Spot Counting

Spot counting is used to count phagocytosed particles, viral load, nuclear foci, micronuclei, FISH spots, granules, single cells, autophagolysosomes or any component in an image that can be individually masked. The Spot Count feature enumerates the number of individual mask components in an image, and is dependent on accurate masking of the feature you want to count. Complex masking schemes such as combining a Range mask with a Peak mask with a Spot mask will help the feature accurately identify the critical components, and enumerate them. There are times when the Intensity or Threshold masks may also be helpful.

To Generate the Spot Counting Mask:

1. Follow the instructions for finding **single, focused, cells**.
2. **Tag** a population of cells that include a range of spot sizes and intensities.
    a. Click on the **tagging** tool.
    b. **Add** images to the population by double clicking on images in the image gallery.
    c. Click **Save**.
    d. **Name** the population and click OK.
    e. Choose the new population to **view** in the image gallery.
3. Create a **Spot mask**. The radius of the largest spots to mask is required.
    a. **Choose** a **representative cell** with large spots.
    b. Under **Analysis** select **Masks**.
    c. Click **New**.
    d. Click **Function** and choose **Spot**. Leave Bright selected.
    e. Under mask select a starting mask. This is usually the default channel mask or if high background persists, use either a threshold or intensity mask to limit masking to spots well above the background.
    f. In the channel drop down select the **channel** of interest.
    g. Enter the **radius**. This can be determined by moving the cursor across the spot and reading the x,y coordinates in the upper left of the image. The radius is half the diameter and generally around 3 or 4 pixels for small spots.
    h. Adjust the **spot to cell background** on a dim spot by increasing the ratio until only the spots of interest are masked.
    i. **Save** the mask by clicking OK, OK.
4. Refine the Spot mask using the **Peak**. This will separate two close spots with one mask into two.
    a. In the Mask manager Click **New** then **Function**.
    b. Select **Peak**.
    c. Select the spot mask from the previous step.
    d. **Save** the mask by clicking OK, OK.
5. Refine the **Peak_Spot** mask using the **Range** mask. This will limit the mask to spots of a certain morphology.
    a. In the mask manager click New then Function.
    b. Select **Range**
    c. Select the **Peak_Spot** mask from the previous step.
    d. Set the **minimum spot area** to eliminate tiny nonspecific masks, generally 4.
    e. When appropriate set the **aspect ratio limits** to identify the shape of the spot you need. For round spots a minimum 0.5 and a maximum of 1 generally will work.
    f. Save the mask by clicking OK, OK.

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6. **Verify** the Range_Peak_Spot mask by displaying the new mask on the hand tagged population of cells with good spots.
    a. Under **population view** select the hand tagged population from step 1.
    b. Select the **image gallery display properties** button.
    c. Select the **views** tab.
    d. Create a new view.
    e. Select **add column** and select brightfield image.
    f. Select add column and choose the spot image.
    g. Below the image input change the **mask** to the new Range_Peak_Spot mask.
7. Adjust the mask as needed to accommodate the variation in spot morphology. This can be done by eliminating background using the Threshold or Intensity mask as mentioned above or by adjusting the radius and background ratio values. Once the best possible mask is generated, calculate the relevant features and plot them.

To create the Spot Count Feature:

1. Under Analysis select Features.
2. Click New.
3. Select **Spot Count** and the new mask.
4. Click **Set Default Name**.
5. Change the default name to RPS_Spot Count_channel x.
6. Click **OK** to put the feature in the list.
7. Click **Close** to save and **calculate** the feature for all objects.
8. To apply the new spot count feature, create a new **histogram** using the focused cells, or use a bivariate plot with a second feature.