

Analysis Sequence "High resolution downstream analysis"

Input Image	Input		
	Channel group : 1 Sequences : ALL Flatfield Correction : Basic Brightfield Correction Stack Processing : 3D Analysis		
Find Image Region	Input	Method	Output
	Channel : SPY555-DNA ROI : None	Method : Local Threshold Threshold : <u>0.5</u> Region Scale : <u>10</u> μm Join touching Fragments Volume : > <u>100000</u> μm^3	Output Population : Image Region Output Region : Image Region
Select Population	Input	Method	Output
	Population : Image Region	Method : Common Filters Remove Objects : Touching Side Faces Region : Image Region	Output Population : Image Region 2
Modify Population	Input	Method	Output
	Population : Image Region 2 Region : Image Region	Method : Cluster by Distance Distance : <u>10</u> μm Volume : > 0 μm^3	Output Population : Organoid Output Region : Organoid
Filter Image	Input	Method	Output
	Channel : SPY555-DNA	Method : Smoothing Filter : Gaussian Width : <u>5</u> μm	Output Image : Gaussian Smoothed
Calculate Image	Input	Method	Output
		Method : By Formula Formula : A-B Channel A : SPY555-DNA Channel B : Gaussian Smoothed Negative Values : Set to Zero Undefined Values : Set to Zero	Output Image : Calculated Image
Filter Image (2)	Input	Method	Output

	Channel : Calculated Image	Method : Smoothing Filter : Gaussian Width : <u>1</u> px	Output Image : Gaussian Smoothed (2)
Calculate Image (2)	Input	Method	Output
		Method : By Formula Formula : $A*2+B$ Channel A : Calculated Image Channel B : Gaussian Smoothed (2) Negative Values : Set to Zero Undefined Values : Set to Zero	Output Image : Calculated Image (2)
Find Nuclei	Input	Method	Output
	Channel : Calculated Image ROI : Organoid ROI Region : Organoid	Method : C Common Threshold : <u>0</u> Volume : > <u>150</u> μm^3 Splitting Coefficient : <u>2.8</u> Individual Threshold : <u>0.05</u> Contrast : > <u>-1</u> Accuracy / Speed : Standard / Standard	Output Population : Nuclei
Calculate Intensity Properties (3)	Input	Method	Output
	Channel : SPY555-DNA Population : Nuclei Region : Nucleus	Method : Standard Mean	Property Prefix : Intensity SPY555 Nuclei
Calculate Intensity Properties	Input	Method	Output
	Channel : ef450 Population : Nuclei Region : Nucleus	Method : Standard Mean	Property Prefix : Intensity ef450 Nuclei
Calculate Intensity Properties (2)	Input	Method	Output
	Channel : Alexa 647 Population : Nuclei Region : Nucleus	Method : Standard Mean	Property Prefix : Intensity AF647 Nuclei
Select Population (2)	Input	Method	Output

	Population : Nuclei	Method : Filter by Property Intensity ef450 Nuclei Mean : > <u>250</u>	Output Population : ef450+ Nuclei (T cell)
Select Population (3)	Input	Method	Output
	Population : Nuclei	Method : Filter by Property Intensity ef450 Nuclei Mean : <= <u>250</u>	Output Population : ef450- (iPSC-aLO)
Select Population (8)	Input	Method	Output
	Population : ef450- (iPSC-aLO)	Method : Filter by Property Intensity SPY555 Nuclei Mean : > <u>350</u>	Output Population : 555+ ef450- Nuclei (iPSC-aLO)
Select Population (4)	Input	Method	Output
	Population : 555+ ef450- Nuclei (iPSC-aLO)	Method : Filter by Property Intensity AF647 Nuclei Mean : > <u>400</u>	Output Population : iPSC-aLO Casp pos
Select Population (5)	Input	Method	Output
	Population : 555+ ef450- Nuclei (iPSC-aLO)	Method : Filter by Property Intensity AF647 Nuclei Mean : <= <u>400</u>	Output Population : iPSC-aLO Casp neg
Select Population (6)	Input	Method	Output
	Population : ef450+ Nuclei (T cell)	Method : Filter by Property Intensity AF647 Nuclei Mean : > <u>400</u>	Output Population : T Cell Casp pos
Select Population (7)	Input	Method	Output
	Population : ef450+ Nuclei (T cell)	Method : Filter by Property Intensity AF647 Nuclei Mean : <= <u>400</u>	Output Population : T cell Casp neg
Calculate Properties	Input	Method	Output
	Population : Organoid	Method : By Related Population Related Population : Nuclei	Property Suffix : Number Nuclei Organoid

		Number of Nuclei	
Calculate Properties (2)	Input	Method	Output
	Population : Organoid	Method : By Related Population Related Population : iPSC-aLO Casp pos Number of iPSC-aLO Casp pos	Property Suffix : per Organoid
Calculate Properties (3)	Input	Method	Output
	Population : Organoid	Method : By Related Population Related Population : iPSC-aLO Casp neg Number of iPSC-aLO Casp neg	Property Suffix : per Organoid
Calculate Properties (4)	Input	Method	Output
	Population : Organoid	Method : By Related Population Related Population : T Cell Casp pos Number of T Cell Casp pos	Property Suffix : per Organoid
Calculate Properties (5)	Input	Method	Output
	Population : Organoid	Method : By Related Population Related Population : T cell Casp neg Number of T cell Casp neg	Property Suffix : per Organoid
Calculate Properties (6)	Input	Method	Output
	Population : Organoid	Method : By Formula Formula : A+B Variable A : Number of T Cell Casp pos- per Organoid Variable B : Number of T cell Casp neg- per Organoid	Output Property : Number T cells
Calculate Properties (9)	Input	Method	Output
	Population : Organoid	Method : By Formula Formula : A+B Variable A : Number of iPSC-aLO Casp pos- per Organoid	Output Property : Number iPSC-aLO cells

		Variable B : Number of iPSC-aLO Casp neg- per Organoid	
Calculate Properties (7)	Input	Method	Output
	Population : Organoid	Method : By Formula Formula : $(A/(A+B))*100$ Variable A : Number of iPSC-aLO Casp pos- per Organoid Variable B : Number of iPSC-aLO Casp neg- per Organoid	Output Property : %Casp pos iPSC-aLO cells
Calculate Properties (8)	Input	Method	Output
	Population : Organoid	Method : By Formula Formula : $(A/(A+B))*100$ Variable A : Number of T Cell Casp pos- per Organoid Variable B : Number of T cell Casp neg- per Organoid	Output Property : %Casp pos T cells

Define Results	Results
	Method : List of Outputs Population : Image Region Number of Objects Population : Image Region 2 Number of Objects Population : ef450+ Nuclei (T cell) Number of Objects Population : Nuclei Number of Objects Population : ef450- (iPSC-aLO) Number of Objects Population : 555+ ef450- Nuclei (iPSC-aLO) Number of Objects Population : iPSC-aLO Casp pos Number of Objects Population : iPSC-aLO Casp neg Number of Objects Population : T Cell Casp pos Number of Objects Population : T cell Casp neg Number of Objects Population : Organoid Number of Objects

Number of Nuclei- Number Nuclei Organoid : Mean
Number of iPSC-aLO Casp pos- per Organoid : Mean
Number of iPSC-aLO Casp neg- per Organoid : Mean
Number of T Cell Casp pos- per Organoid : Mean
Number of T cell Casp neg- per Organoid : Mean
Number T cells : Mean
Number iPSC-aLO cells : Mean
%Casp pos iPSC-aLO cells : Mean
%Casp pos T cells : Mean

Method : Formula Output

Formula : a/b

Population Type : Objects

Variable a : Image Region - Number of Objects

Variable b : Image Region - Number of Objects

Output Name : Estimated No of Lung cells

Object Results

Population : Image Region : ALL

Population : Image Region 2 : ALL

Population : ef450+ Nuclei (T cell) : ALL

Population : Nuclei : ALL

Population : ef450- (iPSC-aLO) : ALL

Population : 555+ ef450- Nuclei (iPSC-aLO) : None

Population : iPSC-aLO Casp pos : ALL

Population : iPSC-aLO Casp neg : ALL

Population : T Cell Casp pos : ALL

Population : T cell Casp neg : ALL

Population : Organoid : ALL

Acapella version: 5.4.1.131587. Timestamp: 2025-05-07 11:08:23 +0200.