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08

Characteristic Quantities of Microvascular Structures in CLSM Volume Datasets

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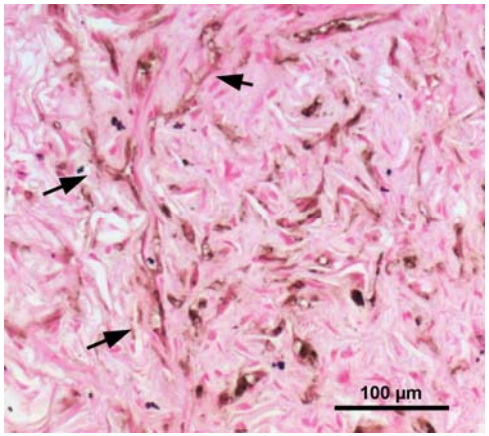
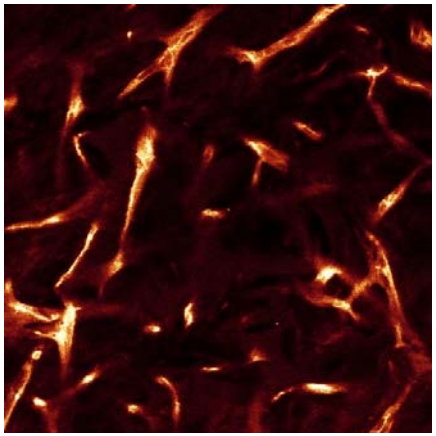
Background

- Models for “microvascular engineering” in vitro
  - Long term goals
    - Integration of a supplying vessel construct (“feeder donor vessel”)
    - Functional microvascular networks
  - Short term goals
    - Models, imaging, quantification
    - Functional analysis (ESR, oxygenation, pH, etc.)



Histologic section, CD31 (DAB, brown)



Confocal laser scanning microscopy (CLSM), UEA-TRITC

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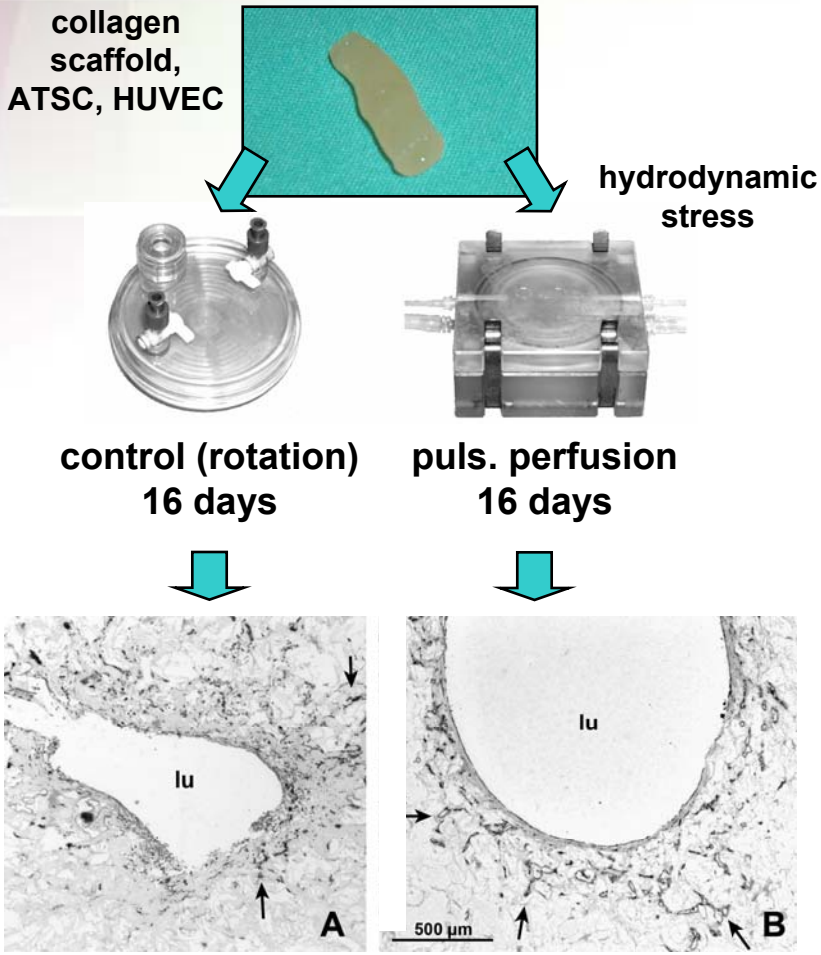
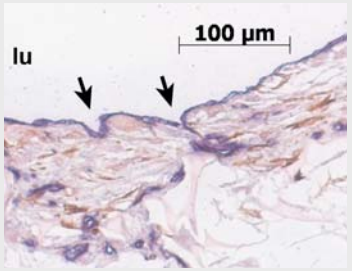
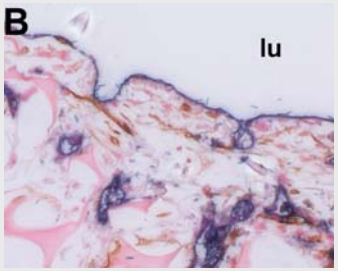
Background

- 3D in vitro vessel model with capillary structures

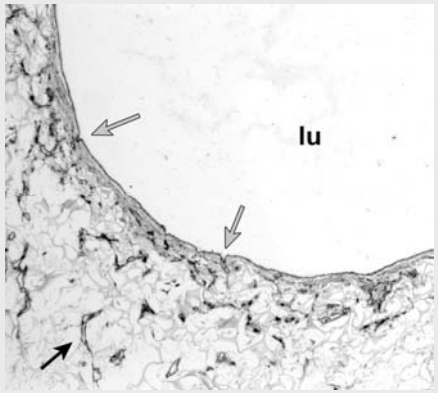


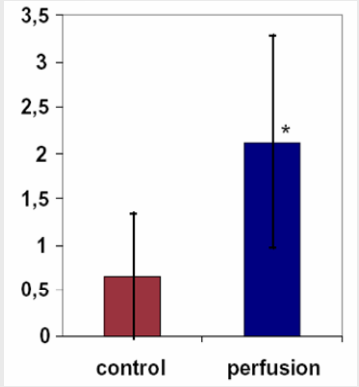
branches from central lumen





CD31 (endothelial cells, blue)
α-actin (perivascular cells, DAB, brown)

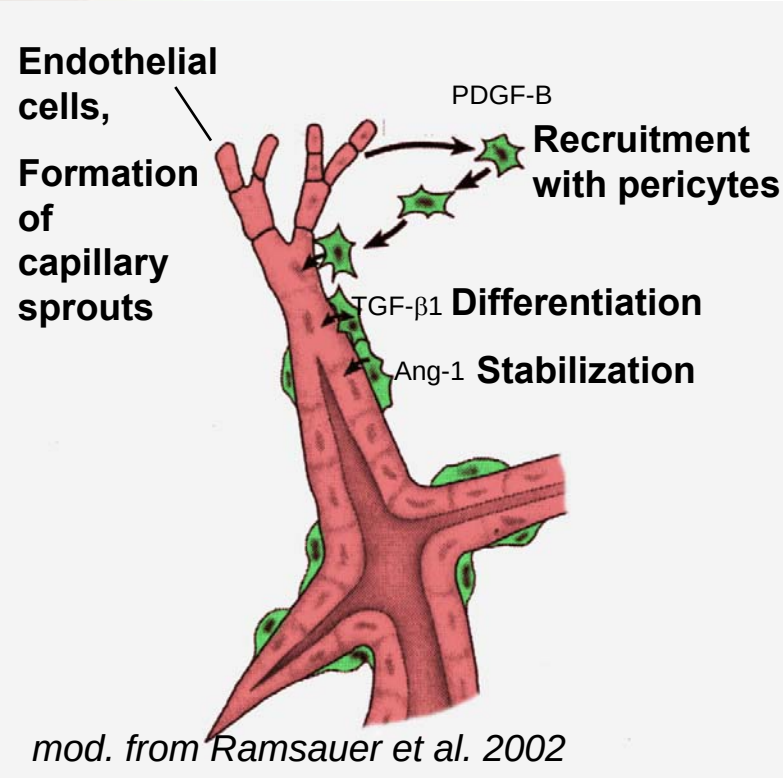




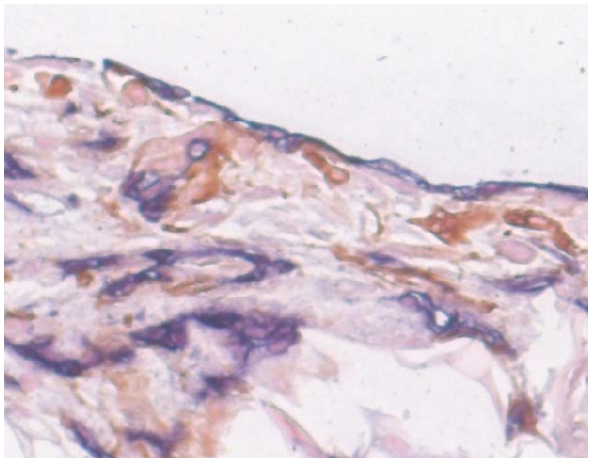
B. Frerich, K. Zückmantel, A. Hemprich Microvascular engineering in perfusion culture. *Head Face Med, 2006; 2(1):26*

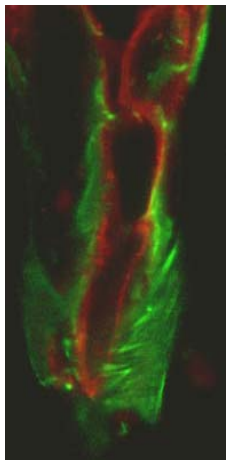
Background

- Stabilization and maturation of newly formed capillaries



mod. from Ramsauer et al. 2002





Morphological parameters, e.g.

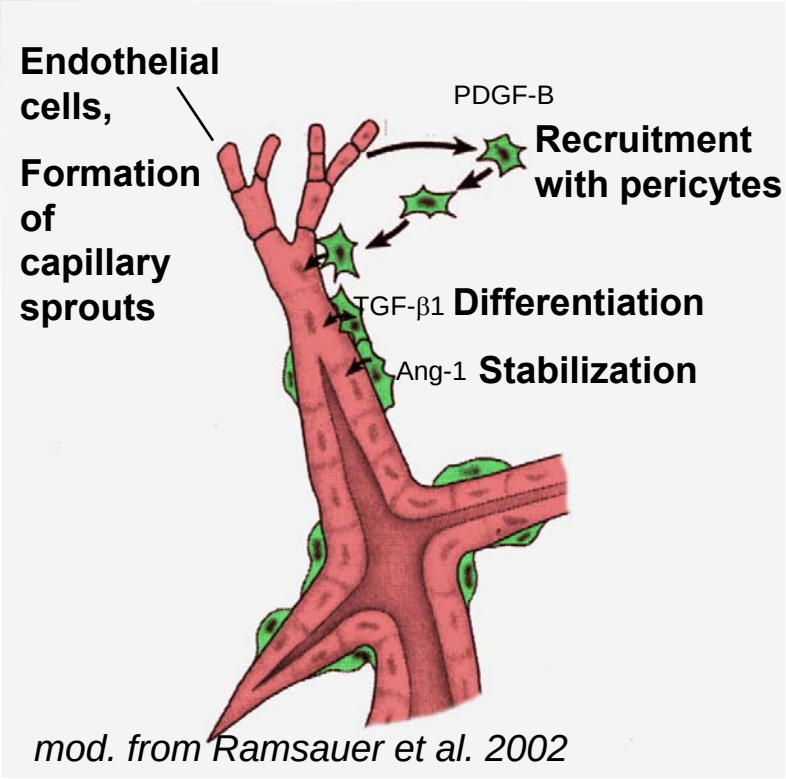
- Recruitment with α-actin-positive cells
- Length, information about microvascular networks

➔ Histomorphometry

➔ Image analysis of CLSM-data

Background

- Stabilization and maturation of newly formed capillaries



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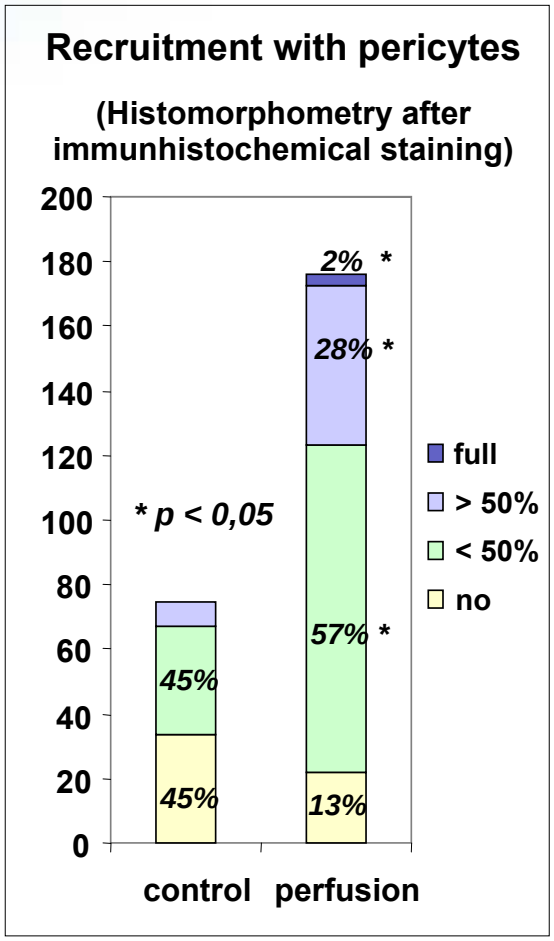
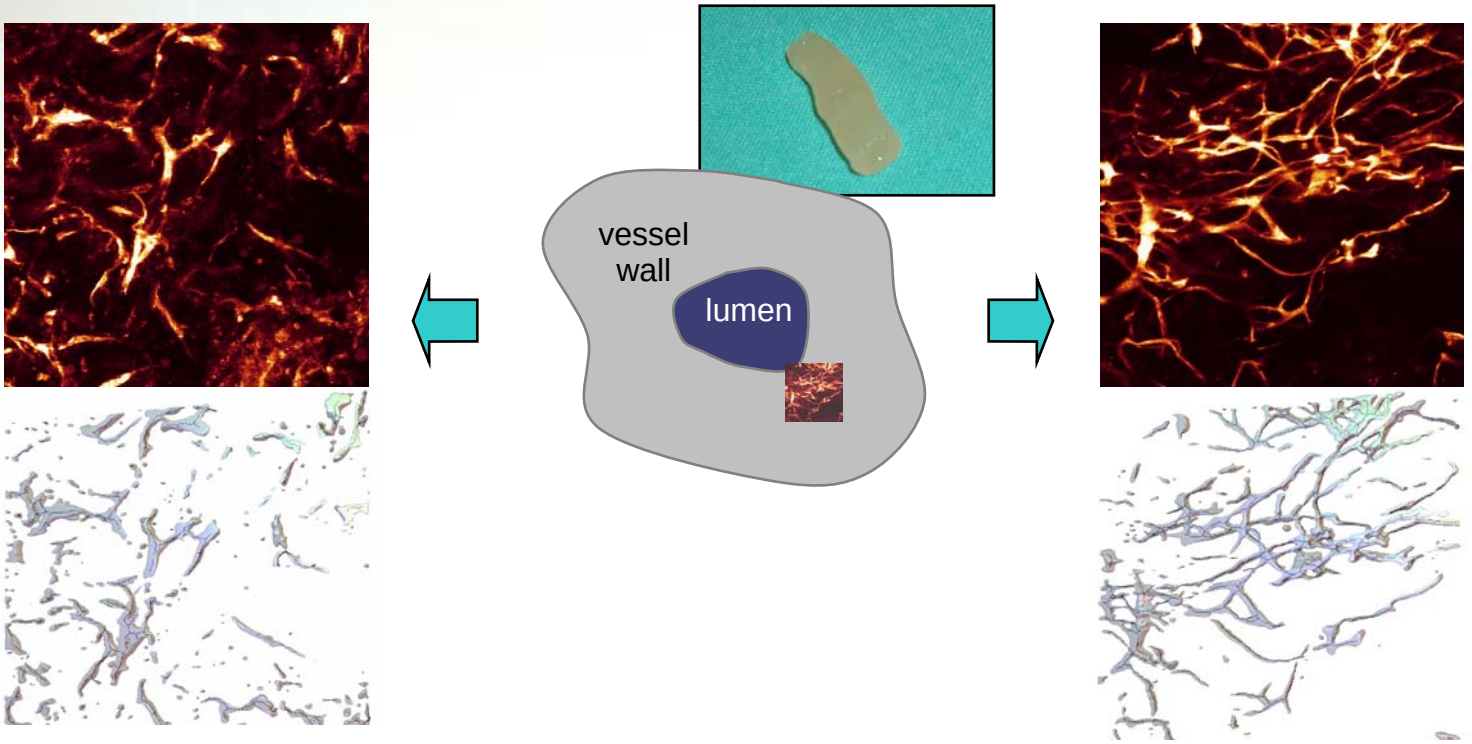


B. Frerich, K. Zückmantel, S. Müller, A. Hemprich
Maturation of capillary-like structures in a tube-like construct in perfusion and rotation culture.
Int J Oral Maxillofac Surg, accepted and in press

3D non-destructive imaging with CLSM

- Influence of hydrodynamic stress on vessel formation



control (rotation)
(low mechanic stress)

perfusion
(high mechanic stress)

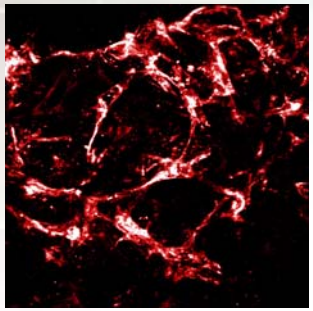
- Need for comprehensive quantification

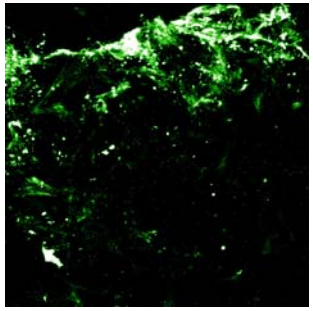
Quantification

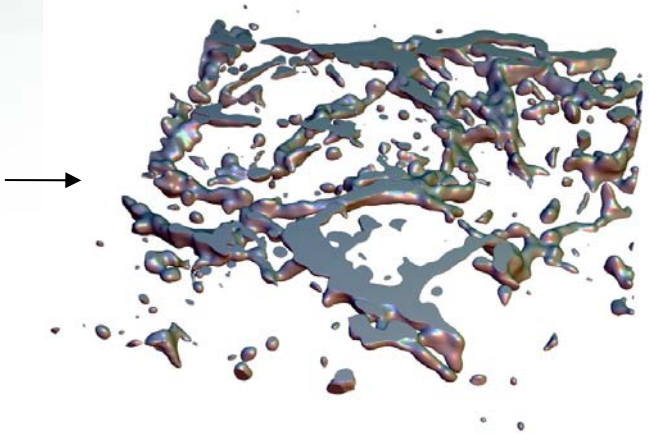
- Method for fully automated morphological and topological analysis of microvascular structures
  - Calculation of several “characteristic quantities” for characterization and comparison of microvascular networks
  - Degree of vessel maturation and stability, recruitment with perivascular cells
  - Extracted c.q. provide information for advanced tissue engineering, in vitro angiogenesis and vessel formation of metabolically active tissues

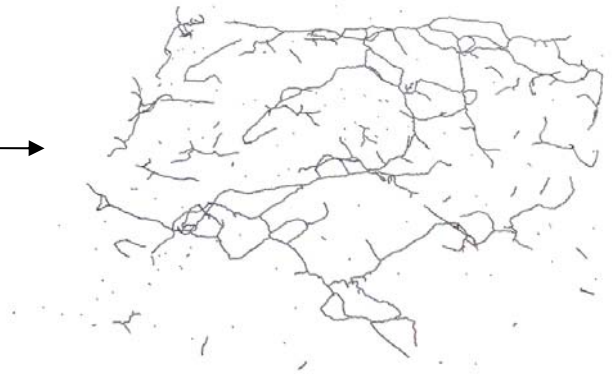
Quantification

- Step-by-step quantification of CLSM datasets









Reifungsparameter des analysierten Endotheldatensatzes:

betrachtetes Gewebevolumen	0.03929794413 mm^3	angewandter Schwellwert	4.5
Objektkomponenten	138	Bedeckungsgrad	26.43%
Hintergrundkomponenten vor dem Füllen	2	Linienzüge im Skelett	523
von Endothelzellen belegtes Gewebevolumen	12.07%	Endpunkte im Skelett	408
Gesamtoberfläche der Komponenten	813229.48 μm^2	Kreuzungspunkte im Skelett	211
Gesamtvolumen der Komponenten	4742758.99 μm^3	3er/4er/5er Kreuzungen	206/5/0
ø gewichtete Kompaktheit der Komponenten	0.074	Gesamtlänge des Skelettes	12031.87 μm

Daten der zehn größten Komponenten des Endotheldatensatzes:

Voxelanzahl	Volumen in μm^3	Fläche in μm^2	Kompaktheit	Gesamtlänge in μm	Linien	Endpunkte	Kreuzungen	3er	4er
938326	3469706.06	544557.96	0.0084	8835.23	302	102	166	162	4
69386	254327.19	35701.47	0.1608	537.03	35	18	17	16	1
56487	208179.71	36600.85	0.1	584.31	21	9	11	11	0
48270	179126.11	24548.48	0.2453	195.25	5	4	2	2	0
42112	155727.83	24428.81	0.1881	230.49	5	4	2	2	0
13504	50668.29	9196.85	0.3733	94.39	3	3	1	1	0
12033	43649.36	12484.98	0.1107	219.46	3	3	1	1	0
11714	41914.83	11980.93	0.1155	210.33	7	5	3	3	0
7219	26820.68	6467.85	0.3007	69.13	5	4	2	2	0
7503	26028.96	8044.54	0.1472	151.93	5	4	2	2	0

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Quantification

- Series of image processing steps for fully automatic image analysis and extraction of characteristic quantities from CLSM datasets
- Visualization of endothelial structures

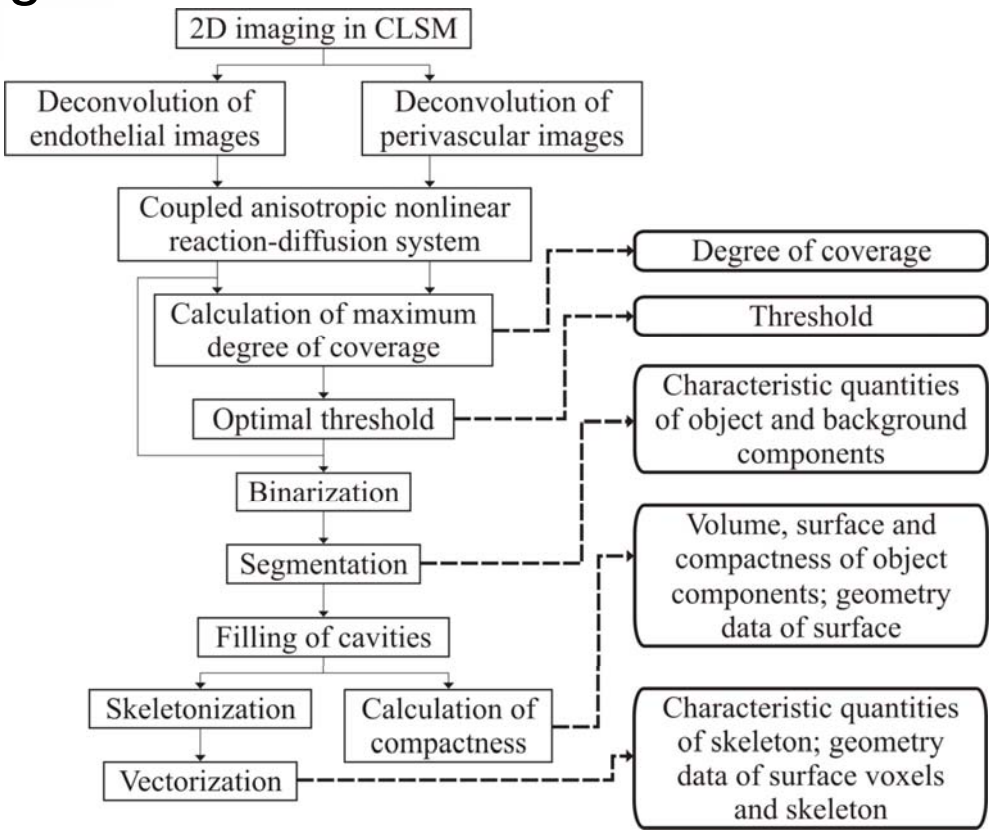


Image preprocessing - Deconvolution

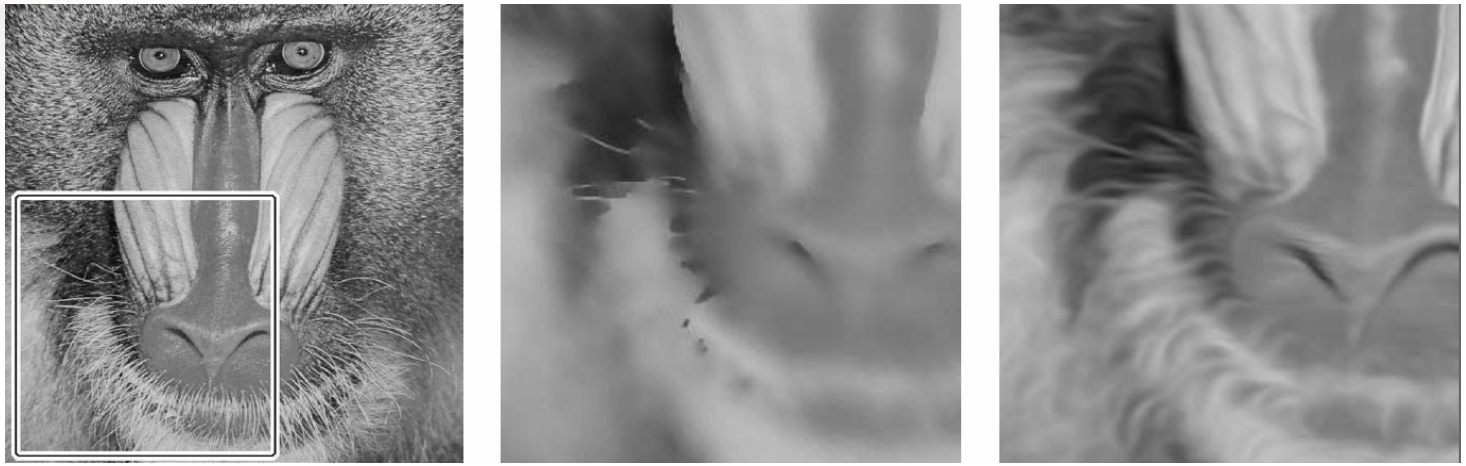
- Image quality suffers from optical aberration, a wide range of noise sources (detector noise, laser noise, shot noise of the light) and shading effects
- Mathematical interpretation: convolution of the source signal (actual image) with an interfering signal (PSF of the CLSM)

$$B\left(\vec{r}\right) = O(\vec{r}) \otimes PSF(\vec{r})$$

- Restoration of the original image by deconvolution
- Implementation of the Richardson-Lucy deconvolution algorithm

Image preprocessing - Coupled anisotropic nonlinear reaction-diffusion system

- Removes noise from datasets and strengthens thin endothelial and perivascular structures
- Preservation of edges since diffusion occurs perpendicularly to grayscale gradients

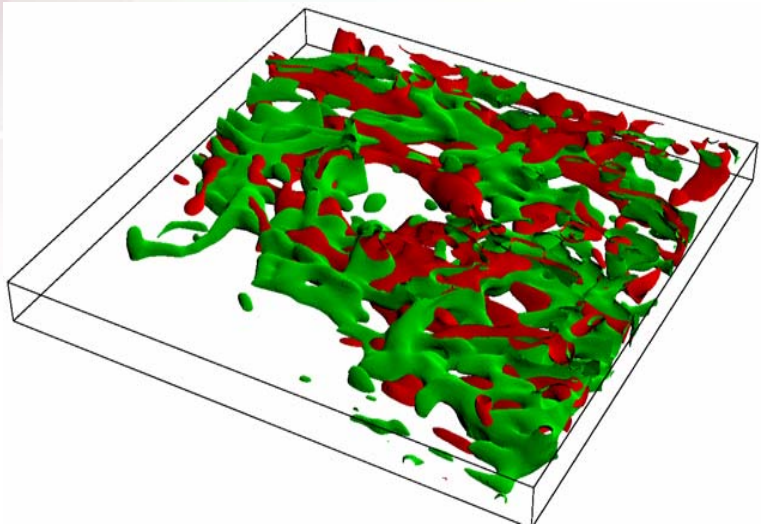


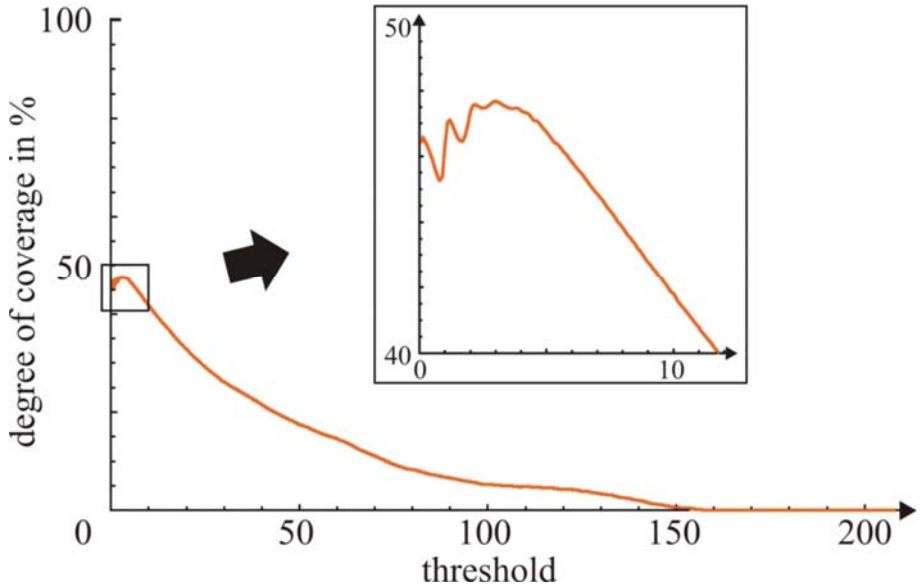
isotropic (middle) vs. anisotropic (right) nonlinear diffusion

- Spatial separation of endothelial and perivascular structures by means of a catalyzed decomposition instead of a simple masking operation

Image analysis – Recruitment with perivascular cells

- Computation of the real contact surface of endothelial and perivascular structures by using a variable threshold





- Maximum degree of coverage corresponds to the optimum threshold for subsequent segmentation of the endothelial dataset

Image analysis – Compactness

- Important characteristic morphological quantity
- Computation of surface and volume from segmented data with a modified Marching Tetrahedron algorithm

$$C = \frac{36\pi V^2}{S^3}$$

$$C_\emptyset = \sum_{n=1}^{\#OC} \frac{C_n \times V_n}{V_{tot}}$$

- Triangulation of the threshold depending iso-surface provides data for visualization

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Image analysis – Compactness

- Some synthetic objects and their compactness

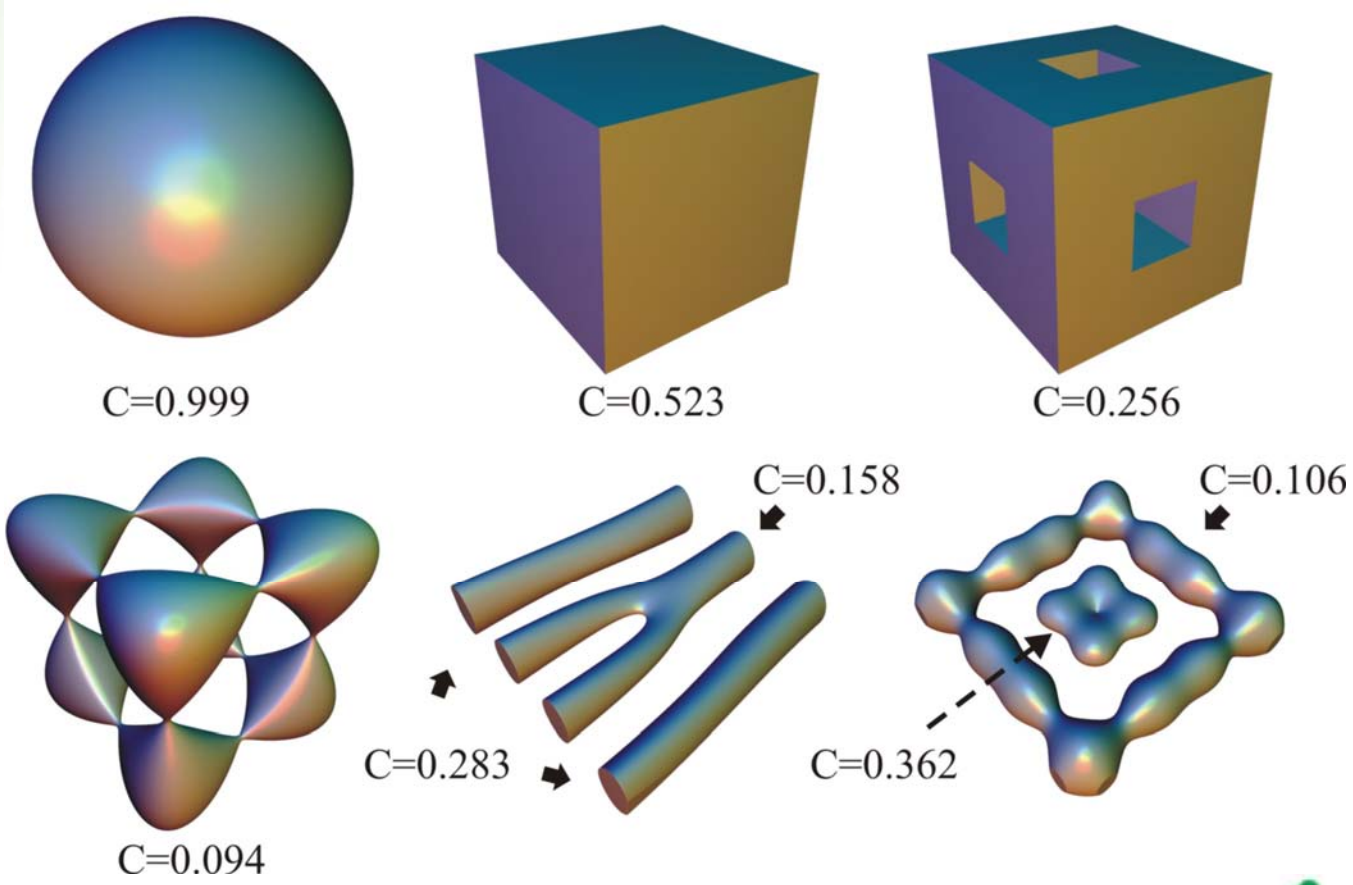

Image analysis – Skeletonization and vectorization

- Development of an anisotropic skeletonization algorithm for segmented endothelial data, location of medial axes
- Computation of length and identification of junction / line end points of the skeleton
- Analysis of connectivity and branching
- Important characteristic topological quantities

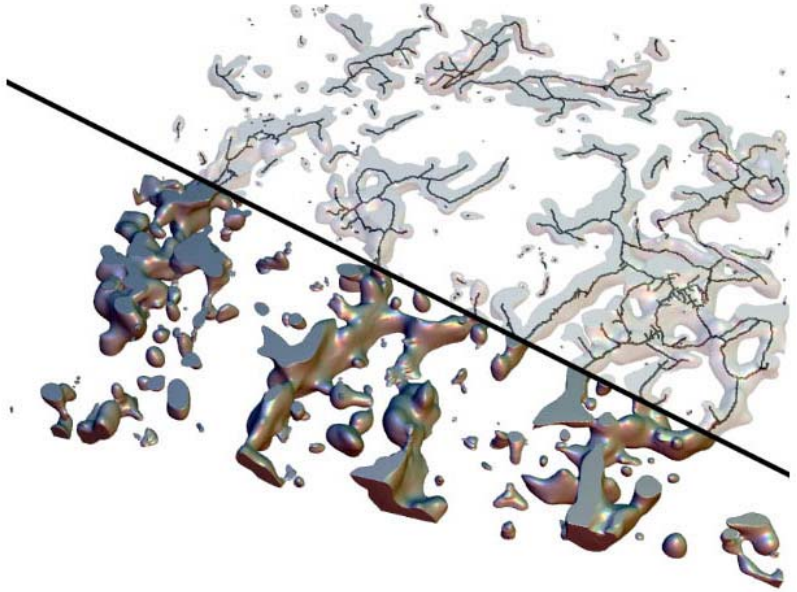

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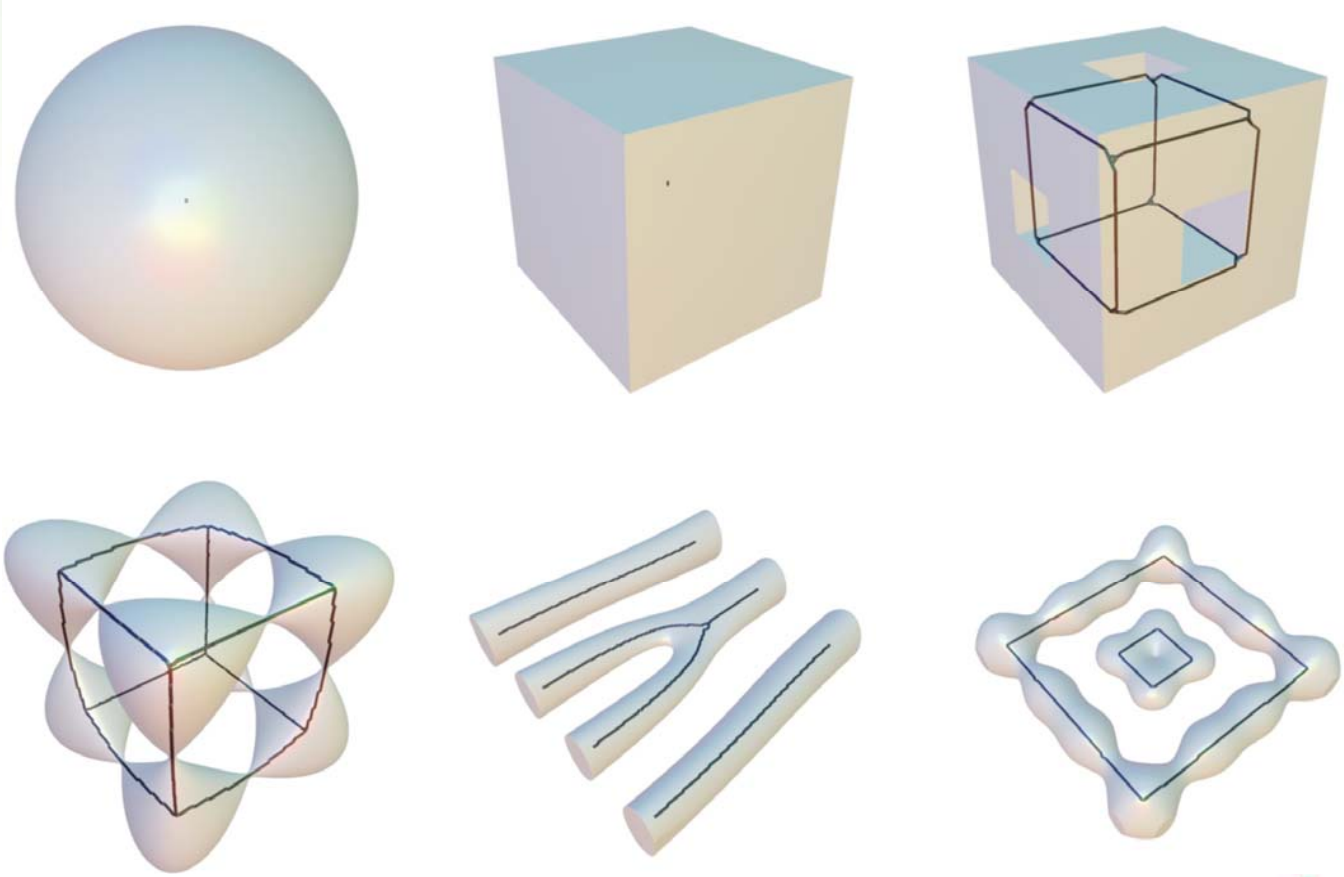
Image analysis – Skeletonization and vectorization

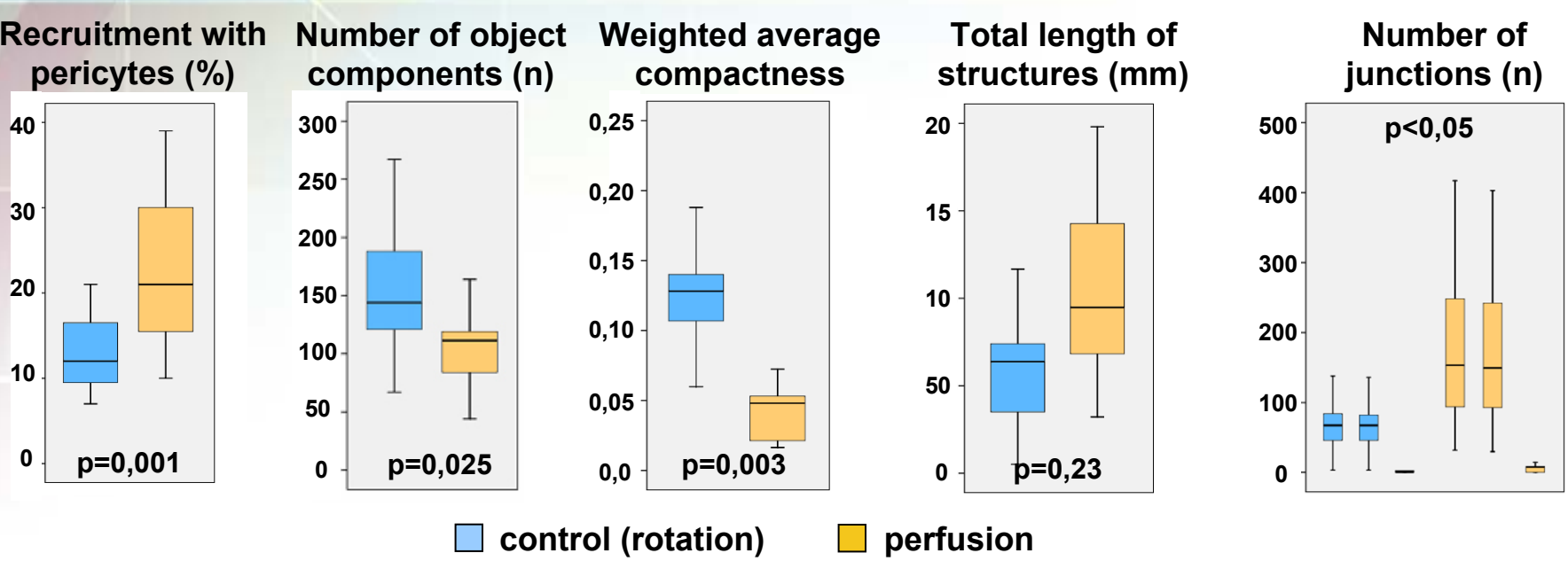
- Some synthetic objects and their skeleton



Characteristic quantities

Symbol	Description
%DC	Maximum degree of coverage
θ	Threshold
#OC	Number of object components
#BC	Number of background components
V	Volume of a single component
V_{tot}	Volume of all components
S	Surface of a single component
S_{tot}	Surface of all components
C	Compactness of a single component
$C_{\emptyset}$	Average weighted compactness of all components
#LEV	Number of line end voxels of a single component
$\#LEV_{tot}$	Number of line end voxels of all components
#JV	Number of junction voxels of a single component
$\#JV_{tot}$	Number of junction voxels of all components
#LS	Number of line segments of a single component
$\#LS_{tot}$	Number of line segments of all components
L	Length of a skeletonized component
L_{tot}	Length of all skeletonized components

Results



characteristic quantity	p-value	characteristic quantity	p-value
%DC	**0.001**	L_{tot}	0.230
θ	0.679	$\#LS_{tot}$	0.311
#OC	**0.025**	$\#LEV_{tot}$	0.200
#BC	0.830	$\#JV_{tot}$	**0.034**
V_{tot}	0.936	$\#JV_3$	**0.041**
S_{tot}	0.522	$\#JV_4$	**0.033**
$C_{\emptyset}$	**0,003**		

K. Winter, L. Metz, J.-P. Kuska, B. Frerich Characteristic Quantities of Microvascular Structures in CLSM Volume Data Sets. IEEE Trans Med Imaging 2007, 26:1103-14

Conclusion

- Method for analysis and visualization of microvascular structures in CLSM volume datasets
- Algorithms are universal, they can be used for quantification of other structures and networks from different modalities (i.e. macrovascular structures, neurites, airways, etc.)
- Extracted characteristic quantities are transferable and can be used to analyze multimodal volumetric datasets
- Also allow comparison of arbitrary structures to each other

Acknowledgements

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Thanks for your attention!

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