

Analysis Sequence "JoVE Image Analysis Protocol"

Input Image	Input		
	Channel group : 1 Sequences : ALL Flatfield Correction : Advanced Brightfield Correction Stack Processing : Individual Planes		
Find Nuclei	Input	Method	Output
	Channel : DAPI ROI : None	Method : B Common Threshold : 0.4 Area : > 30 μm^2 Splitting Coefficient : 7.0 Individual Threshold : 0.4 Contrast : > <u>0.13</u>	Output Population : Nuclei
Find Cytoplasm	Input	Method	Output
	Channel : CellMask Deep Red Nuclei : Nuclei	Method : A Individual Threshold : <u>0.02</u>	
Select Population	Input	Method	Output
	Population : Nuclei	Method : Common Filters Remove Border Objects Region : Cell	Output Population : Whole cells
Find Image Region	Input	Method	Output
	Channel : mCherry ROI : Whole cells ROI Region : Cell	Method : Absolute Threshold Lowest Intensity : \geq <u>300</u> Highest Intensity : \leq inf Split into Objects Area : > 0 px^2	Output Population : Bacteria in whole cells Output Region : Bacteria
Calculate Properties (2)	Input	Method	Output
	Population : Whole cells	Method : By Related Population Related Population : Bacteria in whole cells Number of Bacteria in whole cells	Property Suffix : per Cell
Select Population (2)	Input	Method	Output

	Population : Whole cells	Method : Filter by Property Number of Bacteria in whole cells- per Cell : > 0	Output Population : Infected whole cells
Select Population (3)	Input	Method	Output
	Population : Whole cells	Method : Filter by Property Number of Bacteria in whole cells- per Cell : == 0	Output Population : Non-infected cells
Calculate Intensity Properties	Input	Method	Output
	Channel : mCherry Population : Infected whole cells Region : Bacteria	Method : Standard Mean	Property Prefix : Intensity Bacteria
Calculate Morphology Properties	Input	Method	Output
	Population : Whole cells Region : Cell	Method : Standard Area Ratio Width to Length	Property Prefix : Whole cells
Calculate Morphology Properties (3)	Input	Method	Output
	Population : Infected whole cells Region : Cell	Method : Standard Area Ratio Width to Length	Property Prefix : Infected whole cells
Calculate Morphology Properties (4)	Input	Method	Output
	Population : Non-infected cells Region : Cell	Method : Standard Area Ratio Width to Length	Property Prefix : Cell
Calculate Morphology Properties (2)	Input	Method	Output
	Population : Infected whole cells Region : Bacteria	Method : Standard Area	Property Prefix : Bacteria region
Define Results	Results		
	Method : List of Outputs Population : Nuclei Apply to All : None		

Population : Bacteria in whole cells

Number of Objects

Population : Whole cells

Number of Objects

Whole cells Area [μm^2] : Mean+StdDev

Whole cells Ratio Width to Length : Mean+StdDev

Population : Non-infected cells

Number of Objects

Cell Area [μm^2] : Mean+StdDev

Cell Ratio Width to Length : Mean+StdDev

Population : Infected whole cells

Number of Objects

Intensity Bacteria Mean : Sum

Infected whole cells Area [μm^2] : Mean+StdDev

Infected whole cells Ratio Width to Length : Mean+StdDev

Bacteria region Area [μm^2] : Sum

Object Results

Population : Nuclei : None

Population : Bacteria in whole cells : ALL

Population : Whole cells : ALL

Population : Non-infected cells : None

Population : Infected whole cells : ALL