

Deep learning to decipher the progression and morphology of axonal degeneration

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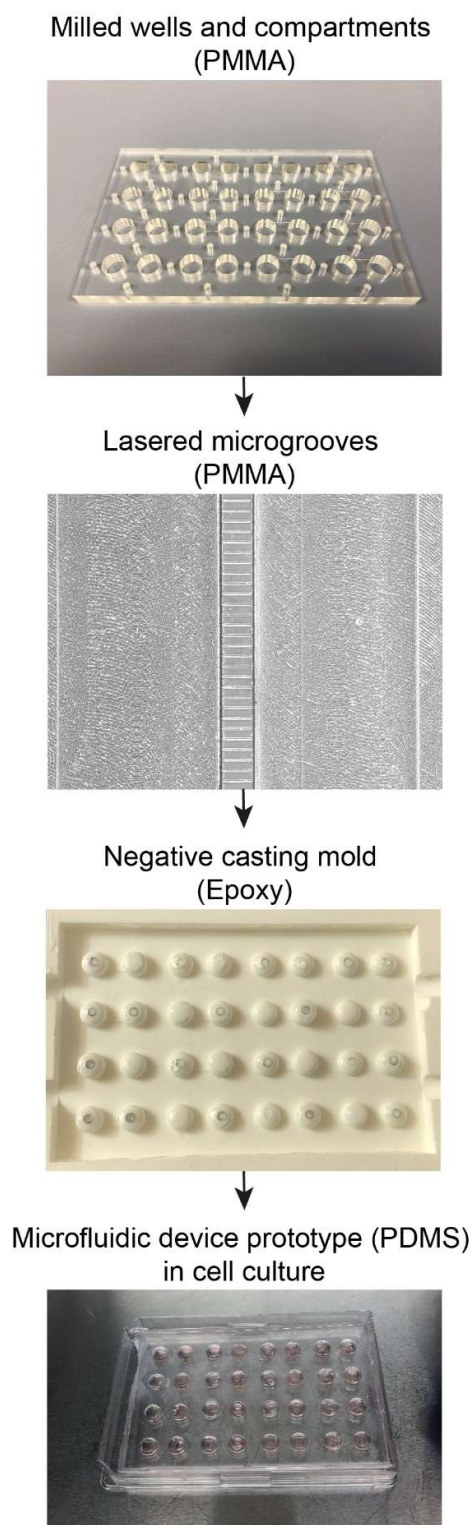
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29

Figure S1. Manufacturing of the microfluidic device for the enhanced throughput cultivation of axons. Individual components of the production process. A milled and lasered polymethyl methacrylate (PMMA) plate was used as a positive imprint to develop a negative casting mold produced from epoxy. Polydimethylsiloxane (PDMS) was poured into the negative casting mold, bonded onto glass and applied in the cell culture.

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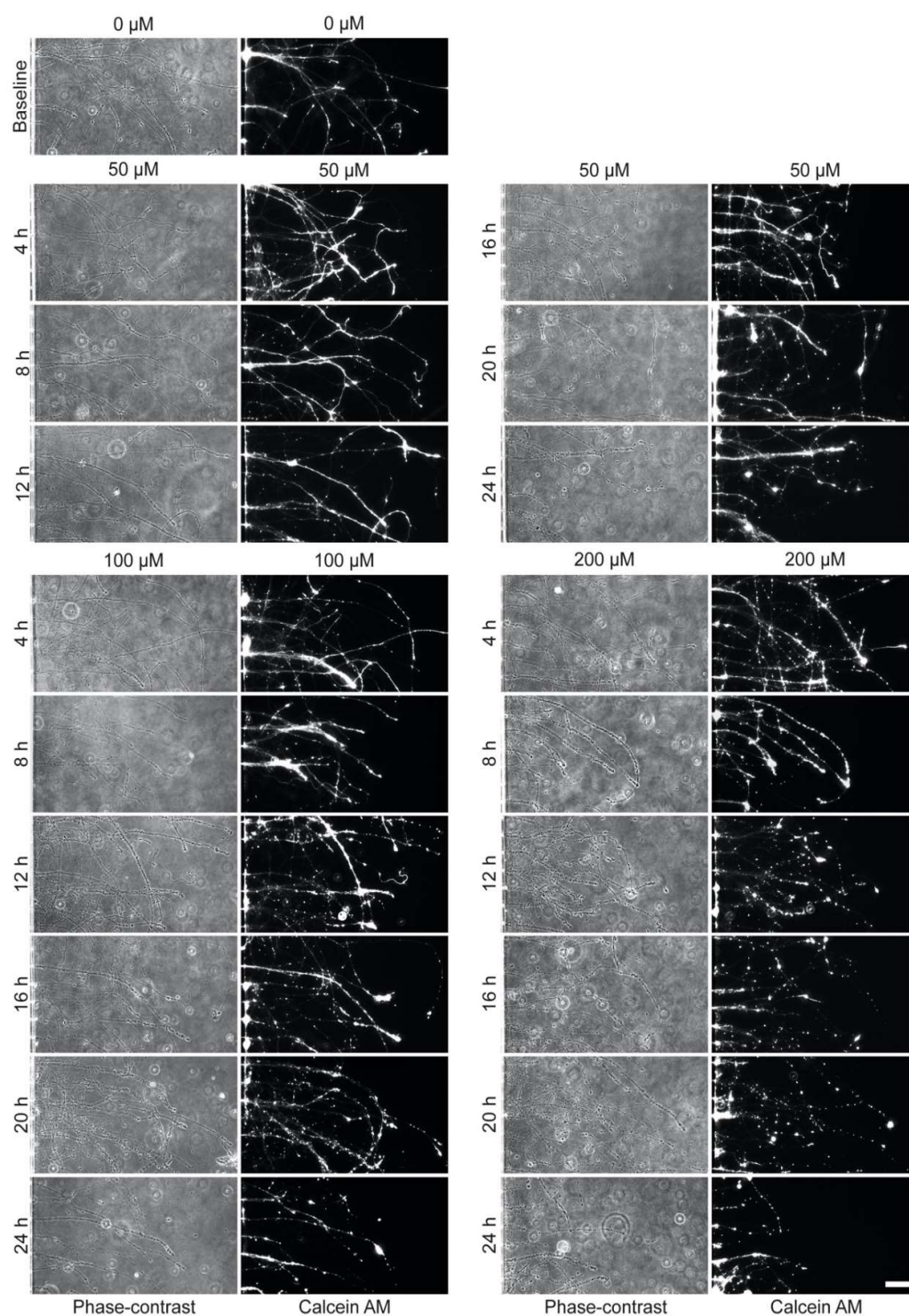


Figure S2. Validation of the time course of AxD by fluorescent live cell marker calcein AM. We determined the start and end of hemin-induced AxD by comparing the appearance of axonal swellings and axonal fragments in phase-contrast and fluorescence (calcein AM) microscopy. Compared to baseline (0 μM hemin), morphological hallmarks of AxD started to appear after 16 hours (50 μM), 12 hours (100 μM), and 8 hours (200 μM) and AxD was observed during the following 4 hours in all concentrations. N = 3 independent cultures of primary cortical neurons. Scale bar: 50 μm.

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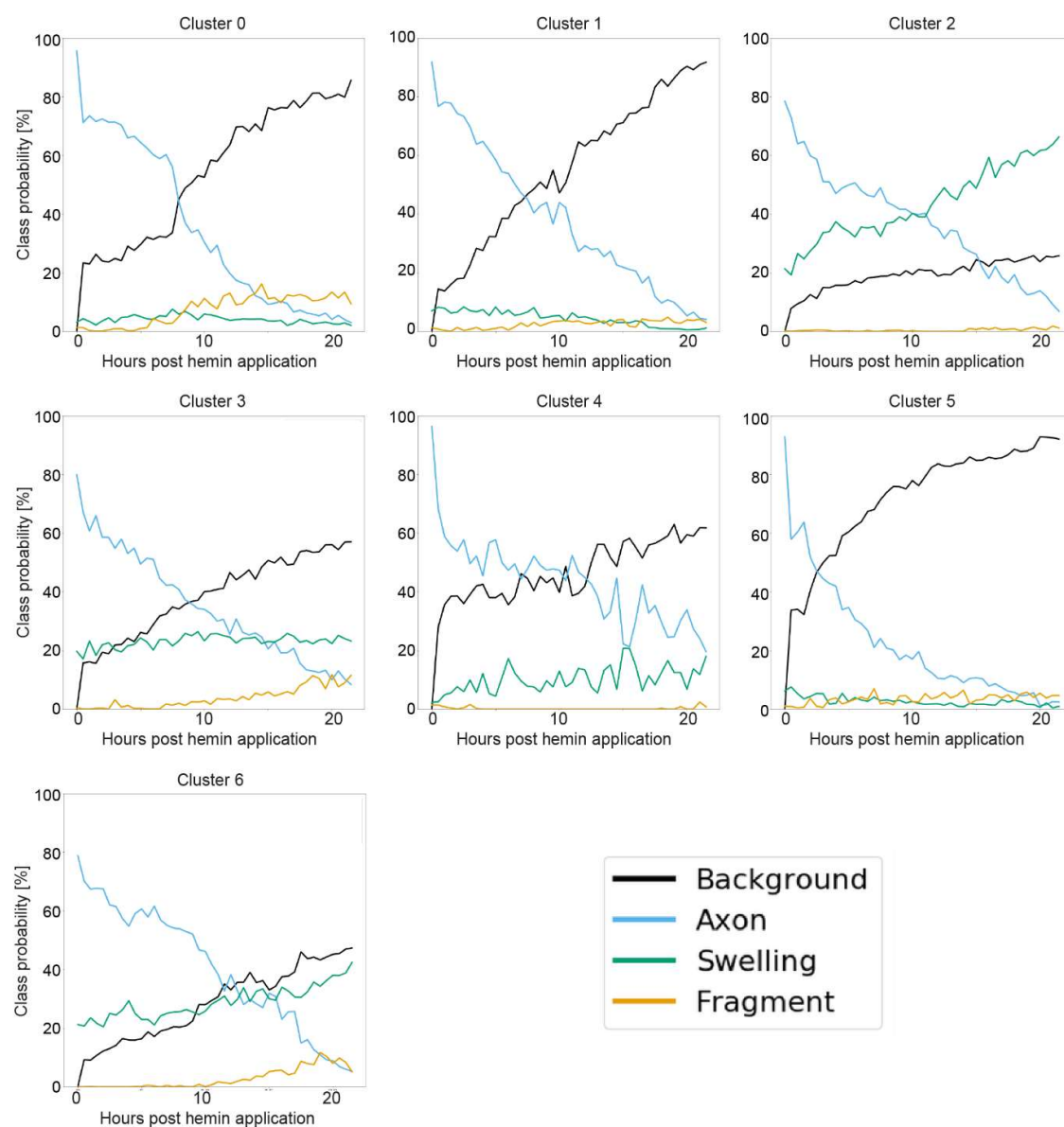


Figure S3. Cluster analysis of the four morphological patterns of AxD based on the changes in class segregation of the pixels. For each time point in comparison to the previous time point, the RNN computed the probability of a change in class for each pixel of an image. Each cluster (0-6) was characterized by an idiosyncratic segmentation pattern over 24 hours.

40
41
42
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Table S1. Chemicals and Reagents.

Product	Company	Catalogue number
B-27™ Plus Neuronal Culture System	Thermo Fisher Scientific	A3653401
Boric acid	Merck	925K12283365
Bovin serum albumin	Sigma Aldrich	A9418
Calcein AM	Santa Cruz	sc-203865
Chicken trypsin inhibitor	Sigma Aldrich	T9253
DAPI	Roche	10236276001
DNAse	Sigma Aldrich	D5025-15KU
Earle's balanced salt solution	Sigma Aldrich	E7510
Epoxy solution Smooth-Cast 310/1	KauPo	09202-003-000002
Ethanol 70%	Carl Roth	9065.5
Ethylenediaminetetraacetic acid	Carl Roth	8043.3
Fetal calf serum	Thermo Fisher Scientific	A15-151
Hemin	Sigma Aldrich	H9039
Horse serum	GE Health Life Sciences	SH30074.03
Laminin	Sigma Aldrich	L2020
L-cysteine	Carl Roth	1693.2
MEM, GlutaMAX™ Supplement	Thermo Fisher Scientific	41090028
Papain	Carl Roth	8933.1
Penicillin/Streptomycin	Biochrom	A2212
Phosphate buffered saline	Thermo Fisher Scientific	14200083
Polydimethylsiloxane	Biesterfeld Spezialchemie GmbH	5498840000
Poly-d-lysine	Sigma Aldrich	P6407
Polymethylmethacrylate	Kongsback	Customized
Sodium acetate	Carl Roth	X891.1
Sodium bicarbonate	Sigma Aldrich	S5761
Sodium pyruvate	Thermo Fisher Scientific	11360070
Sodium tetraborate	Merck	1.06308.1000
Triton-X-100	Fluka	93420

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46

Table S2. Antibodies.

Antibody	Company	Catalogue number	RRID
Polyclonal rabbit anti-microtubule-associated protein 2 (MAP2)	Abcam	ab32454	AB_776174
Monoclonal mouse anti-synaptophysin	Thermo Fisher Scientific	MA1-213	AB_2723681
Goat anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 546	Thermo Fisher Scientific	A-11030	AB_2534089
Goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 488	Thermo Fisher Scientific	A-11034	AB_2576217

Table S3. Time-dependent AxD in an *in vitro* model of hemorrhagic stroke.

Readout	Mauchly test	Omnibus Test	Posthoc Test
Axon area	[chi]2 is undefined because the number of repeated measurements is greater than the sample size; hence, sphericity was not assumed and the Greenhouse-Geisser correction was applied	one-way ANOVA with Greenhouse-Geisser correction ($\epsilon = 0.045$) across time*group: $F(6.109) = 19.758$, $P < 0.001$, partial- $\eta^2 = 0.748$	Post hoc Bonferroni $P = 0.018$ for 50 μM from 15 hours ($P < 0.001$ from 19 hours), $P = 0.040$ for 100 μM from 14 hours ($P < 0.001$ from 18.5 hours), $P = 0.020$ for 200 μM from 11.5 hours ($P < 0.001$ from 15 hours) vs. 0 μM
Axonal swelling area		one-way ANOVA with Greenhouse-Geisser correction ($\epsilon = 0.042$) across time*group: $F(5.703) = 3.201$, $P = 0.013$, partial- $\eta^2 = 0.324$	Post hoc Bonferroni $P = 0.030$ for 50 μM from 8 hours, $P = 0.019$ for 100 μM from 6 hours, $P = 0.010$ for 200 μM from 6 hours until 18.5 hours vs. 0 μM
Axonal fragment area		one-way ANOVA with Greenhouse-Geisser correction ($\epsilon = 0.026$) across time*group: $F(3.522) = 9.115$, $P < 0.001$, partial- $\eta^2 = 0.578$	Post hoc Bonferroni $P = 0.044$ for 100 μM from 17 hours, $P = 0.037$ for 200 μM from 9 hours vs. 0 μM

Table S4. AUC analyses of hemin-mediated AxD.

	Kolmogorov-Smirnov test	Levené test	Omnibus Test	Posthoc Test
Axon	Z = 0.093, P = 0.200	F(3,20) = 0.116, P = 0.949	one-way ANOVA F(3,20) = 8.547, P = 0.001, partial- η^2 = 0.562	Post hoc Bonferroni P = 0.026 for 50 μ M, P = 0.018 for 100 μ M, and P < 0.001 for 200 μ M vs. 0 μ M
Axonal swelling	Z = 0.104, P = 0.200	F(3,20) = 0.993, P = 0.416	one-way ANOVA F(3,20) = 6.721, P = 0.003, partial- η^2 = 0.502	Post hoc Bonferroni P = 0.012 for 50 μ M, P = 0.005 for 100 μ M, and P = 0.016 for 200 μ M vs. 0 μ M
Axonal fragment	Z = 0.245, P = 0.001	F(3,20) = 5.800, P = 0.005	Kruskal-Wallis Test [chi]2(3,N = 24) = 16.393, P = 0.001, η^2 = 0.713	Post hoc Mann-Whitney test with Bonferroni-Holm correction, P = 0.037 for 50 μ M, P = 0.008 for 100 μ M and 200 μ M vs. 0 μ M

Table S5. Comparison of the slopes of the linear regression of the four morphological patterns of AxD in an *in vitro* model of hemorrhagic stroke.

	Comparison of the slopes with Bonferroni-Holm correction for multiple comparisons
Granular degeneration vs. retraction degeneration	F(1,44) = 6.971, P = 0.034
Granular degeneration vs. swelling degeneration	F(1,44) = 0.202, P = 1.000
Granular degeneration vs. transport degeneration	F(1,44) = 8.865, P = 0.026
Retraction degeneration vs. swelling degeneration	F(1,44) = 7.842, P = 0.030
Retraction degeneration vs. transport degeneration	F(1,44) = 0.406, P = 1.000
Swelling degeneration vs. transport degeneration	F(1,44) = 9.074, P = 0.026

Supplementary Movies	57
	58
Video S1 (separate file). Time-lapse video of axons treated with 0 μM hemin.	59
Primary cortical axons were treated with vehicle (0 μ M) for 24 hours and recorded by time-lapse	60
microscopy in a 30-minutes interval and shown in 7 frames/second. Scale bar: 50 μ m.	61
Video S2 (separate file). Time-lapse video of axons treated with 50 μM hemin.	62
Primary cortical axons were treated with 50 μ M hemin for 24 hours and recorded by time-lapse	63
microscopy in a 30-minutes interval and shown in 7 frames/second. Scale bar: 50 μ m.	64
Video S3 (separate file). Time-lapse video of axons treated with 100 μM hemin.	65
Primary cortical axons were treated with 100 μ M hemin for 24 hours and recorded by time-lapse	66
microscopy in a 30-minutes interval and shown in 7 frames/second. Scale bar: 50 μ m.	67
Video S4 (separate file). Time-lapse video of axons treated with 200 μM hemin.	68
Primary cortical axons were treated with 200 μ M hemin for 24 hours and recorded by time-lapse	69
microscopy in a 30-minutes interval and shown in 7 frames/second. Scale bar: 50 μ m.	70
Video S5 (separate file). Time-lapse video of granular degeneration induced by hemin.	71
Granular degeneration of primary cortical axons treated with 200 μ M hemin for 24 hours	72
and recorded by time-lapse microscopy in a 30-minutes interval and shown in 7	73
frames/second. Scale bar: 20 μ m.	74
Video S6 (separate file). Time-lapse video of retraction degeneration induced by hemin.	75
Retraction degeneration of primary cortical axons treated with 200 μ M hemin for 24 hours	76
and recorded by time-lapse microscopy in a 30-minutes interval and shown in 7	77
frames/second. Scale bar: 20 μ m.	78
Video S7 (separate file). Time-lapse video of swelling degeneration induced by hemin.	79
Swelling degeneration of primary cortical axons treated with 200 μ M hemin for 24 hours	80
and recorded by time-lapse microscopy in a 30-minutes interval and shown in 7	81
frames/second. Scale bar: 20 μ m.	82
Video S8 (separate file). Time-lapse video of transport degeneration induced by hemin.	83
Transport degeneration of primary cortical axons treated with 200 μ M hemin for 24 hours	84
and recorded by time-lapse microscopy in a 30-minutes interval and shown in 7	85
frames/second. Scale bar: 20 μ m.	86
Video S9 (separate file). Time-lapse video of the segmentation by the recurrent	87
neuronal networks of AxD induced by hemin.	88
Segmentation of degenerating primary cortical axons treated with 200 μ M hemin for 24 hours and recorded by time-lapse	89
microscopy in a 30-minutes interval and shown in 7 frames/second. Scale bar: 100 μ m.	90