Azure Cielo:
Adding Dyes and Color Compensation

Introduction

The Azure Cielo is able to detect multiple dyes per channel, as long as the excitation and emission spectra of the dyes fall within the excitation light source and emission filter settings for the channel. Due to the overlap of the emission spectra of some dyes, fluorescent crosstalk may occur during multiplex experiments. Crosstalk is defined as one channel detecting signals from another channel that can lead to misinterpretation of the data. In order to eliminate the crosstalk, it is recommended to run the Color Compensation Protocol after adding new dyes and while running multiplex reactions.

This technical note explains how to add new dyes and describes how to run the Color Compensation Protocol to correct for potential crosstalk following a data run.

Figure 1.

Add Dye Protocol

In order to add a new dye as an option to select during a Cielo experiment, users must complete the Add Dye Protocol followed by the Color Compensation Protocol within the Azure Cielo Manager software.

1. Navigate to the Thermal Profile area of the Experiment Setup group. Click on the Dye Manager tab, then click on the Add Dye tab. Click the + button to the right of the Add New Dye text (Figure 1). Fields for Channel # and Dye Name will appear (Figure 2).

2. Select the appropriate Channel # for your new Dye and enter the new Dye Name into the text field and click the "Add Dye(s)" button.

3. Complete the Color Compensation Protocol (see below) for each new dye added, making sure to select the new dye from the Dye/Target section of the plate setup.
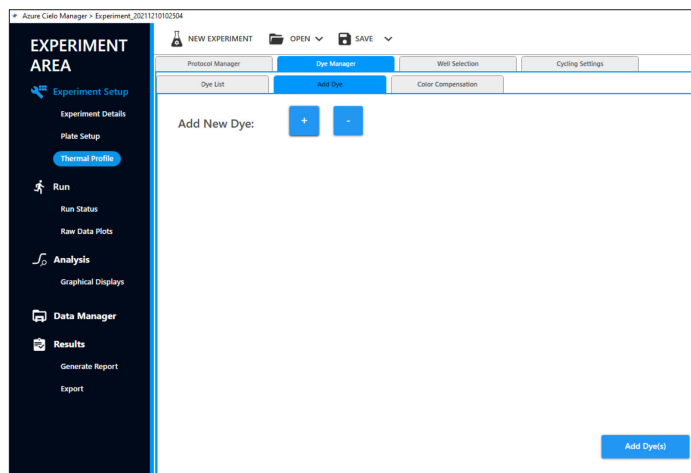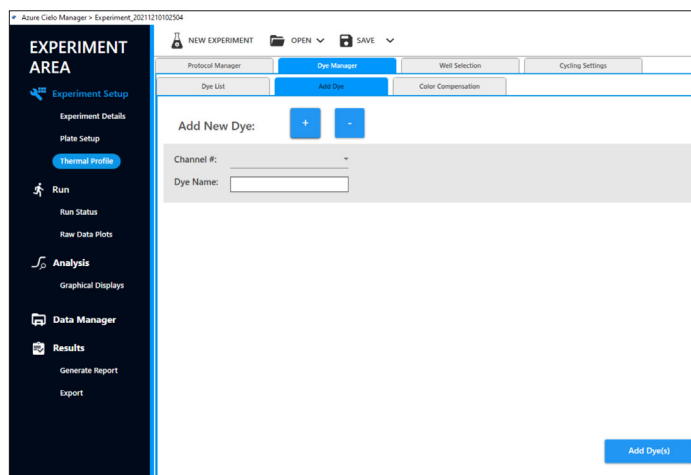

Figure 2.

Color Compensation Protocol

In order to account for potential fluorescent crosstalk, users will need to run the Color Compensation Protocol to generate new color compensation coefficients that will correct the crosstalk from your data. This will need to be done anytime a new dye is used on the instrument or after crosstalk has been observed in the data. It is also best practice to complete this protocol anytime a new combination of dyes is being optimized for a multiplex reaction.

This protocol requires the user to run simplex reactions for each dye/probe that will be used in the multiplex reaction. Triplicates of each simplex reaction should be run in non-adjacent wells. Following the run, the simplex reactions will be labeled on the plate map as "New Dye" before generation of the color compensation coefficients. It is also recommended to run the multiplex reactions on the same plate.

1. Prepare the qPCR run with simplex reactions for each dye/probe, set-up in non-adjacent wells (Figure 1). Also include a set of multiplex reactions for assessment of the elimination of the crosstalk.

2. Run the simplex and multiplex reactions according to reaction protocols.

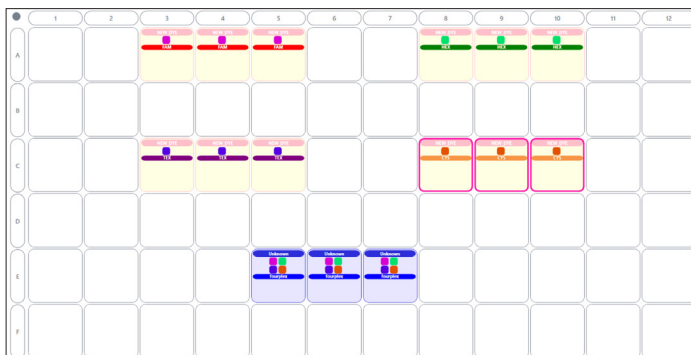3. Extract the AZD file from the instrument and launch in Azure Cielo Manager.

Figure 1. Triplicate simplex reactions of FAM, HEX, Texas Red, and Cy5 in non-adjacent wells, and a set of triplicate multiplex reactions.

4. Properly label the simplex reactions as Well type: "NEW_DYE", Sample: dye name, and the channel for the new dye. (also, see Figure 1). Label the multiplex reactions as Well type: "Unknown", Sample: multiplex, and all channels included in the multiplex reaction.

5. Navigate to Experiment Setup > Thermal Profile. Click on the Dye Manager tab. Click on the Color Compensation tab (Figure 2).

6. Click the "load" button. Then click the "Calculate Color Compensation Coefficients" button (Figure 3).
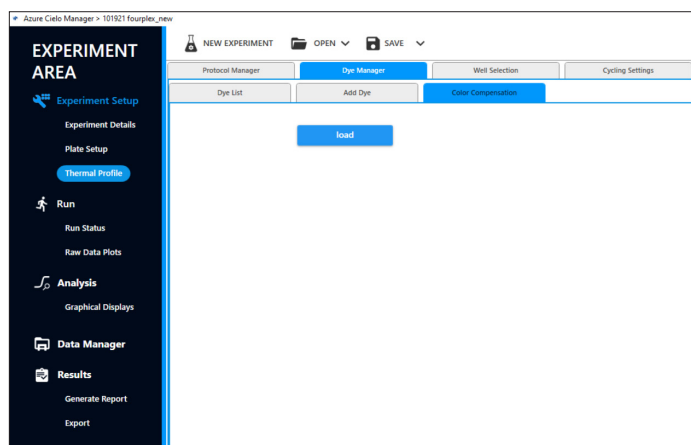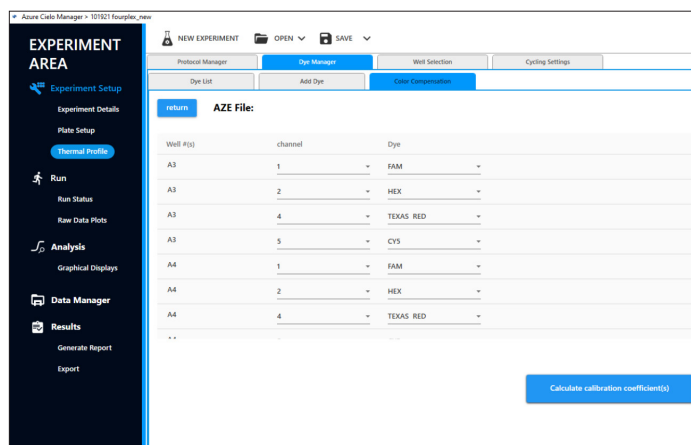

Figure 2.

Figure 3.

7. Click save (Figure 4). Color compensation settings will automatically be saved and applied.

8. Navigate to Analysis > Graphical Displays.
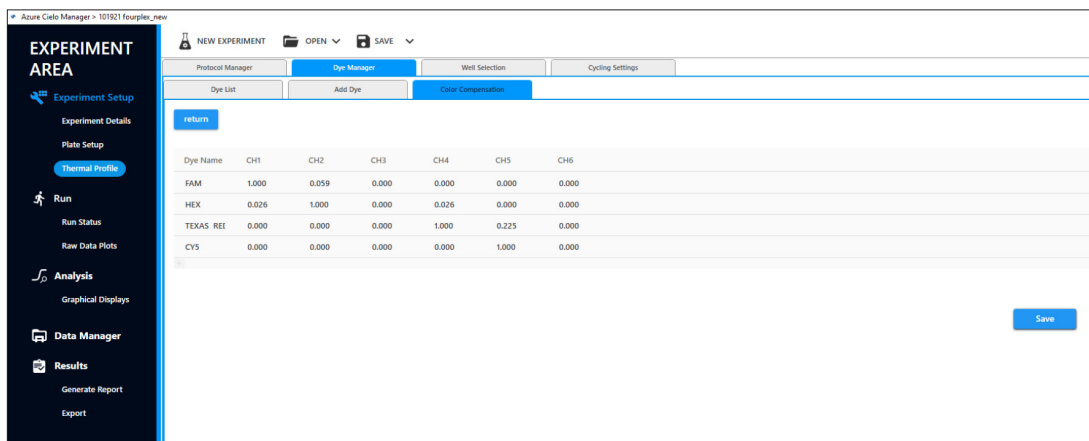
9. Analyze multiplex reactions with eliminated crosstalk.

Figure 4.

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