Evaluation Strategies in Reference-Free 3-D Histological Image Reconstruction

Segmentation Colloquium, 20.01.2014

Simone Gaffling
Pattern Recognition Lab
Friedrich-Alexander-Universität Erlangen-Nürnberg

Outline

- 3-D Histology and Why go reference-free?
- Co-occurrence matrices
- Evaluation strategies
  - Guest and Baldock (1995)
  - Baheerathan (1998)
  - Wirtz (2004)
  - Ju (2006)
  - Tan (2007)
  - Bagci (2010)
  - Cifor (2011)
- What to do?
- Remaining problem

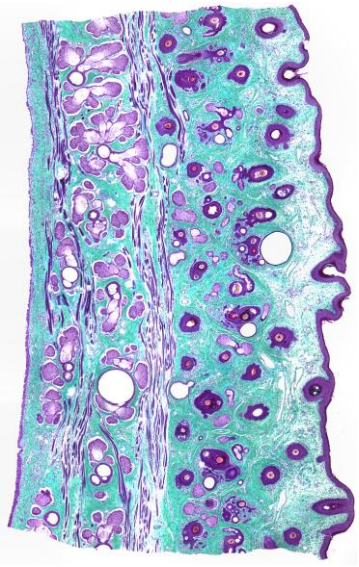
Outline

- **3-D Histology and Why go reference-free?**
- Co-occurrence matrices
- Evaluation strategies
  - Guest and Baldock (1995)
  - Baheerathan (1998)
  - Wirtz (2004)
  - Ju (2006)
  - Tan (2007)
  - Bagci (2010)
  - Cifor (2011)

- What to do?
- Remaining problem

3-D Histology

- Histology valuable investigation procedure for small objects
- Very high resolution
- Histological staining enhances contrast of structures



Problem:
Cutting and processing destroys connectivity!

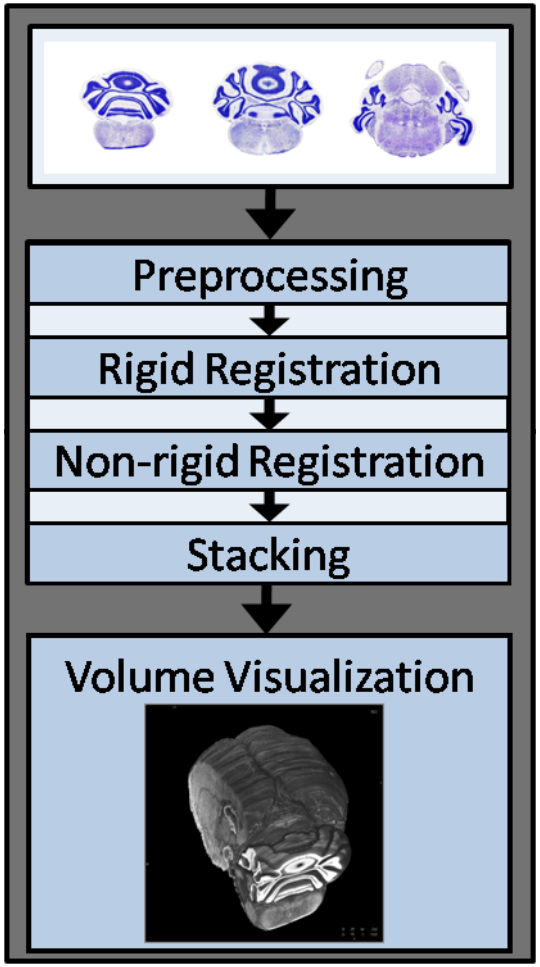
Sometimes hard to infer prior spatial properties of tissue from a slice sequence.

Idea:
3-D reconstruction of digital slice sequence might help!

3-D Histology

Problem: Conventional histology destroys 3-D structure severely!

Fixation + Embedding	• Shrinking
Slicing	• Slice loss • Deformations
Object plate	• Sorting
Staining	• Intensity offsets
Digitizing	• Rigid Transforms • Bias Field
Stitching	• Several bias fields • Black borders



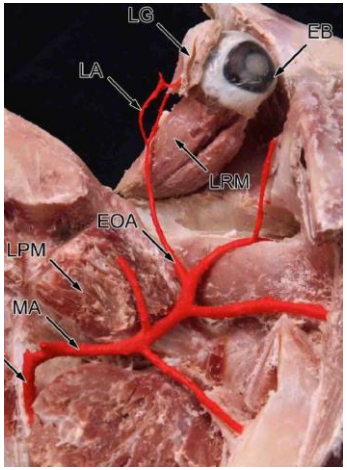
3-D Histology

Example:

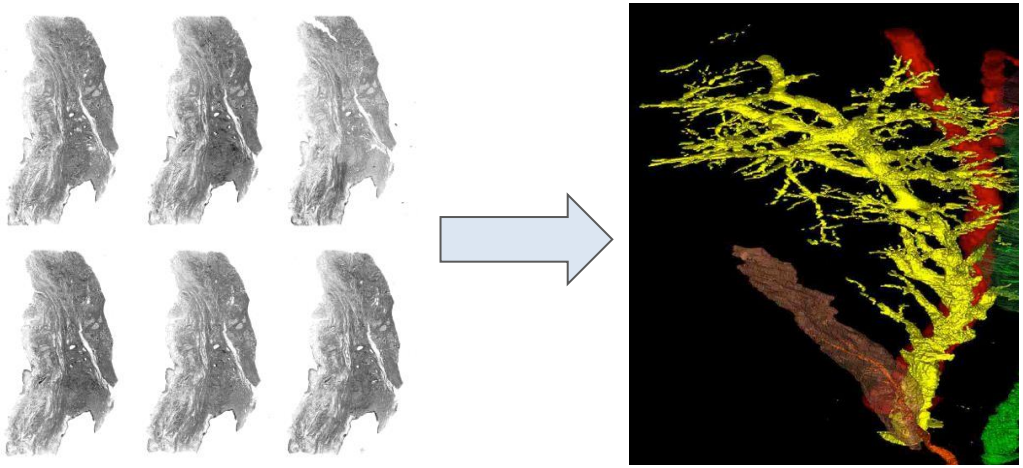
Are tear ducts from pigs in principle suitable as human xenograft?

→ Investigate 3-D structure of blood supply and tear duct system

Vascular system:



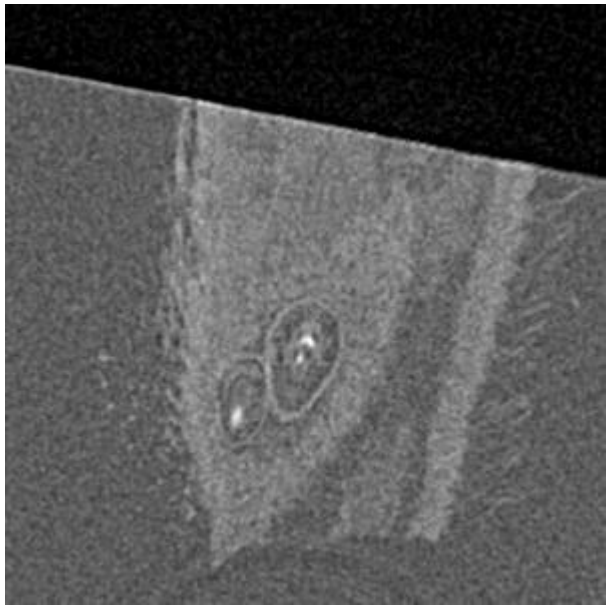
Lacrimal gland: Too small!

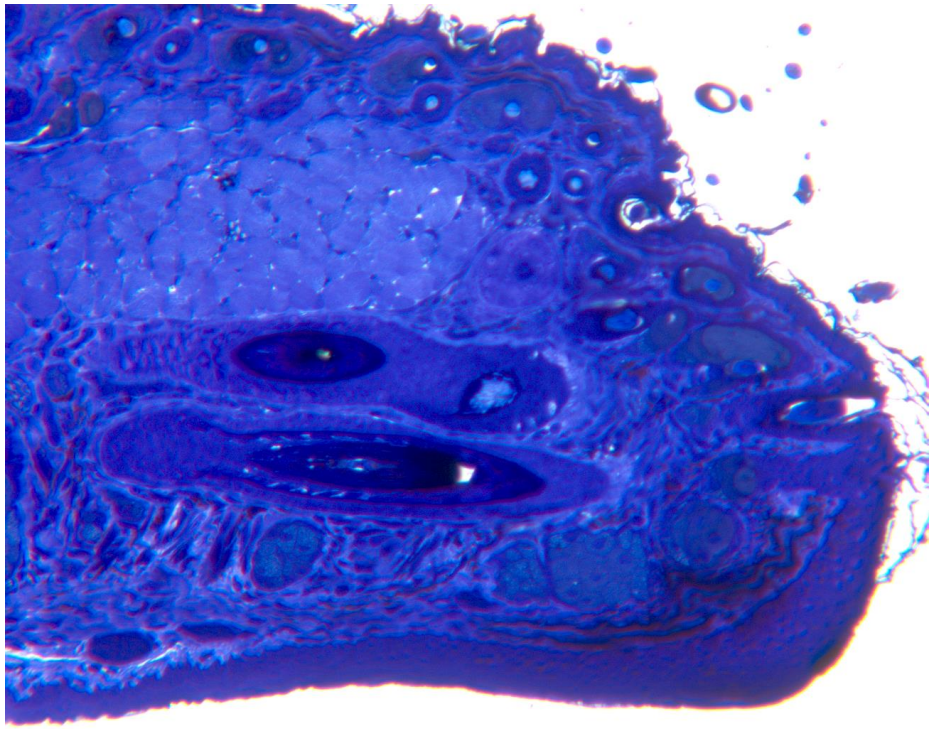


Why go reference-free?

Different possibilities for references:

- Prior acquisition of 3-D volume (e.g., µ-CT/MR)
  ➔ Device not available, resolution for relevant structures too small

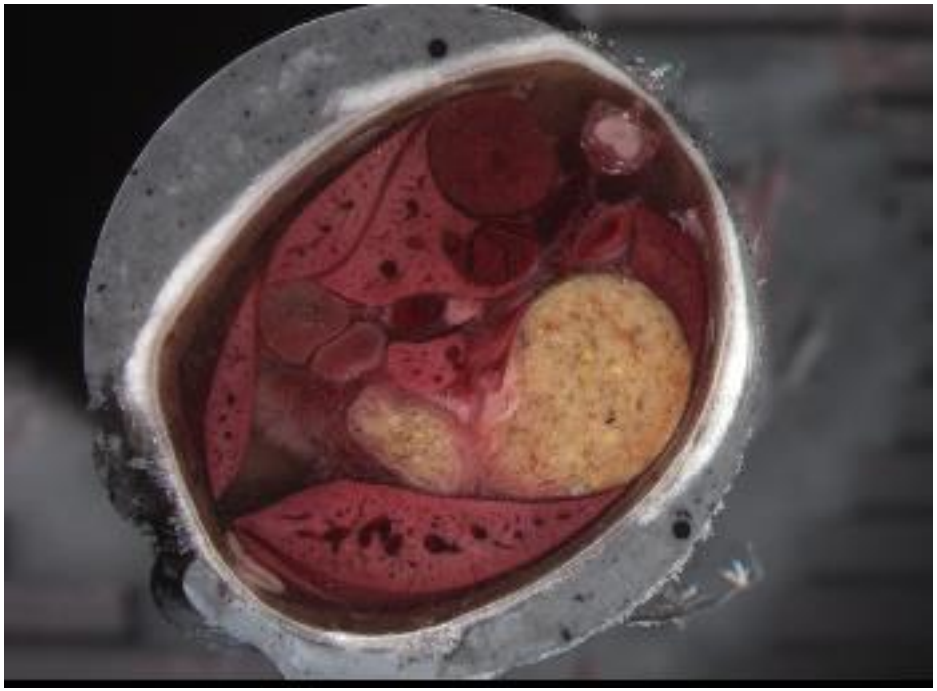




Why go reference-free?

Different possibilities for references:

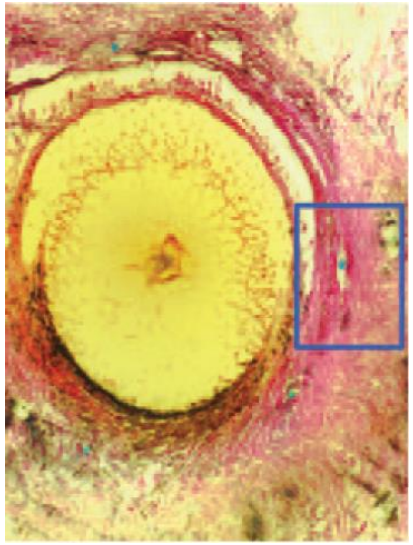
- Blockface photographs:
  Take photograph of block surface prior to cutting
  Apply staining on-the-fly
  ➔ Tedious, bad workflow, only for certain types of staining



Why go reference-free?

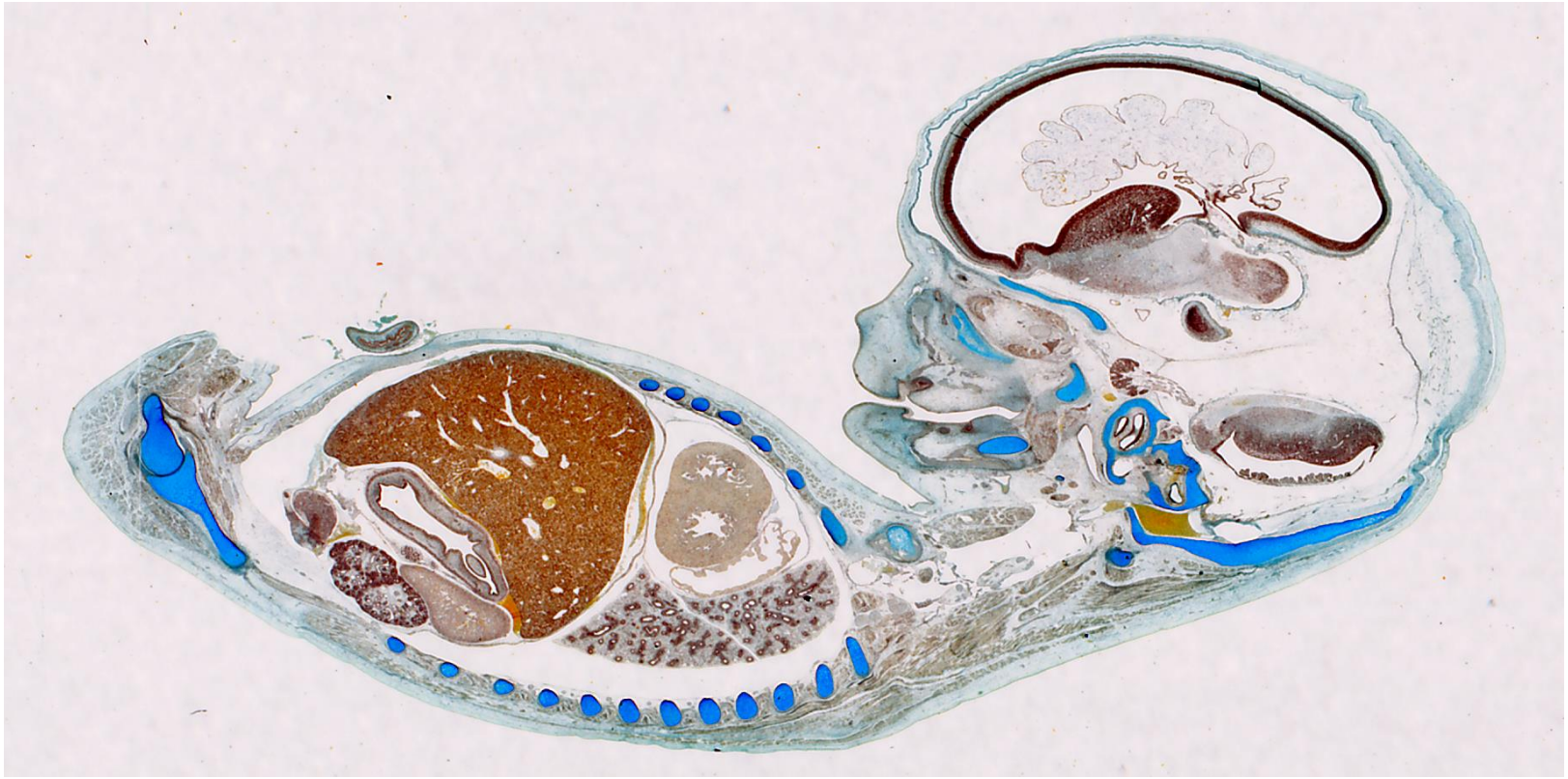
Different possibilities for references:

- External markers:
  Insert markers into tissue and/or block for later reference
  ➔ Not always applicable (for small structures) destroys tissue, does not help with unwarping



Why go reference-free?

- Historical slice sequences which are already cut
- Ex.: Human embryo, 5 weeks gestational age, cut in the 1970s (embryo might be much older)



Outline

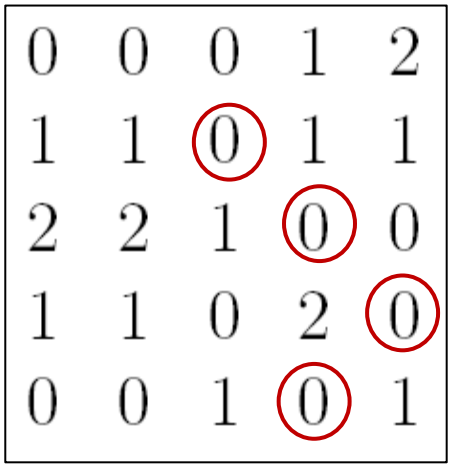
- 3-D Histology and Why go reference-free?
- **Co-occurrence matrices**
- Evaluation strategies
  - Guest and Baldock (1995)
  - Baheerathan (1998)
  - Wirtz (2004)
  - Ju (2006)
  - Tan (2007)
  - Bagci (2010)
  - Cifor (2011)
- What to do?
- Remaining problem

Gray-level Co-Occurrence matrix (GLCM)

from: http://code.ucsd.edu/pcosman/glcm.pdf

- Introduced by Haralick et al. in 1973
- Captures relationships between spatial pairs of pixels
- Definition of „spatial“ can be provided

Ex.: 1 pixel to the right and 1 pixel down



Image

p_{ij}

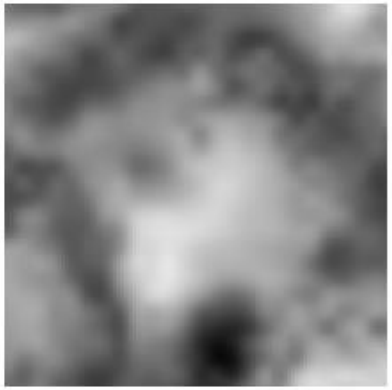
$$C = \frac{1}{16} \begin{bmatrix} 4 & 2 & 1 \\ 2 & 3 & 2 \\ 0 & 2 & 0 \end{bmatrix}$$

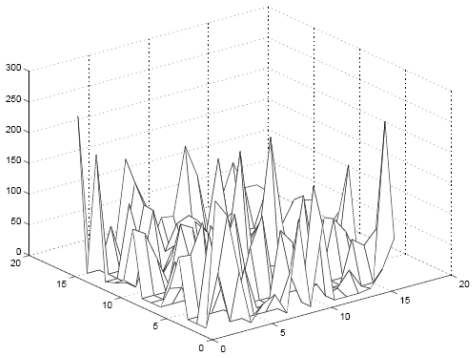
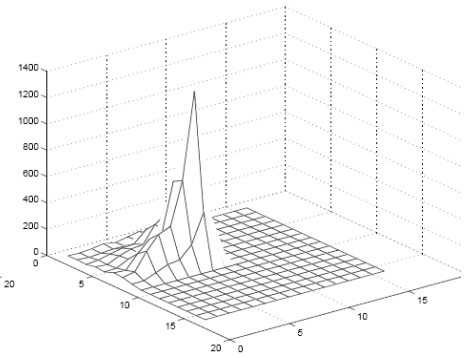
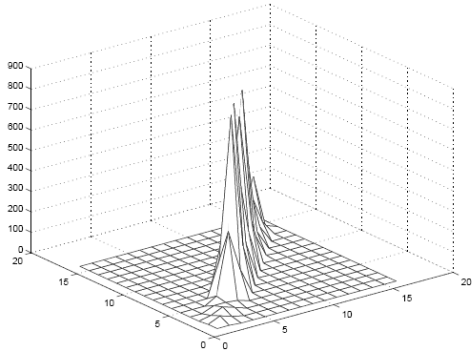
0 1 2 (columns)

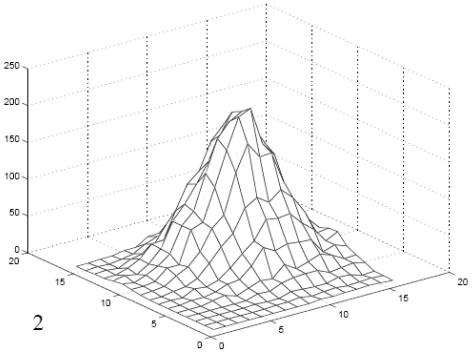
0 1 2 (rows)

GLCM

Example plots for GLCMs



- ➔ GLCMs can provide more statistical information about structure

Outline

- 3-D Histology and Why go reference-free?
- Co-occurrence matrices
- **Evaluation strategies**
  - Guest and Baldock (1995)
  - Baheerathan (1998)
  - Wirtz (2004)
  - Ju (2006)
  - Tan (2007)
  - Bagci (2010)
  - Cifor (2011)
- What to do?
- Remaining problem

Guest, Baldock (1995) (BioImaging)

- Method: FEM method: model each section as elastic plate, connect with springs at distinct points, let go and see how it settles

Evalution:

1. Residual displacement (on test images with known deformations): should ideally be zero

$$\mathrm{RD} = \sum_{\text{all pixels}} (\text{applied displacement}) + (\text{calculated displacement}).$$

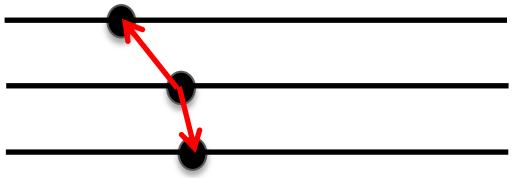
Guest, Baldock (1995) (BioImaging)

- (1 - correlation)
  - Correlation:

$$C(I_1, I_2) = \frac{I_1 \odot I_2}{\sqrt{(I_1 \odot I_1)(I_2 \odot I_2)}},$$

$$I_1 \odot I_2 = \sum_{i,j} I_1(i, j) I_2(i, j),$$

- Correspondence Alignment Measure (CAM):
  - Calculate corresponding points (through def-field) in neighbor-images
  - Calculate confidence that points are really correspondences
  - Calculate the sum of the vectors, and add the square of the resultant to the cumulative sum. Normalize.



Baheerathan (1998) Journal of Microscopy

- Method: Choose control points, polynomial mapping of control points

Evaluation:

Correlation coefficient $$C = \frac{\sum_x \sum_y A(x,y)B(x,y)}{\left[\sum_x \sum_y A(x,y)^2\right]^{1/2}\left[\sum_x \sum_y B(x,y)^2\right]^{1/2}}$$

Overlap index C_0:
N_A,N_B,N_{AB} = Areas of images A,B and overlap

$$C_0 = \frac{2N_{AB}}{N_A + N_B}$$

- C, C_0 calculated over segmented images!

Baheerathan (1998) Journal of Microscopy

- 3-D GLCM (Gray-level Co-occurrence matrices)

- 14 features from GLCM proposed by Haralick et al. (1973)
- Most important: visual textural characteristics:
  - Angular Second Moment (ASN)
    Measure of homogeneity of an image
    High for homogeneous
    $$= \sum_i \sum_j p[i,j]^2$$
  - Contrast (CON)
    Local variation
    High if high variation
    $$= \sum_{n=0}^{Ng-1} n^2 \left\{ \sum_{i=1}^{Ng} \sum_{j=1}^{Ng} p[i,j] \right\}, \text{ where } |i-j| = n$$
  - Correlation (COR)
    Gray-level linear dependencies
    High for homogeneous image
    $$= \frac{\sum_{i=1}^{Ng} \sum_{j=1}^{Ng} (ij) p[i,j] - \mu_x \mu_y}{\sigma_x \sigma_y}$$

http://en.wikipedia.org/wiki/Image_texture#Co-occurrence_Matrices

Wirtz 2004

- Method: „normal“ non-rigid registration, using SSD and elastic regularization expanded for image series

Evaluation:

Magnitude of the distance measure: more similar ➔ lower measure

$$\mathcal{D}[R;u] \quad := \quad \frac{1}{2}\sum_{\nu=2}^{M}\int_{\Omega}\left(R^{(\nu)}\circ\varphi^{(\nu)} - R^{(\nu-1)}\circ\varphi^{(\nu-1)}\right)^2 dx$$

Ju (2006)

- Method: Calculate deformation fields, use binomiallly-weighted sum from *n* neighbors to correct section image

Evaluation:

Synthetic histology from MRI (artificial deformations)

- L_2-norm between original MR slice and distorted, and finally the unwarped slice (and sum over entire stack).
  Measures how close reconstructed volume is to real volume.

Ju (2006)

Real histology (same mouse brain data)

- Comparison to atlas images (Paxinos Atlas)
- Quantitative validation: 2-step procedure

1. Correspondence evaluation:
   l2-norm between successive images: measures quality of pairwise warps that establish correspondence between adjacent images

2. Smoothness (similar to CAM):
   Calculation of smoothness S_k on each section:
   how close does a point $A(i, j)$ lie to the mid-point of its two corresponding points on the neighboring sections

$$S_k = \frac{\sum_{i=0}^{n}\sum_{j=0}^{m}(B(i, j) + C(i, j) - 2A(i, j))^2}{nm}$$

Tan 2007 (ISBI)

- Method:
  Identify corresponding points, approximate by NURBS-curve, find new positions, and approximate affine transform

Evaluation:

- Comparison of adjacent slices. Error:

$$err = \frac{\sum_{i=1}^{n-1} \|I(i) - I(i+1)\|}{\sum_{i=1}^{n-1} \|I(i)\|}$$

„I(i) indicates the density distribution of the i-th slice“ = SSD??

Bagci 2010 (TMI)

- Method:
  Transform image to feature space, use features for registration

Evaluation:

- CAM: if point is perfectly aligned, it lies midway between its corresponding points on neighboring slices
- One CAM-value for each section
- Mean and standard-deviation of CAM values are measure of reconstruction quality

Bagci 2010 (TMI)

- Standard Deviation Map (SDM):
  2-D image template showing the spread of intensity values over the reconstructed volume (on the same pixel position!)

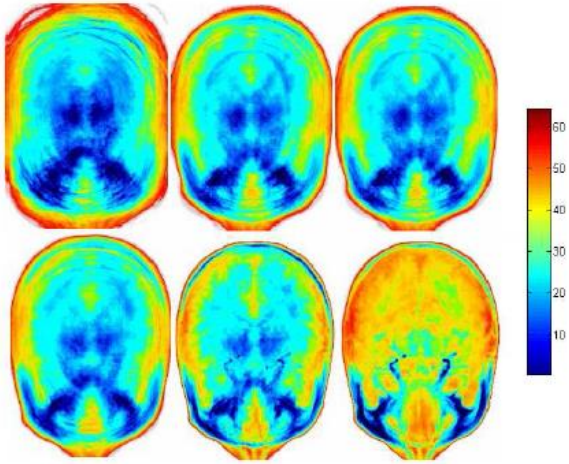


Fig. 10. SDMs for warped volumes spanning high-to-low levels of deformation.

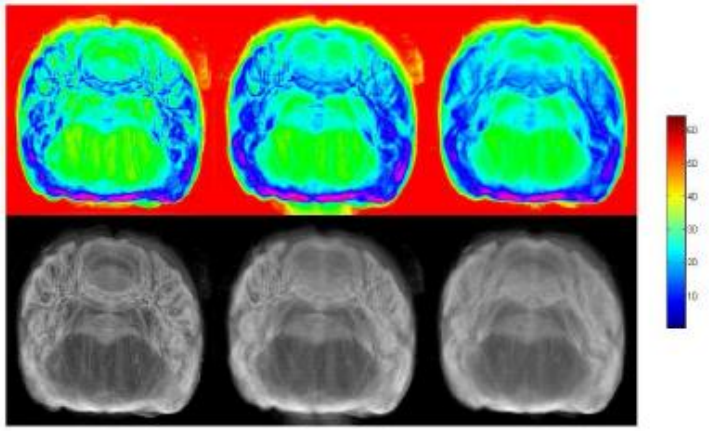
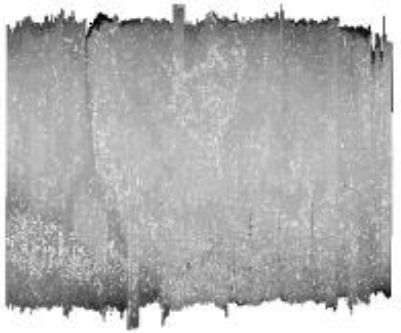


Fig. 11. SDMs for stacks reconstructed using rigid, affine, and LAGS registration methods.



Cifor 2011 (NeuroImage)

- Method:
  Extract boundaries of structure, evolve under min-max curvature flow, extract coarse to dense deformation fields

Evaluation:

Contrast as smoothness quantifier

- Contrast-GLCM around extracted surface rather than whole volume

$$f_2 = \sum_{i-j=0}^{N-1} (i-j)^2 \left(\sum_{i=0}^{N-1} \sum_{j=0}^{N-1} p(i,j) \right)$$

Outline

- 3-D Histology and Why go reference-free?
- Co-occurrence matrices
- Evaluation strategies
  - Guest and Baldock (1995)
  - Baheerathan (1998)
  - Wirtz (2004)
  - Ju (2006)
  - Tan (2007)
  - Bagci (2010)
  - Cifor (2011)

- **What to do?**
- Remaining problem

What to do?

- Use smoothness (→ CAM measure seems useful)
- Use convergence over iterations

To prevent improvement of measure with stronger banana-effect:

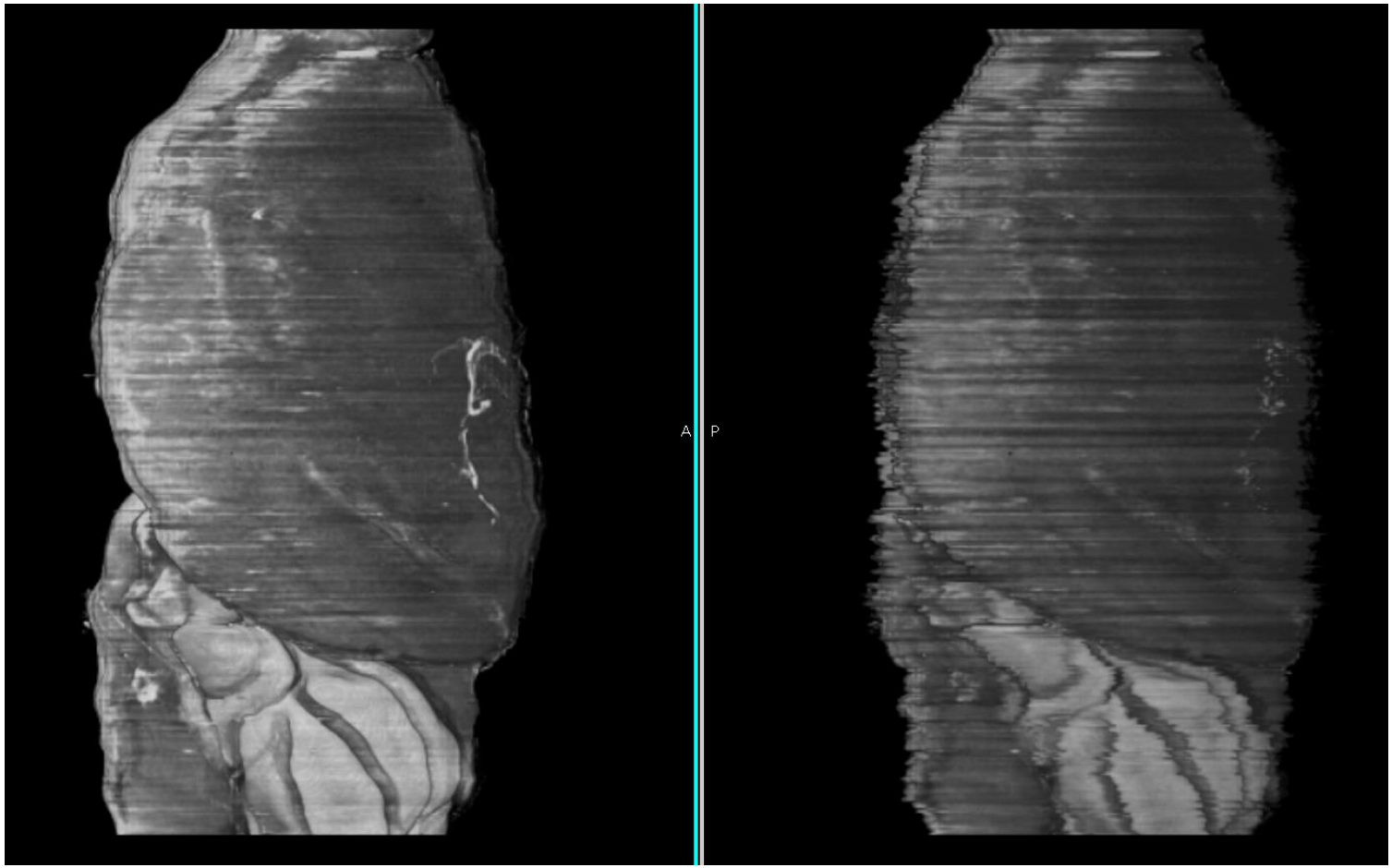
- Weight smoothness with global deformation

Outline

- 3-D Histology and Why go reference-free?
- Co-occurrence matrices
- Evaluation strategies
  - Guest and Baldock (1995)
  - Baheerathan (1998)
  - Wirtz (2004)
  - Ju (2006)
  - Tan (2007)
  - Bagci (2010)
  - Cifor (2011)
- What to do?
- **Remaining problem**

Remaining problem: global alignment

- Rigid registration has to provide true global shape



Thank you for listening!

FRIEDRICH-ALEXANDER
UNIVERSITÄT
ERLANGEN-NÜRNBERG

TECHNISCHE FAKULTÄT