

# **Supplementary Information to Chapter 3 Opera Phenix phenotyping**

The tables here provide details associated with **Chapter 3**.

Species	Isolate ID	Coating
S. Typhimurium	NCTC 13347	Thick Collagen
S. Typhimurium	NCTC 13348	Matrigel
S. Typhimurium	D23580	Vitronectin

**Table B.1. Final plate coatings chosen for isolates used in Opera Phenix imaging.**

<b>Input Image</b>	Input		
	<b>Flatfield Correction:</b> Basic Brightfield Correction <b>Stack Processing:</b> Maximum Projection ( <i>for non-adherent isolates, individual planes were analysed</i> ) <b>Min. Global Binning:</b> Dynamic		
<b>Filter Image</b>	Input	Method	Output
	<b>Channel :</b> DAPI	<b>Method :</b> Texture SER Filter : SER Ridge Scale : 1 px Normalization by : Kernel	Output Image : SER Ridge
<b>Find Image Region</b>	Input	Method	Output
	<b>Channel:</b> SER Ridge <b>ROI:</b> None	Method : Common Threshold Threshold : 0.4 Split into Objects Area : > 100 px <sup>2</sup>	Output Population : Image Region Output Region : Image Region
<b>Calculate Intensity Properties</b>	Input	Method	Output
	<b>Channel :</b> SER Ridge <b>Population :</b> Image Region <b>Region :</b> Image Region	<b>Method :</b> Standard Mean	Property Prefix : Intensity Image Region SER Ridge
<b>Select Population</b>	Input	Method	Output
	<b>Population :</b> Image Region	<b>Method</b> Filter by Property Intensity Image Region SER Ridge Mean : > 0.02	Output Population : Image Region Selected
<b>Select Region</b>	Input	Method	Output
	<b>Population :</b> Image Region Selected <b>Region :</b> Image Region	<b>Method :</b> Resize Region [ $\mu\text{m}/\text{px}$ ] Outer Border : $-4\mu\text{m}$ Restrictive Population : None Restrictive Region : Keep Image Border Inner Border : INF $\mu\text{m}$	Output Population : Image Region Resized
<b>Select Region (2)</b>	Input	Method	Output
	<b>Population :</b> Image Region Selected <b>Region :</b> Image Region Resized	<b>Method :</b> Standard Border Filled Region : INF $\mu\text{m}^2$	Property Prefix : Image Region Resized

Select Region (3)	Input	Method	Output
	<b>Population</b> : Image Region Selected <b>Region</b> : Image Region Resized Filled	<b>Method</b> : Resize Region [ $\mu\text{m}/\text{px}$ ] Outer Border : 4 px Restrictive Population : None Restrictive Region : Keep Image Border Inner Border : INF px	Output Population : Image Region Resized Filled Resized
Modify Population	Input	Method	Output
	<b>Population</b> : Image Region Selected Region : Image Region Resized Filled Resized	<b>Method</b> : Cluster by Distance Distance : 0 px Area : $> 0 \text{ px}^2$	Output Population : Modified Image Region Selected Output Region : Modified Image Region
Select Population (2)	Input	Method	Output
	<b>Population</b> : Modified Image Region Selected	<b>Method</b> : Common Filters Remove Border Objects Region : Modified Image Region	Output Population : Modified Image Region Selected Border Removed
Calculate Image	Input	Method	Output
		<b>Method</b> : By Formula Formula : $100*(A-300)+100*(B-300)$ Channel A : DAPI Channel B : FM4-64 Negative Values : Set to Zero Undefined Values : Set to Local Average	Output Image : Calculated Image
Find Spots	Input	Method	Output
	<b>Channel</b> : Calculated Image <b>ROI</b> : Modified Image Region Selected Border Removed <b>ROI Region</b> : Modified Image Region	<b>Method</b> : D Detection Sensitivity : 0.5 Splitting Sensitivity : 0.1 Background Correction : 0.5 Calculate Spot Properties	Output Population : Spots
Calculate Morphology Properties	Input	Method	Output
	<b>Population</b> : Spots <b>Region</b> : Spot	<b>Method</b> : Standard Area Roundness	Property Prefix : Spot
Select Population (3)	Input	Method	Output
	<b>Population</b> : Spot	<b>Method</b> Filter by Property Spot Area [ $\text{px}^2$ ] : $> 1$	Output Population : bacteria
Calculate Morphology Properties (2)	Input	Method	Output
	<b>Population</b> : bacteria <b>Region</b> : Spot	<b>Method</b> : Standard Area Roundness Width Length Ratio Width to Length	Property Prefix : bacteria
Calculate Morphology Properties (3)	Input	Method	Output
	<b>Population</b> : bacteria <b>Region</b> : Spot	<b>Method</b> : STAR Channel : DAPI Symmetry Threshold Compactness	Property Prefix : Bacteria DAPI

		Axial Radial Profile Profile Width : 3 px <b>Sliding Parabola</b> Curvature : 10 <b>Texture SER</b> Scale : 1 px Normalization by : Kernel	
Calculate Morphology Properties (4)	Input	Method	Output
	<b>Population</b> : bacteria <b>Region</b> : Spot	<b>Method</b> : STAR Channel : FM4-64 Symmetry Threshold Compactness Axial Radial Profile Profile Width : 3 px <b>Sliding Parabola</b> Curvature : 10 <b>Texture SER</b> Scale : 1 px Normalization by : Kernel	Property Prefix : Bacteria FM4-64
Calculate Morphology Properties (5)	Input	Method	Output
	<b>Population</b> : bacteria <b>Region</b> : Spot	<b>Method</b> : STAR Channel : SYTOX green Symmetry Threshold Compactness Axial Radial Profile Profile Width : 3 px <b>Sliding Parabola</b> Curvature : 10 <b>Texture SER</b> Scale : 1 px Normalization by : Kernel	Property Prefix : Bacteria SYTOX green
Calculate Intensity Properties (3)	Input	Method	Output
	<b>Channel</b> : SYTOX green <b>Population</b> : bacteria <b>Region</b> : Spot	<b>Method</b> : Standard Mean Standard Deviation	Property Prefix : Intensity Spot SYTOX green
Select Population (4)	Input	Method	Output
	<b>Population</b> : bacteria	<b>Method</b> Linear Classifier Number of Classes : 3 Relative Spot Intensity Corrected Spot Intensity Uncorrected Spot Peak Intensity Spot Contrast Spot Background Intensity Spot Area [px <sup>2</sup> ] Region Intensity Spot to Region Intensity Spot Area [μm <sup>2</sup> ] Spot Roundness bacteria Area [μm <sup>2</sup> ] bacteria Roundness bacteria Width [μm] bacteria Length [μm] bacteria Ratio Width to Length Bacteria DAPI Symmetry 02 Bacteria DAPI Symmetry 03 Bacteria DAPI Symmetry 04 Bacteria DAPI Symmetry 05 Bacteria DAPI Symmetry 12 Bacteria DAPI Symmetry 13 Bacteria DAPI Symmetry 14	Output Population A : Single Cells Output Population B : Dividing Cells Output Population C : Other

Bacteria DAPI Symmetry 15  
 Bacteria DAPI Threshold  
 Compactness 30%  
 Bacteria DAPI Threshold  
 Compactness 40%  
 Bacteria DAPI Threshold  
 Compactness 50%  
 Bacteria DAPI Threshold  
 Compactness 60%  
 Bacteria DAPI Axial Small Length  
 Bacteria DAPI Axial Length Ratio  
 Bacteria DAPI Radial Mean  
 Bacteria DAPI Radial Relative  
 Deviation  
 Bacteria DAPI Profile 1/2  
 Bacteria DAPI Profile 2/2  
 Bacteria FM4-64 Symmetry 02  
 Bacteria FM4-64 Symmetry 03  
 Bacteria FM4-64 Symmetry 04  
 Bacteria FM4-64 Symmetry 05  
 Bacteria FM4-64 Symmetry 12  
 Bacteria FM4-64 Symmetry 13  
 Bacteria FM4-64 Symmetry 14  
 Bacteria FM4-64 Symmetry 15  
 Bacteria FM4-64 Threshold  
 Compactness 30%  
 Bacteria FM4-64 Threshold  
 Compactness 40%  
 Bacteria FM4-64 Threshold  
 Compactness 50%  
 Bacteria FM4-64 Threshold  
 Compactness 60%  
 Bacteria FM4-64 Axial Small  
 Length  
 Bacteria FM4-64 Axial Length  
 Ratio  
 Bacteria FM4-64 Radial Mean  
 Bacteria FM4-64 Radial Relative  
 Deviation  
 Bacteria FM4-64 Profile 1/2  
 Bacteria FM4-64 Profile 2/2  
 Bacteria Sytox Symmetry 02  
 Bacteria Sytox Symmetry 03  
 Bacteria Sytox Symmetry 04  
 Bacteria Sytox Symmetry 05  
 Bacteria Sytox Symmetry 12  
 Bacteria Sytox Symmetry 13  
 Bacteria Sytox Symmetry 14  
 Bacteria Sytox Symmetry 15  
 Bacteria Sytox Threshold  
 Compactness 30%  
 Bacteria Sytox Threshold  
 Compactness 40%  
 Bacteria Sytox Threshold  
 Compactness 50%  
 Bacteria Sytox Threshold  
 Compactness 60%  
 Bacteria Sytox Axial Small Length  
 Bacteria Sytox Axial Length Ratio  
 Bacteria Sytox Radial Mean  
 Bacteria Sytox Radial Relative  
 Deviation  
 Bacteria Sytox Profile 1/2  
 Bacteria Sytox Profile 2/2  
 Bacteria Sytox Symmetry 02 SP-  
 Filter  
 Bacteria Sytox Symmetry 03 SP-  
 Filter  
 Bacteria Sytox Symmetry 04 SP-  
 Filter  
 Bacteria Sytox Symmetry 05 SP-  
 Filter  
 Bacteria Sytox Symmetry 12 SP-  
 Filter

		Bacteria Sytox Symmetry 13 SP-Filter Bacteria Sytox Symmetry 14 SP-Filter Bacteria Sytox Symmetry 15 SP-Filter Bacteria Sytox Threshold Compactness 30% SP-Filter Bacteria Sytox Threshold Compactness 40% SP-Filter Bacteria Sytox Threshold Compactness 50% SP-Filter Bacteria Sytox Threshold Compactness 60% SP-Filter Bacteria Sytox Axial Small Length SP-Filter Bacteria Sytox Axial Length Ratio SP-Filter Bacteria Sytox Radial Mean SP-Filter Bacteria Sytox Radial Relative Deviation SP-Filter Bacteria Sytox Radial Mean Ratio SP-Filter Bacteria Sytox Profile 1/2 SP-Filter Bacteria Sytox Profile 2/2 SP-Filter Intensity Spot SYTOX green Mean Intensity Spot SYTOX green StdDev	
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**Table B.2. Gram-negative rods analysis pipeline (Harmony v4.9).**

Species	ID	Ampicillin	Azithromycin	Trimethoprim-sulfamethoxazole	Ciprofloxacin	Gentamicin	Rifampicin	Merope- nem	Tigecycline	Cefuroxime	Oxacillin	Vancomycin
<i>S. Typhimurium</i>	NC	0.5	2	0.25	0.012	2	16	0.125	0.19	3	ND	ND
	TC 133 47											
<i>S. Typhimurium</i>	NC	>256*	3	0.25	0.012	3	24	0.064	0.19	3	ND	ND
	TC 133 48											

**Table B.3. Antimicrobials and determined MICs used for Opera Phenix imaging optimization.**

Parameter	Z'
Single_cells - Bacteria_CSA Symmetry 14 - StdDev per Well	0.953
Single_cells - Bacteria_CSA Symmetry 04 - StdDev per Well	0.942
Single_cells - FITC_dead Radial Mean - Mean per Well	0.939
Single_cells - Bacteria_CSA Symmetry 15 - Mean per Well	0.938
Single_cells - Bacteria DAPI Symmetry 15 - Mean per Well	0.935
Single_cells - Bacteria_CSA Symmetry 04 - Mean per Well	0.934
Single_cells - Bacteria_CSA Radial Relative Deviation - Mean per Well	0.933
Single_cells - Bacteria_CSA Symmetry 02 - Mean per Well	0.93
Single_cells - Bacteria_CSA Symmetry 12 - Mean per Well	0.921
Single_cells - Bacteria_CSA Symmetry 14 - Mean per Well	0.918
Single_cells - Bacteria_CSA Threshold Compactness 60% - Mean per Well	0.918
Single_cells - Bacteria_CSA Threshold Compactness 40% - Mean per Well	0.917
Single_cells - Bacteria_CSA Axial Length Ratio - Mean per Well	0.916
Single_cells - Bacteria_CSA Threshold Compactness 30% - Mean per Well	0.913
Single_cells - Bacteria_CSA Radial Mean - Mean per Well	0.9
Single_cells - Bacteria_CSA Threshold Compactness 50% - Mean per Well	0.897
Single_cells - bacteria Roundness - Mean per Well	0.896
Single_cells - Spot Roundness - Mean per Well	0.896
Single_cells - Bacteria DAPI Symmetry 14 - StdDev per Well	0.893
Single_cells - FITC_dead Symmetry 04 - StdDev per Well	0.892
Single_cells - Bacteria DAPI Threshold Compactness 60% - Mean per Well	0.891
Single_cells - bacteria Length [ $\mu\text{m}$ ] - Mean per Well	0.889
Single_cells - FITC_dead Symmetry 15 - Mean per Well	0.886
Single_cells - bacteria Ratio Width to Length - Mean per Well	0.878
Single_cells - Bacteria DAPI Symmetry 05 - Mean per Well	0.875
Single_cells - Bacteria DAPI Radial Mean - Mean per Well	0.874
Single_cells - Bacteria DAPI Symmetry 04 - StdDev per Well	0.871
Single_cells - Bacteria DAPI Threshold Compactness 40% - Mean per Well	0.869
Single_cells - Bacteria DAPI Threshold Compactness 50% - Mean per Well	0.862
Single_cells - FITC_dead Threshold Compactness 60% - Mean per Well	0.859
Single_cells - FITC_dead Radial Relative Deviation - Mean per Well	0.855
Single_cells - FITC_dead Symmetry 14 - StdDev per Well	0.838
Single_cells - Bacteria DAPI Symmetry 04 - Mean per Well	0.837
Single_cells - Bacteria DAPI Threshold Compactness 30% - Mean per Well	0.836
Single_cells - Bacteria DAPI Symmetry 14 - Mean per Well	0.827
Single_cells - Bacteria DAPI Symmetry 03 - Mean per Well	0.826
Single_cells - FITC_dead Symmetry 05 - StdDev per Well	0.823
Single_cells - Bacteria DAPI Axial Length Ratio - Mean per Well	0.818
Single_cells - Bacteria DAPI Radial Relative Deviation - Mean per Well	0.794
Single_cells - Bacteria DAPI Symmetry 12 - Mean per Well	0.792
Single_cells - Bacteria DAPI Symmetry 02 - Mean per Well	0.789
Single_cells - Bacteria_CSA Symmetry 05 - Mean per Well	0.767

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Single_cells - FITC_dead Symmetry 14 - Mean per Well	0.763
Single_cells - FITC_dead Axial Length Ratio - Mean per Well	0.763
Single_cells - Bacteria DAPI Radial Relative Deviation - StdDev per Well	0.757
Single_cells - FITC_dead Symmetry 04 - Mean per Well	0.746
Single_cells - FITC_dead Threshold Compactness 50% - Mean per Well	0.745
Single_cells - bacteria Area [ $\mu\text{m}^2$ ] - Mean per Well	0.744
Single_cells - Spot Area [ $\mu\text{m}^2$ ] - Mean per Well	0.744
Single_cells - FITC_dead Symmetry 03 - StdDev per Well	0.724
Single_cells - FITC_dead Symmetry 05 - Mean per Well	0.723
Single_cells - FITC_dead Symmetry 12 - Mean per Well	0.719
Single_cells - FITC_dead Threshold Compactness 40% - Mean per Well	0.698
Single_cells - Bacteria DAPI Symmetry 15 - StdDev per Well	0.692
Single_cells - FITC_dead Symmetry 02 - Mean per Well	0.679
Single_cells - Bacteria_CSA Symmetry 15 - StdDev per Well	0.668
Single_cells - FITC_dead Radial Relative Deviation - StdDev per Well	0.659
Single_cells - bacteria Ratio Width to Length - StdDev per Well	0.653
Single_cells - FITC_dead Threshold Compactness 30% - Mean per Well	0.639
Single_cells - Bacteria_CSA Symmetry 02 - StdDev per Well	0.638
Single_cells - Bacteria DAPI Threshold Compactness 60% - StdDev per Well	0.59
Single_cells - Bacteria_CSA Threshold Compactness 60% - StdDev per Well	0.585
Single_cells - Bacteria_CSA Threshold Compactness 50% - StdDev per Well	0.556
Single_cells - Bacteria DAPI Threshold Compactness 50% - StdDev per Well	0.543
Single_cells - Bacteria DAPI Threshold Compactness 40% - StdDev per Well	0.499
Single_cells - Bacteria_CSA Radial Relative Deviation - StdDev per Well	0.486
Single_cells - Bacteria DAPI Symmetry 05 - StdDev per Well	0.47
Single_cells - FITC_dead Threshold Compactness 40% - StdDev per Well	0.436
Single_cells - FITC_dead Threshold Compactness 30% - StdDev per Well	0.427
Single_cells - Bacteria_CSA Symmetry 05 - StdDev per Well	0.425
Single_cells - Bacteria DAPI Threshold Compactness 30% - StdDev per Well	0.395
Single_cells - FITC_dead Threshold Compactness 60% - StdDev per Well	0.394
Single_cells - Spot Roundness - StdDev per Well	0.383
Single_cells - bacteria Roundness - StdDev per Well	0.383
Single_cells - Bacteria_CSA Threshold Compactness 30% - StdDev per Well	0.366
Single_cells - Bacteria_CSA Profile 1/2 - Mean per Well	0.351
Round_cells - Number of Objects	0.346
Single_cells - Bacteria_CSA Threshold Compactness 40% - StdDev per Well	0.34
Single_cells - FITC_dead Profile 2/2 - Mean per Well	0.314

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Single_cells - Bacteria DAPI Symmetry 02 - StdDev per Well	0.269
Single_cells - Bacteria DAPI Profile 2/2 - Mean per Well	0.266
Single_cells - FITC_dead Symmetry 03 - Mean per Well	0.263
Single_cells - FITC_dead Threshold Compactness 50% - StdDev per Well	0.252
Single_cells - FITC_dead Symmetry 15 - StdDev per Well	0.184
Round_cells - bacteria Ratio Width to Length - Mean per Well	0.178
Single_cells - Bacteria_CSA Profile 2/2 - Mean per Well	0.049
Single_cells - FITC_dead Symmetry 02 - StdDev per Well	0.029
Single_cells - Bacteria DAPI Profile 1/2 - StdDev per Well	0.024
Single_cells - bacteria Area [ $\mu\text{m}^2$ ] - StdDev per Well	-0.018
Single_cells - Spot Area [ $\mu\text{m}^2$ ] - StdDev per Well	-0.018
Single_cells - bacteria Length [ $\mu\text{m}$ ] - StdDev per Well	-0.038
Single_cells - Bacteria DAPI Symmetry 13 - Mean per Well	-0.055
Single_cells - Bacteria_CSA Symmetry 13 - Mean per Well	-0.06
Single_cells - Bacteria_CSA Profile 2/2 - StdDev per Well	-0.077
Single_cells - Intensity Spot Fluorescein (FITC) StdDev - StdDev per Well	-0.116
Spots Selected - Number of Objects	-0.123
Spots - Number of Objects	-0.158
Single_cells - Bacteria_CSA Axial Length Ratio - StdDev per Well	-0.238
Single_cells - Bacteria DAPI Profile 1/2 - Mean per Well	-0.259
Single_cells - FITC_dead Axial Length Ratio - StdDev per Well	-0.26
Single_cells - Bacteria_CSA Radial Mean - StdDev per Well	-0.381
Single_cells - Bacteria DAPI Profile 2/2 - StdDev per Well	-0.386
Single_cells - Bacteria_CSA Symmetry 03 - Mean per Well	-0.463
Single_cells - FITC_dead Profile 1/2 - Mean per Well	-0.494
Other (2) - Number of Objects	-0.505
Single_cells - FITC_dead Symmetry 13 - Mean per Well	-0.577
Single_cells - Bacteria DAPI Radial Mean - StdDev per Well	-0.589
Single_cells - Number of Objects	-0.638
Round_cells - bacteria Length [ $\mu\text{m}$ ] - Mean per Well	-0.659
Single_cells - Bacteria_CSA Symmetry 12 - StdDev per Well	-0.713
Single_cells - Single_cells_live - Mean per Well	-0.719
Single_cells - Bacteria DAPI Axial Length Ratio - StdDev per Well	-0.755
Single_cells_live - Number of Objects	-0.755
Single_cells - Intensity Spot Fluorescein (FITC) Mean - StdDev per Well	-0.776
Single_cells - Bacteria_CSA Symmetry 03 - StdDev per Well	-0.793
Single_cells - bacteria Width [ $\mu\text{m}$ ] - StdDev per Well	-0.797
Single_cells - Single_cells_live - StdDev per Well	-0.83
Single_cells - FITC_dead Profile 2/2 - StdDev per Well	-1.201
Single_cells - FITC_dead Radial Mean - StdDev per Well	-1.284
Single_cells - Intensity Spot DAPI StdDev - Mean per Well	-1.446
Round_cells - bacteria Ratio Width to Length - StdDev per Well	-1.684
Single_cells - Intensity Spot DAPI StdDev - StdDev per Well	-1.733

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Single_cells - FITC_dead Symmetry 12 - StdDev per Well	-1.938
Single_cells - Bacteria_CSA Symmetry 13 - StdDev per Well	-2.106
Single_cells - Bacteria DAPI Symmetry 13 - StdDev per Well	-2.551
Round_cells - bacteria Length [ $\mu\text{m}$ ] - StdDev per Well	-2.788
Single_cells - bacteria Width [ $\mu\text{m}$ ] - Mean per Well	-2.998
Single_cells - Bacteria_CSA Profile 1/2 - StdDev per Well	-3.416
Single_cells - Intensity Spot DAPI Mean - StdDev per Well	-3.708
Single_cells - Bacteria DAPI Symmetry 03 - StdDev per Well	-3.92
Single_cells - Bacteria DAPI Axial Small Length - Mean per Well	-3.931
bacteria - Number of Objects	-3.98
Single_cells - FITC_dead Profile 1/2 - StdDev per Well	-4.04
Single_cells - FITC_dead Axial Small Length - Mean per Well	-4.552
Single_cells - FITC_dead Symmetry 13 - StdDev per Well	-4.962
Round_cells - bacteria Width [ $\mu\text{m}$ ] - Mean per Well	-6.17
Round_cells - bacteria Area [ $\mu\text{m}^2$ ] - StdDev per Well	-6.651
Single_cells - Bacteria_CSA Axial Small Length - Mean per Well	-9.78
Round_cells - bacteria Width [ $\mu\text{m}$ ] - StdDev per Well	-10.228
Round_cells - bacteria Area [ $\mu\text{m}^2$ ] - Mean per Well	-10.389
Single_cells - Intensity Spot Fluorescein (FITC) StdDev - Mean per Well	-13.553
Single_cells - Intensity Spot DAPI Mean - Mean per Well	-14.798
Single_cells - Bacteria_CSA Axial Small Length - StdDev per Well	-30.091
Single_cells - Bacteria DAPI Symmetry 12 - StdDev per Well	-50.256
Single_cells - Bacteria DAPI Axial Small Length - StdDev per Well	-53.794
Single_cells - Intensity Spot Fluorescein (FITC) Mean - Mean per Well	-844.663
Single_cells - FITC_dead Axial Small Length - StdDev per Well	-3258.365

**Table B.4. Z' statistics of *S. Typhimurium* D23580 treated for 2 h with 2x MIC ciprofloxacin versus no treatment.**

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