

# Stereologické hodnocení tkání a biomateriálů s vláknitou a trámčitou stavbou – překryv mezi optickou mikroskopií a mikro-CT

**Zbyněk Tonar, Miroslav Jiřík, Tereza Kubíková, Martin Bartoš, Jana Horáková,  
David Lukáš, Tomáš Suchý, Pavla Sauerová, Marie Hubálek Kalbáčová**

Ústav histologie a embryologie a Laboratoř kvantitativní histologie Biomedicínského centra  
Lékařská fakulta Univerzity Karlovy v Plzni

# Spolupráce a inspirace:

- **Technická univerzita v Liberci**
  - Katedra netkaných textilií a nanovláknenných materiálů
- **Ústav struktury a mechaniky hornin AV ČR, v.v.i.**
  - Oddělení kompozitních a uhlíkových materiálů
- **Ústav experimentální medicíny AV ČR, v.v.i**
  - Oddělení tkáňového inženýrství
- **1. lékařská fakulta Univerzity Karlovy v Praze a Všeobecná fakultní nemocnice v Praze**
  - Stomatologická klinika
- **Biomedicínské centrum, Lékařská fakulta Univerzity Karlovy v Plzni**
  - Laboratoř studia interakcí buněk s materiálem
  - Laboratoř nádorové léčby a regenerace tkáně

*projekty:*  
*AZV 15-25813A*  
*NPU LO10503*

# Odkud pocházíme a kde pracujeme

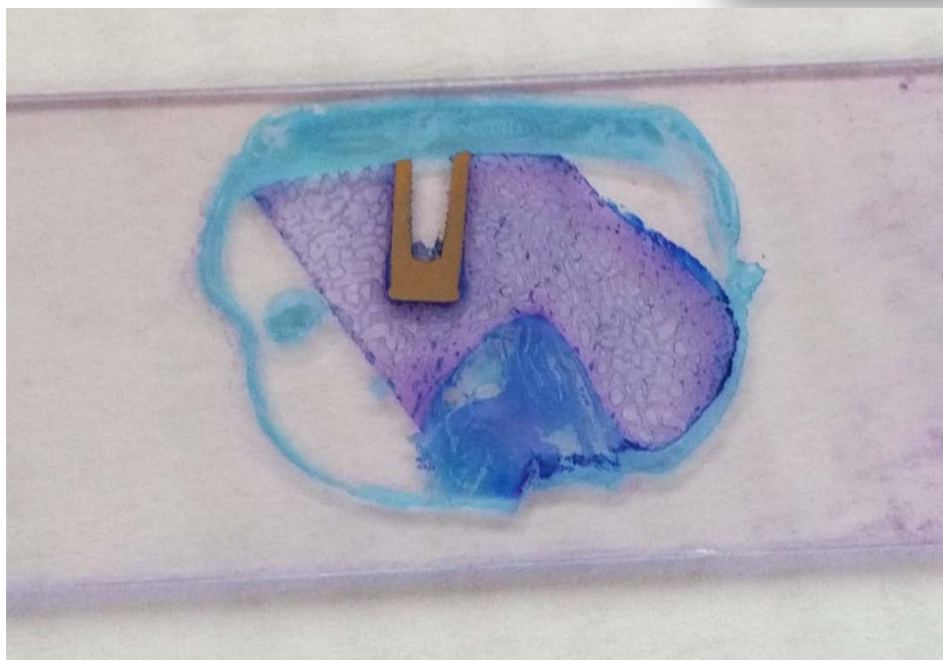
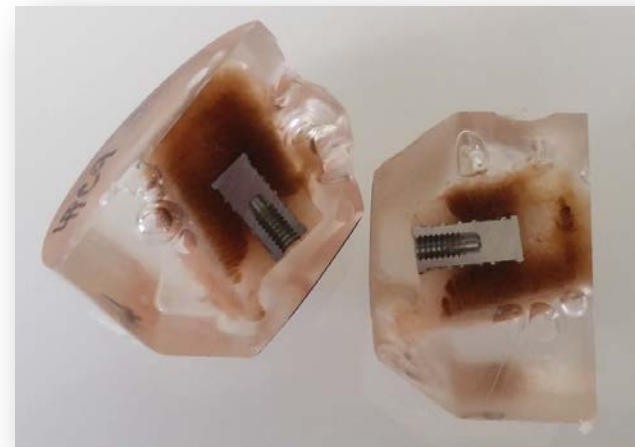
Ústav histologie a embryologie a Biomedicínské centrum LF UK v  
Plzni

Laboratoř kvantitativní histologie



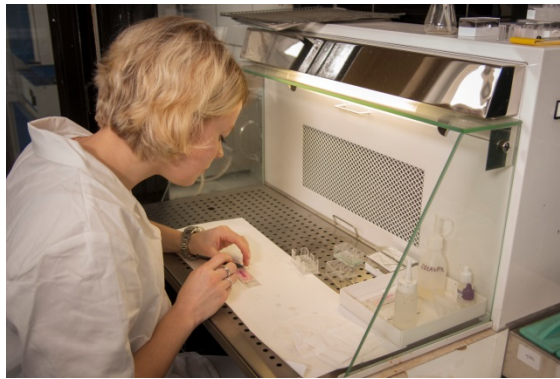


# Zpracování tvrdých tkání





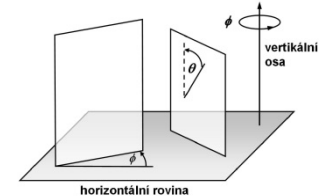
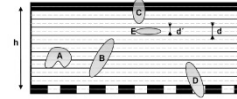
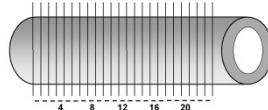
# Měkké tkáně - parafinové řezy, imunohistochemie



# Základní strategie: Unbiased stereology

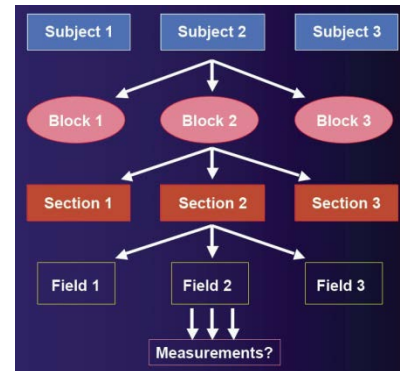
Randomizace **orientace** a **víceúrovňové systematické** a **nestranné vzorkování** při

- **odběru** orgánů a bločků
- **výběru** histologických řezů a mikroskopických zorných polí



**Složení vzorků** je vizualizováno

- přehlednými metodami
- imunohistochemicky



→ Popis složení  
vzorků pomocí  
**spojitých**  
**kvantitativních**  
**proměnných**

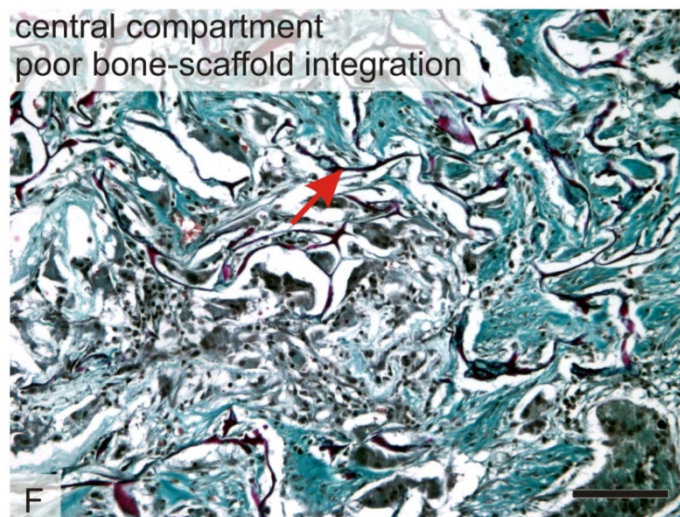
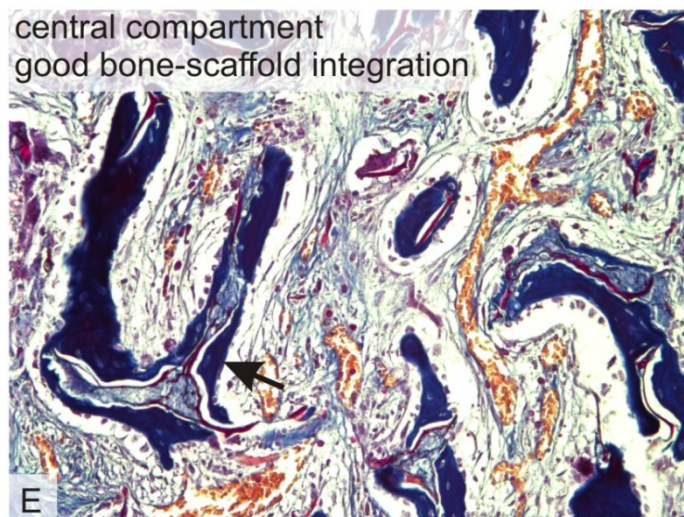
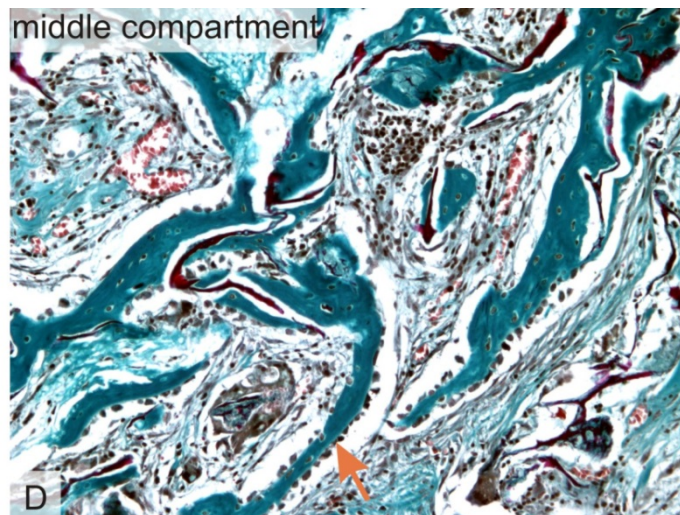
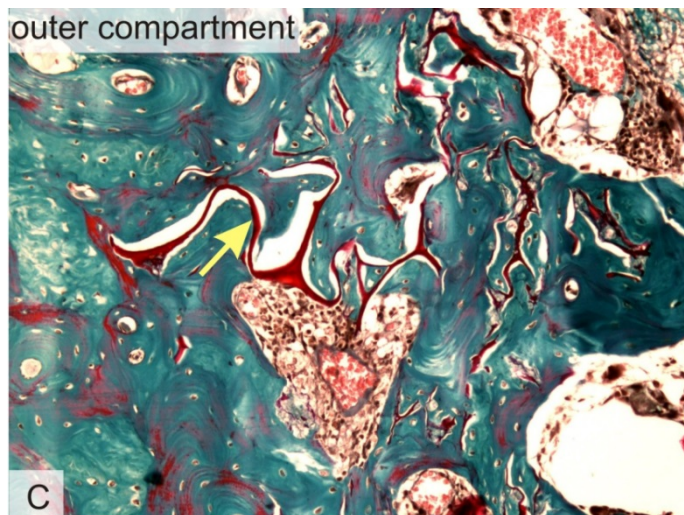
**Stereologie:** Popis 3D objektů na základě studia 2D řezů

- počítání mikroskopických objektů (disektor, frakcionátor)
- odhady a měření délek, ploch, objemů
- hodnocení hustoty mikrocév
- plošné a objemové podíly vícefázových struktur
- tortuozita vláknitých struktur
- shlukování objektů

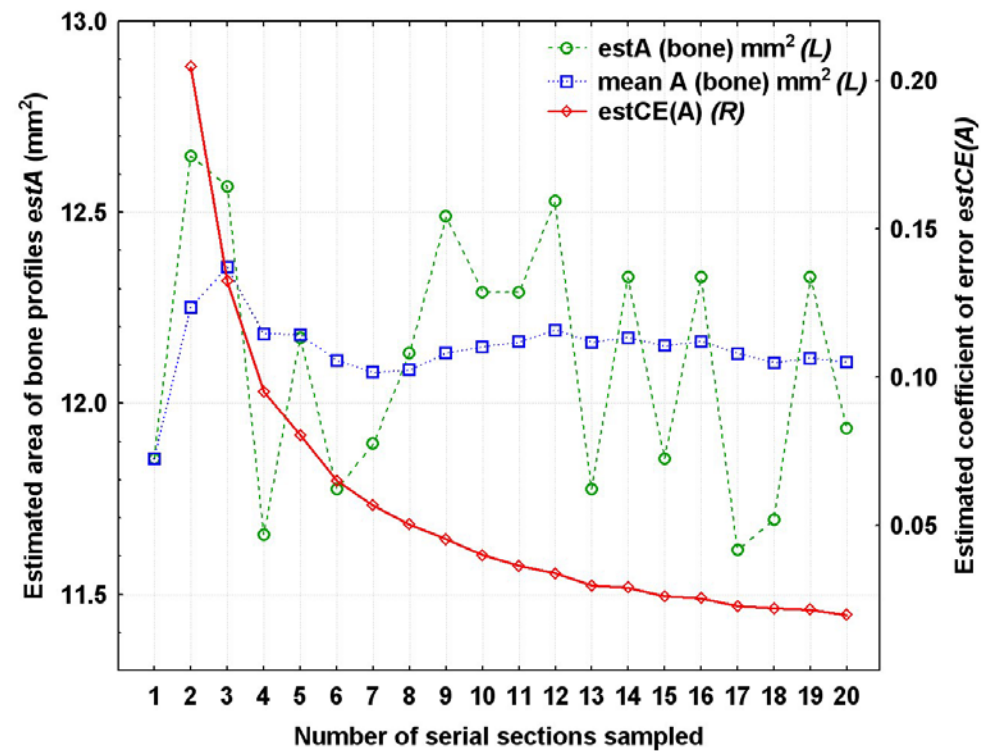
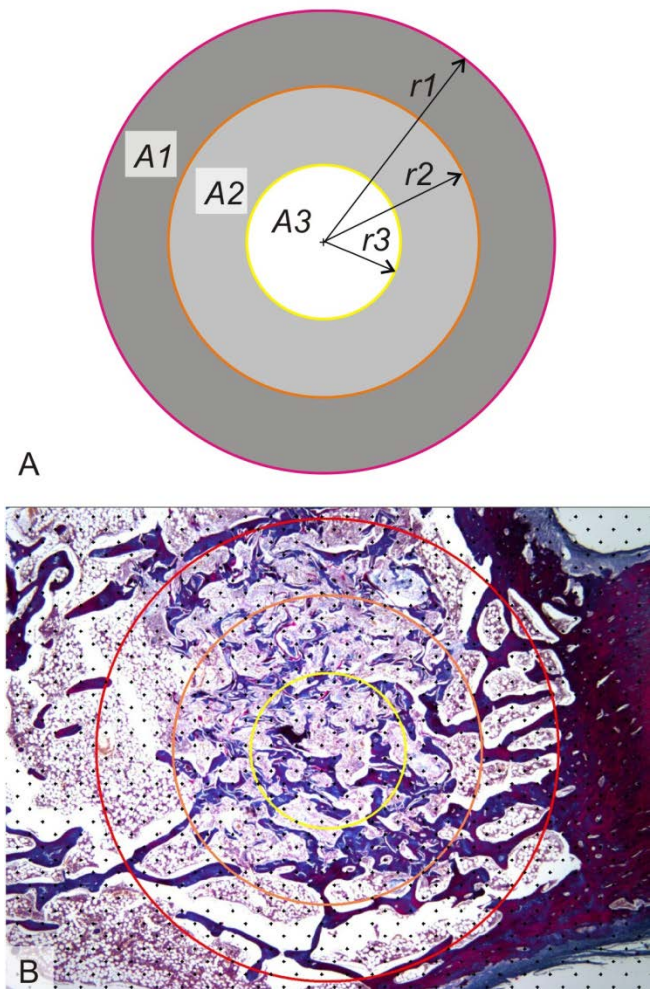


# Příklady řešených výzkumných otázek

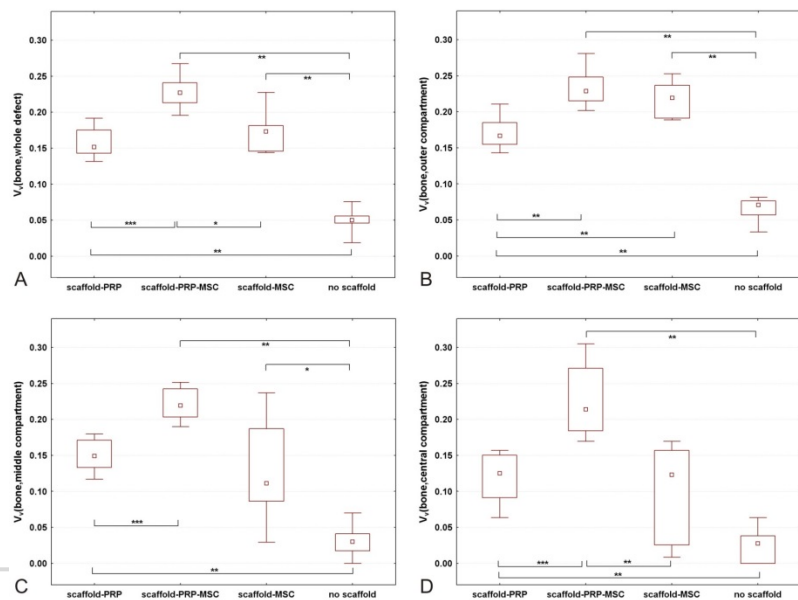
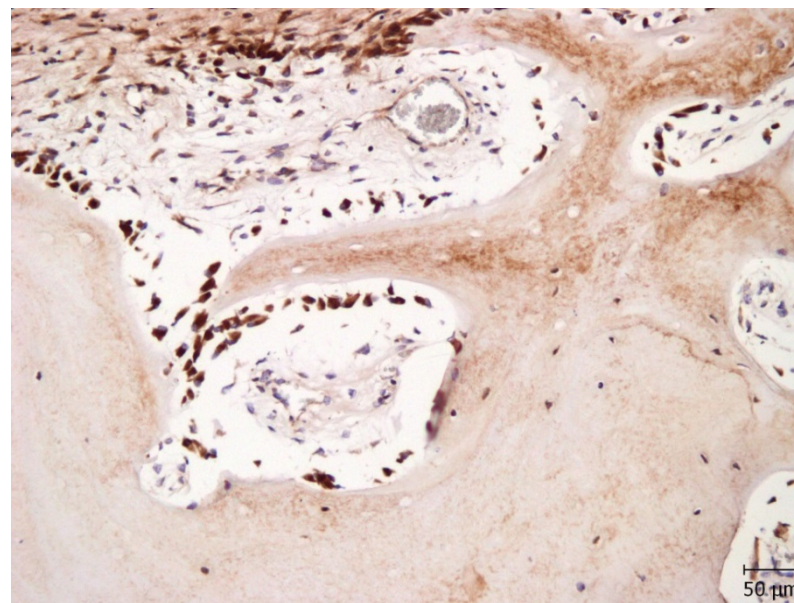
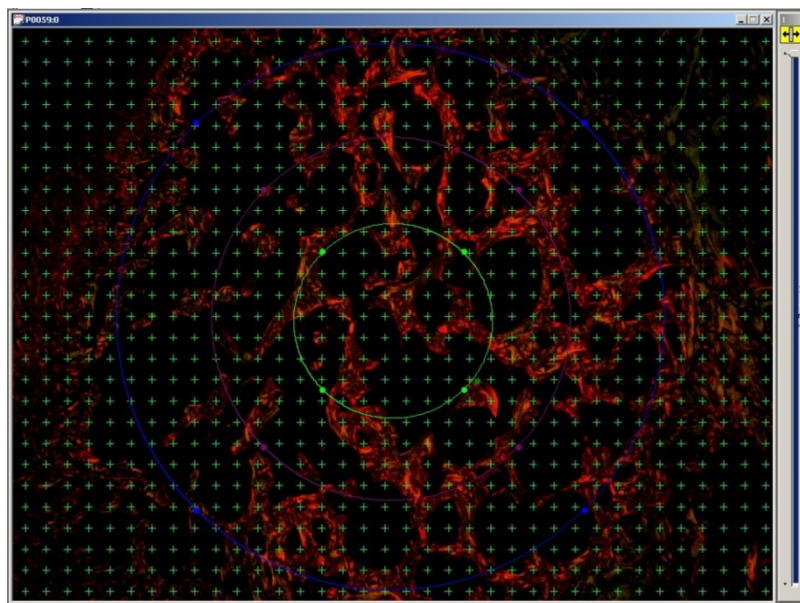
## Objem a rovnoměrnost distribuce novotvořené kosti v experimentálním defektu







Parameter	Compartment		
	Outer	Middle	Central
Circle diameter (mm)	6	4	2
Area per section (mm <sup>2</sup> )	15.71	9.42	3.14
Area label	A1	A2	A3
Volume in 4 s (mm <sup>3</sup> )	3.142	1.884	0.628
Volume fraction	0.552	0.331	0.114



## Collagen/hydroxyapatite scaffold enriched with polycaprolactone nanofibers, thrombocyte-rich solution and mesenchymal stem cells promotes regeneration in large bone defect in vivo

E. Prosecká,<sup>1,2,3</sup> M. Rampichová,<sup>2,4</sup> A. Litvinec,<sup>2</sup> Z. Tonar,<sup>5</sup> M. Králíčková,<sup>5</sup> L. Vojtová,<sup>6</sup> P. Kochová,<sup>7</sup> M. Plencner,<sup>1,2</sup> M. Buzgo,<sup>1,2,4</sup> A. Mičková,<sup>1,2,4</sup> J. Jančár,<sup>6,8</sup> E. Amler,<sup>1,2,9</sup>

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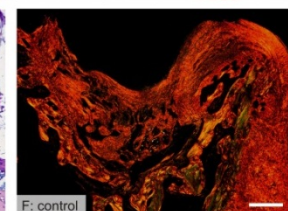
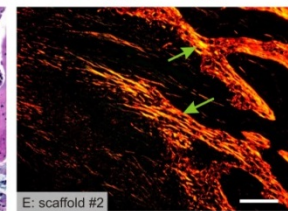
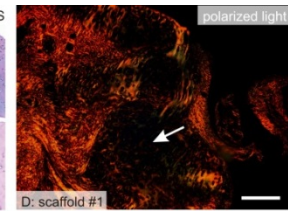
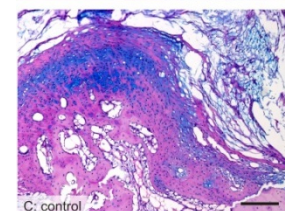
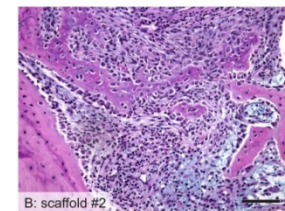
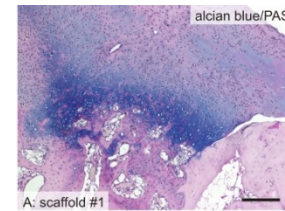
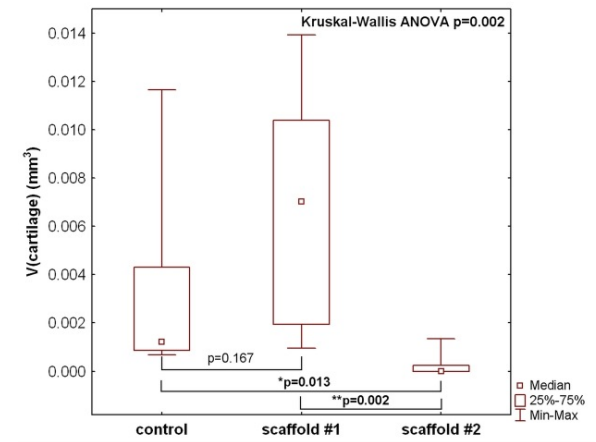
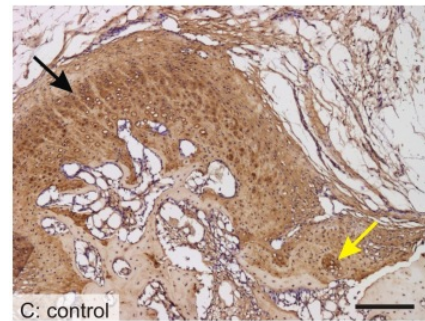
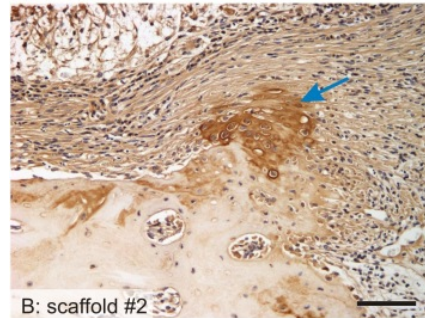
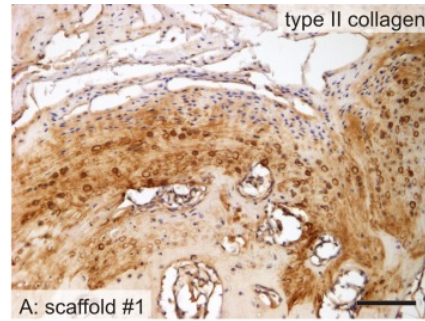
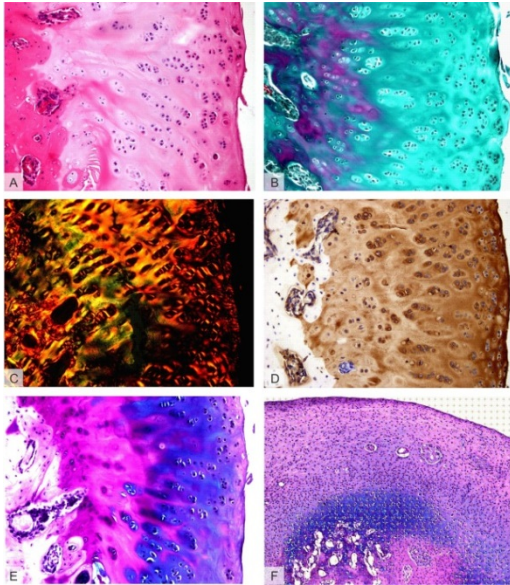
<sup>7</sup>European Centre of Excellence-New Technologies for Information Society, Faculty of Applied Sciences, University of West Bohemia, Univerzitní 22, 306 14 Pilsen, Czech Republic

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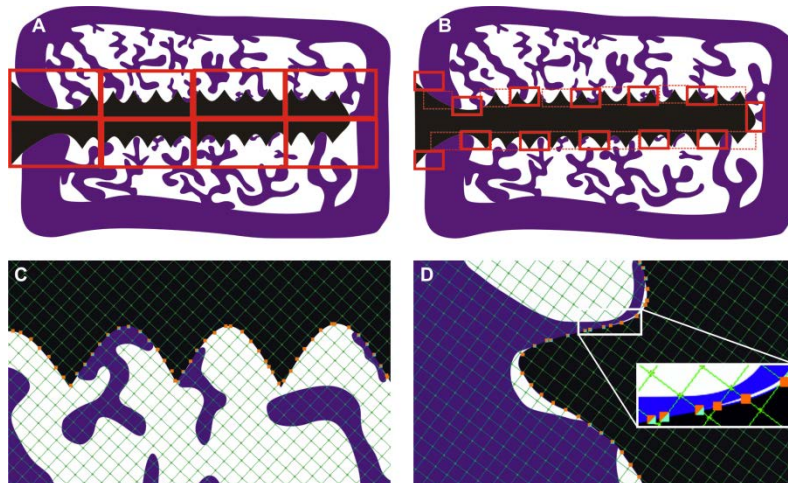
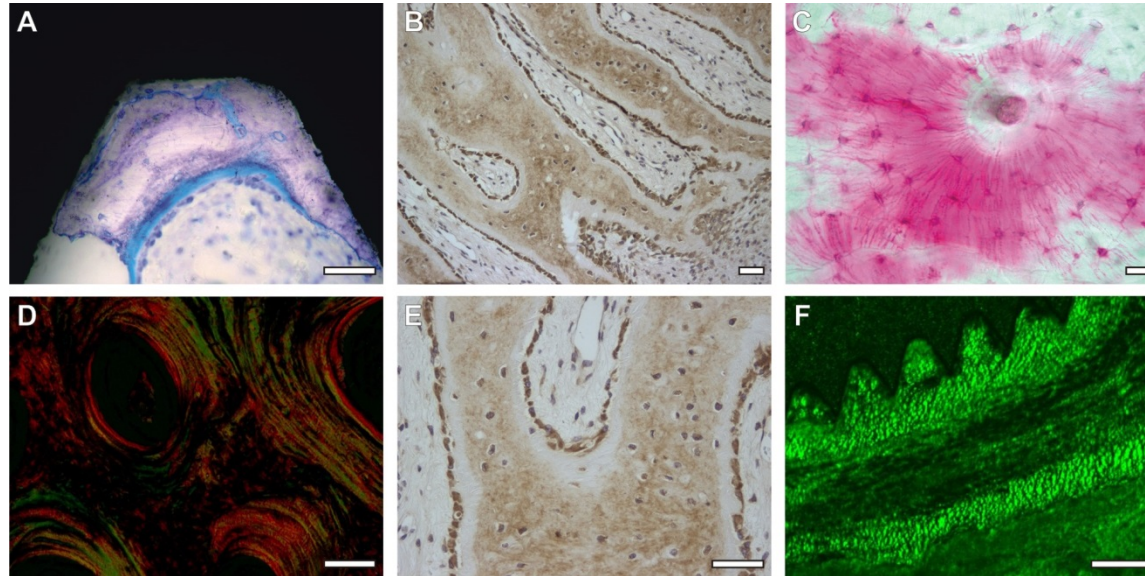


# Diferenciace hyalinní chrupavky v osteochondrálním defektu





# Oseointegrace nanostrukturovaných titanových implantátů u zvířecích modelů



## Evaluating the osseointegration of nanostructured titanium implants in animal models: Current experimental methods and perspectives (Review)

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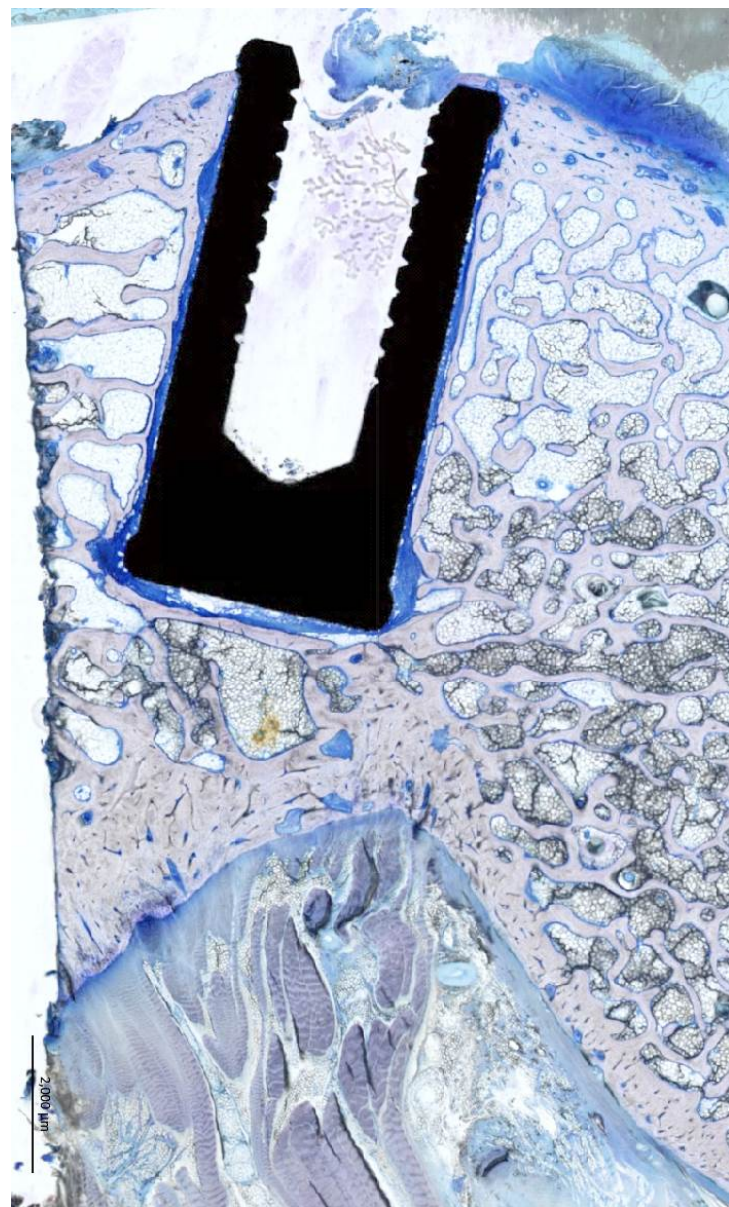
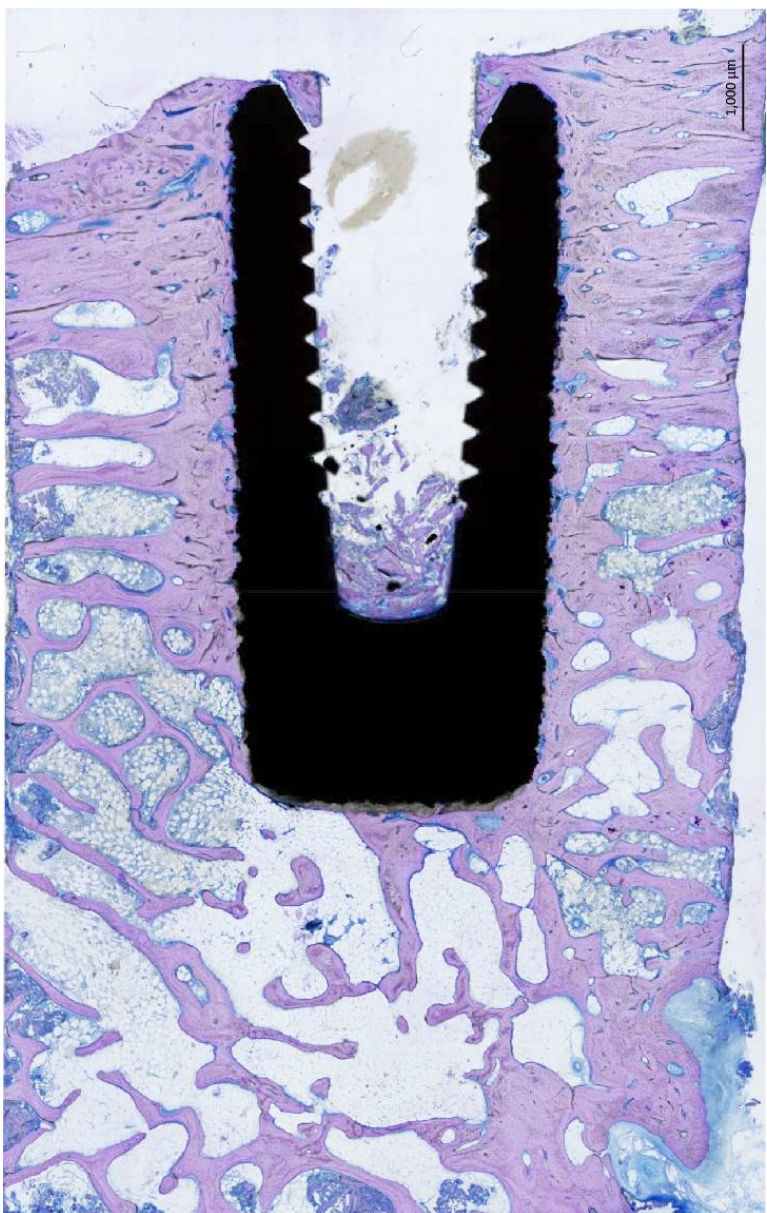
Daniel Hrusak

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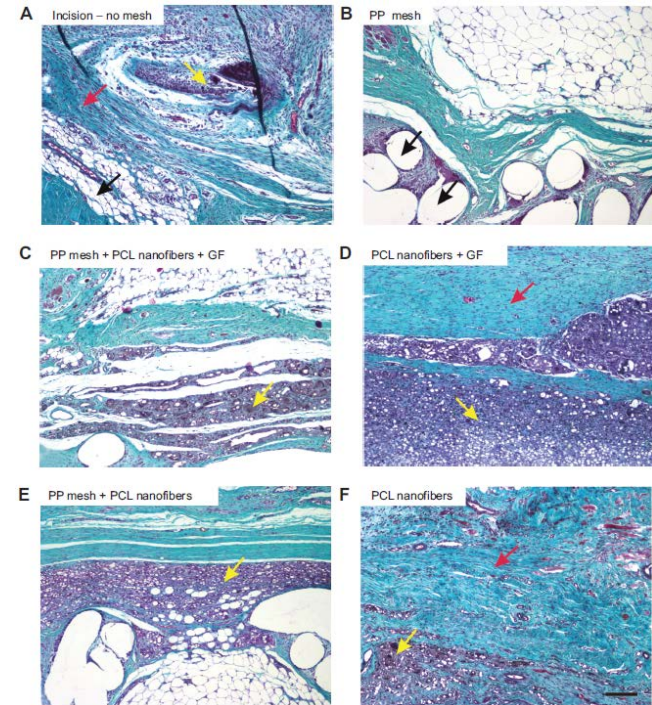
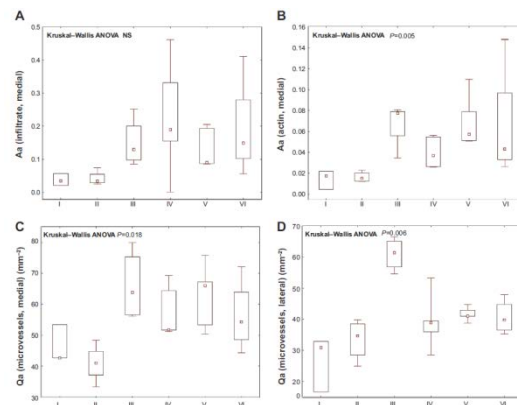
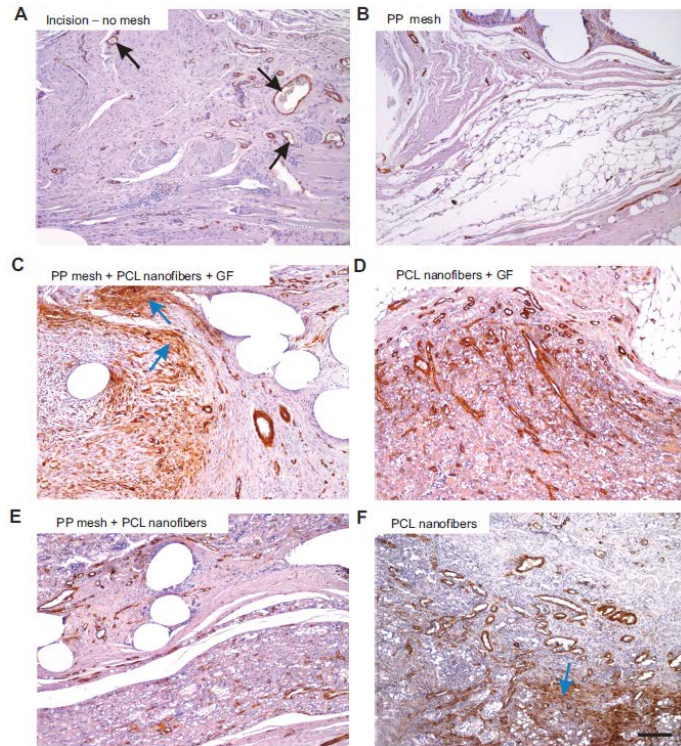
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# Kvalita jizvy břišní stěny při aplikaci nanovláknenné sítě



International Journal of Nanomedicine

Dovepress

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ORIGINAL RESEARCH

## Abdominal closure reinforcement by using polypropylene mesh functionalized with poly-ε-caprolactone nanofibers and growth factors for prevention of incisional hernia formation

This article was published in the following Dove Press journal:  
International Journal of Nanomedicine  
9 July 2014  
Number of times this article has been viewed

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Tomáš Krejčí<sup>7</sup>  
Andrej Litvinec<sup>2,7</sup>  
Matej Buzgo<sup>2,4</sup>  
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Alois Nečas<sup>8</sup>  
Jiří Hoch<sup>1</sup>  
Evžen Amler<sup>1,2,9</sup>

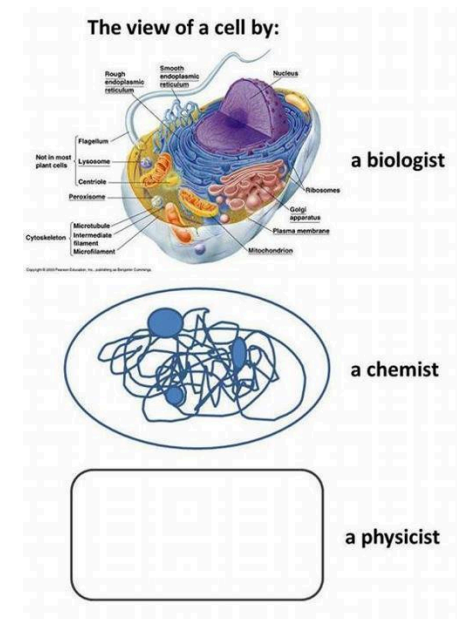
**Abstract:** Incisional hernia affects up to 20% of patients after abdominal surgery. Unlike other types of hernia, its prognosis is poor, and patients suffer from recurrence within 10 years of the operation. Currently used hernia-repair meshes do not guarantee success, but only extend the recurrence-free period by about 5 years. Most of them are nonresorbable, and these implants can lead to many complications that are in some cases life-threatening. Electrospun nanofibers of various polymers have been used as tissue scaffolds and have been explored extensively in the last decade, due to their low cost and good biocompatibility. Their architecture mimics the natural extracellular matrix. We tested a biodegradable polyester poly-ε-caprolactone in the form of nanofibers as a scaffold for fascia healing in an abdominal closure-reinforcement model for prevention of incisional hernia formation. Both in vitro tests and an experiment on a rabbit model showed promising results.

**Keywords:** nanofibers, growth factors, surgical mesh, hernia regeneration, in vivo



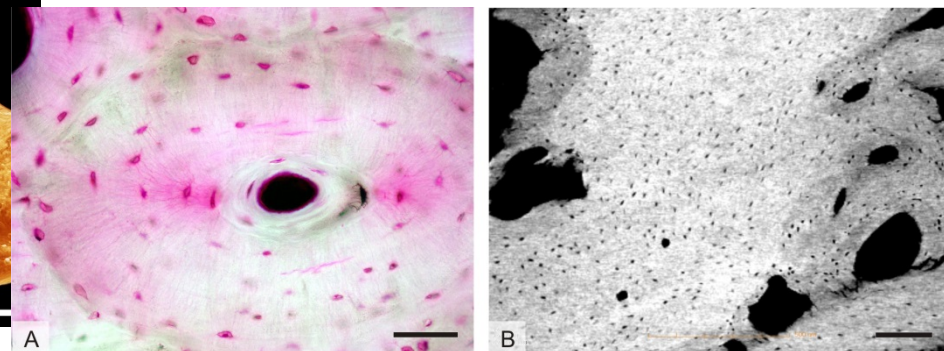
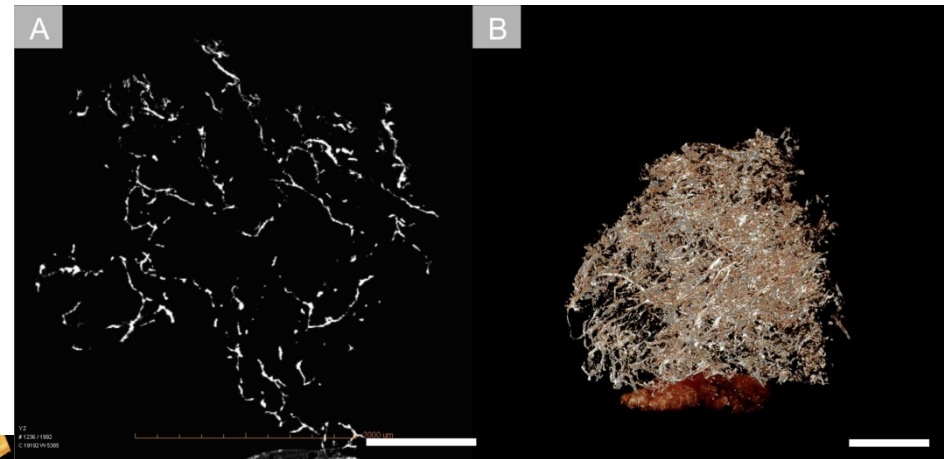
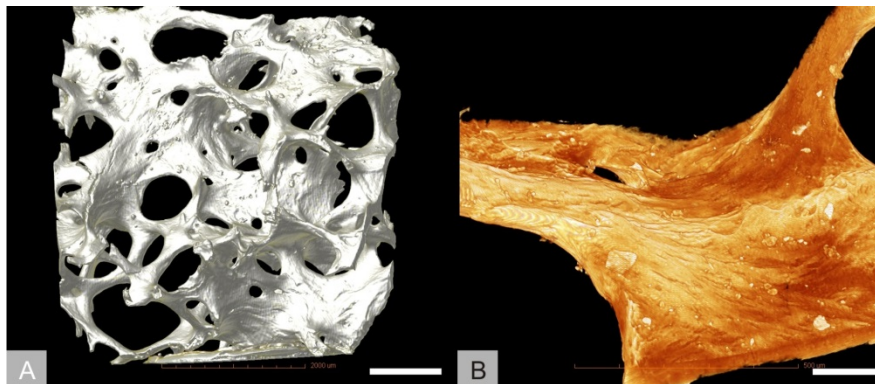
# Co histologie „umí říci“ a co ne

- **kvalita a kvantita** mikroanatomického **složení**
- buněčný **fenotyp**
- zastoupení **buněk** a složek **mezibuněčné hmoty**
- **přestavba** tkáně oproti původnímu stavu, „**historie**“ vzorku
- **rozmístění** útvarů, buněk, matrix, antigenů... na vhodně orientovaných řezech v měřítku **1  $\mu\text{m}$ -10<sup>3</sup> $\mu\text{m}$**  (xhomogenizace u biochemických vyšetření)
- x 3D informace v omezené míře (cf. mikro-CT)
- x **vysvětlení mechanismů** změn na buněčné úrovni  
→ paralelně biochemická (molek. biol.) analýza
- x **extrapolace strukturálního obrazu** do **mechanických** vlastností → paralelně biomechanické testování

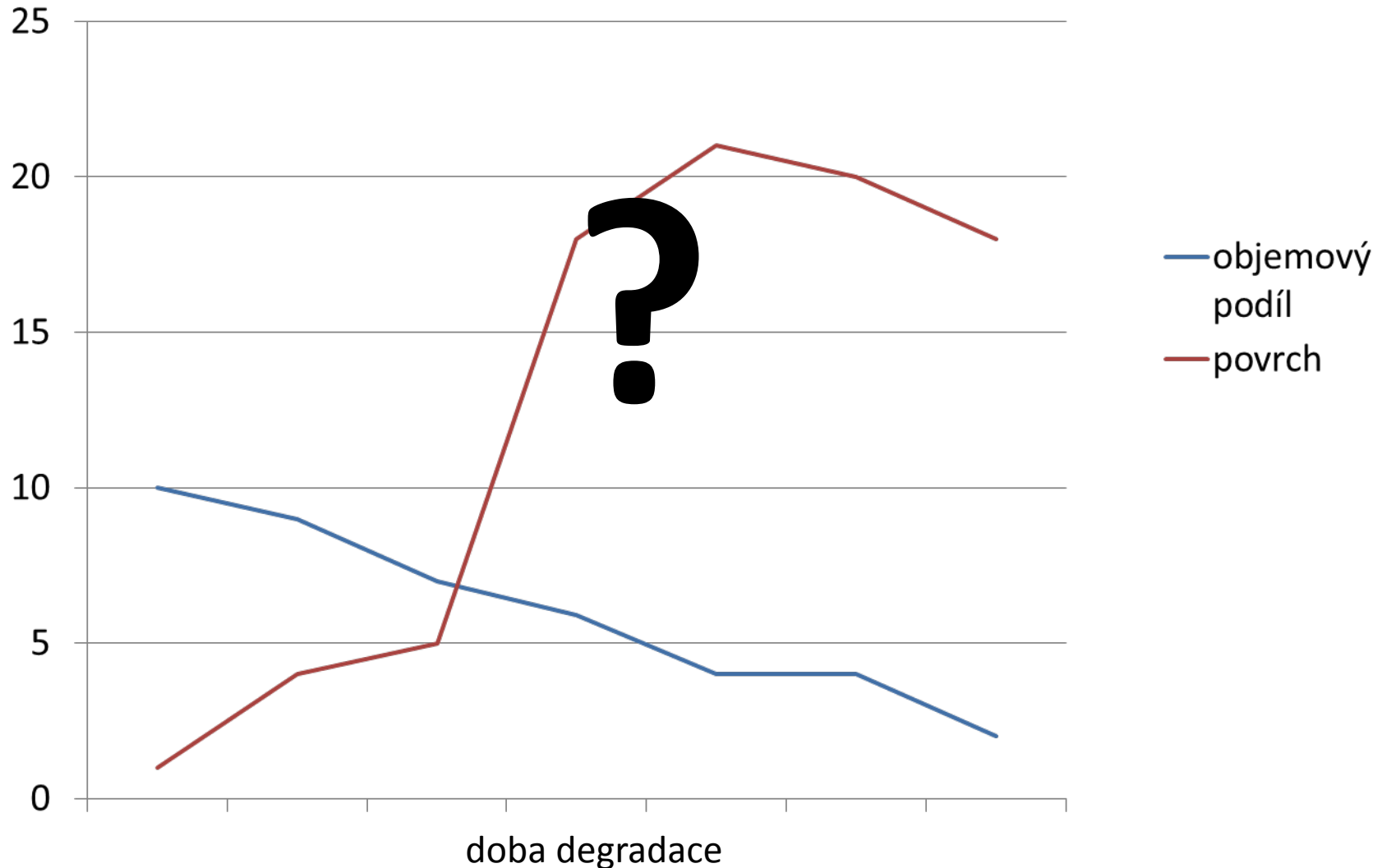


# Výhody $\mu$ -CT

- nativně 3D → minimální vzorkovací chyba
- obejde histologické artefakty
  - teplota
  - rozpouštědla
  - smrštění
  - demineralizace
  - ztráta materiálu řezáním a krájením...)



# mikro-CT jako slibná metoda pro posouzení *in vivo* degradace biomateriálu?





# Mikro-CT: kritické shrnutí možností kvantifikace

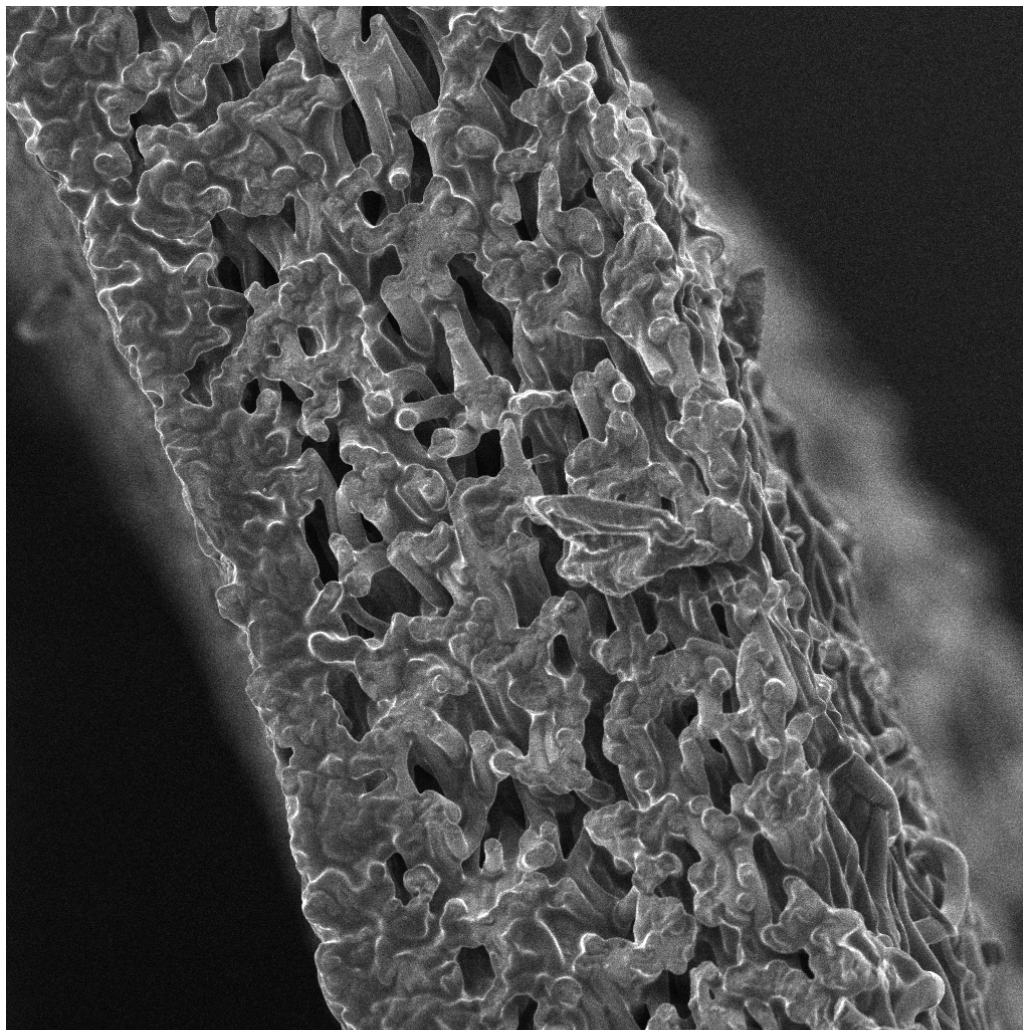
**status: September 2016**

**location: Herbertov**



- objemové podíly vláken i porozit jsou bezesporné
- CAVE: měření **povrchů** a **délek** silně **závisí** na **způsobu segmentace**
- Návrh: provést **analýzu citlivosti** a **doporučit nejvhodnější postup**

**Ponaučení:** U některých charakteristik nemáme „**zlatý standard**“ ani **kalibrační nástroje**!



SEM HV: 5.0 kV

SEM MAG: 1.00 kx

VEGA3 TESCAN

WD: 12.22 mm

Det: SE

50  $\mu$ m

View field: 276  $\mu$ m

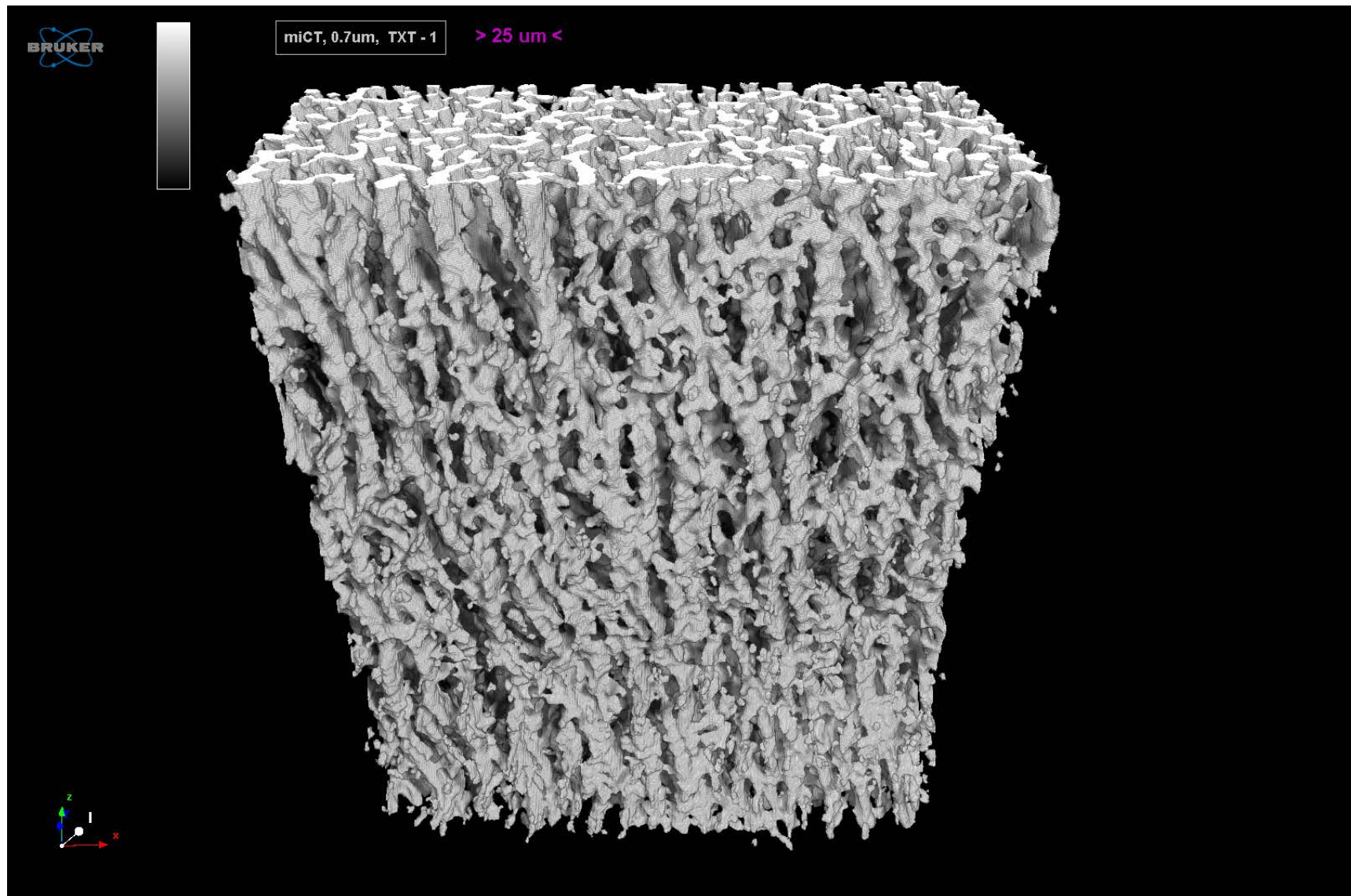
Date(m/d/y): 08/15/14

FT TUL Librec

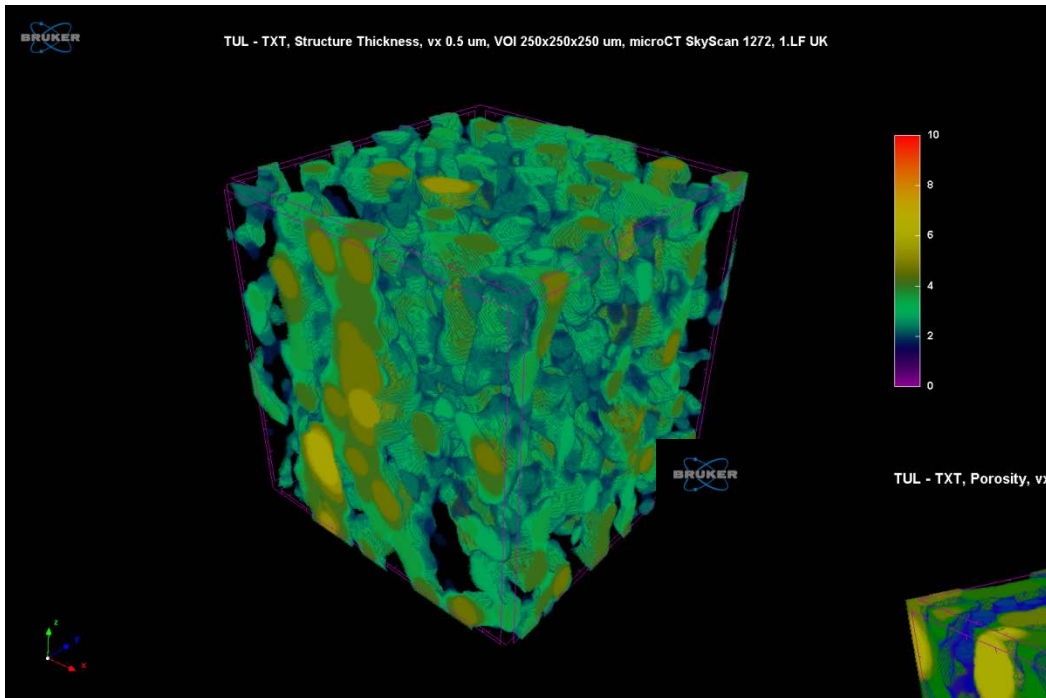




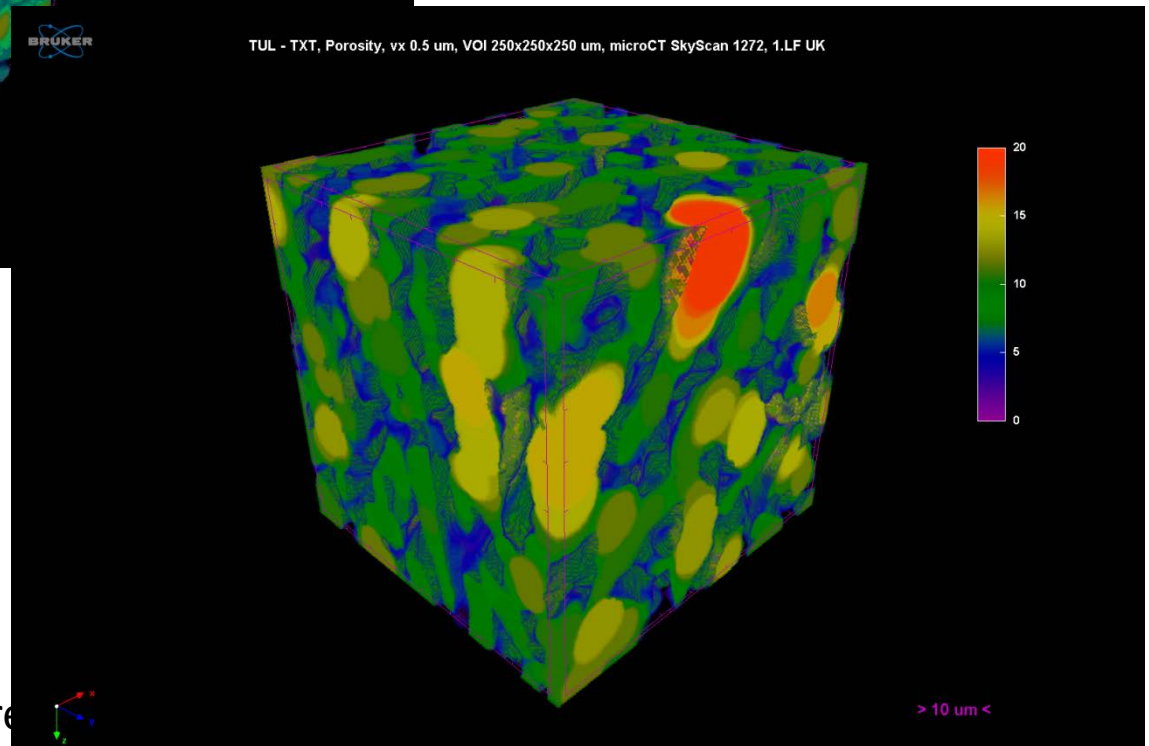
# Mikro-CT, rozlišení 0,5 $\mu\text{m}$



# Mikro-CT – vizualizace textilie i porozity



structure



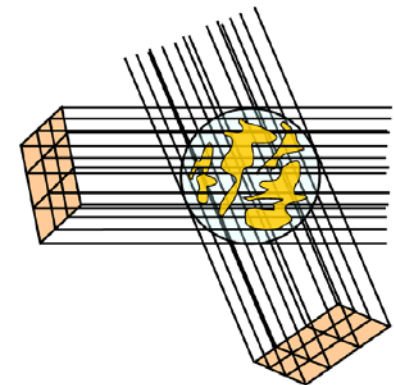


# Mikro-CT

- vyžaduje **binarizaci**
- běžná **morfometrie** ve **2D i 3D**
- charakteristiky **celku i jednotlivých objektů** v něm
- **an/izotropie** (míra 3D symetrie, analýza preferenčních orientací)
- **konektivita** (redundance spojek bránících rozpadu na samostatné objekty) pomocí Euler-Poincarého čísla

$$\chi = \Sigma (\text{connected components}) - \Sigma (\text{bridges, „handles“, loops}) + \Sigma (\text{holes, voids})$$

- analýza **porozit**
- uplatnění **stereologie** v analýze  $\mu$ -CT obrazů



**Figure 2.** For the MIL analysis, a grid of lines is sent through the volume over a large number of 3D angles (just two are illustrated here). The MIL for each angle is calculated as the average for all the lines of the grid.

# Mikro-CT – automatická kvantifikace pomocí SW QuantAn

Int J CARS  
DOI 10.1007/s11548-016-1378-3

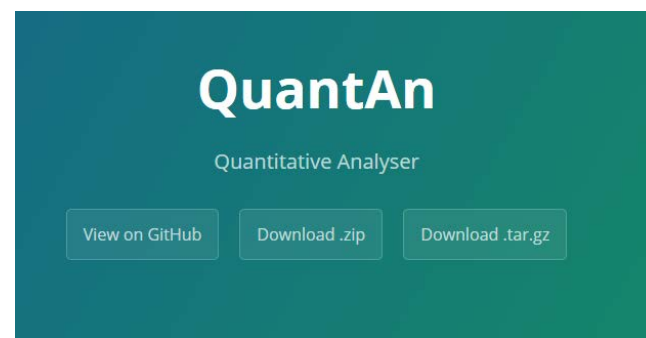
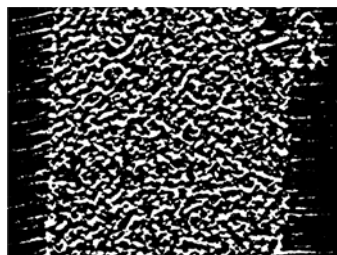


ORIGINAL ARTICLE

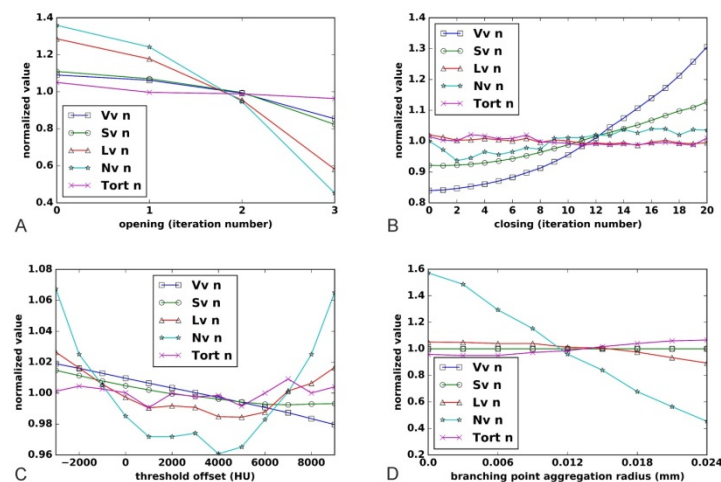
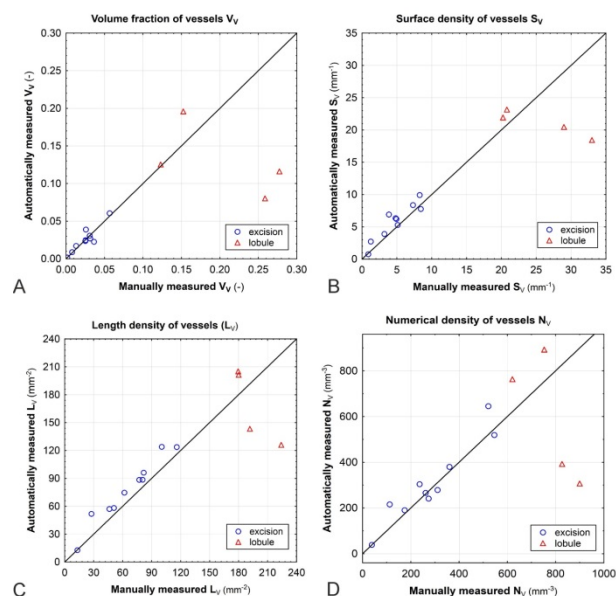
**Stereological quantification of microvessels using semiautomated evaluation of X-ray microtomography of hepatic vascular corrosion casts**

Miroslav Jířík<sup>1,6</sup> · Zbyněk Tonar<sup>1</sup> · Anna Králíčková<sup>2</sup> · Lada Eberlová<sup>3,6</sup> · Hyněk Mírka<sup>4,6</sup> · Petra Kochová<sup>1</sup> · Tomáš Gregor<sup>5</sup> · Petr Hošek<sup>6</sup> · Miroslava Svobodová<sup>6</sup> · Eduard Rohan<sup>1</sup> · Milena Králíčková<sup>2,6</sup> · Václav Liška<sup>6,7</sup>

Received: 12 November 2015 / Accepted: 8 March 2016  
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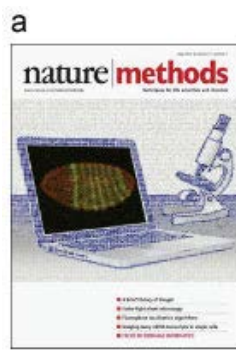
<http://mjirik.github.io/quantan/>



manuální validace - Wilcoxon n.s.

analýza citlivosti





RoiManager3D 3.83

027  
32/100 (slice000062); 300x300 pixels; 8-bit; 8.6MB

027-3Dseg  
32/100; 300x300 pixels (300x300); 16-bit; 17MB

Log

File Edit Font

Checking installation...  
Java3D not installed.  
Installation OK  
Starting RoiManager3D  
Adding image : min-max 1 33  
33 objects added. Total of 33 objects  
Adding image : min-max 1 33  
12 objects added. Total of 45 objects  
Adding image : min-max 1 33  
7 objects added. Total of 52 objects

Menu

Nb	Obj	Label	CX (pix)	CY (pix)	CZ (pix)	CX (unit)	CY (unit)	CZ (unit)	Xmin (pix)	Ymin (pix)	Zmin (pix)	Xmax (pix)	Ymax (pix)	Zmax (pix)	VolBoundin...	RatioVolbox	Vol (unit)	Vol (pix)	Surf (unit)	Surf (pix)	SurfCorr (pix)	Comp (p
0	1	obj1-val1	178.001	278.628	20.168	178.001	278.628	20.168	166	259	11	190	298	29	19,000	0.389	7,396	7396	3,202	3,202	2,108.596	0.1
1	2	obj2-val2	196.022	218.45	8.352	196.022	218.45	8.352	184	201	0	209	235	16	15,470	0.444	6,861	6861	2,918	2,918	1,938.613	0.2
2	3	obj3-val3	205.079	57.49	19.604	205.079	57.49	19.604	186	21	1	224	94	38	109,668	0.369	40,497	40497	10,240	10,240	6,816.292	0.1
3	4	obj4-val4	95.415	165.895	13.287	95.415	165.895	13.287	77	142	1	114	190	26	48,412	0.504	24,420	24420	6,548	6,548	4,361.291	0.1
4	5	obj5-val5	230.838	46.657	90.144	230.838	46.657	90.144	213	17	78	248	84	99	53,856	0.361	19,458	19458	6,632	6,632	4,593.186	0.1
5	6	obj6-val6	174.907	165.351	12.288	174.907	165.351	12.288	159	143	1	191	188	23	34,914	0.468	16,331	16331	5,154	5,154	3,448.905	0.1
6	7	obj7-val7	281.999	173.514	90.113	281.999	173.514	90.113	271	149	79	294	208	99	29,232	0.339	9,908	9908	4,336	4,336	2,946.86	0.1
7	8	obj8-val8	182.025	92.556	12.068	182.025	92.556	12.068	172	70	2	192	115	22	20,288	0.37	7,512	7512	3,424	3,424	2,280.674	0.1
8	9	obj9-val9	39.086	154.644	17.394	39.086	154.644	17.394	19	119	2	59	190	33	94,464	0.385	36,351	36351	9,440	9,440	6,341.611	0.1
9	10	obj10-val10	184.083	36.306	92.789	184.083	36.306	92.789	163	10	82	205	74	99	50,310	0.483	24,320	24320	7,474	7,474	5,395.225	0.1
10	11	obj11-val11	248.602	213.237	20.799	248.602	213.237	20.799	238	176	2	259	250	39	62,700	0.254	15,905	15905	6,410	6,410	4,222.169	0.1
11	12	obj12-val12	236.206	167.246	93.245	236.206	167.246	93.245	207	134	83	266	213	99	81,600	0.476	38,836	38836	11,070	11,070	8,275.81	0.1
12	13	obj13-val13	160.251	180.08	92.456	160.251	180.08	92.456	148	160	83	172	206	99	19,975	0.429	8,578	8578	3,670	3,670	2,540.34	0.1
13	14	obj14-val14	41.463	258.953	92.776	41.463	258.953	92.776	28	238	83	55	289	99	24,752	0.369	9,130	9130	3,984	3,984	2,745.094	0.1
14	15	obj15-val15	275.287	51.13	18.751	275.287	51.13	18.751	263	22	3	288	80	34	49,088	0.271	13,323	13323	5,306	5,306	3,438.085	0.1
15	16	obj16-val16	197.178	176.837	51.247	197.178	176.837	51.247	165	132	27	229	222	75	289,835	0.448	129,888	129888	20,634	20,634	13,718.707	0.2
16	17	obj17-val17	75.446	65.87	37.553	75.446	65.87	37.553	50	26	17	101	106	58	176,904	0.388	68,588	68588	14,010	14,010	9,298.542	0.1
17	18	obj18-val18	244.309	100.455	96.436	244.309	100.455	96.436	231	87	92	257	119	99	7,128	0.5	3,561	3561	2,120	2,120	1,545.23	0.1
18	19	obj19-val19	162.904	231.698	93.491	162.904	231.698	93.491	144	210	84	182	260	99	31,824	0.487	15,499	15499	5,290	5,290	3,782.103	0.1
19	20	obj20-val20	208.003	257.504	55.163	208.003	257.504	55.163	190	223	40	226	292	71	82,880	0.417	34,556	34556	9,090	9,090	6,085.57	0.1
20	21	obj21-val21	43.34	91.248	84.384	43.34	91.248	84.384	4	39	62	80	169	99	383,306	0.424	162,361	162361	26,848	26,848	19,188.304	0.1
21	22	obj22-val22	61.981	207.225	97.619	61.981	207.225	97.619	47	192	95	76	223	99	4,800	0.48	2,302	2302	1,932	1,932	1,465.287	0.0
22	23	obj23-val23	118.474	243.381	44.254	118.474	243.381	44.254	85	192	19	152	295	69	360,672	0.471	169,873	169873	24,944	24,944	16,800.12	0.1
23	24	obj24-val24	170.338	60.74	70.338	170.338	60.74	70.338	157	7	30	184	115	83	164,808	0.184	30,308	30308	10,858	10,858	7,155.182	0.0
24	25	obj25-val25	263.042	232.155	69.096	263.042	232.155	69.096	243	164	44	285	297	91	276,576	0.222	61,506	61506	18,584	18,584	12,337.963	0.0
25	26	obj26-val26	81.357	175.808	55.828	81.357	175.808	55.828	62	123	31	101	229	81	218,280	0.261	56,947	56947	14,576	14,576	9,632.142	0.1
26	27	obj27-val27	282.444	91.037	61.493	282.444	91.037	61.493	267	54	45	298	128	78	81,600	0.308	25,142	25142	8,018	8,018	5,357.022	0.1
27	28	obj28-val28	28.785	245.919	55.071	28.785	245.919	55.071	0	196	31	57	298	79	287,042	0.439	126,070	126070	21,164	21,164	14,448.724	0.1
28	29	obj29-val29	138.397	78.032	44.943	138.397	78.032	44.943	109	32	22	164	124	68	244,776	0.422	103,346	103346	18,368	18,368	12,280.447	0.1
29	30	obj30-val30	106.996	64.607	94.169	106.996	64.607	94.169	87	40	86	131	97	99	36,540	0.515	18,823	18823	6,412	6,412	4,723.384	0.1
30	31	obj31-val31	249.189	110.184	21.454	249.189	110.184	21.454	230	87	10	268	133	33	43,992	0.481	21,142	21142	6,032	6,032	4,032.621	0.1
31	32	obj32-val32	223.607	82.087	69.415	223.607	82.087	69.415	187	40	49	260	124	90	264,180	0.473	124,924	124924	19,512	19,512	13,189.784	0.2
32	33	obj33-val33	18.326	84.414	22.557	18.326	84.414	22.557	0	60	11	37	109	34	45,600	0.496	22,621	22621	6,352	6,352	4,264.929	0.2

# Mikro-CT – interaktivní kvantifikace ve stereologickém software Ellipse

- jiné techniky binarizace a skeletonizace

## Měření binárního obrazu

*ROI volume:* 1e+006

*Volume:* 334953

*Surface area:* 198346

*Length:* 5503.67

*Euler characteristics:* -647

## Skeleton binárního obrazu

: Thresh, Skeletonization3D 10, 20;

ResampleGeom1D 5

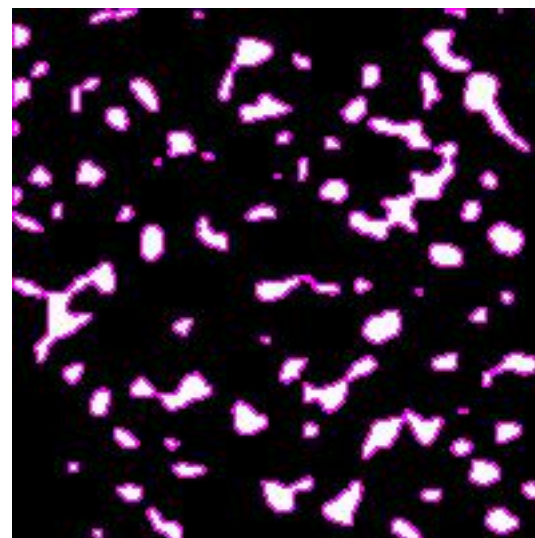
*Total length:* 16310.7

*Total curvature:* 2231.46 radians

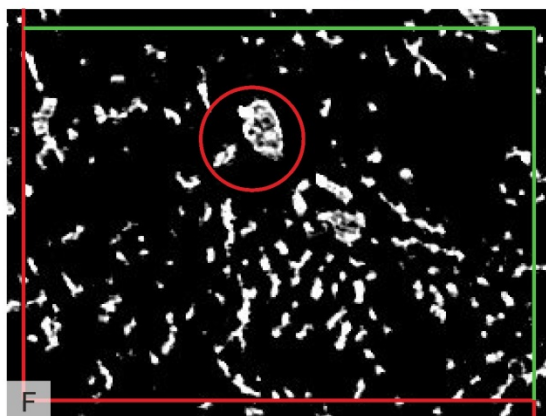
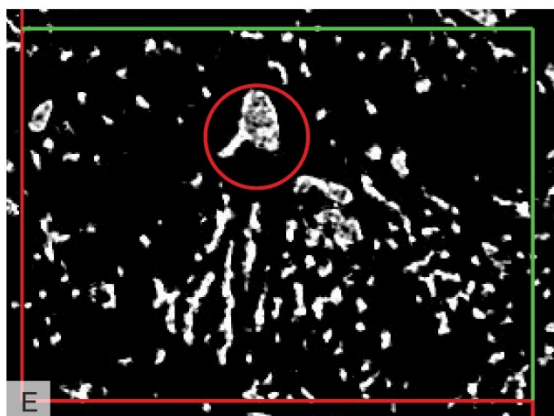
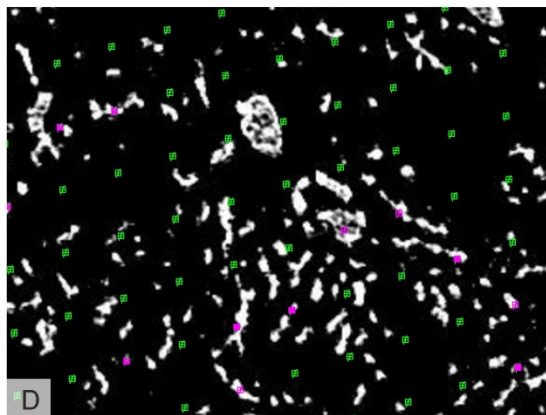
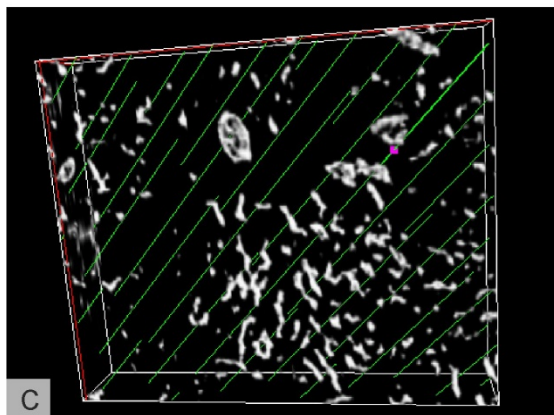
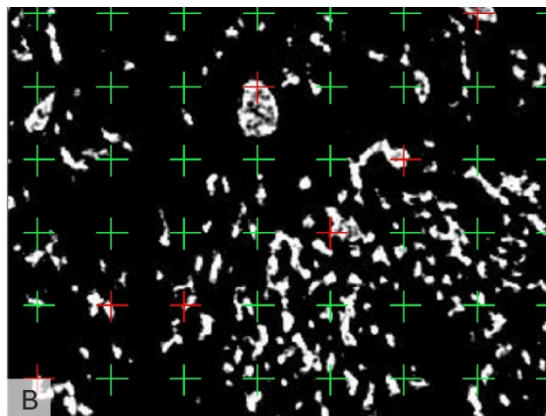
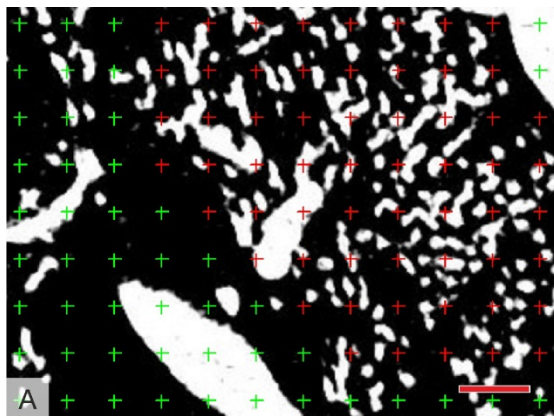
*No. of branchings:* 1022

*No. of ends:* 362




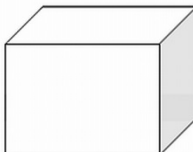

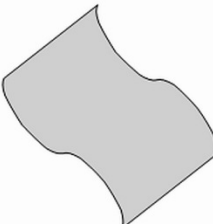

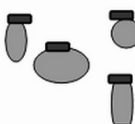
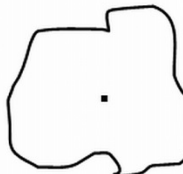
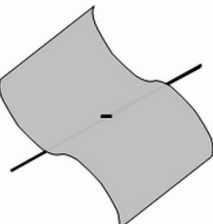
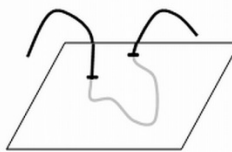
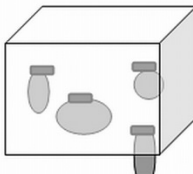
*Euler characteristics:* -416







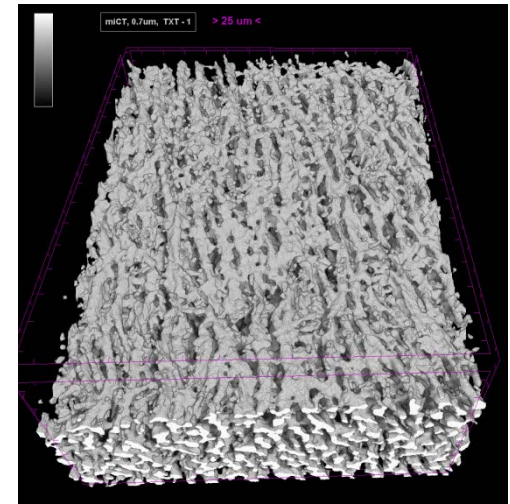
# Rozměry hodnocené veličiny a stereologické sondy

Sonda	 bodová (0D)	 lineární (1D)	 rovinná (2D)	 objemová(3D)
$n$ Veličina	 objem (3D)	 plocha (2D)	 délka (1D)	 počet (0D)
$=$ počítané události	 objem (3D)	 plocha (2D)	 délka (1D)	 objemová(3D)



# Problémy s délkou – interaktivní měření

- binární obraz je **silně ovlivněn kontaktem vláken**
- záporná 3D Eulerova charakteristika se projeví **podceněním odhadu délky**, který se počítá z 2D Eulerových charakteristik ve 3 kolmých rovinách
- **x**přístup založený na skeletonizaci naopak nadhodnocuje délku **o nadbytečné spojky**
- různými druhy filtrace lze před binarizací získat výrazně větší a kladnou Eulerovu charakteristiku a **odhad délky ze zvýší**
- **x**skeletonizací a vyhlazením dostáváme pak o něco **kratší délku**



# Software Teigen

<https://mjirik.github.io/teigen/>

## Teigen

Test Image Generator

[View the Project on GitHub](#)

[mjirik/teigen](#)

Download  
**ZIP File**

Download  
**TAR Ball**

View On  
**GitHub**

This project is maintained by [mjirik](#)

Hosted on GitHub Pages — Theme by [orderedlist](#)

## teigen

Test Image Generator

## Installation

```
conda install -c mjirik -c simpleitk teigen
```

## Windows

[Windows installer](#)



# Flowchart – generátor neprotínajících se válců a koulí

## vstupní parametry

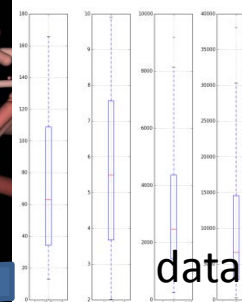
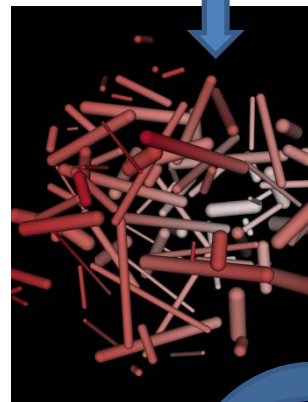
- počet objektů
- objemový podíl
- distribuce průměrů válců/koulí
- distribuce délek válců
- profily intenzit uvnitř/na okraji objektů
- izotropie/anizotropie
- rozlišení
- gaussovské rozostření
- parametrický generátor náhodného šumu
- počty iterací
- seed
- ...

load

save

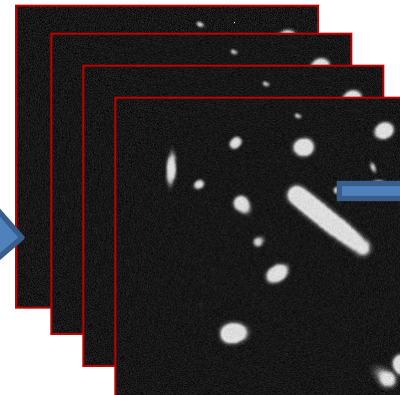
# YAML konfigurační soubor

## generate skeleton



data, graphs, tables, histograms

generate  
and save  
volumetric  
data

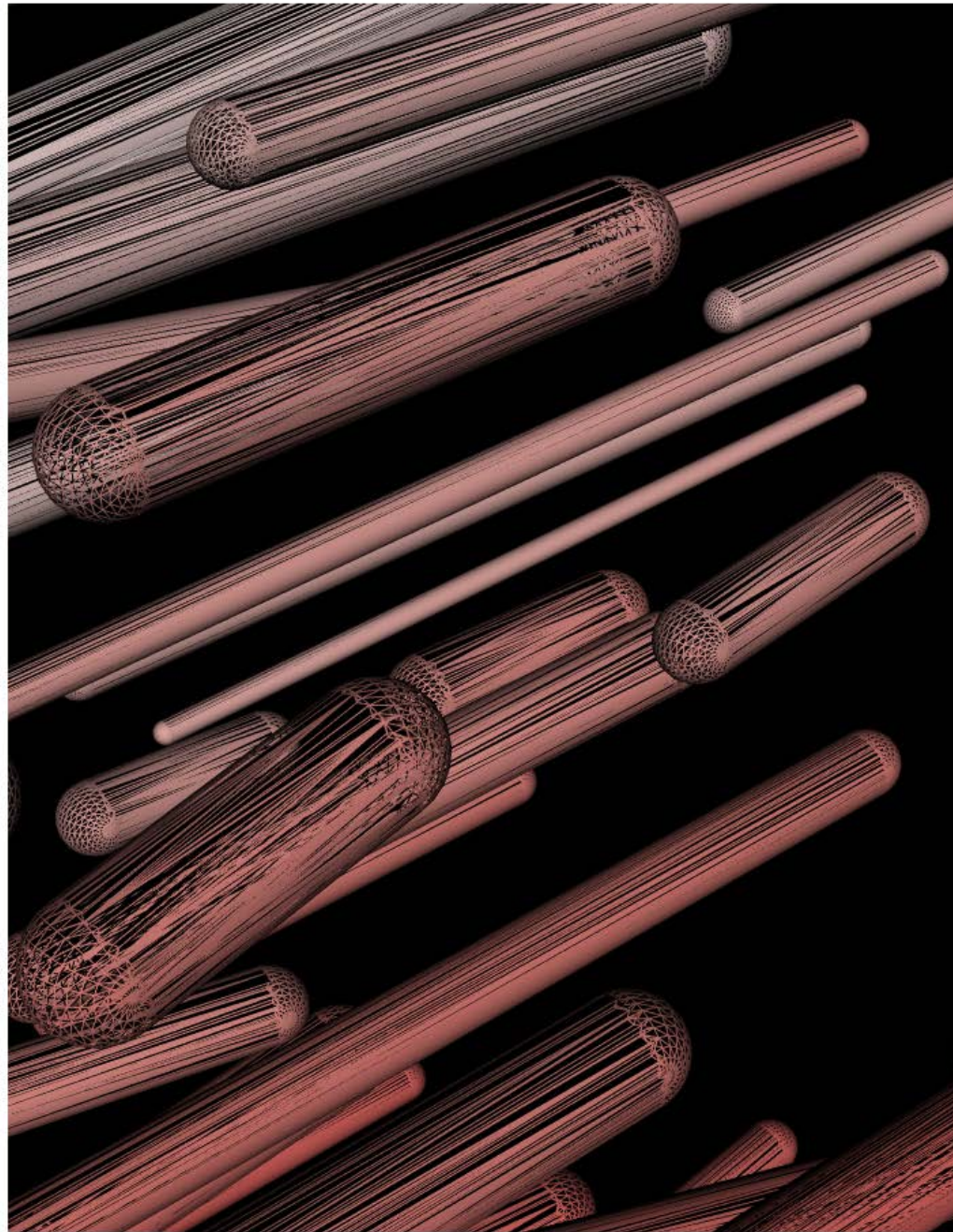


- micro-CT SW
- ImageJ
- interaktivní stereologie
- ...

```
[*] file:///c:/Users/.../engineering/kivotoso_civtech_pabhai_TIA Software generated... 85 %
```

```
Souder Upvrett Madnoti Kivotoso Niganda  
appearance: {show_surface: true}  
areasampling:  
    size_min: [300.0, 300.0, 300.0]  
    size_max: [300, 300, 300]  
    noise_min: [1.0, 1.0, 1.0]  
    noise_max: C:\Users\Tomar\external_data\serless\BdM\Slice\BdM.jpg  
filepattern series_number: 27  
generator_id: 1  
generators: *tpython/object/application/collections.OrderedDict  
- - Cylinder generator  
    * tpython/object/application/collections.OrderedDict  
        - {element_number, 30}  
        - {uniform_radius_distribution, true}  
        - {normal_radius_distribution, false}  
        - {fixed_radius_distribution, false}  
        - {radius_distribution_minimum, 2.0}  
        - {radius_distribution_maximum, 10.0}  
        - {radius_distribution_standard_deviation, 5.0}  
        - {radius_profile_radius  
            + idBdM [0.8, 0.7, 1.0, 1.3]  
        - {intensity_profile_intensity  
            + idBdM [195, 190, 200, 200]  
        - {random_generator_seed, 0}  
- - Gensel generator  
    * tpython/object/application/collections.OrderedDict  
        - {n_objects, 10}  
- - Cylinder continues  
    * tpython/object/application/collections.OrderedDict  
        - {element_number, 30}  
        - {uniform_radius_distribution, true}  
        - {normal_radius_distribution, false}  
        - {fixed_radius_distribution, false}  
        - {radius_distribution_minimum, 2.0}  
        - {radius_distribution_maximum, 10.0}  
        - {radius_distribution_standard_deviation, 5.0}  
        - {radius_profile_radius  
            + idBdM [0.8, 0.7, 1.0, 1.3]  
        - {intensity_profile_intensity  
            + idBdM [195, 190, 200, 200]  
        - {random_generator_seed, 0}  
- - Unconnected cylinders  
    * tpython/object/application/collections.OrderedDict  
        - {element_number, 50}  
        - {uniform_radius_distribution, true}  
        - {normal_radius_distribution, false}  
        - {fixed_radius_distribution, false}  
        - {radius_distribution_minimum, 10.0}  
        - {radius_distribution_maximum, 50.0}  
        - {radius_distribution_standard_deviation, 5.0}  
        - {length_distribution_mean, 100.0}  
        - {length_distribution_standard_deviation, 30.0}  
        - {intensity_profile_radius  
            + [0.8, 0.7, 1.0, 1.3]  
        - {intensity_profile_intensity  
            + [195, 190, 200, 200]  
        - {orientation_anisotropic, false}  
        - {orientation_axis  
            + [(1.0, 1.0, 0.0)]  
        - {orientation_variation_rad, 0.1}  
        - {volume_fraction, 0.5}  
        - {maximum_1000_iteration_number, 10}  
        - {random_generator_seed, 0}  
postprocessing: *tpython/object/application/collections.OrderedDict  
+ {gaussian_blur, true}
```





Teigen 0.2.17

Parameter

Value

Batch processing

Run batch

Add

Area Sampling

voxelsize\_mm

01mm

11mm

21mm

areazsize\_mm

0300mm

1300mm

2300mm

areazsize\_px

0300px

1300px

2300px

Appearance

show\_surface

Postprocessing

gaussian\_blur

gaussian\_filter\_sigma\_mm

1

add\_noise

noise\_preview

limit\_negative\_intensities

noise\_random\_generator\_seed

0

exponent

0.0001

lambda\_start

0.1

lambda\_stop

3

noise\_amplitude

40

noise\_mean

30

surface\_measurement

measurement\_multiplier

-1

measurement\_resolution

20

output\_dtype

uint8

negative

Cylinder generator

Gensei generator

Cylinder continues

Unconnected cylinders

element\_number

100

uniform\_radius\_distribution

normal\_radius\_distribution

fixed\_radius\_distribution

radius\_distribution\_minimum

2.000000

radius\_distribution\_maximum

10.000000

radius\_distribution\_mean

2.000000

radius\_distribution\_standard\_deviation

5.000000

length\_distribution\_mean

150.000000

length\_distribution\_standard\_deviation

10.000000

intensity\_profile\_radius

0.400000

0.700000

1.000000

1.300000

intensity\_profile\_intensity

195

190

200

30

orientation\_anisotropic

orientation\_main

1.000000

1.000000

0.000000

orientation\_variance\_rad

0.100000

volume\_fraction

0.150000

maximum\_1000\_iteration\_number

10

random\_generator\_seed

1000

last\_element\_can\_be\_smaller

output directory

C:\Users\tonar\teigen\_data\{seriesn:03d}\data\{:05d}.jpg

Set dir

C:\Users\tonar\teigen\_data\005\data\_parameters.yaml

Save parameters

Save parameters and add to batch

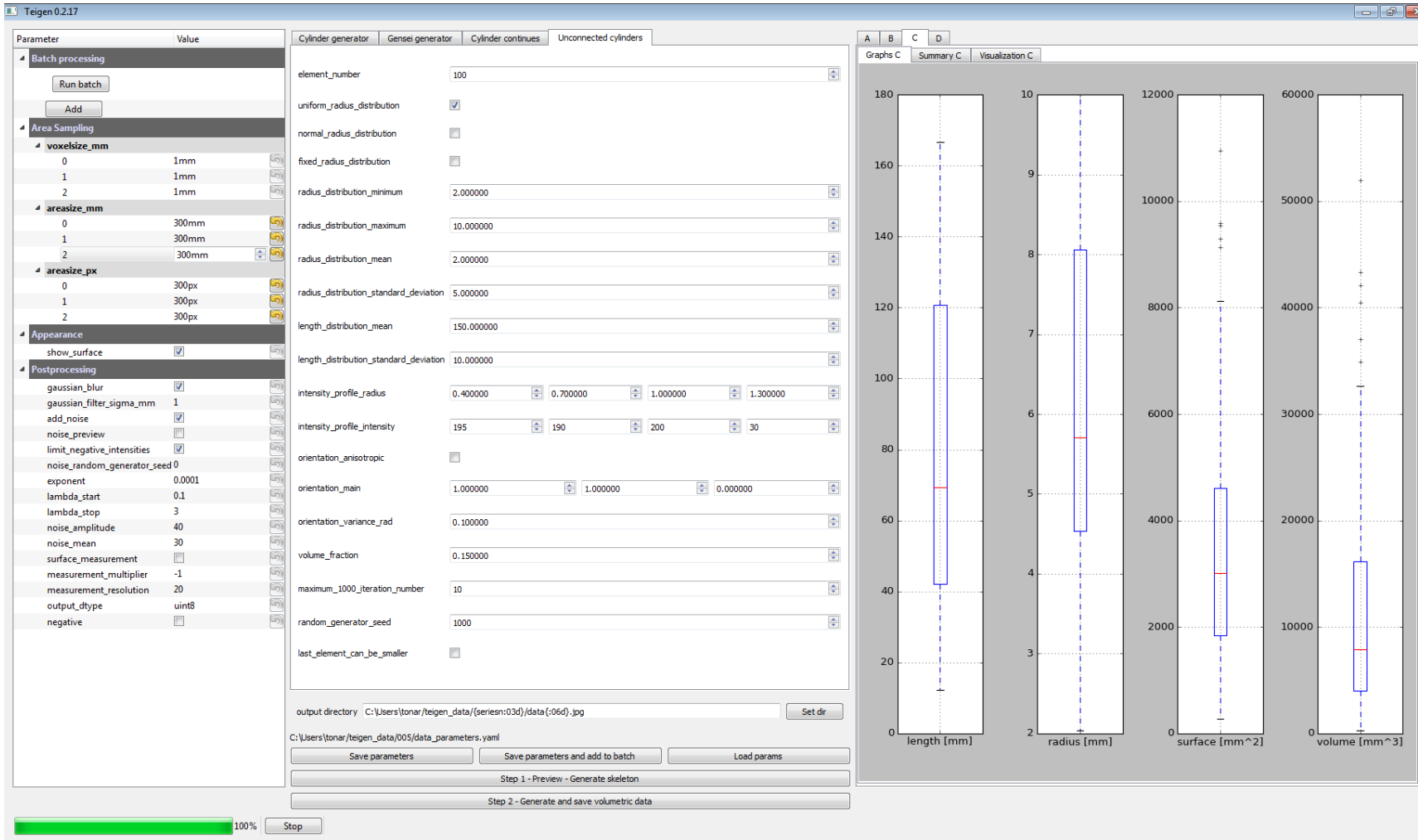
Load params

Step 1 - Preview - Generate skeleton

Step 2 - Generate and save volumetric data

100%

Stop





Teigen 0.2.17

Parameter

Value

Batch processing

Run batch

Add

Area Sampling

voxelsize\_mm

0

1mm

1

1mm

2

1mm

aresize\_mm

0

300mm

1

300mm

2

300mm

aresize\_px

0

300px

1

300px

2

300px

Appearance

show\_surface

Postprocessing

gaussian\_blur

gaussian\_filter\_sigma\_mm

1

add\_noise

noise\_preview

limit\_negative\_intensities

noise\_random\_generator\_seed

0

exponent

0.0001

lambda\_start

0.1

lambda\_stop

3

noise\_amplitude

40

noise\_mean

30

surface\_measurement

measurement\_multiplier

-1

measurement\_resolution

20

output\_dtype

uint8

negative

Cylinder generator

Gensei generator

Cylinder continues

Unconnected cylinders

element\_number

100

uniform\_radius\_distribution

☒

normal\_radius\_distribution

☐

fixed\_radius\_distribution

☐

radius\_distribution\_minimum

2.000000

radius\_distribution\_maximum

10.000000

radius\_distribution\_mean

2.000000

radius\_distribution\_standard\_deviation

5.000000

length\_distribution\_mean

150.000000

length\_distribution\_standard\_deviation

10.000000

intensity\_profile\_radius

0.400000

0.700000

1.000000

1.300000

intensity\_profile\_intensity

195

190

200

30

orientation\_anisotropic

☐

orientation\_main

1.000000

1.000000

0.000000

orientation\_variance\_rad

0.100000

volume\_fraction

0.150000

maximum\_1000\_iteration\_number

10

random\_generator\_seed

1000

last\_element\_can\_be\_smaller

☐

output\_directory

C:\Users\tonar\teigen\_data\seriesn:03d\data(:06d).jpg

Set dir

C:\Users\tonar\teigen\_data\005\data\_parameters.yaml

Save parameters

Save parameters and add to batch

Load params

Step 1 - Preview - Generate skeleton

Step 2 - Generate and save volumetric data

100%

Stop

A

B

C

D

Graphs C

Summary C

Visualization C

	length [mm]	radius [mm]	surface [mm^2]	volume [mm^3]
mean	81.9487545497	6.12591035536	3587.68376712	11764.7184661
std	47.8644398978	2.23079639823	2306.27586275	10799.0898717
min	12.3253913783	2.03469356959	276.65291985	278.223579392
25%	42.1977806325	4.53746024993	1845.30088212	3989.75168567
50%	69.4306301508	5.71180473516	3014.58355404	7910.38532356
75%	120.77496663	8.05536676887	4616.51613452	16134.9908616
max	166.713334465	9.99921444204	10948.0755644	51947.9719283

ength d. [mm^-2]	volume d. []	urface d. [mm^-1]
0.00030351390574	0.043573031356	0.013287717656

length [mm]	volume [mm^3]	surface [mm^2]	ea volume [mm^4]	count []	neric volume [mm]	neric surface
8194.87545497	1176471.84661	358768.376712	27000000.0	100.0	1156246.93267	357007.509415

Save in one row

Teigen 0.2.17

Parameter

Value

Batch processing

Run batch

Add

Area Sampling

voxelsize\_mm

0

1mm

1

1mm

2

1mm

areasize\_mm

0

300mm

1

300mm

2

300mm

areasize\_px

0

300px

1

300px

2

300px

Appearance

show\_surface

☒

Postprocessing

gaussian\_blur

☒

gaussian\_filter\_sigma\_mm

1

add\_noise

☒

noise\_preview

☐

limit\_negative\_intensities

☒

noise\_random\_generator\_seed

0

exponent

0.0001

lambda\_start

0.1

lambda\_stop

3

noise\_amplitude

40

noise\_mean

30

surface\_measurement

☐

measurement\_multiplier

-1

measurement\_resolution

20

output\_dtype

uint8

negative

☐

Cylinder generator

Gensei generator

Cylinder continues

Unconnected cylinders

element\_number

100

uniform\_radius\_distribution

☒

normal\_radius\_distribution

☐

fixed\_radius\_distribution

☐

radius\_distribution\_minimum

2.000000

radius\_distribution\_maximum

10.000000

radius\_distribution\_mean

2.000000

radius\_distribution\_standard\_deviation

5.000000

length\_distribution\_mean

150.000000

length\_distribution\_standard\_deviation

10.000000

intensity\_profile\_radius

0.400000

0.700000

1.000000

1.300000

intensity\_profile\_intensity

195

190

200

30

orientation\_anisotropic

☐

orientation\_main

1.000000

1.000000

0.000000

orientation\_variance\_rad

0.100000

volume\_fraction

0.150000

maximum\_1000\_iteration\_number

10

random\_generator\_seed

1000

last\_element\_can\_be\_smaller

☐

output\_directory

C:\Users\tonar\teigen\_data\seriesn:03d\data{:06d}.jpg

Set dir

C:\Users\tonar\teigen\_data\005\data\_parameters.yaml

Save parameters

Save parameters and add to batch

Load params

Step 1 - Preview - Generate skeleton

Step 2 - Generate and save volumetric data

100%

Stop

A

B

C

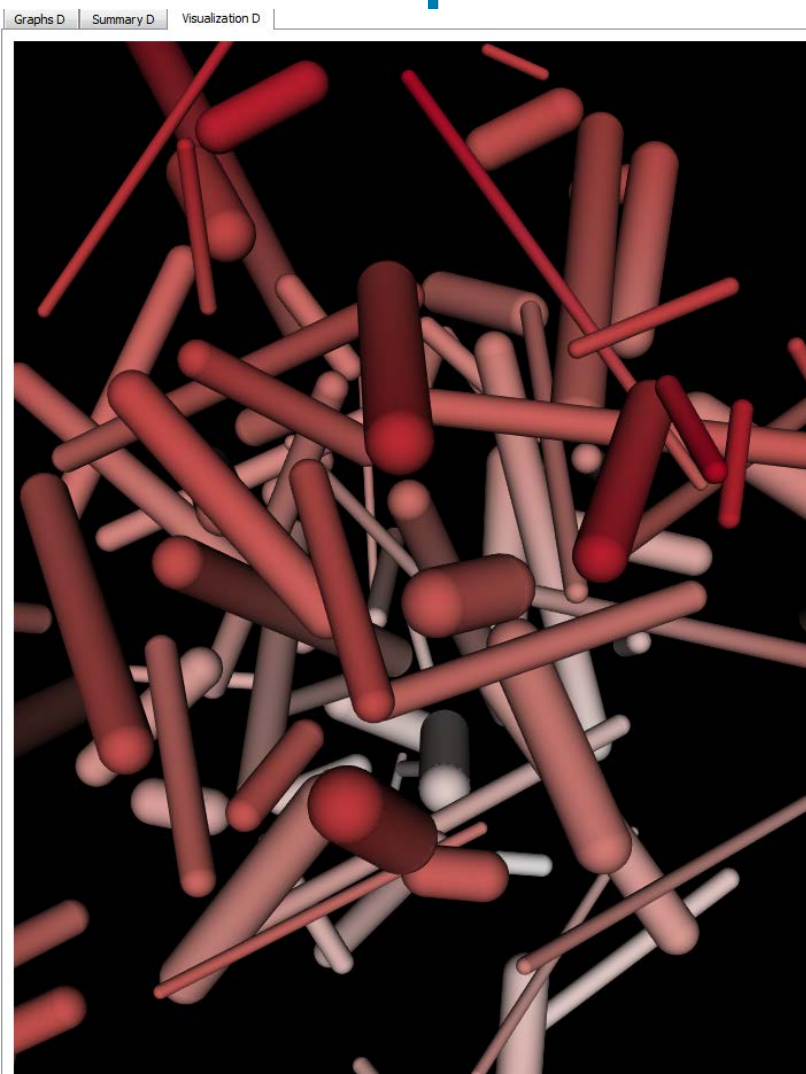
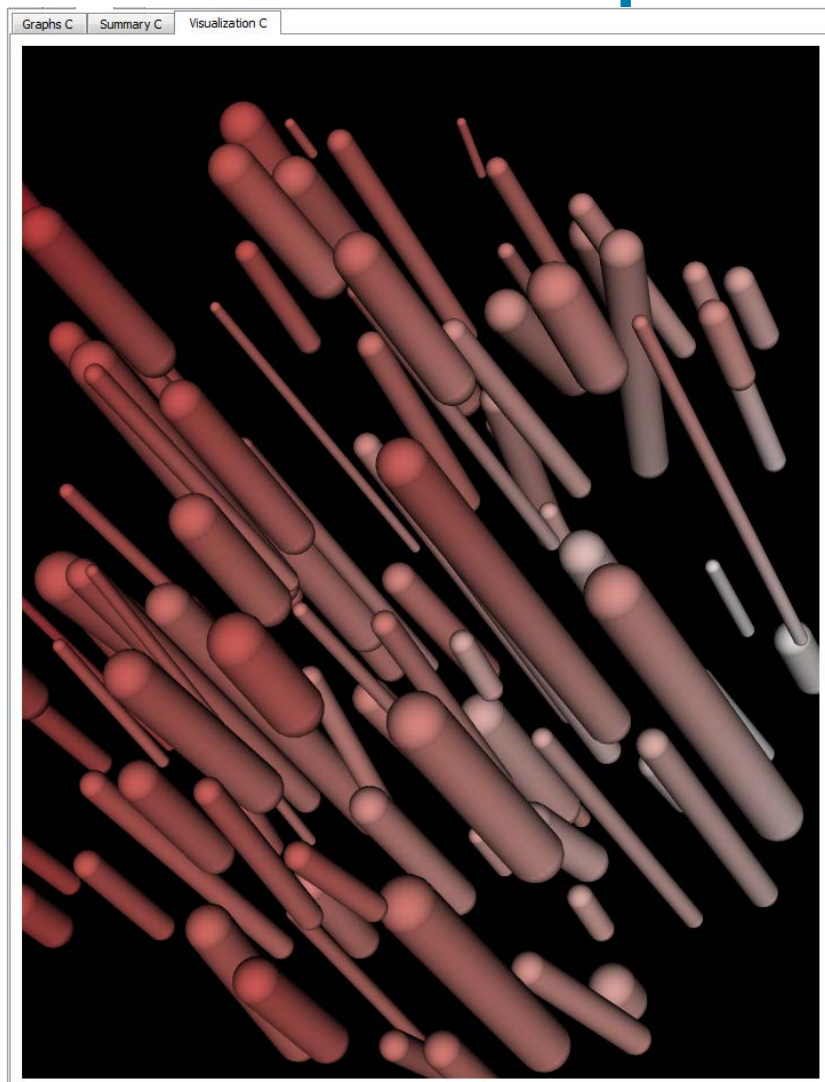
D

Graphs C

Summary C

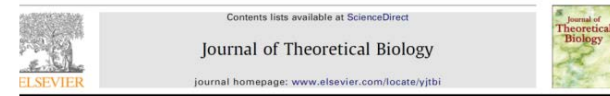
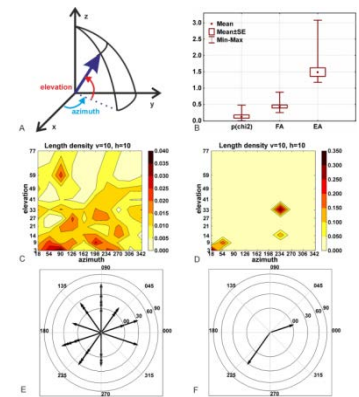
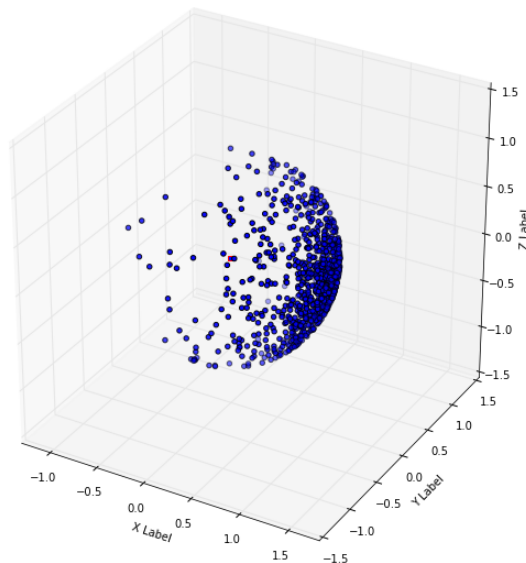
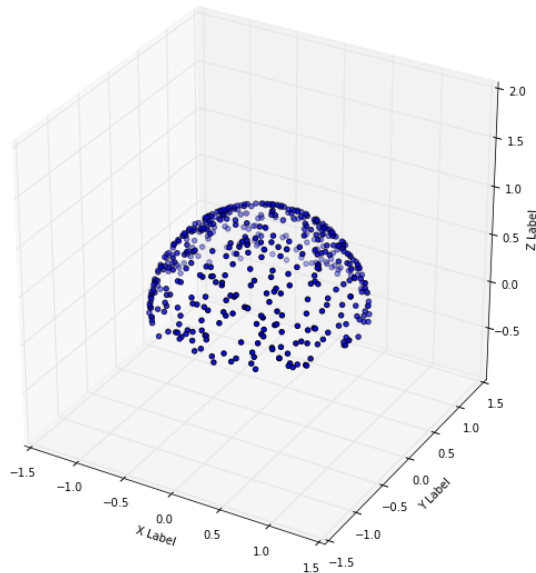
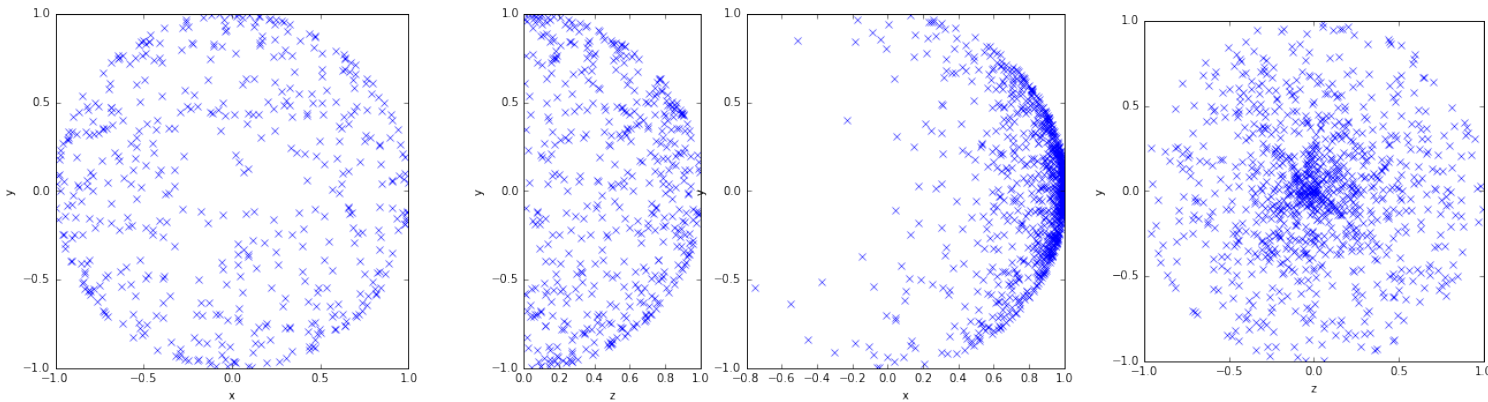
Visualization C

# Izotropie a anizotropie





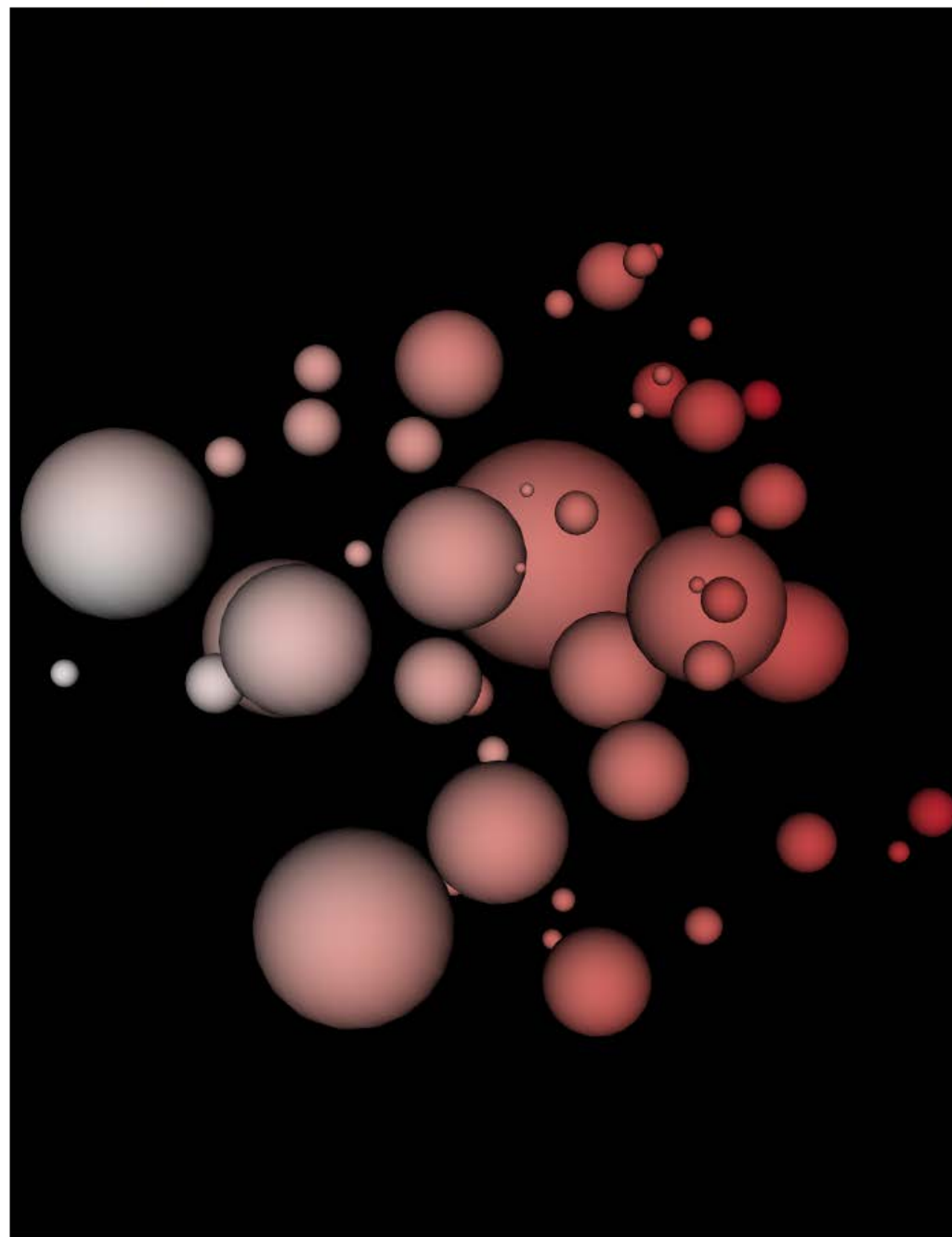
# Izotropie a anizotropie



How to assess, visualize and compare the anisotropy of linear structures reconstructed from optical sections—A study based on histopathological quantification of human brain microvessels

Petra Kochová<sup>a,\*</sup>, Robert Cimrman<sup>a</sup>, Jiří Janáček<sup>b</sup>, Kirsti Witter<sup>c</sup>, Zbyněk Tonar<sup>d,e</sup>

<sup>a</sup>Department of Mechanics, Faculty of Applied Sciences, University of West Bohemia, Univerzitní 8, 306 14 Pilsen, Czech Republic;  
<sup>b</sup>Institute of Physiology, Academy of Sciences of the Czech Republic, Videlská 1083, 142 20 Prague, Czech Republic;  
<sup>c</sup>Institute of Anatomy, Histology and Embryology, Department for Pathobiology, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria;  
<sup>d</sup>European Centre of Excellence NTD—New Technologies for Information Society, Faculty of Applied Sciences, University of West Bohemia, Univerzitní 22, 306 14 Pilsen, Czech Republic;  
<sup>e</sup>Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University in Prague, Karlovarská 48, 301 66 Pilsen, Czech Republic



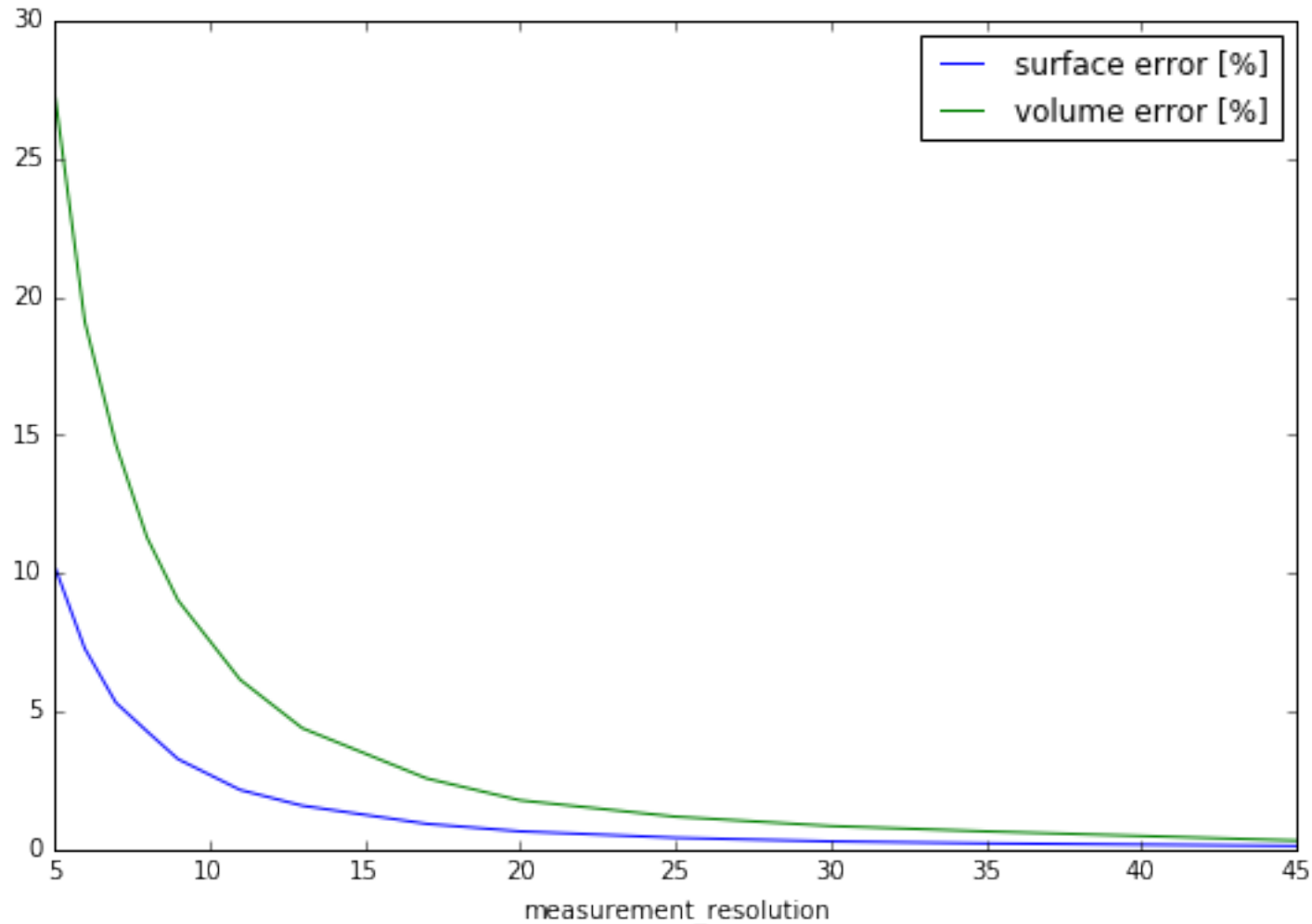
# Míra shody při použití různých SW a metod

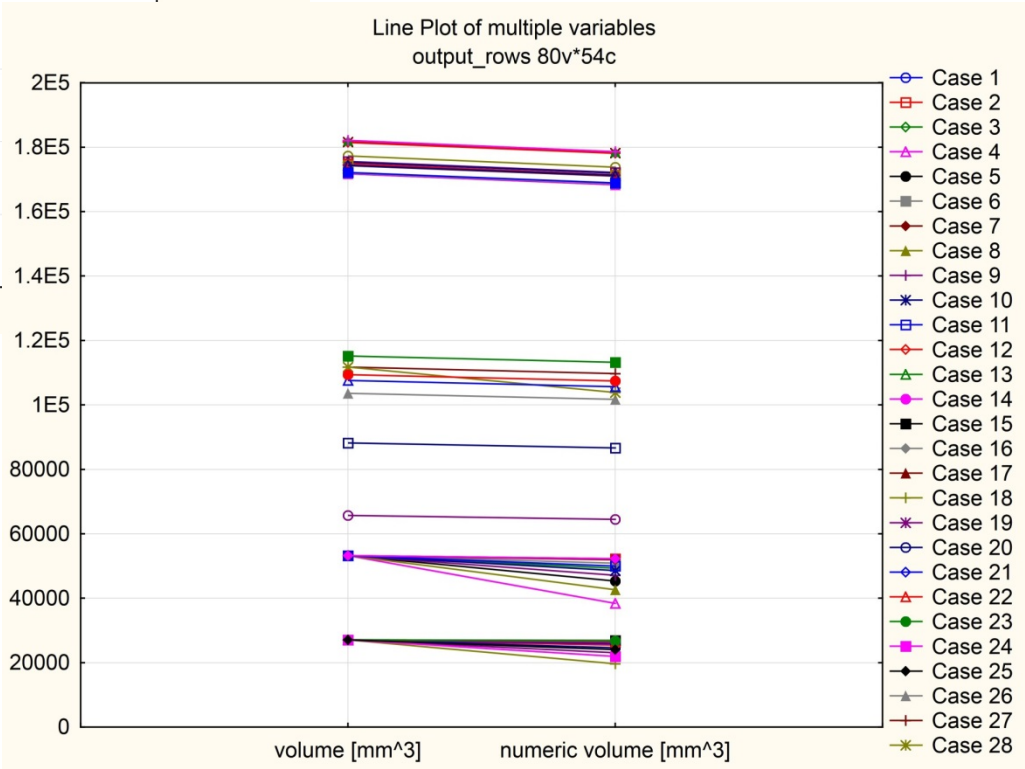
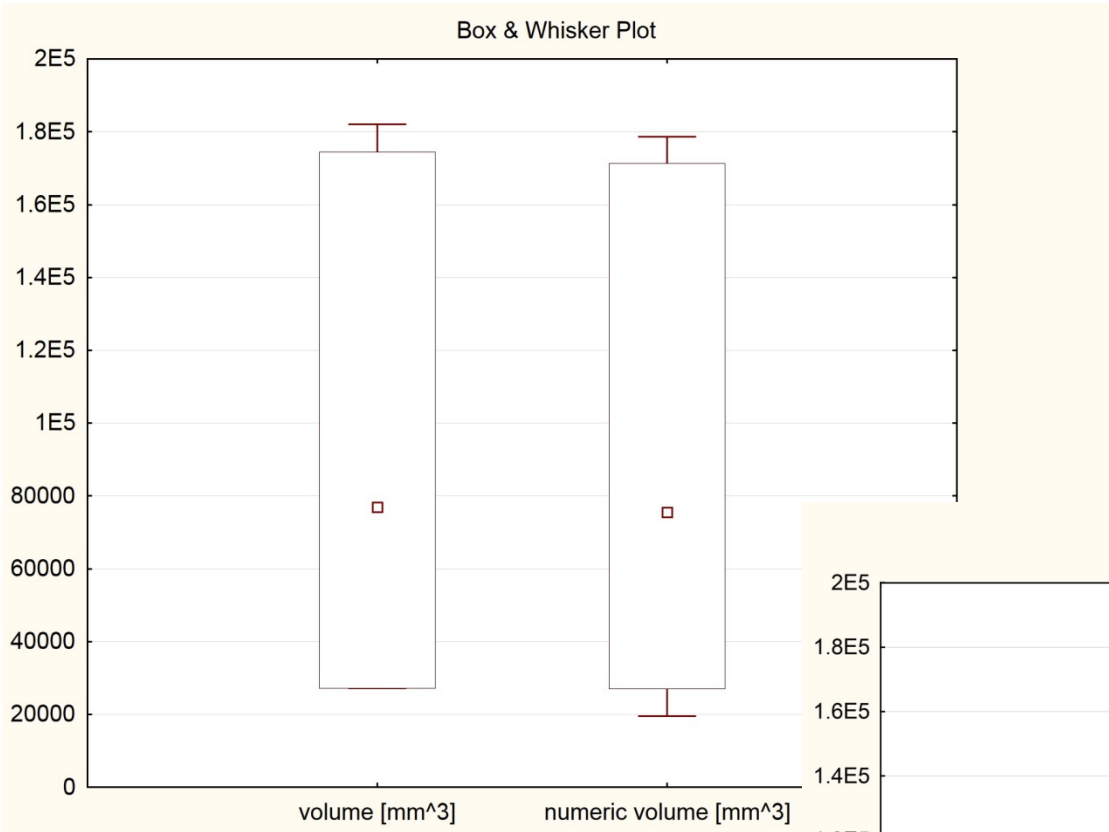
	volume (mm <sup>3</sup> )	<i>relative error</i>	surface (mm <sup>2</sup> )	<i>relative error</i>
known (analytic geometry)	4105593.235	-	436448.9461	-
calculated (Teigen triangulation)	4029915	<b>1.84</b>	433681	<b>0.63</b>
estimated using Fiji 3D Manager (ImageJ)	4405818	<b>7.31</b>	453426	<b>3.89</b>
estimated using pointgrid in Ellipse SW	4388875	<b>6.90</b>	-	-
estimated using thresholding in Ellipse SW	4390000	<b>6.95</b>	470919	<b>7.90</b>
estimated using Bruker micro-CT SW	4271889	<b>4.05</b>	486237	<b>11.41</b>

relative percent error =  $100 * |(value - estimate) / value|$

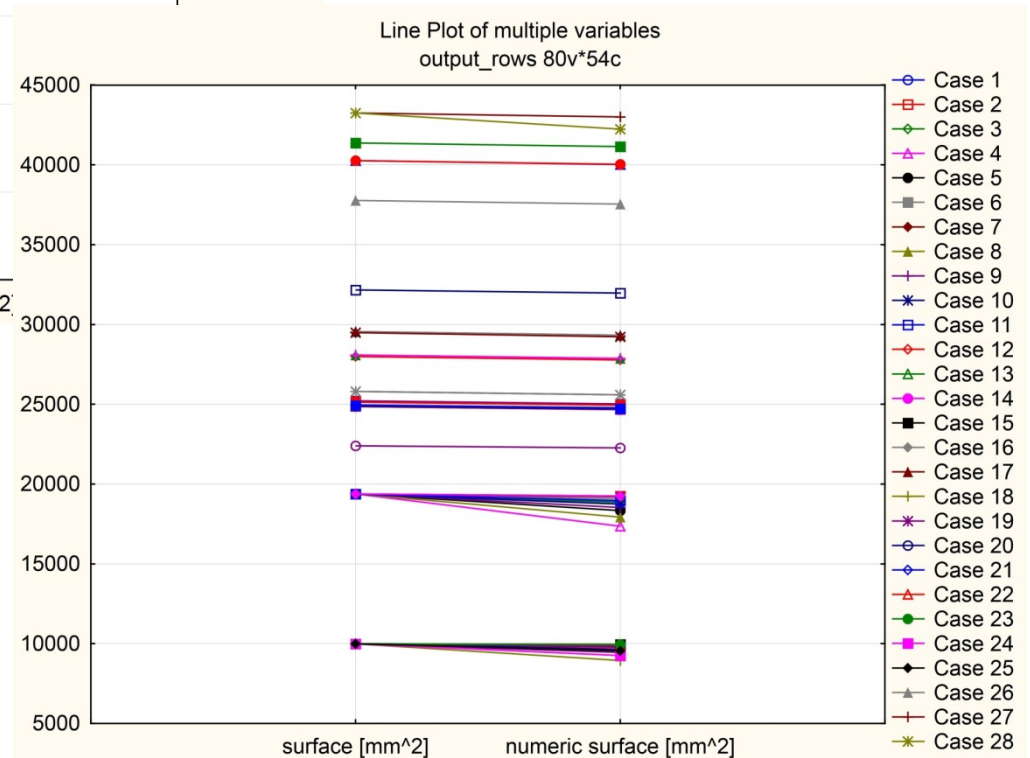
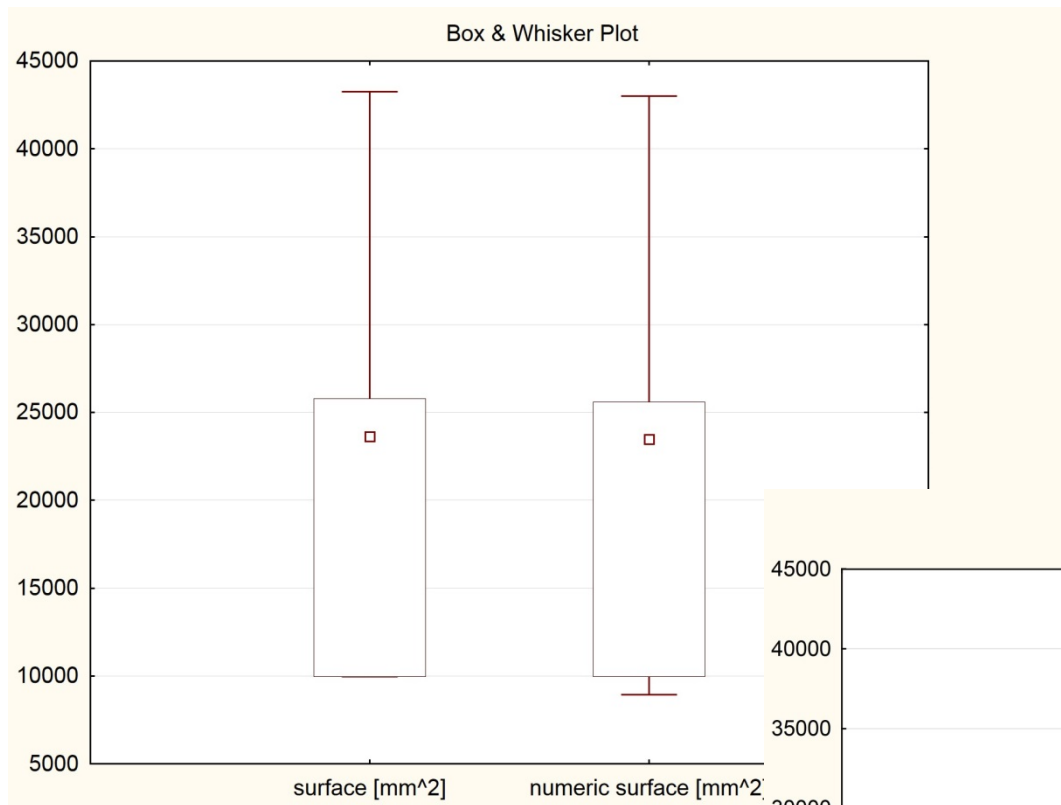


# Analytické počítání povrchů a objemů geometrických primitiv vs. numerická triangulace komplikovaných tvarů



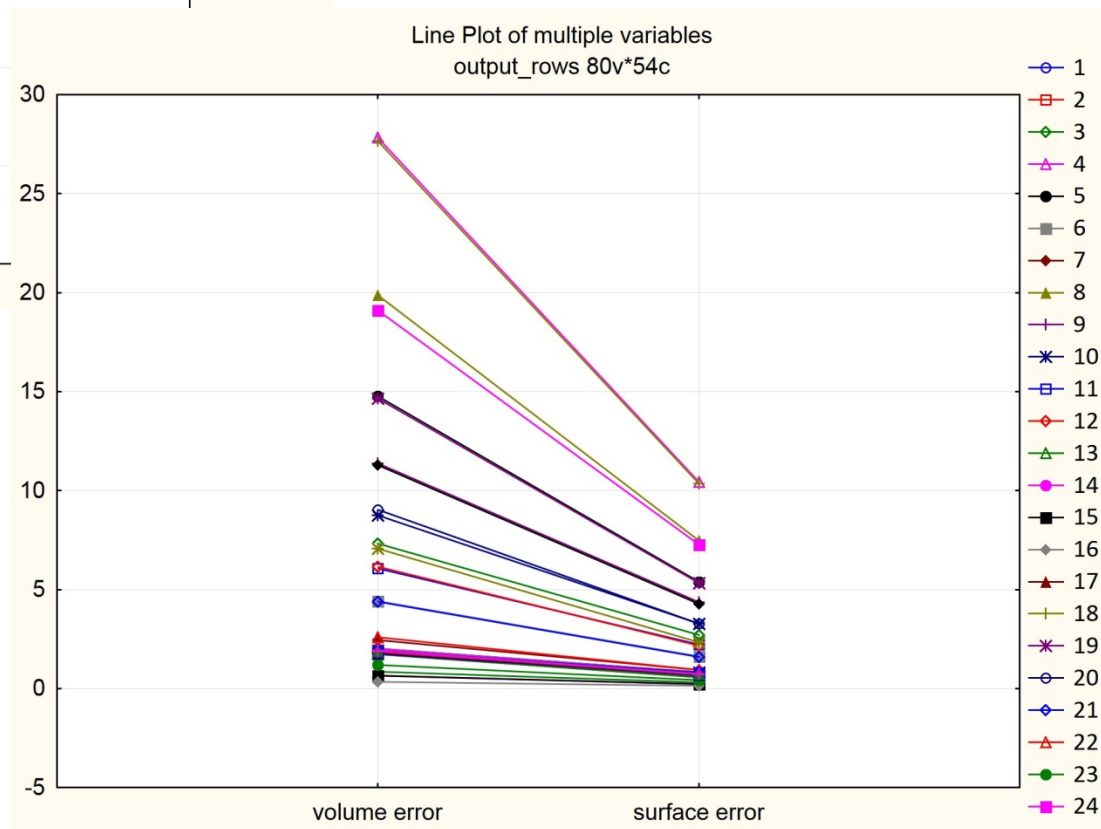
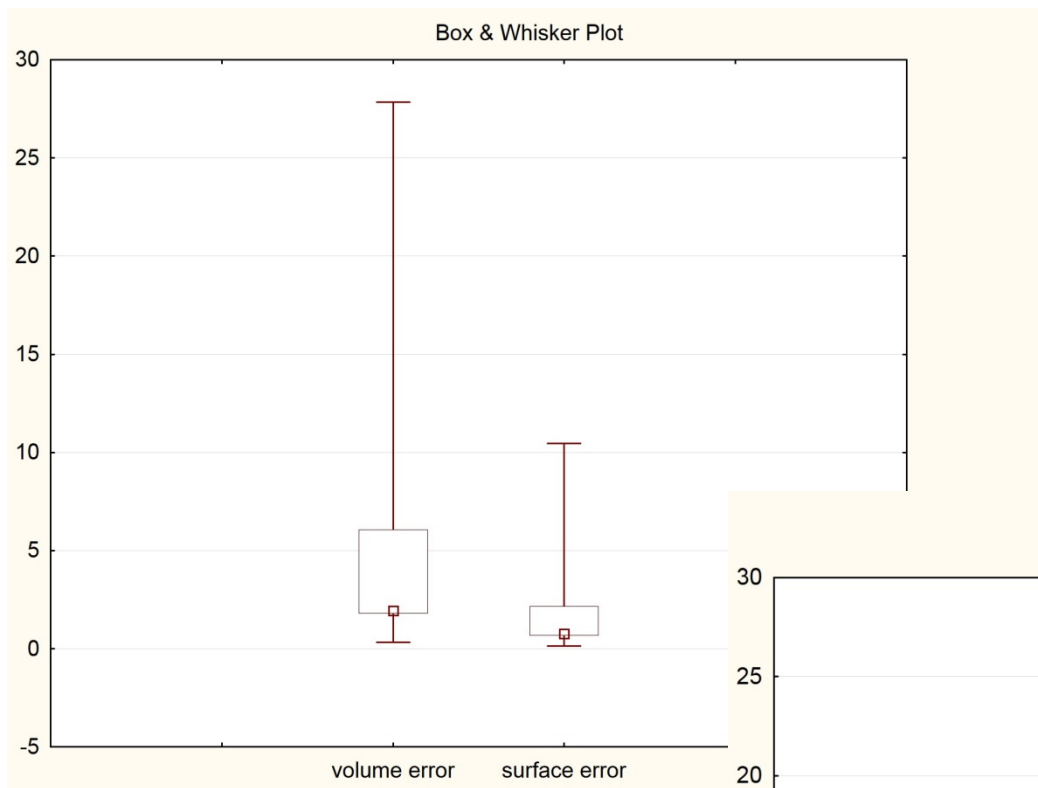


Wilcoxon matched pairs test  $p < 0.001$



Wilcoxon matched pairs test  $p < 0.001$



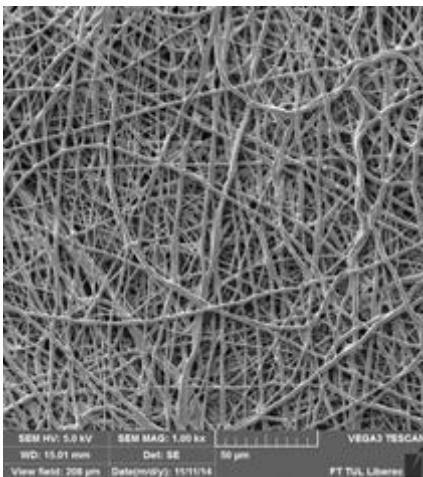


# Distribuce průměrů realistická, ale zaplnění prostoru dosud nedostižné...

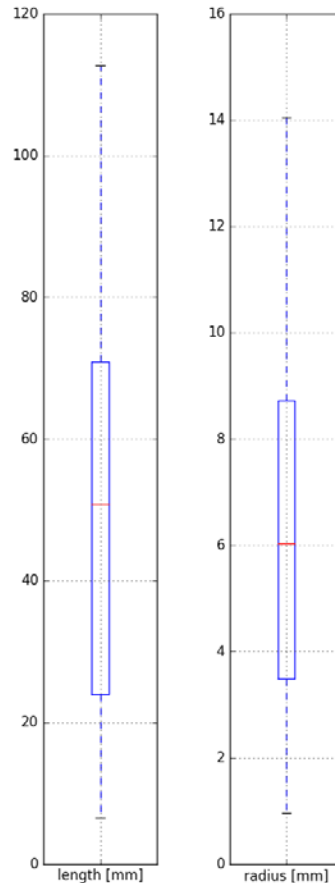
- zadání: průměr vláken

tubular PLC:

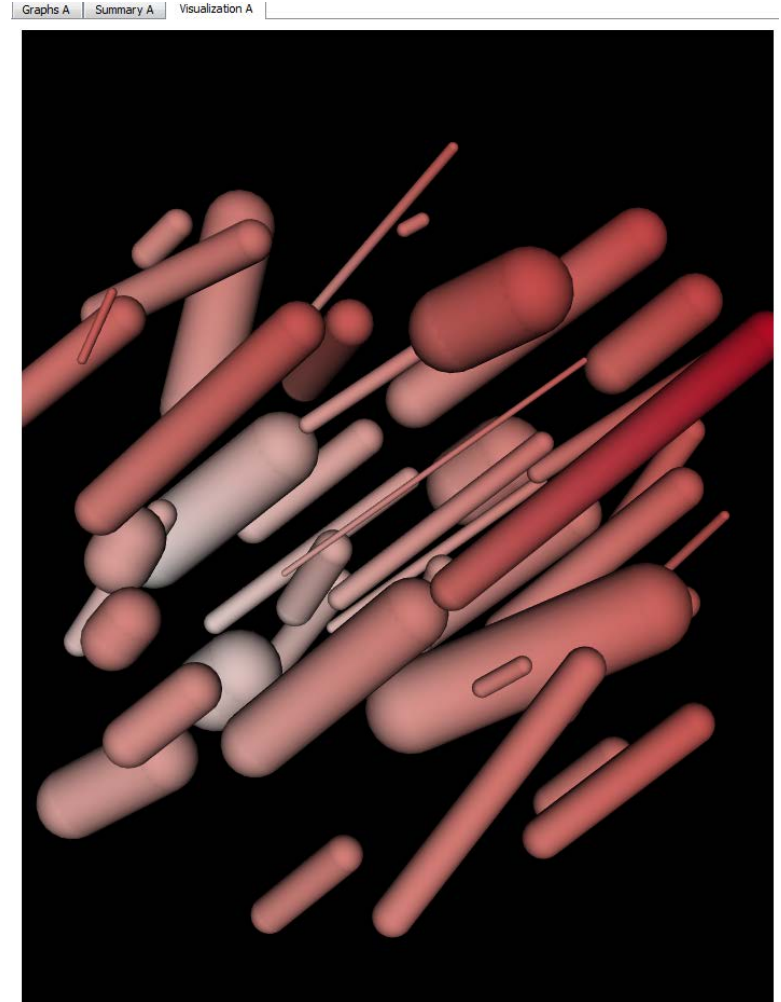
- Min 0,91  $\mu\text{m}$
- Max 14,05  $\mu\text{m}$
- Průměr 5,4  $\mu\text{m}$
- SD 2,1  $\mu\text{m}$
- Medián 5,5  $\mu\text{m}$



zaplnění prostoru cca 80 %



- simulace



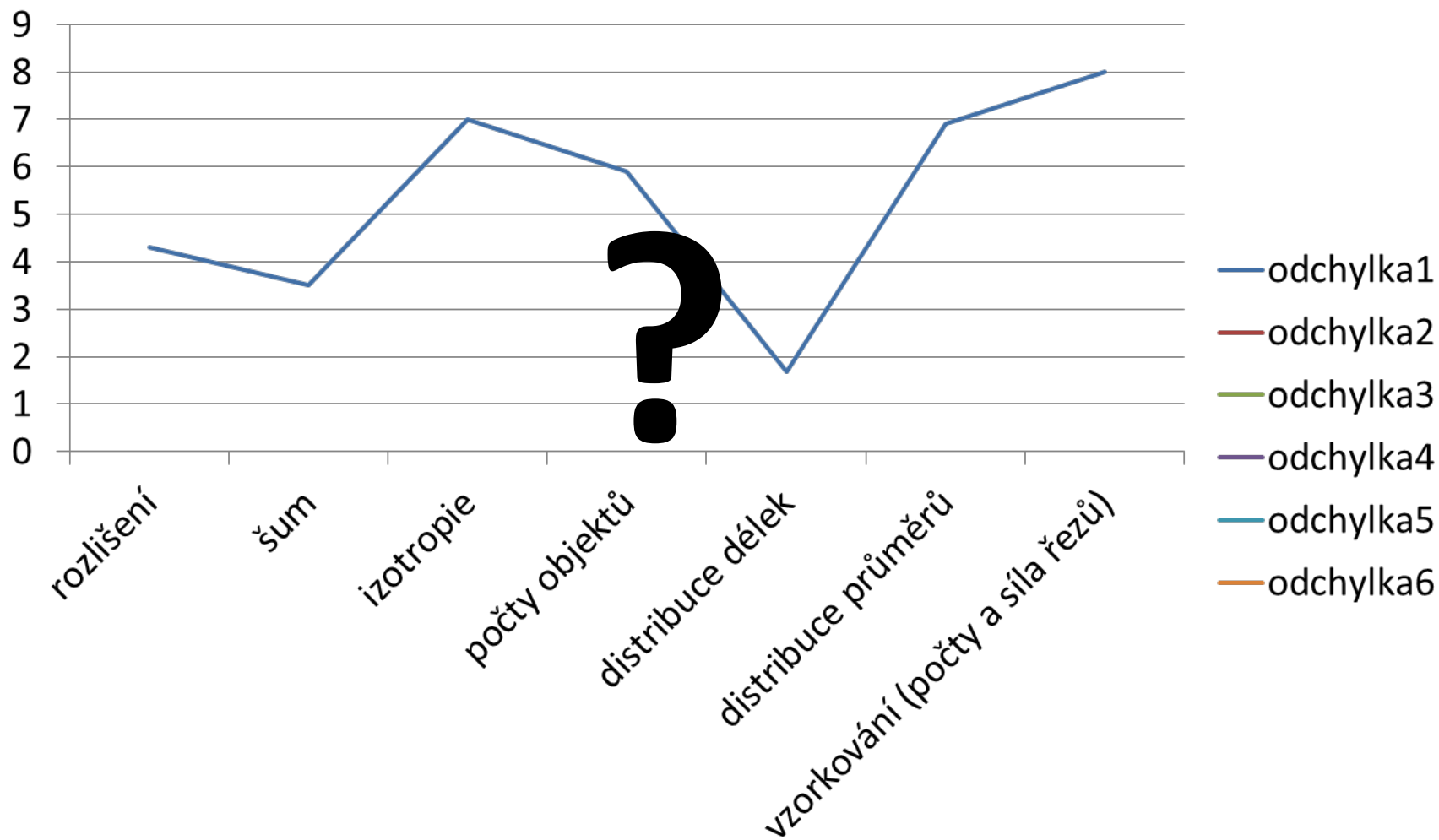
zaplnění prostoru 6,03 %

## Práce pokračuje...

- generátor **kolidujících** a **větvených** struktur
- problém s vyšším **zaplněním prostoru** srovnatelným s **reálnými** materiály
- generátor **porozit**
- ***ukazatel průběhu***
- **dávkové zpracování**



# nutno provést analýzu citlivosti



# Shrnutí – software Teigen

- nezávislý na **platformě**
- **volně dostupný** pro nekomerční použití
- s analyticky počítaným i verifikovaným **numerickým** odhadem **objemů, povrchů a délek**
- bude následovat
  - implementace dalších generátorů
    - **kolidující objekty** s vlivem vzájemných kontaktů, **větvení**
    - **porozity**
  - analýza **citlivosti**
  - popis **doporučených postupů** pro **minimalizaci chyb** při segmentaci dat v mikro-CT
  - nalezení a popis **rizikových postupů** ohrožujících validitu vyhodnocování obrazových dat v mikro-CT

# Děkuji Vám za pozornost!

