Virtual Microscopy as a Research Tool in Medical Informatics:

Image Analysis for Microscopy – Activities at University Leipzig

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Overview

Morphometric Description and Quantification of

⇒ ***Carcinoma growth*** (uterine cervix, prostate, skin)

⇒ *Regeneration processes* (neurites, joint cartilage, tissue formation)

using (virtual) transmitted light microscopy and tailored image analysis.
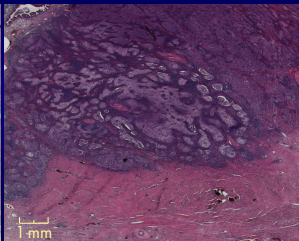
Carcinoma growth

- Malignant growth and invasiveness of cancers:
  - $\rightarrow$ intratumoral and stromal factors

- Shape of the tumor invasion front:
  - $\rightarrow$ accessibility to nutrients, oxygen and growth factors
  - $\rightarrow$ stromal composition, interference with the immune system

- Supposed growth pattern-related prognostic differences or surgical relevance
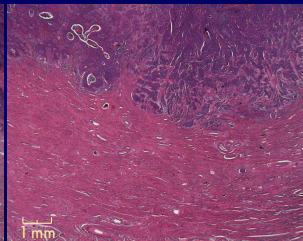
Carcinoma growth: Uterine cervix

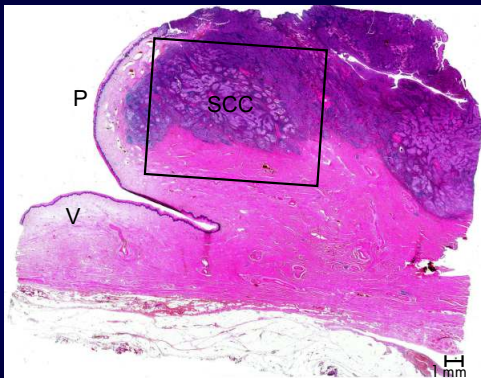- Phenomenon: Same tumor stage – different invasion pattern
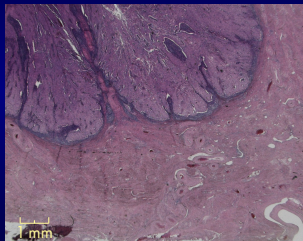
"closed"   "finger-like"   "diffuse"

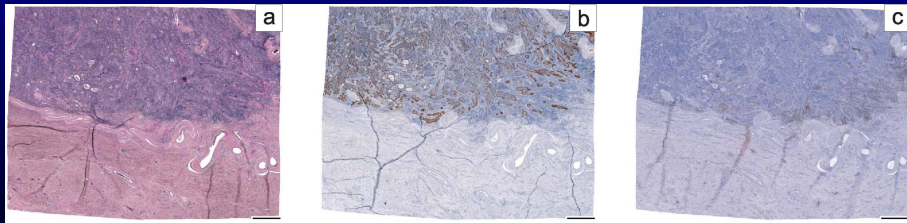# Carcinoma growth: Uterine cervix

Carcinoma growth: Uterine cervix

- Specific question:
  - $\rightarrow$ the spatial organization of a cervical cancer
  - $\Rightarrow$ the relation of the tumor invasion front vs. the infiltration with $CD3^+$ T-cells.

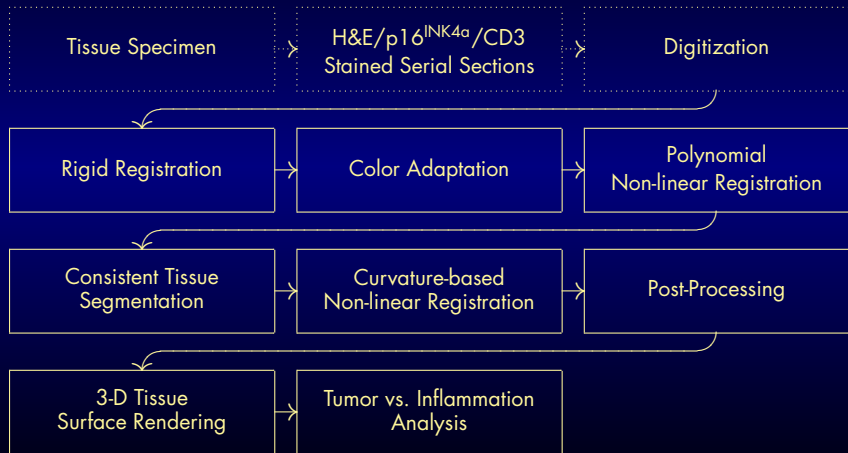# Carcinoma growth: Uterine cervix

- Cervical squamous cell carcinoma specimen
  - → serial section with 84 slices
  - → three interleaving subsets stained with
    - a H&E (routine reference stain)
    - b the cervical carcinoma biomarker p16^{INK4a}
    - c the T-cell marker CD3

⇒ Image processing chain ⤳ 3-D reconstructed tissue volume
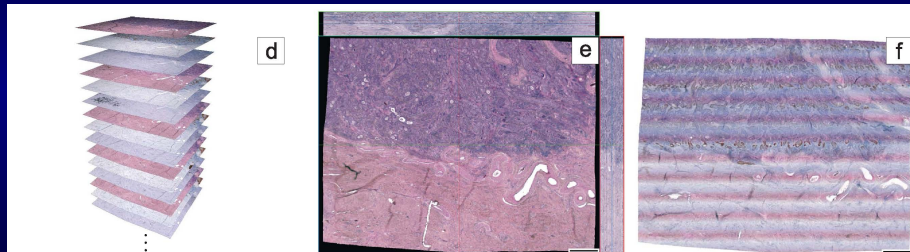
Carcinoma growth: Uterine cervix

- Adapted Image Processing Chain

| Tissue Specimen	H&E/p16^{INK4a}/CD3 Stained Serial Sections	Digitization
Rigid Registration	Color Adaptation	Polynomial Non-linear Registration
Consistent Tissue Segmentation	Curvature-based Non-linear Registration	Post-Processing
3-D Tissue Surface Rendering	Tumor vs. Inflammation Analysis	

Carcinoma growth: Uterine cervix

- 3-D Tissue Reconstruction

Carcinoma growth: Uterine cervix

- 3-D Tissue Reconstruction
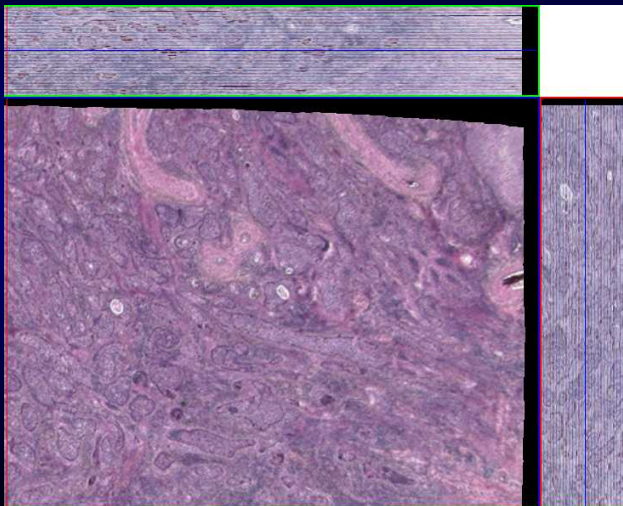
Carcinoma growth: Uterine cervix

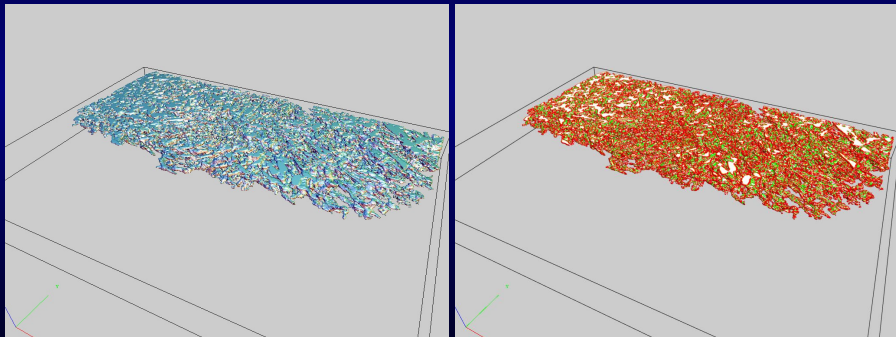- 3-D Tissue Reconstruction
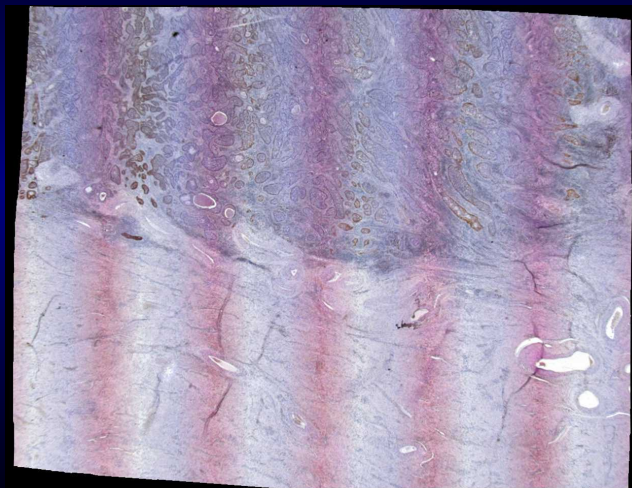
Movie

Carcinoma growth: Uterine cervix

- How to do a *local* tumor invasion front analysis:

  Mean surface curvature, related to

  $\rightarrow$ the respective local minimum tumor to T-cell distance

  $\rightarrow$ a T-cell originated diffusing substance's concentration at the tumor surface

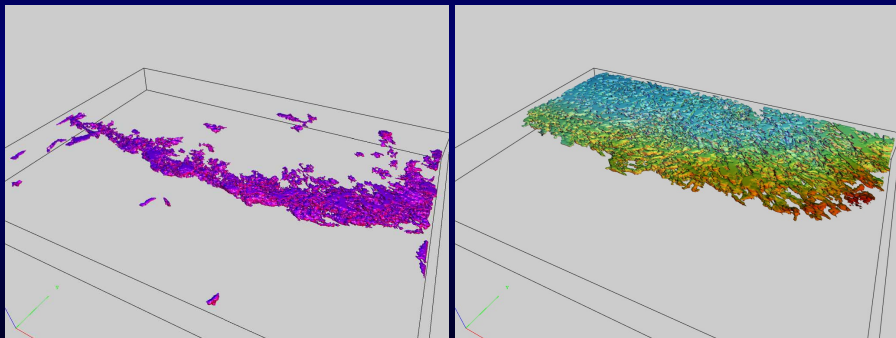# Carcinoma growth: Uterine cervix

- Mean curvature of tumor surface

Carcinoma growth: Uterine cervix

- 3-D Reconstruction results: T-Cell $\leftrightarrow$ Tumor Distances
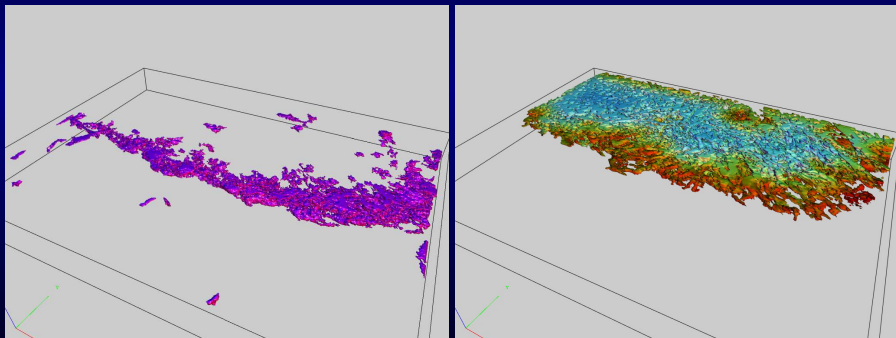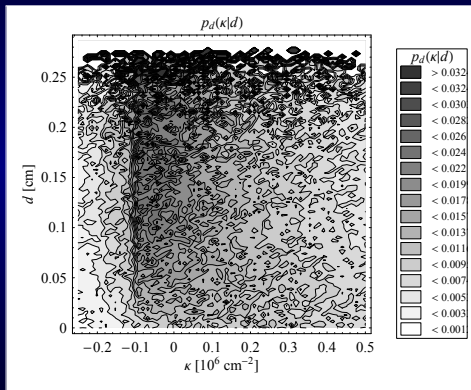
Carcinoma growth: Uterine cervix

- 3-D Reconstruction results: T-Cell $\rightarrow$ Tumor Diffusion
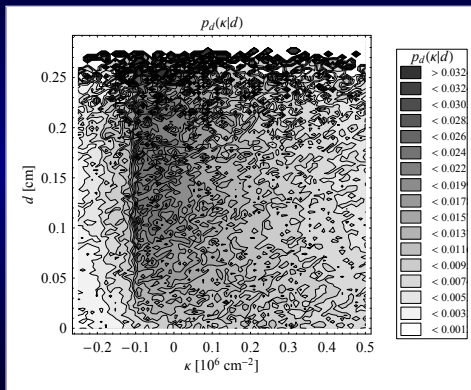
Carcinoma growth: Uterine cervix

- Conditional probability density $p_d(\kappa|d)$ for the mean curvature κ at a certain distance d from the T-cells

- the longer d, the more surface regions with a high magnitude of κ occur (neg. κ: convex curv.)
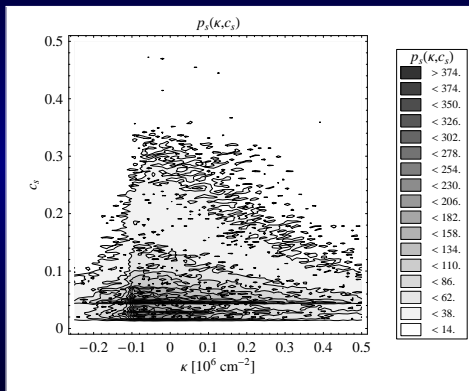
Carcinoma growth: Uterine cervix

- Conditional probability density $p_d(\kappa|d)$ for the mean curvature κ at a certain distance d from the T-cells

$\leadsto$ T-cells seem to cause a smoothing of the tumor surface (the smaller the d)

Carcinoma growth: Uterine cervix

- Probability density $p_s(\kappa, c_s)$ for curvature κ and substance concentration c_s (subst. const. emitted by T-cells)

- at low c_s, a broad range of curvatures κ occurs (expressing an irregular tumor surface shape)

Carcinoma growth: Uterine cervix

- Probability density $p_s(\kappa, c_s)$ for curvature κ and substance concentration c_s (subst. const. emitted by T-cells)
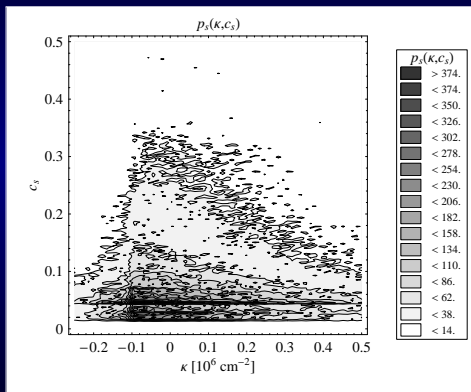
- with rising c_s, this range shrinks to low $|\kappa|$ (increasing tumor smoothness)

Movie

Carcinoma growth: Prostate

- Malignant glandular changes:
  - → de-differentiation of prostate glands

- Conventional grading system in uropathology:
  Gleason score (1966)
  - → visual inspection-based numerical description
  - → growing dissatisfaction with prognostic performance/precision

- Presently still missing:
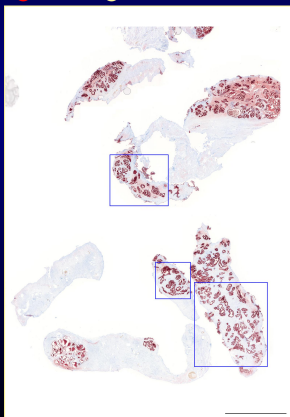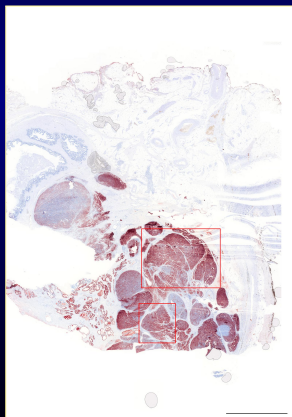 *Automated quantification method* well reflecting the various tumour grades

Carcinoma growth: Prostate

- Our digital microscopy device: Zeiss Mirax Midi

Carcinoma growth: Prostate

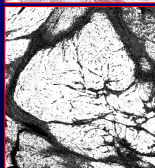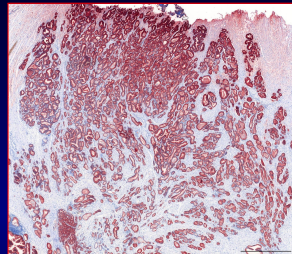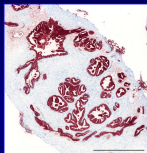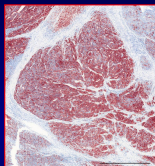- Samples out of 26 virtual slides from prostate specimens immunohistochemically stained for PSA (scale bar: 5mm)

- ROIs: normal/malignant glandular tissue
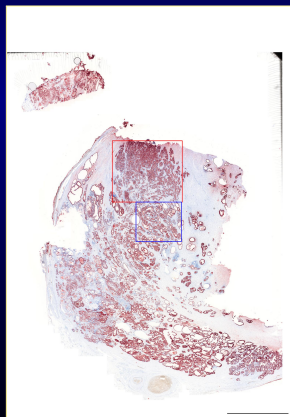
Carcinoma growth: Prostate

- 3 ROIs in detail (scale bar: 1mm)

- C-means-based gland segmentation
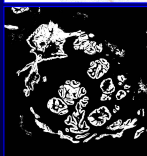
Carcinoma growth: Prostate

- 95 ROIs (random samples) defined in the 26 virtual slides

- Gleason score occurrences (assigned by a reference pathologist):
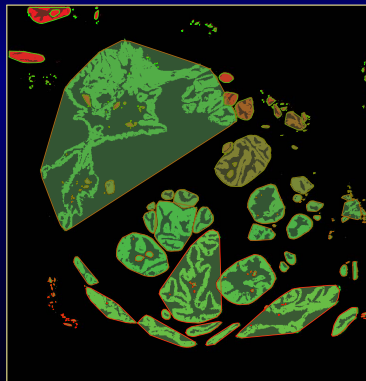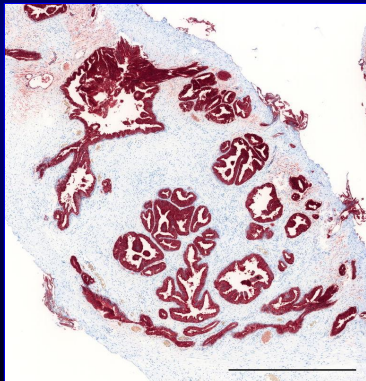
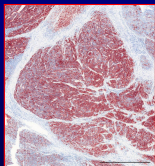"0": 27×

4: 7×

5: 3×

6: 31×

7: 15×

8: 7×

9: 2×

10: 3×

Carcinoma growth: Prostate

- Morphometry approach: gland shape solidities $s_i = \dfrac{H_i}{A_i}$

- Weighted solidity: $S = \dfrac{\sum_i A_i \, s_i}{\sum_i A_i} = \dfrac{\sum_i H_i}{\sum_i A_i}$
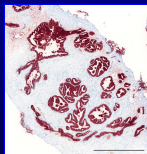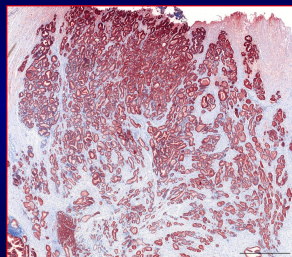
Carcinoma growth: Prostate

- Examples for weighted solidity assessment for 3 ROIs
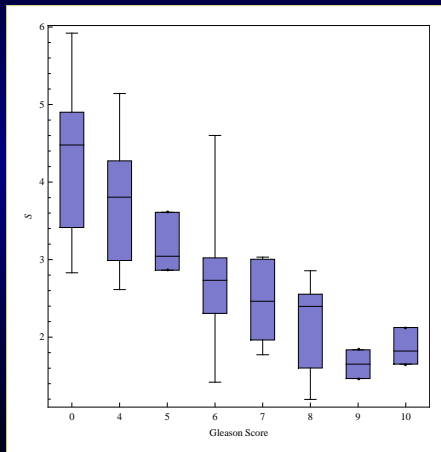

S=2.43/Gleason 6   S=4.51/Gleason "0"   S=3.04/Gleason 5

Carcinoma growth: Prostate

- How is the agreement with *Gleason*?

Carcinoma growth: Prostate

- Automated morphometry for malignant prostate changes still ongoing work

- First results suggest to consider weighted solidity a good indicator for glandular un-differentiation.

- Present work: how does solidity-based assessments improve prognosis?

Carcinoma growth: Skin

- Basal cell carcinoma (BCC):
  - → slow growing malignant tumor
  - → high risk of local recurrences
  - → underestimated (?) subclinical infiltration

- Surgical treatment:
  - → directional growth analysis
  - → consequences for safety margin

- Previously never done: a 3-D reconstruction of the BCCs tumor invasion front
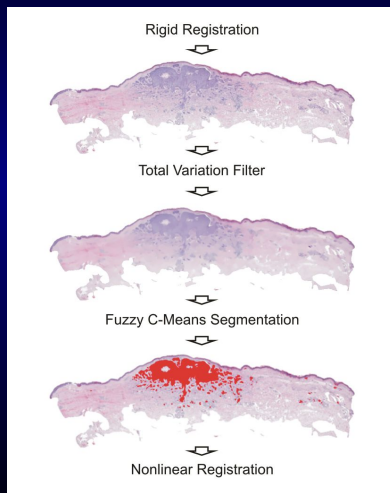
- Clarification of BCCs tumor-stroma-interface

Carcinoma growth: Skin

- 180 consecutively sliced sections form a BCC's VOI

- perpendicular to skin surface

- H&E staining

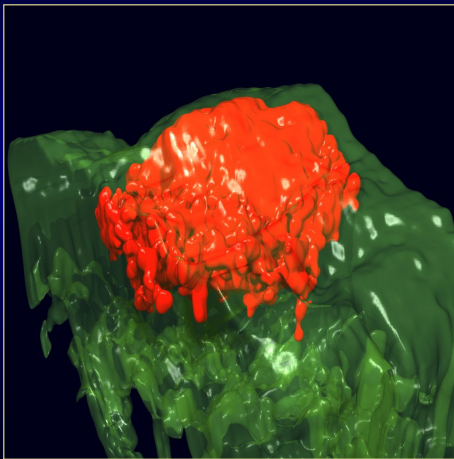- basically analogous to the uterine cervix case(s)

Carcinoma growth: Skin
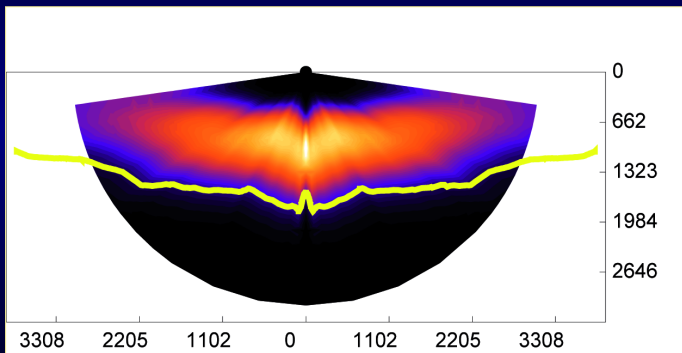
- Adapted image processing chain:

Carcinoma growth: Skin

- View onto the 3-D reconstructed VOI of a BCC:

Carcinoma growth: Skin

- Estimation of a directional probability density distribution:

Carcinoma growth: Skin

- BCC growth analysis is also ongoing work
- Directional growth analysis needs to be further refined
- Required: systematic study including the whole clinical spectrum of BCCs

Regeneration processes

- Spatio-temporal self-organisation of tissues
  - → geometrical/topological aspects
  - → biomechanics
  - → cellular gene expression
  - → signalling

- Quantification of regeneration
  - → architecture and spatiality
  - → experimental regeneration outcomes (standardisation!)
  - → enables systems-biological models of tissue formation (translational aspects)
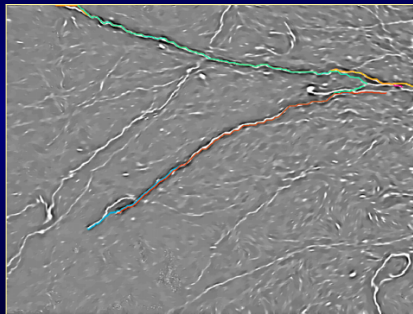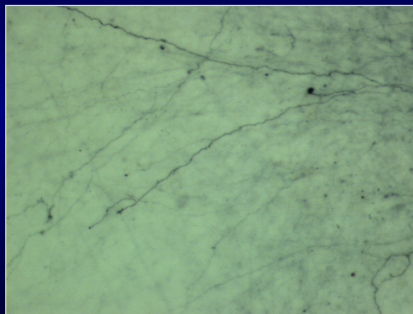
Regeneration processes: Neurites

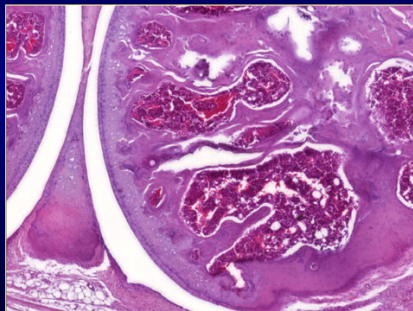- From a astrocyte image to an abstract representation

 $\Rightarrow$ $\Rightarrow$ $\Rightarrow$ $\Rightarrow$
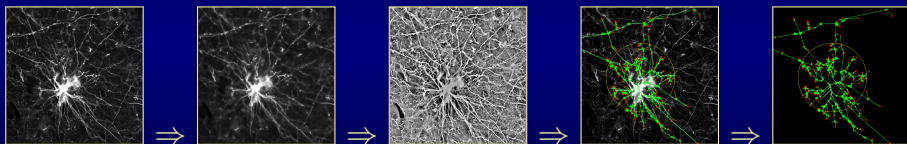
Regeneration processes: Neurites

- Growth in organotypic co-cultures

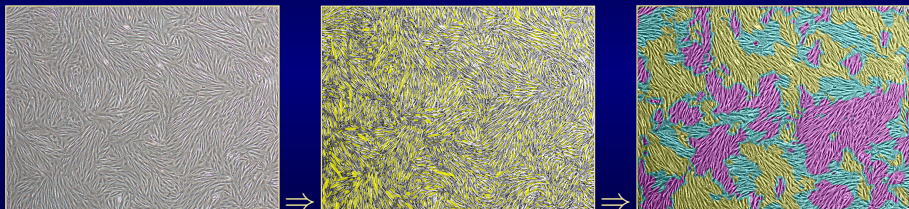$\Rightarrow$

Regeneration processes: Joint cartilage

- Quantification of mouse knee-joint regeneration after stem cell therapy

$\Rightarrow$

Regeneration processes: Tissue formation
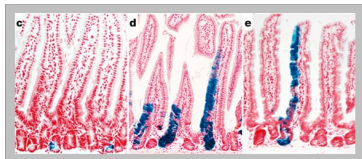
- Quantification of cells, assemblies
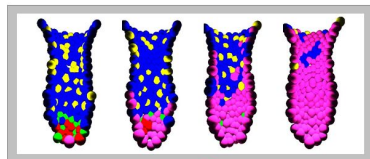
Regeneration processes: Intestinal crypt

- Which way imaging and simulation should be linked for systems biology:

Direct comparison between theory and experiment
From histology to model simulation of intestinal crypt organisation

Clevers et al., Nature 2007

Quantitative image analysis

Galle et al., 2008

Thank you!