

Getting started with Fluorospot plates.

This document will guide you through the first steps in FluoroSpot assays analysis, which can be done with the AID iSpot and the AID iSpot Spectrum machine. It is assumed that you are working with either a two-color Cy3/FITC plate or a three-color Cy3/FITC/DAPI plate (e.g. Mabtech, FluoroSpot). You should be familiar with the basic handling of the AID software.



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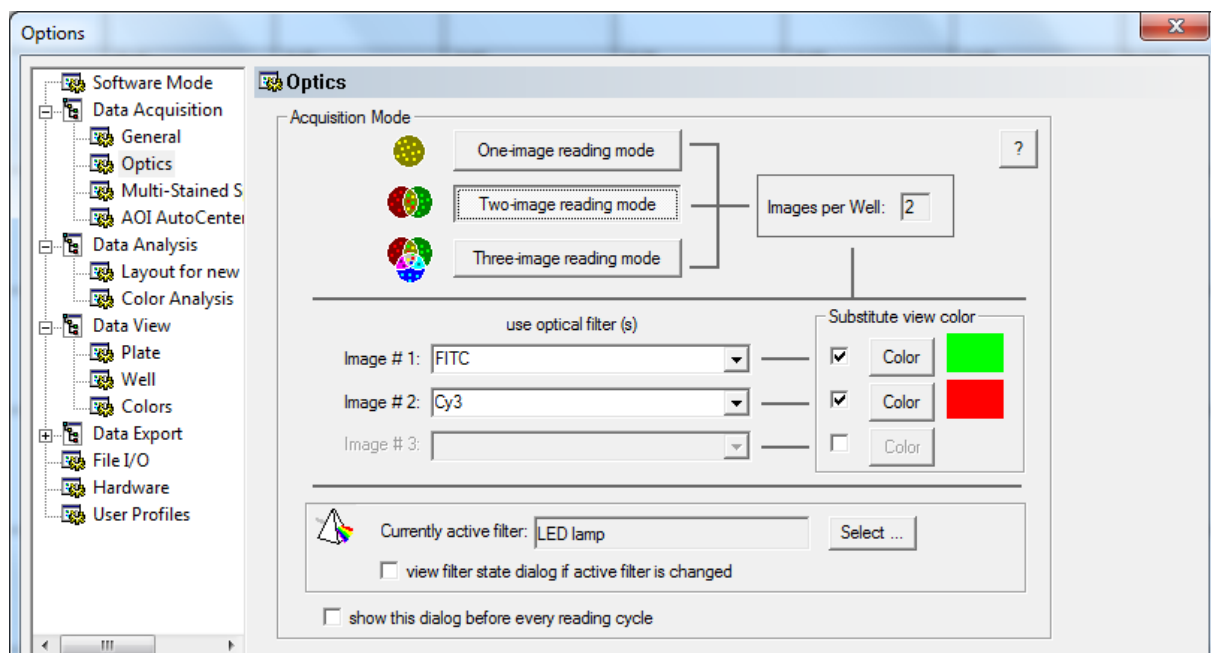
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1. Set up the machine for fluorescent counting

Switch off the LED light and turn on the external Xenon lamp. The Xenon light source is ready-to-operate immediately. Be careful, since the box might get very hot over time.

Change the user in the user management to “2 Color” or “3-Color”. The user “2 Color” is prepared from AID especially to analyse FITC-Cy3 double color plates.

If you want to set up the needed settings by yourself open the “Optics” dialog in the “Options” section of the software (Tools – Options – Data Acquisition – Optics) and select either “Two-image reading mode” or “Three-image reading mode”

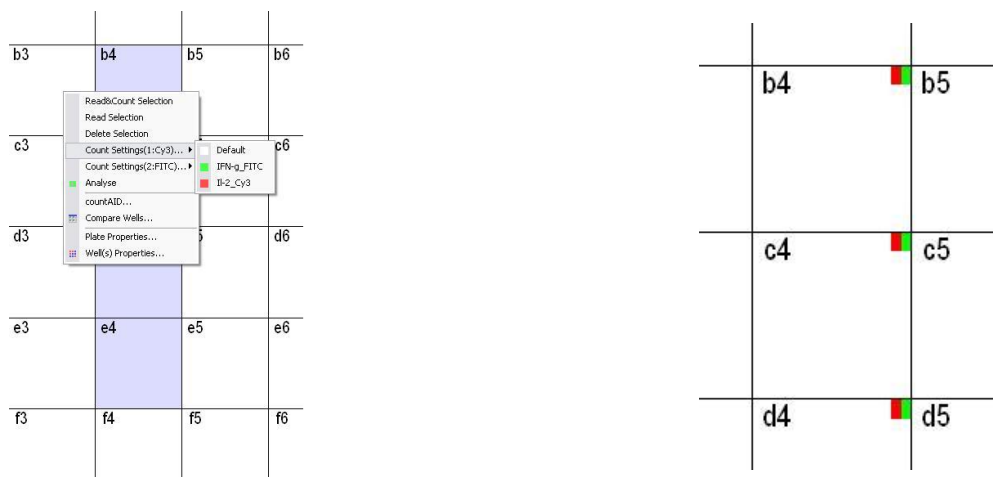


Set up the two dyes (e.g. Cy3 and FITC) in the combo-boxes below. It does not matter which one will give Image 1 and Image 2 and which one is the active filter.

Once set up in this manner the iSpot machine will make two images of each well and overlay them for the final result (e.g. determination of double-stained spots).

2. Scan a plate with two different settings

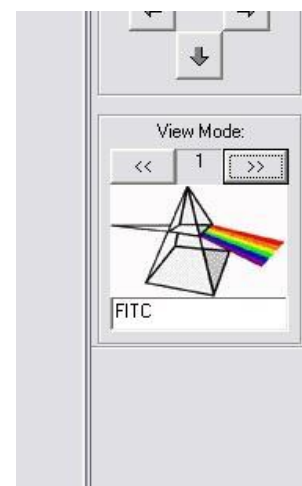
Highlight some wells, right click on the area and choose the appropriate count-settings. Note that in the two-image modus you'll find two different settings for each well (left figure). Choose the correct one for each dye. The wells will look as follows (right figure):



Read and count selection as usual. After the iSpot has finished reading and counting the selection or the plate, open a well of choice.

You will find a new graphic on the lower right side of the one-well few, allowing to switch between four different well pictures.

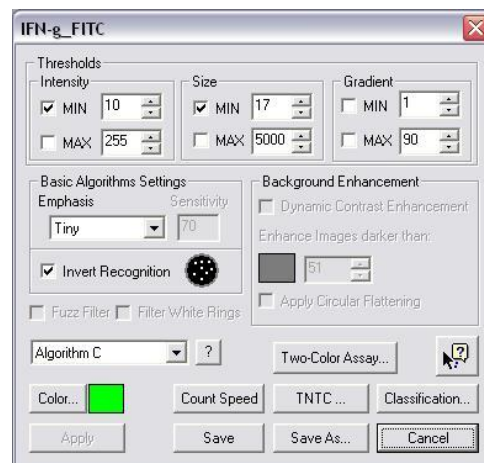
1. First Image (Cy3 or FITC)
2. Second Image (FITC or Cy3)
3. Overlaid picture
4. Artificial picture highlighting the double stained spots only
5. All above images in one view



3. Find new count settings

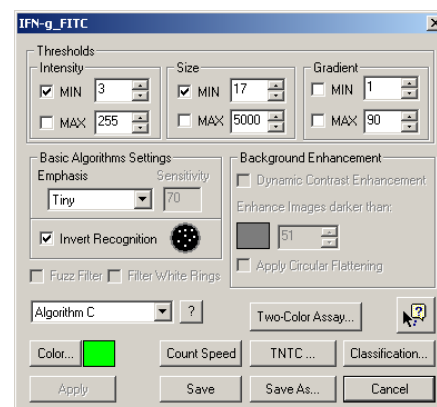
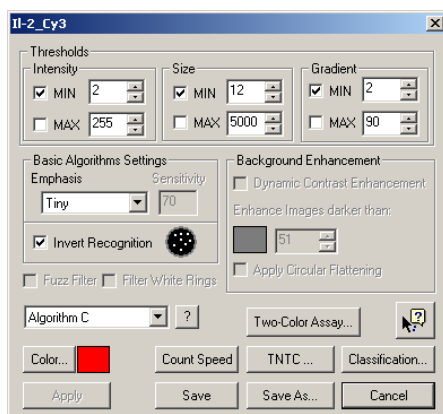
If desired you may set up new count-settings at this stage.

Creating count settings is nearly the same as in the enzymatic assays. One difference is that you should enable the check box “Invert recognition”. The software will then interpret bright structures (e.g. red and green) on a dark background.



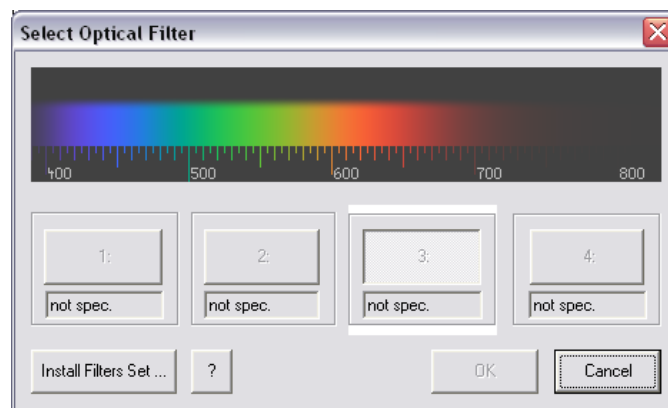
The other difference is that you should set up individual count-settings for each dye. In case of the FITC/Cy3 plate you would need two count settings. Select the corresponding dye with the buttons described under section 2 of this manual.

Suggested starting settings for Cy3 and FITC (highly depending on shutter and gain in the camera settings, see next paragraph)



4. Camera settings

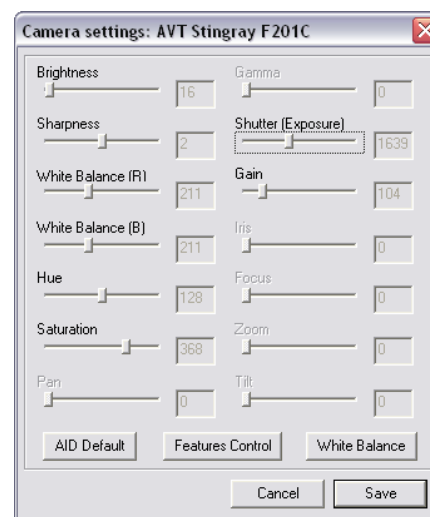
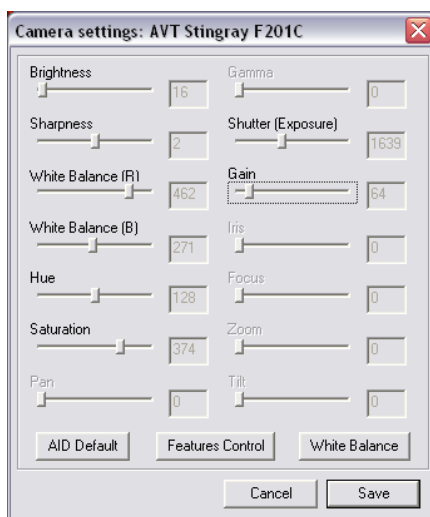
For each dye different camera settings are needed. Change them as follows: First move the stage to a well where spots are present, then open the filter dialog (Tools – Optics – select optical filters) in the main menu of the program.



Select the desired filter and press OK to quit the dialog. Finally open the Camera dialog (Tools – Change camera settings) and find the best settings. A starting point is given below. Save the settings.

Change the filter as before and find the best camera settings for the other dye.

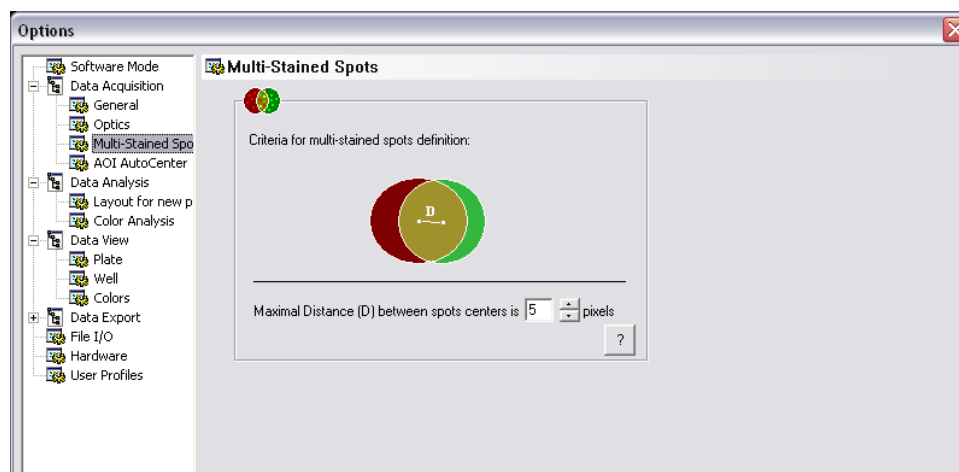
Suggested camera-settings for an Cy3 (left figure) /FITC (right figure) FluoroSpot plate



Note: Especially the FITC dye is exposed to rapid bleaching. If you analyse an older plate you might need to increase the exposure time (shutter). Avoid increasing “Gain” too much, since this will cause pixelated images.

5. Definition of double-stained spots

The AID iSpot software finds double- or multiple stained spots by position on the plate rather than by the color. You will be able to define which amount of overlay between the different colored spots is needed to be interpreted as double-stained spot. In the “Tools” section of the Main menu go to “Options— Data Acquisition – Multiple-stained spots”.



If you want that a double-stained spot is only recognized if there is a 100% overlap between both signals, select “0” pixels in the dialog box.

6. Get support from AID

In case having trouble with the machine or with the software functions you could contact us at support@elispot.com. We will also be able to help you via **Citrix GotoAssist** online meeting.