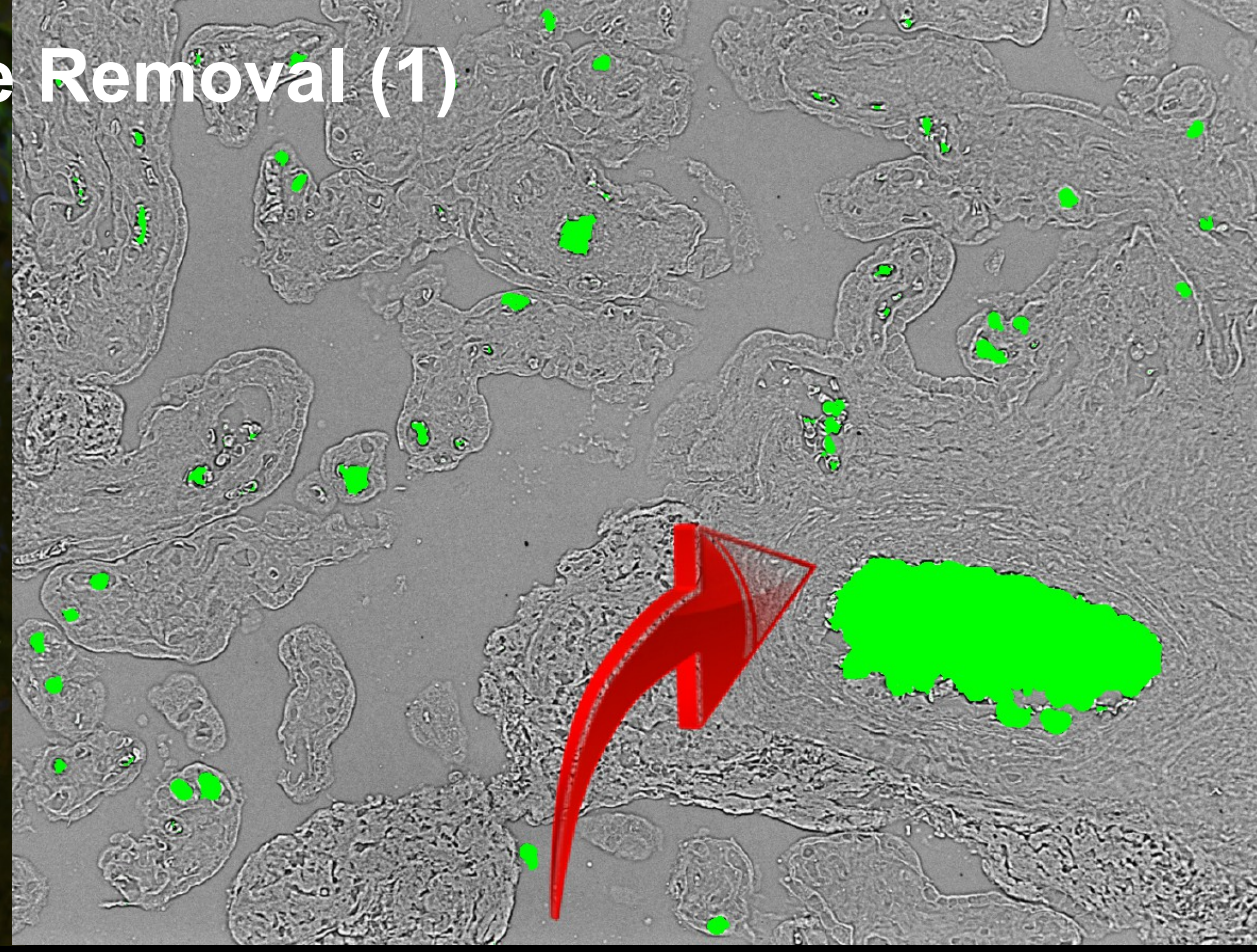
10

Figure 8-10

The total amount of RAGE proteins quantified in PE and CO placentas is shown in Figure 8, respectively localization within the villi in Figure 10. RAGE can be found in STB (red arrowheads) or other cell types (blue arrowheads) visualized in Figure 9.

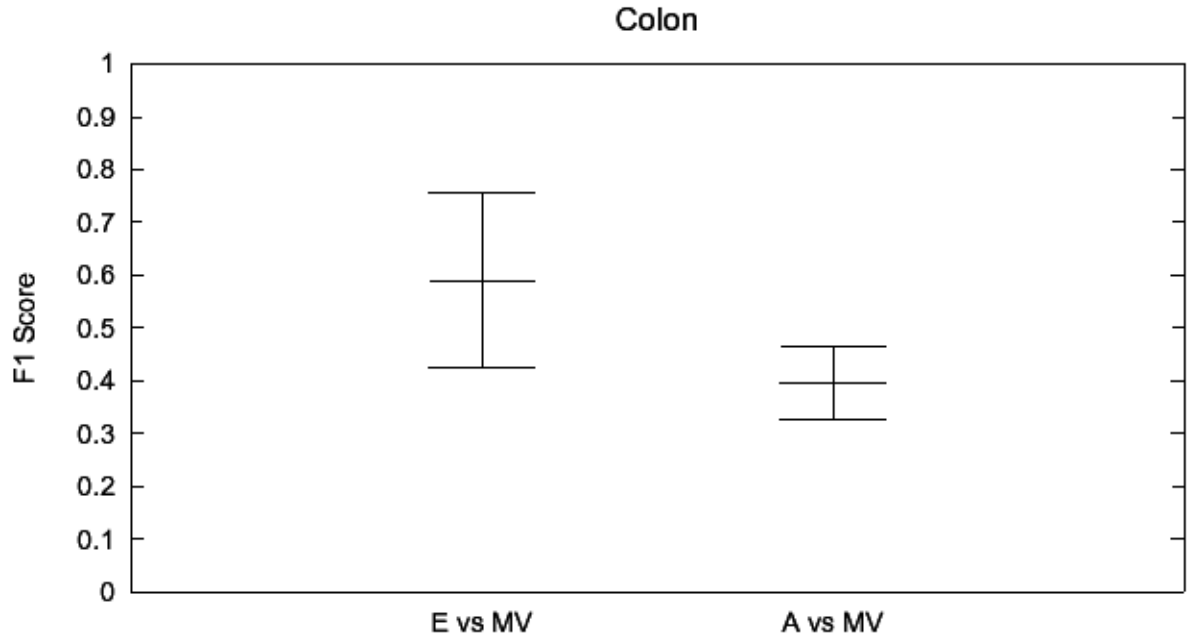
Erythrocyte Removal (1)

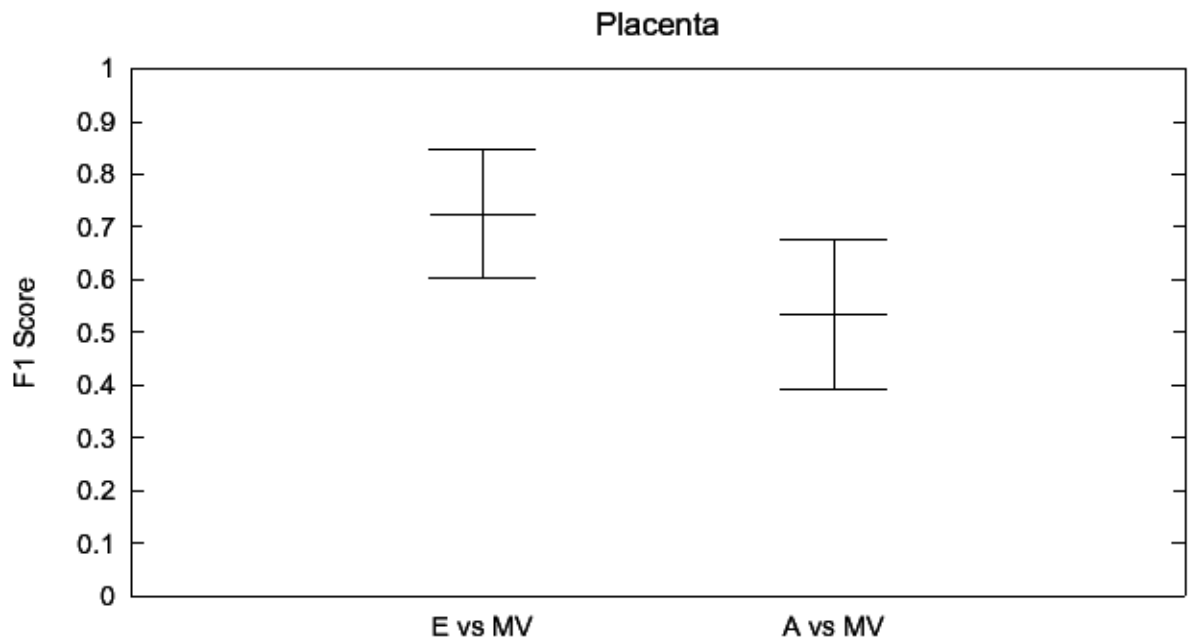


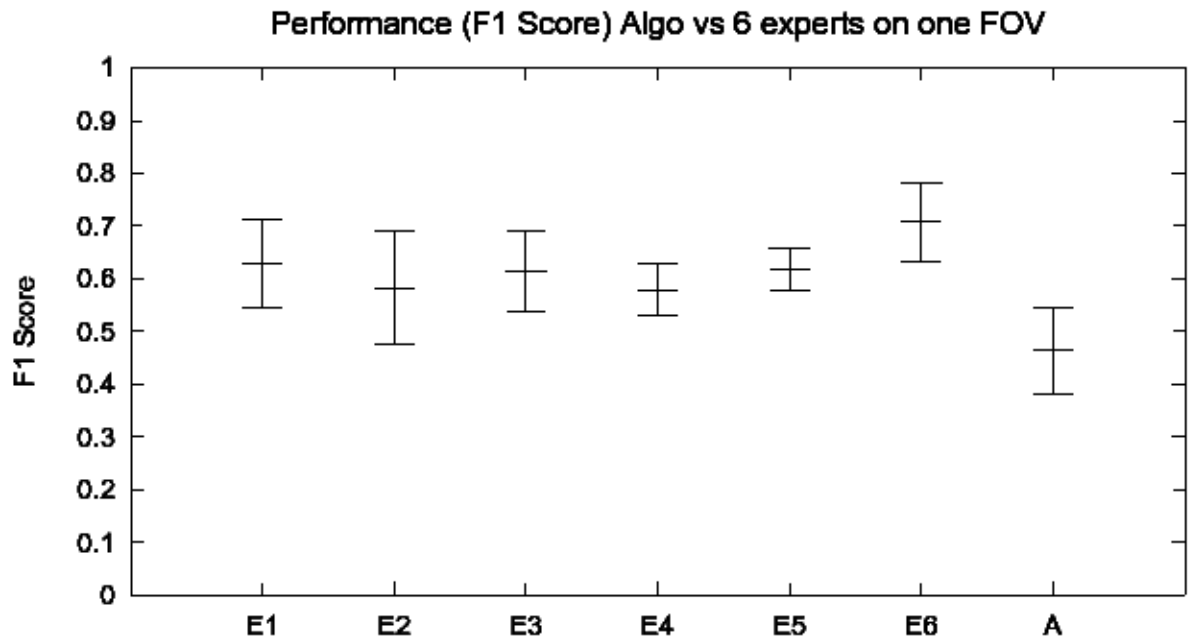


Erythrocytes have high autofluorescence and may lead to noisy immunofluorescent measurements. **Using only ground-truth data, we „taught“ the computer to remove erythrocytes from images ([Viola & Jones, 2001] = OpenCV Haartraining)**

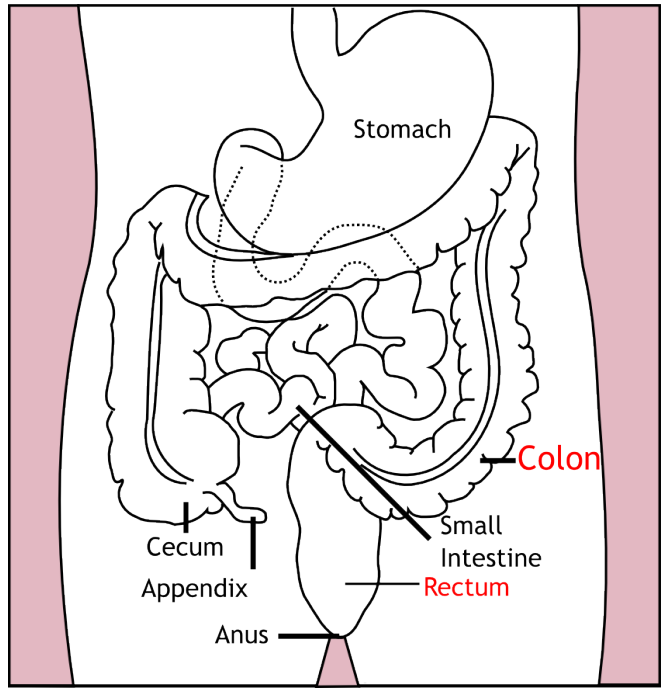
Essential for RAGE analysis from previous slide!


Colon
F1 Score
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
E vs MV
A vs MV


Placenta
F1 Score
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
E vs MV
A vs MV


Performance (F1 Score) Algo vs 6 experts on one FOV
F1 Score
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
E1
E2
E3
E4
E5
E6
A

Colorectal Cancer (1)



Colorectal cancer (CRC) is the second most frequent cause of cancer-related mortality in the Western world. Increasing evidence supports the role of the tumor microenvironment in influencing tumor progression (e.g. epithelium-to-stroma ratio) **We built a system to identify stroma, epithelium and lumen in colon tissue as a first step towards automated diagnosis and prognosis.**

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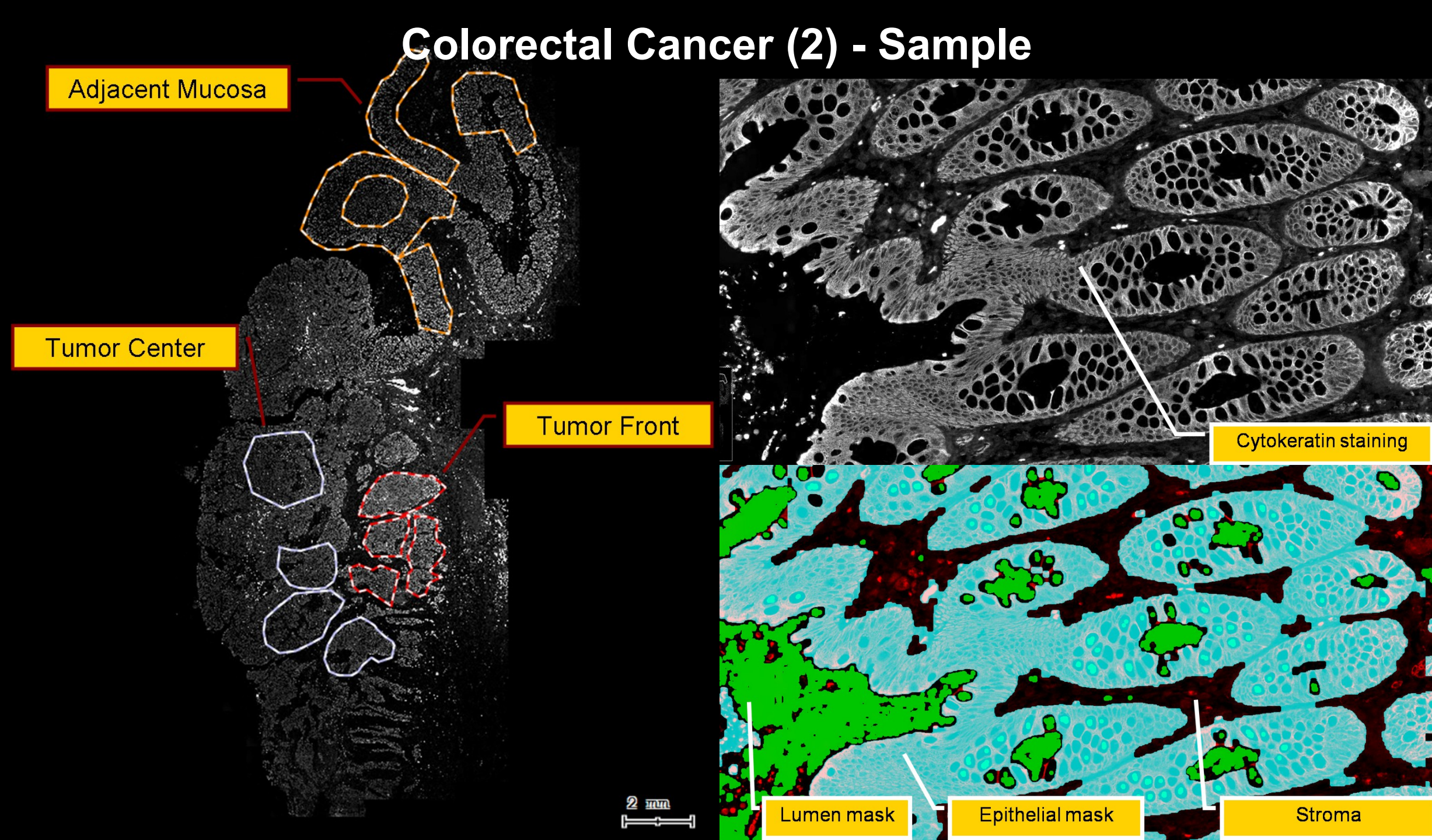
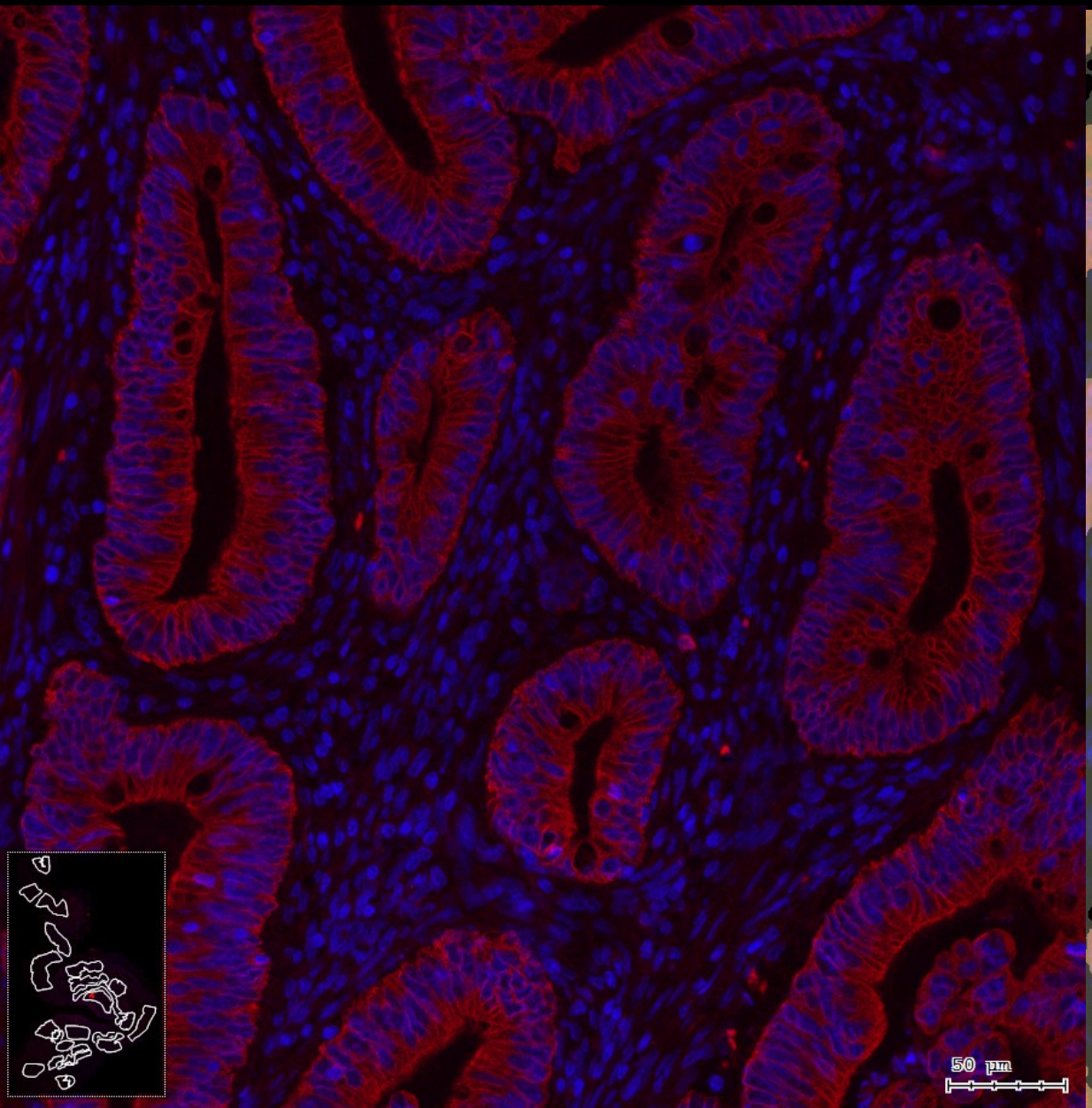
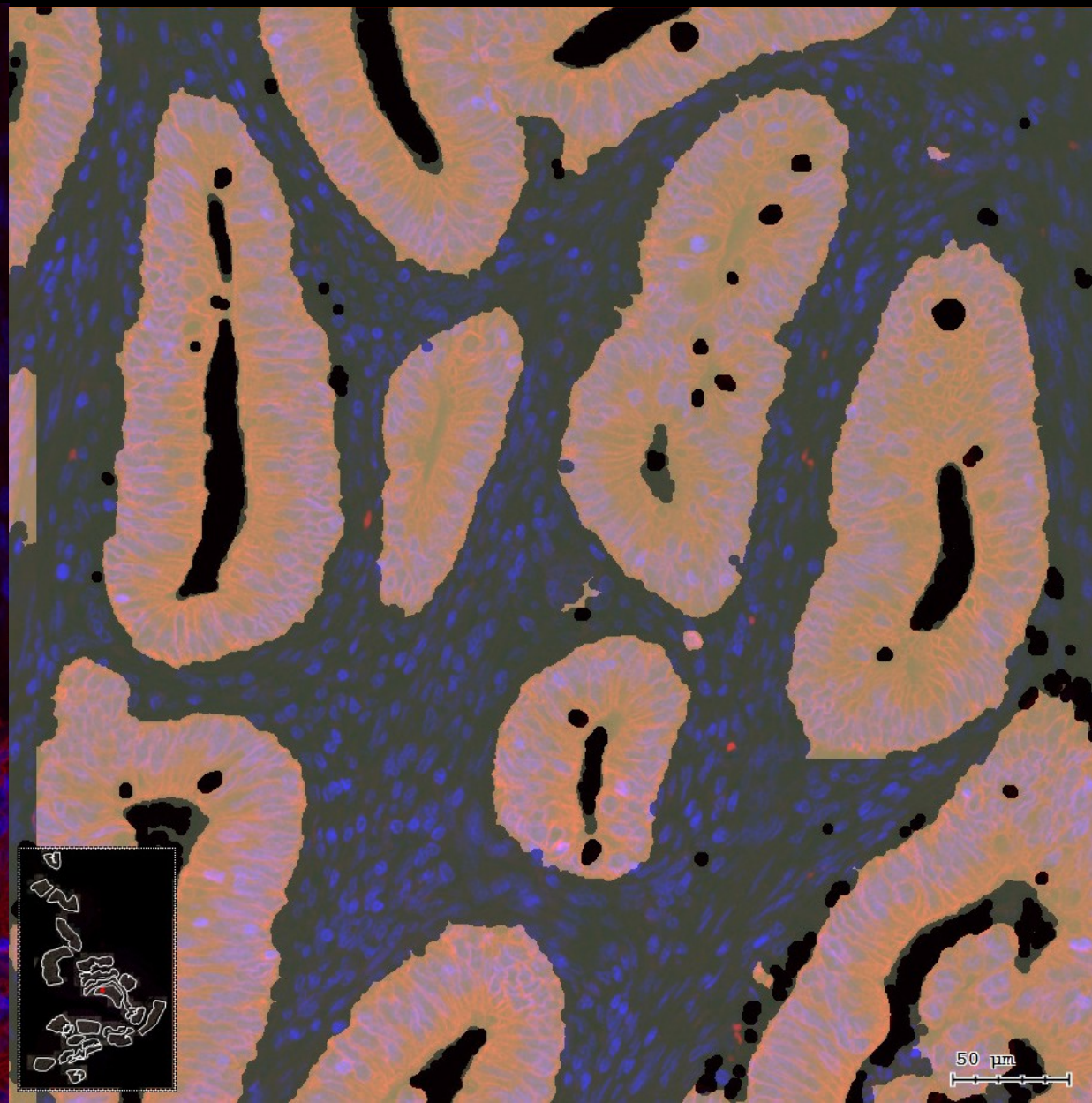
Colorectal Cancer (2) - Sample



Colorectal Cancer (3) - Results





Colorectal Cancer (3) - Results

CONCLUSIONS AND OUTLOOK

1) The strong reduction of the epithelium/stroma ratio at the invasive front of colon cancer tissue of patients with liver metastasis confirms the benefit to use this parameter as an additional information in CRC diagnosis.

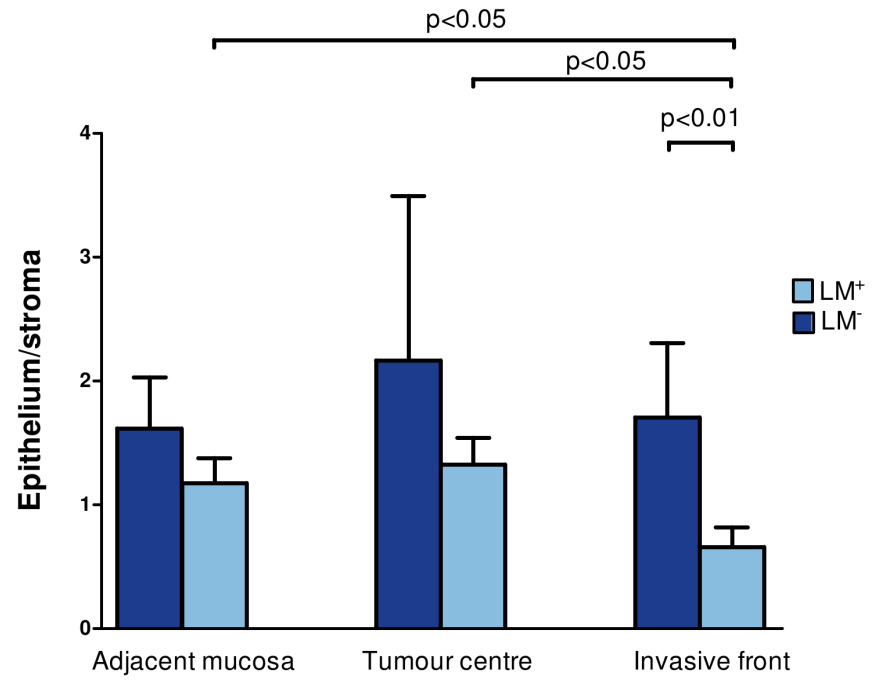
2) Our results are in agreement with previous studies that used visual methods.

3) The implemented software can be used for detailed morphometric analysis of CRC tissue, as well as for the quantitative evaluation of markers expressed in the epithelial or stromal area for a better characterization of epithelium-stroma interaction in tumour progression.

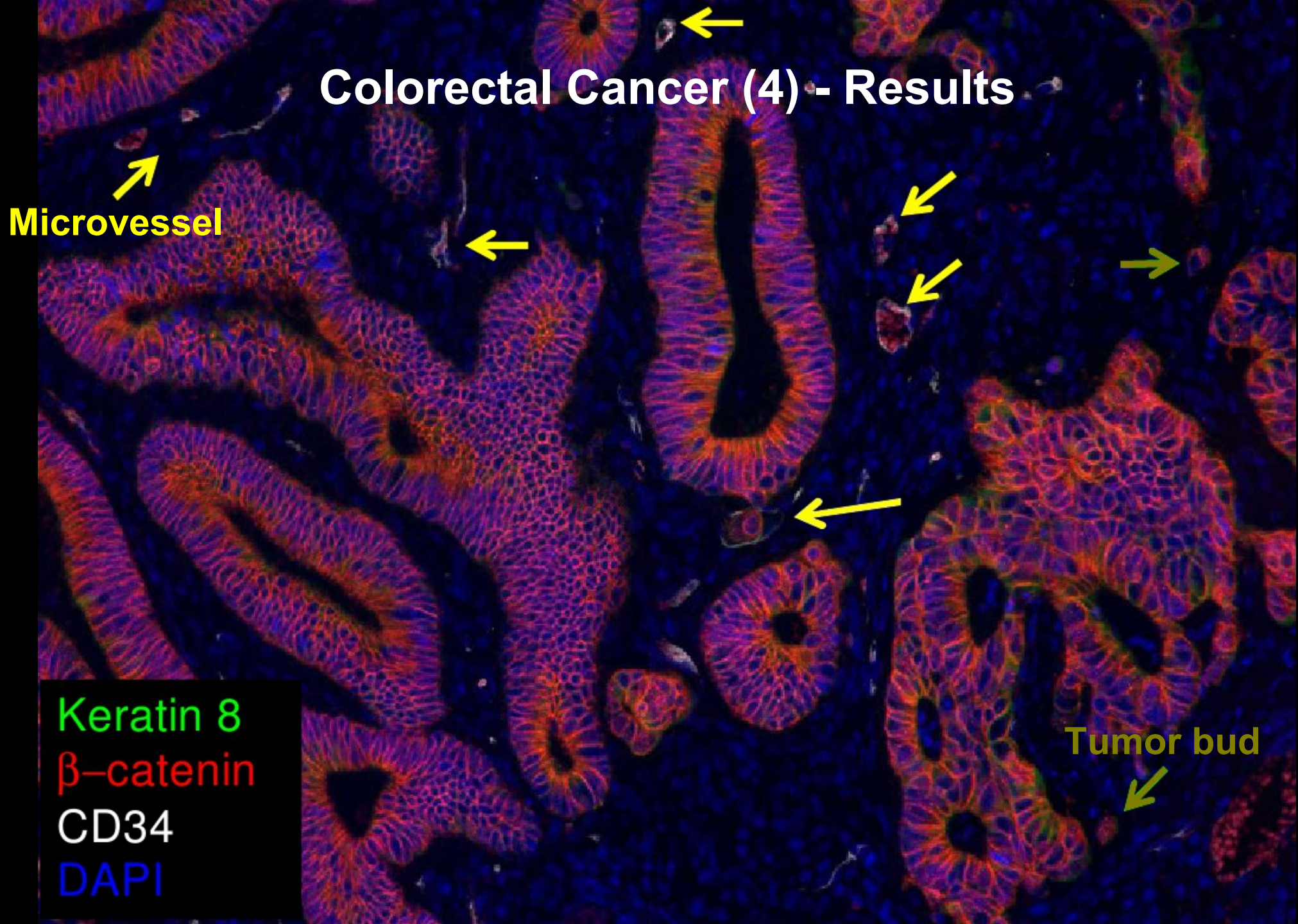
50 µm

50 µm

Figure 3 – Epithelium/stroma ratio in different sub-regions in colorectal cancer patients with and without liver metastasis



Colorectal Cancer (4) - Results



Colorectal Cancer (4) - Results

Microvessel

Keratin 8
β–catenin
CD34
DAPI

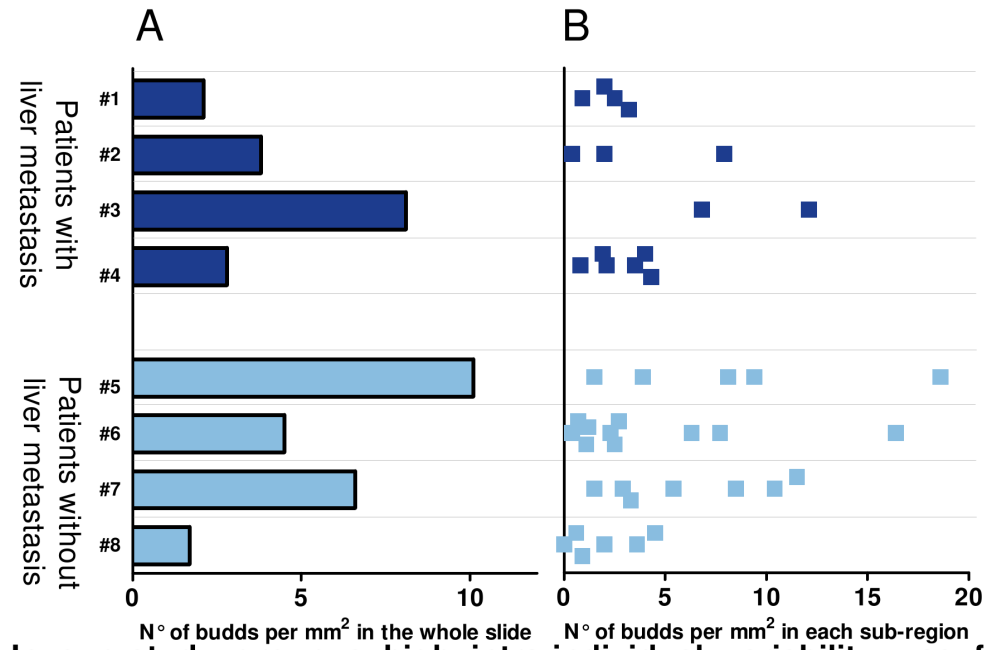


Figure 4. Intra-tumoral budding was manually assessed in tissue specimens of 4 colorectal cancer patients with liver metastasis (#1 - #4) and 4 without liver metastasis (#5 - #8). Results are presented as buds per mm^2. **(A)** The total number of buds counted in each specimen was related to the overall tumor center area. **(B)** Each tumor center sub-region is represented by a square.

In our study group a high intra-individual variability was found, more pronounced in patients without liver metastasis.

CONCLUSION AND FUTURE DIRECTIONS

Our data suggest no correlation between intra-tumoral budding and the presence of liver metastasis in patients with colorectal cancer grade 2. This might me due to the limited number of patients. Therefore, we propose assessing intra-tumoral budding in a larger cohort of patients. Furthermore, we aim to evaluate budding in relation to other factors such as distance to blood and/or lymphatic vessels.

Watching C. Elegans Think

Basic research project in Systems Neuroscience

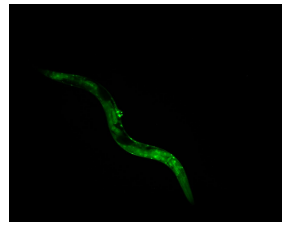
Four Objectives

- Engineering — *Real-time tracking nerve cells*
- Methodological — *Validate nervous cell models*
- Holistic — *Understand complete N.S.*
- Insight — *Better learning algorithms*

Model organism: C. elegans

~ 1000 cells, ~ 300 nerve cells

Might be feasible to simulate



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C. Elegans Protein Localization (1)

Seewald AK, Cypser J, Mendenhall A, Johnson T (2010) Quantifying Phenotypic Variation in Isogenic Caenorhabditis elegans Expressing Phsp-16.2::gfp by Clustering 2D Expression Patterns, PLoS ONE 5(7): e11426. doi:10.1371/journal.pone.0011426.

Quantifying Phenotypic Variation...

Analyzing changes in appearance / phenotype...

in Isogenic Caenorhabditis elegans...

in small nematodes (worms) which all have the same genetic code (clones)

Expressing Phsp-16.2::gfp...

which express a GFP reporter that binds to heatshock protein 16 (transgenic)

by Clustering 2D Expression Patterns

by extracting 2D expression patterns that are independent of worm pose AND clustering these patterns using hierarchical clustering methods.

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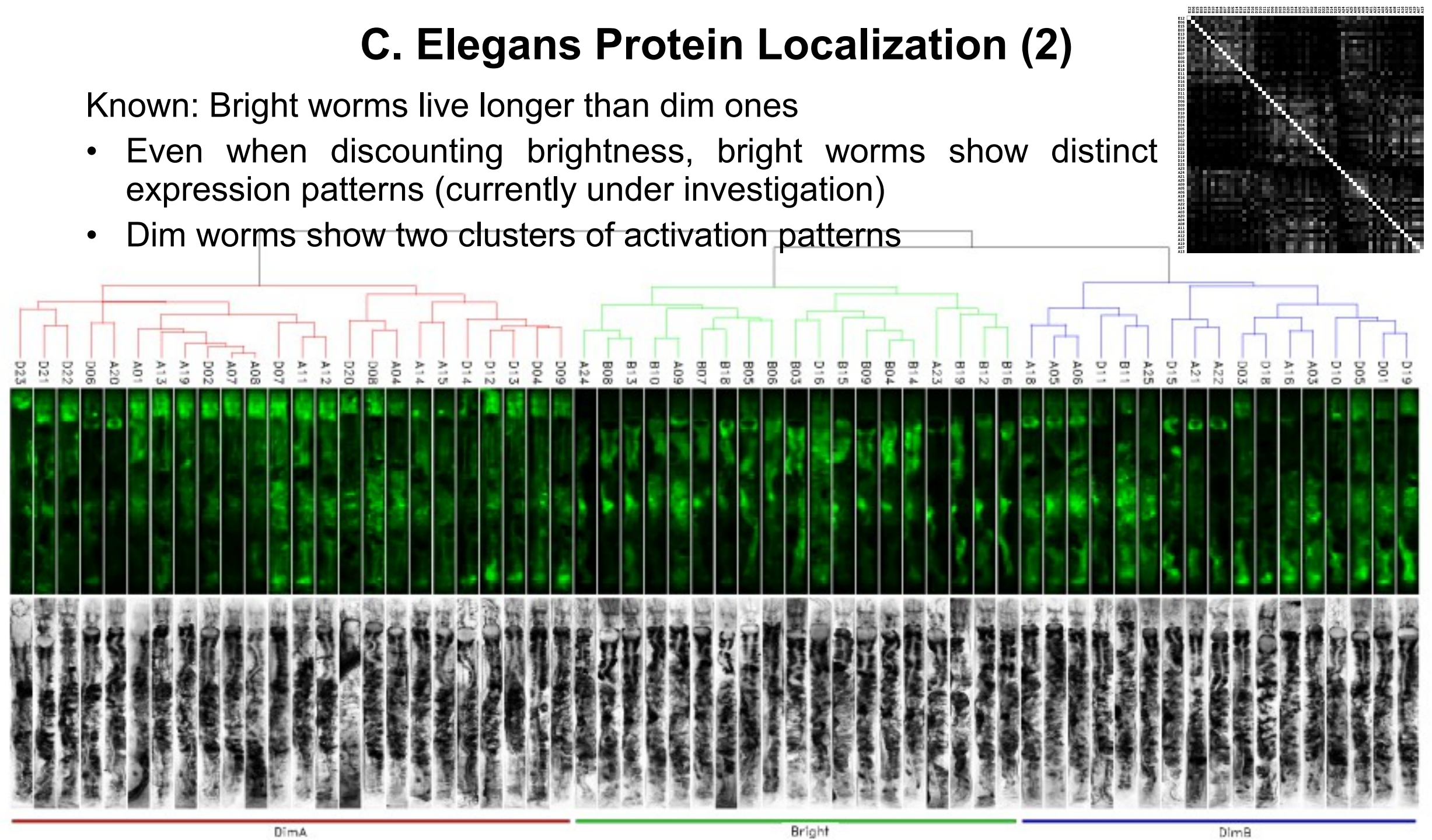
C. Elegans Protein Localization (2)

Known: Bright worms live longer than dim ones

- Even when discounting brightness, bright worms show distinct expression patterns (currently under investigation)
- Dim worms show two clusters of activation patterns



C. elegans Intestine Nuclei Detection

(R.Brent & A.Mendenhall, FHCR)

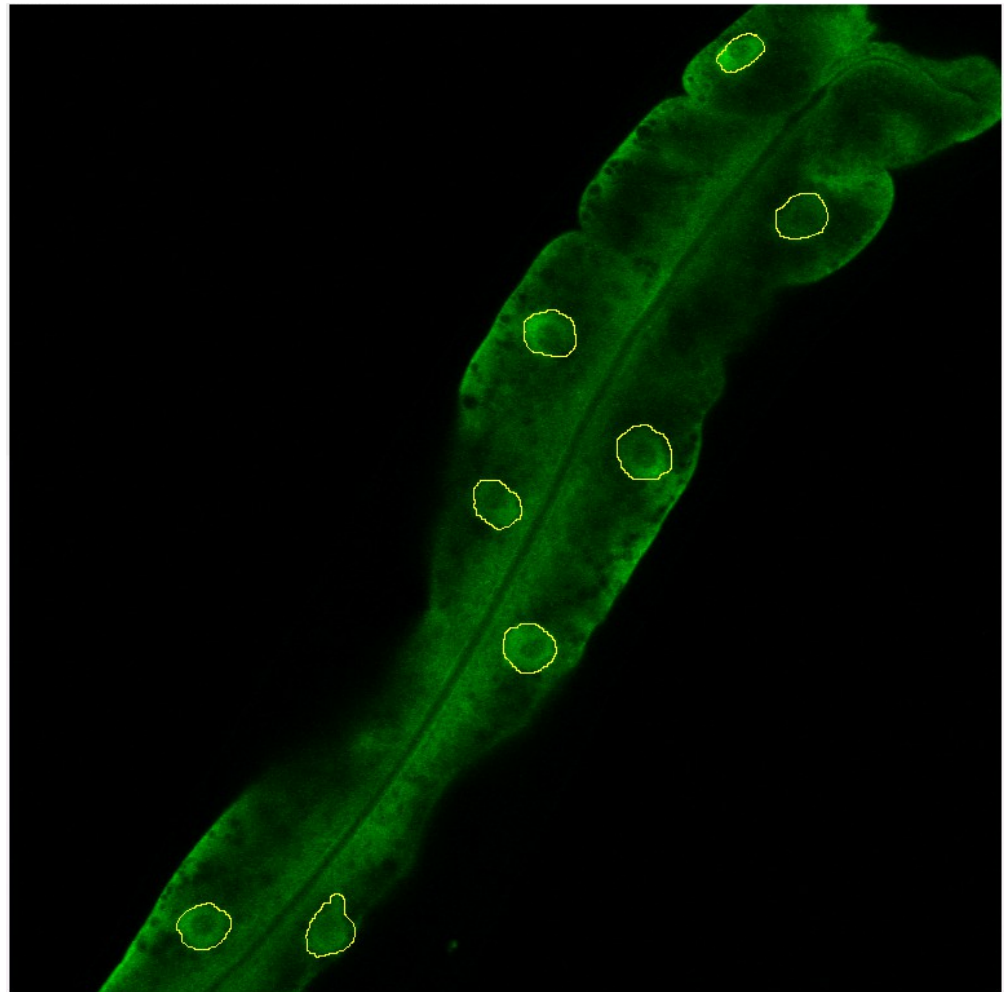
- Intestine nuclei in 3D stacks from point-sampled confocal recordings
- Nuclei borders are very noisy...

Tested three approaches so far..

- ML rule learners to directly predict cut-off point from 1D rays through border
- Avg. profile + dynamic time warping
- Avg. profile + cross-correlation

... first one works best.

TODO: prototype detector; 6-DOF stitching, 3D worm pose normalization



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Video - Worm Dedistorting in Real Time

(data by IMP Vienna, M. Zimmer's lab)

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Conclusion

Designing Image Analysis Systems is tough and takes time...

- Four systems designed within three year FFG Bridge project (2.5 outside)
  2 PhD students, 1 CompSci researcher, 3 biological res., ~7 additional students
- Obtaining useful images and reliable ground-truth data is the key!
  Sometimes tasks are ill-defined and/or experts disagree on classification
- Image Machine Learning techniques can speed up implementation significantly but are currently applicable only to restricted imaging tasks (e.g. *Ery.Removal*)
- Parameter optimization & algorithm finetuning with ground truth data can dramatically improve even simple systems.

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Thanks for listening!

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