Smart Lipid Droplet Assay^{LIVE}

NANOLIVE
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METRIC CATEGORY: STANDARD - PER LIPID DROPLET

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Area	Area [µm²]	Lipid_droplet_metrics.csv	Lipid droplets	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	Lipid_droplet_metrics.csv	Lipid droplets	-	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	Lipid_droplet_metrics.csv	Lipid droplets	-	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	Lipid_droplet_metrics.csv	Lipid droplets	-	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 in different directions will have a value closer to 0.
Form factor	FormFactor	Lipid_droplet_metrics.csv	Lipid droplets	-	Calculated as (4*π*Area)/Perimeter². It equals 1 for a perfectly circular object and approaches 0 as the shape deviates from circularity and becomes elongated, with spaghetti-like shapes and minimal discernible area.
Perimeter	Perimeter [µm]	Lipid_droplet_metrics.csv	Lipid droplets	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	Lipid_droplet_metrics.csv	Lipid droplets	-	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	Lipid_droplet_metrics.csv	Lipid droplets	-	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.

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Dry mass	DryMass [pg]	Lipid_droplet_metrics.csv	Lipid droplets	pg	Dry mass reflects the content of the object 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]	Lipid_droplet_metrics.csv	Lipid droplets	pg/µm³	The object volume is estimated by multiplying the measured cell area by 3.2µm. Consequently, the dry mass density is expressed in pg/µm³, obtained by dividing the dry mass by the estimated object volume.

METRIC CATEGORY: STANDARD - LIPID DROPLETS PER CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Count	Count [#]	Lipid_droplet_per_cell_metrics.csv	Cells	#	Number of lipid droplets in the parent cell.
Total area	TotalArea [µm²]	Lipid_droplet_per_cell_metrics.csv	Cells	µm²	Area of a single object 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]	Lipid_droplet_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]	Lipid_droplet_per_cell_metrics.csv	Cells	µm²	Mean area of a single object 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).
Mean compactness	Compactness	Lipid_droplet_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. Here the mean compactness of single objects in the parent cell is calculated. Compactness is calculated as Perimeter²/(4*π*Area).
Mean eccentricity	Eccentricity	Lipid_droplet_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.

METRIC CATEGORY: STANDARD - LIPID DROPLETS PER CELL

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Mean extent	Extent	Lipid_droplet_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	Lipid_droplet_per_cell_metrics.csv	Cells	-	Calculated as $(4*\pi*Area)/Perimeter^2$. 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]	Lipid_droplet_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.
Mean granularity	Granularity_5px	Lipid_droplet_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	Lipid_droplet_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]	Lipid_droplet_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]	Lipid_droplet_per_cell_metrics.csv	Cells	pg/µm³	The object volume is estimated by $4/3*\pi*radius^3$. 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 desnity of single objects in the parent cell is calculated.
Mean radial distance index	RadialDistanceIndex	Lipid_droplet_per_cell_metrics.csv	Cells	-	Describes the relative distance of objects from the parent cell's center. Values closer to 0 indicate lipid droplets located closer to the cell center, while values closer to 1 reflect lipid droplets 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)

METRIC CATEGORY: STANDARD - PER FOV

NAME IN NANOPLOT	METRIC NAME IN CSV	CSV NAME	POPULATION	UNIT	DESCRIPTION
Lipid droplet confluency	Lipid_droplet_Confluency [%]	FoV_metrics.csv	Lipid droplets	%	Total object area over total FoV area.
Lipid droplet count	Lipid_droplet_Count [#]	FoV_metrics.csv	Lipid droplets	#	Total number of objects.
Lipid droplet total dry mass	Lipid_droplet_TotalDryMass [pg]	FoV_metrics.csv	Lipid droplets	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.