Guide to Configuring Vectra® 3 and inForm® for Compatibility with the DISCOVERY 5-Plex Procedure from Roche Tissue Diagnostics

Introduction

Follow this step-by-step guide to configuring your Vectra® 3 hardware and software to accommodate Chroma's AQUA filter cube and the DISCOVERY fluorophores for imaging and analysis of your DISCOVERY 5-Plex immunofluorescent samples.

AKOYA BIOSCIENCES

Vectra®

1. Physically add AQUA filter cube (Chroma, 49302 – ET – Aqua FISH) to the microscope.

   a. Using the microscope controller handset, move the Texas Red filter cube into the light path.

   b. Power off the microscope controller and use the microscope's screwdriver to remove the epi-filter turret. (Figure 1).
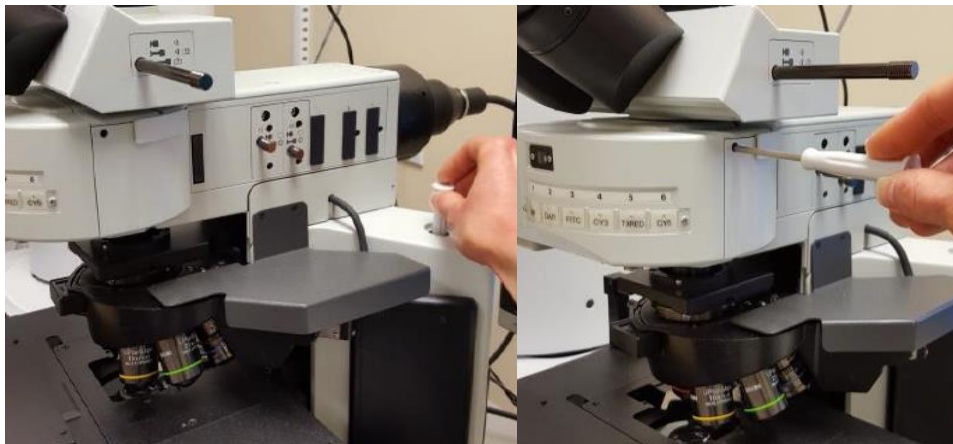

Figure 1. (a) Remove screwdriver from base of microscope and (b) use it to loosen and remove the epi-filter turret from the microscope

   c. Unscrew the Texas Red filter cube from the turret and pull it out. Slide the AQUA filter cube in its place and tighten the screw. (Figure 2).

Figure 2. Place the epi-filter turret upside down and loosen the screw that is holding the Texas red filter in place. Slide the filter up to remove.

   d. Replace the epi-filter turret into the microscope and tighten the screw. Power the microscope controller back on.

2. Add the AQUA filter and imaging band to the Vectra Hardware Setup.

   a. Open the Vectra Hardware Setup application (C:\Program Files\PerkinElmer\Vectra\3.0.3\VectraHardwareSetup.exe).

   b. Go to the "Epi-Filters" tab and select "Add."

c. Enter the filter information into the "Add Epi-Filter window" (see Figure 3 for appropriate set-up). Select "OK."

Figure 3. Enter in the above filter information in the "Add Epi-Filter" window.

d. The "Create Default Bands" window will appear. Name the band and keep the Band start (nm) at 460 and the Band stop (nm) at 500. (Figure 4).
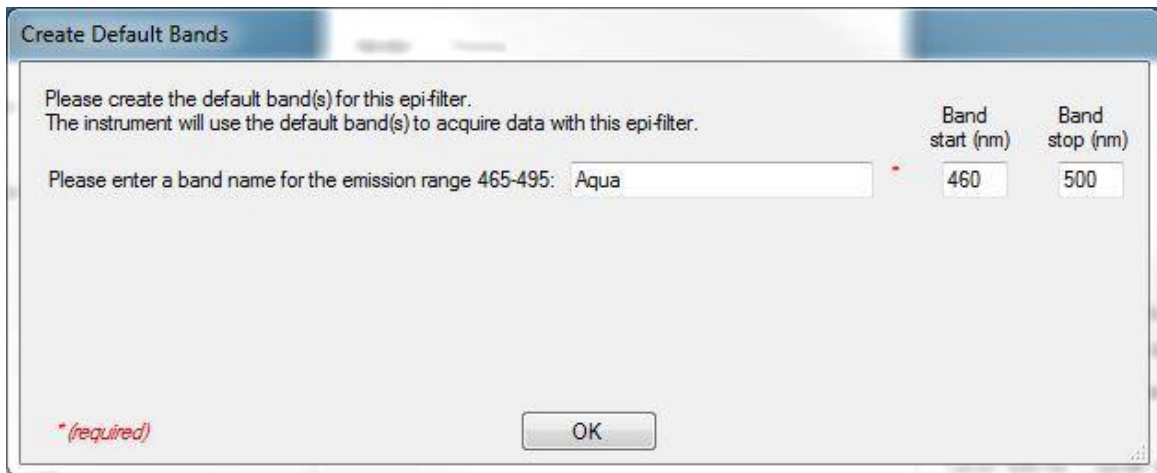
Figure 4. Name the imaging band for the AQUA filter in the "Create Default Bands" window.

     e. Change Epi-Filter Position #5 from Texas Red to AQUA.

     f. Close the Vectra Hardware Setup application.

3. Acquire Fluorescence References for the AQUA filter.

     a. Start up the Vectra 3.0.3 software and go to the "Check Dashboard." Manually place the blue reference slide onto the stage.

     b. Select "Fluorescence References" and select the "AQUA" filter. Choose "Acquire References for AQUA." Move to a clear area on the blue reference slide and select "OK." When finished close the Fluorescence References window.

     c. Vectra 3.0.3 is now ready for imaging with the AQUA filter.

inForm®

1. Add the Roche Tissue Diagnostics fluorophores to the inForm Stain Store.

     a. Within inForm, go to Tools from the upper toolbar and select "Stain Store Manager." In the Stain Store Management window, select "Choose Which Types of Stains and Fluors Your Lab Uses."

     b. From the list under "Family Name" on the left hand side, select "Other (User Specified)."

     c. Select "New" and in the "Create a New Custom Stain or Fluor" window, enter a Name, choose "Fluorescent" for the Imaging Mode, and select "OK." (Figure 5).
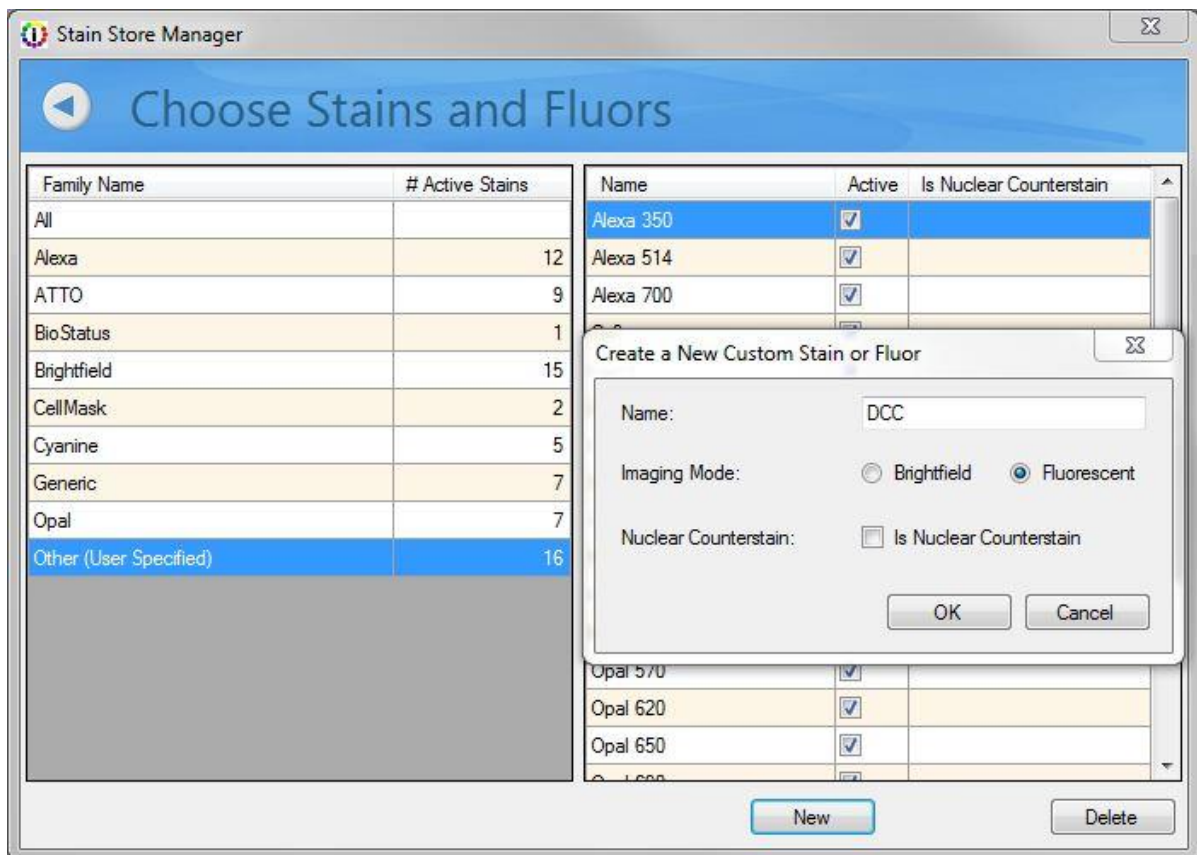
Figure 5. Use the "Create a New Custom Stain or Fluor" window to name a new fluor and select the imaging mode.

    d. Repeat this for all new fluorophores and then close the Stain Store Manager.

2. Extract spectra for these fluorophores from single-stained library samples imaged using the new Vectra filter configuration. (Figure 6).

    a. Within inForm, go to the "Build Libraries" tab.

    b. Use the "Browse" button to load one single-stained library image.

    c. Select the appropriate "Fluor" from the drop-down selection.

    d. Select the appropriate "Signal band" for this fluor (where you expect to detect signal, i.e. Aqua for the DCC fluor).

    e. Select the appropriate "Dark band" for this fluor (where you expect to not detect any signal, i.e. Cy5 for the DCC fluor and DAPI or Aqua for the Cy5 fluor).

    f. f. Select "Extract" and assess the resulting spectrum and unmixed result. If results look good, select "Save to Store" and choose an appropriate color and Group (or make a new group), then "Save."
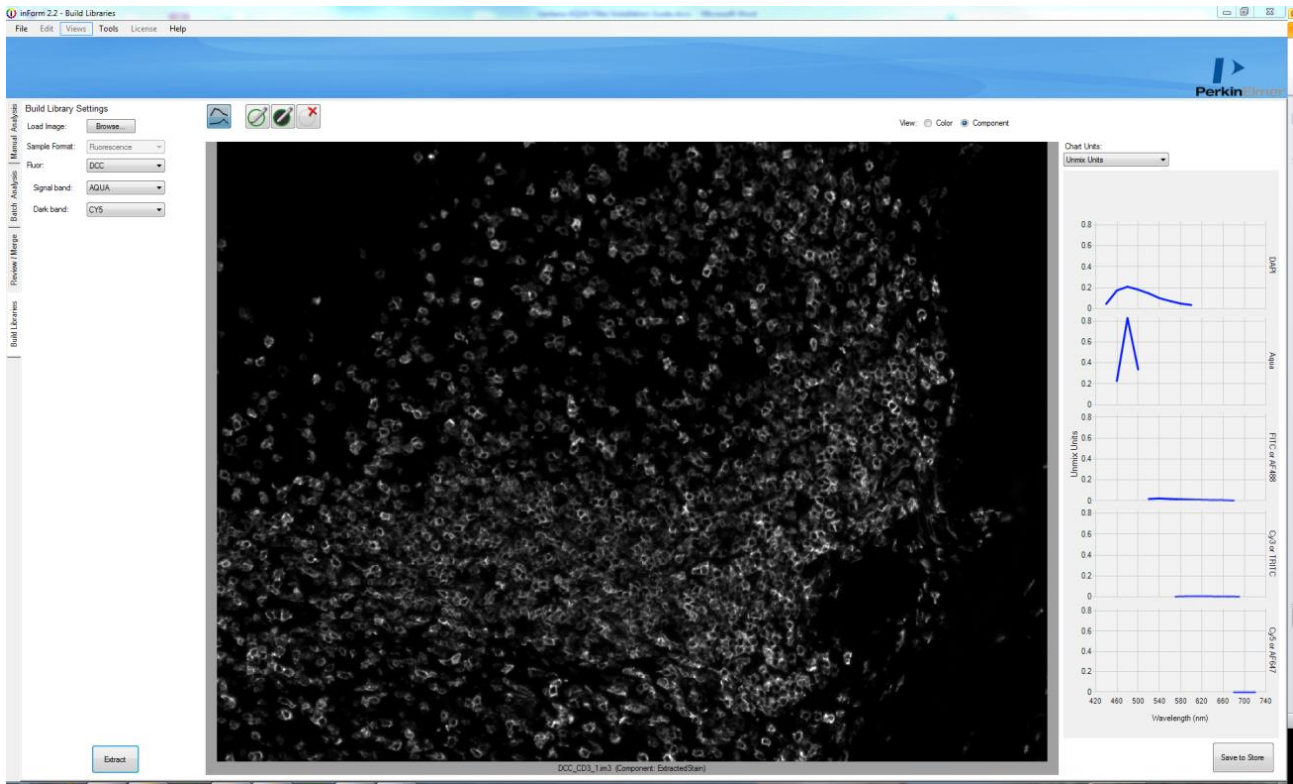
Figure 6. In the Build Libraries tab, load each single-stained library image in one-by-one and select appropriate Fluor, Single band and Dark band. Extract and save the spectra to the Stain Store.

        g.   Repeat for all single-stained library images.

Conclusion

Please reach out to your Akoya Biosciences, Inc. representative with any questions.