

Analysis Sequence "Proliferation Index 3D sample"

Input Image	Input		
	Flatfield Correction : None Brightfield Correction Stack Processing : 3D Analysis Min. Global Binning : Dynamic		
Find Image Region	Input	Method	Output
	Channel : Alexa 488 ROI : None	Method : Local Threshold Threshold : 0.4 Region Scale : 15 μm Filling : Fill Plane-Wise Volume : > <u>2000</u> μm^3	Output Population : Spheroid Output Region : Spheroid
Find Nuclei	Input	Method	Output
	Channel : HOECHST 33342 - extended ROI : Spheroid ROI Region : Spheroid	Method : B Common Threshold : 0.4 Volume : > 120 μm^3 Splitting Coefficient : 7 Individual Threshold : 0.4 Contrast : > 0.1 Accuracy / Speed : Standard / Standard	Output Population : Hoechst +
Find Nuclei (2)	Input	Method	Output
	Channel : Alexa 488 ROI : Hoechst + ROI Region : Nucleus	Method : C Common Threshold : 0.4 Volume : > <u>50</u> μm^3 Splitting Coefficient : 7.0 Individual Threshold : 0.4 Contrast : > 0.1 Accuracy / Speed : Standard / Standard	Output Population : Ki67+ pockets
Calculate Intensity Properties	Input	Method	Output
	Channel : Alexa 488 Population : Ki67+ pockets Region : Nucleus	Method : Standard Mean	Property Prefix : Intensity 488
Calculate Properties	Input	Method	Output
	Population : Hoechst +	Method : By Related Population Related Population : Ki67+ pockets Number of Ki67+ pockets Intensity 488 Mean	Property Suffix : per Cell
Select Population	Input	Method	Output
	Population : Hoechst +	Method : Filter by Property Number of Ki67+ pockets- per Cell : > <u>1</u> Intensity 488 Mean- Mean per Cell : > <u>1000</u> Boolean Operations : F1 and F2	Output Population : Nuclei en division
Define Results	Results		
	Method : List of Outputs Population : Spheroid Number of Objects Method : Formula Output Formula : (a/b)*100 Population Type : Objects Variable a : Hoechst +/- Dividing Nuclei Sum Variable b : Hoechst +/- Number of Objects Output Name : % Dividing Cells Object Results Population : Hoechst + : None Population : Spheroid : None Population : Ki67+ pockets : None Population : Dividing Nuclei : None		