Supplementary information

Behavioural immune landscapes of inflammation

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This PDF file includes:

Other Supplementary Materials:

Supplementary Table 1. List of mouse strains used in the behavioral screening

Gene	Protein	Mutation	Driver	Specificity	Strain	Source
Selplg$^{-/-}$	PSGL1	KO	--	Not sp.	C57BL/6	Hidalgo (CNIC)
Fpr1$^{-/-}$	Fpr1	KO	--	Not sp.	C57BL/6	Soehnlein (U Münster)
Fcrg/ Dap12$^{-/-}$	FcγR/ DAP12	KO	--	Not sp.	C57BL/6	Zarbock (U Münster)
Cxcr2^{ΔN}/ Cxcr2$^{-/-}$	CXCR2	Floxed/ KO	Mrp8-Cre	Neu / Not sp.	C57BL/6	C. Weber (LMU)
Fgr$^{-/-}$	Fgr	KO	--	Not sp.	C57BL/6	J.A. Enríquez (CNIC)
Rap1a^{ΔM}	Rap1	Floxed	LysM-Cre	Myeloid	C57BL/6	Zarbock (U Münster)
Prex1/2$^{-/-}$	Prex	KO	--	Not sp.	C57BL/6	Welch (Babraham)
Rac1^{ΔM}	Rac1	KO	LysM-Cre	Myeloid	C57BL/6	Zarbock (U Münster)
Dap12$^{-/-}$	Dap12	KO	--	Not sp.	C57BL/6	Zarbock (U Münster)
Pik3cb$^{KD/+}$	PI3Kβ	Kinase Dead	--	Not sp.	C57BL/6	Graupera (IJC)
Fpr2$^{-/-}$	Fpr2	KO	--	Not sp.	C57BL/6	Soehnlein (U Münster)
Hck$^{-/-}$	Hck	KO	--	Not sp.	C57BL/6	A. Mocsai (Semmelweis)
Fcrg$^{-/-}$	FcγR	KO	--	Not sp.	C57BL/6	Zarbock (U Münster)
Lyn$^{-/-}$	Lyn	KO	--	Not sp.	C57BL/6	A. Mocsai (Semmelweis)
Syk^{ΔN}	Syk	Floxed	Mrp8-Cre	Neu	C57BL/6	Hidalgo (CNIC)
Cxcr4^{ΔN}	CXCR4	Floxed	Mrp8-Cre	Neu	C57BL/6	Hidalgo (CNIC)
Btk$^{-/-}$	Btk	KO	--	Not sp.	C57BL/6	R.W. Hendriks (Erasmus MC)
Mapk13$^{-/-}$	P38δ	KO	--	Not sp.	C57BL/6	G. Sabio (CNIC)
Rac2$^{-/-}$	Rac2	KO	--	Not sp.	C57BL/6	Welch (Babraham)
Cdc42^{ΔM}	Cdc42	Floxed	LysM-Cre	Myeloid	C57BL/6	Zarbock (U Münster)
Mapk14$^{-/-}$	P38γ	KO	--	Not sp.	C57BL/6	G. Sabio (CNIC)
Pik3cd$^{KD/KD}$	PI3Kδ	Kinase Dead	--	Not sp.	C57BL/6	Graupera (IJC)
Pik3cg$^{KD/KD}$	PI3Kγ	Kinase Dead	--	Not sp.	C57BL/6	E. Hirsch (U Torino)
Mst1$^{-/-}$	Mst1	KO	--	Not sp.	C57BL/6	M. Sperandio (LMU Munich)
Tnfr$^{-/-}$	TNFR	KO	--	Not sp.	C57BL/6	Soehnlein (U Münster)

IJC, Insitute Josep Carreras; LMU, Ludwig-Maximilians University
Not sp, not specific; Neu, neutrophil-specific
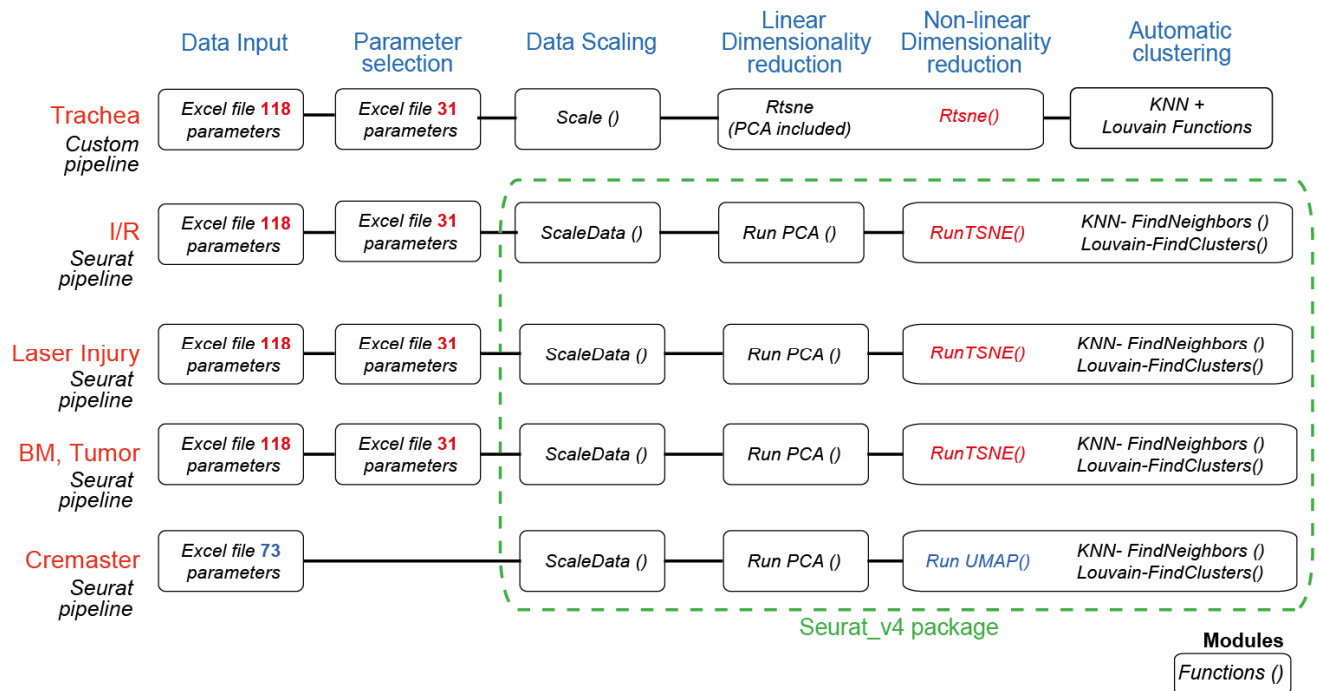
Supplementary Table 2. Imaging parameters

Influenza-infected trachea	
Microns per pixel (µm)	0.854
Step Size (Z)(µm)	3
Total Z (µm)	54
Scan frequency (sec)	30
total time (hh:mm)	00:30
Laser burn injury (skin)	
Microns per pixel (µm)	0.87
Step Size (Z)(µm)	4
Total Z (µm)	44
Scan frequency (sec)	60
total time (hh:mm)	02:33
Ischemia/reperfusion (skin)	
Microns per pixel (µm)	0.99
Step Size (Z)(µm)	4
Total Z (µm)	84
Scan frequency (sec)	300
total time (hh:mm)	02:55
Cremaster muscle	
Microns per pixel (µm)	0.667
Step Size (Z)(µm)	2
Total Z (µm)	26
Scan frequency (sec)	8.8
total time (hh:mm)	00:06
Bone Marrow	
Microns per pixel (µm)	0.774
Step Size (Z)(µm)	4
Total Z (µm)	56
Scan frequency (sec)	20
total time (hh:mm)	00:40
Tregs vs. CTLs (tumor)	
Microns per pixel (µm)	1.20
Step Size (Z)(µm)	5
Total Z (µm)	45
Scan frequency (sec)	15
total time (hh:mm)	00:08
CTLs in tumor regions	
Microns per pixel (µm)	1.20
Step Size (Z)(µm)	4
Total Z (µm)	52
Scan frequency (sec)	30
total time (hh:mm)	00:15

Supplementary Table 3. Summary of imaging acquisition and data analysis workflows

Experiment	Microscopy	Image analysis	Representation
Trachea	2PM microscopy	Imaris	RStudio / tSNE
Skin-I/R	2PM microscopy	Imaris	Seurat_v4 / tSNE
Skin-laser injury	2PM microscopy	Imaris	Seurat_v4 / tSNE
Skin-CT26 tumor	2PM microscopy	Imaris	Seurat_v4 / tSNE
BM-CXCL1	2PM microscopy	Imaris	Seurat_v4 / tSNE
Cremaster 4D	Spinning-disk confocal	ACME	Seurat_v4 / UMAP
Cremaster 2D	Epifluorescence	Fiji_cell tracking	unidimensional plot GraphPad
Cremaster 3D	Spinning-disk confocal	Imaris	unidimensional plot GraphPad

BM, bone marrow; I/R, ischemia-reperfusion injury

Supplementary Table 4. General analytical workflow and modules for the imaging datasets

	Data Input	Parameter selection	Data Scaling	Linear Dimensionality reduction	Non-linear Dimensionality reduction	Automatic clustering
Trachea *Custom pipeline*	Excel file **118** parameters	Excel file **31** parameters	Scale ()	Rtsne (PCA included)	Rtsne()	KNN + Louvain Functions
I/R *Seurat pipeline*	Excel file **118** parameters	Excel file **31** parameters	ScaleData ()	Run PCA ()	RunTSNE()	KNN- FindNeighbors () Louvain-FindClusters()
Laser Injury *Seurat pipeline*	Excel file **118** parameters	Excel file **31** parameters	ScaleData ()	Run PCA ()	RunTSNE()	KNN- FindNeighbors () Louvain-FindClusters()
BM, Tumor *Seurat pipeline*	Excel file **118** parameters	Excel file **31** parameters	ScaleData ()	Run PCA ()	RunTSNE()	KNN- FindNeighbors () Louvain-FindClusters()
Cremaster *Seurat pipeline*	Excel file **73** parameters		ScaleData ()	Run PCA ()	Run UMAP()	KNN- FindNeighbors () Louvain-FindClusters()

Seurat_v4 package

Modules
Functions ()

Supplementary Table 5. List and ranking of image-extracted parameters

Selected Variables for Imaris

Variable # (after selection)	Parameter (Short name)	Parameter full name	ARI (Adjusted Rand Index)	Type (Track/Object)	Morpho/ kinetic	Description
1	total distance	Total distance	0.4084	T	K	Indicates total distance travelled (i.e. the total length of displacements within the Track).
2	max speed	Maximum speed	0.3739	T	K	
3	mean speed	Mean speed	0.3889	T	K	
4	meander ratio	Meander ratio	0.3545	T	K	Calculated as the total distance divided by the displacement length of the track. When the index is between 1 and 1.5 the track is sinuous, but if between 1.5 and 4, then meandering. Index of 1 (straight).
5	tortuosity	Tortuosity	0.3832	T	K	Tortuosity is a property of a curve being tortuous. The inverse of meander ratio.
6	angle vs stream	Angle between the trajectory and the steam	0.3677	T	K	Angle between the trajectory (straight line that goes from the first position to the last position of the trajectory) and the bloodstream.
7	mean volume	Mean volume	0.4219	T	M	
8	mean surface area	Mean surface area	0.4111	T	M	
9	mean height	Mean height respect to the wall	0.3930	T	M	
10	mean max length	Mean of the maximum length	0.3685	T	M	
11	mean H/L ratio	Mean of the height/length ratio	0.4240	T	M	
12	mean sphericity	Mean sphericity	0.3598	T	M	
13	mean ellip prolate	Mean ellipticity prolate	0.4376	T	M	
14	mean ellip oblate	Mean ellipticity prolate	0.3893	T	M	
15	mean length A axis	Mean length principal axis (A)	0.4401	T	M	
16	mean length B axis	Mean length second principal axis (B)	0.3981	T	M	
17	mean length C axis	Mean length third principal axis (C)	0.4260	T	M	
18	mean extent	Mean extent	0.3448	T	M	
19	mean solidity	Mean solidity	0.4012	T	M	
20	mean equivalent diam	Mean equivalent diameter	0.4705	T	M	
21	volume Std	Standard deviation of the Volume	0.4151	T	M	
22	surface area Std	Standard deviation of the surface area	0.4788	T	M	

23	max area Std	Standard deviation of the maximum area	0.3767	T	M	
24	max length Std	Standard deviation of the maximum length	0.4406	T	M	
25	H/L ratio Std	Standard deviation of the height/length ratio	0.3206	T	M	
26	sphericity Std	Standard deviation of sphericity	0.2178	T	M	
27	ellip prolate Std	Standard deviation of ellipticity prolate	0.4573	T	M	
28	ellip oblate Std	Standard deviation of ellipticity oblate	0.4370	T	M	
29	length A axis Std	Standard deviation of length principal axis (A)	0.5391	T	M	
30	length B axis Std	Standard deviation of length principal axis (B)	0.5119	T	M	
31	length C axis Std	Standard deviation of length principal axis (C)	0.4781	T	M	
32	extent Std	Standard deviation of the extent	0.4620	T	M	
33	solidity Std	Standard deviation of the solidity	0.4417	T	M	
34	equivalent diam Std	Standard deviation of the equivalent diameter	0.5305	T	M	
35	mean polar radius	Mean of the polar radius	0.1925	T	K	
36	mean polar angle	Mean of the polar angle	0.3787	T	K	
37	polar radius Std	Standard deviation of the polar radius	0.1094	T	K	
38	polar angle Std	Standard deviation of the polar angle	0.2835	T	K	
39	ori X mean	Mean orientation respect to steam (X)	0.3533	T	K	
40	ori Y mean	Mean orientation respect to steam (Y)	0.3886	T	K	
41	ori Z mean	Mean orientation respect to steam (Z)	0.3287	T	K	
42	ori X Std	Standard deviation of the orientation respect to steam (X)	0.4321	T	K	
43	ori Y Std	Standard deviation of the orientation respect to steam (Y)	0.5238	T	K	

44	ori Z Std	Standard deviation of the orientation respect to steam (Z)	0.4246	T	K	
45	mean flattening	Mean distance of the centre of neutrophil to the wall (flatenning)	0.2995	T	M	
46	mean min dist to wall	Mean of the minimum distance to wall	0.3522	T	K	The mean of minimum distance of the neutrophil in each time step to the blood vessel surface.
47	track min dist to wall	Minimun distance of the trayectory to wall	0.00529	T	K	The minimum distance of a trajectory to blood vessel surface.
48	mean net score	Mean neural network score	0.2261	T	M	
49	max net score	Maximum neural network score	0.2225	T	M	
50	min net score	Minimum neural network score	0.3640	T	M	
51	network score range	Neural network score range	0.3761	T	M	
52	volume	Volume	0.06198	O	M	Number of voxels of the neutrophil.
53	surface area	Surface area	0.07241	O	M	Superficial area of the neutrophil in pixels.
54	height	Height respect to the wall	0.00595	O	M	Height of the neutrophil respect to the vessel wall.
55	max length	Maximum length	0.02487	O	M	Maximum width of an object
56	H/L ratio	height/length ratio	0.03479	O	M	height/length ratio of a neutrophil (object)
57	sphericity	Sphericity	0.01640	O	M	Sphericity measures how closely the shape of an object resembles that of a perfect sphere. Any particle that is not a sphere will have sphericity less than 1.
58	ellipticity prolate	Ellipticity prolate	0.01645	O	M	An oblate spheroid is the shape of the Earth and some other planets. It is like a sphere squashed from the top so the circumference around the poles is less than the circumference around the equator. Shapes of this type are called ellipsoids.
59	ellipticity oblate	Ellipticity oblate	0.01220	O	M	Prolate spheroids — A *spheroid* has circular symmetry. If the ellipse is rotated about its major axis, the result is a *prolate* (elongated) *spheroid*, shaped like an American football or rugby ball.
60	length A axis	Length principal axis (A)	0.01676	O	M	Length of the longest principal axis of the best-fitted ellipsoid into the segmented object (neutrophil)
61	length B axis	Length second principal axis (B)	0.04391	O	M	Length of the second principal axis of the best-fitted ellipsoid into the segmented object (neutrophil). B is perpendicular to A and C.
62	length C axis	Length third principal axis (C)	0.05992	O	M	Length of the third principal axis of the best-fitted ellipsoid into the segmented object (neutrophil). C is perpendicular to A and B.
63	extent	Extent	0.01455	O	M	Pixel ratio between the neutrophil and the bounding box that contains it (it can be an indicator of the quality of the

						segmentation, since a good segmentation must be compact).
64	solidity	Solidity	0.01917	O	M	Pixel ratio between the region and the smallest convex polygon that encompasses the region (as above, it can be an indicator of the compactness of the segmentation).
65	equivalent diam	Equivalent diameter	0.06950	O	M	The equivalent diameter is defined as the diameter of a circle with an equal aggregate sectional area, which is calculated by d = 2 Area / π .
66	ori X stream	Orientation respect to steam (X)	0.01707	O	K	3D orientation of the neutrophil with respect to the direction of the bloodstream along the trajectory (X axis).
67	ori Y stream	Orientation respect to steam (Y)	0.02472	O	K	3D orientation of the neutrophil with respect to the direction of the bloodstream along the trajectory (Y axis).
68	ori Z stream	Orientation respect to steam (Z)	0.01141	O	K	3D orientation of the neutrophil with respect to the direction of the bloodstream along the trajectory (Z axis).
69	polar radius	Polar radius	0.03839	O	K	3D position of the neutrophil with respect to the cross section of the vessel in polar coordinates: distance from the center (between 0 and 1).
70	polar angle	Polar angle	0.02997	O	K	3D position of the neutrophil with respect to the cross section of the vessel in polar coordinates: angle (between 0 and 2Pi).
71	flattening	Distance centre of neutrophil to the wall (flattening)	0.00482	O	M	Distance between the center of the neutrophil (object) and the vessel wall.
72	min distance to wall	Minimum distance to wall	0.00157	O	K	Minimum distance between the neutrophil (object) and the vessel surface (that of the voxel closest to the vessel).
73	network score	Neural network score	0.01591	O	M	The neural network includes a module that provides a probability value between 0 and 1 for each of the 3D regions resulting from the segmentation. This value indicates the reliability of each region based on its size and shape (for example, compact regions with a size similar to that of the neutrophils of interest will be more reliable).

Ranking (LRI/ARI) of the Selected Variables for Imaris

Variable # (after selection)	Parameter (full name)	Trachea LRI	Laser injury LRI	Ischemia reperfusion ARI	Geometric mean	Morpho/kinetic	Description
97	Track Speed Variation	0.9139	0.00326	0.08261	0.06267	K	Ratio of the Track Standard Deviation to the Track Speed Mean value; i.e. variability 'normalized' to its own speed.
64	Track Area Mean	0.7569	0.1047	0.2427	0.2679	M	Mean of surface area of the object along the track.

100	Track Volume Mean	0.7237	0.2745	0.3797	0.4225	M	The mean volume of all Surfaces along the tracks.
83	Track Ellipsoid Axis Length B Mean	0.6445	0.2048	0.1039	0.2394	M	Mean of the length of the second longest principal axis of the best-fitted ellipsoid into the objects that forms the track.
82	Track Ellipsoid Axis Length A Mean	0.6358	0.2527	0.1553	0.2922	M	Mean of the length of the shortest principal axis of the best-fitted ellipsoid into the objects that forms the track.
8	Area	0.6309	0.05402	0.1176	0.1588	M	Surface area of the object.
56	Volume	0.6029	0.1663	0.2478	0.2918	M	Quantification of how much space an object occupies.
84	Track Ellipsoid Axis Length C Mean	0.5808	0.1664	0.2965	0.3060	M	Mean of the length of the longest principal axis of the best-fitted ellipsoid into the objects that forms the track.
40	Ellipsoid Axis Length B	0.4981	0.09786	0.02034	0.0997	M	Length of the second longest principal axes of the best-fitted ellipsoid into the surface.
39	Ellipsoid Axis Length A	0.4848	0.1207	0.05635	0.1490	M	Length of the shortest principal axes of the best-fitted ellipsoid into the surface.
95	Track Speed Mean	0.4546	0.5140	0.2759	0.4010	K	Mean of the value of the object's speed on the track.
41	Ellipsoid Axis Length C	0.4405	0.08559	0.1316	0.1706	M	Length of the longest principal axes of the best-fitted ellipsoid into the surface.
71	Track Displacement Length	0.4323	0.00042	0.1040	0.02663	K	The object's Track Displacement is the distance between the first and last object's position.
46	Speed	0.3443	0.1131	0.05282	0.1272	K	instantaneous speed of the object (μm/s)
99	Track Straightness	0.3319	0.1581	0.03790	0.1258	K	Equals Track Displacement/ Track Length
21	Displacement 2	0.2841	0.00221	0.06798	0.03495	K	The square of the distance of the object from the starting point of the track (also known as mean square displacement: MSD) when plotted against the relative time since the beginning of the track.
72	Track Duration	0.2299	0.02112	0.1106	0.08128	K	The Track Duration is the duration between the first and last time point within the track.
25	Displacement Delta Length	0.2130	0.1763	0.08985	0.1500	K	The length of the position difference of the current object and the previous object of the same track.
117	TrackX Sphericity Std	0.1830	0.2667	0.06521	0.1471	M	Describes the SD of Surfaces Sphericity along the tracks.
98	Track Sphericity Mean	0.1516	0.2102	0.3604	0.2256	M	Describes the mean Surfaces Sphericity along the tracks.
115	TrackX Ellipticity(prolate) Std	0.1467	0.09620	0.09357	0.1097	M	This parameter defines the SD of the Ellipsoid

						Prolate for the track (see below for the detailed prolate definition).
113	TrackX Ellipticity(oblate) Std	0.1266	0.1218	0.04042	0.08542	M	This parameter defines the SD of the Ellipsoid Oblate for the track (see below for the detailed oblate definition)
47	Sphericity	0.1054	0.1060	0.2082	0.1325	M	Sphericity is a measure of how spherical an object is. When the value = 1, the volume is a sphere.
4	Acceleration	0.05262	0.11977	0.1047	0.0870	K	Acceleration is the change in the Surface velocity over time. The acceleration at the first and last time-point is 0.
103	TrackX BoundingBoxOOLengthB Std	0.02733	0.01059	0.06822	0.02702	M	BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation. SD of the length of the second longest principal axis of the best-fitted ellipsoid into the objects that forms the track.
101	TrackX BoundingBoxOOLengthA Std	0.01131	0.00088	0.06758	0.00876	M	BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation. SD of the length of the shortest principal axis of the best-fitted ellipsoid into the objects that forms the track.
105	TrackX BoundingBoxOOLengthC Std	0.00863	0.00496	0.1145	0.01699	M	BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation. Mean of the length of the longest principal axis of the best-fitted ellipsoid into the objects that forms the track.
86	Track Ellipticity Prolate Mean	0.00142	0.01135	0.08283	0.01101	M	This parameter defines the mean Ellipsoid Prolate for the track (see below for the detailed prolate definition).
43	Ellipticity (prolate)	0.00069	0.00163	0.01615	0.00263	M	A prolate spheroid is a sphere (or ellipsoid) squashed from the equator so that ellipses around the poles are longer than the

							circumference around the equator.
85	Track Ellipticity Oblate Mean	0.00027	0.02884	0.1305	0.01005	M	This parameter defines the mean Ellipsoid Oblate for the track (see below for the detailed oblate definition)
42	Ellipticity (oblate)	0.00014	0.00481	0.03579	0.00288	M	An oblate spheroid is a sphere (or ellipsoid) squashed from the top so that ellipses around the poles are shorter than the circumference around the equator.

Full List of the Imaris-extracted parameters (Trachea experiment)

Variable #	Parameter (full name)	LRI (Likelihood Ratio Index)	Morpho/kinetic
1	PosX	0.02821	-
2	PosY	0.00291	-
3	PosZ	0.3517	-
4	Acceleration	0.05262	K
5	Acceleration_VectorX	7.58316×10^{-6}	K
6	Acceleration_VectorY	9.43658×10^{-8}	K
7	Acceleration_VectorZ	0.00026954	K
8	Area	0.6309	M
9	BoundingBoxAA_LengthX	0.4927	M
10	BoundingBoxAA_LengthY	0.3789	M
11	BoundingBoxAA_LengthZ	0.3112	M
12	BoundingBoxOO_LengthX	0.5675	M
13	BoundingBoxOO_LengthY	0.5660	M
14	BoundingBoxOO_LengthZ	0.5449	M
15	Center_of_Homogeneous_MassX	0.02821	-
16	Center_of_Homogeneous_MassY	0.00291	-
17	Center_of_Homogeneous_MassZ	0.3517	-
18	DisplacementX	0.00868	K
19	DisplacementY	0.00032552	K
20	DisplacementZ	0.05285	K
21	Displacement_2	0.2841	K
22	Displacement_DeltaX	0.00503	K
23	Displacement_DeltaY	0.000512577	K
24	Displacement_DeltaZ	0.00256	K
25	Displacement_Delta_Length	0.2130	K
26	Displacement_Length	0.3133	K
27	Distance_from_Origin	0.03962	K
28	Distance_to_Image_Border_XYZ_Img_1	0.05569	K
29	Distance_to_Image_Border_XY_Img_1	0.08841	K
30	Ellipsoid_Axis_AX	0.01498	M
31	Ellipsoid_Axis_AY	0.000109567	M

32	Ellipsoid_Axis_AZ	0.00064845	M
33	Ellipsoid_Axis_BX	0.00574	M
34	Ellipsoid_Axis_BY	0.000484091	M
35	Ellipsoid_Axis_BZ	0.00114	M
36	Ellipsoid_Axis_CX	0.0000146000	M
37	Ellipsoid_Axis_CY	0.0000121000	M
38	Ellipsoid_Axis_CZ	0.00661	M
39	Ellipsoid_Axis_Length_A	0.4848	M
40	Ellipsoid_Axis_Length_B	0.4981	M
41	Ellipsoid_Axis_Length_C	0.4405	M
42	Ellipticity_(oblate)	0.0001350000	M
43	Ellipticity_(prolate)	0.0006930000	M
44	Number_of_Triangles	0.6031	M
45	Number_of_Voxels_Img_1	0.5757	M
46	Speed	0.3443	K
47	Sphericity	0.1054	M
48	Time	0.0003900000	-
49	Time_Since_Track_Start	0.08998	-
50	VelocityX	0.00767	K
51	VelocityY	0.0008400000	K
52	VelocityZ	0.00483	K
53	Velocity_AngleX	0.00191	K
54	Velocity_AngleY	0.0002500000	K
55	Velocity_AngleZ	0.0003640000	K
56	Volume	0.6029	M
57	TrPosMeanX	0.02831	-
58	TrPosMeanY	0.00292	-
59	TrPosMeanZ	0.3601	-
60	Track_Ar1X	0.01449	K
61	Track_Ar1Y	0.01898	K
62	Track_Ar1Z	0.02467	K
63	Track_Ar1_Mean	0.01920	K
64	Track_Area_Mean	0.7569	M
65	Track_Center_of_Homogeneous_Mass_MeanX	0.02768	-
66	Track_Center_of_Homogeneous_Mass_MeanY	0.00304	-
67	Track_Center_of_Homogeneous_Mass_MeanZ	0.3723	-
68	Track_DisplacementX	0.03247	K
69	Track_DisplacementY	0.00483	K
70	Track_DisplacementZ	0.05722	K
71	Track_Displacement_Length	0.4323	K
72	Track_Duration	0.2299	K
73	Track_Ellipsoid_Axis_A_MeanX	0.06204	M
74	Track_Ellipsoid_Axis_A_MeanY	0.0004600000	M
75	Track_Ellipsoid_Axis_A_MeanZ	0.00224	M

76	Track_Ellipsoid_Axis_B_MeanX	0.02249	M
77	Track_Ellipsoid_Axis_B_MeanY	0.00136	M
78	Track_Ellipsoid_Axis_B_MeanZ	0.00584	M
79	Track_Ellipsoid_Axis_C_MeanX	0.0000488000	M
80	Track_Ellipsoid_Axis_C_MeanY	0.0000557000	M
81	Track_Ellipsoid_Axis_C_MeanZ	0.01544	M
82	Track_Ellipsoid_Axis_Length_A_Mean	0.6358	M
83	Track_Ellipsoid_Axis_Length_B_Mean	0.6445	M
84	Track_Ellipsoid_Axis_Length_C_Mean	0.5808	M
85	Track_Ellipticity_Oblate_Mean	0.0002730000	M
86	Track_Ellipticity_Prolate_Mean	0.00142	M
87	Track_Length	0.07954	K
88	Track_Number_of_Surfaces	0.2346	-
89	Track_Number_of_Triangles	0.5072	M
90	Track_Number_of_Voxels_Img_1	0.5342	M
91	Track_Position_StartX	0.02457	-
92	Track_Position_StartY	0.00319	-
93	Track_Position_StartZ	0.2734	-
94	Track_Speed_Max	0.2260	K
95	Track_Speed_Mean	0.4546	K
96	Track_Speed_StdDev	0.1878	K
97	Track_Speed_Variation	0.9139	K
98	Track_Sphericity_Mean	0.1516	M
99	Track_Straightness	0.3319	K
100	Track_Volume_Mean	0.7237	M
101	TrackX_BoundingBoxOO_LengthX_Std	0.01131	M
102	TrackX_BoundingBoxOO_LengthX_Vrn	0.2979	M
103	TrackX_BoundingBoxOO_LengthY_Std	0.02733	M
104	TrackX_BoundingBoxOO_LengthY_Vrn	0.2112	M
105	TrackX_BoundingBoxOO_LengthZ_Std	0.00863	M
106	TrackX_BoundingBoxOO_LengthZ_Vrn	0.2423	M
107	TrackX_Ellipsoid_Axis_Length_A_Std	0.0000800000	M
108	TrackX_Ellipsoid_Axis_Length_A_Vrn	0.2630	M
109	TrackX_Ellipsoid_Axis_Length_B_Std	0.00656	M
110	TrackX_Ellipsoid_Axis_Length_B_Vrn	0.1742	M
111	TrackX_Ellipsoid_Axis_Length_C_Std	0.00534	M
112	TrackX_Ellipsoid_Axis_Length_C_Vrn	0.1830	M
113	TrackX_Ellipticity_(oblate)_Std	0.1266	M
114	TrackX_Ellipticity_(oblate)_Vrn	0.1009	M
115	TrackX_Ellipticity_(prolate)_Std	0.1467	M
116	TrackX_Ellipticity_(prolate)_Vrn	0.09417	M
117	TrackX_Sphericity_Std	0.1830	M
118	TrackX_Sphericity_Vrn	0.08196	M

Full List of the Imaris-extracted parameters (I/R model)

Variable #	Parameter (full name)	ARI (Adjusted Rand Index)	Morpho/kinetic
1	PosX	0.199812	-
2	PosY	0.0134792	-
3	PosZ	0.0959785	-
4	Acceleration	0.104706	K
5	Acceleration_VectorX	-0.0011867	K
6	Acceleration_VectorY	0.00383366	K
7	Acceleration_VectorZ	0.0030997	K
8	Area	0.117614	M
9	BoundingBoxAA_LengthX	0.0227233	M
10	BoundingBoxAA_LengthY	0.0376718	M
11	BoundingBoxAA_LengthZ	0.191715	M
12	BoundingBoxOO_LengthX	0.0561999	M
13	BoundingBoxOO_LengthY	0.0161615	M
14	BoundingBoxOO_LengthZ	0.16488	M
15	Center_of_Homogeneous_MassX	0.199812	-
16	Center_of_Homogeneous_MassY	0.0134792	-
17	Center_of_Homogeneous_MassZ	0.0959785	-
18	DisplacementX	0.0400756	K
19	DisplacementY	0.0400721	K
20	DisplacementZ	0.0209411	K
21	Displacement_2	0.067975	K
22	Displacement_DeltaX	0.0526806	K
23	Displacement_DeltaY	0.0436453	K
24	Displacement_DeltaZ	0.045846	K
25	Displacement_Delta_Length	0.089845	K
26	Displacement_Length	0.0684016	K
27	Distance_from_Origin	0.191899	K
28	Distance_to_Image_Border_XYZ_Img_1	0.053547	K
29	Distance_to_Image_Border_XY_Img_1	0.0010355	K
30	Ellipsoid_Axis_AX	0.00390094	M
31	Ellipsoid_Axis_AY	0.00265001	M
32	Ellipsoid_Axis_AZ	0.0197113	M
33	Ellipsoid_Axis_BX	0.00596838	M
34	Ellipsoid_Axis_BY	0.0215016	M
35	Ellipsoid_Axis_BZ	0.0587948	M
36	Ellipsoid_Axis_CX	0.0364135	M
37	Ellipsoid_Axis_CY	0.0511461	M
38	Ellipsoid_Axis_CZ	0.0695831	M
39	Ellipsoid_Axis_Length_A	0.0563467	M
40	Ellipsoid_Axis_Length_B	0.0203416	M
41	Ellipsoid_Axis_Length_C	0.131645	M
42	Ellipticity_(oblate)	0.0357881	M

43	Ellipticity_(prolate)	0.0161523	M
44	Number_of_Triangles	0.12309	M
45	Number_of_Voxels_Img_1	0.227189	M
46	Speed	0.0528194	K
47	Sphericity	0.208156	M
48	Time	0.00847723	-
49	Time_Since_Track_Start	0.0274705	-
50	VelocityX	0.0318647	K
51	VelocityY	0.0267809	K
52	VelocityZ	0.0164558	K
53	Velocity_AngleX	0.00461156	K
54	Velocity_AngleY	0.00834365	K
55	Velocity_AngleZ	0.00674175	K
56	Volume	0.247763	M
57	TrPosMeanX	0.201936	-
58	TrPosMeanY	0.0293915	-
59	TrPosMeanZ	0.1278	-
60	Track_Ar1X	0.0195397	K
61	Track_Ar1Y	0.0273641	K
62	Track_Ar1Z	0.00942567	K
63	Track_Ar1_Mean	0.0280121	K
64	Track_Area_Mean	0.242658	M
65	Track_Center_of_Homogeneous_Mass_MeanX	0.194032	-
66	Track_Center_of_Homogeneous_Mass_MeanY	0.037175	-
67	Track_Center_of_Homogeneous_Mass_MeanZ	0.130334	-
68	Track_DisplacementX	0.0872252	K
69	Track_DisplacementY	0.092103	K
70	Track_DisplacementZ	0.0549932	K
71	Track_Displacement_Length	0.104001	K
72	Track_Duration	0.110579	K
73	Track_Ellipsoid_Axis_A_MeanX	0.0509026	M
74	Track_Ellipsoid_Axis_A_MeanY	0.0689269	M
75	Track_Ellipsoid_Axis_A_MeanZ	0.0486089	M
76	Track_Ellipsoid_Axis_B_MeanX	0.0421736	M
77	Track_Ellipsoid_Axis_B_MeanY	0.0522001	M
78	Track_Ellipsoid_Axis_B_MeanZ	0.0917855	M
79	Track_Ellipsoid_Axis_C_MeanX	0.0596638	M
80	Track_Ellipsoid_Axis_C_MeanY	0.104145	M
81	Track_Ellipsoid_Axis_C_MeanZ	0.153649	M
82	Track_Ellipsoid_Axis_Length_A_Mean	0.155325	M
83	Track_Ellipsoid_Axis_Length_B_Mean	0.103889	M
84	Track_Ellipsoid_Axis_Length_C_Mean	0.296532	M
85	Track_Ellipticity_Oblate_Mean	0.130508	M
86	Track_Ellipticity_Prolate_Mean	0.0828341	M

87	Track_Length	0.25157	K
88	Track_Number_of_Surfaces	0.104596	-
89	Track_Number_of_Triangles	0.153894	M
90	Track_Number_of_Voxels_Img_1	0.248616	M
91	Track_Position_StartX	0.194621	-
92	Track_Position_StartY	0.0312247	-
93	Track_Position_StartZ	0.093343	-
94	Track_Speed_Max	0.269933	K
95	Track_Speed_Mean	0.275931	K
96	Track_Speed_StdDev	0.195366	K
97	Track_Speed_Variation	0.082606	K
98	Track_Sphericity_Mean	0.360418	M
99	Track_Straightness	0.0378991	K
100	Track_Volume_Mean	0.379741	M
101	TrackX_BoundingBoxOO_LengthX_Std	0.0675782	M
102	TrackX_BoundingBoxOO_LengthX_Vrn	0.0547006	M
103	TrackX_BoundingBoxOO_LengthY_Std	0.0682231	M
104	TrackX_BoundingBoxOO_LengthY_Vrn	0.0734061	M
105	TrackX_BoundingBoxOO_LengthZ_Std	0.114533	M
106	TrackX_BoundingBoxOO_LengthZ_Vrn	0.0528588	M
107	TrackX_Ellipsoid_Axis_Length_A_Std	0.0605932	M
108	TrackX_Ellipsoid_Axis_Length_A_Vrn	0.0521802	M
109	TrackX_Ellipsoid_Axis_Length_B_Std	0.110174	M
110	TrackX_Ellipsoid_Axis_Length_B_Vrn	0.0918706	M
111	TrackX_Ellipsoid_Axis_Length_C_Std	0.0879851	M
112	TrackX_Ellipsoid_Axis_Length_C_Vrn	0.0528872	M
113	TrackX_Ellipticity_(oblate)_Std	0.0404199	M
114	TrackX_Ellipticity_(oblate)_Vrn	0.131004	M
115	TrackX_Ellipticity_(prolate)_Std	0.0935684	M
116	TrackX_Ellipticity_(prolate)_Vrn	0.0393121	M
117	TrackX_Sphericity_Std	0.0652086	M
118	TrackX_Sphericity_Vrn	0.0968026	M

Full List of the Imaris-extracted parameters (Laser injury model)

Variable #	Parameter (full name)	LRI (Likelihood Ratio Index)	Morpho/kinetic
1	PosX	0.0168261	-
2	PosY	0.0392471	-
3	PosZ	0.0138623	-
4	Acceleration	0.119743	K
5	Acceleration_VectorX	9.26003×10^{-10}	K
6	Acceleration_VectorY	1.55634×10^{-7}	K
7	Acceleration_VectorZ	0.0000282144	K

8	Area	0.05402	M
9	BoundingBoxAA_LengthX	0.215489	M
10	BoundingBoxAA_LengthY	0.208612	M
11	BoundingBoxAA_LengthZ	0.00346372	M
12	BoundingBoxOO_LengthX	0.12183	M
13	BoundingBoxOO_LengthY	0.11817	M
14	BoundingBoxOO_LengthZ	0.0656389	M
15	Center_of_Homogeneous_MassX	0.0168261	-
16	Center_of_Homogeneous_MassY	0.0392471	-
17	Center_of_Homogeneous_MassZ	0.0138623	-
18	DisplacementX	0.000276114	K
19	DisplacementY	0.000866898	K
20	DisplacementZ	0.00315104	K
21	Displacement_2	0.00221264	K
22	Displacement_DeltaX	$3.13805*10^{-6}$	K
23	Displacement_DeltaY	0.000203754	K
24	Displacement_DeltaZ	0.0000149753	K
25	Displacement_Delta_Length	0.176337	K
26	Displacement_Length	0.00305491	K
27	Distance_from_Origin	0.0273929	K
28	Distance_to_Image_Border_XYZ_Img_1	0.0174722	K
29	Distance_to_Image_Border_XY_Img_1	0.212328	K
30	Ellipsoid_Axis_AX	0.000705328	M
31	Ellipsoid_Axis_AY	0.000503991	M
32	Ellipsoid_Axis_AZ	0.00081884	M
33	Ellipsoid_Axis_BX	0.000711085	M
34	Ellipsoid_Axis_BY	0.0086879	M
35	Ellipsoid_Axis_BZ	0.00313848	M
36	Ellipsoid_Axis_CX	0.00147093	M
37	Ellipsoid_Axis_CY	0.0148913	M
38	Ellipsoid_Axis_CZ	0.113218	M
39	Ellipsoid_Axis_Length_A	0.120737	M
40	Ellipsoid_Axis_Length_B	0.0978613	M
41	Ellipsoid_Axis_Length_C	0.0855932	M
42	Ellipticity_(oblate)	0.00480699	M
43	Ellipticity_(prolate)	0.00163375	M
44	Number_of_Triangles	0.0970691	M
45	Number_of_Voxels_Img_1	0.140969	M
46	Speed	0.113132	K
47	Sphericity	0.106042	M
48	Time	0.00457403	-
49	Time_Since_Track_Start	0.0144588	-
50	VelocityX	$2.31073*10^{-6}$	K
51	VelocityY	0.000439806	K

52	VelocityZ	4.40869*10^-7	K
53	Velocity_AngleX	0.0000672533	K
54	Velocity_AngleY	0.00042618	K
55	Velocity_AngleZ	0.0000981268	K
56	Volume	0.166302	M
57	TrPosMeanX	0.0175163	-
58	TrPosMeanY	0.0408592	-
59	TrPosMeanZ	0.0200071	-
60	Track_Ar1X	1.42425*10^-6	K
61	Track_Ar1Y	1.04298*10^-8	K
62	Track_Ar1Z	6.51116*10^-6	K
63	Track_Ar1_Mean	1.46908*10^-6	K
64	Track_Area_Mean	0.104663	M
65	Track_Center_of_Homogeneous_Mass_MeanX	0.0173689	-
66	Track_Center_of_Homogeneous_Mass_MeanY	0.0416838	-
67	Track_Center_of_Homogeneous_Mass_MeanZ	0.023701	-
68	Track_DisplacementX	0.0000219335	K
69	Track_DisplacementY	0.00159672	K
70	Track_DisplacementZ	0.00326279	K
71	Track_Displacement_Length	0.000417457	K
72	Track_Duration	0.0211158	K
73	Track_Ellipsoid_Axis_A_MeanX	0.00720058	M
74	Track_Ellipsoid_Axis_A_MeanY	0.00518807	M
75	Track_Ellipsoid_Axis_A_MeanZ	0.00746173	M
76	Track_Ellipsoid_Axis_B_MeanX	0.00934467	M
77	Track_Ellipsoid_Axis_B_MeanY	0.10254	M
78	Track_Ellipsoid_Axis_B_MeanZ	0.0359771	M
79	Track_Ellipsoid_Axis_C_MeanX	0.0101858	M
80	Track_Ellipsoid_Axis_C_MeanY	0.115632	M
81	Track_Ellipsoid_Axis_C_MeanZ	0.337947	M
82	Track_Ellipsoid_Axis_Length_A_Mean	0.252659	M
83	Track_Ellipsoid_Axis_Length_B_Mean	0.20483	M
84	Track_Ellipsoid_Axis_Length_C_Mean	0.166433	M
85	Track_Ellipticity_Oblate_Mean	0.0288364	M
86	Track_Ellipticity_Prolate_Mean	0.0113473	M
87	Track_Length	0.288055	K
88	Track_Number_of_Surfaces	0.00284488	-
89	Track_Number_of_Triangles	0.0367711	M
90	Track_Number_of_Voxels_Img_1	0.0749882	M
91	Track_Position_StartX	0.0118363	-
92	Track_Position_StartY	0.0362313	-
93	Track_Position_StartZ	0.0034983	-
94	Track_Speed_Max	0.587187	K
95	Track_Speed_Mean	0.513951	K

96	Track_Speed_StdDev	0.582588	K
97	Track_Speed_Variation	0.00325858	K
98	Track_Sphericity_Mean	0.2102	M
99	Track_Straightness	0.158131	K
100	Track_Volume_Mean	0.274455	M
101	TrackX_BoundingBoxOO_LengthX_Std	0.000879673	M
102	TrackX_BoundingBoxOO_LengthX_Vrn	0.163121	M
103	TrackX_BoundingBoxOO_LengthY_Std	0.0105878	M
104	TrackX_BoundingBoxOO_LengthY_Vrn	0.222923	M
105	TrackX_BoundingBoxOO_LengthZ_Std	0.00496087	M
106	TrackX_BoundingBoxOO_LengthZ_Vrn	0.154153	M
107	TrackX_Ellipsoid_Axis_Length_A_Std	0.00203799	M
108	TrackX_Ellipsoid_Axis_Length_A_Vrn	0.136433	M
109	TrackX_Ellipsoid_Axis_Length_B_Std	0.00585157	M
110	TrackX_Ellipsoid_Axis_Length_B_Vrn	0.153317	M
111	TrackX_Ellipsoid_Axis_Length_C_Std	0.00241157	M
112	TrackX_Ellipsoid_Axis_Length_C_Vrn	0.0895463	M
113	TrackX_Ellipticity_(oblate)_Std	0.121768	M
114	TrackX_Ellipticity_(oblate)_Vrn	0.0380257	M
115	TrackX_Ellipticity_(prolate)_Std	0.0962038	M
116	TrackX_Ellipticity_(prolate)_Vrn	0.122993	M
117	TrackX_Sphericity_Std	0.26669	M
118	TrackX_Sphericity_Vrn	0.337505	M

Cremaster- Full List of ACME-extracted parameters

Variable # (after selection)	Parameter (Short name)	Parameter full name	ARI (Adjusted Rand Index)	Type (Track/ Object)	Morpho / kinetic	Description
1	total distance	Total distance	0.4084	T	K	Indicates total distance travelled (i.e. the total length of displacements within the Track).
2	max speed	Maximum speed	0.3739	T	K	
3	mean speed	Mean speed	0.3889	T	K	
4	meander ratio	Meander ratio	0.3545	T	K	Calculated as the total distance divided by the displacement length of the track. When the index is between 1 and 1.5 the track is sinuous, but if between 1.5 and 4, then meandering. Index of 1 (straight).
5	tortuosity	Tortuosity	0.3832	T	K	Tortuosity is a property of a curve being tortuous. The inverse of meander ratio.
6	angle vs stream	Angle between the trajectory and the steam	0.3677	T	K	Angle between the trajectory (straight line that goes from the first position to the last position of the trajectory) and the bloodstream.
7	mean volume	Mean volume	0.4219	T	M	
8	mean surface area	Mean surface area	0.4111	T	M	

9	mean height	Mean height respect to the wall	0.3930	T	M	
10	mean max length	Mean of the maximum length	0.3685	T	M	
11	mean H/L ratio	Mean of the height/length ratio	0.4240	T	M	
12	mean sphericity	Mean sphericity	0.3598	T	M	
13	mean ellip prolate	Mean ellipticity prolate	0.4376	T	M	
14	mean ellip oblate	Mean ellipticity prolate	0.3893	T	M	
15	mean length A axis	Mean length principal axis (A)	0.4401	T	M	
16	mean length B axis	Mean length second principal axis (B)	0.3981	T	M	
17	mean length C axis	Mean length third principal axis (C)	0.4260	T	M	
18	mean extent	Mean extent	0.3448	T	M	
19	mean solidity	Mean solidity	0.4012	T	M	
20	mean equivalent diam	Mean equivalent diameter	0.4705	T	M	
21	volume Std	Standard deviation of the Volume	0.4151	T	M	
22	surface area Std	Standard deviation of the surface area	0.4788	T	M	
23	max area Std	Standard deviation of the maximum area	0.3767	T	M	
24	max length Std	Standard deviation of the maximum length	0.4406	T	M	
25	H/L ratio Std	Standard deviation of the height/length ratio	0.3206	T	M	
26	sphericity Std	Standard deviation of sphericity	0.2178	T	M	
27	ellip prolate Std	Standard deviation of ellipticity prolate	0.4573	T	M	
28	ellip oblate Std	Standard deviation of ellipticity oblate	0.4370	T	M	
29	length A axis Std	Standard deviation of length principal axis (A)	0.5391	T	M	

30	length B axis Std	Standard deviation of length principal axis (B)	0.5119	T	M	
31	length C axis Std	Standard deviation of length principal axis (C)	0.4781	T	M	
32	extent Std	Standard deviation of the extent	0.4620	T	M	
33	solidity Std	Standard deviation of the solidity	0.4417	T	M	
34	equivalent diam Std	Standard deviation of the equivalent diameter	0.5305	T	M	
35	mean polar radius	Mean of the polar radius	0.1925	T	K	
36	mean polar angle	Mean of the polar angle	0.3787	T	K	
37	polar radius Std	Standard deviation of the polar radius	0.1094	T	K	
38	polar angle Std	Standard deviation of the polar angle	0.2835	T	K	
39	ori X mean	Mean orientation respect to steam (X)	0.3533	T	K	
40	ori Y mean	Mean orientation respect to steam (Y)	0.3886	T	K	
41	ori Z mean	Mean orientation respect to steam (Z)	0.3287	T	K	
42	ori X Std	Standard deviation of the orientation respect to steam (X)	0.4321	T	K	
43	ori Y Std	Standard deviation of the orientation respect to steam (Y)	0.5238	T	K	
44	ori Z Std	Standard deviation of the orientation respect to steam (Z)	0.4246	T	K	
45	mean flattening	Mean distance of the centre of neutrophil to the wall (flatenning)	0.2995	T	M	
46	mean min dist to wall	Mean of the minimum distance to wall	0.3522	T	K	The mean of minimum distance of the neutrophil in each time step to the blood vessel surface.

47	track min dist to wall	Minimun distance of the trayectory to wall	0.00529	T	K	The minimum distance of a trajectory to blood vessel surface.
48	mean net score	Mean neural network score	0.2261	T	M	
49	max net score	Maximum neural network score	0.2225	T	M	
50	min net score	Minimum neural network score	0.3640	T	M	
51	network score range	Neural network score range	0.3761	T	M	
52	volume	Volume	0.06198	O	M	Number of voxels of the neutrophil.
53	surface area	Surface area	0.07241	O	M	Superficial area of the neutrophil in pixels.
54	height	Height respect to the wall	0.00595	O	M	Height of the neutrophil respect to the vessel wall.
55	max length	Maximum length	0.02487	O	M	Maximum width of an object
56	H/L ratio	height/length ratio	0.03479	O	M	height/length ratio of a neutrophil (object)
57	sphericity	Sphericity	0.01640	O	M	Sphericity measures how closely the shape of an object resembles that of a perfect sphere. Any particle which is not a sphere will have sphericity less than 1.
58	ellipticity prolate	Ellipticity prolate	0.01645	O	M	An oblate spheroid is the shape of the Earth and some other planets. It is like a sphere squashed from the top so the circumference around the poles is less than the circumference around the equator. Shapes of this type are called ellipsoids.
59	ellipticity oblate	Ellipticity oblate	0.01220	O	M	Prolate spheroids — A *spheroid* has circular symmetry. If the ellipse is rotated about its major axis, the result is a *prolate* (elongated) *spheroid*, shaped like an American football or rugby ball.
60	length A axis	Length principal axis (A)	0.01676	O	M	Length of the longest principal axis of the best-fitted ellipsoid into the segmented object (neutrophil)
61	length B axis	Length second principal axis (B)	0.04391	O	M	Length of the second principal axis of the best-fitted ellipsoid into the segmented object (neutrophil). B is perpendicular to A and C.
62	length C axis	Length third principal axis (C)	0.05992	O	M	Length of the third principal axis of the best-fitted ellipsoid into the segmented object (neutrophil). C is perpendicular to A and B.
63	extent	Extent	0.01455	O	M	Pixel ratio between the neutrophil and the bounding box that contains it (it can be an indicator of the quality of the segmentation, since a good segmentation must be compact).
64	solidity	Solidity	0.01917	O	M	Pixel ratio between the region and the smallest convex polygon that encompasses the region (as above, it can be an indicator of the compactness of the segmentation).
65	equivalent diam	Equivalent diameter	0.06950	O	M	The equivalent diameter is defined as the diameter of a circle with an equal aggregate sectional area, which is calculated by d = 2 Area / π .
66	ori X stream	Orientation respect to steam (X)	0.01707	O	K	3D orientation of the neutrophil with respect to the direction of the

						bloodstream along the trajectory (X axis).
67	ori Y stream	Orientation respect to steam (Y)	0.02472	O	K	3D orientation of the neutrophil with respect to the direction of the bloodstream along the trajectory (Y axis).
68	ori Z stream	Orientation respect to steam (Z)	0.01141	O	K	3D orientation of the neutrophil with respect to the direction of the bloodstream along the trajectory (Z axis).
69	polar radius	Polar radius	0.03839	O	K	3D position of the neutrophil with respect to the cross section of the vessel in polar coordinates: distance from the center (between 0 and 1).
70	polar angle	Polar angle	0.02997	O	K	3D position of the neutrophil with respect to the cross section of the vessel in polar coordinates: angle (between 0 and 2Pi).
71	flattening	Distance centre of neutrophil to the wall (flattening)	0.00482	O	M	Distance between the center of the neutrophil (object) and the vessel wall.
72	min distance to wall	Minimum distance to wall	0.00157	O	K	Minimum distance between the neutrophil (object) and the vessel surface (that of the voxel closest to the vessel).
73	network score	Neural network score	0.01591	O	M	The neural network includes a module that provides a probability value between 0 and 1 for each of the 3D regions resulting from the segmentation. This value indicates the reliability of each region based on its size and shape (for example, compact regions with a size similar to that of the neutrophils of interest will be more reliable).

Video S1 caption. Multiphoton imaging of leukocytes in influenza-infected trachea. Representative 3D image sequence of inflamed trachea from a CD11c^{YFP} mouse at 3 days post-infection with PR8 influenza virus. The left panel shows raw imaging data capturing the shape and movement of DCs (red) and adoptively transferred CK6^{ECFP} neutrophils (green) within the tracheal collagen fibers (SHG, blue). The video at right shows the corresponding 3D surface reconstructions of the cells (also referred to as 'objects', grey) and their tracks (red), which combined were used for the morpho-kinetic analysis in Figure 1B. Tracks only show the trajectories of the cells during the last 7 time points to avoid crowding. Scale bar, 50µm. Total elapsed time: 30 min. The video is played twice.

Video S2 caption. Multiphoton imaging of leukocytes during ischemia-reperfusion injury. Dynamic imaging of the ear skin of LyzM^{GFP}; CD11c^{YFP} mice subjected to I/R. 4D intravital images were acquired after pressing magnets were removed (4h post reperfusion). The top panel corresponds to raw images where DCs are visualized in red, neutrophils in green and macrophages in light green. SHG shown in blue corresponds to the collagen matrix. The I/R area is at the left side of the image where neutrophil density exponentially increases after reperfusion. The bottom panel shows the corresponding 3D surface reconstruction of cells ('objects', grey) and their tracks (red), which were used for the morpho-kinetic analysis represented in Figure 1H. Tracks represent the trajectories of cells during the last 7 time points. Scale bar, 20µm. Total elapsed time: 2 h 55 min. The video is played twice.

Video S3 caption. Multiphoton imaging of DCs and neutrophils during laser-burn injury. 3D image sequences of the ear skin of LyzM^{GFP}; CD11c^{YFP} mice after laser-induced focal sterile injury. The video at left displays raw imaging data with DCs in red and neutrophils in green. SHG is shown in blue (collagen) and blood vessels and the burnt area are in grey (Evans blue and autofluorescence). The right panel shows the corresponding 3D surface reconstruction of cells ('objects', grey) and their tracks (red), which were used for the morpho-kinetic analysis in Figure 1I. Tracks represent the trajectories of cells during the last 7 time points. Scale bar, 50µm. Total elapsed time: 2h 30 min. The video is played twice.

Video S4 caption. Spinning-disk imaging of inflamed venules for behavioral analysis of intravascular neutrophils. Representative 3D image sequence of an inflamed cremaster venule from a LyzM^{GFP} mouse showing leukocytes rolling and adhering (grey) to the vessel wall (blue, CD31). The tracks of crawling neutrophils are depicted in red for a maximum period of 7 time points. The morpho-kinetic information for intravascular neutrophils was extracted from a set of similar recordings and were used for the analyses shown in Figure 2D. Scale bar, 20µm. Total elapsed time: 6 min. The video is played twice.

Video S5 caption. Three behavioral states for intravascular neutrophils within inflamed venules. 4D image sequences of representative intravascular neutrophils (red) from behavioral groups 1-3 defined in Figure 2E. Behavior 1 is associated with small, egg-shaped neutrophils sessile on the vascular wall (blue, CD31). Behavior 2 neutrophils are characterized by ameboid-shape and faster motion, with frequent changes in shape. Behavior 3 neutrophils are large and sessile, and in intimate contact with the vascular wall. Scale bar, 5µm. Total elapsed time: 6 min. The video is played twice.

Video S6. Filtering out subcellular fragments. Cell segmentation in the trachea imaging experiments showing DCs, neutrophils and subcellular fragments. These fragments are defined by setting a filter of 40 voxel (~90 μm^3), and were not included in the behavioral analyses to avoid over-representation of "false" cells.