Contents

Introduction ................................................................................................................................................ 5
Detecting Cores ........................................................................................................................................ 7
Loading and Configuring Solutions ................................................................................................ 13
The Export Function .............................................................................................................................. 17
Running and Reviewing Analyses ........................................................................................................ 19
Introduction

This tutorial will guide you through the steps required to perform an analysis using the tissue microarray (TMA) functions of Definiens Tissue Studio®.

You will learn how to detect cores on the TMA slide and to perform detailed nuclear marker detection on each of these cores. You will also get an impression of the customized export function, which can extract information on a cell-by-cell basis. This tutorial will also show how you can use your configured solution in batch mode on several slides.

1 Start Definiens Tissue Studio® in the Tissue Studio® (TMA) portal.

You will see that, in addition to the Workflow and Workspace windows, a third one is available — TMA Grid View. This is used to detect cores and match them to their locations on the slide.

2 Select the Load tab and click on the Import Folders button. The image files you need are located in 2_BF_TMA_MembranesCells\images. Select Aperio ScanScope – with resolution as the scanner type.

3 Type a name for the workspace and save it to a convenient location (use the prefix WS_ to distinguish the workspace folder easily from other folders in the same directory) and click Finish.

Three TMA scans will appear in the newly created workspace in the Tissue Slides folder, one for each type of stain.

4 Double-click on the project Tissue Slides.TMA_Membrane in the Workspace pane to open it (see Figure 1).

Our images are xenograft TMAs, treated with either a nuclear, membrane or cytoplasmic marker stain. In this tutorial we will work on a TMA stained with a membrane marker. The task is to correctly allocate each core, then to configure an analysis solution for the detailed cell detection based on the membrane stain and the nuclei. We will then classify cells according to stain intensity.

To allow for a rapid inspection of the image details, you may activate the Whole Slide Review mode by clicking the checkbox in the lower-right corner of the main window.

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.
Figure 1: Overview of the imported TMA image
Detecting Cores

To free up space for the TMA Grid View window, we recommend closing the Workspace window; to re-activate it, use View > Workspace in the main menu.

1. Make sure you are in the Layout mode of the TMA Grid View. Ensure Whole Slide Review mode is not activated.

2. Under Grid Layout Templates (Figure 2), click on New and name the grid in the Grid Parameters field.

3. Normally you will need to count the rows and columns on the slide – including the free spaces between cores – and enter the values in the Rows and Columns fields (in this example there are 24 rows and 17 columns – see Figure 2).

The grid layout in the TMA Grid View will update based on your settings. By default, all fields are labeled as 'normal'. To simplify the core detection and to aid clarity, you can label cells as 'empty', 'positive control' or 'negative control'.

To display the entire grid, double-click the title bar of the TMA Grid View and resize the window (you can restore it by double-clicking the title bar again).

We will now exclude all the empty cells in the microarray.

4. Click the ‘3’ above the third column of the grid, to select the whole column. Click on the Empty cell button (the black circle) under Core Type.

5. Do the same for columns 6, 11, 16 and 21, and rows F and L (Figure 3).

You can only select single columns one at a time; therefore you will need to select a single column, press Empty cell, then repeat.

6. To select only part of a row or col-
umn, click on a cell and drag the selection, or click on individual cells while holding down the Ctrl key.

7 Do this for cell columns D1-Q1 and B2-Q2.

This step is not mandatory, but it increases the chances of Tissue Studio® calculating the correct outlines.

8 Press the Confirm button and change to Matching mode.

9 Under Grid Layout select the newly created layout from the drop-down box, then press Assign to apply it to the currently open image.

10 Under Core Detection keep the default settings and click Detect Cores (this may take several seconds).

Most of the cores are detected properly, but the grid matching needs some refinement. You can rearrange the cores directly in the TMA Grid View using drag and drop, but a more convenient way is to use the built-in adjustment features.

Matching the Grid to the TMA

1 Click on the Matching button to activate the grid for configuration (this will open a Help window that contains a short description of available configuration shortcuts).

If the grid does not display first time, press the Reset button.

2 Start to match the grid using the commands listed in “Shortcuts for Grid Matching” on page 9.

TIP: Don’t spend a lot of time trying to match every core by painstakingly adjusting the grid; it’s better to match most of the cores and assign the remainder manually.

Figure 4: Result of the initial core detection
The best way to familiarize yourself with the grid tools is simply by experimentation; however here are a few ideas with regard to this particular grid:

- Because of the orientation of the cores, the matching process may benefit from turning the whole grid slightly anti-clockwise.
- The right-hand columns are skewed upwards a little; you may want to hold down Ctrl-Shift and skew the bottom-right and top-right of the grid horizontally.

3 Click **Optimize** for automatic adjustments. Repeat the adjustment as necessary (Figure 5, left).

The manual adjustments do not have to be perfect, as the **Optimize** function usually takes care of the fine-tuning. Not every core has to be exactly within a cell to be recognized.

4 Click **Confirm Changes** to automatically match the cores to the configured grid (Figure 5, right).

The majority of cores will be matched but, depending on your manual ad-

---

**Shortcuts for Grid Matching**

- To rescale or rotate the entire grid, hold down the Ctrl key while double-clicking any node. Keeping the Ctrl key held down, click on another node and rotate and resize by dragging the mouse.
- To move the complete grid, hold the Ctrl key while moving it into position.
- To resize to a single square, double-click on a core within a grid square.
- To move the frame of single square, click any node and move it to the desired position.
- To move the frames of one intersecting row and column, hold down the Shift key while clicking a node and moving the grid into position.
- To move the frames of all squares within the grid, hold down the Ctrl and Shift keys while clicking a node and moving the grid into position.
justment, a small number – in this example, the cores in the TMA Grid View arranged under row Q in Figure 5 – will need to be assigned manually.

When you click on a core in the TMA Grid View, the corresponding core will be highlighted in the TMA image. If the core in the Grid View is empty, you can drag an unassigned core with the mouse. You can select multiple cores on the grid by holding down the Ctrl button and moving them as a group.

**Manually Assigning Cores**

Although we have matched most of the cores, some cores may have been recognized incompletely, single cores may have been identified as multiple cores, or some cores may have been missed (see ).

1. Change to the Editing tab, then click on the Cores button.

2. To identify a missing core, activate the first button (Add New Cores / ROI), draw a rectangle around it and press Confirm to save it.

   Up to now, there is no tissue detected in the newly created core, so it would not be considered during the batch analysis.

3. Click Detect tissue in all empty cores to perform a tissue-background separation for the manually annotated core.

   The core appears in Grid View and can be dropped into the respective cell. Alternatively, you can click the Matching button followed by Confirm Changes to automatically assign the newly detected core to its place in the grid (which is especially helpful when you have to match several cores).

4. If you need to change the dimensions of the bounding box of a core, or move it, press the Resize / Move button

5. To delete a core, press the Select Core / ROIs button, select the bounding box and press the Delete button.
Every core has been established as one independent project in the **Workspace**. The cores in the **Grid View** and in the image are linked and can be opened by double-clicking on them.

Make sure the entire slide image, and not a single core, is active before you proceed with the cellular analysis.

Figure 8: TMA Grid View with all cores matched
Loading and Configuring Solutions

1. In the Workflow pane switch to the Configure tab. Press Load Solution.

2. In the Load Solution dialog select the solution TMA > Tissue > Nuclei, Membranes & Cells.

3. Go back to the Load tab, activate the original whole slide image, then switch to the Load tab again.

   You can now begin to configure the solution. Use the arrows on the right to switch the position of actions and the + and – buttons to add or remove actions.

4. Click on the General Settings action to activate its widgets (the active action is indicated by a yellow frame).

   Move the cursor over the different widgets to display a short explanation of each item.

5. Configure the settings according to the analysis task (in this example, IHC Brown Chromogen for the Stain Combination, and Membrane for the IHC Marker).

6. Change to the Initialize Cellular Analysis action. Set the Magnification to 20x and press Select. Choose up to 12 subsets for configuration (see Figure 9). Use the Deselect button if you need to remove any selections.

   The more subsets the longer the configuration time, but the more heteroge-

Figure 9: Subset selection on downsampled image, with four subsets selected
neity can be covered.

14

7 Press **View Subsets** to visualize the selected subsets (see Figure 10). Use the **Deselect** button to change your selection if required.

8 Switch to the **Nucleus Detection** action. Keep the default settings and press the Preview Thresholds button. Adjust the thresholds until you get a satisfying result (here: 0.26/0.3).

9 Press the **Select Samples** button and click on some standard nuclei to find the approximate nucleus size (here: 55). Click the **Preview** button (see Figure 10).

10 Switch to the **Membranes and Cells** action.

11 Adjust the **IHC threshold** to define the region in which the cell simulation should be performed (here: 0.15).

12 Set the **Membrane Thickness** (here: 2) and press **Preview** (see Figure 11).

   The cells will only be detected inside the area that has been defined via the IHC Threshold parameter of this action. The system automatically removes cells that are too big or are otherwise not correctly found.

13 Switch to the **Cell Classification** action. Select **IHC Marker Intensity** and **Membrane** as classification parameters.

   You can group the cells into four stain classes: negative, low, medium and

![Figure 10: Subset selection. Right-hand image shows a subset following nucleus detection](image)
high. You can disable certain classes by setting the slider to the minimum or maximum.

14 Adjust the slider until you get a satisfying result (here: 0.13 / 0.27 / 0.58) – see Figure 12.

In addition to the classification, you can also exclude cells from the analysis based on up to three different criteria. In the present example, we will exclude all cells from the analysis that are adjacent to either unclassified or background areas, or are too big.

15 Activate the Use Exclusion checkbox.

16 For Feature 1, select Class-Related Features > Relations to neighbor objects > Number of > Unclassified.

17 For Feature 2, select Class-Related Features > Relations to neighbor objects > Number of > Background.

18 For Feature 3, select Object Features > Geometry > Extent > Area (µm²). We will exclude anything bigger than 300µm², so set the criteria to => 300.

19 Press the Preview button to get the exclusion classification. Activate the Remove Excluded Cells checkbox and press Preview again to compare the results (Figure 13).
The Export Function

The export function generates screenshots of the classification results and creates a results file, containing all information about the identified nucleus objects.

1. Select the Default Export action.

2. In this example, copy the settings displayed in Figure 14.

   In addition to Default Export, you can also customize data to be exported. In the present example we will export the mean stain intensity of the cytoplasm for each cell and for each cell group (low, medium or high), to get information of membrane stain leakage. Of course, there are plenty other possibilities to customize the data output.

3. Click on the + in the blue bar on the left-hand side of the window. Add the action Custom Export (Cellular Analysis).

   Custom Export actions can be added to the Analysis Builder multiple times.

4. Select the class Cell from the Class drop-down box – this will consider all cell types for export.

5. Click the Features button. Go to Class-Related features > Relation to sub-objects > Intensity > Cytoplasm Subobject Brown Chromogen – double-click on the feature to move it to the Selected pane. Press OK.

6. Under Select Type of Export, select Per Single Objects, Average/ Mean, Minimum and Maximum.

7. Press Save Solution in the Workflow pane to save it as a Definiens Analysis Solution (dax) file.

   TIP: If you want to reuse the solution, we recommend storing it under C:\ProgramData\Definiens\TissueStudio\Data\Solutions. This way it is directly accessible via the Load Solution button.
Runing and Reviewing Analyses

When you run the analysis, a read-only .dax file of the solution with date and time of the analysis will be saved to the results folder.

1. Switch to the Run tab in the Workspace window.

2. In the Workspace window, click on the folder Tissue Slides in the left-hand pane, to display the image list.

3. Mark the original image you want to submit to the analysis (not the core projects) and click Analyze Selected Images.

You can follow the status of the analysis in the Workspace window (projects will switch from created to licensing to processing and finally to processed) or in the Job Scheduler (for information on how to use the Job Scheduler, refer to the Definiens XD Installation Guide).

When the analysis is done, switch to the Review tab and click on the Open Results Folder button.

Here you will find all exported results (screenshots, statistics and solution .dax file) — see Figure 15.

In the TMA Grid View window, the cores can also be displayed in Heat Map mode (see Figure 16) by selecting a feature in the Show drop-down list in the bottom-left of the window. To export the Heat Map view, right-click in the TMA Grid View and select Copy to Clipboard.

Figure 15: Results folder contents
Figure 16: Heat Map view of cores