Analysis Sequence "Ehlen_Farrera_Tumoroids_ImagingMask"

Input Image	Input
	Channel group : 1 **Sequences :** ALL **Flatfield Correction :** None Brightfield Correction **Stack Processing :** Maximum Projection

Find Cells	Input	Method	Output
	Channel : NucRed live **ROI :** None	**Method :** C Common Threshold : 0.4 Area : $> 100\ \mu m^2$ Splitting Coefficient : 33.8 Individual Threshold : 0.4 Contrast : > 0.1	Output Population : Organoids_raw

Calculate Morphology Properties	Input	Method	Output
	Population : Organoids_raw **Region :** Cell	**Method :** Standard Area Roundness Perimeter Width Length Ratio Width to Length	Property Prefix : Organoids_raw

Calculate Intensity Properties	Input	Method	Output
	Channel : ef450 **Population :** Organoids_raw **Region :** Cell	**Method :** Standard Mean	Property Prefix : Intensity Orgraw ef450

Calculate Intensity Properties (2)	Input	Method	Output
	Channel : NucRed dead **Population :** Organoids_raw **Region :** Cell	**Method :** Standard Mean	Property Prefix : Intensity Orgraw NucRed dead

Calculate Intensity Properties (3)	Input	Method	Output
	Channel : NucRed live **Population :** Organoids_raw	**Method :** Standard Mean	Property Prefix : Intensity Orgraw NucRed live

	Region : Cell		

Select Population	Input	Method	Output
	Population : Organoids_raw	**Method :** Linear Classifier Number of Classes : 2 Cell Area [µm²] Cell Roundness Cell Perimeter [µm] Cell Width [µm] Cell Length [µm] Cell Ratio Width to Length Intensity Cell ef450 Mean Intensity Cell NucRed dead Mean Intensity Cell NucRed live Mean	Output Population A : IntactOrg_final Output Population B : Discarded_cells

Calculate Morphology Properties (2)	Input	Method	Output
	Population : IntactOrg_final **Region :** Cell	**Method :** Standard Area Roundness	Property Prefix : IntactOrg_final

Find Nuclei	Input	Method	Output
	Channel : NucRed dead **ROI :** IntactOrg_final **ROI Region :** Cell	**Method :** M Diameter : 13 µm Splitting Sensitivity : 0.4 Common Threshold : 0.4	Output Population : DeadCells_in_IntactOrg

Calculate Morphology Properties (3)	Input	Method	Output
	Population : DeadCells_in_IntactOrg **Region :** Nucleus	**Method :** Standard Area	Property Prefix : DeadCells

Define Results	Results
	Method : List of Outputs **Population : Organoids_raw** Number of Objects **Population : IntactOrg_final** Number of Objects IntactOrg_final Area [µm²] : Sum **Population : DeadCells_in_IntactOrg** Number of Objects DeadCells Area [µm²] : Sum **Method :** Formula Output Formula : a-b Population Type : Objects Variable a : IntactOrg_final - IntactOrg_final Area [µm²] Sum Variable b : DeadCells_in_IntactOrg - DeadCells Area [µm²] Sum Output Name : Viable_Org_Area

Object Results
Population : Organoids_raw : None
Population : Discarded_cells : None
Population : IntactOrg_final : None
Population : DeadCells_in_IntactOrg : None

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