

## Preparation Procedures for Tissue Microarray Construction.

1. Decide why you want to make a tissue microarray (TMA) and what you intend to learn from it.
2. If needed, obtain IRB approval before going forward. IRB approval is needed to query most Hopkins databases including PDS.
3. Determine the number of cases that will provide a statistically significant dataset.

If you will be using pathology archival human tissue blocks to make your TMA, query the PDS or TMAJ database to ascertain that the numbers of cases needed exist.

4. Determine the core size needed on the TMA.

The TMA core offers three core sizes for TMA construction, 0.6mm, 1.0mm and 1.5mm. The maximum dimensions for each is listed in the chart below.

Core Size	Number of Row	Number of Columns	Total Number of Cores
0.6 mm	19	25	475
1.0 mm	12	18	216
1.5 mm	8	14	112

The number of cores and the size of the core needed ideally will represent the entire targeted tissue type of each case. The coring size that best preserves the original donor blocks is the 0.6 mm size with three or four replicates. It has been found that when arraying tissues with high lipid content, the 1.0 mm core size will adhere to the slide a little better. Many pathologists prefer to use the 1.5mm core in order to see more architecture of the tissue. It may be better to make 2 TMAs with larger core sizes if needed than use too small a core size to get more samples on the slide.

5. Internal JHU TMA projects must be performed with a pathologist faculty member at Johns Hopkins.

In order to make a TMA with pathology archival materials you must work with a Johns Hopkins Board Certified Pathologist. Only JH Pathologists will have access to the surgical pathology paraffin blocks.

6. You can obtain an estimate of the cost of generating a TMA.

Ask if you need an estimate of the costs to construct the TMA with the desired number of samples. In order to get an estimate we need to know how many cases and how many cores per case and what core size will be used. Additionally we need to know if you want normal human control tissues added to the TMA and how many columns of controls you will incorporate into the TMA. The normal controls are added in to serve as navigation and orientation markers and also may add some interesting IHC staining results. It would be a great asset if you had cell lines available to add to the TMA's.

7. Request slides from Archives and review.

If using JHU archival materials, initially request all the necessary slides. Then confirm the following. Are all slides present? Is the slide with the most significant desired tissue type present with the case? Will tumor and normal be used from each case? Is it important to note the location of the benign tissue? Is it adjacent to or away from the tumor? Once satisfied you will have enough cases to use for a TMA, request blocks from the archives.

8. Request blocks from Archives for review and markup.

Blocks will be sent to the Reference Histology lab on the 7<sup>th</sup> floor of the pathology building. Confirm that all of the blocks requested match the case slides. No blocks are allowed to leave that

laboratory. Examine the block face to make sure the block and slide match and check the block designation as well. Be sure to examine the target area to assess the amount of tissue that is remaining in the block. Ideally the depth of the tissue should be > 2mm deep. Less depth will mean a lesser number of TMA slides that the constructed TMA will be able to produce. Circle the area of interest on the slide that you want to be cored. Use only a light touch to circle the area of interest on the block face. You can use a xylene free marker or a Sharpie. The marker tips will get clogged with paraffin while you do this. Keep a paper towel nearby to wipe the tip of the marker off. Keep several pens on hand and switch from one to another as they stop working. Do not press into the paraffin. It is not necessary to circle the area for every core. Just circle the area to be cored. Arrange the blocks and slides in order that they will go into the array. The block should be in front of the slide. The array is built from left to right all the way to the end of the row and then down to the left of the second row. And so on until the array is complete.

#### 9. Meet with Helen Fedor.

Meet with Helen to get a login to TMAJ and learn how to input the data in to the TMAJ Database. The OTS TMA Core labs require that the TMA is designed using the ArrayBuilder Application in the TMAJ Software. Java must be installed on the computer in order to use the software.

#### 10. Enter data into the Array Builder Application in TMAJ.

You are now ready to put the data into the ArrayBuilder Application of TMAJ. TMAJ restricts access of cases that have already been used by someone else. To avoid running into this problem you can send a list of cases that will be utilized to Helen and she can give you access to the cases before you get started. If you send a list ahead they need to be formatted correctly. TMAJ reads all input as txt. Therefore the formatting must appear with 9 characters. S00-00000. If the case has less than 5 digits after the dash then leading zeros need to be added to achieve this formatting. TMAJ will not recognize that S00-00002, S00-0002, S00-02 are all the same case. This is most important if you would like to read the surgical pathology reports in TMAJ. It will be difficult to fix if the case is entered in multiple formats. Helen can download the cases from PDS and put them into TMAJ. TMAJ will strip out the patient's name from the gross description and make the data more HIPPA friendly.

#### 11. Pictorial Tutorial on how to enter data into TMAJ.

Open TMAJ>Click on ArrayBuilder Application>Highlight the Array that you want to work on>click OPEN

The screenshot displays the TMAJ Software Project website in a web browser. The browser's address bar shows the URL <http://tmaj.pathology.jhmi.edu/>. The website features a header with the logo and the text "The TMAJ Software Project" and "http://tmaj.pathology.jhmi.edu". Below the header, there are several navigation links: "Launch TMAJ", "Documentation", "Demo", "Download", "Contact", "Links", and "About". A large purple button labeled "Launch TMAJ" is positioned below these links. The main content area includes a section titled "Start TMAJ Application" with a link and a list of instructions. Overlaid on the website are several application windows. The "TMAJ" window is a login screen with buttons for "Login", "Reset", "About", "Logout", and "Exit". The "ArrayBuilder" window shows a list of "My ArrayBlocks" with one entry: "ArrayBlockID#198 -- Pseudo Array Block (De Marzo)". The "Scans" window displays a grid of image thumbnails. A black pen is drawn over the "Start TMAJ Application" link and the "ArrayBuilder" window.

The array template form for the TMA will open up with the number of columns and rows that have been specified for the requested design. It is not a problem if all of the columns and rows are not filled in, but the empty rows and columns should be the last ones on the map, unless your design includes blank rows and columns.

Highlight the cell (1)>click express entry(2)> fill in all data fields (3 to 8).

ArrayBlockID#198 -- Pseudo Array Block (De Marzo)

File View Help

2 Express Entry...  Control TissueDiagnosisID:  Apply

	1	2	3	4	5
1					
2					
3			1		
4					
5					

Express Entry

Specimen

SpecimenID:

Surg Path Number: T00-00218

Hospital: unknown

Donor Block

BlockID:

Block Designation: LCP

Part: 3

Tissue Diagnosis

TissueDiagnosisID:

Lesion Letter is:  Auto Assigned  Specified

Lesion Letter: T1

Tissue Type: Adrenal

Enter Close

9

Tissue Diagnosis

TissueDiagnosisID:

Lesion Letter: None

Outside TD Number: 0

Tissue Type: Other

CellLineExperimentID:

Block

BlockID: 1

Block: (none)

Part: 0

OutsideBlockNum:

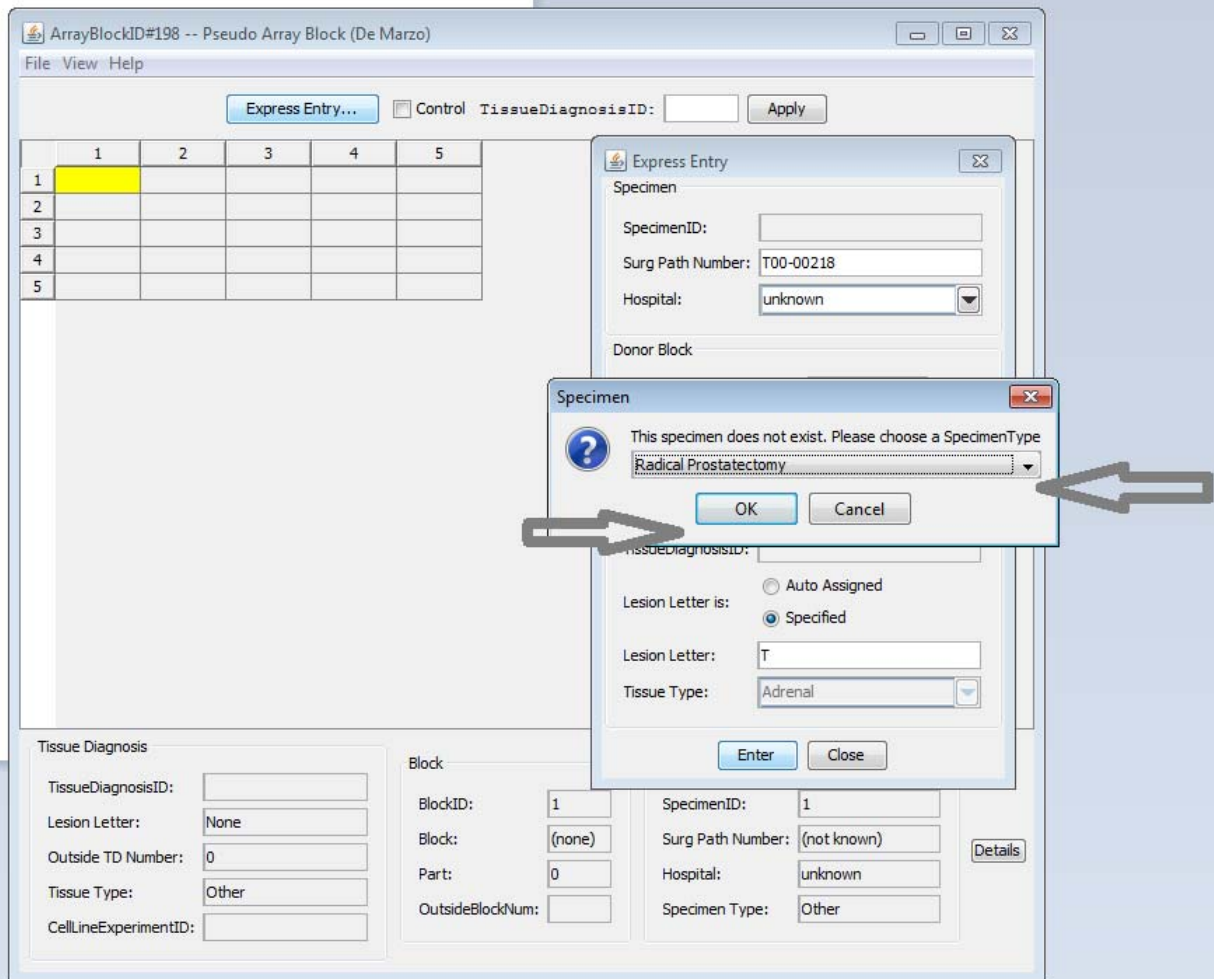
SpecimenID: 1

Surg Path Number: (not known)

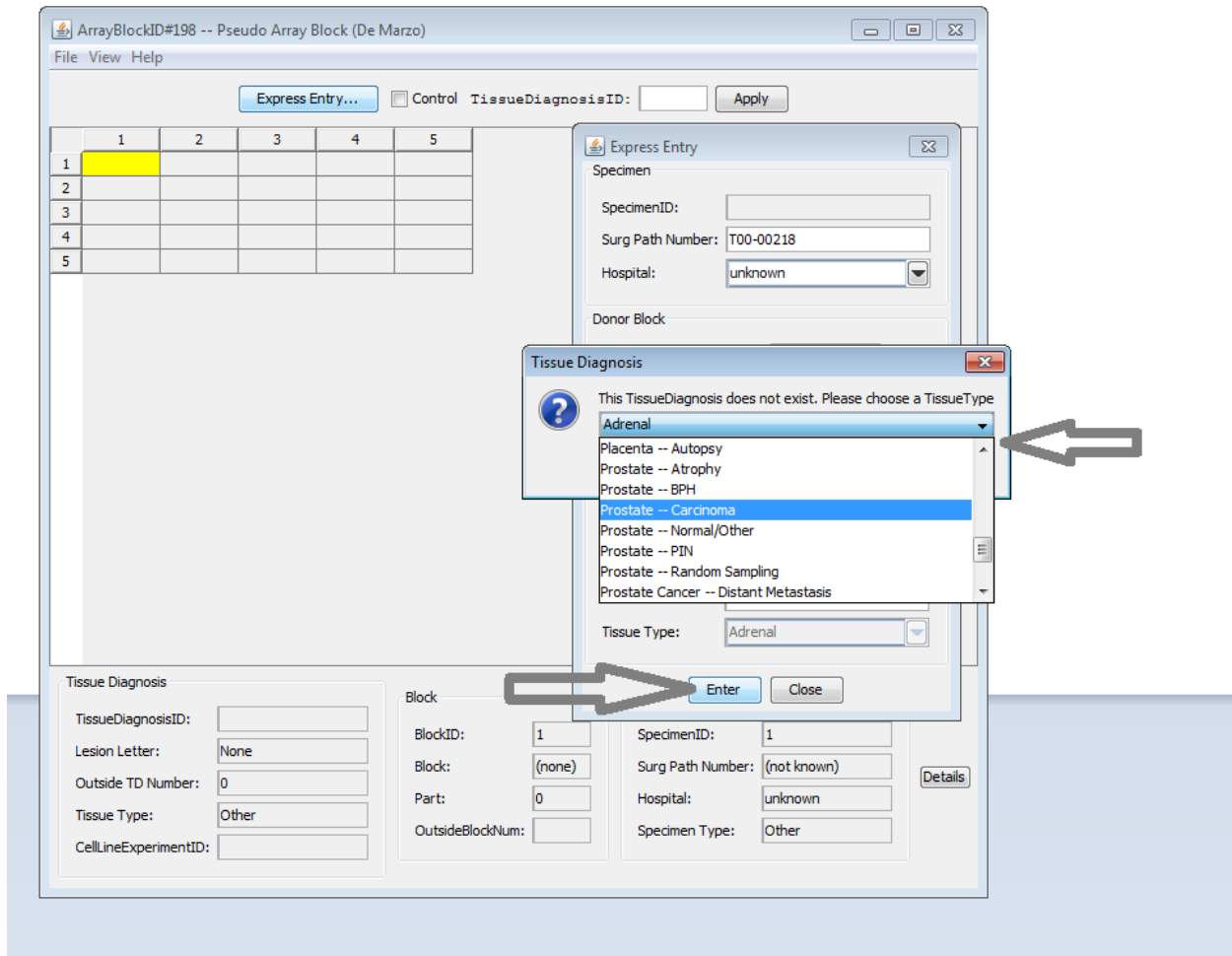
Hospital: unknown

Specimen Type: Other

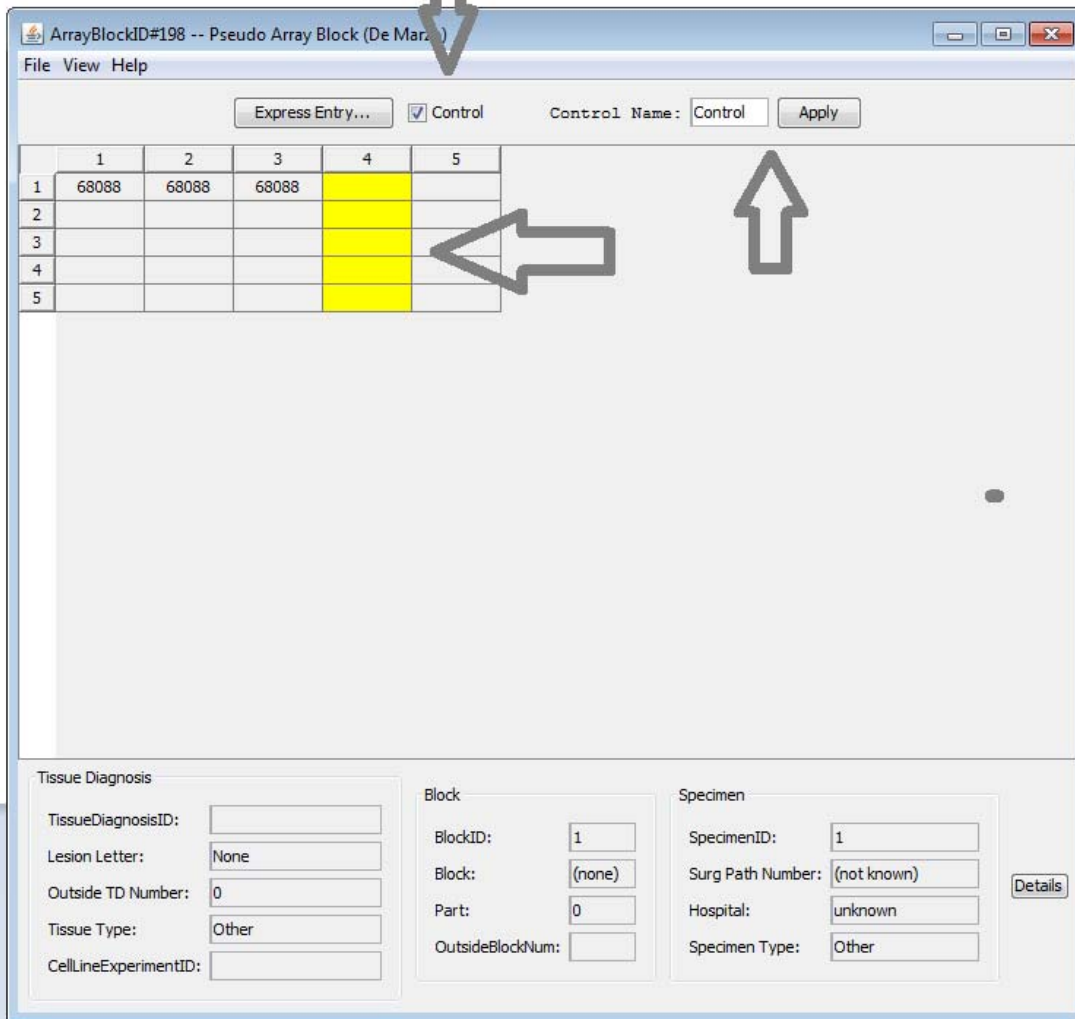
Details



A message will appear that says the Specimen does not exist. One of the required fields in TMAJ is Specimen type. You must pick from the drop down menu and then press enter.



Another message will appear saying the TissueDiagnosis does not exist. You must choose from the drop down menu and press enter. After you press enter TMAJ will generate a number in the TissueDiagnosisID field on the bottom left of the screen. Write the TissueDiagnosisID onto the slide near the area circled. This should be in the same color as the circled area. That way if you have more than one area of interest on the block there will be less confusion as to which of the areas goes into which position on the TMA block. Place the block and slide combination into a cardboard blocks sleeve with the block in front of the slide. Repeat this process until the entire TMA map is filled in.



To fill in a column of controls select the cell>hold down the control key> highlight all cells that will contain controls> click the control box>type “control” in the Control Name box and >click apply.

Give the arranged blocks and slides to Marcia in the Pathology 7 lab. She needs to record what blocks are leaving the lab and then she will alert Helen that the project is ready to be picked up.

12. Need more information? Try these useful materials.

There is literature available on our website to help you learn about TMA’s.

[http://tmalab.jhmi.edu/tma\\_construction.html](http://tmalab.jhmi.edu/tma_construction.html) Scroll down on this page of our website to get access to the information listed below.

- [Procedure for making a cell block for TMA studies](#)



- [Example of using a cell line for IHC studies in TMAs.](#)
- [Book Chapter on TMAs](#) a book chapter describing practical methods for tissue microarray construction
- [Tissue Microarrays: A Valuable Clinical & Research Resource](#) (National Society for Histotechnology, 2010, Helen Fedor)
- [Imaging and Image Analysis of Tissue Microarrays](#) (National Society for Histotechnology, 2009, Kristen Lecksell )
- [TMAJ](#) TMAJ is open-source software designed to support information and images related to tissue micro-arrays. It contains support for multiple organ systems, multiple users, image analysis, and is designed to be compliant with HIPPA regulations.
- [NIH Tissue Array Research Program \(TARP\)](#)
- [The Tissue Microarray Data Exchange Specification](#) a community-based, open source tool for sharing tissue microarray data.