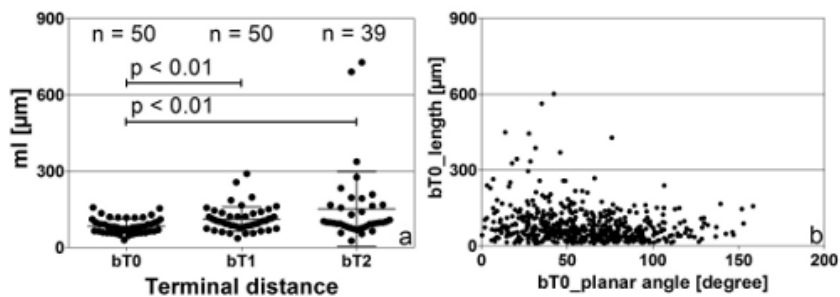


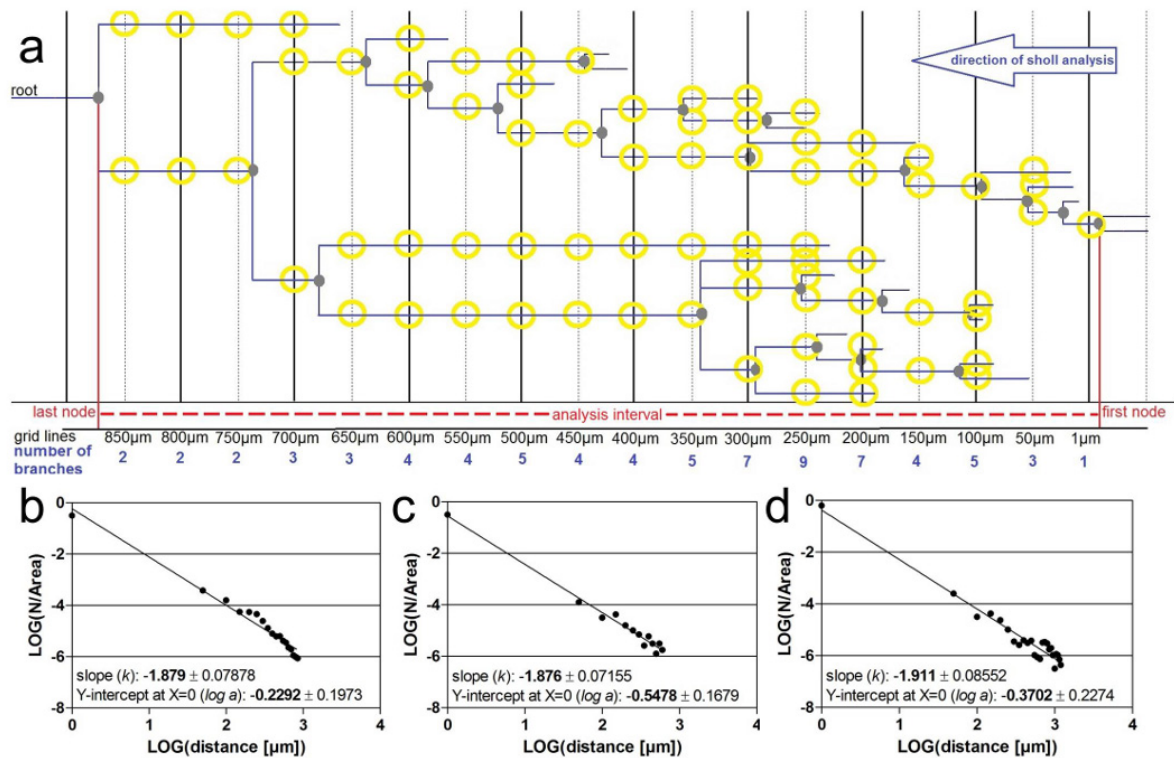
Novel 3D Microscopic Analysis of Human Placental Villous Trees Reveals Unexpected Significance of Branching Angles

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Supplementary Figures



Supplementary Figure S1: Very long terminal branches of the villous tree of the human placenta have a small planar branching angle. (a) Each dot represents the mean length of branches of a single isolated villous tree. The mean, standard deviation and mean lengths (dots) of bT0 branches (bT0_ml), bT1 branches (bT1_ml), and bT2 branches (bT2_ml) are shown. The mean length of the bT0 branches was statistically significantly different from the mean length of the bT1 and bT2 branches. (b) Mean length of the bt0 branches as a function of their planar angle..



Supplementary Figure S2: Sholl analysis of 3D data extracted from the villous trees of human placentas. (a) Illustration of the process of data extraction and subsequent Sholl analysis. The data extraction was based on the nodes (grey points) and branches (lines) of the dendrogram view with its x-scale in μm . The direction of analysis was defined from terminal end to the root and confined to the interval (analysis interval; red dashed line) between the first node (red solid line on the right; most distal branching point) and the last node (red solid line on the left; node most proximal to the root). Measuring lines were placed every $50\mu\text{m}$ and perpendicular to the branches (vertical black lines; $100\mu\text{m}$ intervals straight lines, $50\mu\text{m}$ dotted lines). The grid of measuring lines started at the terminal end of the villous tree. The first of the measuring lines falling into the analysis interval was placed at $1\mu\text{m}$, and the following lines were arranged in $50\mu\text{m}$ intervals. At each measuring line the number of intersections (yellow circles) with branches of the dendrogram was determined (blue numbers at the very bottom). The position of each measuring line in the analysis interval in μm and the number of branches at the respective measuring line were the raw data used for the Sholl-analysis. (b-d) Log/Log plots of three exemplary Sholl analyses. N/area is on the y-axis and formed by the number of intersections (N) and by the area, which is defined by the distance of the respective grid line in μm taken to the square and multiplied with π ($\frac{N}{\text{area}} = \frac{N}{\text{distance}^2 \cdot \pi}$). The distance of the grid line in μm is on the x-axis. The straight lines in (b to d) were defined by linear regression, with their slope being the Sholl regression coefficient k with its y intersection being $\log a$.

Supplementary Tables

Supplementary Table S1: Descriptive statistics of parameters of the villous tree of the human placenta obtained by 3D analysis.

Parameter	n	mean	SD
bT0_mpa [degree]	50	58.78	10.907
bT1_mpa [degree]	50	44.17	13.317
bT2_mpa [degree]	33	32.11	17.185
bT0_md [μm]	50	38.91	6.273
bT1_md [μm]	50	45.80	9.943
bT2_md [μm]	39	50.56	11.513
bT0_n	50	11.10	5.140
bT1_n	50	7.70	3.824
bT2_n	39	1.74	1.019
bT0_ml [μm]	50	84.36	27.716
bT1_ml [μm]	50	110.79	48.119
bT2_ml [μm]	39	150.96	146.023
bT0_ms [μm^2]	50	9988.39	4062.634
bT1_ms [μm^2]	50	15961.31	8105.258
bT2_ms [μm^2]	39	24688.72	28400.659
bT0_mv [μm^3]	50	97887.70	58713.082
bT1_mv [μm^3]	50	194404.61	143229.858
bT2_mv [μm^3]	39	335151.75	474652.064
<i>sholl</i> K-value	50	2.00	0.243
<i>sholl</i> Log a	50	0.104	0.156

Parameters are number (n), mean, and standard deviations (SD) of Neurolucida-derived parameters determined in the present study. Normal human placentas (n=50, one isolated peripheral villous tree per placenta) were investigated. Positions were encoded by (i) bT0: branch (b) in terminal position (T0), (ii) bT1: branch one node apart from the nearest terminal, and (iii) bT2: branch two nodes apart from the nearest terminal. Other parameters were expressed by the position code combined with: _mpa, mean planar branching angle; _md, mean diameter; _n, number; _ml, mean length; _ms, mean surface area; _mv, mean volume. Parameters determined by Sholl analysis were labelled with *sholl*. In 11 analyzed peripheral villous trees, bT2 positions were not found (reducing n to 39 for bT2_md and bT2_ml). In another 6 investigated peripheral villous trees, the branching angle of bT2 could not be determined because no previous segment was present (reducing n to 33 for bT2_mpa).

Supplementary Table S2: Clinical data of the two placenta collections used in the present study.

Parameter	3D Analysis			Histology		
	n	mean	SD	n	mean	SD
delivery mode	50			50		
—vaginal	21			26		
—sectio	29			24		
gestational week	50	39.34	1.08	50	39.65	1.20
birth weight [g]	50	3463	589	50	3412	426
placenta weight [g]	50	523	121	50	503	94
PW/BW ratio	50	0.150	0.023	50	0.149	0.028
thickness [cm]	50	1.73	0.35	50	1.59	0.43

3D Analysis: Cohort of placentas analyzed by Neurolucida-assisted 3D analysis. Histology: Cohort of placentas analyzed by thin 2D histological sections. The number (n) of placentas, mean and standard deviation of each parameter are given. The t-test with paired variables show no statistically significant differences between the two collections were observed.

Supplementary Table S3: Estimation of total processing time in hours [h] per sample and per step.

Process Steps	Processing Time in [h]	
	3D Analysis	Histology
Sampling of Tissue	0.3	0.3
Fixation	12	24
Bleaching/Rinsing	1	—
Dehydration/Embedding	—	48
Cutting/Deparaffination	—	2
Staining/Mounting	6	0.3
Computer-Assisted Analysis	0.5	1.5
Σ Processing Time/Sample	19.8	76.1

Three-dimensional analysis with Neurolucida software (3D Analysis) was compared to the analysis of thin 2D histological sections using Stereo Investigator software (Histology). Hematoxylin staining (3D Analysis) or hematoxylin/eosin staining (HE, Histology) were used. The estimated total processing time in hours is given as Σ Processing Time/Sample. The absence of a certain step from one of the work flows is indicated (—).

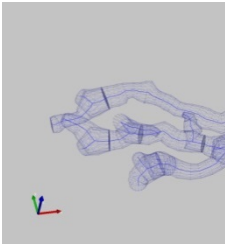
Supplementary Table S4: Descriptive statistics of parameters obtained from thin 2D histological sections of villous trees of human placentas.

Structure	n	mean Vol. [ml]	SD	mean Vol.Fr.	SD
Villous Tree	50	197.03	48.62	39.41	7.41
— <i>Villous Stroma</i>	50	102.50	34.18	20.59	6.03
— <i>Vessel Lumen</i>	50	52.59	23.09	10.37	3.77
— <i>Endothelium</i>	50	15.51	8.62	3.18	1.65
— <i>Syncytiotrophoblast</i>	50	26.43	9.73	5.26	1.60
Fibrinoid	50	43.01	28.40	8.66	5.18
Intervillous Space	50	263.97	69.08	51.65	8.85

Parameters were number (n), mean absolute volume (mean Vol.) with standard deviation (SD), dimensionless mean volume fraction (mean Vol.Fr.) with SD. The data were stratified by structural components (villous tree, fibrinoid, intervillous space); substructures (villous stroma, vessel lumen, endothelium, syncytiotrophoblast) are indented (—). For each normal human placenta one systematically and randomly sampled tissue block and one thin 2D histological section per tissue block were investigated.

Supplementary Movies

a) Still images for supplementary movies:



Still image 1: The still image shows a single frame of supplementary movie 1.



Still image 2: The still image shows a single frame of supplementary movie 2.



Still image 3: The still image shows a single frame of supplementary movie 3.



Still image 4: The still image shows a single frame of supplementary movie 4.

- b) Captions for supplementary movies 1-4; the movies are provided as separate files

Supplementary Movie S1: The movie shows the NeuroLucida-based three-dimensional reconstruction of a villous tree of a human placenta. The reconstructed tree turns around its x-axis (red). In a first phase of the movie, the entire reconstructed peripheral villous tree is shown. The second phase of the movie enlarges the most peripheral part of the villous tree.

Supplementary Movie S2: The movie shows an example of the visualization of the three-dimensional (3D) data set obtained by the analysis of a single isolated peripheral villous tree of a human placenta using NeuroLucida software. The single isolated peripheral villous tree is shown in a blue skeletonized view and turns clockwise to provide insight into the 3D nature of the data.

Supplementary Movie S3: The movie shows an example of the visualization of the three-dimensional (3D) data set obtained by the analysis of a single isolated peripheral villous tree of a human placenta using NeuroLucida software. The single isolated peripheral villous tree is shown in a blue skeletonized view and turns clockwise to provide insight into the three-dimensional nature of the data.

Supplementary Movie S4: The movie shows an example of the visualization of the three-dimensional data set obtained by the analysis of a single isolated peripheral villous tree of a human placenta using NeuroLucida software. The single isolated peripheral villous tree is shown in a blue skeletonized view and turns clockwise to provide insight into the three-dimensional nature of the data.