METRIC CATEGORY: STANDARD - PER FOV

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Mitochondria confluency	Mitochondria_Confluency [%]	FoV_metrics.csv	Mitochondria	%	Total object area over total FoV area.
Mitochondria count	Mitochondria_Count [#]	FoV_metrics.csv	Mitochondria	#	Total number of objects.
Mitochondria total dry mass	Mitochondria_TotalDryMass [pg]	FoV_metrics.csv	Mitochondria	pg	Sum of the dry mass of all objects in the FoV. The more objects are included and the higher their dry mass content, the higher the total dry mass is.

LIVE T Cell Assay

METRIC CATEGORY: STANDARD - PER TARGET CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Area	Area [µm²]	Target_cell_metrics.csv	Target cells	µm²	Area of a single object in µm²: The number of pixels in the object of interest, multiplied by the area of a pixel (200 nm x 200 nm = 0.04µm²) resulting in the surface area when seen from above (along the vertical z axis).
Compactness	Compactness	Target_cell_metrics.csv	Target cells	-	Compactness measures how densely the object area is concentrated around its center of gravity. The fewer protrusions an object has and the more rounded it becomes, the closer its compactness score is to 1 (the value of a perfect circle), whereas an object with multiple protrusions extending into different directions will have a value greater than 1. Compactness is calculated as Perimeter²/(4*π*Area).
Eccentricity	Eccentricity	Target_cell_metrics.csv	Target cells	-	Eccentricity measures how much an ellipsoid area deviates from being circular. The algorithm first approximates the cell shape with an ellipse of the same area. Eccentricity is then calculated as the ratio of the distance between the foci of the ellipse to the length of its major axis. A value of 0 corresponds to a perfect circle, while increasingly elongated shapes yield values approaching 1.
Extent	Extent	Target_cell_metrics.csv	Target cells	-	The area of the object divided by the area of the bounding box of the object. A rounded cell with no protrusions will have a value closer to 1, whereas a cell with multiple protrusions extending in different directions will have a value closer to 0.
Form factor	FormFactor	Target_cell_metrics.csv	Target cells	-	Calculated as (4*π*Area)/Perimeter². It equals 1 for a perfectly circular object and approaches 0 as the shape deviates from circularity, reaching values close to 0 for extremely elongated, spaghetti-like shapes with minimal discernible area.
Perimeter	Perimeter [µm]	Target_cell_metrics.csv	Target cells	µm	The total number of pixels around the boundary of each region in the image multiplied by 0.2 µm (resolution in xy) to convert to µm. Cells blebbing or beginning to form filopodia will increase their mean perimeter significantly.
Granularity	Granularity_5px	Target_cell_metrics.csv	Target cells	-	Granularity quantifies the existence of fine structures within a region; for cells, it reflects the degree of internal organization. The mean granularity of a single object below 5 pixels describes the amount of fine (<5px) texture. High granularity indicates the presence of well-defined structures with strong refractive index differences within the object compared to their surroundings.
Mean RI intensity	MeanRIIntensity	Target_cell_metrics.csv	Target cells	-	Mean single object intensity in RI values. A higher RI corresponds to greater density. Typically, the highest RI values are observed in cellular lipid storage sites: lipid droplets.

METRIC CATEGORY: STANDARD - PER TARGET CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Dry mass	DryMass [pg]	Target_cell_metrics.csv	Target cells	pg	Dry mass reflects the content of the cell excluding water, i.e., material like proteins, lipids, etc. There is a direct relationship between the refractive index (RI) measured by holotomography and the dry mass (Philips KG et al., PhysRev Lett., 2012, pmid:23005682; P. A. Sandoz et al., PLOS, 2019): Dry Mass = [(measured RI /1.33) – 1]*(1.33/0.002), where the measured RI is the mean RI of the cell, obtained by dividing the summed RI by the cell area in pixels.
Dry mass density	DryMassDensity [pg/µm³]	Target_cell_metrics.csv	Target cells	pg/µm³	The cell volume is estimated by multiplying the measured cell area by 3.2 µm. Consequently, the dry mass density is expressed in pg/µm³, obtained by dividing the dry mass by the estimated cell volume.

METRIC CATEGORY: CLASSIFICATION - PER TARGET CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Probability living	Probability_Living	Target_cell_classification /Classifications.csv	Target cells	-	Probability that a target cell is alive. Values close to 1 indicate high model confidence in classifying the cell as living.
Probability dead	Probability_Dead	Target_cell_classification /Classifications.csv	Target cells	-	Probability that a target cell is dead. Values close to 1 indicate high model confidence in classifying the cell as dead.

METRIC CATEGORY: STANDARD - PER T CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Area	Area [µm²]	T_cell_metrics.csv	T cells	µm²	Area of a single object in µm²: The number of pixels in the object of interest, multiplied by the area of a pixel (200 nm x 200 nm = 0.04µm²) resulting in the surface area when seen from above (along the vertical z axis).
Compactness	Compactness	T_cell_metrics.csv	T cells	-	Compactness measures how densely the object area is concentrated around its center of gravity. The fewer protrusions an object has and the more rounded it becomes, the closer its compactness score is to 1 (the value of a perfect circle), whereas an object with multiple protrusions extending into different directions will have a value greater than 1. Compactness is calculated as Perimeter²/(4*π*Area).
Eccentricity	Eccentricity	T_cell_metrics.csv	T cells	-	Eccentricity measures how much an ellipsoid area deviates from being circular. The algorithm first approximates the cell shape with an ellipse of the same area. Eccentricity is then calculated as the ratio of the distance between the foci of the ellipse to the length of its major axis. A value of 0 corresponds to a perfect circle, while increasingly elongated shapes yield values approaching 1.
Extent	Extent	T_cell_metrics.csv	T cells	-	The area of the object divided by the area of the bounding box of the object. A rounded cell with no protrusions will have a value closer to 1, whereas a cell with multiple protrusions extending in different directions will have a value closer to 0.
Form factor	FormFactor	T_cell_metrics.csv	T cells	-	Calculated as (4*π*Area)/Perimeter². It equals 1 for a perfectly circular object and approaches 0 as the shape deviates from circularity, reaching values close to 0 for extremely elongated, spaghetti-like shapes with minimal discernible area.
Perimeter	Perimeter [µm]	T_cell_metrics.csv	T cells	µm	The total number of pixels around the boundary of each region in the image multiplied by 0.2 µm (resolution in xy) to convert to µm. Cells blebbing or beginning to form filopodia will increase their mean perimeter significantly.
Granularity	Granularity_5px	T_cell_metrics.csv	T cells	-	Granularity quantifies the existence of fine structures within a region; for cells, it reflects the degree of internal organization. The mean granularity of a single object below 5 pixels describes the amount of fine (<5px) texture. High granularity indicates the presence of well-defined structures with strong refractive index differences within the object compared to their surroundings.
Mean RI intensity	MeanRIIntensity	T_cell_metrics.csv	T cells	-	Mean single object intensity in RI values. A higher RI corresponds to greater density. Typically, the highest RI values are observed in cellular lipid storage sites: lipid droplets.
Dry mass	DryMass [pg]	T_cell_metrics.csv	T cells	pg	Dry mass reflects the content of the cell excluding water, i.e., material like proteins, lipids, etc. There is a direct relationship between the refractive index (RI) measured by holotomography and the dry mass (Philips KG et al., PhysRev-Lett., 2012, pmid:23005682; P. A. Sandoz et al., PLOS, 2019): Dry Mass = [(measured RI /1.33) – 1]*(1.33/0.002), where the measured RI is the mean RI of the cell, obtained by dividing the summed RI by the cell area in pixels.
Dry mass density	DryMassDensity [pg/µm³]	T_cell_metrics.csv	T cells	pg/µm³	The cell volume is estimated by multiplying the measured cell area by 3.2 µm. Consequently, the dry mass density is expressed in pg/µm³, obtained by dividing the dry mass by the estimated cell volume.

METRIC CATEGORY: CLASSIFICATION - PER T CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Probability active	Probability_Active	T_cell_classification/ Classifications.csv	T cells	-	Probability that a T cell is active. Values close to 1 indicate high model confidence in classifying the cell as active.
Probability inactive	Probability_Inactive	T_cell_classification/ Classifications.csv	T cells	-	Probability that a T cell is inactive. Values close to 1 indicate high model confidence in classifying the cell as inactive.
Probability dead	Probability_Dead	T_cell_classification/ Classifications.csv	T cells	-	Probability that a T cell is dead. Values close to 1 indicate high model confidence in classifying the cell as dead.

METRIC CATEGORY: STANDARD - PER FOV

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Target cell confluency	Target_cell_Confluency [%]	FoV_metrics.csv	Target cells	%	Total object area over total FoV area.
Target cell count	Target_cell_Count [#]	FoV_metrics.csv	Target cells	#	Total number of objects.
Target cell total dry mass	Target_cell_TotalDryMass [pg]	FoV_metrics.csv	Target cells	pg	Sum of the dry mass of all target cells in the FoV. The more target cells included and the higher their dry mass content, the higher the total dry mass will be.
T cell confluency	T_cell_Confluency [%]	FoV_metrics.csv	T cells	%	Total object area over total FoV area.
T cell count	T_cell_Count [#]	FoV_metrics.csv	T cells	#	Total number of objects.
T cell total dry mass	T_cell_TotalDryMass [pg]	FoV_metrics.csv	T cells	pg	Sum of the dry mass of all T cells in the FoV. The more T cells included and the higher their dry mass content, the higher the total dry mass will be.

METRIC CATEGORY: CLASSIFICATION - PER FOV

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Lethal fraction	Lethal fraction	Target_cell_classification/ Classification_metrics.csv	Target cells	-	Target cell death fraction takes into account the detachment and disappearance of dead target cells. It is defined as: [1-((living target cells at timepoint $_t$)/(total number of living target cells at timepoint $_t$+max dead target cells from timepoints $_{1-t}$))].
Cell death rate	Cell death rate [lethal fraction/hour]	Target_cell_classification/ Classification_metrics.csv	Target cells	lethal fraction/hour	Dead target cell fraction per hour, calculated as: (lethal fraction of target cells at timepoint $_t$ - lethal fraction of target cells at timepoint $_{t-1}$)*frequency(hours-^{1}).
Health index	Health index	Target_cell_classification/ Classification_metrics.csv	Target cells	-	The probability of target cells to be living, averaged over all target cells in the FoV. Values close to 1 represent healthy target cells, and values close to 0 represent stressed or dead cells.

METRIC CATEGORY: CLASSIFICATION - PER FOV

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Living cells	Living cells [#]	Target_cell_classification/ Classification_metrics.csv	Target cells	#	Number of target cells whose probability of being alive is assessed to be at 50% or higher by the model.
Living cells	Living cells [%]	Target_cell_classification/ Classification_metrics.csv	Target cells	%	Number of living target cells divided by the total number of target cells, multiplied by 100.
Dead cells	Dead cells [#]	Target_cell_classification/ Classification_metrics.csv	Target cells	#	Number of target cells whose probability of being dead is assessed to be at 50% or higher by the model.
Dead cells	Dead cells [%]	Target_cell_classification/ Classification_metrics.csv	Target cells	%	Number of dead target cells divided by the total number of target cells, multiplied by 100.
Lethal fraction	Lethal fraction	T_cell_classification/ Classification_metrics.csv	T cells	-	T cell lethal fraction takes into account the disappearance of dead T cells. It is defined as: [1-((living T cells at timepoint t)/(total number of living T cells at timepoint t+max dead T cells from timepoints $_{1-t}$))].
Cell death rate	Cell death rate [lethal fraction/hour]	T_cell_classification/ Classification_metrics.csv	T cells	lethal fraction/hour	T cell death fraction per hour, calculated as: (lethal fraction of T cells at timepoint t - lethal fraction of T cells at timepoint $_{t-1}$)*frequency (hours^{-1}).
Health index	Health index	T_cell_classification/ Classification_metrics.csv	T cells	-	The probability of T cells to be living, averaged over all target cells in the FoV. Values close to 1 represent healthy target cells, and values close to 0 represent stressed or dead cells.
Living cells	Living cells [#]	T_cell_classification/ Classification_metrics.csv	T cells	#	Number of T cells whose probability of being alive is assessed to be at 50% or higher by the model.
Living cells	Living cells [%]	T_cell_classification/ Classification_metrics.csv	T cells	%	Number of living T cells divided by the total number of T cells, multiplied by 100.
Dead cells	Dead cells [#]	T_cell_classification/ Classification_metrics.csv	T cells	#	Number of T cells whose probability of being dead is assessed to be at 50% or higher by the model.
Dead cells	Dead cells [%]	T_cell_classification/ Classification_metrics.csv	T cells	%	Number of dead T cells divided by the total number of T cells, multiplied by 100.

METRIC CATEGORY: CLASSIFICATION - PER FOV

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Active cells	Active cells [#]	T_cell_classification/ Classification_metrics.csv	T cells	#	Number of T cells whose probability of being active is assessed to be at 50% or higher by the model.
Active cells	Active cells [%]	T_cell_classification/ Classification_metrics.csv	T cells	%	Number of active T cells divided by the total number of T cells, multiplied by 100.
Inactive cells	Inactive cells [#]	T_cell_classification/ Classification_metrics.csv	T cells	#	Number of T cells whose probability of being inactive is assessed to be at 50% or higher by the model.
Inactive cells	Inactive cells [%]	T_cell_classification/ Classification_metrics.csv	T cells	%	Number of inactive T cells divided by the total number of T cells, multiplied by 100.

METRIC CATEGORY: FINDING/BINDING - PER FOV

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
T cells in contact with target cells	T cells in contact with target cells [#]	Interaction_metrics.csv	Target cells + T cells	#	Number of T cells that are in contact with at least one target cell.
Ratio of T cells in contact with target cells	Ratio of T cells in contact with target cells [%]	Interaction_metrics.csv	Target cells + T cells	%	Percentage of T cells that are in contact with at least one target cell.
Target cell area covered by T cells	Target cell area covered by T cells [%]	Interaction_metrics.csv	Target cells + T cells	%	Area of target cells that is covered by T cells over total target cell area.
T cell area covering target cells	T cell area covering target cells [%]	Interaction_metrics.csv	Target cells + T cells	%	Area of T cells that cover target cells over total T cell area.
Average min distance T cell - target cell	Average min distance T cell - target cell [µm]	Interaction_metrics.csv	Target cells + T cells	µm	Mean Euclidean distance from each T cell to its closest target cell, calculated from the T cell border to target cell border and averaged across all T cells in the image. For each image : sum (for each T cell) of the minimum pixel-to-pixel distance between a T cell and its closest target cell / total number of T cells.
T cell velocity	T cell velocity [µm/s]	Interaction_metrics.csv	Target cells + T cells	µm/s	Mean velocity of T cells, calculated by estimating the T cell movement between timepoints. It is defined as: (1-similarity)*(image area in pixels*0.03)/(average time interval * total number of T cells). Similarity is determined by measuring the structural similarity of T cells between the current timepoint and adjacent timepoints using a sliding window of 8 minutes. The similarity is then converted to µm/s by multiplying it by experimental variables (the conversion factor from similarity to velocity has been manually calibrated).

METRIC CATEGORY: FLUORESCENCE - PER TARGET CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Total intensity - channel	TotalIntensity - channel	channel_target_cell_ metrics.csv	Target cells	-	Total fluorescent signal in a single object.
Mean intensity - channel	MeanIntensity - channel	channel_target_cell_ metrics.csv	Target cells	-	Mean fluorescent signal intensity in a single object.
Std intensity - channel	StdIntensity - channel	channel_target_cell_ metrics.csv	Target cells	-	Standard deviation of fluorescent signal intensity in a single object.
10th percentile intensity - channel	10thPercentileIntensity - channel	channel_target_cell_ metrics.csv	Target cells	-	10th percentile of fluorescent signal intensity in a single object.
25th percentile intensity - channel	25thPercentileIntensity - channel	channel_target_cell_ metrics.csv	Target cells	-	25th percentile of fluorescent signal intensity in a single object.
Median intensity - channel	MedianIntensity - channel	channel_target_cell_ metrics.csv	Target cells	-	Median of fluorescent signal intensity in a single object.
75th percentile intensity - channel	75thPercentileIntensity - channel	channel_target_cell_ metrics.csv	Target cells	-	75th percentile of fluorescent signal intensity in a single object.
90th percentile intensity - channel	90thPercentileIntensity - channel	channel_target_cell_ metrics.csv	Target cells	-	90th percentile of fluorescent signal intensity in a single object.

METRIC CATEGORY: FLUORESCENCE - PER T CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Total intensity - channel	TotalIntensity - channel	channel_T_cell_ metrics.csv	T cells	-	Total fluorescent signal in a single object.
Mean intensity - channel	MeanIntensity - channel	channel_T_cell_ metrics.csv	T cells	-	Mean fluorescent signal intensity in a single object.
Std intensity - channel	StdIntensity - channel	channel_T_cell_ metrics.csv	T cells	-	Standard deviation of fluorescent signal intensity in a single object.
10th percentile intensity - channel	10thPercentileIntensity - channel	channel_T_cell_ metrics.csv	T cells	-	10th percentile of fluorescent signal intensity in a single object.
25th percentile intensity - channel	25thPercentileIntensity - channel	channel_T_cell_ metrics.csv	T cells	-	25th percentile of fluorescent signal intensity in a single object.
Median intensity - channel	MedianIntensity - channel	channel_T_cell_ metrics.csv	T cells	-	Median of fluorescent signal intensity in a single object.
75th percentile intensity - channel	75thPercentileIntensity - channel	channel_T_cell_ metrics.csv	T cells	-	75th percentile of fluorescent signal intensity in a single object.
90th percentile intensity - channel	90thPercentileIntensity - channel	channel_T_cell_ metrics.csv	T cells	-	90th percentile of fluorescent signal intensity in a single object.