Smart Mitochondrial Assay^{LIVE}

METRIC CATEGORY: STANDARD - PER MITOCHONDRION

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Area	Area [µm²]	Mitochondria_metrics.csv	Mitochondria	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	Mitochondria_metrics.csv	Mitochondria	-	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	Mitochondria_metrics.csv	Mitochondria	-	Eccentricity measures how much an ellipsoid area deviates from being circular.The algorithm first approximates the object 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	Mitochondria_metrics.csv	Mitochondria	-	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 an object with multiple protrusions extending into different directions will have a value closer to 0.
Form factor	FormFactor	Mitochondria_metrics.csv	Mitochondria	-	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]	Mitochondria_metrics.csv	Mitochondria	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.
Granularity	Granularity_5px	Mitochondria_metrics.csv	Mitochondria	-	Granularity quantifies the existence of fine structures within a region. 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	Mitochondria_metrics.csv	Mitochondria	-	Mean single object intensity in RI values. A higher RI corresponds to greater density.

METRIC CATEGORY: STANDARD - PER MITOCHONDRION

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Dry mass	DryMass [pg]	Mitochondria_metrics.csv	Mitochondria	pg	Dry mass reflects the content of the cell excluding water. There is a direct relationship between the refractive index (RI) measured by holotomography and the dry mass (Philips KG et al., PhysRevLett., 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 object, obtained by dividing the summed RI by the object area in pixels.
Dry mass density	DryMassDensity [pg/µm³]	Mitochondria_metrics.csv	Mitochondria	pg/µm³	The object volume is estimated by multiplying the measured mitochondrial area by 0.2µm. Consequently, the dry mass density is expressed in pg/µm³, obtained by dividing the dry mass by the estimated volume.
Branches	Branches [#]	Mitochondria_metrics.csv	Mitochondria	#	Number of branches per single mitochondrion. Branches are defined as skeleton pixel groups that have exactly 2 neighbours. (Figure 2)
Endpoints	Endpoints [#]	Mitochondria_metrics.csv	Mitochondria	#	Number of endpoints per single mitochondrion. Endpoints are defined as skeleton pixels that have exactly 1 neighbour. (Figure 2)
Junctions	Junctions [#]	Mitochondria_metrics.csv	Mitochondria	#	Number of junctions per single mitochondrion. Junctions are defined as skeleton pixel groups that have 3 or more neighbours. (Figure 2)
Mean branch length	MeanBranchLength [µm]	Mitochondria_metrics.csv	Mitochondria	µm	Mean length of branches per single mitochondrion in µm.
Longest branch length	LongestBranchLength [µm]	Mitochondria_metrics.csv	Mitochondria	µm	Maximum length of branches per single mitochondrion in µm.
Total length	MitochondriaLength [µm]	Mitochondria_metrics.csv	Mitochondria	µm	Total length of mitochondrion in µm. Defined as the total skeleton length.
Mean radius	MeanRadius [µm]	Mitochondria_metrics.csv	Mitochondria	µm	Describes mitochondria thickness. Defined as the mean distance of any pixel in the object to the closest pixel outside of the object and it is the mean of the mean radius per mitochondria in the parent cell.

METRIC CATEGORY: STANDARD - MITOCHONDRIA PER CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Count	Count [#]	Mitochondria_per_cell_metrics.csv	Cells	#	Total number of objects in the parent cell.
Total area	TotalArea [µm²]	Mitochondria_per_cell_metrics.csv	Cells	µm²	Total area of objects in µm² in the parent cell: 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).
Total dry mass	TotalDryMass [pg]	Mitochondria_per_cell_metrics.csv	Cells	pg	Sum of the dry mass of all objects in the parent cell. The more objects are included, the higher the total dry mass is.
Mean area	Area [µm²]	Mitochondria_per_cell_metrics.csv	Cells	µm²	Mean area of objects in the parent cell 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).
Mean compactness	Compactness	Mitochondria_per_cell_metrics.csv	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). Here the mean compactness of single objects in the parent cell is calculated.
Mean eccentricity	Eccentricity	Mitochondria_per_cell_metrics.csv	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. Here the mean eccentricity of single objects in the parent cell is calculated.
Mean extent	Extent	Mitochondria_per_cell_metrics.csv	Cells	-	The area of the object divided by the area of the bounding box of the object. A rounded object with no protrusions will have a value closer to 1, whereas an object with multiple protrusions extending into different directions will have a value closer to 0. Here the mean extent of single objects in the parent cell is calculated.
Mean form factor	FormFactor	Mitochondria_per_cell_metrics.csv	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. Here the mean form factor of single objects in the parent cell is calculated.
Mean perimeter	Perimeter [µm]	Mitochondria_per_cell_metrics.csv	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. Here the mean perimeter of single objects in the parent cell is calculated.

METRIC CATEGORY: STANDARD - MITOCHONDRIA PER CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Mean granularity	Granularity_5px	Mitochondria_per_cell_metrics.csv	Cells	-	Granularity quantifies the existence of fine structures within a region. 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. Here the mean granularity of single objects in the parent cell is calculated.
Mean mean RI intensity	MeanRIIntensity	Mitochondria_per_cell_metrics.csv	Cells	-	Mean single object intensity in RI values. A higher RI corresponds to greater density. Here the mean of the mean RI of single objects in the parent cell is calculated.
Mean dry mass	DryMass[pg]	Mitochondria_per_cell_metrics.csv	Cells	pg	Dry mass reflects the content of the cell excluding water. There is a direct relationship between the refractive index (RI) measured by holotomography and the dry mass (Philips KG et al., PhysRevLett., 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 object, obtained by dividing the summed RI by the object area in pixels. Here the mean dry mass of single objects in the parent cell is calculated.
Mean dry mass density	DryMassDensity [pg/µm³]	Mitochondria_per_cell_metrics.csv	Cells	pg/µm³	The object volume is estimated by multiplying the measured mitochondrial area by 0.2µm. Consequently, the dry mass density is expressed in pg/µm³, obtained by dividing the dry mass by the estimated volume. Here the mean dry mass density of single objects in the parent cell is calculated.
Mean radial distance index	RadialDistanceIndex	Mitochondria_per_cell_metrics.csv	Cells	-	Describes the relative distance of objects from the parent cell's center. Values closer to 0 indicate mitochondria located closer to the cell center, while values closer to 1 reflect mitochondria positioned near the cell edges. For each cell a normalized distance map is generated, with the center assigned a value of 0 and the borders a value of 1. This distance map is then masked by the organelle mask, and the mean of the non-zero values is then computed. Here the mean radial distance index of single objects in the parent cell is calculated. (Figure 3)
Mean branches	Branches [#]	Mitochondria_per_cell_metrics.csv	Cells	#	Mean number of branches per single mitochondrion in the parent cell. Branches are defined as skeleton pixel groups that have exactly 2 neighbours. (Figure 2)
Mean endpoints	Endpoints [#]	Mitochondria_per_cell_metrics.csv	Cells	#	Mean number of endpoints per single mitochondrion in the parent cell. Endpoints are defined as skeleton pixels that have exactly 1 neighbour. (Figure 2)
Mean junctions	Junctions [#]	Mitochondria_per_cell_metrics.csv	Cells	#	Mean number of junctions per single mitochondrion in the parent cell. Junctions are defined as skeleton pixel groups that have 3 or more neighbours. (Figure 2)

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Mean mean branch length	MeanBranchLength [µm]	Mitochondria_per_cell_metrics.csv	Cells	µm	Average of the mean branch length per mitochondrion in the parent cell.
Mean longest branch length	LongestBranchLength [µm]	Mitochondria_per_cell_metrics.csv	Cells	µm	Mean maximum branch length for all mitochondria in the parent cell.
Mean total length	MitochondriaLength [µm]	Mitochondria_per_cell_metrics.csv	Cells	µm	Mean length of mitochondria in the parent cell.
Mean mean radius	MeanRadius [µm]	Mitochondria_per_cell_metrics.csv	Cells	µm	Describes mitochondria thickness. Defined as the mean distance of any pixel in the object to the closest pixel outside of the object and it is the mean of the mean radius per mitochondrion in the parent cell.

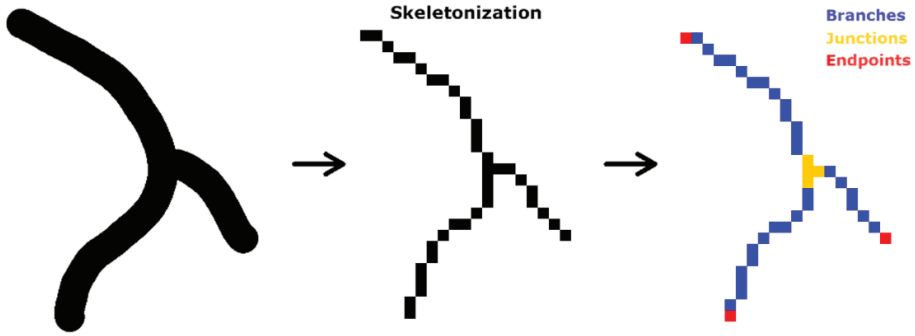


Figure 2. Skeletonization of mitochondrial masks. The process of skeletonization reduces binary objects to 1 pixel wide representations in order to calculate the object's topology. This example displays 3 branches, 3 endpoints and 1 junction



Figure 3. Radial distance index calculation method