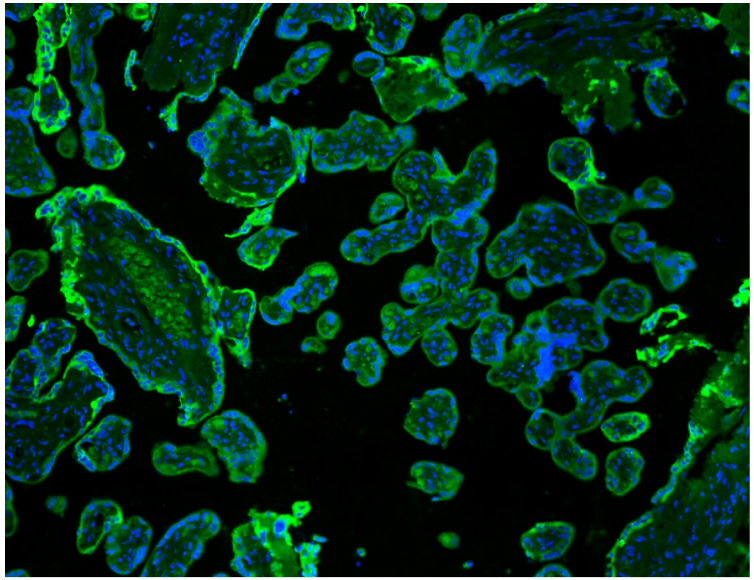
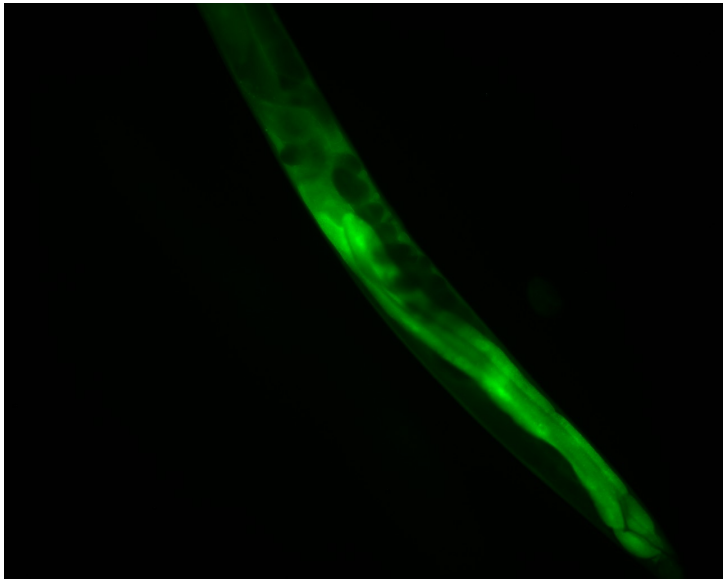
Image Data Analysis in H.sapiens and C. elegans





Dr. Alexander K. Seewald

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Collaborations (1)

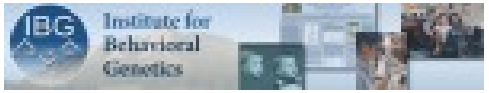
2009-2012

Inst.f.Pathophysiology & Allergy Research, Medical University Vienna, Austria

Tissue Gnostics GmbH, Austria

Funded by FFG (Bridge 818094)

2007-2010



Inst.f.Behavioral Genetics, Univ. Colorado, Boulder, USA

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Collaborations (2)

SEEWALD SOLUTIONS


IPP

TISSUE GNOSTICS








IBG Institute for Behavioral Genetics




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Image Data Analysis

Recording good images for image processing

- Slide-based microscopy for high resolution
- Common pitfalls and how to avoid them
  - Uneven illumination
  - Acquisition parameters
  - Image formats
- Ground truth markup
  - Why it is essential
  - What it can be used for
  - How to create it properly
- Erythrocyte removal using a trained system

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Slide-based Microscopy

Tissue features may span several FOVs or a higher resolution may be required.

Slide-based Microscopy uses a movable stage to move the tissue sample, recording hundreds or thousands of different parts of the tissue sample automatically.

These images need to be combined via stitching.

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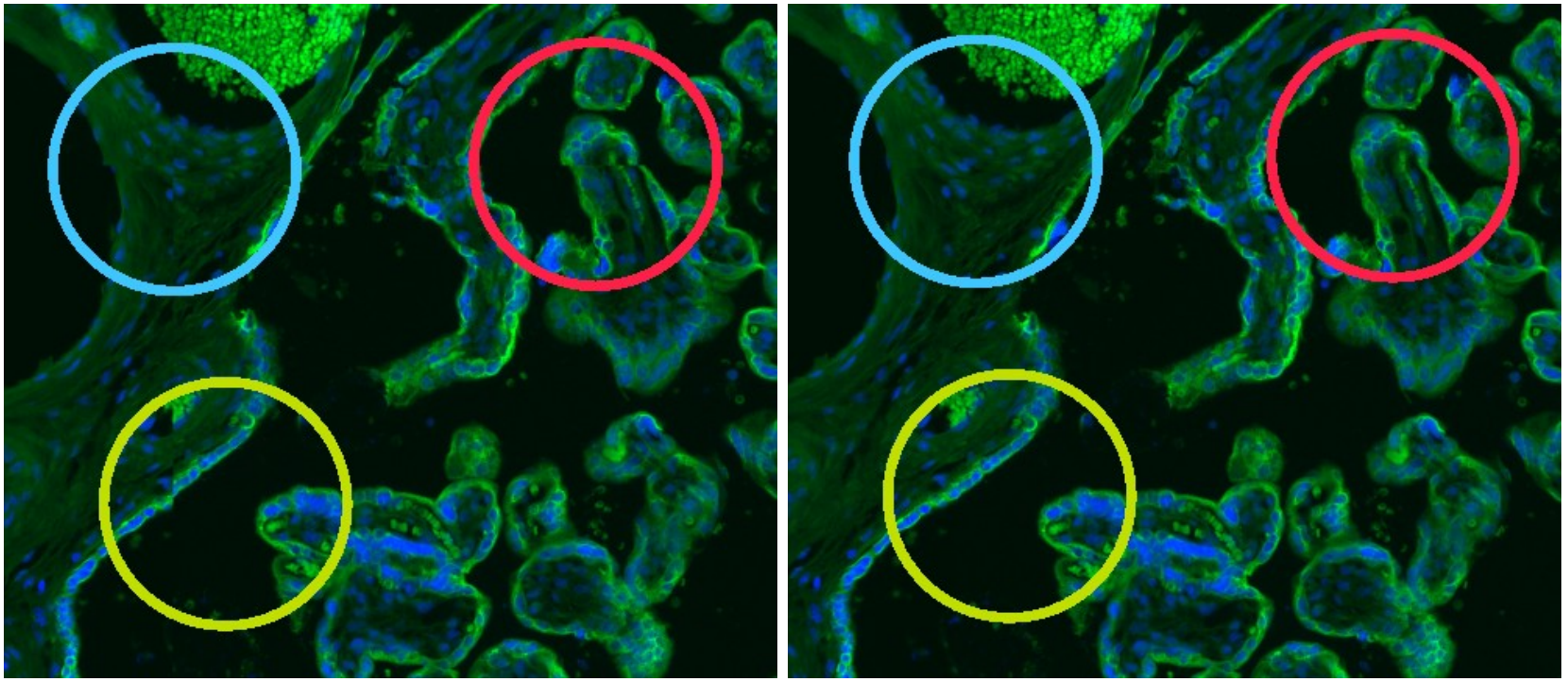
Stitching (1)



- *Stitching* analyzes the overlap between adjacent images to determine more precise combination. **We developed our own algorithm during the FFG Bridge project.**

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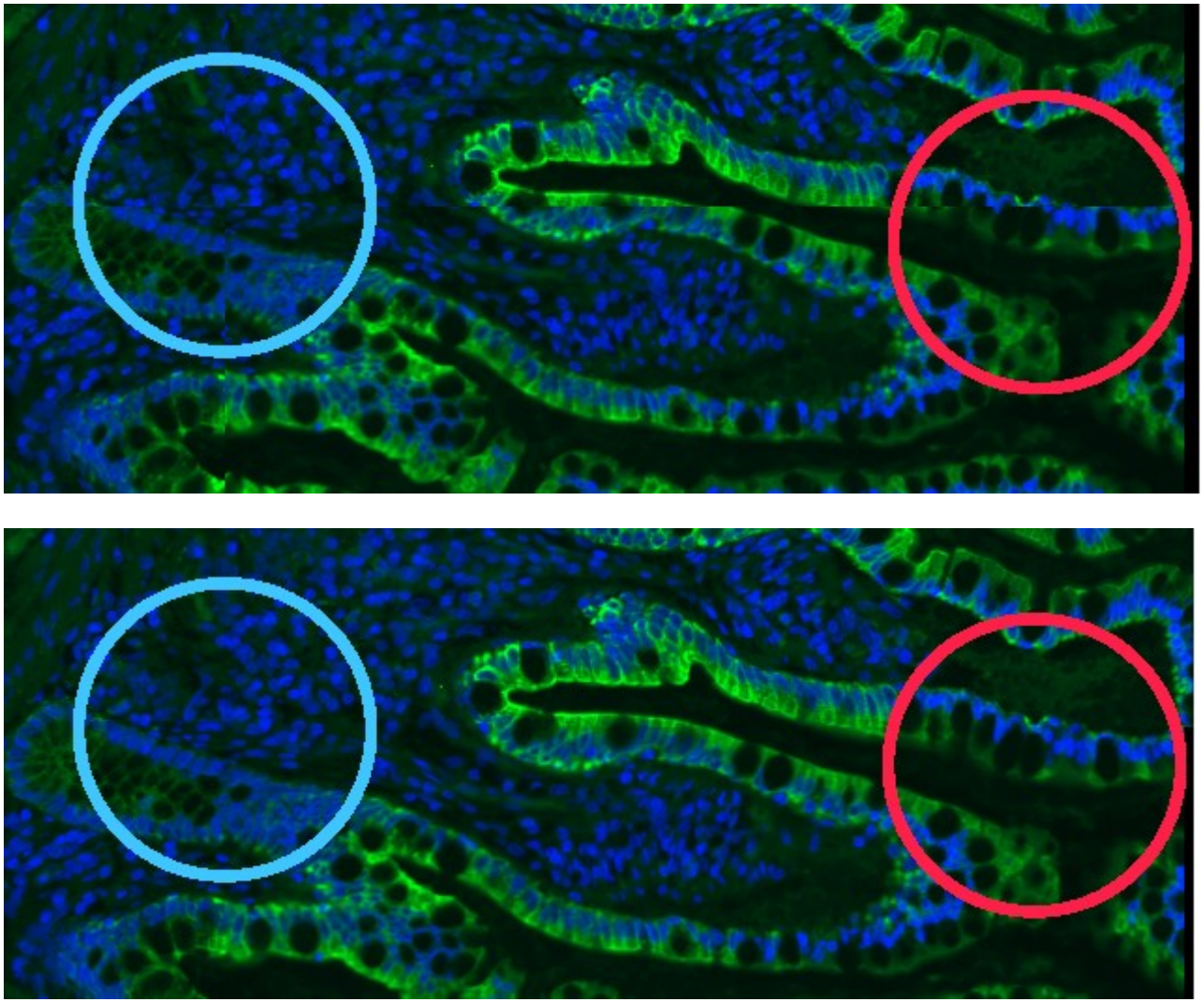
Stitching (2)



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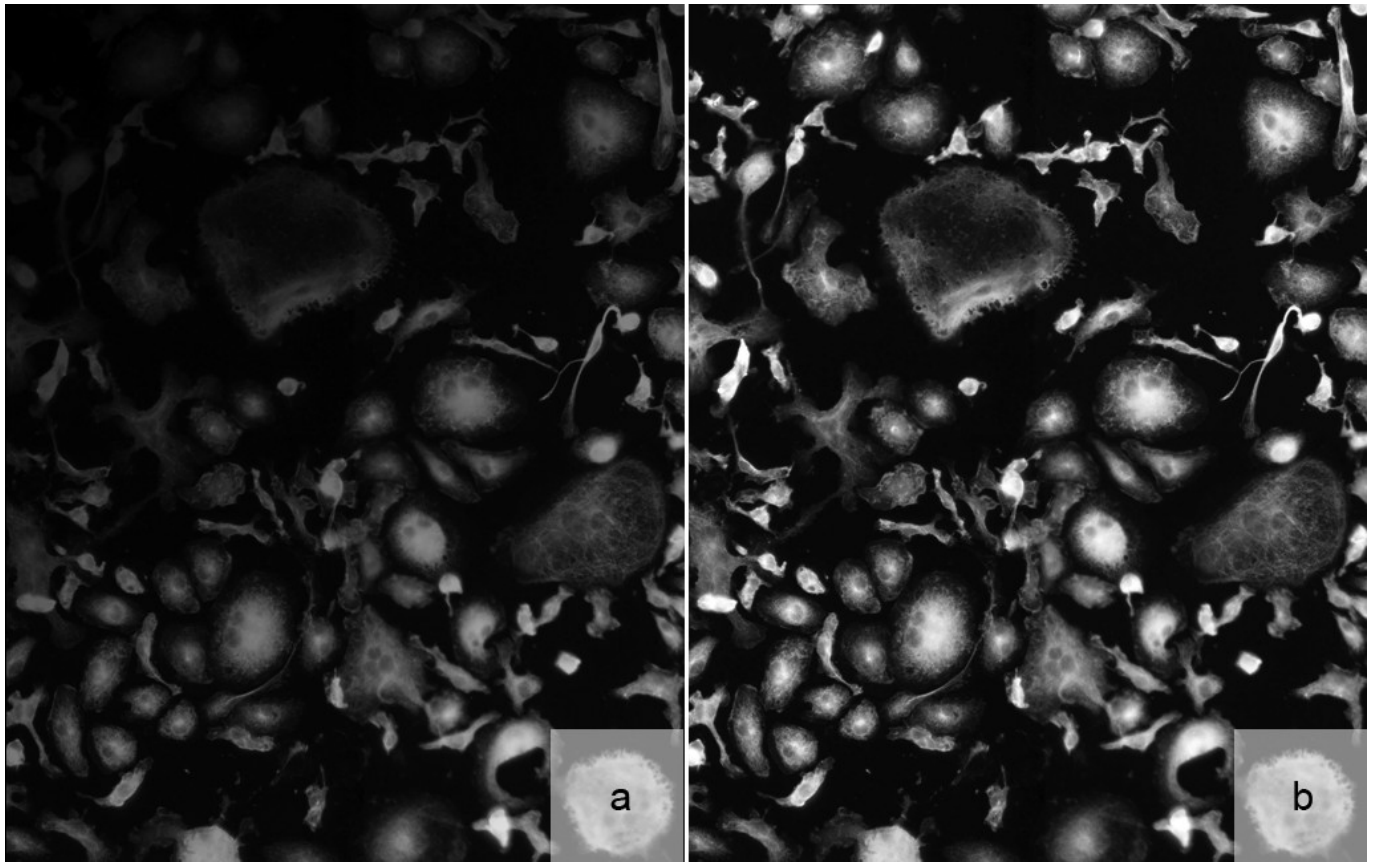
Uneven Illumination



- Camera setup, lamps, condenser, …. software correction is feasible, but should be avoided

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Acquisition Parameters

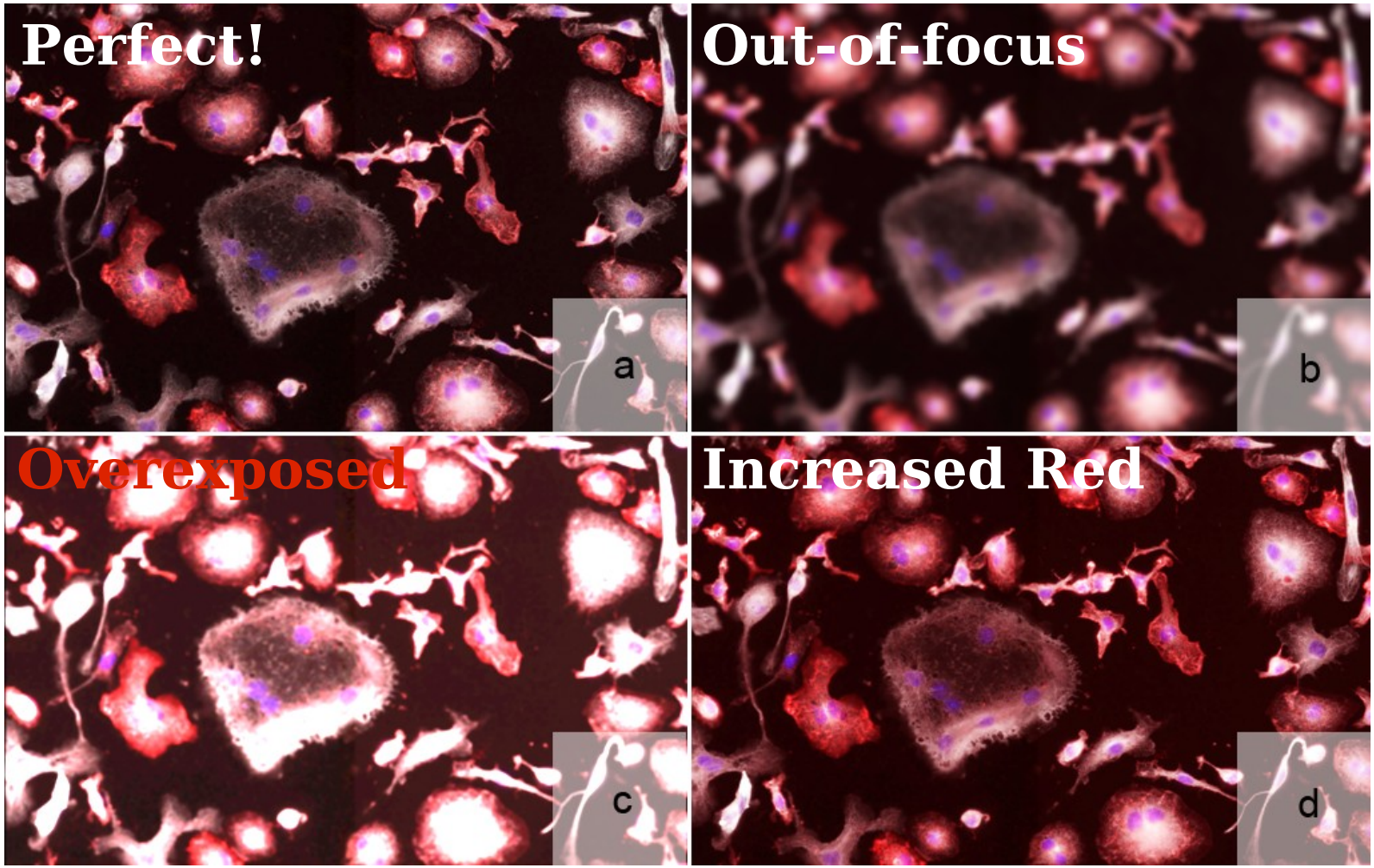

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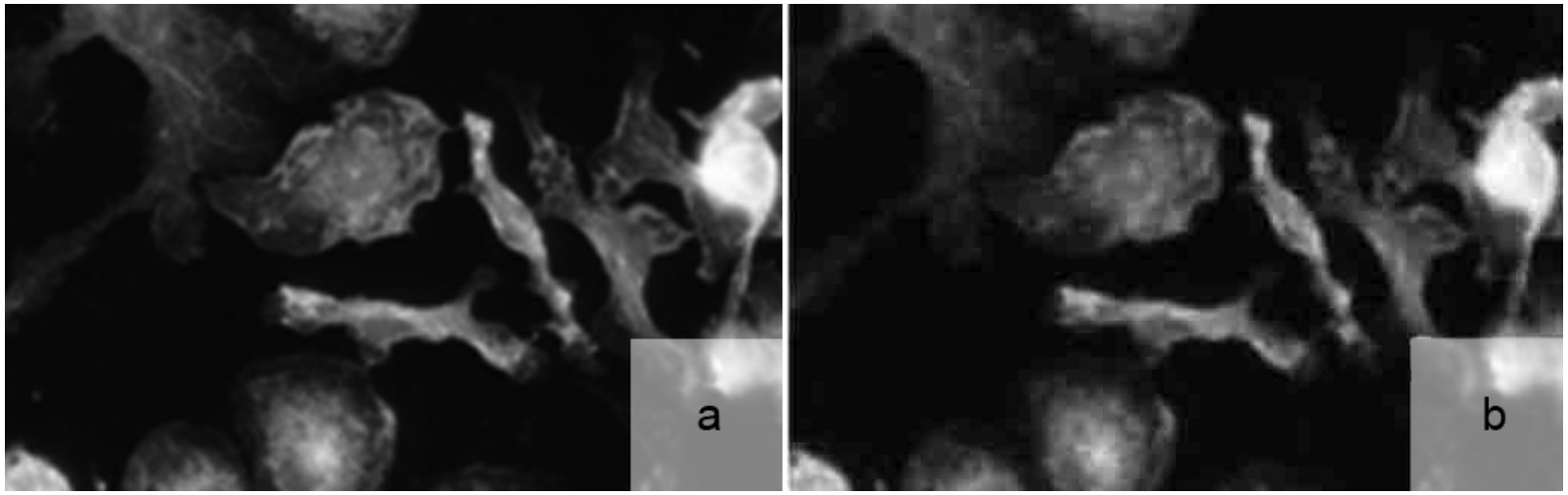
Image Formats

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 on several levels

No normalization of input images!

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Ground-Truth Markup (1)

Image processing systems should be designed by biological experts – not by computer scientists with some biological background! The way to do this is by creating ground-truth markup for all tasks!

- Time-consuming to create (~ hours to days)
- Markups from at least 2-3 experts needed

Very useful in many ways!

- Compute inter-expert variance (~ complexity)
- Properly validate ad-hoc IP systems
- Optimize parameters for ad-hoc IP systems
- Train IP systems w/o writing code ...

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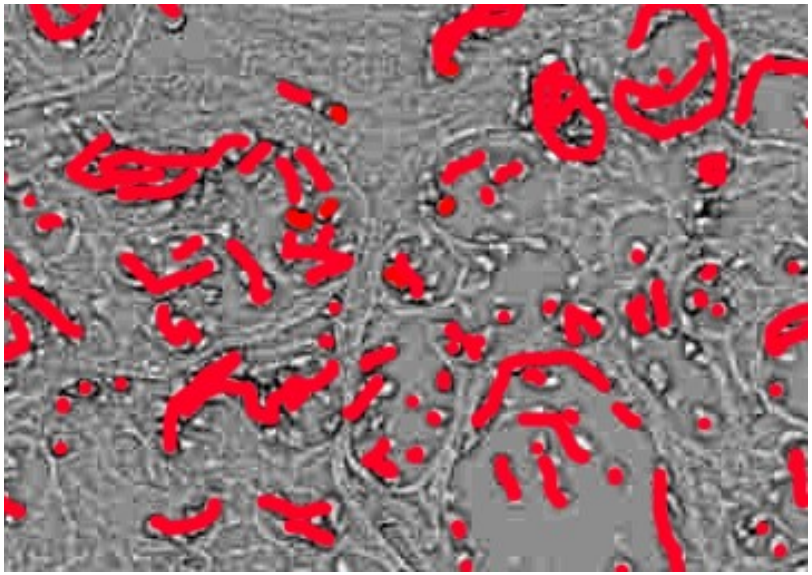
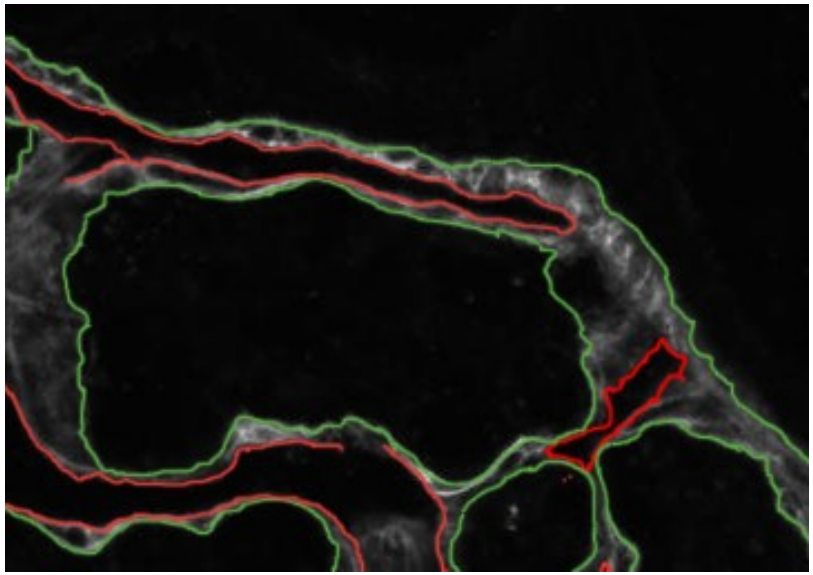
Ground-Truth Markup (2)

Creating Markups

- Use any painting program (Photoshop,GIMP,...)
- Open original image, add new layer for markup
- Save in multi-layered format (TIFF, XCF)
- Digitizer tablets improve speed & accuracy
- Use „average“ images, not the best ones!





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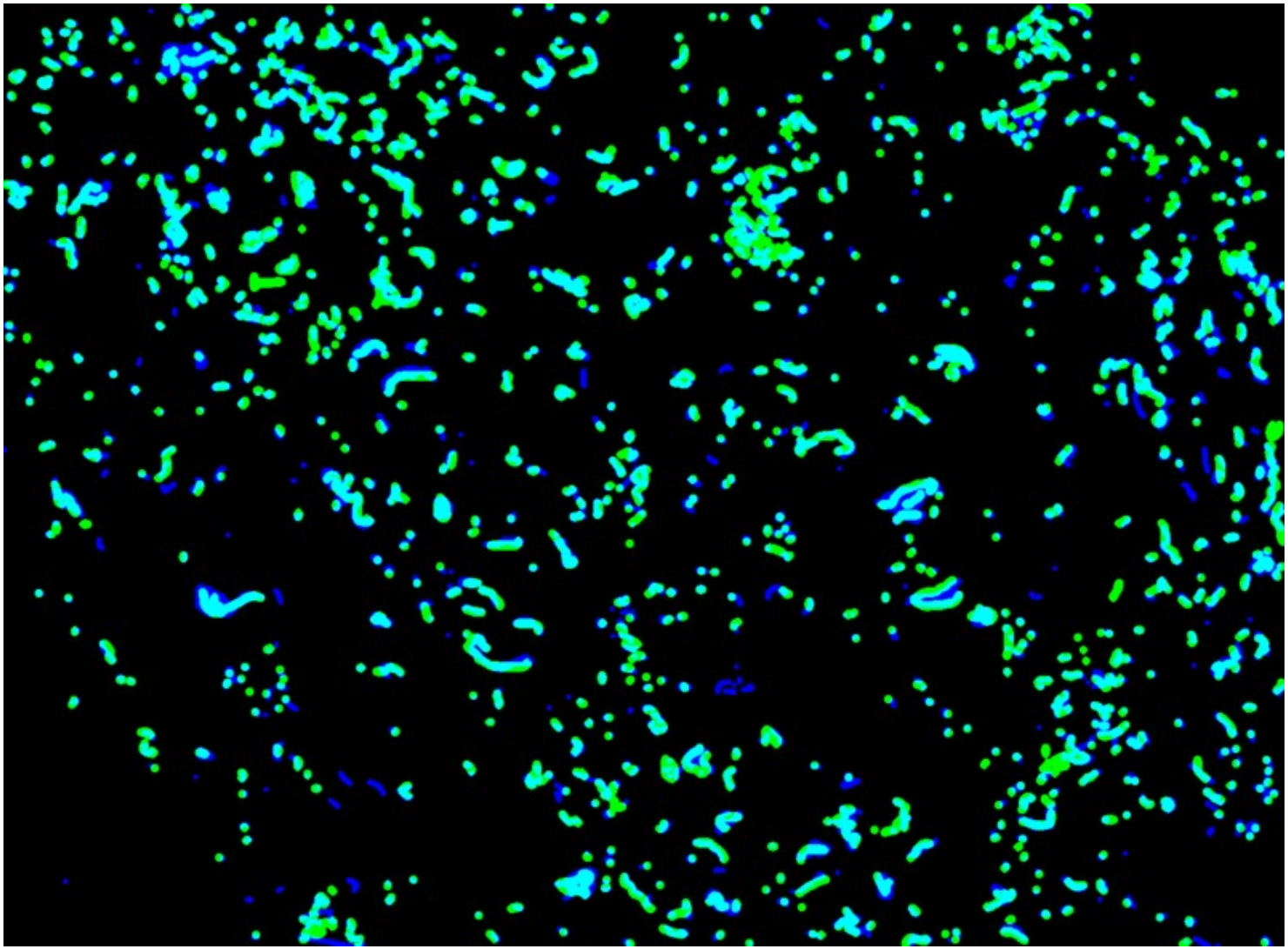
Inter-Expert Variance - Erythrocytes



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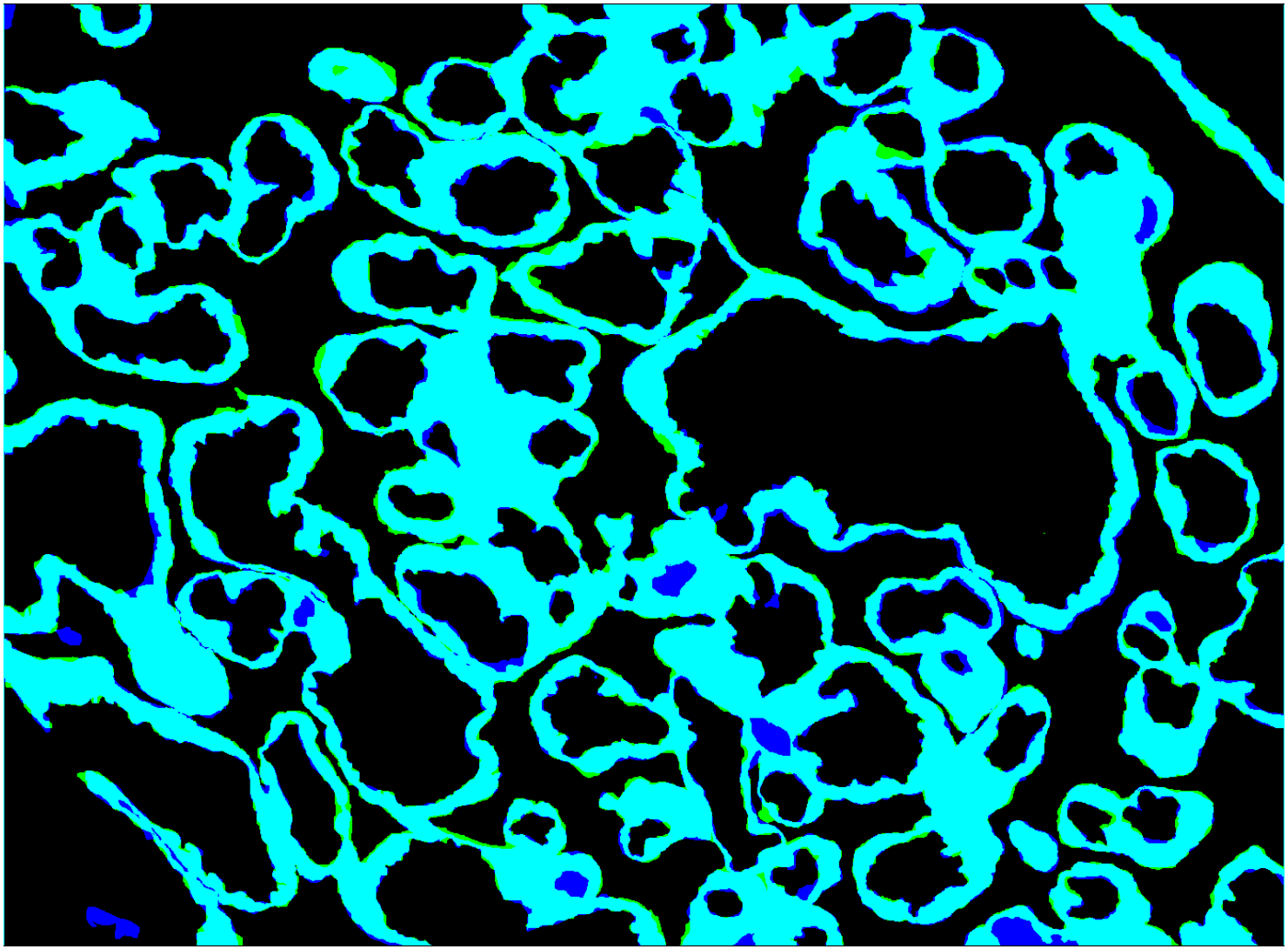
Inter-Expert Variance - Syncytiotrophoblast



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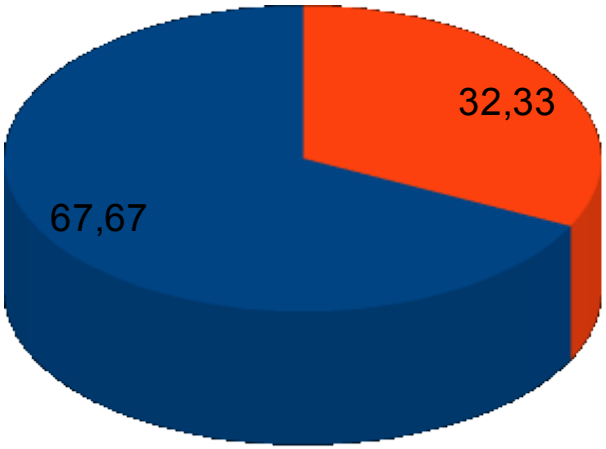
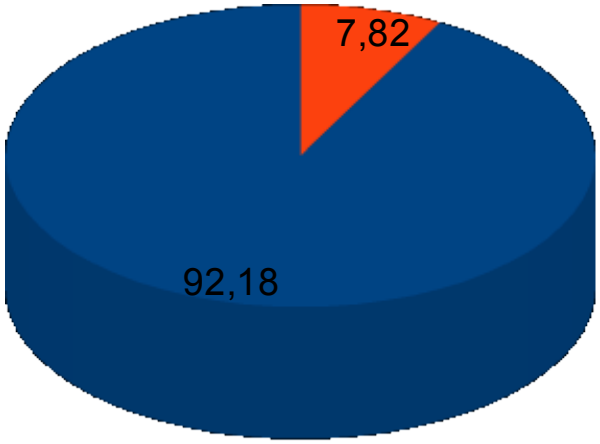
Inter-Expert Variance - Summary

Comparing agreement of expert IE vs. SD

Erythrocytes



Syncytiotrophoblasts



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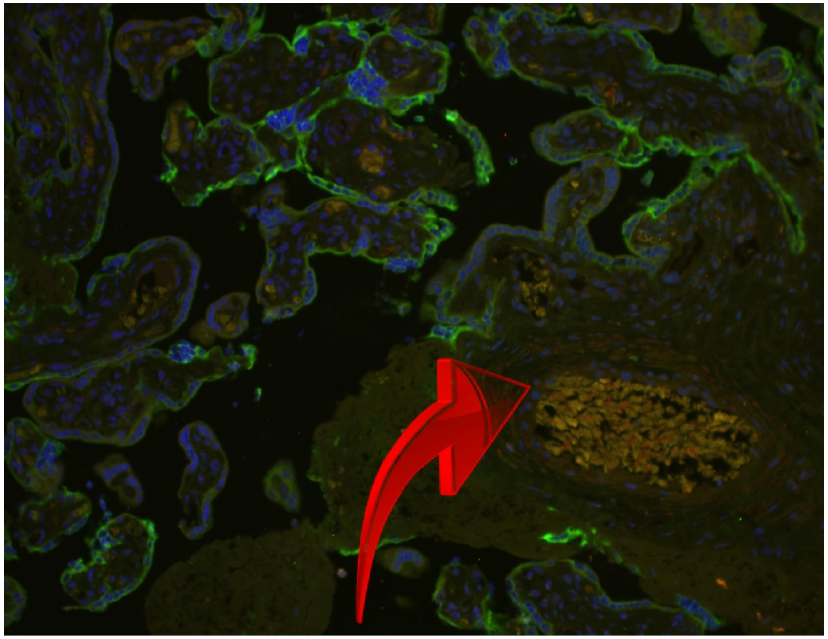
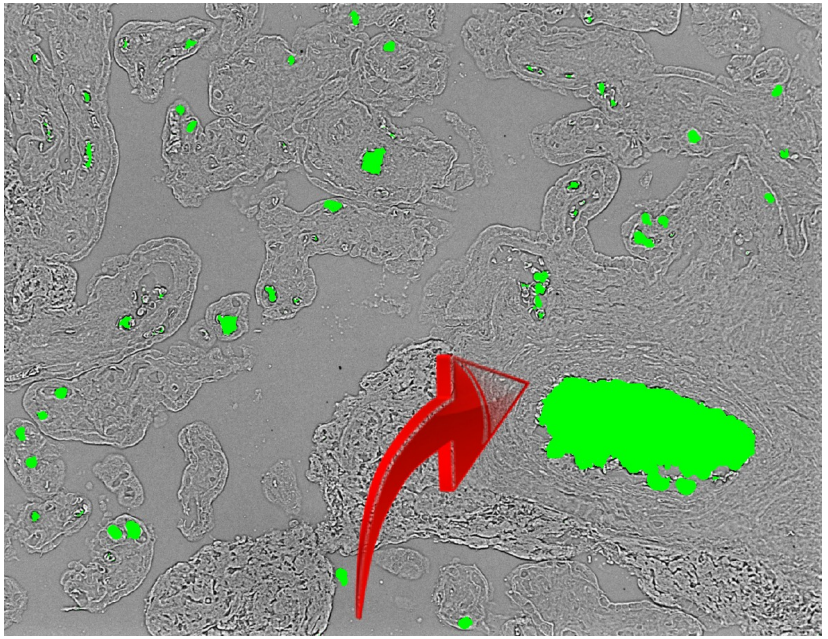
Erythrocyte Removal (1)





Erythrocytes have high autofluorescence. May lead to bad fluorescent measurements.

Using only ground-truth data, we „taught“ the computer to remove erythrocytes from images (Haartraining [Viola & Jones, 2001])

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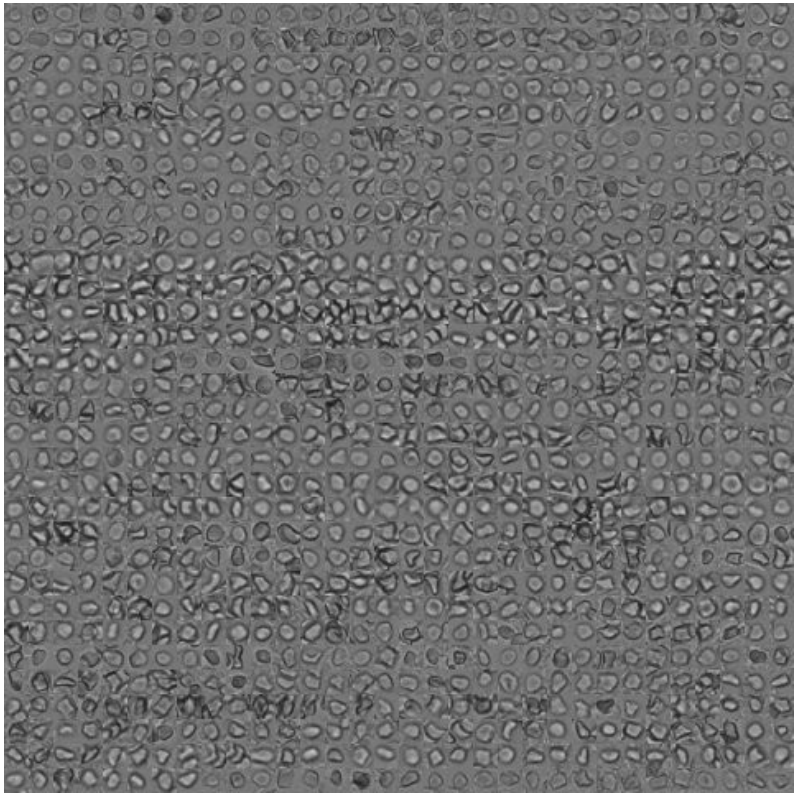
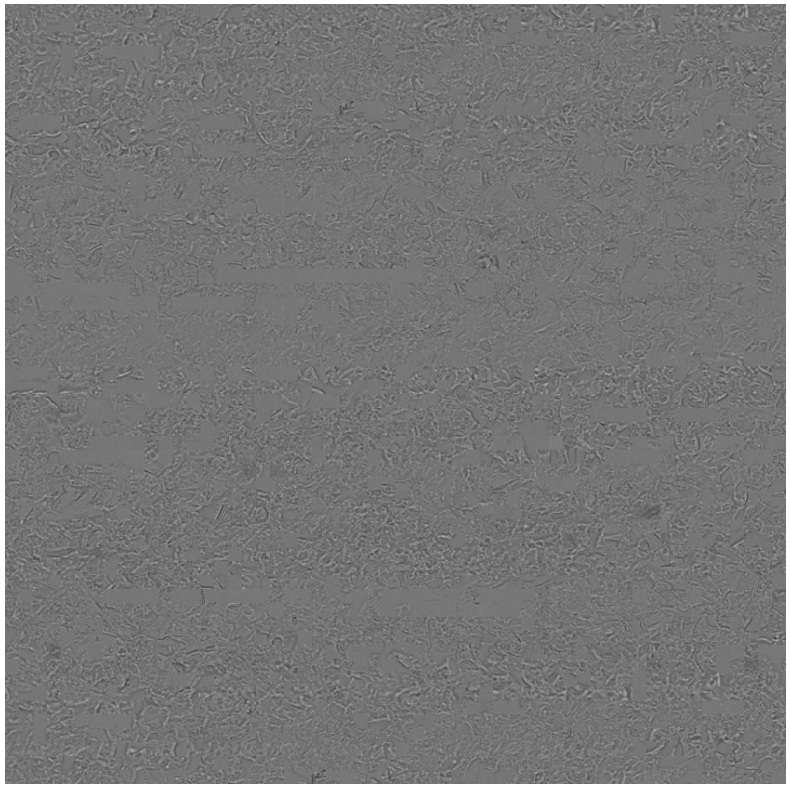
Erythrocyte Removal (2)

Training Data

3764 erythrocytes have been marked up manually as well as...



... *2000* regions containing no erythrocytes (tissue, background)



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Applications

Given good images, what can be achieved?

- Bone (osteoclast and precursor cells)
- Placenta (syncytiotrophoblast)
- C. elegans (localization of HSP::16.2)

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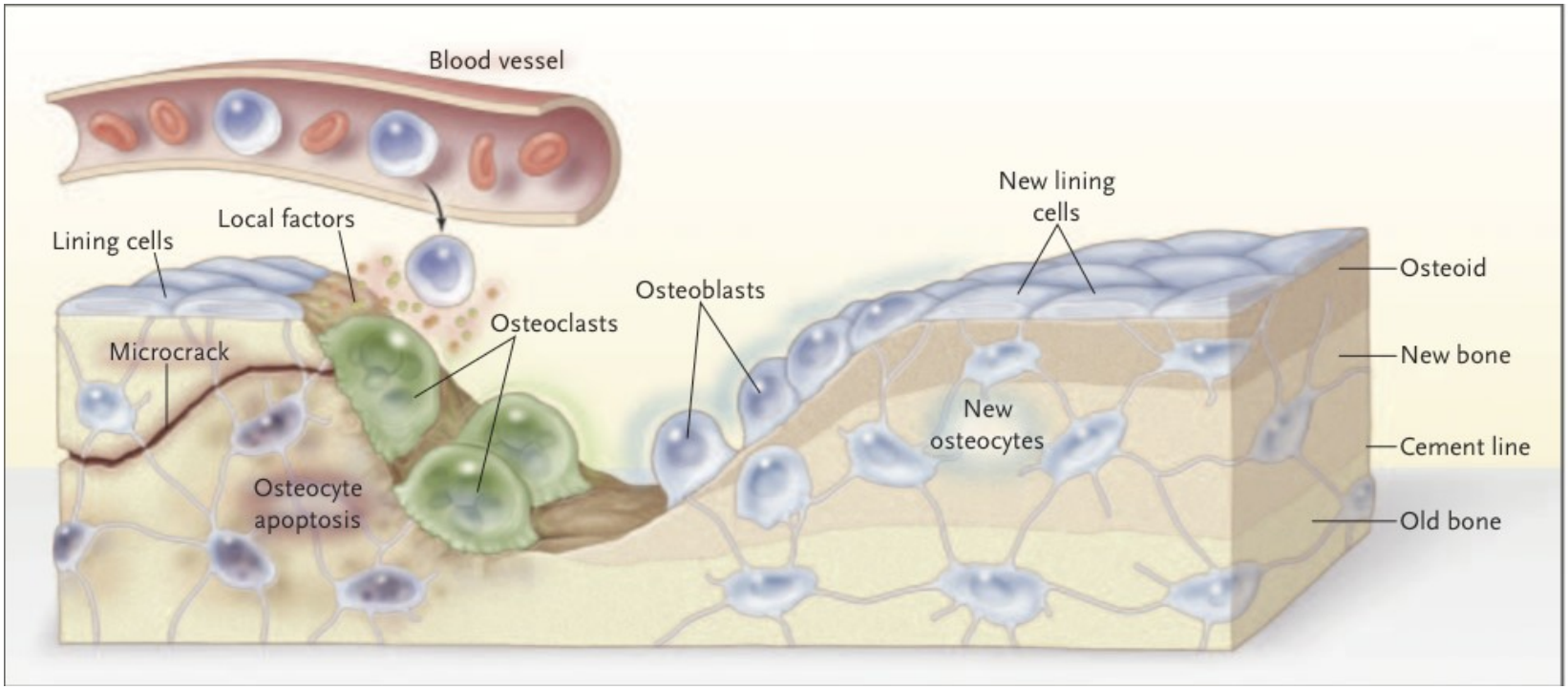
Osteoclast Quantification (1)



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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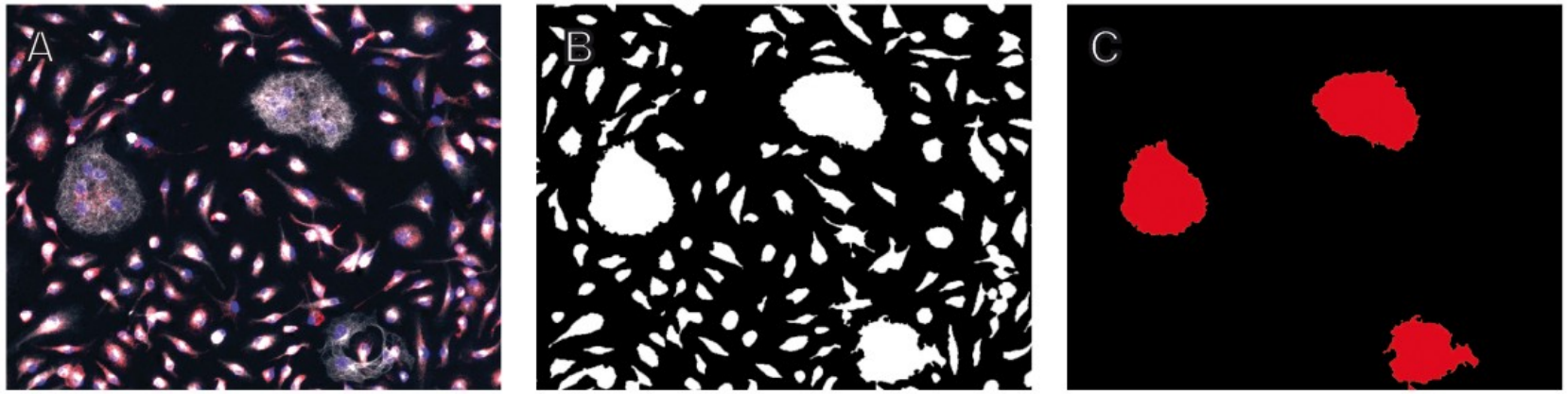


Figure 1: Automated image analysis of osteoclasts in culture. A: Immunofluorescence image (overlay) of OC and precursor cells. Red = F4/80 macrophage marker. White = alpha-tubulin and calcitonin-receptor. Blue = nuclei. B: Detection of all cells by the automated system. C: Identification of the OC among those cells.

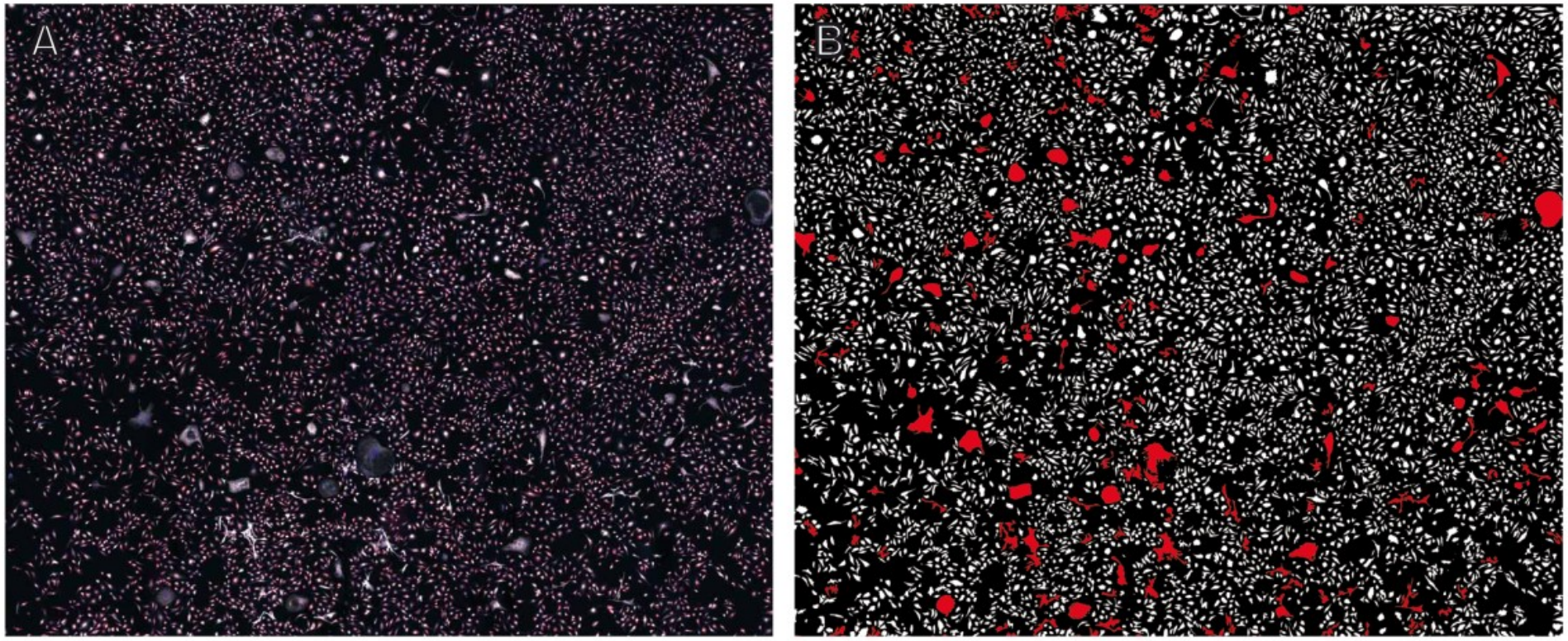
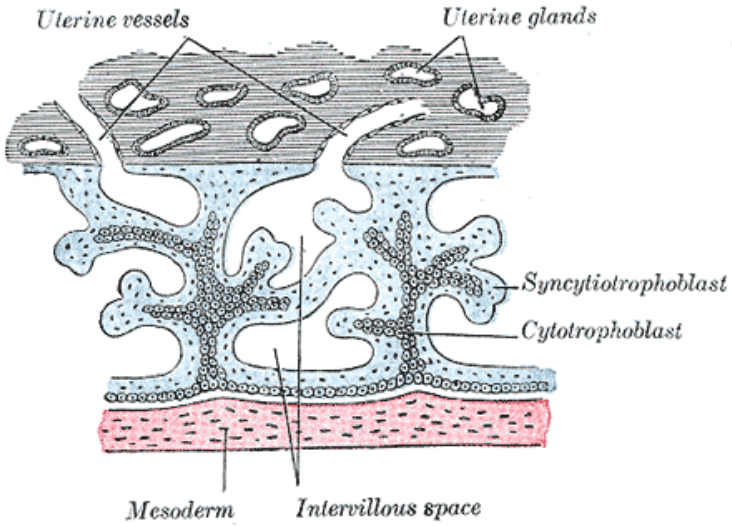


Figure 4: Image analysis of a whole sample-region consisting of 100 (10 x 10) FOVs. A: Immunofluorescence image. B: Result of cell segemntation and analysis: non-OC are marked in white, OC are marked in red.

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 an IP system to segment villi & syncytiotrophoblast and applied it to RAGE quantification.**

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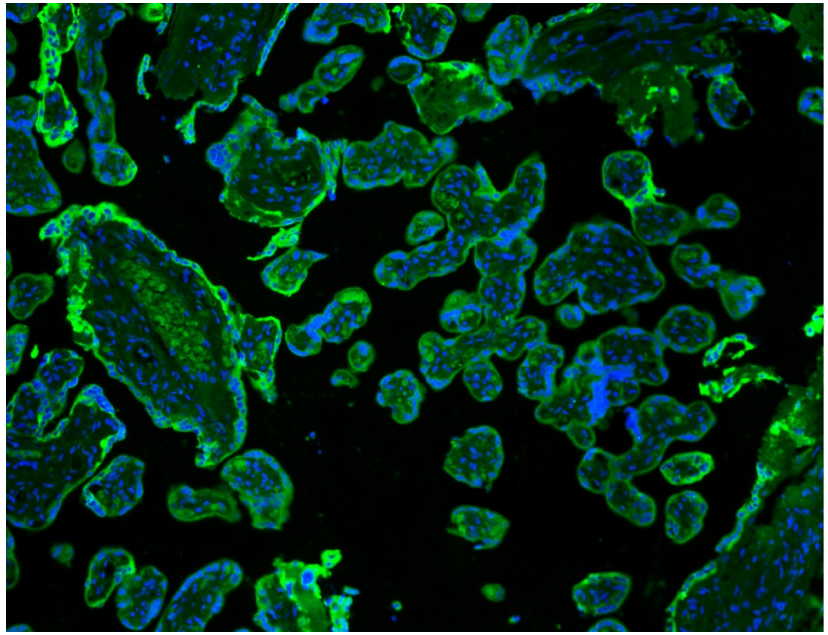
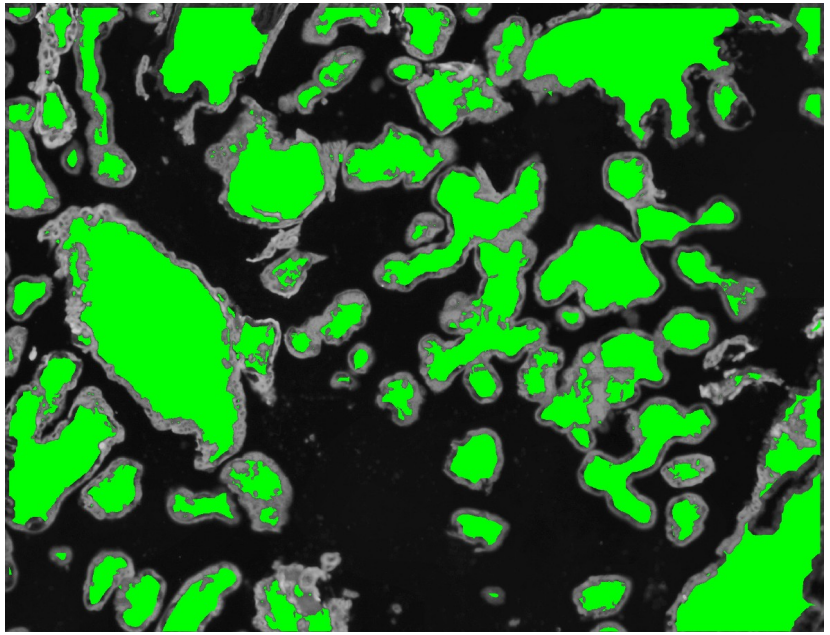
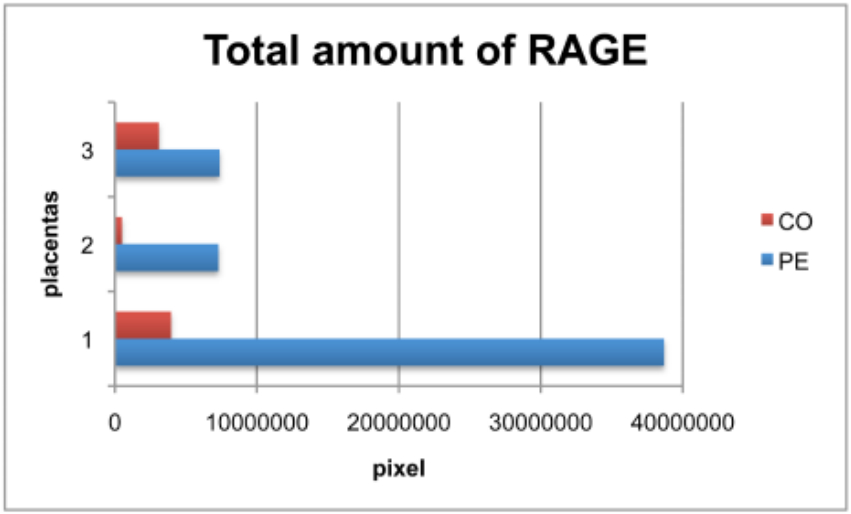
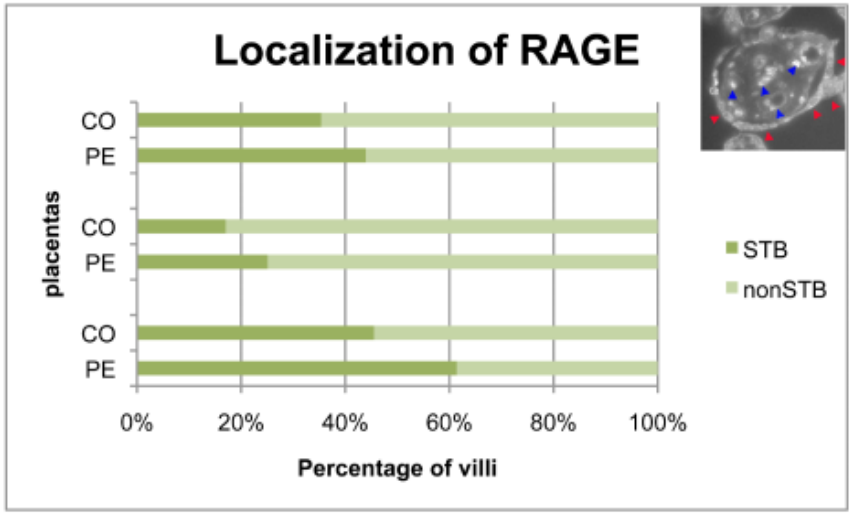
Syncytiotrophoblast (2)







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.

C. Elegans Protein Localization (1)

Quantifying Phenotypic Variation...

Analyzing changes in appearance / phenotype...

in Isogenic Caenorhabditis elegans...

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

Expressing Phsp-16.2::gfp...

which express a GFP reporter that binds to heat shock protein 16 (i.e. transgenic worms)...

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)

Heat Shock Protein 16 – increases expression not only when organism is exposed to high temperatures

HSP are named by molecular weight (=16kD). Expressed in intestine and pharynx. Induced in response to heat shock or other environmental stresses.

Interacts with intra-cellular human beta amyloid peptide (Alzheimer plagues)

High expr. correlates with worm longevity acc. to earlier studies.

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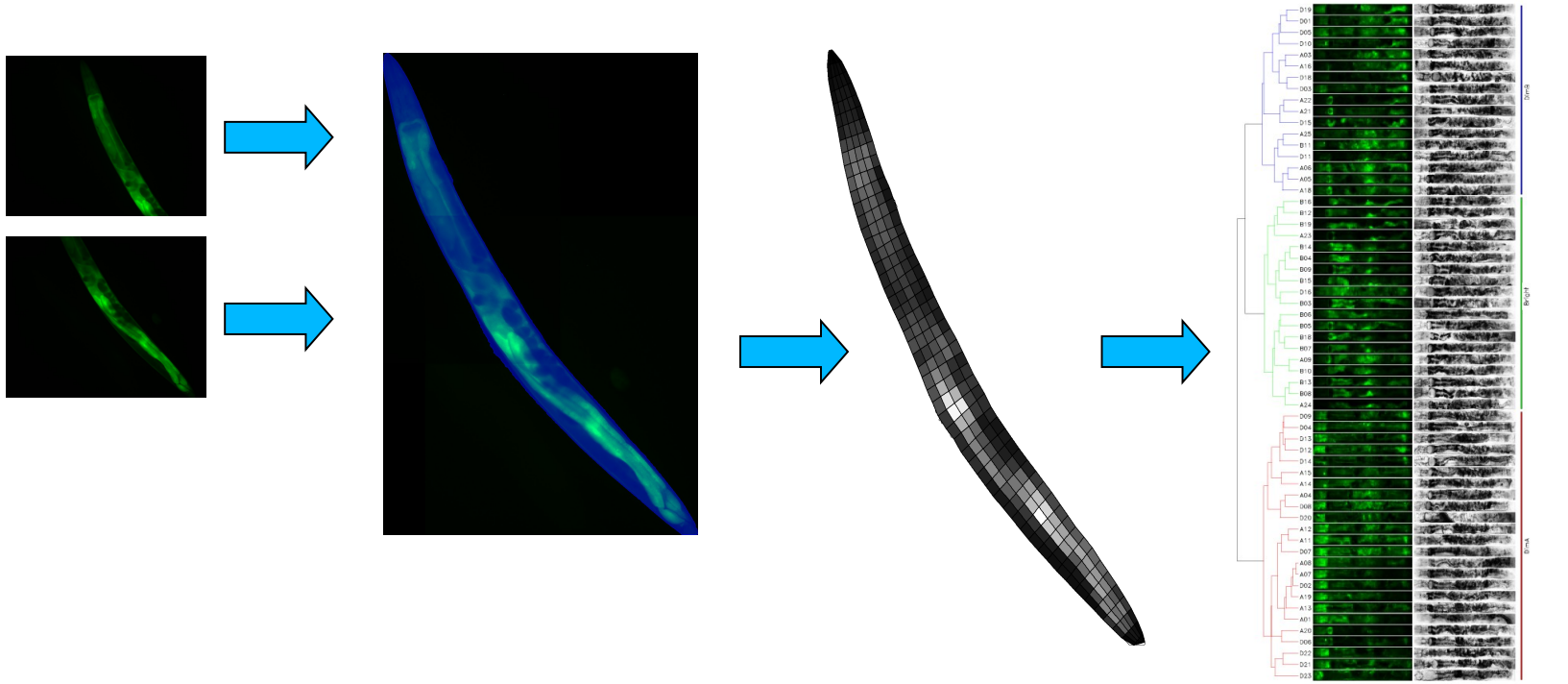
C. Elegans Protein Localization (3)

Automated analysis of C.elegans images



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.

Prototype GPL source code: http://elegans.seewald.at/

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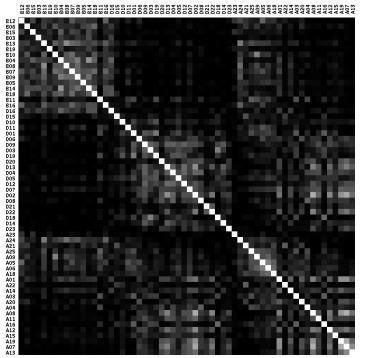
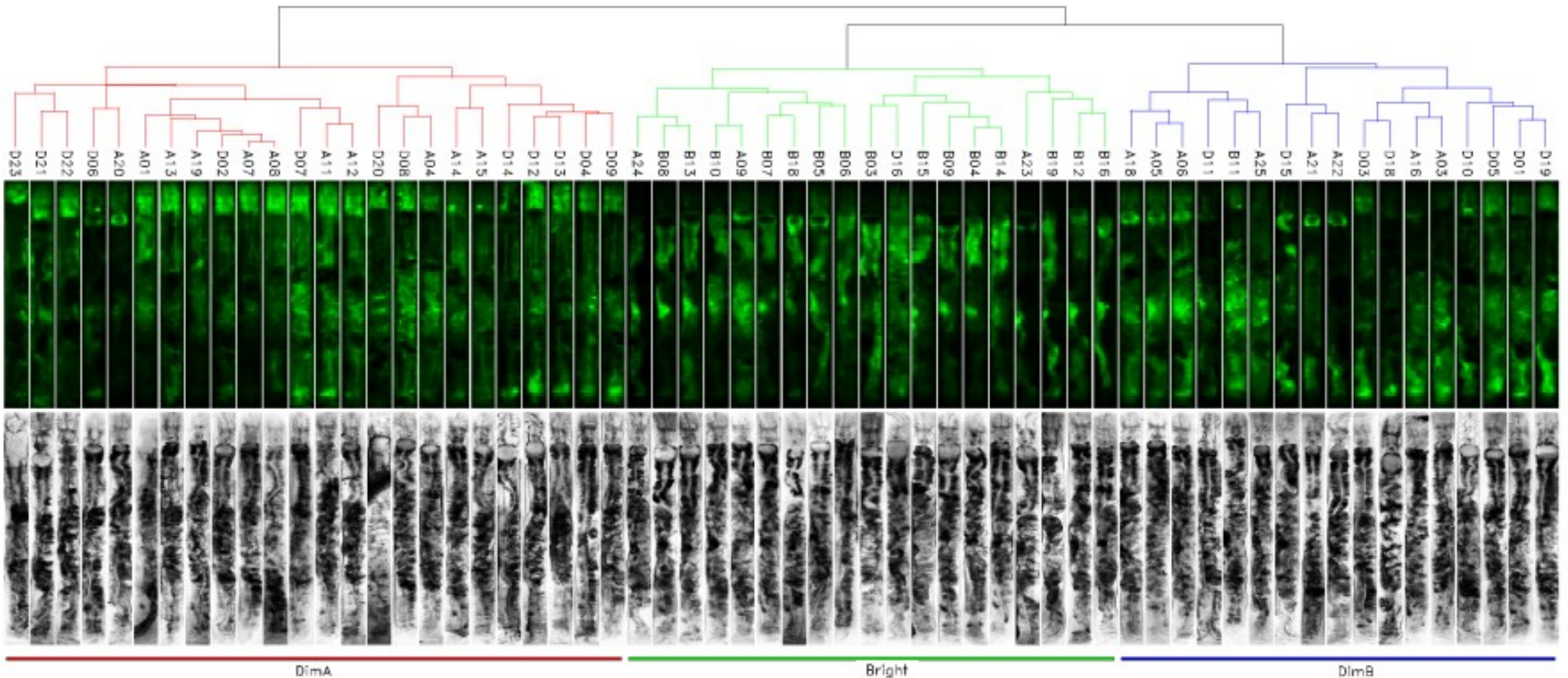
C. Elegans Protein Localization (4)

Results (1)

- Bright worms live longer than dim ones
- Even when discounting brightness, bright worms show distinct expression patterns (currently under investigation)



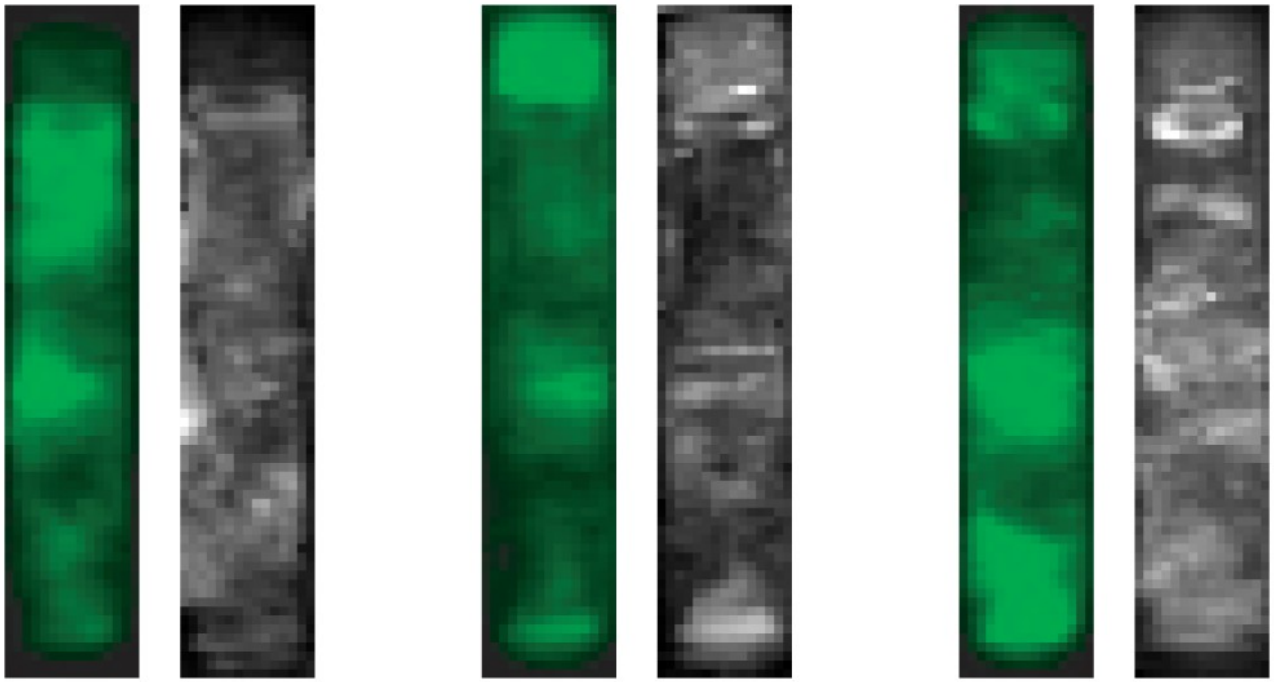


C. Elegans Protein Localization (5)

Results (2)

- Bright worms: one cluster
- Dim worms: two clusters w/ distinct expr. patterns

Bright **DimA** **DimB**



Top: head, bottom: tail, vulva to the right.

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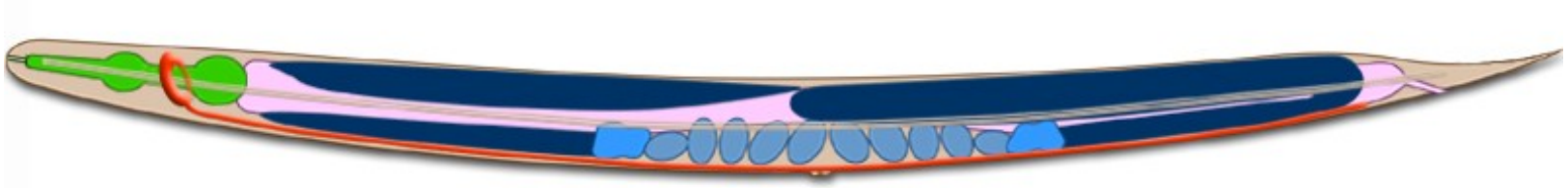
C. Elegans Protein Localization (6)



Open Issues

- Bottleneck is image acquisition – each worm has to be taken from culture medium, anesthesized, 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 in a coupled system)*

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Future Work

- New Collaborations
- Follow-up Projects
  - WWTF Cognitive Science Call
  - Suggestions?
- Addressing other challenging image processing tasks in medical and biological research
  - C. elegans: Cell tracking for neural sub-network (Manuel Zimmer, IMP)
  - Human: Osteoclasts in tissue, Placenta

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Publications

Schepelmann M, Heindl A, Seewald A, Burger K, Rogojanu R, Ecker R, Bises G, Pietschmann P, Ellinger I, Thalhammer T: A novel method for automated quantification of osteoclasts in culture - Advantages, workflow and application. Journal für Mineralstoffwechsel (17) 2010, Sonderheft 2, p.7. Talk held at the annual autumn meeting of the Austrian Society for Bone and Mineral Research, Vienna, Austria. ISSN: 1023-7763, 2010.

Heindl A, Schepelmann M, Seewald A, Burger K, Rogojanu R, Ecker R, Bises G, Pietschmann P, Ellinger I, Thalhammer T: Towards an automated evaluation system of osteoclasts in cultures using a combined image-processing and machine-learning strategy. Journal für Mineralstoffwechsel (17) 2010, Sonderheft 2, p.7. Talk held at the annual autumn meeting of the Austrian Society for Bone and Mineral Research, Vienna, Austria. ISSN: 1023-7763, 2010.

Heindl A, Dekan S, Ellinger I, Seewald A: Towards a Versatile Automated Cell-Detection System for Science and Diagnostics. Proceedings of the 32nd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Buenos Aires, Argentina, p. 3045-3048, IEEE Catalog Number: CFP10EMB-DVD, ISBN: 978-1-4244-4124-2, ISSN: 1557-170X, 2010.

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.

Heindl A, Helmer H, Dekan S, Rogojanu R, Ecker R, Thalhammer T, Bises G, Uhrova H, Seewald A, Ellinger I (2010) Automated detection and analysis of fluorescent biomarkers in huma placental chorionic tissue. Pediatric Research, August 2010, 68 (2), p. 174-179.

Rogojanu R., Mesteri I., Ellinger I., Thalhammer T., Kallay E., Heindl A., Seewald A., Bises G.: Characterization and Quantification of Macrophages in Colorectal Cancer by an Automated Cell Detection System. Poster presentation at the 6th PhD Symposium of the Young Scientist Assocation of the Medical University of Vienna, June 2010, Vienna, Austria. Also available as EJC Supplements, June 2010, Vol.8, Issue 5, Page 107.

Heindl A., Pohl V., Rogojanu R., Ecker R., Thalhammer T., Bises G., Seewald A., Ellinger I.: Towards a Versatile Automated Cell-Detection System for Science and Diagnostics exemplified through receptor for advanced glycated end-products (RAGE) quantification in placental chorionic villi. Poster presentation at the 6th PhD Symposium of the Young Scientist Association of the Medical University of Vienna, June 2010, Vienna, Austria.

Heindl A., Ecker R., Steiner G., Bises G., Thalhammer T., Rogojanu R., Uhrova H., Helmer H., Ellinger E., Seewald A.: Automated cell-detection technologies for science and diagnostics. Placenta 30 (9) 2009: P06.14.

Heindl A., Ecker R., Steiner G., Bises G., Ellinger I., Thalhammer T., Fuchs R., Uhrova H., Seewald A.: Automated cell-detection technologies for science and diagnostics. Poster presentation at the 5th PhD Symposium of the Young Scientist Association of the Medical University of Vienna, June 2009, Vienna, Austria.

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