

## Supporting Information

### Machine learning classifies ferroptosis and apoptosis cell death modalities with TfR1 immunostaining

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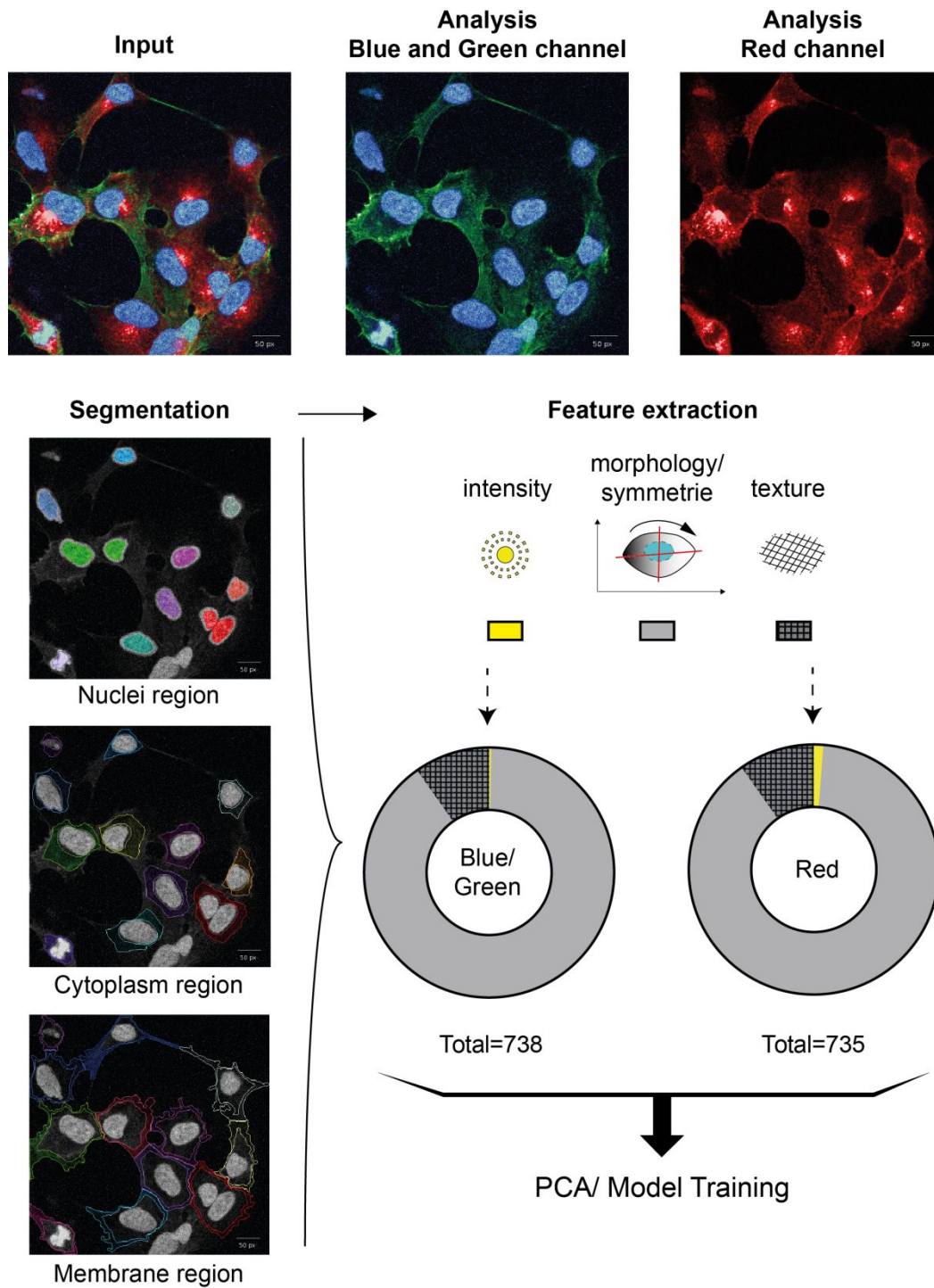
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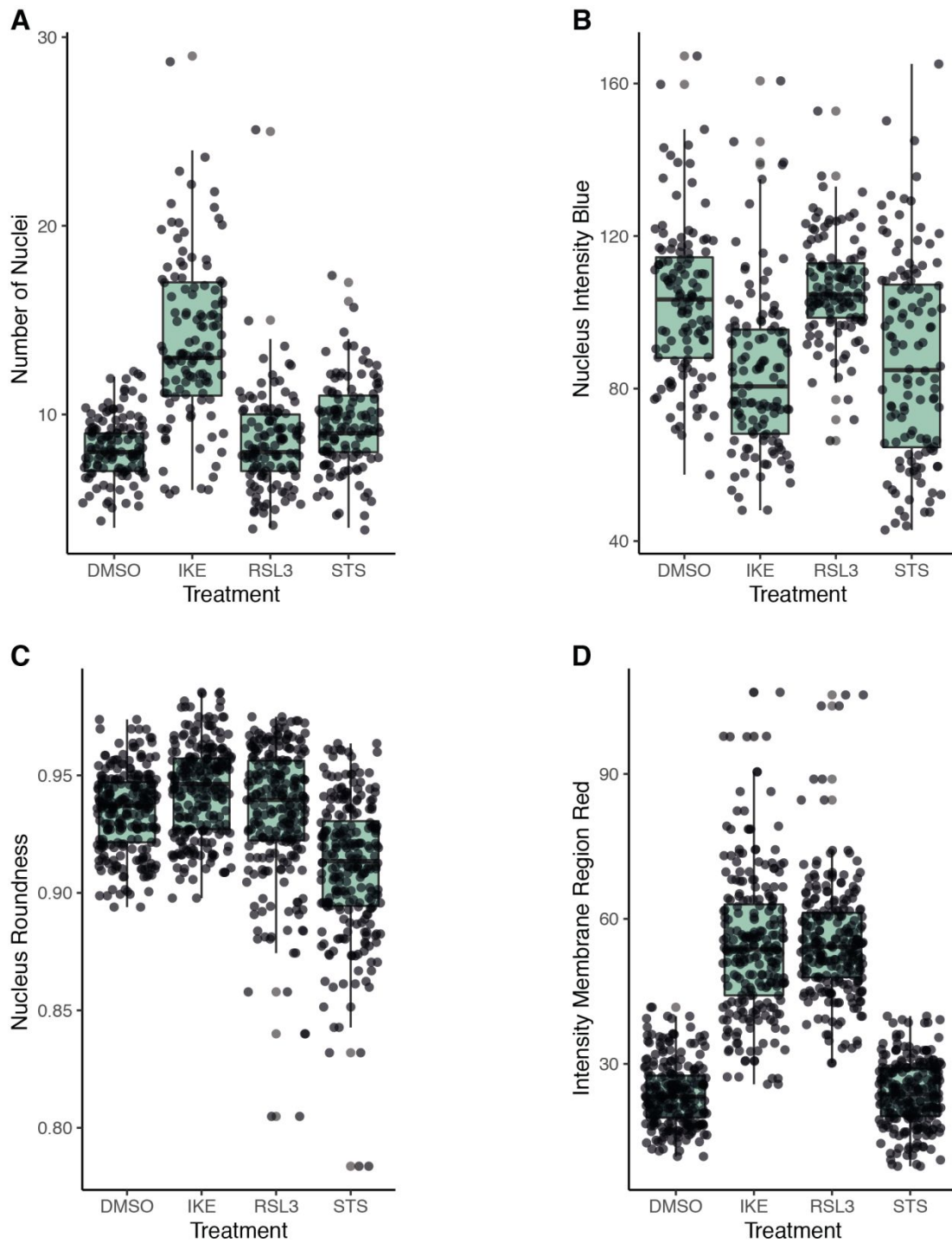
## SUPPORTING FIGURES

Cell death inducer	Treatment time	% cell death (relative to DMSO control)
RSL3	2h	13.2%
	3h	28.2%
	4h	51.5%
IKE	6h	2.7%
	7h	13.3%
	8h	15.5%
STS	6h	16.7%
	7h	25.4%
	8h	30.8%

**Supplementary Figure S1.** Pilot study using CellTiter-Glo viability assay to assess optimal cell death inducer concentration and timepoint. HT-1080 cells were treated with ferroptosis inducers RSL3 (1  $\mu$ M) or IKE (20  $\mu$ M), apoptosis inducer STS (1  $\mu$ M), or DMSO vehicle control, at various timepoints. Using a CellTiter-Glo viability assay, an optimal treatment timepoint (identified above by the highlighted rows) was determined for each treatment when percentage cell death reached between 10-20%.



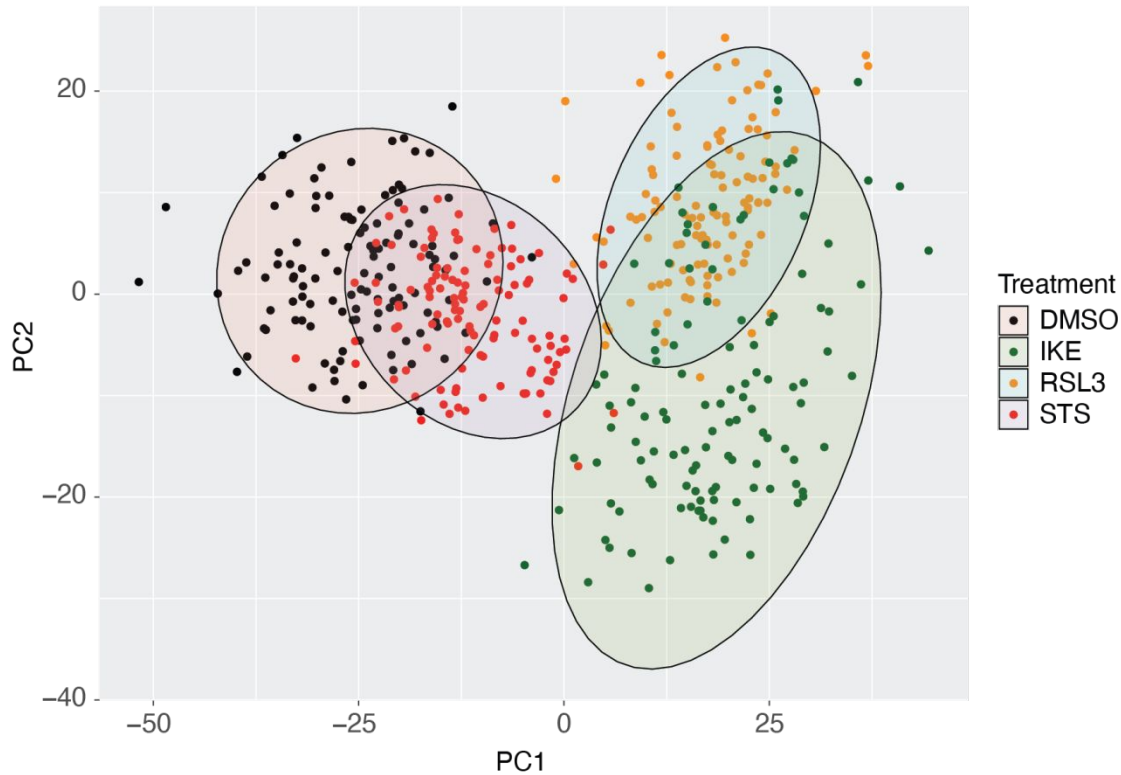
**Supplementary Figure 2.** Illustrative figures with a workflow of the automated image analysis and data extraction. In each image, the nuclei were identified using DAPI staining. The cytoplasm and membrane region were segmented using phalloidin-FITC staining. Subsequently, a large number of features (intensity, morphology/ symmetry, texture) were extracted for each cell segment by processing and analyzing the blue-green and red fluorescence signals. All data were then combined and used for further analyzes. For detailed description see material and methods section.



**Supplementary Figure 3.** Boxplots of representative features. Each dot shows (A) the sum or (B-C) the median value of an image feature. The data were sorted by treatment (DMSO, IKE, RSL3, STS). (A) Number of Nuclei; (B) Nucleus Blue Intensity; (C) Nucleus Roundness; (D) Membrane region red intensity.

**A**

Cell death inducer	% cell death (relative to DMSO control)
RSL3 (1 $\mu$ M, 2.5 h)	13.6%
IKE (20 $\mu$ M, 8 h)	16.8%
STS (1 $\mu$ M, 5.5 h)	12.1%

**test data set****B**

**Supplementary Figure 4.** CTG data and visualization of the extracted data for validation experiment (test data set): (A) CTG values for the different treatments that were generated in parallel to the immunofluorescent staining. (B) Principal component analysis of validation experiment. PC1 = 29,74% and PC2 = 7,96%.

## ANALYSIS SEQUENCES

### Blue-Green:

<b>Input Image</b>	<b>Stack Processing :</b> Individual Planes <b>Flatfield Correction :</b> None		
<b>Filter Image</b>	<b>Channel :</b> Blue	<b>Method :</b> Sliding Parabola Curvature : 10	Output Image : Sliding Parabola
<b>Filter Image (2)</b>	<b>Channel :</b> Sliding Parabola	<b>Method :</b> Smoothing Filter : Median Scale : 6 px	Output Image : Median Smoothed Nuclei
<b>Filter Image (3)</b>	<b>Channel :</b> Green	<b>Method :</b> Smoothing Filter : Median Scale : 3 px	Output Image : Median Smoothed
<b>Find Nuclei</b>	<b>Channel :</b> Median Smoothed Nuclei <b>ROI :</b> None	<b>Method :</b> B Common Threshold : 0.4 Area : > 150 px2 Split Factor : 10 Individual Threshold : 0.4 Contrast : > 0.1	Output Population : Nuclei
<b>Calculate Intensity Properties</b>	<b>Channel :</b> Blue <b>Population :</b> Nuclei <b>Region :</b> Nucleus	<b>Method :</b> Standard Mean	Output Properties : Intensity Nucleus Blue
<b>Calculate Morphology Properties</b>	<b>Population :</b> Nuclei <b>Region :</b> Nucleus	<b>Method :</b> Standard Area Roundness Width Length Ratio Width to Length Output Properties : Nucleus	Output Properties : Nucleus
<b>Select Population</b>	<b>Population :</b> Nuclei	<b>Method :</b> Filter by Property Intensity Nucleus Blue Mean : > 35 Nucleus Roundness : > 0.6 Nucleus Area [px2] : > 300 Boolean Operations : F1 and F2 and F3	Output Population : Nuclei Selected
<b>Find Cytoplasm</b>	<b>Channel :</b> Median Smoothed <b>Nuclei :</b> Nuclei Selected	<b>Method :</b> A Individual Threshold : 0.15	Output Population : Nuclei

<b>Select Population (2) :</b>	<b>Population :</b> Nuclei Selected	<b>Method :</b> Common Filters Remove Border Objects Region : Nucleus	Output Population : Nuclei Selected Selected
<b>Select Cell Region</b>	<b>Population :</b> Nuclei Selected Selected	<b>Method :</b> Resize Region [%] Region Type : Cytoplasm Region Outer Border : 15 % Inner Border : 40 %	Output Region : Cytoplasm Region
<b>Select Cell Region (2)</b>	<b>Population :</b> Nuclei Selected Selected	<b>Method :</b> Resize Region [%] Region Type : Membrane Region Outer Border : -10 % Inner Border : 10 %	Output Region : Membrane Region
<b>Select Cell Region (3)</b>	<b>Population :</b> Nuclei Selected Selected	<b>Method :</b> Resize Region [%] Region Type : Nucleus Region Outer Border : 55 % Inner Border : 100 %	Output Region : Nucleus Region
<b>Calculate Texture Properties (1)</b>	<b>Channel :</b> Blue <b>Population :</b> Nuclei Selected Selected <b>Region :</b> Nucleus Region	<b>Method :</b> SER Features Scale : 0 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Nucleus Region Blue
<b>Calculate Texture Properties (2)</b>	<b>Channel :</b> Blue <b>Population :</b> Nuclei Selected Selected	<b>Method :</b> SER Features Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Nucleus Region Blue
<b>Calculate Texture Properties (3)</b>	<b>Channel :</b> Blue <b>Population :</b> Nuclei Selected Selected <b>Region :</b> Nucleus Region	<b>Method :</b> SER Features Scale : 2 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Nucleus Region Blue

<p><b>Calculate Morphology Properties (2)</b></p>	<p><b>Population :</b> Nuclei Selected Selected <b>Region :</b> Nucleus Region</p>	<p><b>Method :</b> STAR Channel : Blue Symmetry Threshold Compactness Axial Radial Profile Profile Inner Region : Nucleus Profile Width : 10 px <b>Sliding Parabola</b> Sliding Parabola Curvature : 10 Use for Center <b>Texture SER</b> Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark</p>	<p>Output Properties : Nucleus Region</p>
<p><b>Calculate Intensity Properties (2)</b></p>	<p><b>Channel :</b> Green <b>Population :</b> Nuclei Selected Selected <b>Region :</b> Cytoplasm Region</p>	<p><b>Method :</b> Standard Mean</p>	<p>Output Properties : Intensity Cytoplasm Region Green</p>
<p><b>Calculate Morphology Properties (3)</b></p>	<p><b>Population :</b> Nuclei Selected Selected <b>Region :</b> Cytoplasm Region</p>	<p><b>Method :</b> Standard Area Roundness Width Length Ratio Width to Length</p>	<p>Output Properties : Cytoplasm Region</p>
<p><b>Calculate Texture Properties (4)</b></p>	<p><b>Channel :</b> Green <b>Population :</b> Nuclei Selected Selected <b>Region :</b> Cytoplasm Region</p>	<p><b>Method :</b> SER Features Scale : 0 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark</p>	<p>Output Properties : Cytoplasm Region Green</p>
<p><b>Calculate Texture Properties (5)</b></p>	<p><b>Channel :</b> Green <b>Population :</b> Nuclei Selected Selected <b>Region :</b> Cytoplasm</p>	<p><b>Method :</b> SER Features Scale : 1 px Normalization by : Kernel SER Spot SER Hole</p>	<p>Output Properties : Cytoplasm Region Green</p>



	Region	SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	
<b>Calculate Texture Properties (6)</b>	<b>Channel :</b> Green <b>Population :</b> Nuclei Selected Selected <b>Region :</b> Cytoplasm Region	<b>Method :</b> SER Features Scale : 2 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Cytoplasm Region Green
<b>Calculate Morphology Properties (4)</b>	<b>Population :</b> Nuclei Selected Selected <b>Region :</b> Cytoplasm Region	<b>Method :</b> STAR Channel : Green Symmetry Threshold Compactness Axial Radial Profile Profile Inner Region : Nucleus Profile Width : 10 px <b>Sliding Parabola</b> Sliding Parabola Curvature : 10 Use for Center <b>Texture SER</b> Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Cytoplasm Region
<b>Calculate Texture Properties (7)</b>	<b>Channel :</b> Green <b>Population :</b> Nuclei Selected Selected <b>Region :</b> Membrane Region	<b>Method :</b> SER Features Scale : 0 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Hole	Output Properties : Membrane Region Green

		SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	
<b>Calculate Texture Properties (8)</b>	<b>Channel</b> : Green <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Membrane Region	<b>Method</b> : SER Features Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Membrane Region Green
<b>Calculate Texture Properties (9)</b>	<b>Channel</b> : Green <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Membrane Region	<b>Method</b> : SER Features Scale : 2 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Membrane Region Green
<b>Calculate Morphology Properties (5)</b>	<b>Population</b> : Nuclei Selected Selected <b>Region</b> : Membrane Region	<b>Method</b> : STAR Channel : Green Symmetry Threshold Compactness Axial Radial Profile Profile Inner Region : Nucleus Profile Width : 10 px <b>Sliding Parabola</b> Sliding Parabola Curvature : 10 Use for Center <b>Texture SER</b> Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Membrane Region

Red:

<b>Input Image</b>	<b>Stack Processing :</b> Individual Planes <b>Flatfield Correction :</b> None		
<b>Filter Image</b>	<b>Channel :</b> Blue	<b>Method :</b> Sliding Parabola Curvature : 10	Output Image : Sliding Parabola
<b>Filter Image (2)</b>	<b>Channel :</b> Sliding Parabola	<b>Method :</b> Smoothing Filter : Median Scale : 6 px	Output Image : Median Smoothed Nuclei
<b>Filter Image (3)</b>	<b>Channel :</b> Green	<b>Method :</b> Smoothing Filter : Median Scale : 3 px	Output Image : Median Smoothed
<b>Find Nuclei</b>	<b>Channel :</b> Median Smoothed Nuclei <b>ROI :</b> None	<b>Method :</b> B Common Threshold : 0.4 Area : > 150 px <sup>2</sup> Split Factor : 10 Individual Threshold : 0.4 Contrast : > 0.1	Output Population : Nuclei
<b>Calculate Intensity Properties</b>	<b>Channel :</b> Blue <b>Population :</b> Nuclei <b>Region :</b> Nucleus	<b>Method :</b> Standard Mean	Output Properties : Intensity Nucleus Blue
<b>Calculate Morphology Properties</b>	<b>Population :</b> Nuclei <b>Region :</b> Nucleus	<b>Method :</b> Standard Area Roundness Width Length Ratio Width to Length	Output Properties : Nucleus
<b>Select Population</b>	<b>Population :</b> Nuclei	<b>Method :</b> Filter by Property Intensity Nucleus Blue Mean : > 35 Nucleus Roundness : > 0.6 Nucleus Area [px <sup>2</sup> ] : > 300 Boolean Operations : F1 and F2 and F3	Output Population : Nuclei Selected
<b>Find Cytoplasm</b>	<b>Channel :</b> Median Smoothed <b>Nuclei :</b> Nuclei Selected	<b>Method :</b> A Individual Threshold : 0.15	
<b>Select Population (2)</b>	<b>Population :</b> Nuclei Selected	<b>Method :</b> Common Filters Remove Border Objects Region : Nucleus	Output Population : Nuclei Selected Selected
<b>Select Cell Region</b>	<b>Population :</b> Nuclei Selected Selected	<b>Method :</b> Resize Region [%] Region Type : Membrane Region Outer Border : -10 % Inner Border : 10 %	Output Region : Membrane Region
<b>Select Cell Region (2)</b>	<b>Population :</b> Nuclei Selected Selected	<b>Method :</b> Resize Region [%] Region Type : Cytoplasm Region Outer Border : 15 % Inner Border : 40 %	Output Region : Cytoplasm Region
<b>Select</b>	<b>Population :</b> Nuclei	<b>Method :</b> Resize Region [%]	Output Region :

<b>Cell Region (3)</b>	Selected Selected	Region Type : Nucleus Region Outer Border : 55 % Inner Border : 100 %	Nucleus Region
<b>Calculate Intensity Properties (2)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Membrane Region	<b>Method</b> : Standard Mean Median Contrast	Output Properties : Intensity Membrane Region Red
<b>Calculate Intensity Properties (3)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Cytoplasm Region	<b>Method</b> : Standard Mean Median Contrast	Output Properties : Intensity Cytoplasm Region Red
<b>Calculate Intensity Properties (4)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Nucleus Region	<b>Method</b> : Standard Mean Median Contrast	Output Properties : Intensity Nucleus Region Red
<b>Calculate Texture Properties</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Membrane Region	<b>Method</b> : SER Features Scale : 0 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Membrane Region Red
<b>Calculate Texture Properties (2)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Cytoplasm Region	<b>Method</b> : SER Features Scale : 0 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Cytoplasm Region Red
<b>Calculate Texture Properties (3)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Nucleus Region	<b>Method</b> : SER Features Scale : 0 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Nucleus Region Red
<b>Calculate</b>	<b>Channel</b> : Red	<b>Method</b> : SER Features	Output Properties :

<b>Texture Properties (4)</b>	<b>Population</b> : Nuclei Selected Selected <b>Region</b> : Membrane Region	Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Membrane Region Red
<b>Calculate Texture Properties (5)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Cytoplasm Region	<b>Method</b> : SER Features Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Cytoplasm Region Red
<b>Calculate Texture Properties (6)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Nucleus Region	<b>Method</b> : SER Features Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Nucleus Region Red
<b>Calculate Texture Properties (7)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Membrane Region	<b>Method</b> : SER Features Scale : 2 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Membrane Region Red
<b>Calculate Texture Properties (8)</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei Selected Selected <b>Region</b> : Cytoplasm Region	<b>Method</b> : SER Features Scale : 2 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Cytoplasm Region Red
<b>Calculate Texture</b>	<b>Channel</b> : Red <b>Population</b> : Nuclei	<b>Method</b> : SER Features Scale : 2 px	Output Properties : Nucleus

<b>Properties (9)</b>	Selected Selected <b>Region</b> : Nucleus Region	Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Region Red
<b>Calculate Morphology Properties (2)</b>	<b>Population</b> : Nuclei Selected Selected <b>Region</b> : Nucleus Region	<b>Method</b> : STAR Channel : Red Symmetry Threshold Compactness Axial Radial Profile Profile Inner Region : Nucleus Profile Width : 10 px <b>Sliding Parabola</b> Sliding Parabola Curvature : 10 Use for Center <b>Texture SER</b> Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Nucleus Region
<b>Calculate Morphology Properties (3)</b>	<b>Population</b> : Nuclei Selected Selected <b>Region</b> : Cytoplasm Region	<b>Method</b> : STAR Channel : Red Symmetry Threshold Compactness Axial Radial Profile Profile Inner Region : Nucleus Profile Width : 10 px <b>Sliding Parabola</b> Sliding Parabola Curvature : 10 Use for Center <b>Texture SER</b> Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley	Output Properties : Cytoplasm Region

		SER Saddle SER Bright SER Dark	
<b>Calculate Morphology Properties (4)</b>	<b>Population :</b> Nuclei Selected Selected <b>Region :</b> Membrane Region	<b>Method :</b> STAR Channel : Red Symmetry Threshold Compactness Axial Radial Profile Profile Inner Region : Nucleus Profile Width : 10 px <b>Sliding Parabola</b> Sliding Parabola Curvature : 10 Use for Center <b>Texture SER</b> Scale : 1 px Normalization by : Kernel SER Spot SER Hole SER Edge SER Ridge SER Valley SER Saddle SER Bright SER Dark	Output Properties : Membrane Region