**Supplemental Code File**

**BrucellaShadingCorrectionPart1-Ver007.cppipe**

Module (1) “LoadImages” loads images with DAPI and GFP channels.

Modules (2) – (3) “CorrectIlluminationCalculate” create a shading model by reading all images and creating a median-averaged image.

Modules (4) – (5) “SaveImages” save the shading models to the output folder.

**BrucellaEndpointWithShadingCorrectionPart2-Ver007**

Module (1) “LoadImages” loads images with DAPI and GFP channels.

Module (2) “LoadSingleImage” loads the shading models.

Module (3) “CorrectIllumintationApply” applies the shading models to the images.

Modules (4) – (5) “ImageMath” correct 12bit images to use the full dynamic range [0, 1].

Module (6) “MeasureImageIntensity” measures the lower quartile intensity of the DAPI and GFP images. The lower quartile intensity is a robust measure for the image background in this assay.

Modules (7) – (8) “ImageMath” subtract the lower quartile intensity from the image. This is done to remove the background from the images.

Module (9) “ImageMath” subtracts a fraction of the GFP image from the DAPI image. This is done to suppress the *Brucella* signal in the DAPI staining.

Module (10) “IdentifyPrimaryObjects” identifies the nuclei in the DAPI stained images from which the *Brucella* signal was suppressed.

Module (11) “ExpandOrShrinkObjects” creates non-overlapping expanded nuclei with an expansion of 8 pixels.

Module (12) “IdentifyTertiaryObjects” creates peri-nuclear rings around the nuclei from the expanded nuclei, by removing the nuclei from the expanded nuclei.

Module (13) “ExpandOrShrinkObjects” creates a Voronoi cell body around the nuclei. A Voronoi cell body is a non-overlapping expansion of the nucleus by 25 pixels.

Module (14) “MeasureObjectIntensity” measures the intensity of the GFP image at the nuclei.

Module (15) “FilterObjects” filters for nuclei of infected cells based on the minimum mean GFP intensity in the nuclei.

Module (16) “FilterObjects” filters for peri-nuclei of infected cells based on the minimum mean GFP intensity in the peri-nuclei.

Module (17) “FilterObjects” filters for Voronoi cell body of infected cells based on the minimum upper quartile GFP intensity in the Voronoi cell body.

Modules (18) – (23) combine the previously found “infected nuclei”, “infected peri-nuclei” and “infected Voronoi cells” into a single object “InfectedCells”

Module (24) “ExportToSpreadsheet” writes a CSV sheet with the summarized readouts for each site.

Modules (25) – (29) create color overlay PNG images with infected and uninfected cells outlined on the microscope images. These images can be helpful for later inspection, and help in optimization of the analysis.

**BrucellaEntryWithShadingCorrectionPart2-Ver007**

Module (1) “LoadImages” loads images with DAPI and GFP channels.

Module (2) “LoadSingleImage” loads the shading models.

Module (3) “CorrectIllumintationApply” applies the shading model to the images.

Module (4) – (5) “ImageMath” correct 12bit images to use the full dynamic range [0, 1].

Module (6) “IdentifyPrimaryObjects” identifies the nuclei in the DAPI stained images.

Module (7) “ExpandOrShrinkObjects” creates a Voronoi cell body around the nuclei. A Voronoi cell body is a non-overlapping expansion of the nucleus by 25 pixels.

Module (8) “IdentifyPrimaryObjects” identifies the intracellular *Brucella* colonies in the GFP image.

Module (9) “MeasureObjectIntensity” measures the intensity of segmented *Brucella* colonies in the GFP image.

Module (10) “MeasureObjectSizeShape” measures the area of segmented *Brucella* objects.

Module (11) “FilterObjects” filters for segmented *Brucella* colonies that have a minimum area of 2 pixels. Smaller *Brucella* objects are discarded to reduce the effects of pixel noise. The Module subsequently filters for segmented *Brucella* colonies based on the minimum upper quartile intensity.

Module (12) “MeasureObjectIntensity” measures the intensity of the filtered *Brucella* colonies in the GFP image.

Module (13) “RelateObjects” relates the filtered *Brucella* colonies to the Voronoi cell body that they mostly overlap with. We will call this relation a parent-child-relation, where the *Brucella* colonies that relate to a Voronoi cell body are the “children” of this Voronoi cell body.

Module (14) “FilterObjects” filters for infected cells, which are defined to be all cells that have a *Brucella* colony as child of the Voronoi cell body.

Module (15) “FilterObjects” filters for *Brucella* colonies that are children of a Voronoi cell body.

Module (16) “MeasureImageAreaOccupied” measures the integrated area of *Brucella* colonies that are children of a Voronoi cell body.

Module (17) “ExportToSpreadsheet” writes a CSV sheet with the summarized readouts for each site.

Modules (18) – (22) create color overlay PNG images with infected and uninfected cells outlined on the microscope images. These images can be helpful for later inspection, and help in optimization of the analysis.