Quantitative analysis of Alzheimer plaques in mice using virtual microscopy

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- About us
- Image analysis on virtual slides and our work flow
- Image analysis process
- Results
- Conclusions

Work Area - Mission

- We support the colleagues of the Department of Pathology in imaging, image analysis, web publishing and e-learning

- 2005 online histology course based on virtual slides (**www.pathol.uzh.ch/histologycourse**)

Equipment

Virtual microscopy and image analysis

- Hamamatsu NanoZoomer HT (since November 2007)
- Slidepath server with some terabyte disk space
- Microsoft IIS web server

Client requirements

- Web browser, Adobe Flash viewer, Adobe SVG viewer
- e-Mail

Software Development Tools

```
zaehlerseqreader = 0;
sreader = 1;
for (int z =0; z < musteranz
    if (sreader > 0){
        char text5[4];
        _itoa_s(zaehlerseqre
        char text3[5];
        text3[0] = 'H';
        text3[1] = text5[0];
        text3[2] = text5[1];
        text3[3] = text5[2];
        text3[4] = NULL;
        CvStringHashNode* te
        if (testk != NULL){
            CvFileNode* mync
            sh = mynode->dat
            for(int myz3 = 0
                colorbin[zae
                }
            zaehlerseqreader
        }else{ sreader = 0;}
        }
    }
const int train_sample_count
CvMat* trainDatah = cvCreate
CvMat* trainClassesh = cvCre
for (int myz = 0; myz < trai
    for(int myz2 = 0; myz2 <
        cvmSet( trainDatah,
        }
    cvmSet( trainClassesh, m
    }
```

JAVA VB SQL C **C++** XML ASP **HTML** SVG

- Eclipse IDE
- Microsoft Visual Basic Express
- Microsoft Visual C++ Express
- Hamamatsu NDP Read
  (C-library, read virtual slides)
- Intel OpenCV
  (C-library, computer vision and ml)

Image analysis on virtual slides I

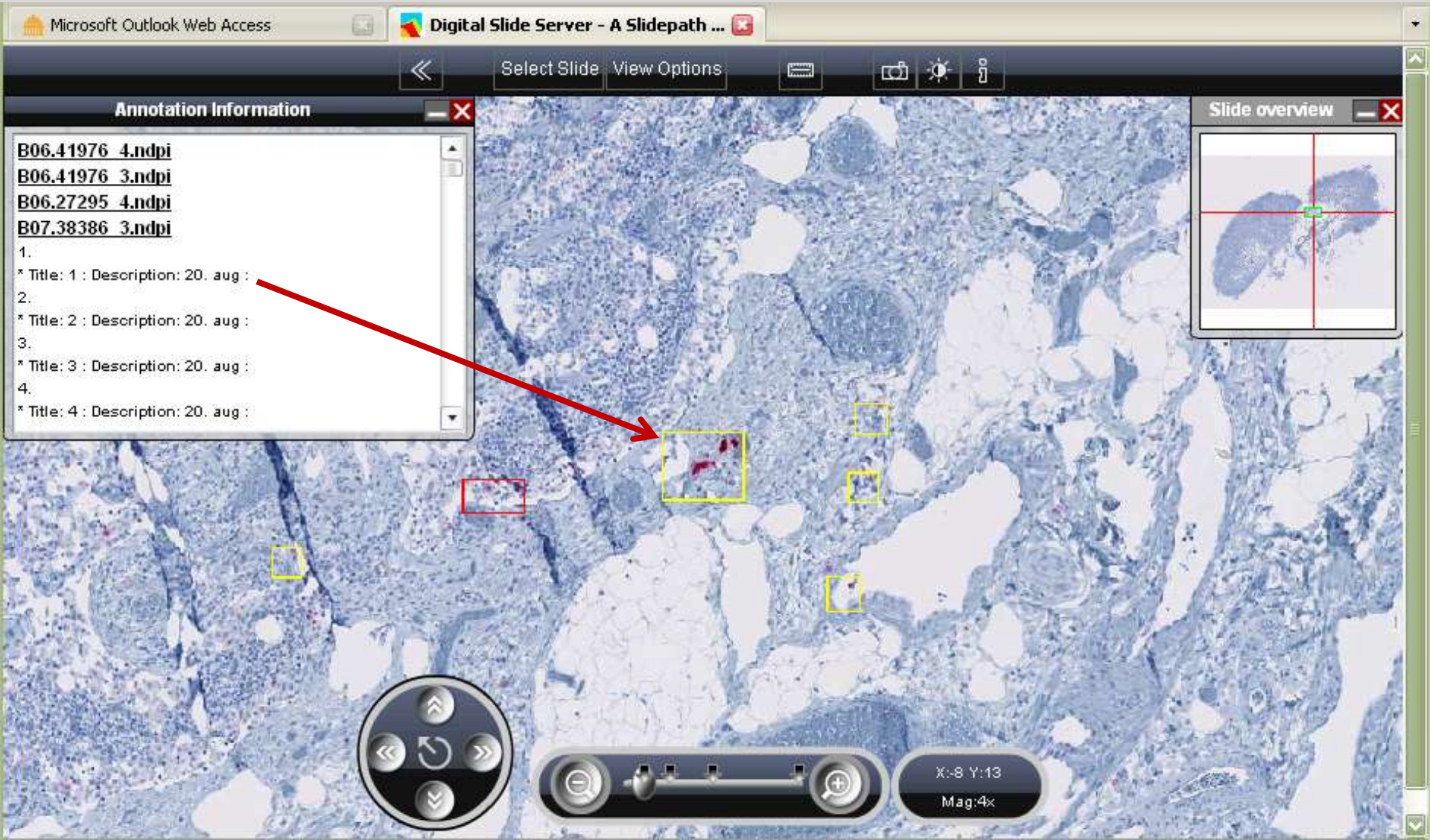
Input
histological slides

Output
annotated virtual slide

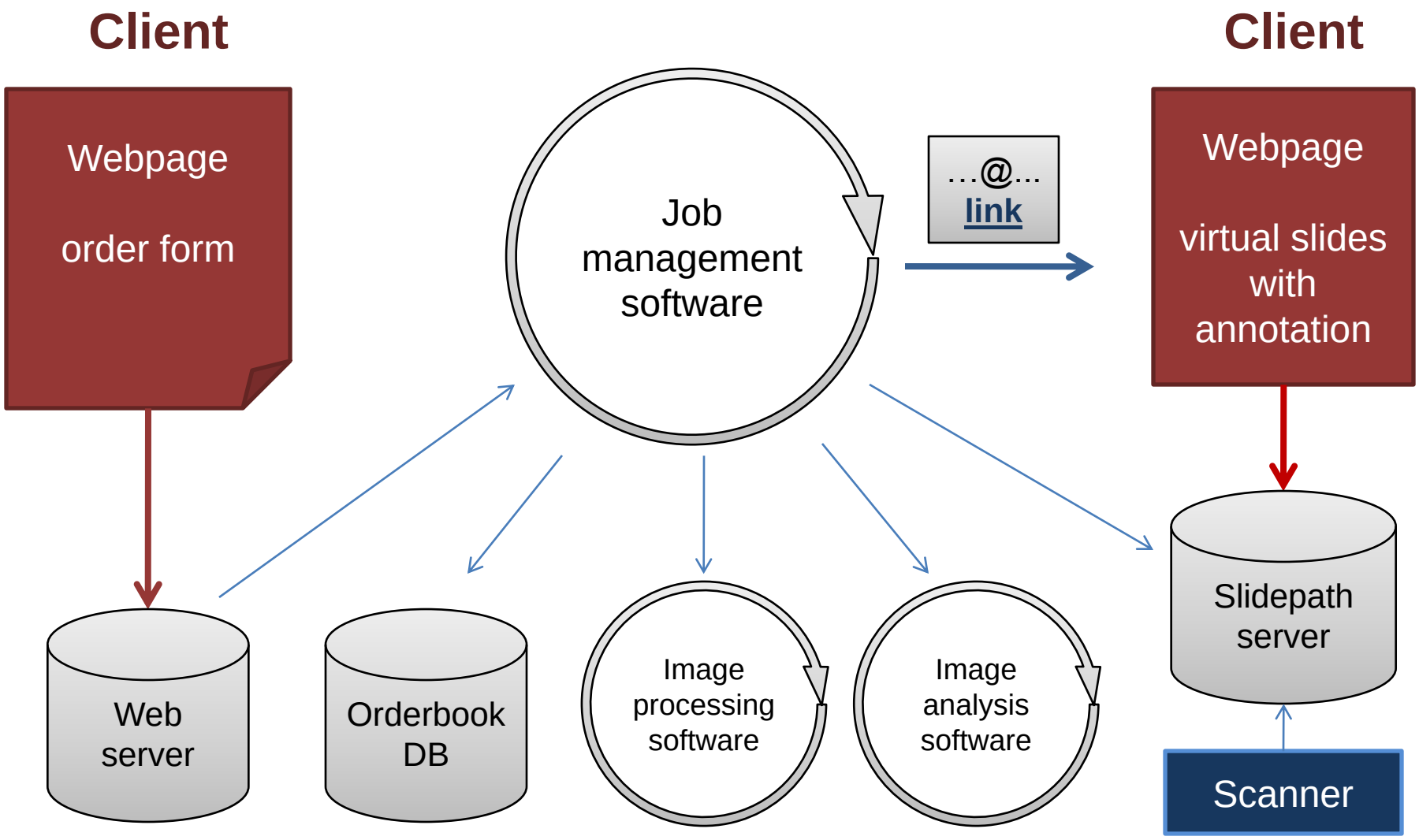
- Detection and classification of melanoma metastasis in lymph nodes

in collaboration with: ETH Zürich

Screening for rare events



Work flow for computer generated annotations in virtual slides



vSlide Order - Windows Internet Explorer bereitgestellt von UniversitätsSpital Zürich

http://www.pty.usz.ch/scripts/_private/Test_biemo/Bestellformular.asp

Datei Bearbeiten Ansicht Favoriten Extras ?

vSlide Order

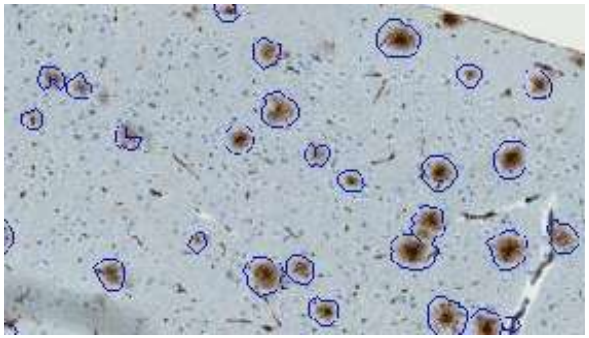
Ihre E-Mail-Adresse	...@...
Wie viele Slides möchten Sie einscannen lassen? (maximal 20 pro Auftrag)	12
vSlides sollen gelöscht werden am: (dd-mm-yyyy)	13 - 8 - 2009
Beschreibung (Projektname oder Stichwort)	Alzheimer 3
Werden die Slides für eine Konferenz gebraucht? Beispiel **Uster** (Sie erhalten ein Username und ein Passwort für Annotations)	☐
Werden die Slides speziell für Übersichten eingescannt? (Anfertigung von zusätzlichen Übersichtsbildern für die Weiterverarbeitung in tif Format mit 4000 Pixel Breite)	☐
Analyse der virtuellen Slides? Anleitungen und Voraussetzung zu den einzelnen Analysen sind hier zu finden Steatosis Quantification (human) Alzheimer Plaque Quantification (mice)	Alzheimer Plaque Quantification

Submit Clear Form

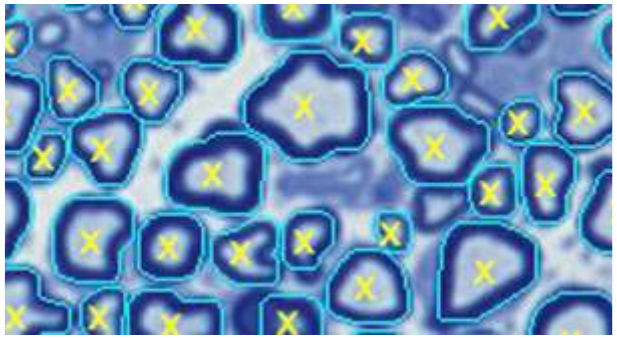
Image analysis on virtual slides II

Input
histological slides

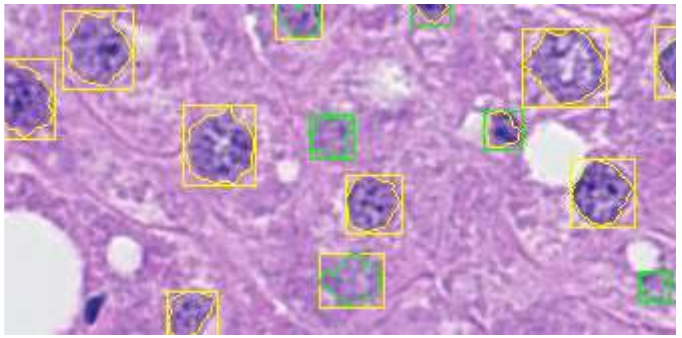
Output
excel sheet with measured values



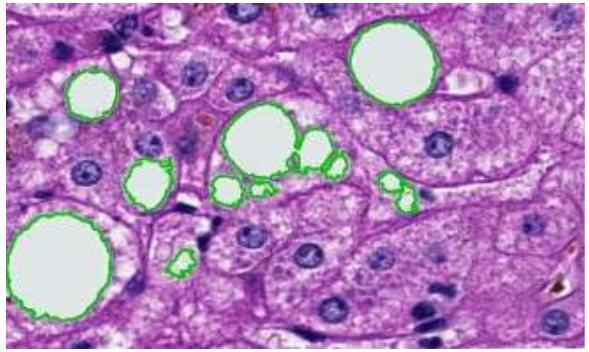
area of Alzheimer plaques
in brain (mouse)



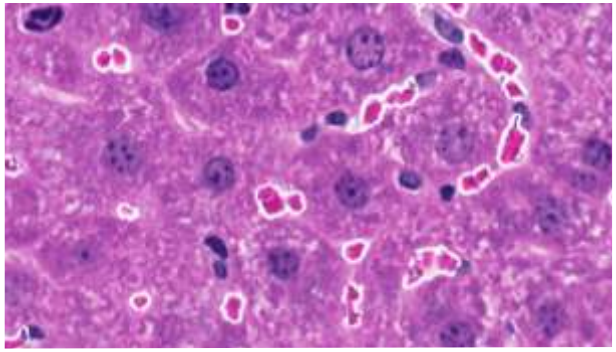
size of axons
in peripheral nerves (mouse)



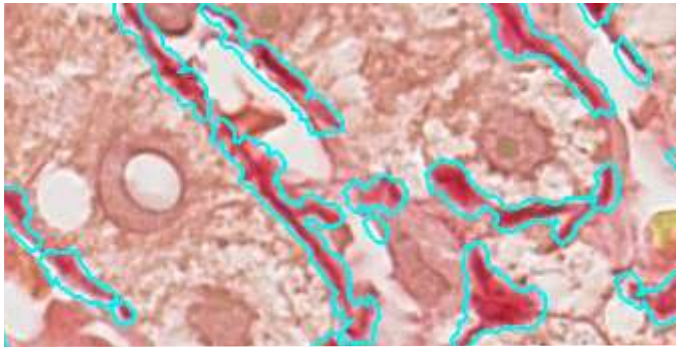
average of cell size
in liver tumours (mouse)



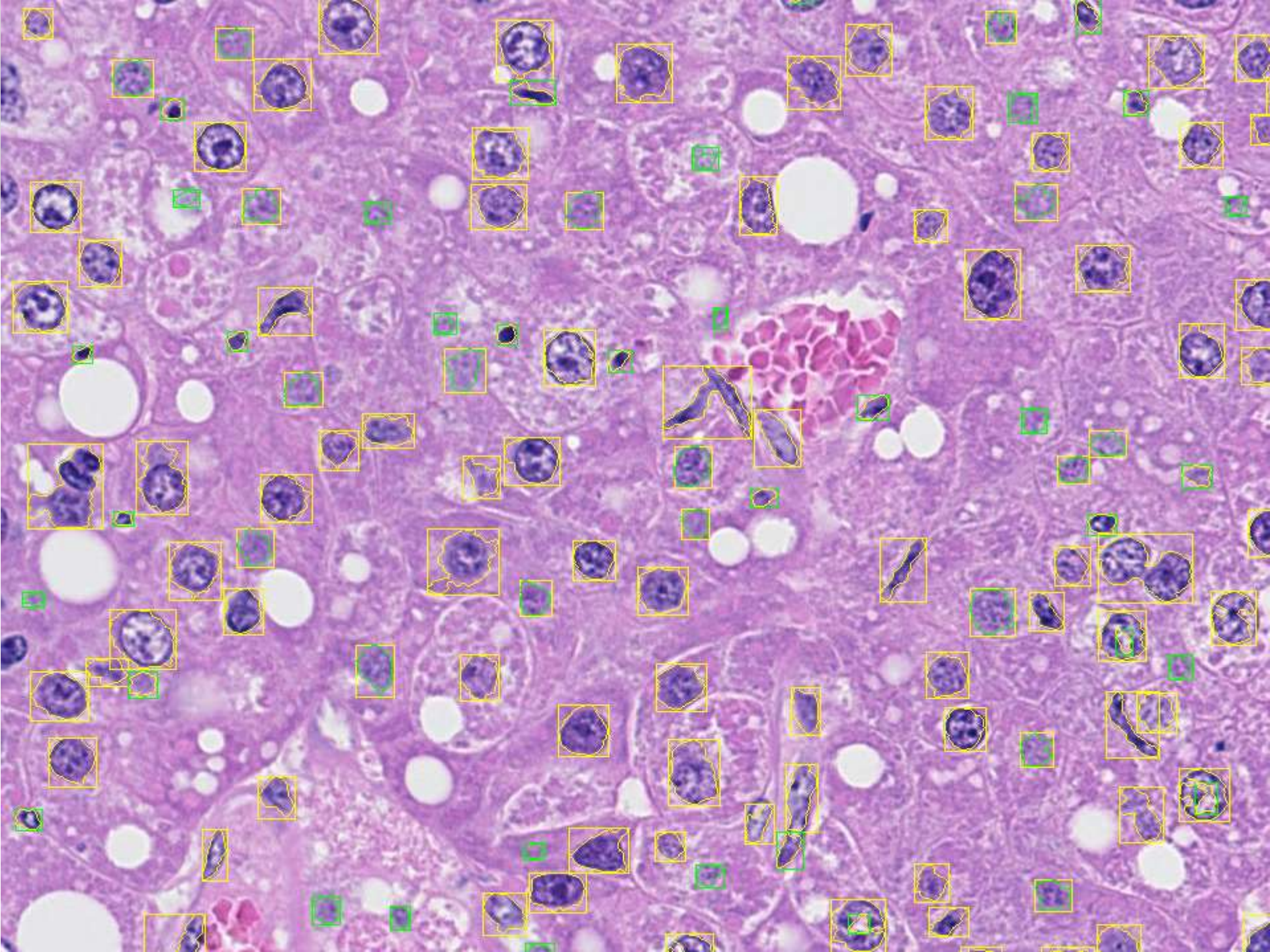
area of fat in liver (human)



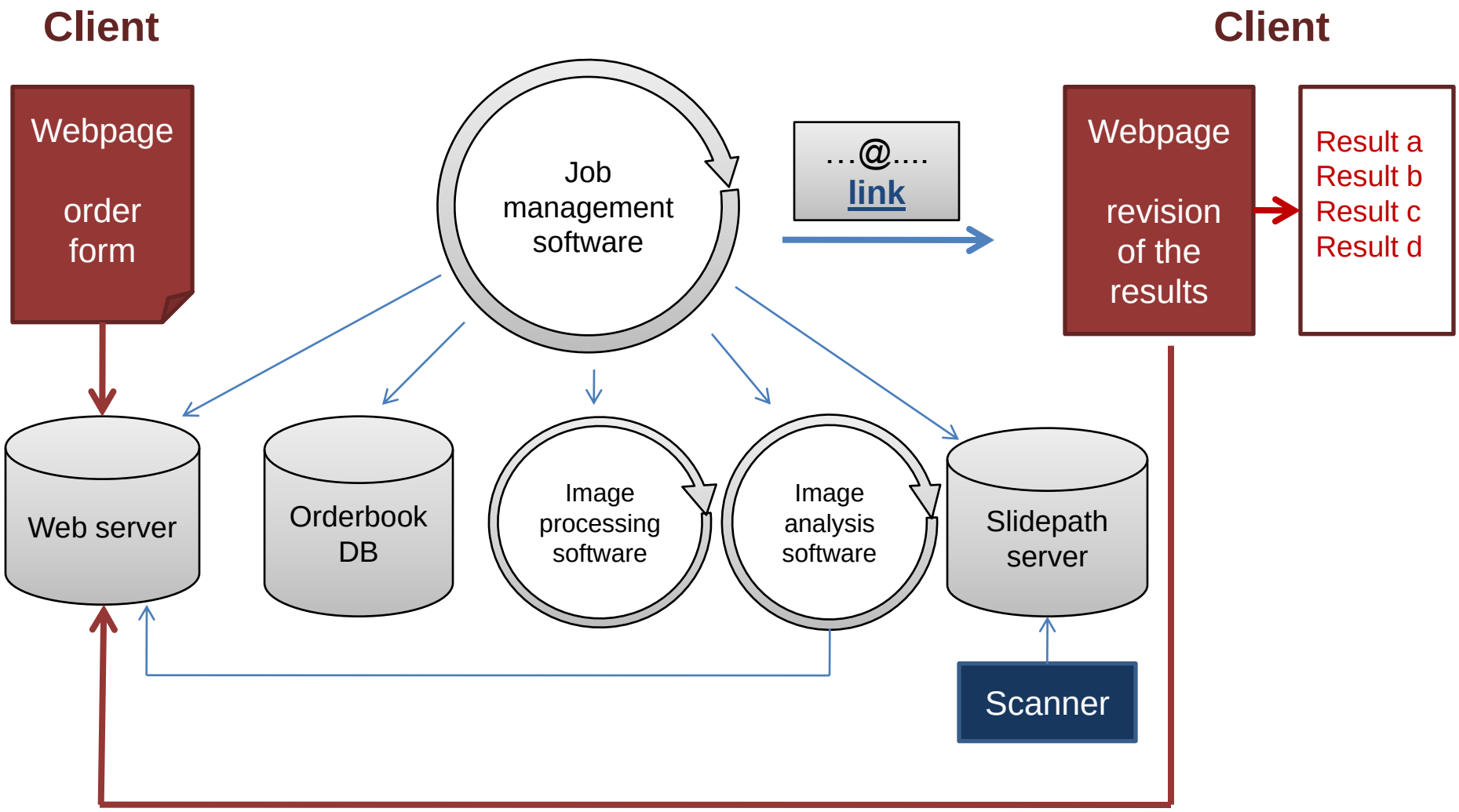
area of blood in liver (mouse)

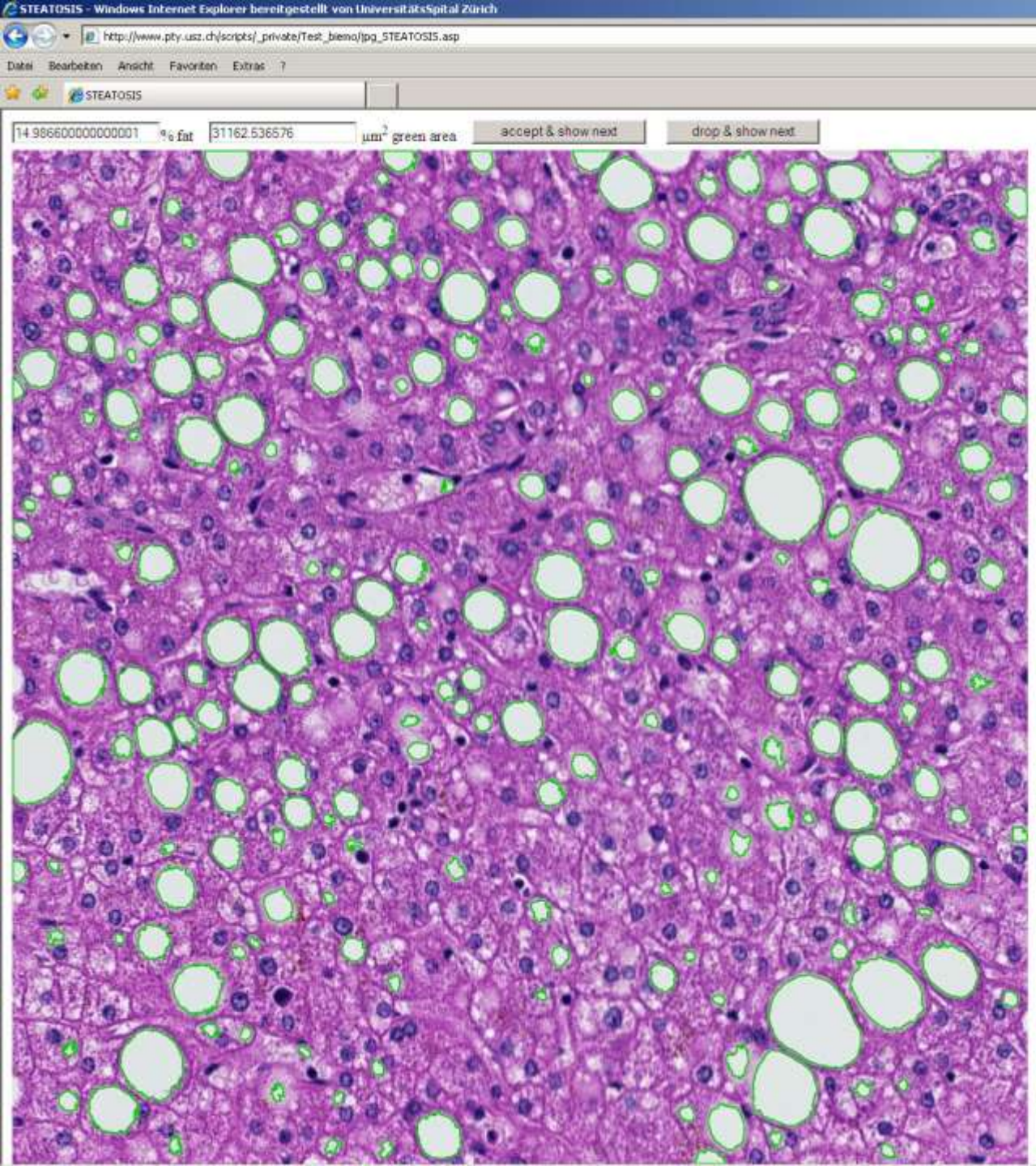


fibrillar collagen in liver (human)



Work flow for value table





- Results are drawn in an overlay for the Adobe SVG-viewer
- Workflow is inflexible for reproducible result

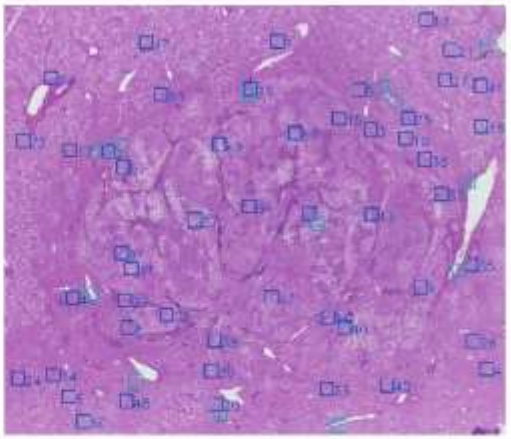
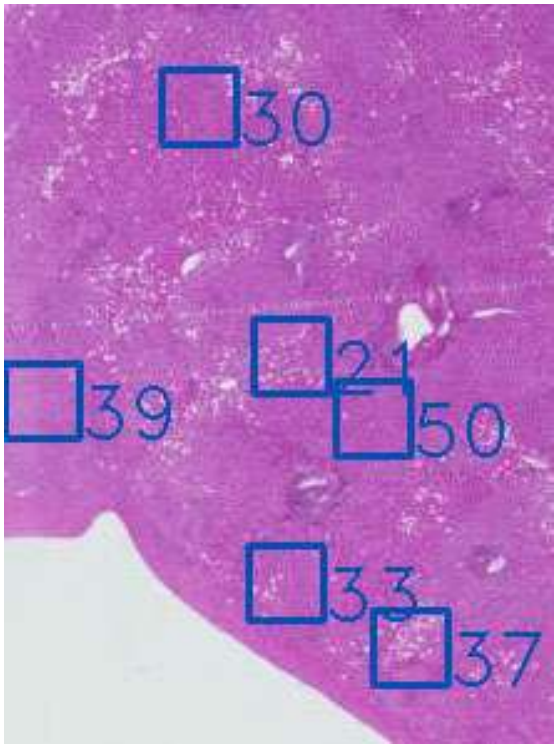
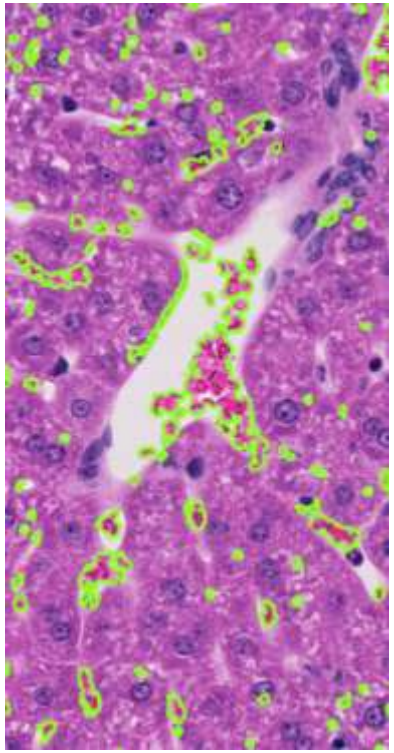


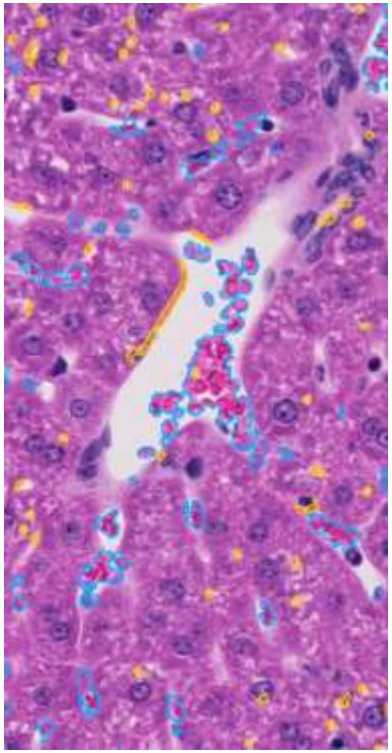
Image analysis process

Object detection

Image acquisition > segmentation > classification > calculation







$$\frac{\text{area objects}}{\text{area tissue}} \times 100$$

Image processing

- Different cropped images
  - overview images (Alzheimer plaques)
  - randomized generation of cropped images within the tissue but without big blood vessels (blood in liver)
  - overlapping tiles of the whole image (lymph node project)
  - calculated from user generated annotations in the Slidepath software (cell size in tumours)
- Adjust colour intensity

Image processing for Alzheimer plaque quantification

- Overview generation
- Reduction to region of interest

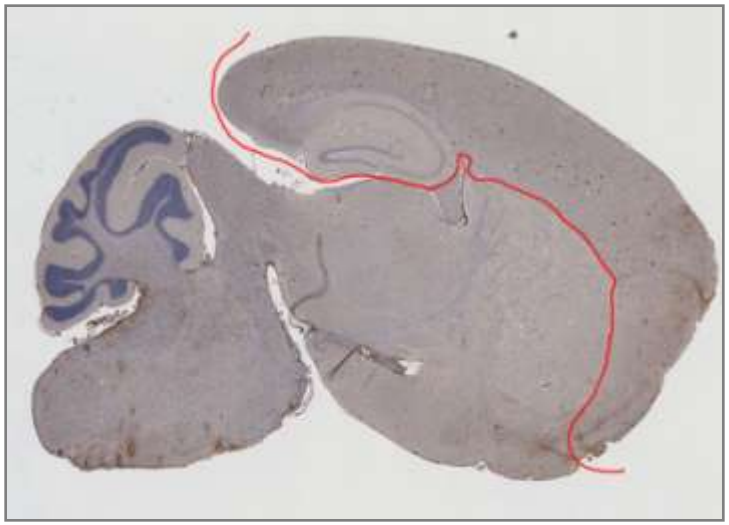
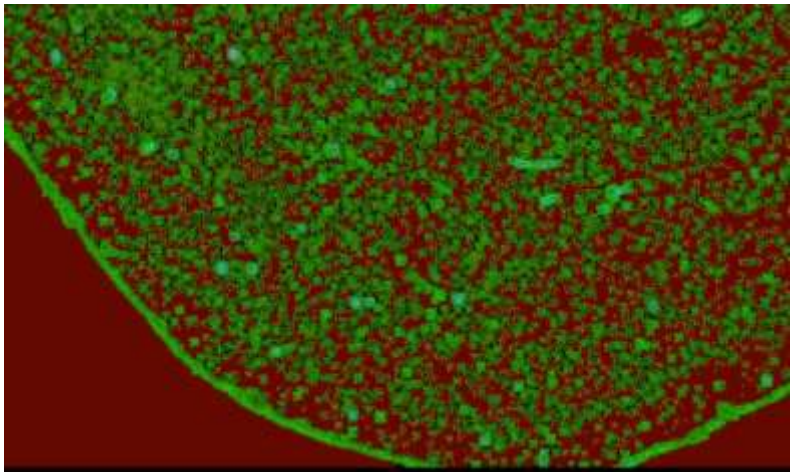


Image segmentation

- Segmentation based on colour threshold
  - by value
  - relative difference between the channels
  - relative to minimum and maximum
- Segmentation/detection based on edges
- Segmentation with pre-trained neuron like algorithm WB08
  - combine information of colour, texture and neighbourhood for each pixel

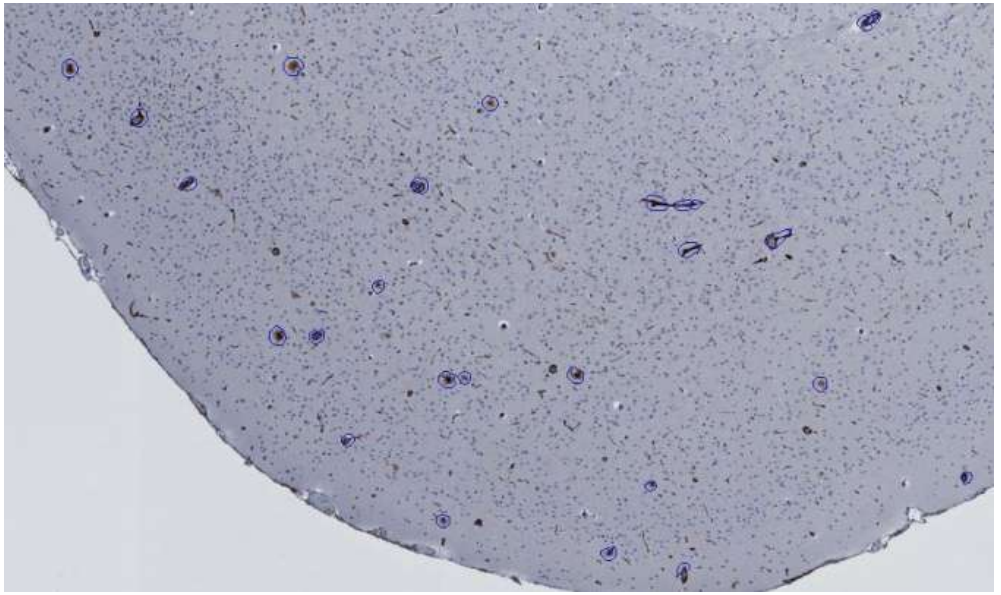
Training of the algorithm

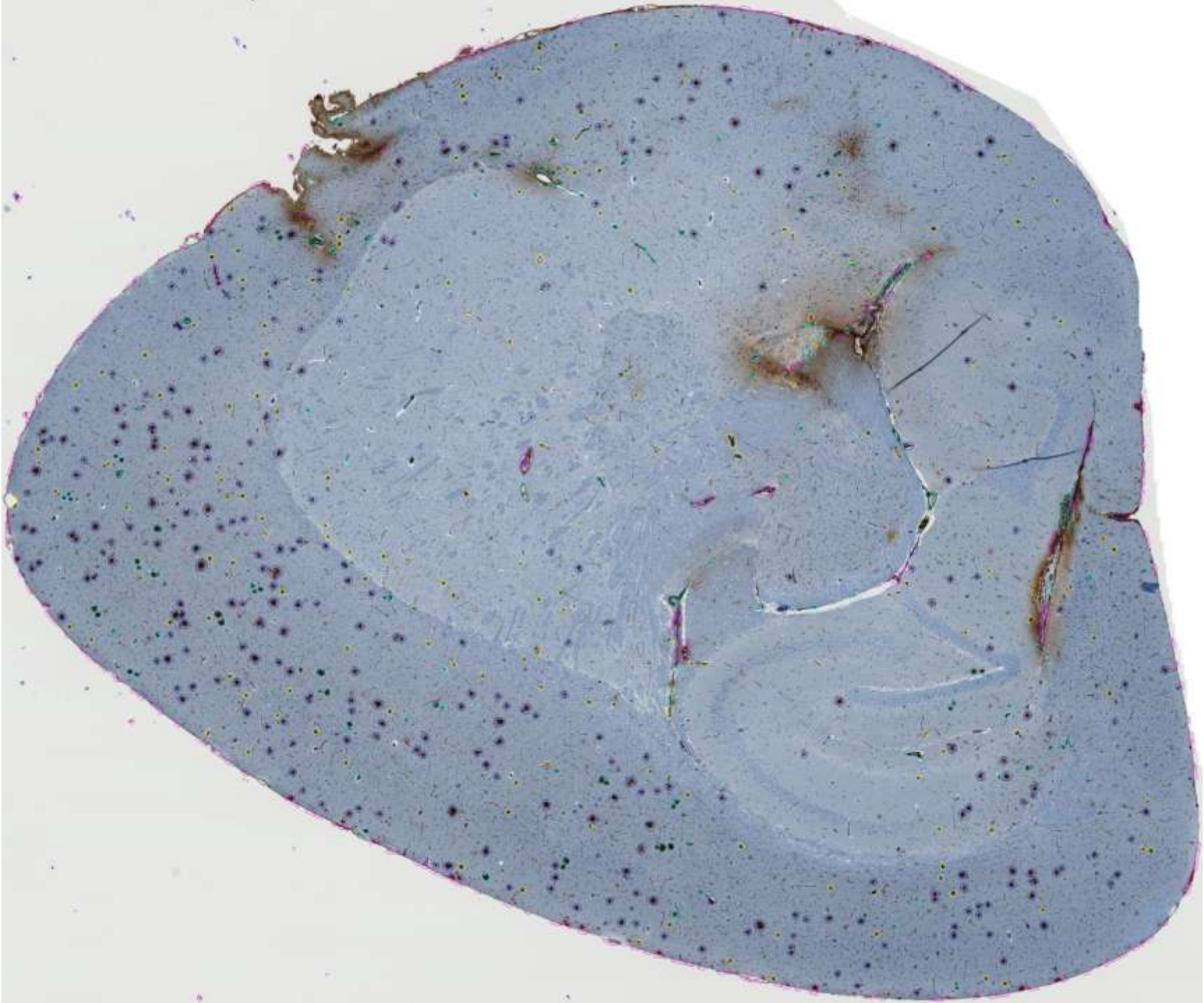
WB08-transformed image



Training mask

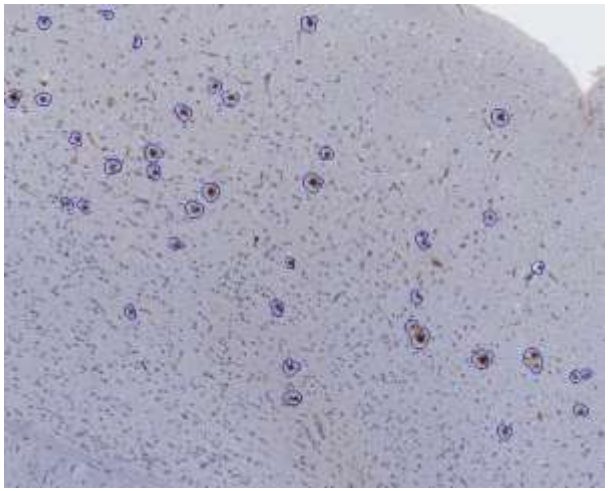
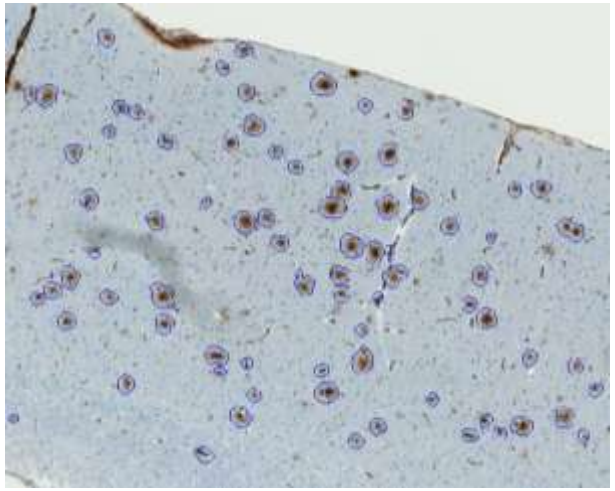
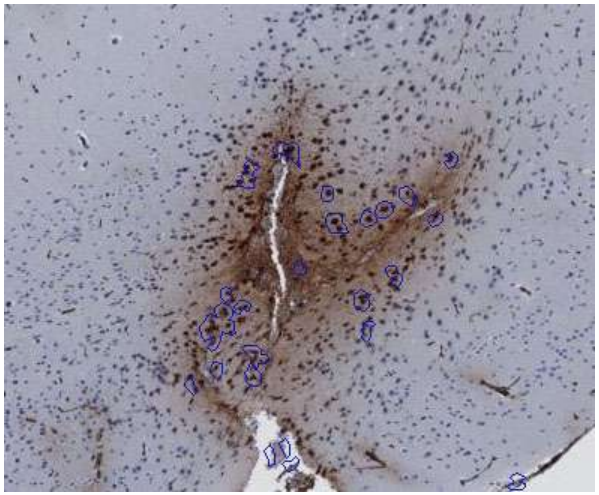
Result on the original



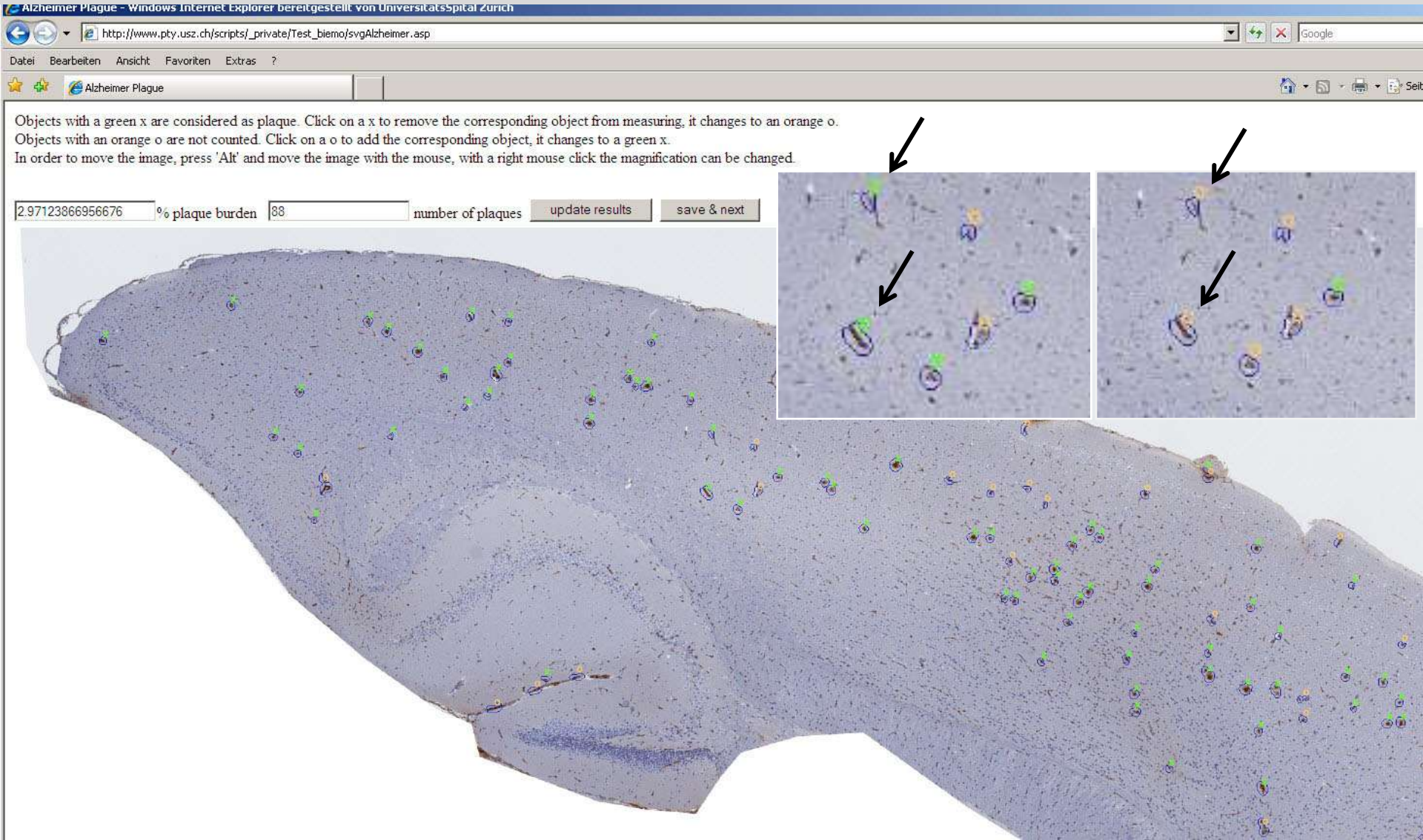


Definitions of quality for segmentation/detection algorithms

- least possible amount of false negatives
- precise contour detection
- robust results even if there are staining differences between the slides

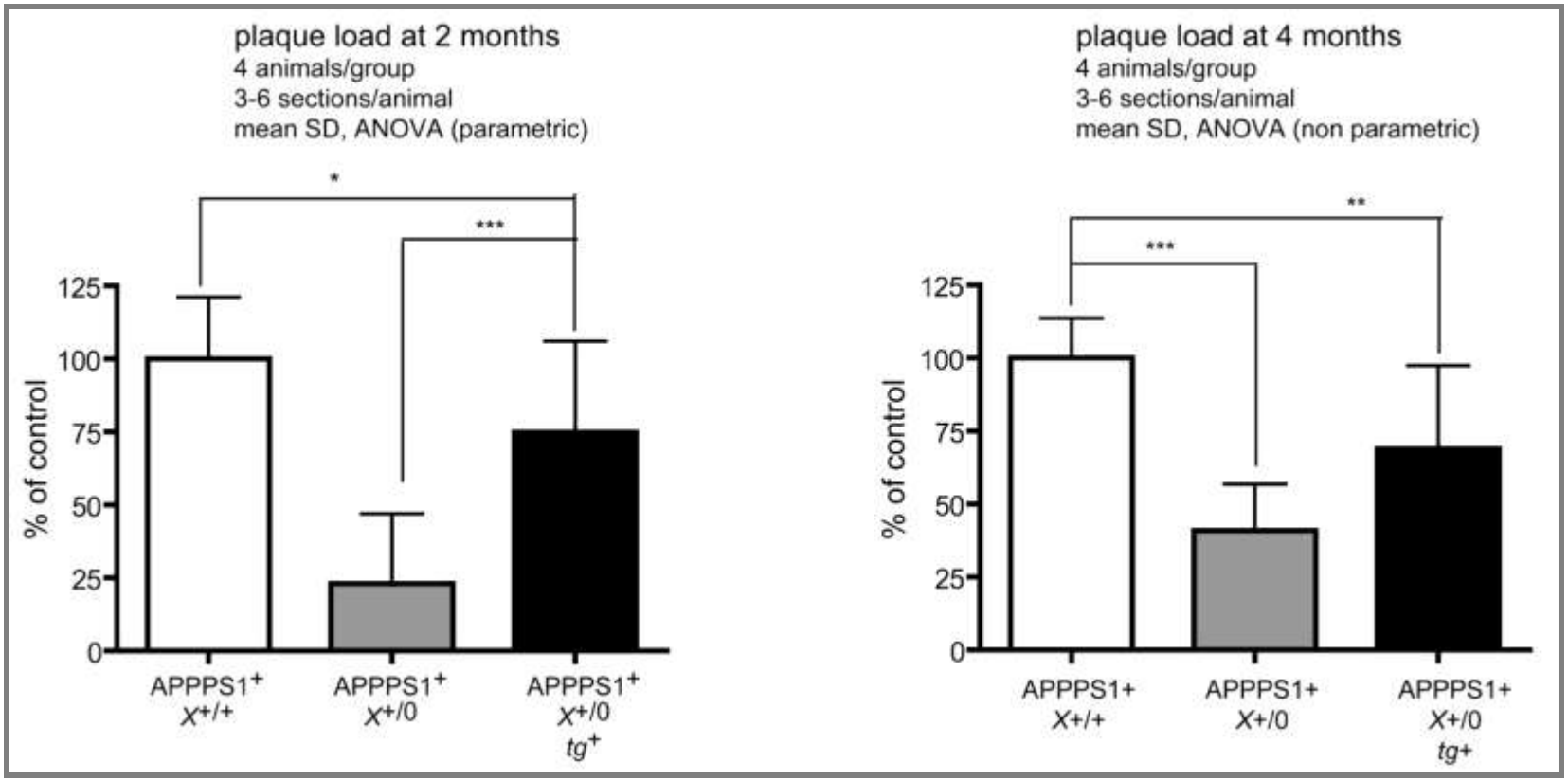




Revision of the detection results



Results of Alzheimer quantification in 24 mice

- 132 slides analyzed
- estimated detection error was about 15% percent before revision



Conclusions I

- Our work flow is efficient and generates easy to use results for the scientists
- The development needs time but it is a valuable investment for the future
- The most common problems in image analysis on virtual slides are:
  - out of focus regions on the virtual slides
  - difference in staining intensity
  - damages in the tissue

Conclusions II

- Improvement of the detection algorithms helps us to generate more valuable results
- We need a test and rating system for image analysis algorithms on histology images to find out the best algorithm
- Standardization of image analysis algorithm for specific application could help to generate comparable values

Acknowledgements

- Monika Bieri (software development)
- André Wethmar (system implementation)
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