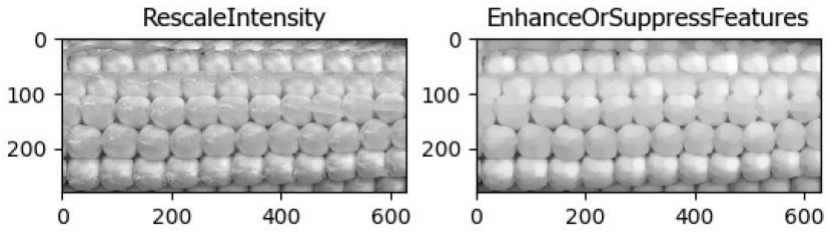
Seed Counting

Goal: The goal is to identify, segment, and count corn kernels from the image of an ear of a corn.
Images: Single color image of an ear of corn.
Pipeline: This pipeline illustrates CellProfiler's™ ability to identify the local maxima from a standard photograph. By identifying local maxima corn kernels can be recognized and counted. The workflow is as follows:

1. Open **CellProfiler**.
2. Click on **Images**. Highlight the image listed. Right click and Clear File list. Go to the downloaded Input images folder, drag and drop the image in the appropriate CellProfiler window. The original image maintains the folder structure of the original computer used to create the pipeline. If the image is not reloaded from your computer an error will occur.
3. **Metadata** information is not used in this example.
4. **NamesAndTypes** is used to name the single color image used in this example as DNA.
5. **ColorToGray** is used to convert the color RGB image into grey scale.
6. **Crop** is used to crop the image so only the region of interest is selected. "Mouse" is selected as the cropping method so a manual crop of the image can be achieved.
7. **RescaleIntensity** is not always necessary but was used in this case to pre-process the image. This rescales the intensity values, increasing contrast, and aids in thresholding the pixels of interest. In cases where signal to background is large the RescaleIntensity step can be skipped.

8. **EnhanceOrSuppressFeatures** is used to suppress bright pixels so the local maxima can be more easily be identified. In this case each corn kernel.

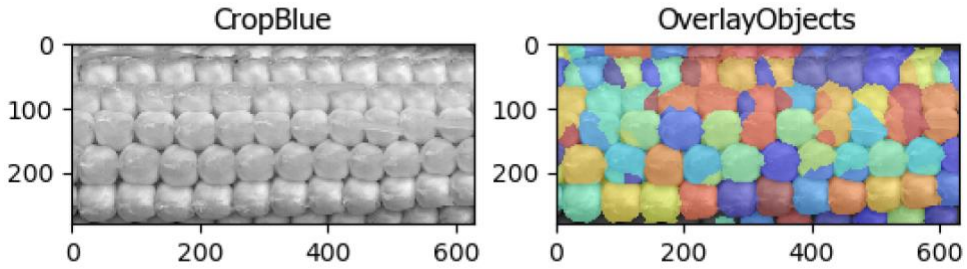


9. **IdentifyPrimaryObjects** is used to identify and segment the cells.
    a. **Typical Diameter of Objects, in pixels** was set to 10-100 since the corn kernels are in a large range. Tightening this range will result in fewer kernals identified, while expanding the range will include more objects. To get an idea of object size, go to the "Images" module, right click on an image and select "Show Selected Image". With the new image window selected, select "Tools", "Measure length" from the toolbar pull down menu. Drag your mouse over an object and view it's size in the lower right hand of the image window.
    b. **Thresholding strategy** Adaptive was selected in this case because the foreground and background intensity values across the image are variable.
    c. **Thresholding method** "Otsu" is the only option for thresholding when the "Adaptive" strategy is used. "Three class thresholding" was selected because of the uneven foreground intensity. This is also why foreground is selected as the middle intensity class.
    d. **Threshold correction factor** was adjusted down slightly to 0.8 to include slightly more pixels.
    e. **Lower and upper bounds on threshold** was adjusted slightly so that the lower bound was raised to 0.34 to reduce the amount of background pixels identified as relevant objects.
    f. **Size of the adaptive window** was set to 50, in the middle of the 10-100 object diameter range.
    g. **Method to identify clumping of objects** was set to "intensity" since we are using local maxima to identify objects.

h. Automatically **calculate size of smoothing filter for declumping** was turned off and set to 10 to give greater control over declumping. The value is set to the lower value of the object diameter range.

i. **Automatically calculate minimum allowed distance between local maxima** was turned off and set to 5 to ensure objects smaller than the minimum object size are segmented . A rough estimate can be calculated using “Measure length” from “Tools” menu.

10. **MeasureObjectSizeShape** was used to quantify the number of corn kernels.
11. **OverlayObjects** was used to overlay the identified objects onto the original image.
12. **SaveImages** saves the OverlayObjects image to your hard drive.



13. **ExportToSpreadsheet** exports all calculated values for identified objects into a .csv file.