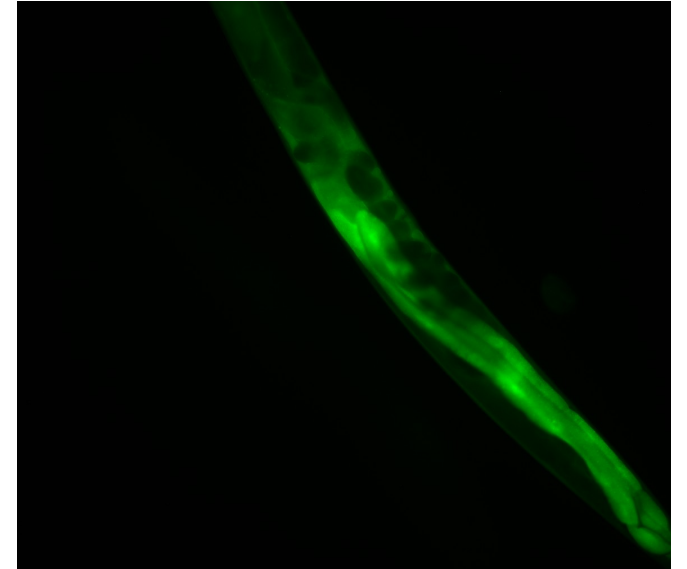
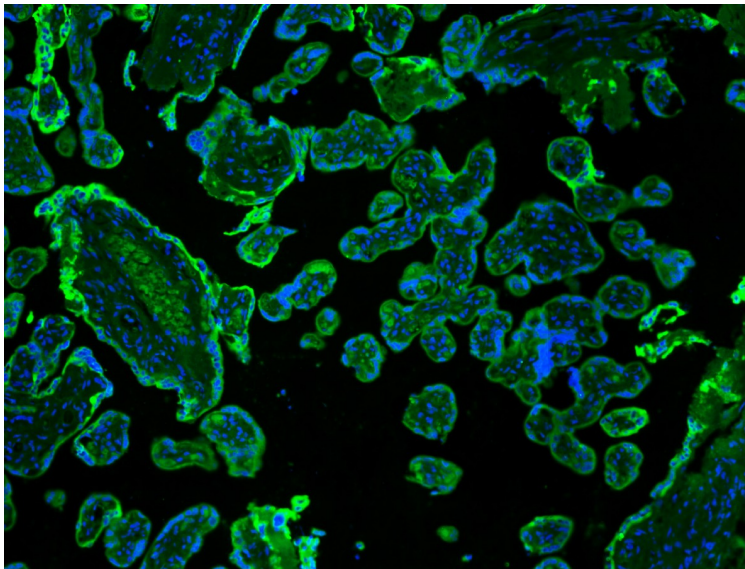


Image Data Analysis in H.sapiens and C.elegans



Dr. Alexander K. Seewald



(Our) Relevant Collaborations for Image Data Analysis

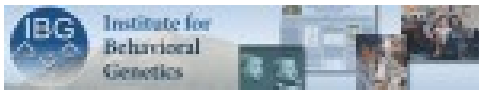


2009-2012

Med.Univ.Vienna, Austria

Tissue Gnostics GmbH

Funded by FFG Bridge



2007-2010

Univ. Colorado@Boulder, USA

Self-funded



2011-

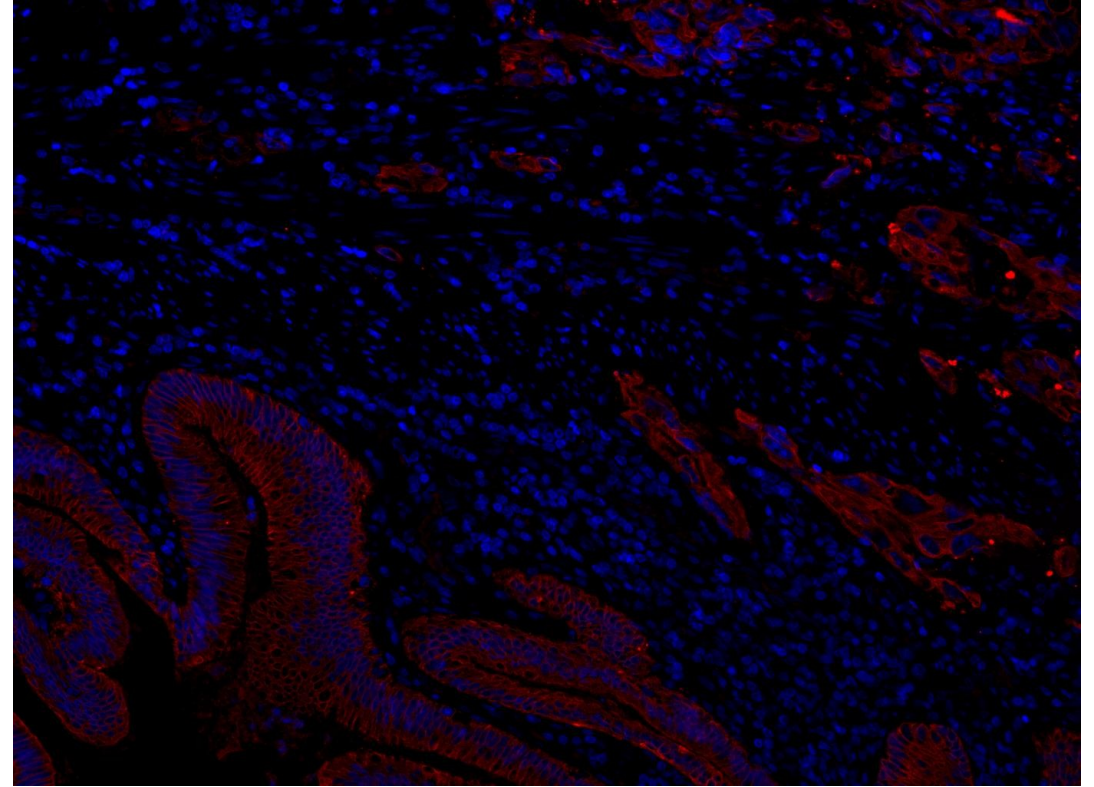
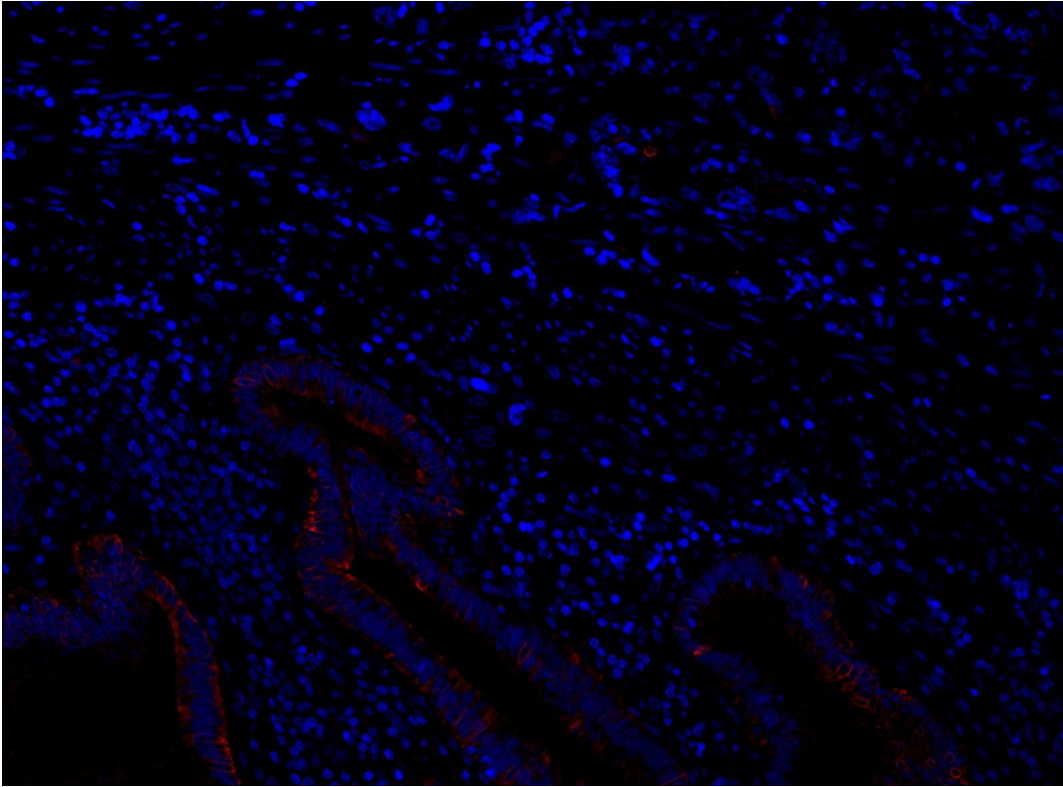
IMP Vienna



Video 1 - How Tissue Samples Are Created



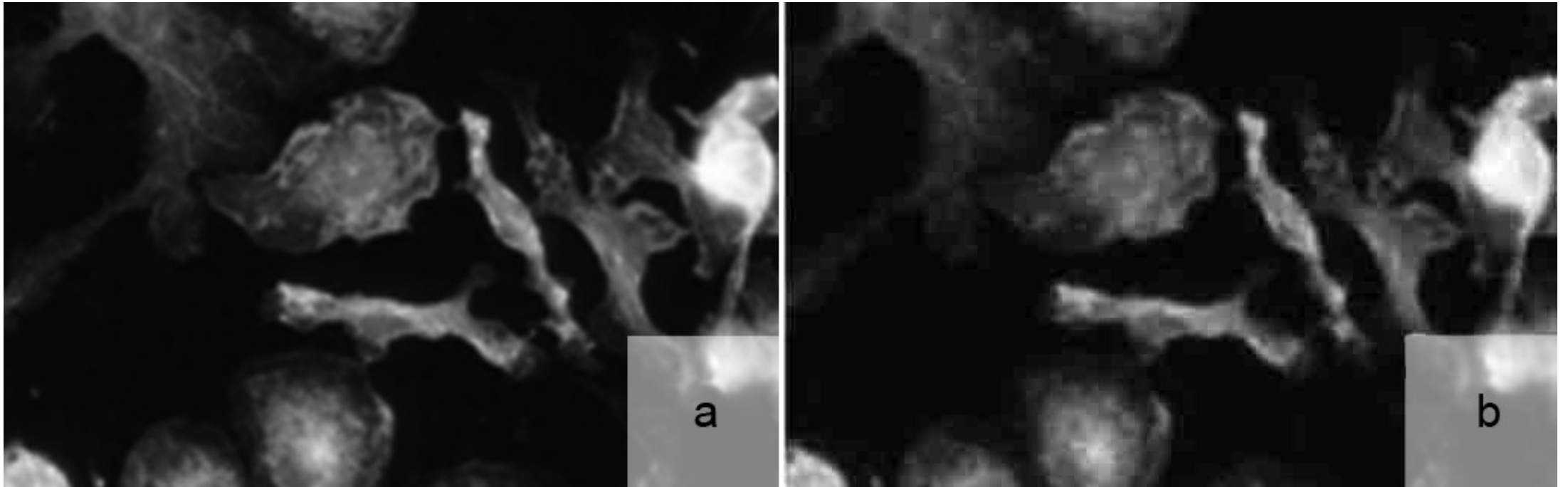
Staining tissue is a complex process!



- Tissue characteristics (thickness, density, age, photobleaching, ...)
- Quality of antibodies (too old, too new, wrong kind, cross-reactions, ..)
- Crosstalk between multiple stainings, Protocols, MTA characteristics...

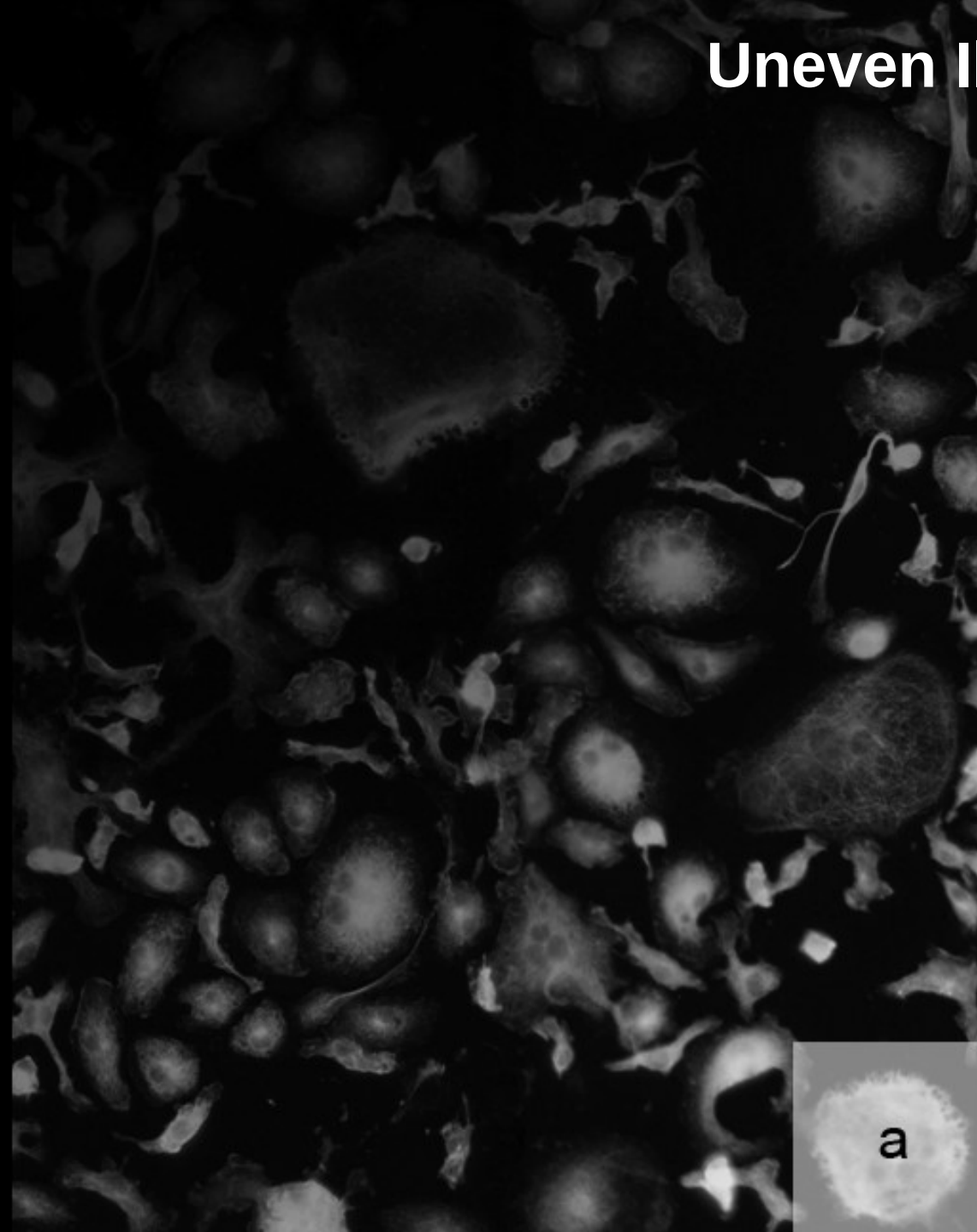
Image Formats

Use only lossless formats (TIFF, PNG)!

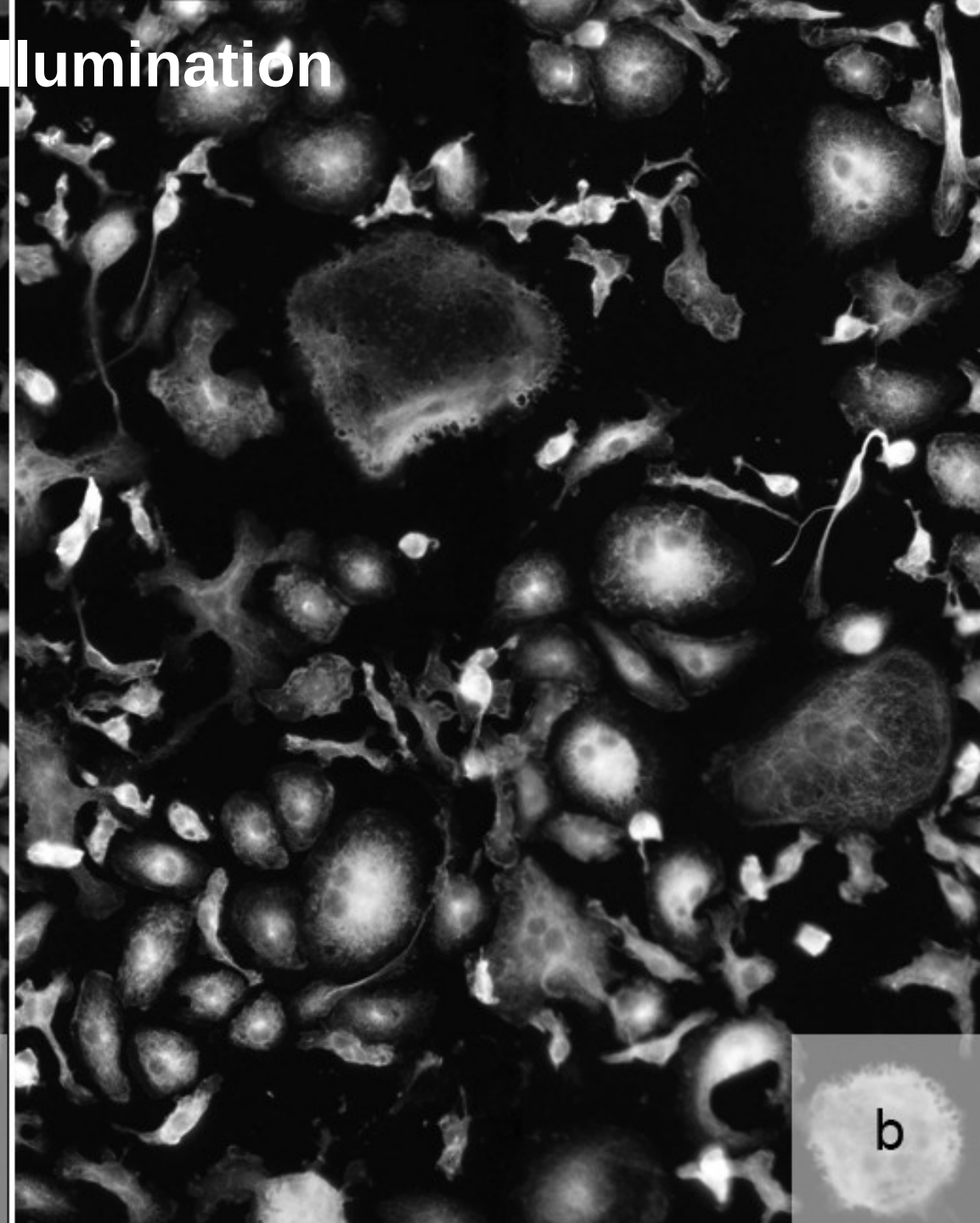


Especially JPEG is to be avoided! Removes information not visible to human eye which is still useful to image processing, introduces artefacts...
Also: No normalization of input images!

Uneven Illumination

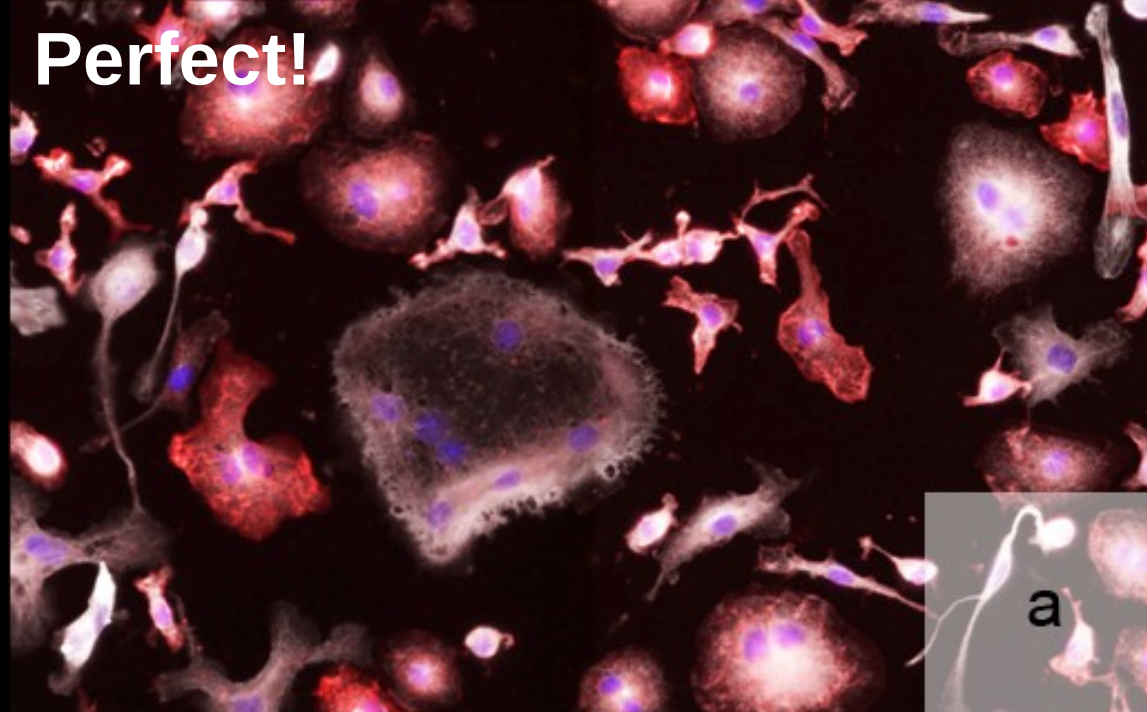


a

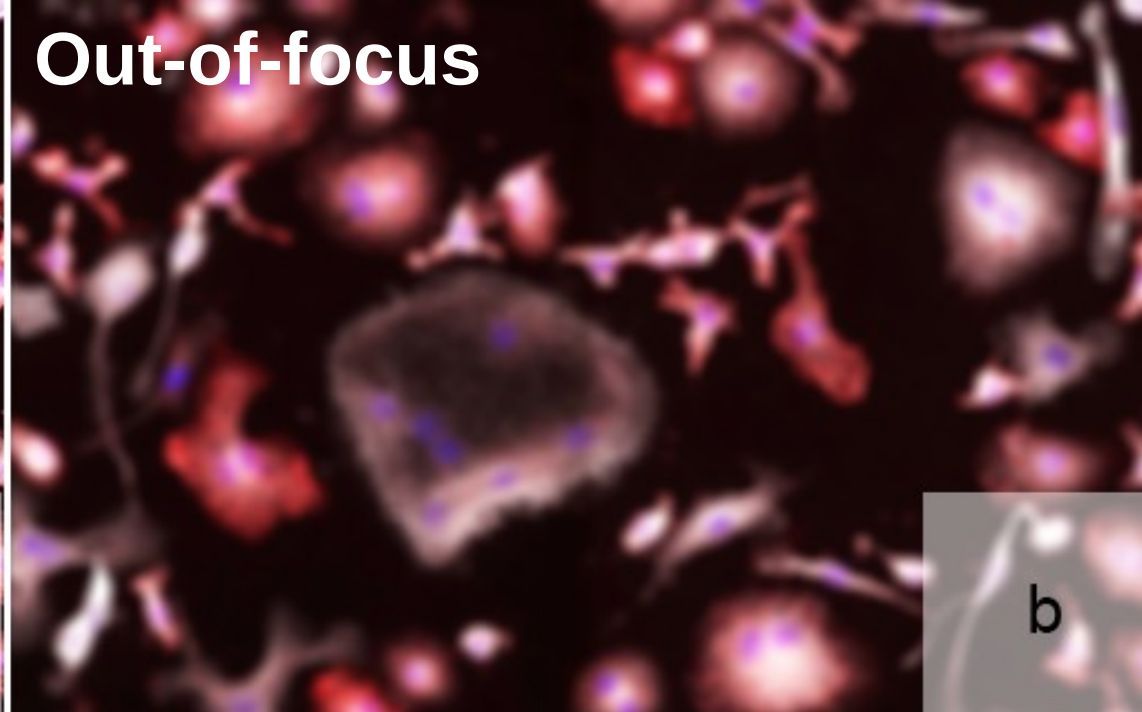


b

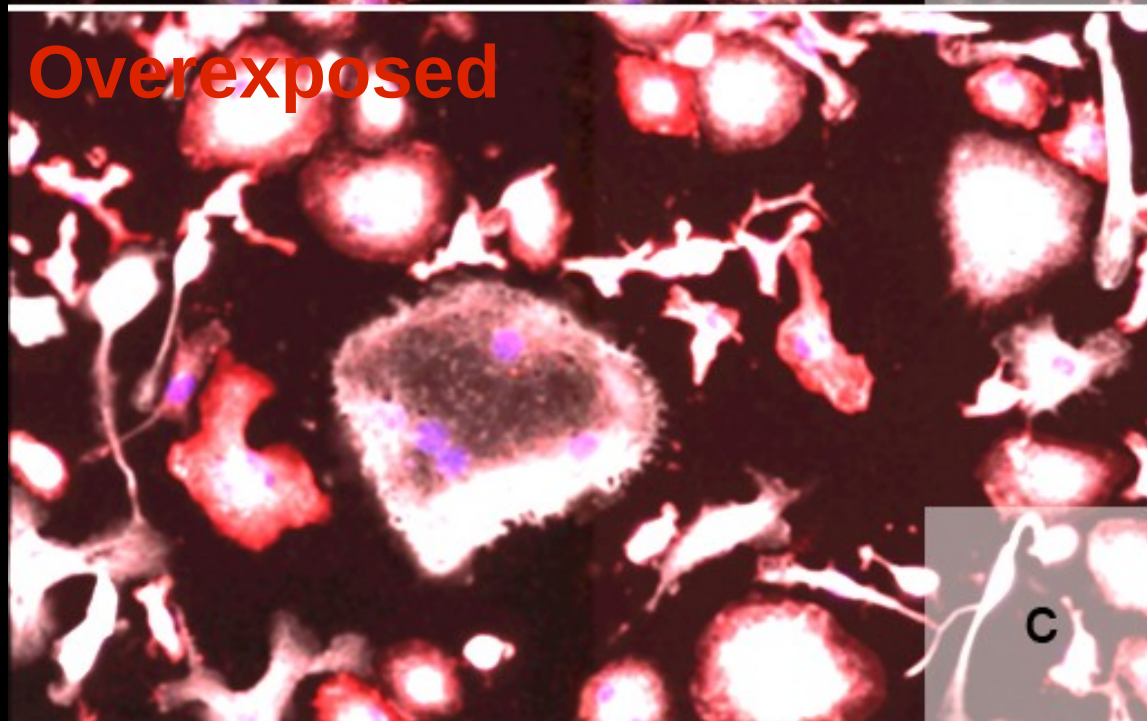
Perfect!



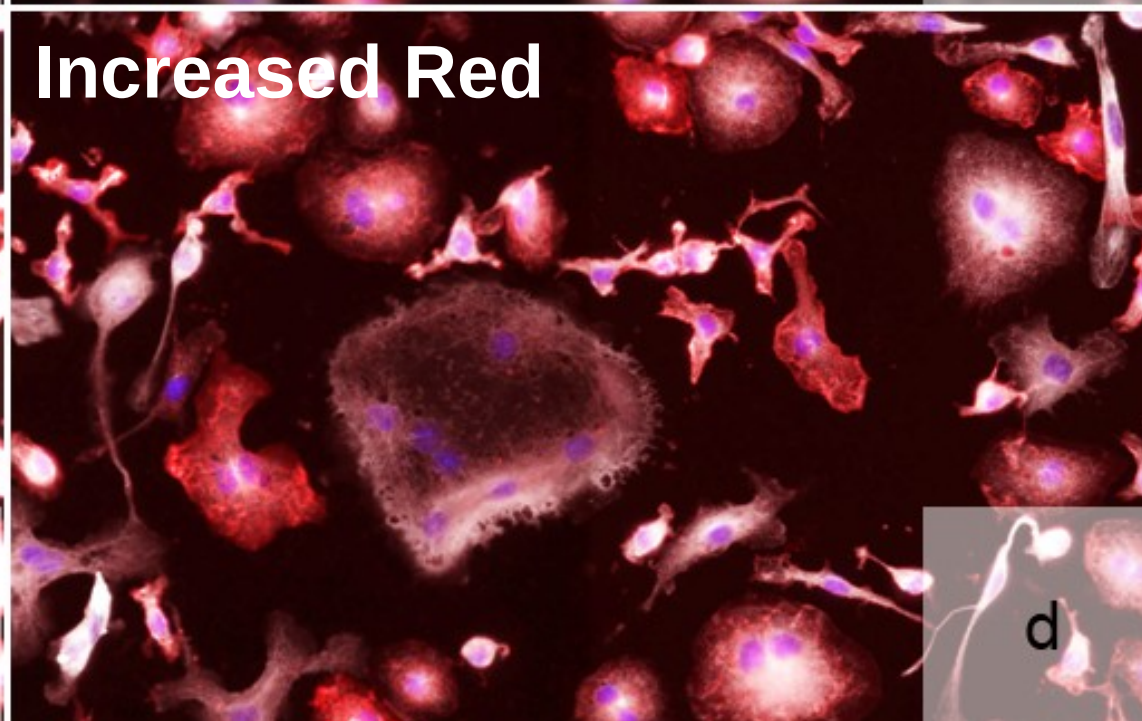
Out-of-focus

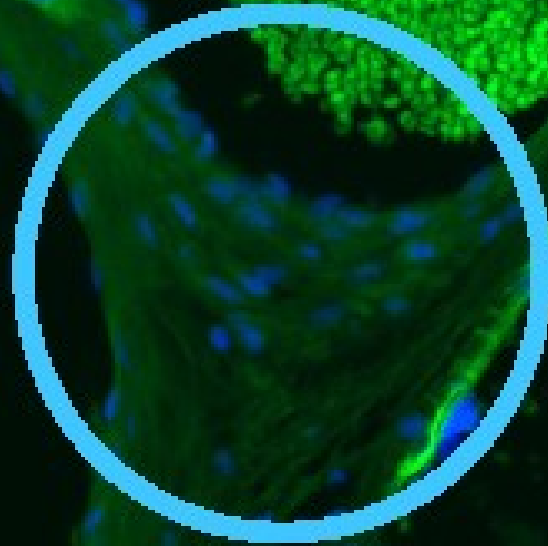
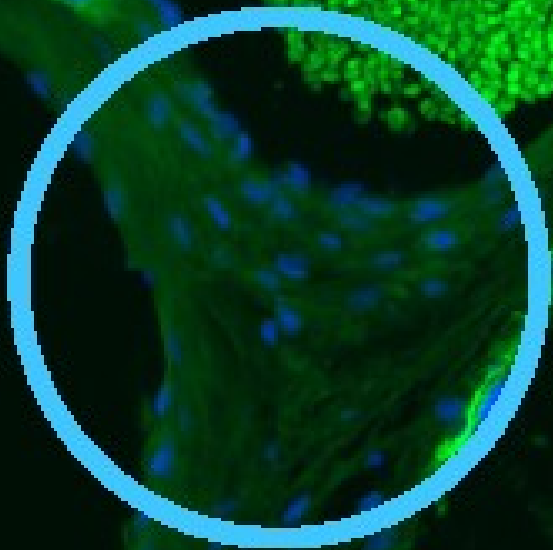


Overexposed



Increased Red

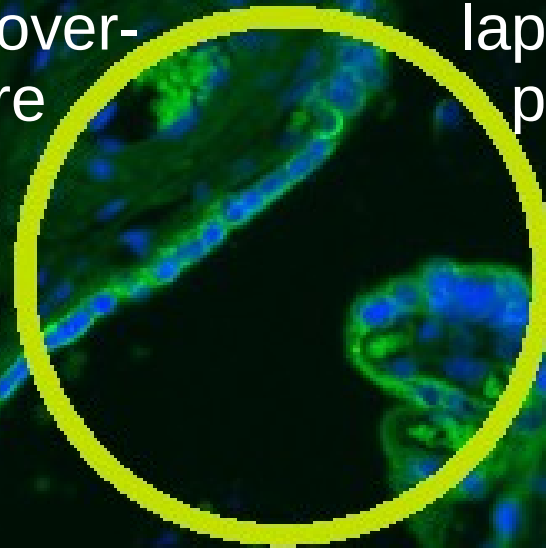




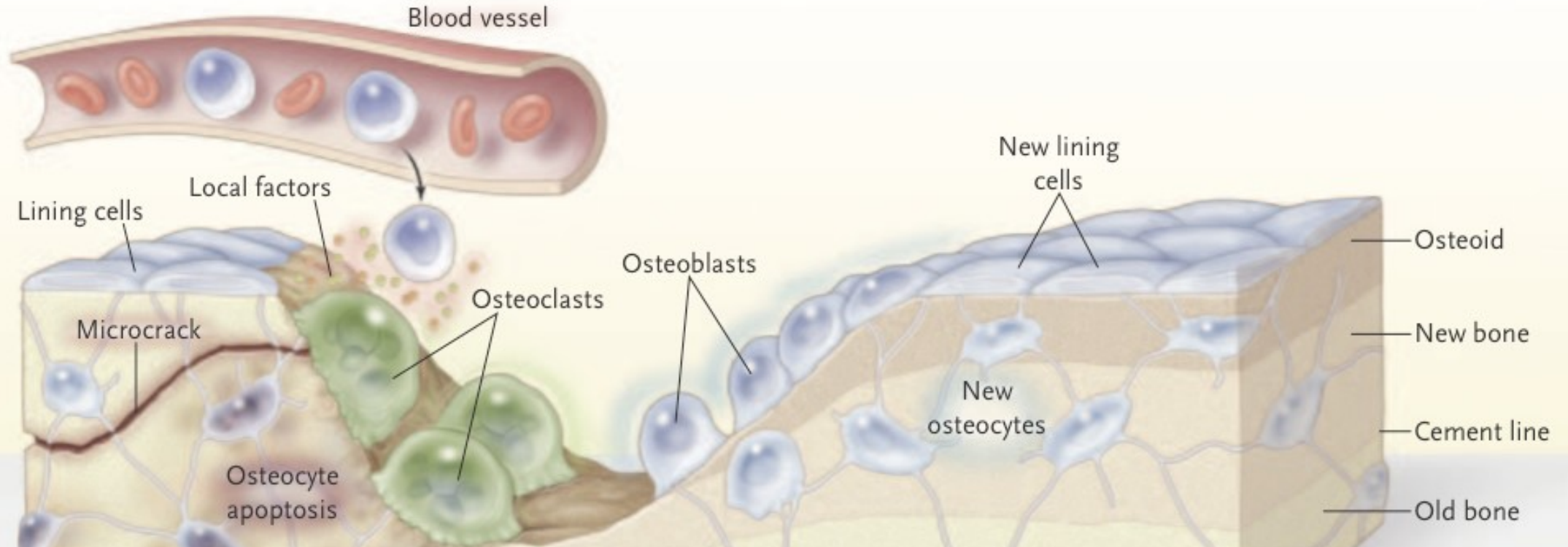
Slide-based microscopy records many images by automatically moving the tissue sample vs. the microscope. Tiling images together may not be sufficient.

Stitching analyzes the overlap between adjacent images to determine a more precise combination. **We had to develop**

our own algorithm.

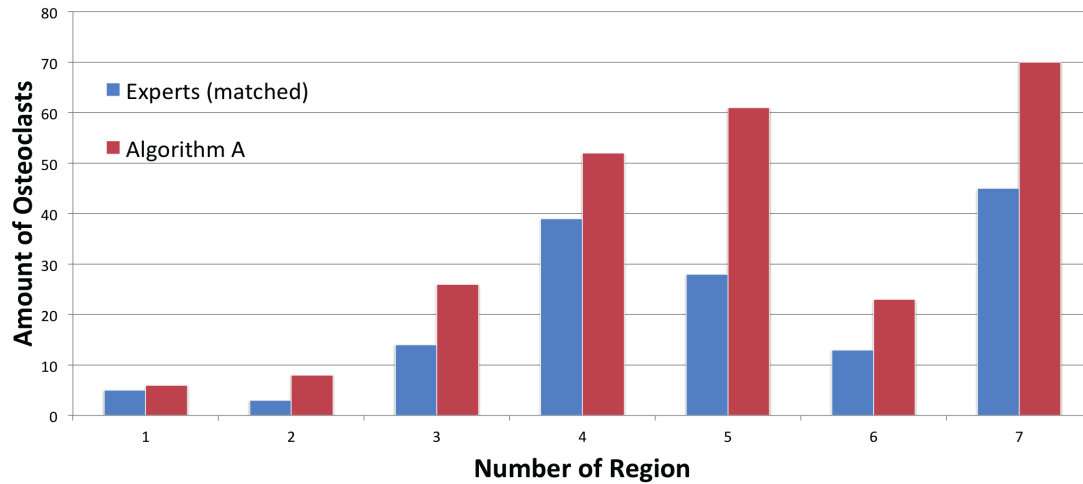


ClastoQuest (1)



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, called ClastoQuest.**

ClastoQuest (3) - Algorithm



•Criterion 1 computes the ratio of the macrophage marker inside the cell versus the total area of the cell

$$\frac{\text{amount}(\text{macrophage marker})}{\text{area of cell}} < T_1$$

•Criterion 2 counts the amount of DAPI staining inside the cell

$$\text{amount}(\text{DAPI}) > T_2$$

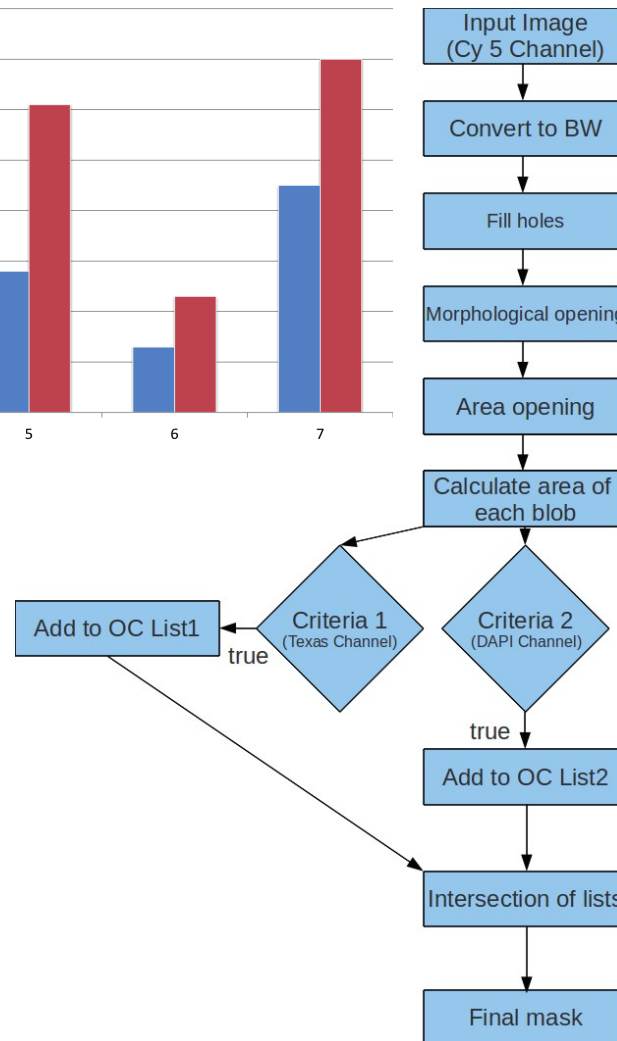


Image processing

Classification

Result

ClastoQuest (4) - Current Work

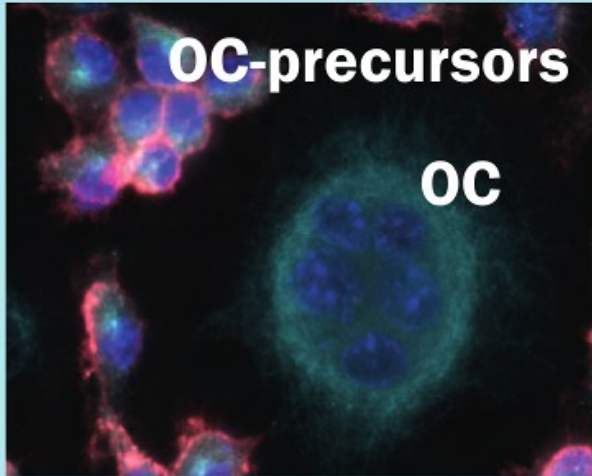


Figure 5

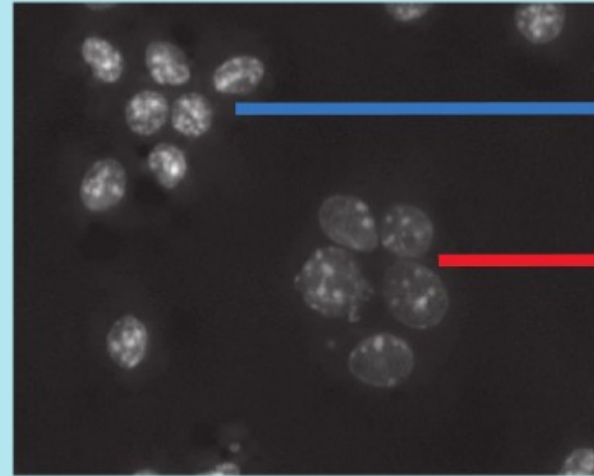
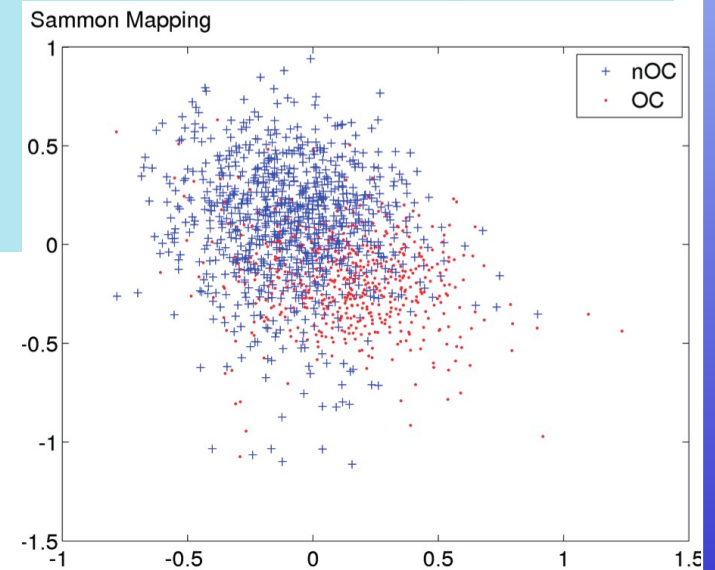
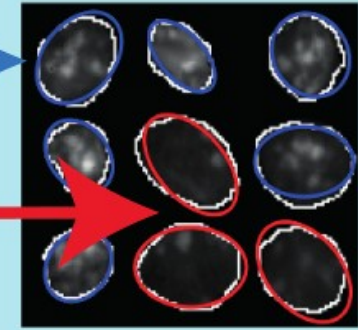


Figure 6



Shape and texture features computed from nuclei of osteoclasts (OC) and non-osteoclasts (nOC) allow to determine class with some confidence (Logistic regression 10CV: 83% accuracy, 75% on holdout test sample) May be combined with ClastoQuest for additional precision. **Dataset**

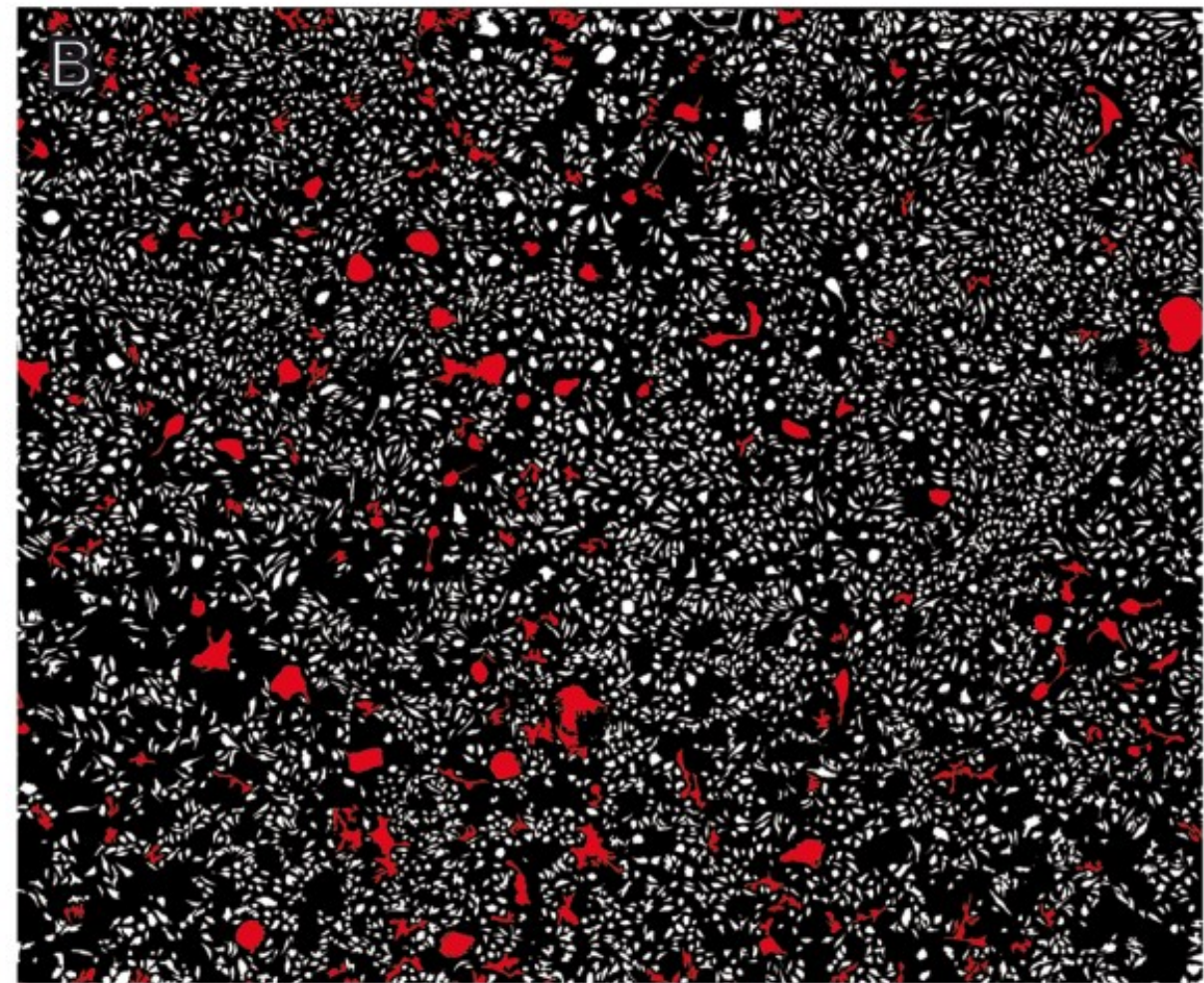
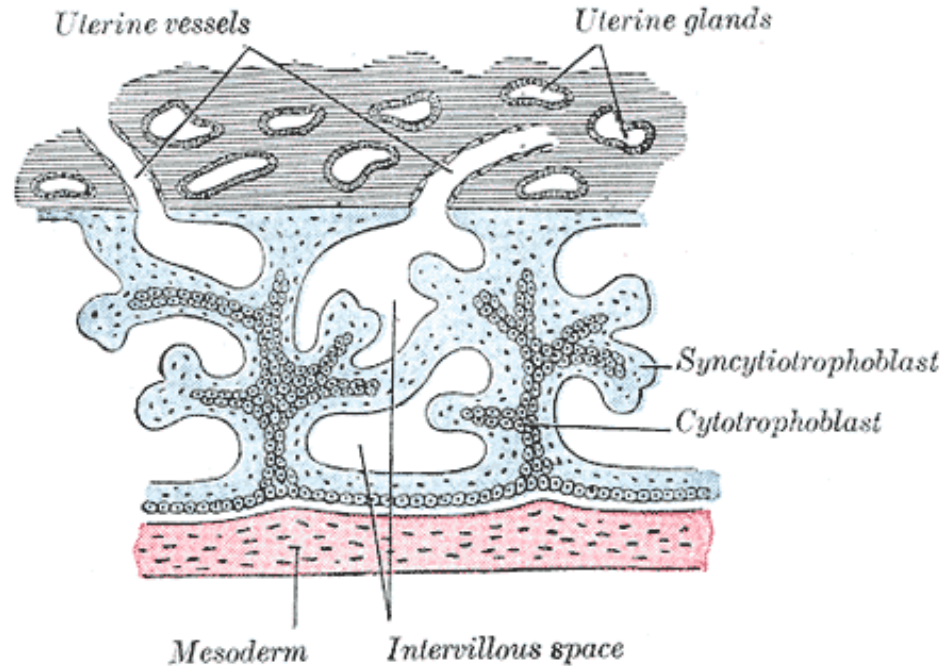


Figure 4: Image analysis of a whole sample-region consisting of 100 (10 x 10) FOVs. A: Immunofluorescence image. B: Result of cell segmentation and analysis: non-OC are marked in white, OC are marked in red.

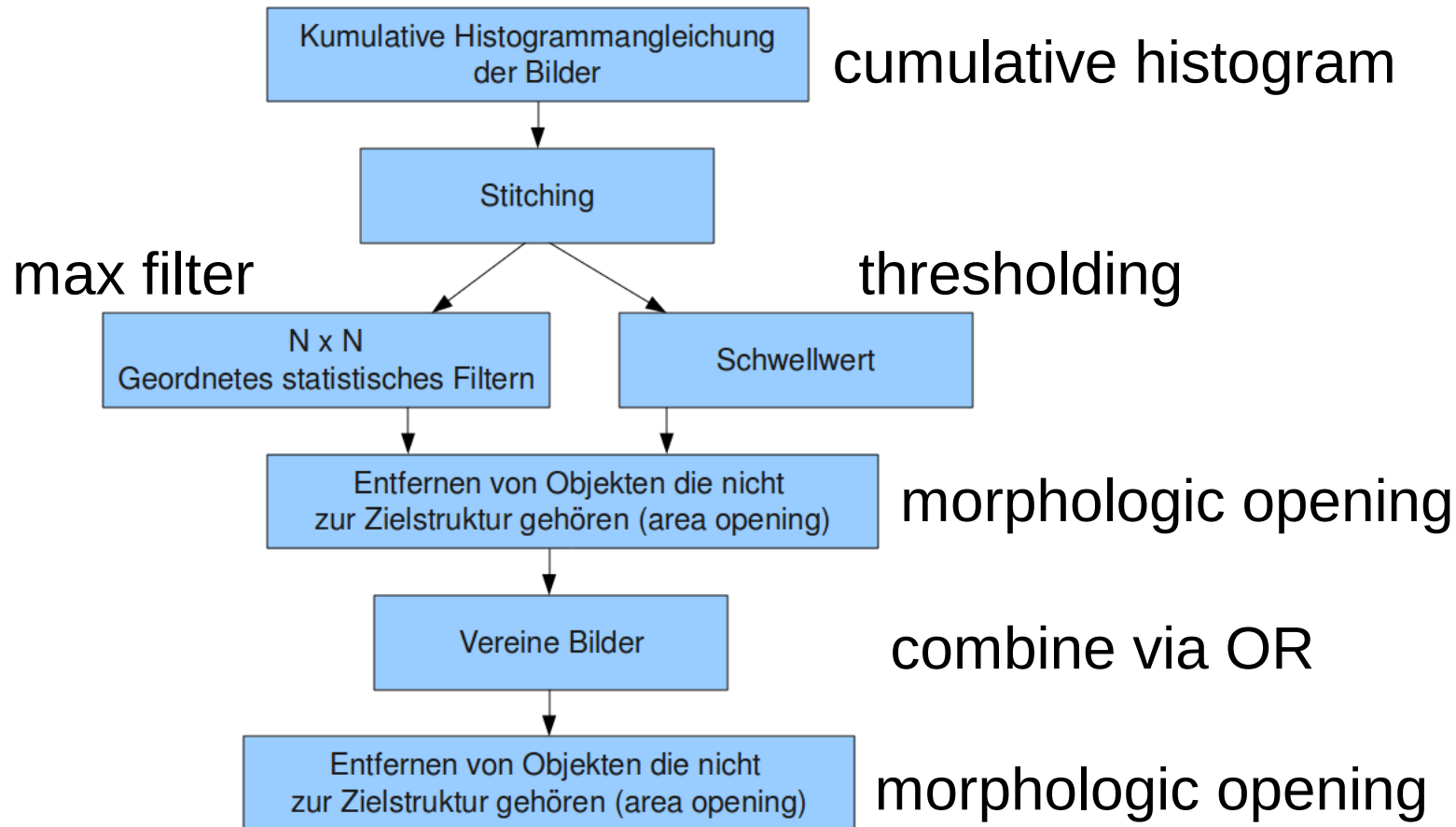
Syncytiotrophoblast (1)

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)**

Syncytiotrophoblast (2) - Algorithm



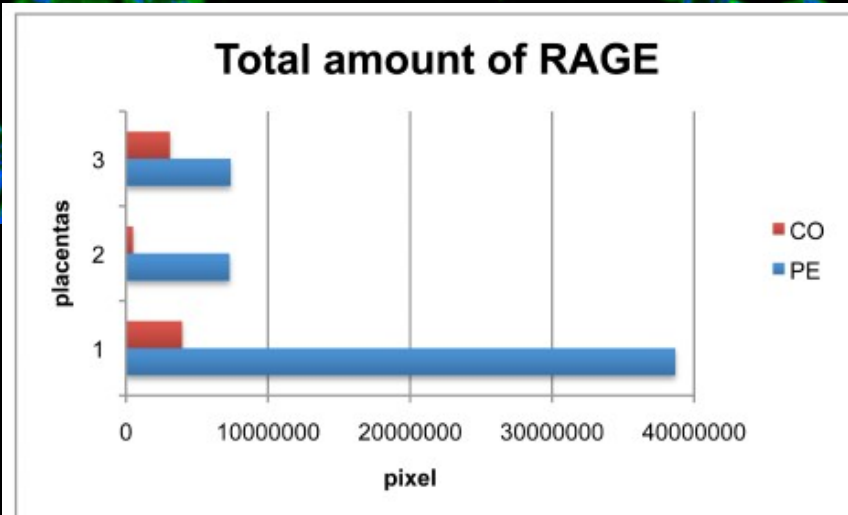
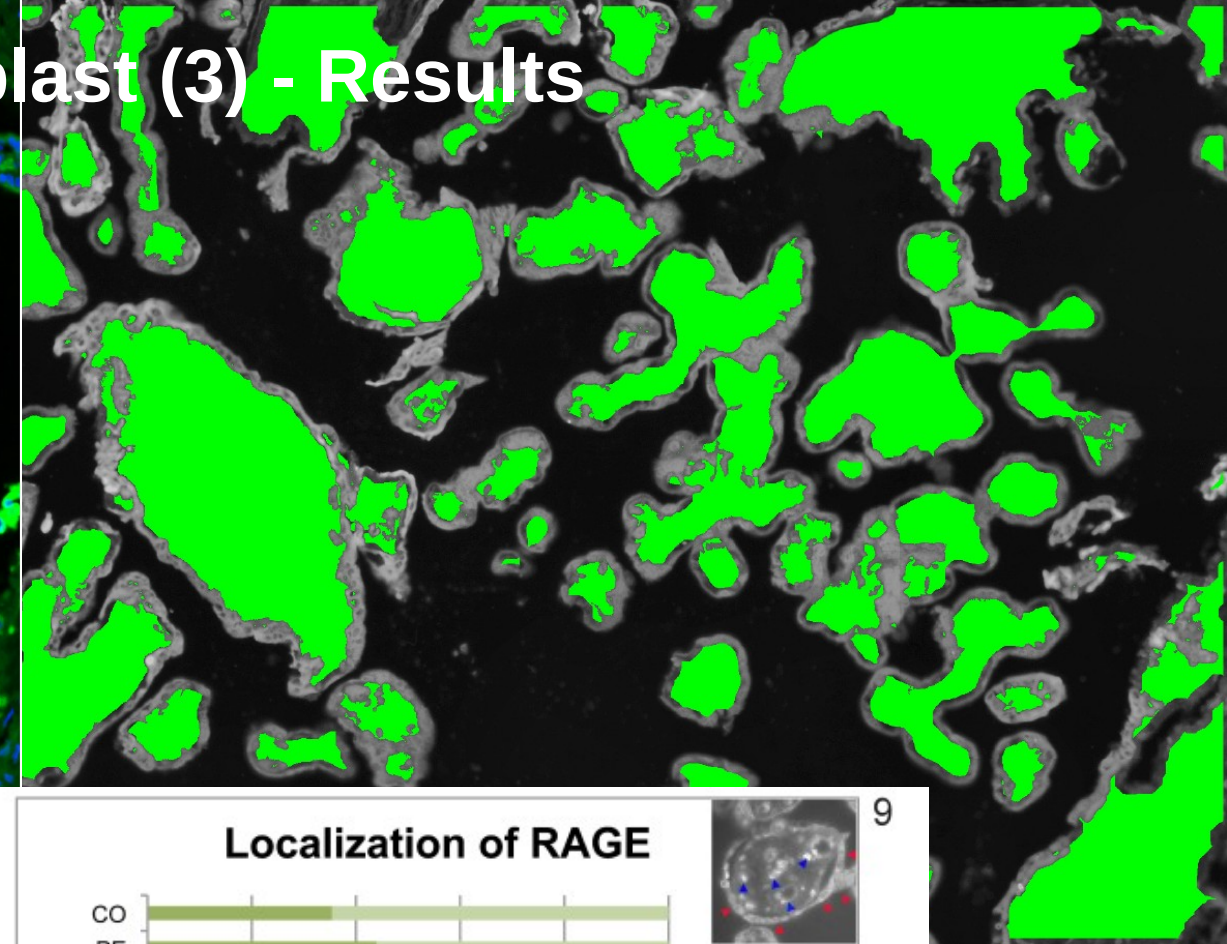
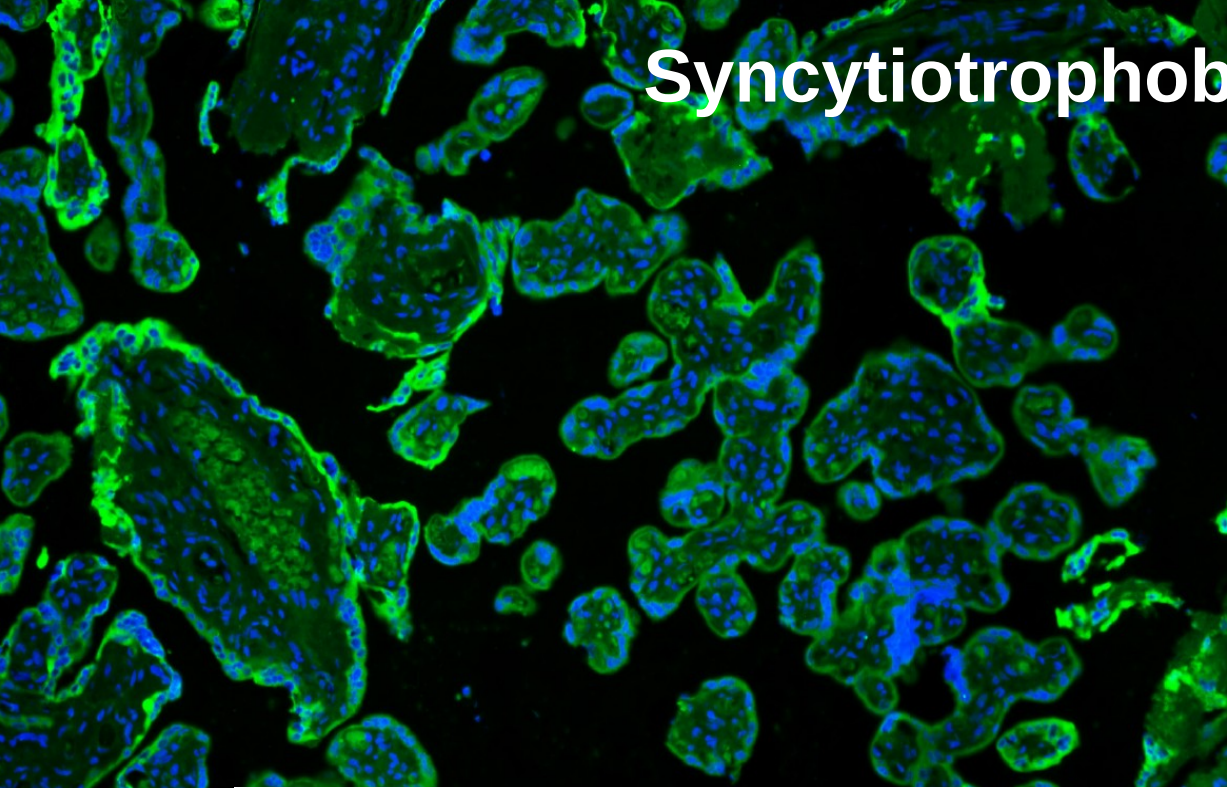
&

Exhaustive
Parameter-
Optimization

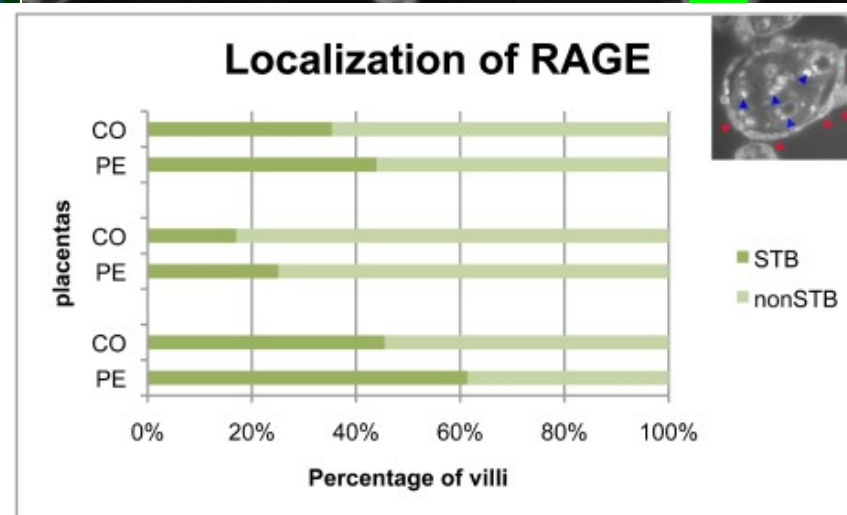
Around 8,100
sets of 7
parameters were
tested.

Improved F1
from 0.78 to 0.93

Syncytiotrophoblast (3) - Results



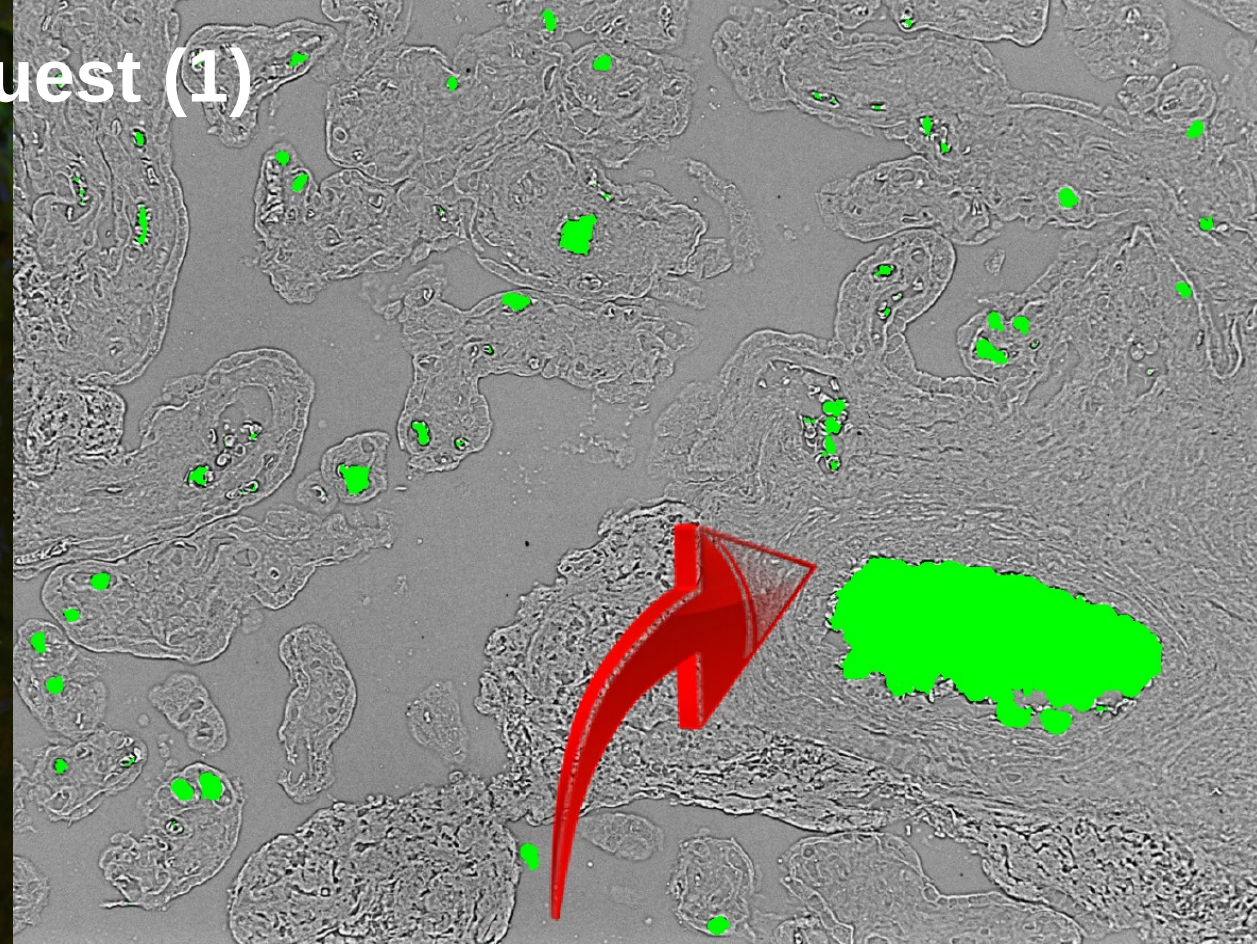
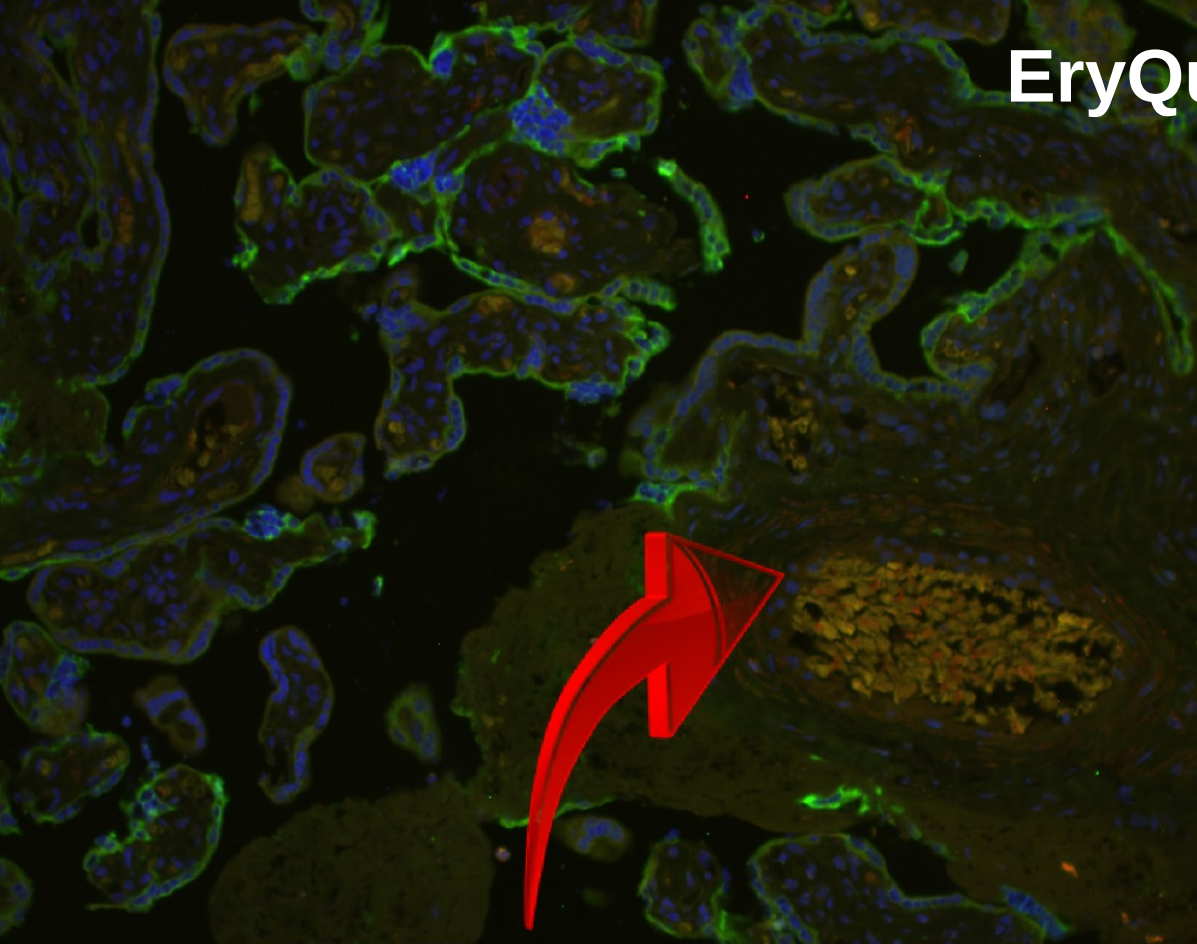
8



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.

EryQuest (1)



Erythrocytes have high autofluorescence. 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!

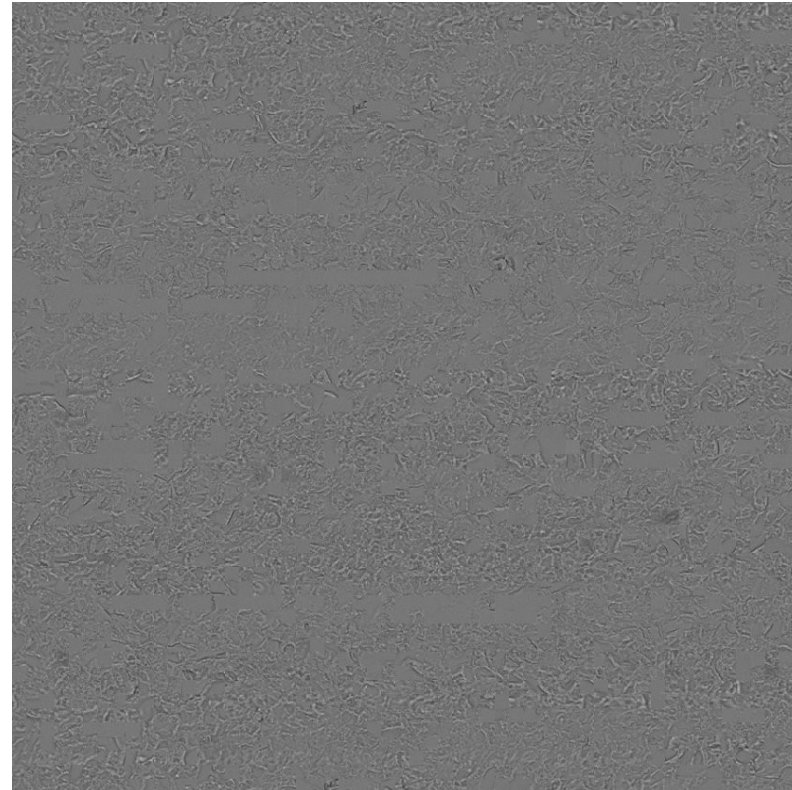
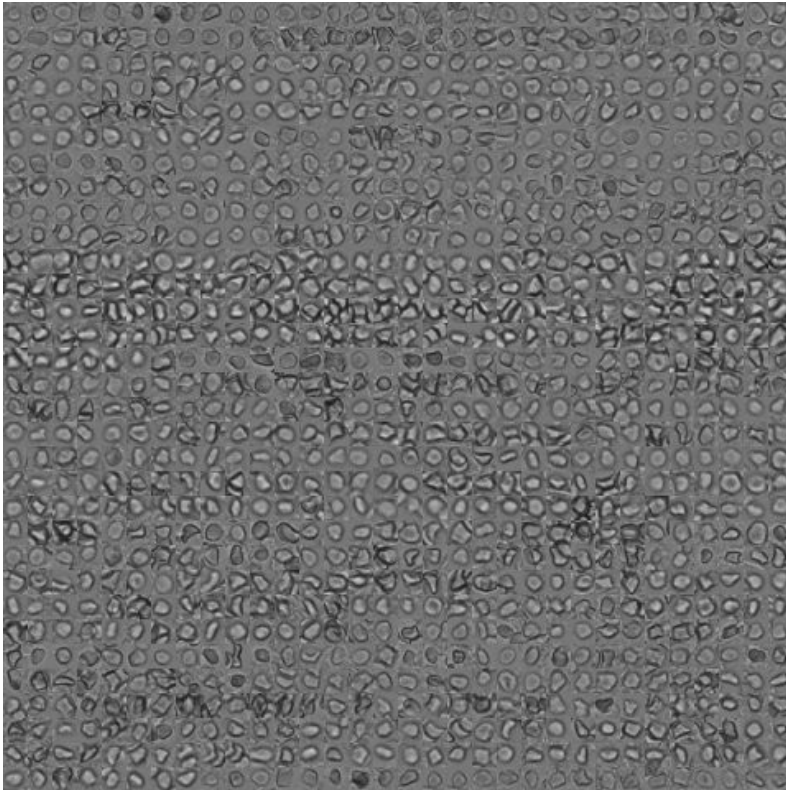
EryQuest (2) - Training

Training Data

>4000 erythrocytes have been marked... up manually as well as...

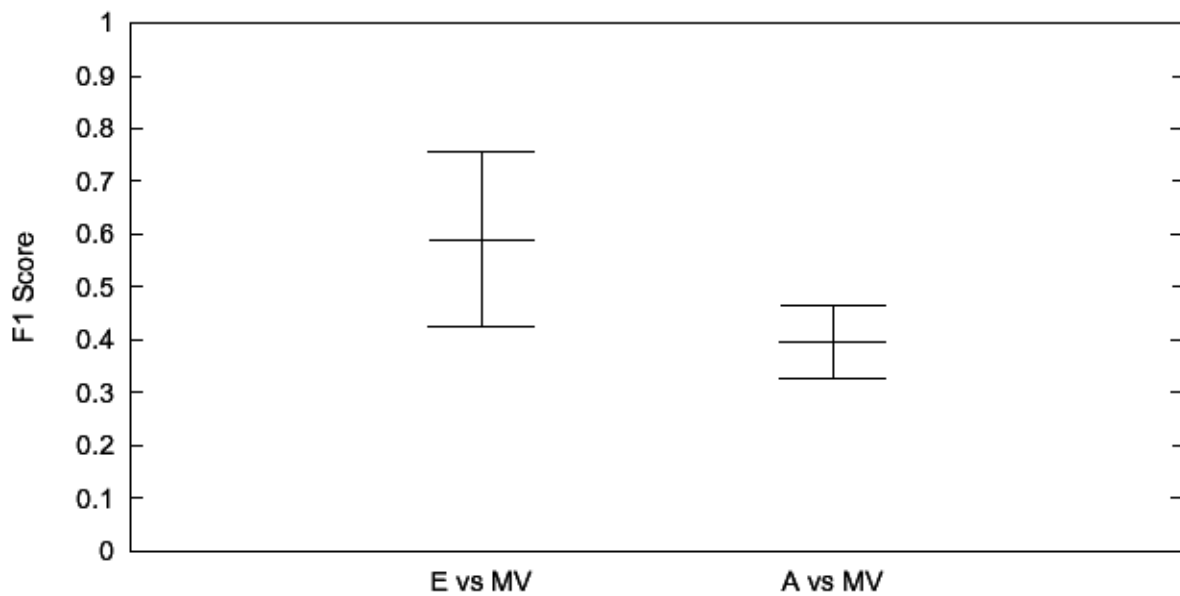
>2000 regions containing no erythrocytes (tissue, background)

&

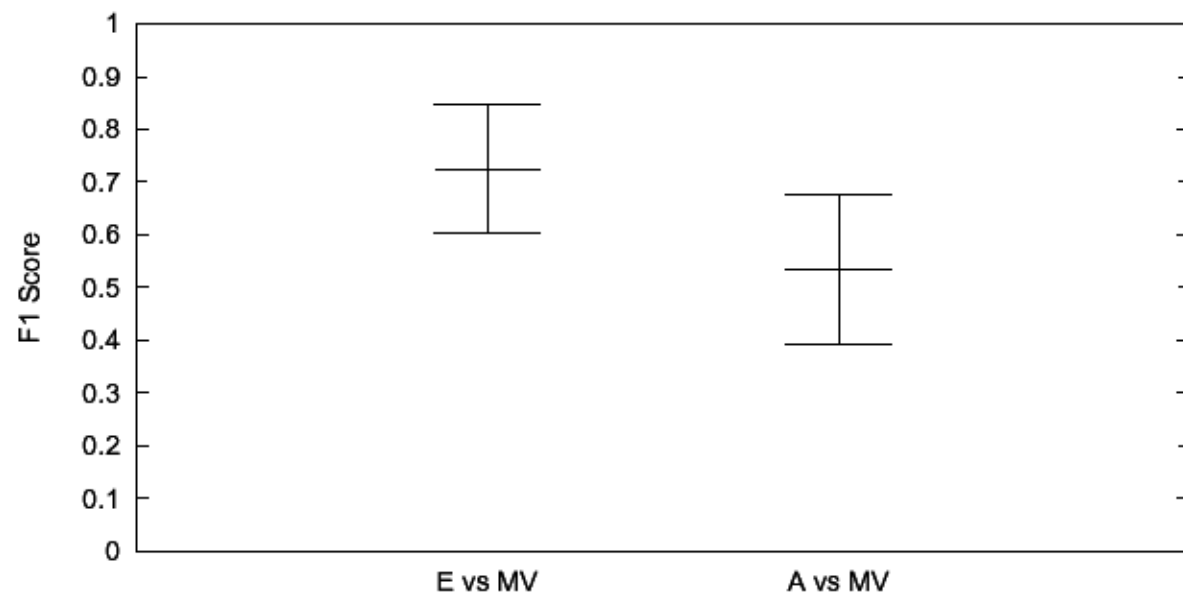


- Exhaustive Haar-Train-Param.Opt (1.8 million masks)
- ≥ 2 Experts for each FOV, many train data variants
- Extend Haar-Test with conf. values
- Run four times with lossless $k \cdot 90^\circ$ rotations...

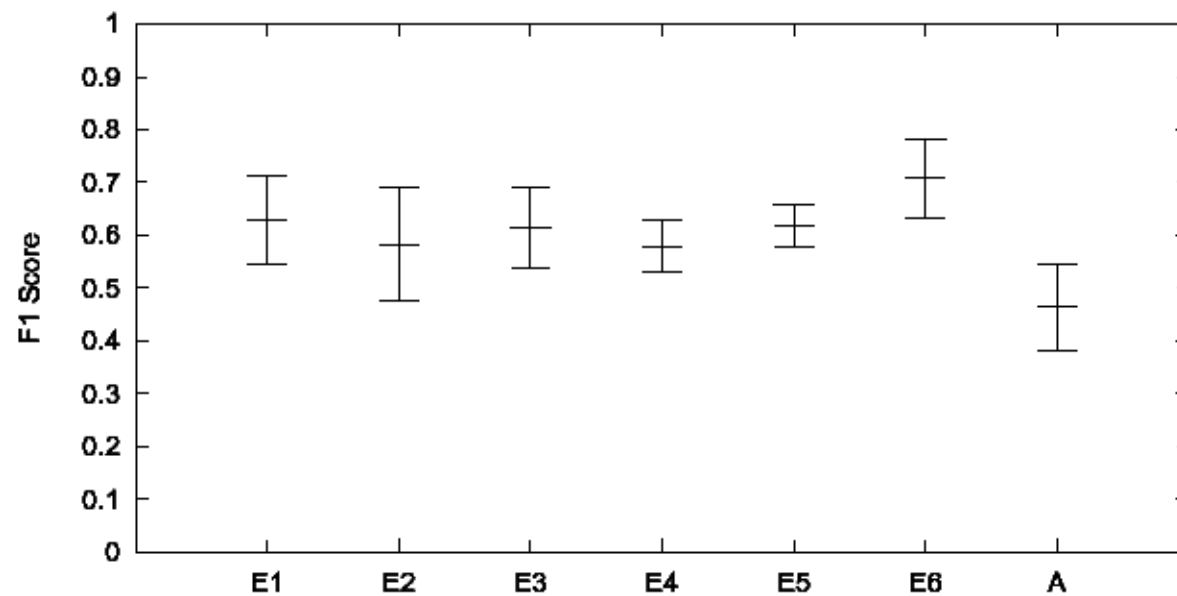
Colon



Placenta



Performance (F1 Score) Algo vs 6 experts on one FOV



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.

C. Elegans Protein Localization (3)

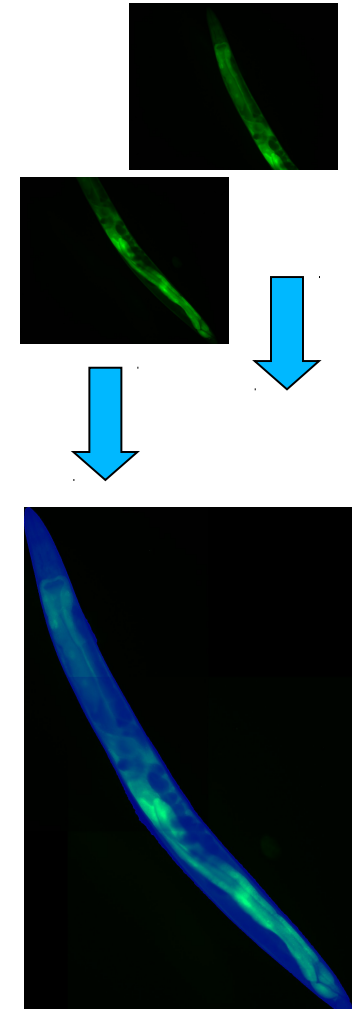
Merging anterior/posterior image & extracting worm

(pixelClassification)

- Machine learning from manually tagged sample images (i.e. „Ground Truth“)
- Threshold optimization by testing minimum circularity and area of largest blob
- Closure (erode, dilate), fill internal holes with circularity below threshold
- Heuristic search for breaks in contour, which are repaired with straight lines and filled on the inside

(meshAB)

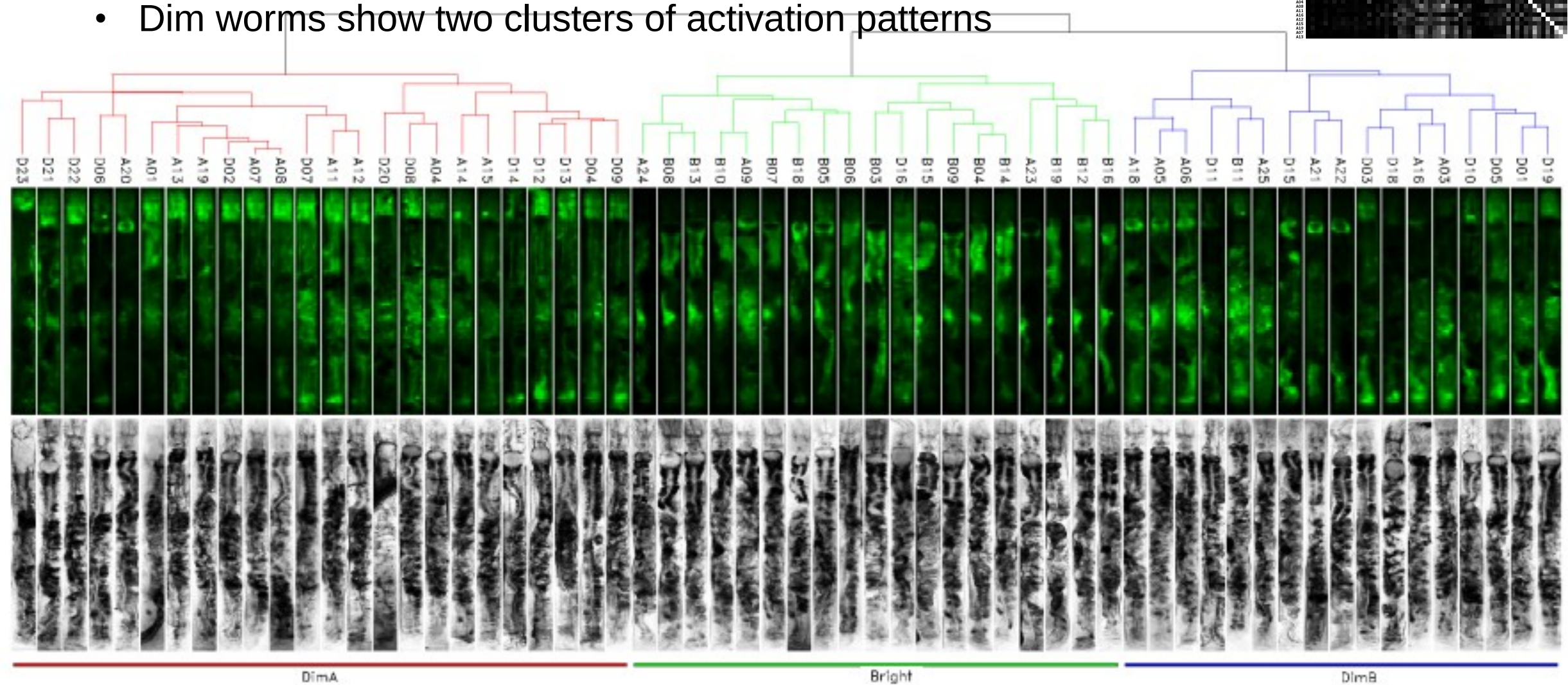
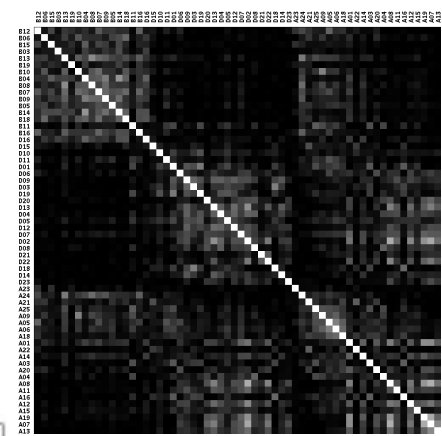
- Image correlation coefficient for combining head and tail images (~ simplified stitching)



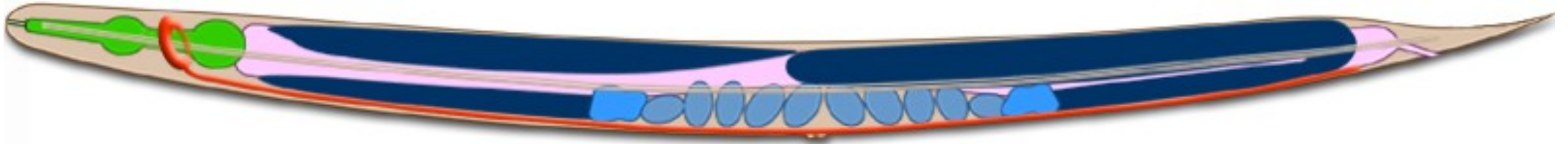
C. Elegans Protein Localization (6)

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



Future Work (1)



- Bottleneck is image acquisition – each worm has to be taken from culture medium, anesthetized, cleaned and imaged (ca. 30min per worm)
- Resolution is too coarse for observing single cells
 - *Culturing worms on chamber-slides, using slide-based microscopy & automated imaging*
- Lots of problems with different microscope settings, air bubbles, finetuning,...
 - *„Closed-loop“ system (microscope, moveable slide and image analysis - coupled system)*

Video 2 - Worm Dedistorting in Real Time



Thanks for listening!



Science2Business 2011 Award (3rd Place)