

Analysis Sequence "Low resolution downstream analysis"

Input Image	Input		
	Channel group : 1 Sequences : ALL Flatfield Correction : Basic Brightfield Correction Stack Processing : Maximum Projection Create Global Image Min. Global Binning : Dynamic		
Find Image Region	Input	Method	Output
	Channel : SPY555-DNA (global) ROI : Imaged Area (global) ROI Region : Imaged Area	Method : Common Threshold Threshold : <u>0.5</u> Split into Objects Area : > <u>3000</u> μm^2 Fill Holes	Output Population : ROI 1 Output Region : Image Region
Select Region	Input	Method	Output
	Population : ROI 1 Region : Image Region	Method : Resize Region [%] Outer Border : <u>42</u> % Outer Population : Imaged Area (global) Outer Region : Imaged Area Inner Border : 100 %	Output Region : ROI 1 Resized
Calculate Texture Properties	Input	Method	Output
	Channel : SPY555-DNA (global) Population : ROI 1 Region : ROI 1 Resized	Method : SER Features Scale : 0 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Property Prefix : ROI 1 Resized SPY555
Calculate Texture Properties (2)	Input	Method	Output
	Channel : SPY555-DNA (global) Population : ROI 1 Region : ROI 1 Resized	Method : Haralick Features Distance : 1 px Haralick Contrast	Property Prefix : ROI 1 Resized SPY555

		Haralick Correlation Haralick Sum Variance Haralick Homogeneity	
Calculate Texture Properties (3)	Input	Method	Output
	Channel : SPY555-DNA (global) Population : ROI 1 Region : ROI 1 Resized	Method : Gabor Features Scale : 2 px Wavelength : 2 Number of Angles : 8 Normalization by : Kernel Gabor Min Gabor Max	Property Prefix : ROI 1 Resized SPY555-DNA (global)
Calculate Texture Properties (4)	Input	Method	Output
	Channel : ef450 (global) Population : ROI 1 Region : ROI 1 Resized	Method : SER Features Scale : 0 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Property Prefix : ROI 1 Resized ef450 (global)
Calculate Texture Properties (5)	Input	Method	Output
	Channel : ef450 (global) Population : ROI 1 Region : ROI 1 Resized	Method : Haralick Features Distance : 1 px Haralick Contrast Haralick Correlation Haralick Sum Variance Haralick Homogeneity	Property Prefix : ROI 1 Resized ef450 (global)
Calculate Texture Properties (6)	Input	Method	Output
	Channel : ef450 (global) Population : ROI 1 Region : ROI 1 Resized	Method : Gabor Features Scale : 2 px Wavelength : 2 Number of Angles : 8 Normalization by : Kernel Gabor Min Gabor Max	Property Prefix : ROI 1 Resized ef450 (global)
Calculate Intensity Properties	Input	Method	Output

	Channel : SPY555-DNA (global) Population : ROI 1 Region : ROI 1 Resized	Method : Standard Mean	Property Prefix : Intensity ROI 1 Resized SPY555-DNA (global)
--	--	-------------------------------	---

Calculate Intensity Properties (2)	Input	Method	Output
	Channel : ef450 (global) Population : ROI 1 Region : ROI 1 Resized	Method : Standard Mean	Property Prefix : Intensity ROI 1 Resized ef450 (global)

Select Population	Input	Method	Output
	Population : ROI 1	Method : Linear Classifier Number of Classes : 2 Organoid Region Resized SPY555-DNA (global) SER Spot 0 px Organoid Region Resized SPY555-DNA (global) SER Hole 0 px Organoid Region Resized SPY555-DNA (global) SER Edge 0 px Organoid Region Resized SPY555-DNA (global) SER Ridge 0 px Organoid Region Resized SPY555-DNA (global) SER Valley 0 px Organoid Region Resized SPY555-DNA (global) SER Saddle 0 px Organoid Region Resized SPY555-DNA (global) SER Bright 0 px Organoid Region Resized SPY555-DNA (global) SER Dark 0 px Organoid Region Resized SPY555-DNA (global) Haralick Correlation 1 px Organoid Region Resized SPY555-DNA (global) Haralick Contrast 1 px Organoid Region Resized SPY555-DNA (global) Haralick Sum Variance 1 px Organoid Region Resized SPY555-DNA (global) Haralick Homogeneity 1 px Organoid Region Resized SPY555-DNA (global) Gabor Min 2 px w2 Organoid Region Resized SPY555-DNA (global) Gabor Max 2 px w2	Output Population A : iPSC-aLO + T cells Output Population B : T cells only

		Image Region ef450 (global) SER Spot 0 px Image Region ef450 (global) SER Hole 0 px Image Region ef450 (global) SER Edge 0 px Image Region ef450 (global) SER Ridge 0 px Image Region ef450 (global) SER Valley 0 px Image Region ef450 (global) SER Saddle 0 px Image Region ef450 (global) SER Bright 0 px Image Region ef450 (global) SER Dark 0 px Organoid Region Resized ef450 (global) Haralick Correlation 1 px Organoid Region Resized ef450 (global) Haralick Contrast 1 px Organoid Region Resized ef450 (global) Haralick Sum Variance 1 px Organoid Region Resized ef450 (global) Haralick Homogeneity 1 px Organoid Region Resized ef450 (global) Gabor Min 2 px w2 Organoid Region Resized ef450 (global) Gabor Max 2 px w2 Intensity Organoid Region Resized SPY555-DNA (global) Mean Intensity Organoid Region Resized ef450 (global) Mean	
--	--	--	--

Calculate Intensity Properties (4)	Input	Method	Output
	Channel : Alexa 647 (global) Population : iPSC-aLO + T cells Region : ROI 1 Resized	Method : Standard Mean	Property Prefix : Intensity Casp iPSC-aLO + T cells

Calculate Properties	Input	Method	Output
	Population : ROI 1	Method : By Related Population Related Population : iPSC-aLO + T cells Intensity Casp iPSC-aLO + T cells Mean : Mean	Property Suffix : per Object

Define Results	Results
----------------	---------

Method : List of Outputs

Population : iPSC-aLO + T cells

Intensity Casp iPSC-aLO + T cells Mean : Mean

Population : ROI 1

Number of Objects

Object Results

Population : T cells only : None

Population : iPSC-aLO + T cells : Use Selected Well Results

Population : ROI 1 : Use Selected Well Results

Acapella version: 5.4.1.131587. Timestamp: 2025-05-07 11:54:57 +0200.