Tutorial 8: Cell Simulation and Classification
Contents

Introduction ................................................................................................................................................ 5
Configure the Solution ............................................................................................................................... 7
Cell Simulation ........................................................................................................................................... 9
Introduction

The Cell Simulation action, which simulates a cell based on the information from the Nucleus Detection action, has been extended in Tissue Studio® 4.0.

This tutorial will cover two of the three new cell simulation features – Simulate Inside Both Stains and Simulate Inside Stains Separately. Both of these features are available when both of your markers are not nuclear, as in this tutorial.

1. Start Tissue Studio® in the Tissue Studio® portal.

2. There are four images associated with this tutorial. Create a workspace from these, then create a training data set (see Figure 1).

   If you have forgotten how to do this, refer back to the Load Images chapter in Tutorial 1.

Figure 1: Training data set

NOTE: If the marker type is nuclear, you have the option to grow the cells within the stain (as in the IHC-single stain case).

NOTE: This book assumes you have already worked through tutorial #1 (Composer and Nuclear Markers) and are familiar with the basic functions and workflow of Tissue Studio®. You may also want to read chapters 1–3 of the Tissue Studio® User Guide.

This tutorial is intended to be an example and will not explain every aspect of the software. For more information refer to the User Guide.
3 Type a name for the workspace and browse to the location where you want to save it. Press Finish.
Configure the Solution

In this section, we will separate background and tissue, and isolate nuclei.

Load the solution Nuclei&GrowthInStainSeparately.dax.

1. Activate the **General Settings** action. Set **Magnification** to 20x and **µm/pixel** to 0.25.

2. In the **Staining Information** pane, select IHC dual Brown/Red Chromogens as the **Stain Combination**, Cytoplasm as the **Red Marker** and Cytoplasm as the **Brown Marker**.

3. Remove the **Draw Polygon** action and add **Tissue-Background Separation**.

4. Select the **Tissue-Background Separation** action. Ensure **Use Auto-thresholds** is selected and set **Tissue Min Size** to 10. Press **Preview**.

5. In the **Initialize Cellular Analysis** action, set the **Magnification** to 20x (which is our recommendation for cell detection). Press **Preview**.

6. Select the **Nucleus Detection** action. Adjust the **Threshold Hematoxylin** value and press **Preview Threshold(s)**, until you are happy with the nucleus detection (see Figure 2).

7. Press the **Select Samples** button and select on or more large nuclei, to select an upper limit. Press **Preview** to view the results.

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**Figure 2:** Nucleus detection, based on Hematoxylin threshold and size selection
Cell Simulation

In this section, we will go through two cell simulation features – Simulate Inside Stains Separately and Simulate Inside Both Stains.

Simulate Inside Stains Separately

1. In the Cell Simulation action, select Simulate Inside Stains Separately from the Simulation Mode drop-down box:

2. Adjust the values of the Red Marker Threshold and the Brown Marker Threshold until you are satisfied with the separation (see Figure 3).

3. You can now select the size of a typical cell. Adjust the value of the Typical Cell Size field and press Preview to view the results (see Figure 4).

   This feature defines the maximum cell size; any smaller cells are accepted.

4. Switch to the Cell Classification action. Select IHC Dual Markers Intensity in the Select Feature field. Ensure Use Exclusion is not selected. Press Preview.

   Cells will be classified as red, brown or ‘negative’ (white) – see Figure 5. They

Figure 3: Cell Simulation using Simulate Inside Stains Separately. Brown and red markers are highlighted (nuclei are in blue)
Figure 4: Cell Simulation preview, following selection for cell size
Figure 5: Classification into red, brown and negative (white)
are classified according to the marker in which they were simulated, during the Cell Simulation action.

**Simulate Inside Both Stains**

1. In the Cell Simulation action, select Simulate Inside Both Stains from the Simulation Mode drop-down box:

2. Adjust the values of the Red Marker Threshold and the Brown Marker Threshold until you are satisfied with the separation (see Figure 6).

3. You can now select the size of a typical cell. Adjust the value of the Typical Cell Size field and press Preview to view the results (see Figure 7).

4. Switch to the Cell Classification action. Select IHC Dual Markers Intensity in the Select Feature field. Ensure Use Exclusion is not selected. Adjust the values of the red and brown marker thresholds.

Cells will be classified as red, brown or ‘negative’ (white) – see Figure 8.

**NOTE:** Simulate Inside Both Stains is available when both markers are not nuclear. We recommend this option when you do not need values for the separate stains, for example when markers are co-localized.

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**Figure 6:** Threshold preview of Cell Simulation using Simulate Inside Both Stains. Regions containing red or brown marker, where the cell will be simulated, are shown in yellow.

**Figure 7:** Cell Simulation preview, following selection for cell size

**Figure 8:** Classification into red, brown and negative (white)