

## Preparation Procedures for Tissue Microarray Construction.

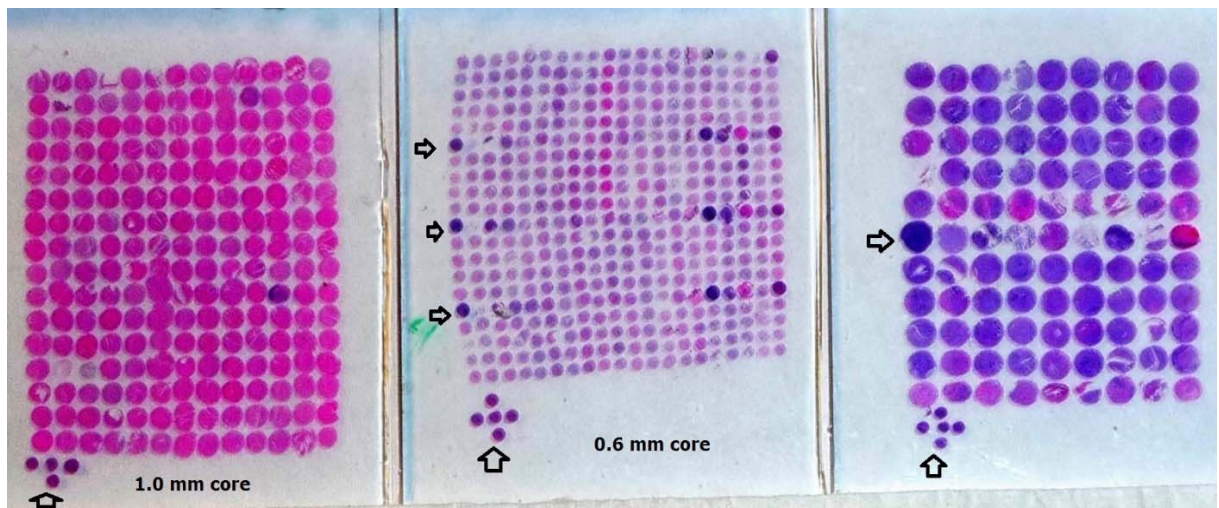
1. Decide why you want to make a tissue microarray (TMA) and what you intend to learn from it.
2. If needed, obtain IRB approval before going forward. IRB approval is needed to query most Hopkins databases including PDS.
3. Determine the number of cases that will provide a statistically significant dataset.

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 generates 99 core TMAs with a 1.5 mm core size, a 208 TMA with a 1mm core size and a 400 core TMA with a 0.6 mm core size. 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.

Core	Number of Row	Number of Columns	Total Number Cores
0.6 mm	20	20	200
1.0 mm	13	16	208
1.5 mm	9	11	99



Images of three TMA slides utilizing the 3 different core sizes, 0.6mm, 1.0mm and 1.5mm cores. Notice the “+” pattern which marks the beginning location of the TMA slide and is always in the upper left corner of JHU OTS constructed cores. This location is 1,1. Also notice the arrows pointing to a column of control tissues. These control tissue serve as orientation and navigation markers. Many TMA labs build in asymmetry into the TMA to help verify the correct orientation of the section 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.

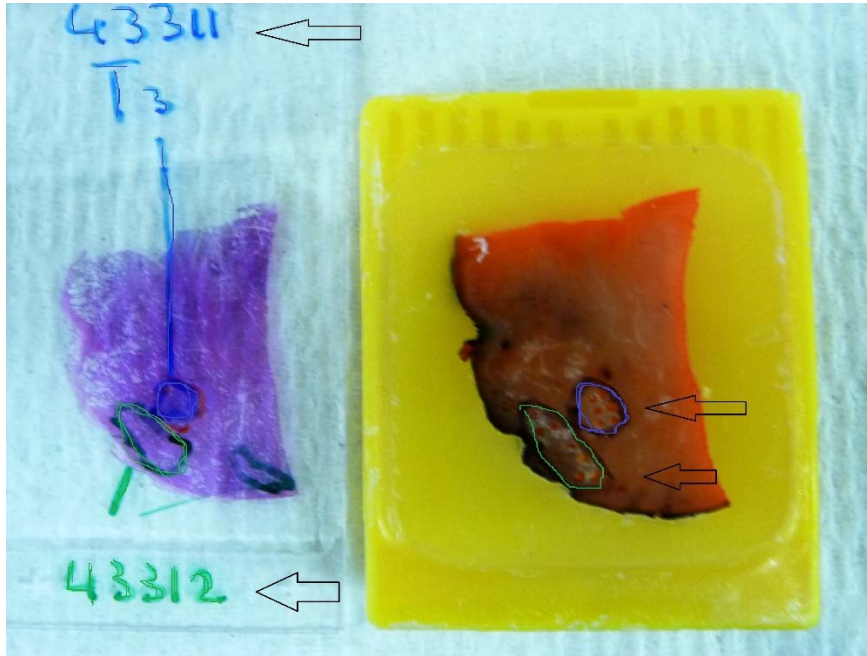
7. Request slides from Archives and review.

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.

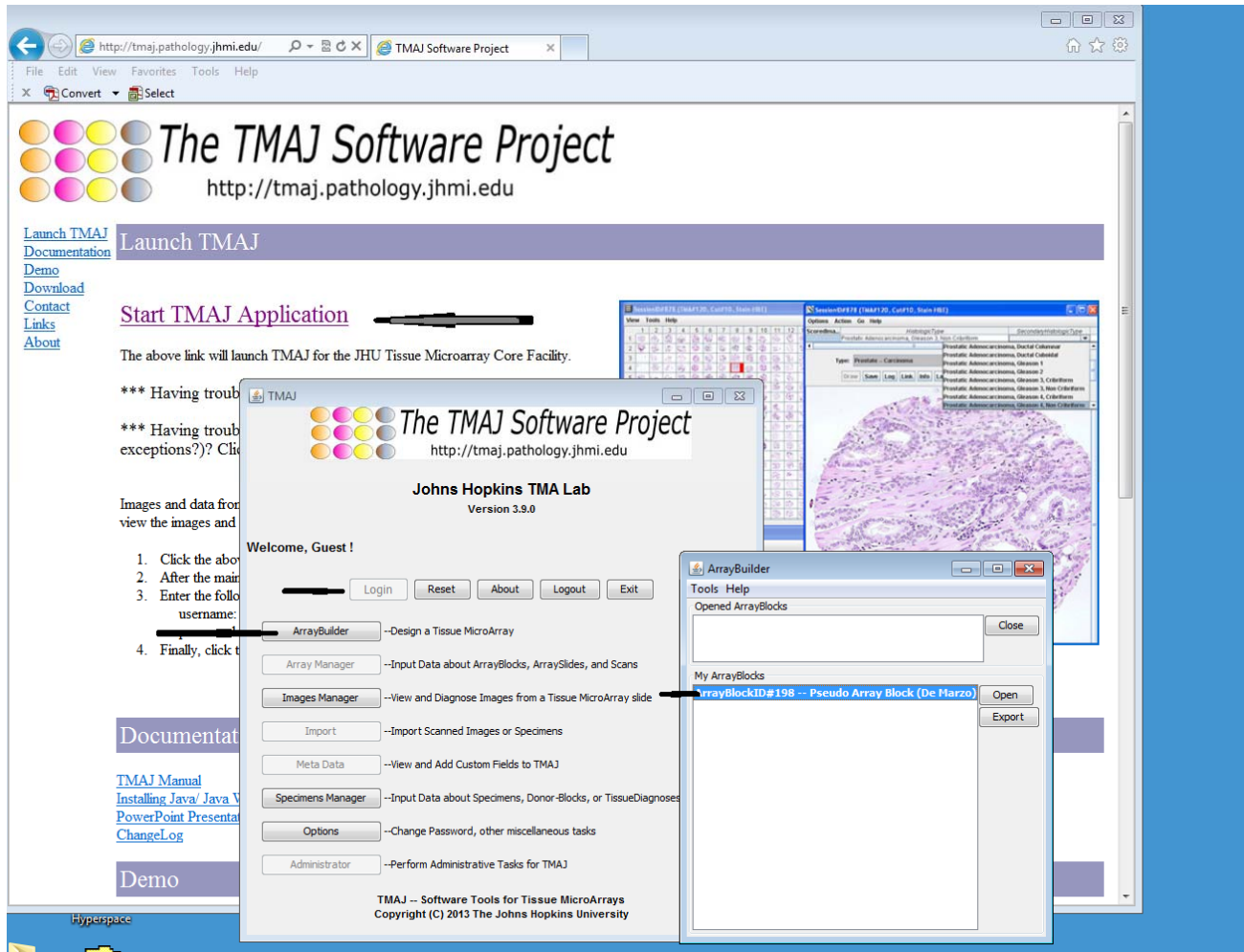
Meet with Helen to get a login to TMAJ and learn how to input the data in to the TMAJ Database. The OTS TMA Core labs require that the TMA is designed using the ArrayBuilder Application in the TMAJ Software. Java must be installed on the computer in order to use the software.

#### 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 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).

The screenshot shows a software interface with a grid of cells and an 'Express Entry' dialog box. The grid has columns 1 to 5 and rows 1 to 5. Cell (1,1) is highlighted in yellow. The 'Express Entry' dialog box is open, showing various fields for data entry. The fields are numbered 1 through 9, corresponding to the instructions in the text.

**Grid:**

	1	2	3	4	5
1	Yellow				
2					
3					
4					
5					

**Express Entry Dialog:**

**Specimen:**

- SpecimenID:
- Surg Path Number:
- Hospital:

**Donor Block:**

- BlockID:
- Block Designation:
- Part:

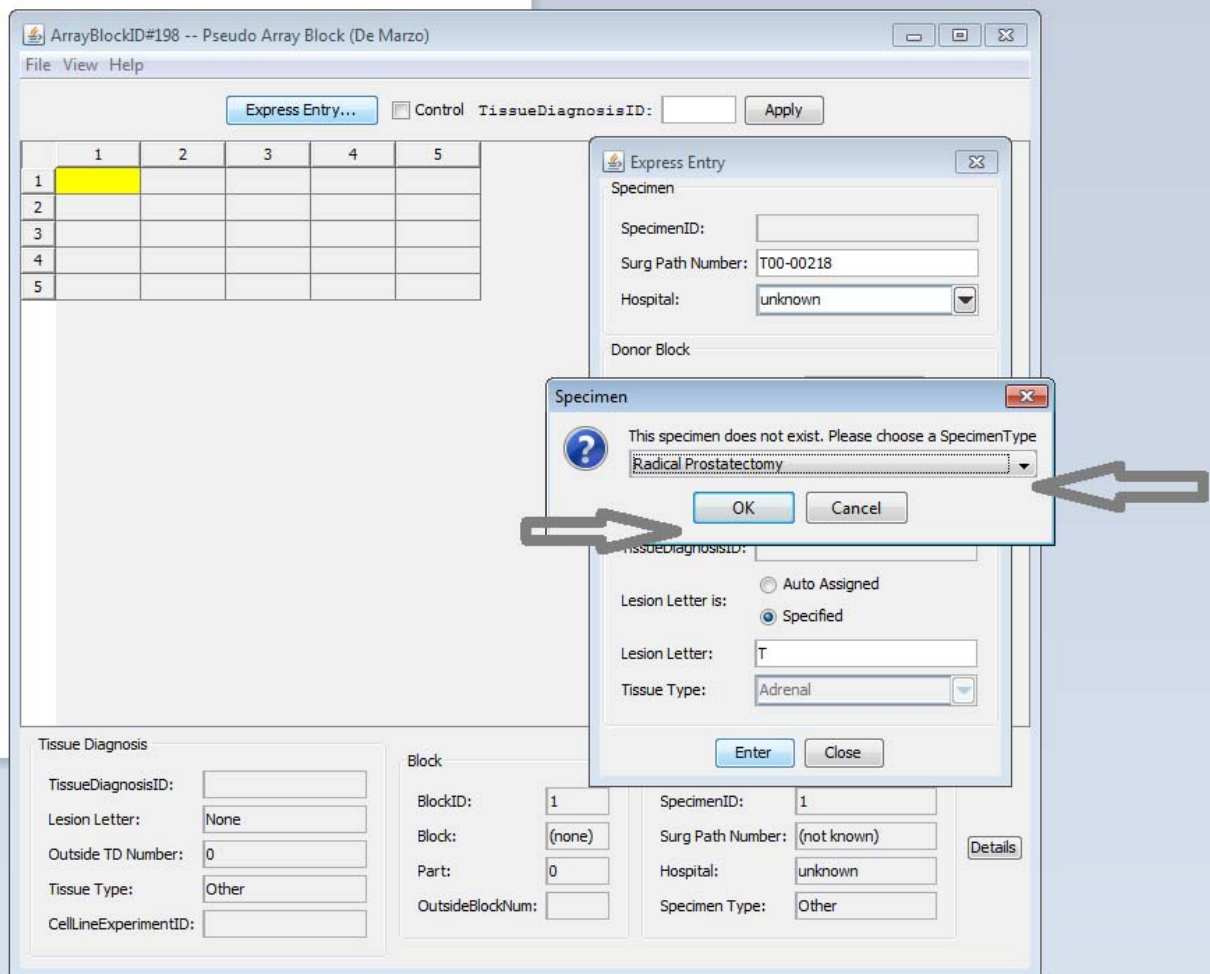
**Tissue Diagnosis:**

- TissueDiagnosisID:
- Lesion Letter is: ☐ Auto Assigned ☒ Specified
- Lesion Letter:
- Tissue Type:

**Buttons:** Enter, Close

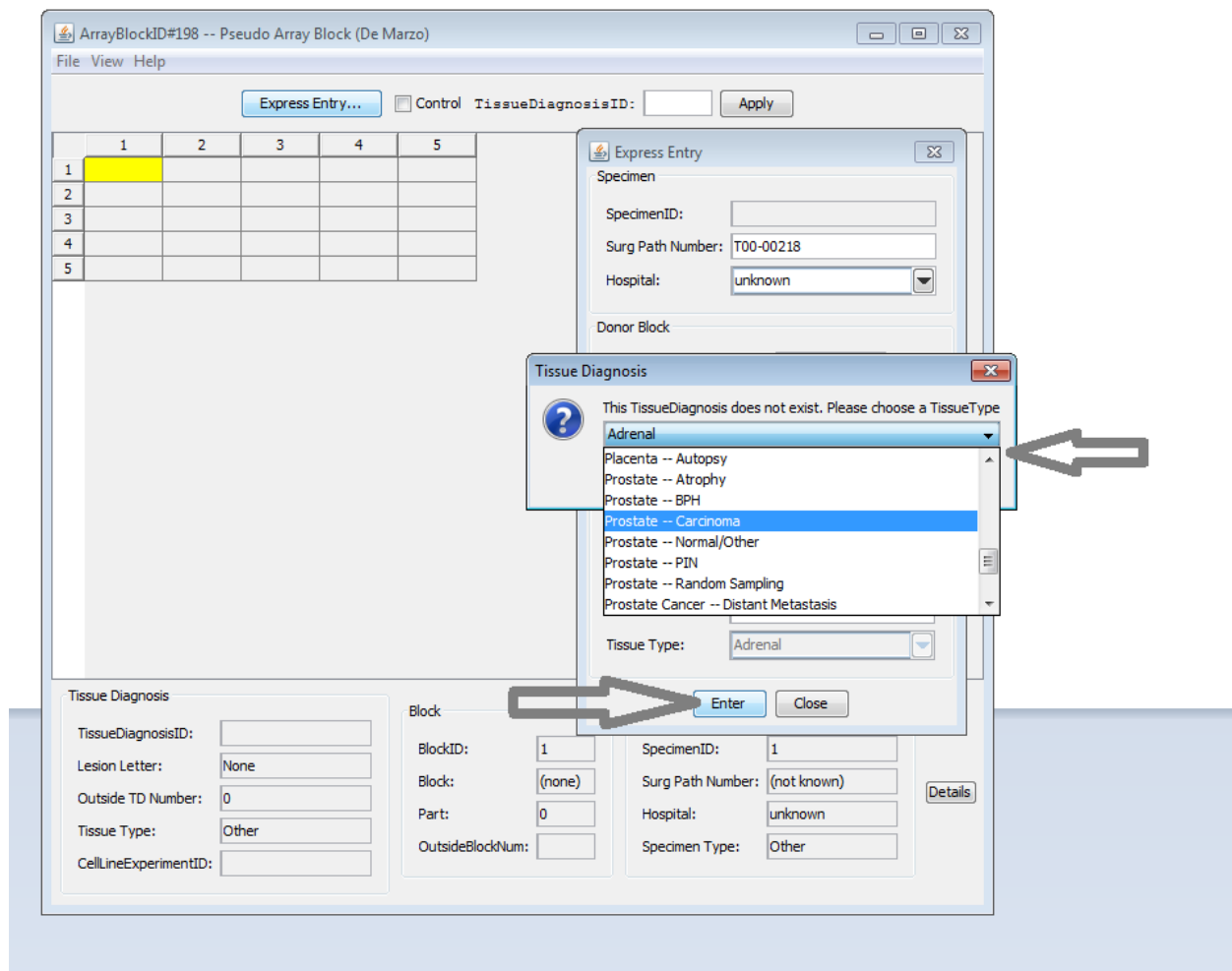
**Annotations:**

- 1: Yellow cell in grid
- 2: Express Entry button
- 3: SpecimenID field
- 4: Surg Path Number field
- 5: Hospital field
- 6: Block Designation field
- 7: Part field
- 8: Lesion Letter is: Specified radio button
- 9: Enter button



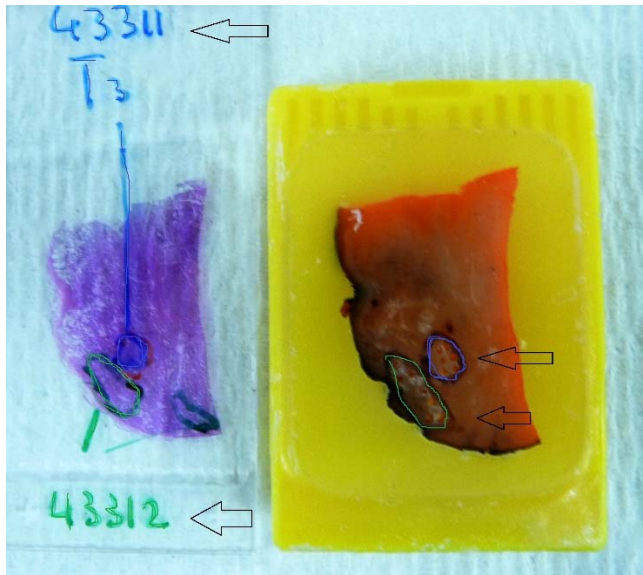
A message will appear that says the Specimen does not exist. One of the required fields in TMAJ is Specimen type. You must pick from the drop down menu and then press enter.





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.



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

File View Help

Express Entry... ☒ Control Control Name: Control Apply

	1	2	3	4	5
1	68088	68088	68088		
2					
3					
4					
5					

Tissue Diagnosis

TissueDiagnosisID:

Lesion Letter:

Outside TD Number:

Tissue Type:

CellLineExperimentID:

Block

BlockID:

Block:

Part:

OutsideBlockNum:

Specimen

SpecimenID:

Surg Path Number:

Hospital:

Specimen Type:

Details

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.

Designing TMA with data input directly to tables.

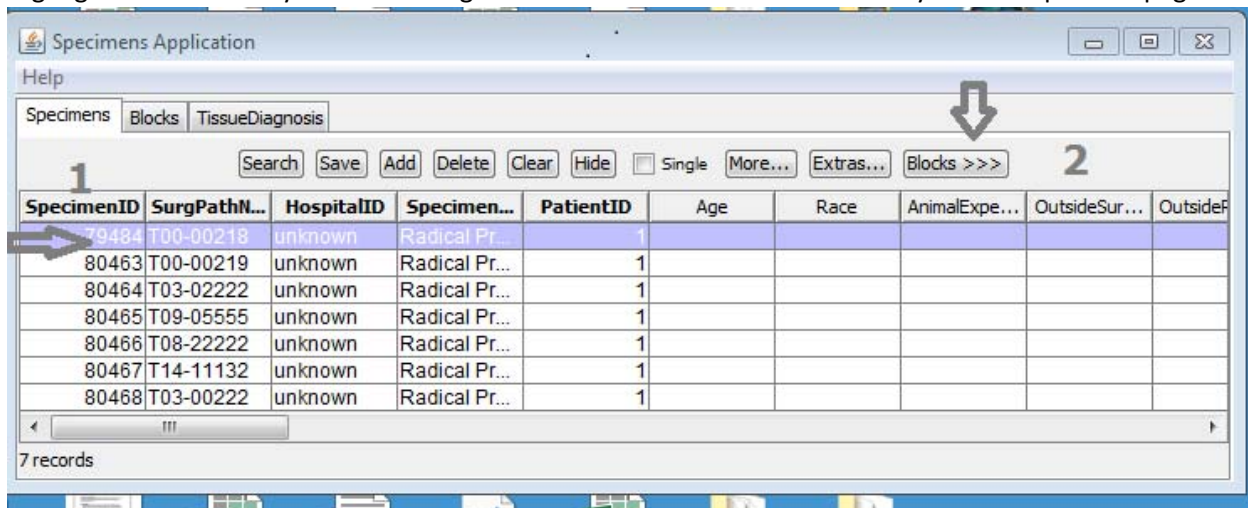
An alternate method of data entry may work better for some projects. These are projects that may take an extended period of time to finalize, or may have a complex target tissue selection processes. TMAJ restricts access to a specific case if another user has already used that case. This is intended to provide some protection to the investigator that is working with a particular set of cases in a project. A TMAJ Administrator can override this. This can be accomplished by providing a list of cases to a TMAJ administrator who can download the Pathology Reports from PDS and also provide access to the cases. The list must be formatted as described previously, with 9 characters (S00-00000). After the cases are downloaded into TMAJ you can access the entire list using the following procedure. Open up the excel file that has the list of cases>Copy the list>Open the Specimens Manager table>Press the search button>Press “Paste Clipboard”>Pick SurgPathNumber from the pull down menu (5) >check box “get type specific fields”>press “Select all”> Press enter.

The screenshot displays the TMAJ Software Project interface. On the left is the main menu with options like ArrayBuilder, Array Manager, Images Manager, Meta Data, Specimens Manager, Options, and Administrator. The central window shows the 'Specimens Application' with a search box and a list of fields to select. On the right, an Excel spreadsheet is open, showing a table of specimen data. Arrows 1 through 8 indicate the steps for searching for a specific case:

- Arrow 1: Copying data from the Excel spreadsheet.
- Arrow 2: Logging in to the TMAJ application.
- Arrow 3: Opening the Specimens Manager.
- Arrow 4: Clicking the Search button.
- Arrow 5: Selecting SurgPathNumber from the dropdown menu.
- Arrow 6: Checking the 'Get Type Specific Fields' checkbox.
- Arrow 7: Clicking the 'Select All' button.
- Arrow 8: Clicking the 'OK' button.

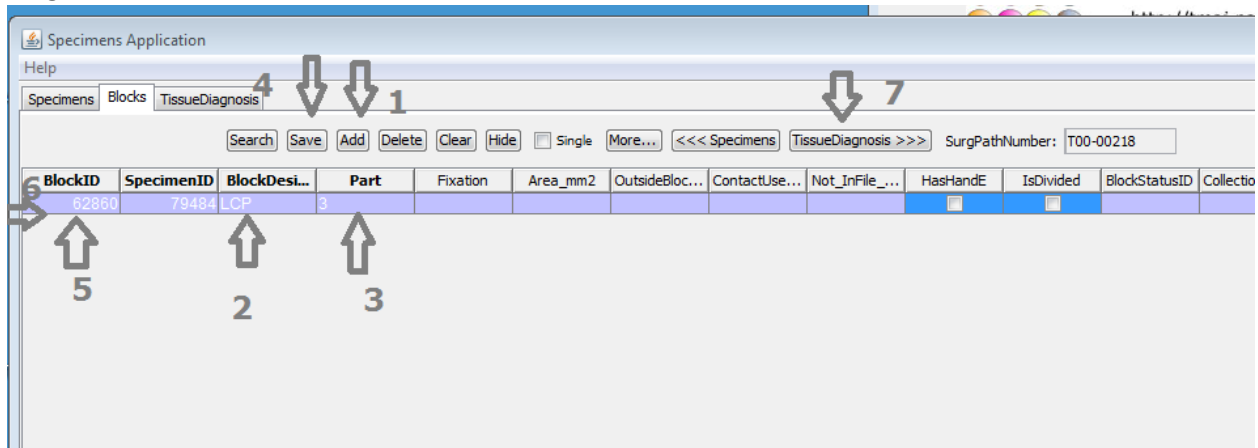
You can also find any one case at a time by typing the number into the search box instead of pasting a list. Make sure that in the drop down list at arrow (5) SurgPathNumber has been selected>press OK.

Highlight the case that you are working with and then click the Block>>> key on the top of the page.



This will take you to the blocks table.

Press add (1) and fill in the block designation and part number (2,3)>save(4). This will generate the BlockID (5) which should be written onto the slide and/or block. It is referred to as the BUI number. Highlight the row (6) and press the TissueDiagnosis>>> button(7). This will take you to the Tissue Diagnosis table.



Press Add (1)>pick a TissueTypeID from the drop down list(2)>add LesionLetter (3), make this something meaningful like T for Tumor or A for adeno carcinoma, N for normal, etc. push the save button(4). The fields will then appear for this type of tissue and you will be able to select from the pull down menus that appear the Histologic types (arrows 5 and 6). Press the save button again (4). Write the TissueDiagnosisId (7) on the slide in the same color of ink that is used to circle the area of interest. If there is more than one type of tissue on the slide/block this process should be repeated and the second TissueTypeID selected.

Specimens Application

P

edimens Blocks TissueDiagnosis 4 ↓ ↓ 1

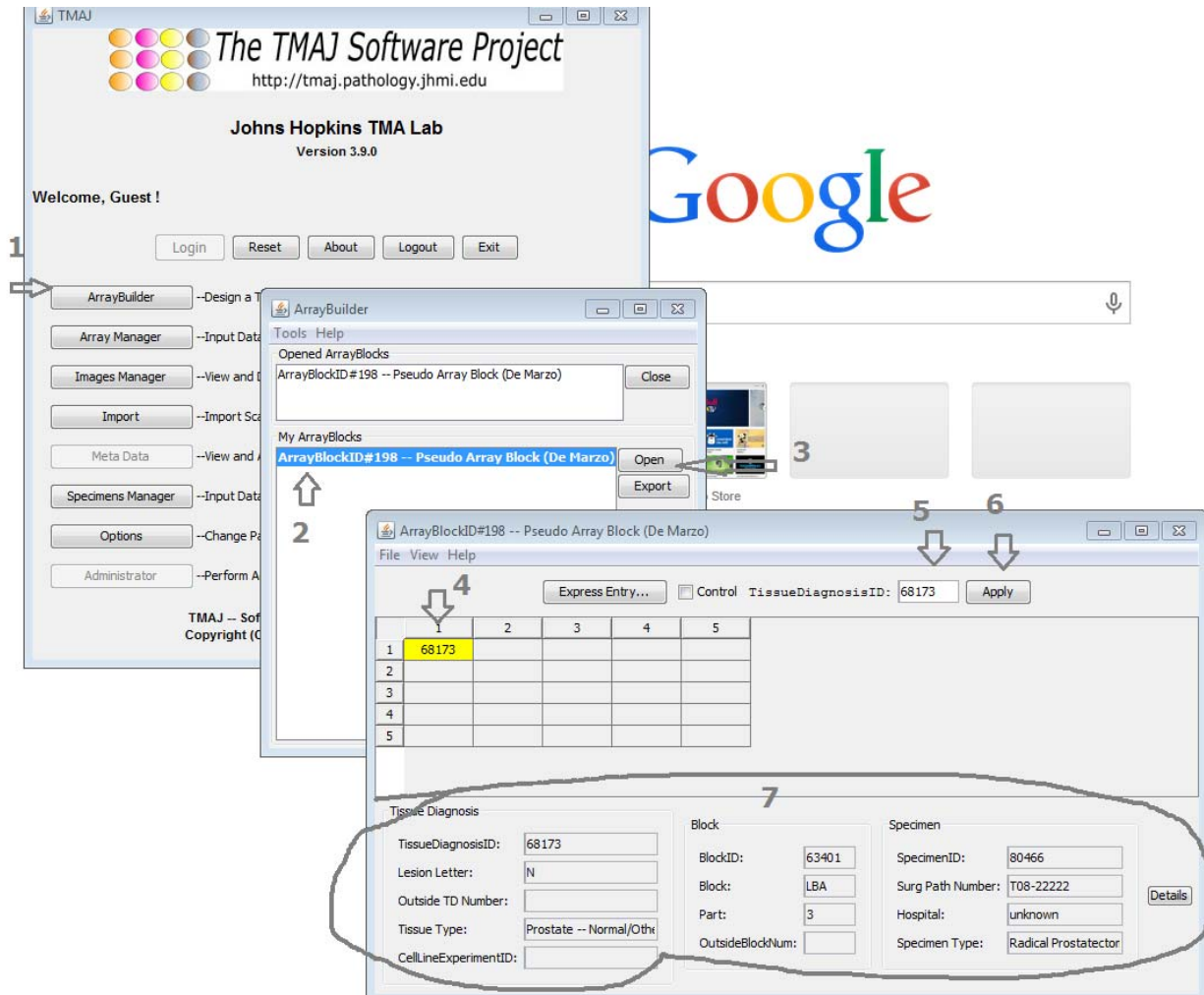
Search Save Add Delete Clear Hide ☐ Single More... <<< Blocks SurgPathNumber: T00-00218 < Cell Lines >

IssueDia...	TissueTypeID	BlockID	LesionLett...	HistologicType	SecondaryHistologicType	Out
68088	Prostate -- Carcinoma	62860	T	Prostatic Adenocarcinoma, Gleason 3, Non Cribriform	Prostatic Adenocarcinoma, Gleason 3, ...	

↑ 7      ↑ 2      ↑ 3      ↑ 5      ↑ 6

## Filling Data into Array Builder Application

After entering all of the data into the three tables in the Specimens Manager application and writing the block Id and the Tissue Diagnosis ID on the blocks and slides that will be used to make the array, you are ready to design the array in the ArrayBuilder application. Open the ArrayBuilder by clicking on it (1). Highlight the array that the data will go in (2). Press Open (3). Highlight the first cell (4). Type the TissueDiagnosis Id that you have written on the slide into the TissueDianosisID cell (5). Press apply (6). The data in the area (7) will be populated automatically. Check to see that the Surg Path Number and block, and tissue type are correct. Hold down the control key and highlight the next cell and press apply



To put in a column of controls check the control box (1), type control in the box (2), highlight a cell where the control tissue will be placed (3), hold down the control key and highlight additional cells, and

press Apply(4). Continue until the entire array map is complete.

**hopkins IMA Lab**

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

File View Help

Express Entry... ☒ Control Control Name: control Apply

	1	2	3	4	5
1	68173	68173	68173	control	
2				control	
3				control	
4				control	
5				control	

Tissue Diagnosis

TissueDiagnosisID: control

Lesion Letter: Control

Outside TD Number: 0

Tissue Type: Control Tissue

CellLineExperimentID: 281

Block

BlockID: 1

Block: (none)

Part: 0

OutsideBlockNum:

Specimen

SpecimenID: 1

Surg Path Number: (not known)

Hospital: unknown

Specimen Type: Other

Details

Press File (1) and press save. Then press file and excel (2) and choose from the pull down list Array Format (Alternate) (3). An Excel file will open up with the array map that you have designed. Save a copy of this and check the data to insure that everything is accurate. You can also get a full details spreadsheet by choosing Full Details from the pull down menu.

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

File View Help

Save

Export

Excel

Close

Array Format (Standard)

Array Format (Secure)

Array Format (Open)

Array Format (Alternate)

Array Format (Cell Line)

Array Format (Typed)

Full Details

Express Entry... ☐ Control TissueDiagnosisID: 68173 Apply

	1	2	3	4	5
1	68173	68173	68173	control	
2				control	
3	68175				
4	68088				
5					

Tissue Diagnosis

TissueDiagnosisID: 68173

Lesion Letter: N

Outside TD Number:

Tissue Type: Prostate -- Normal/Other

CellLineExperimentID:

Block

BlockID: 63401

Block: LBA

Part: 3

OutsideBlockNum:

Specimen

SpecimenID: 80466

Surg Path Number: T08-22222

Hospital: unknown

Specimen Type: Radical Prostatectomy

Details