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Title: **Tissue microarray construction using MiniCore TMA**

Version: **v1**

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Effective from:	01/11/2021
Valid to:	01/11/2023

SOP History		
Number	Date	Reason for Change
v1	01/11/2021	Original

1.0 Purpose –

This SOP describes the current procedures for performing tissue microarray construction using the MiniCore Tissue Micro Arrayer in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff performing tissue microarray construction using the MiniCore Tissue Micro Arrayer in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing tissue microarray construction using the MiniCore Tissue Micro Arrayer in this manner are responsible for ensuring that the methods are followed in accordance with this SOP. All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

4.1 Defining a tissue array using TMADesigner_2

- **Define a block –** defined a type of recipient block. To access the interface 'Define a block', either access through the menu File/New/Recipient Block or by using CTRL+ B.

- **Define a list of tissue types -** A structure is defined as a type of tissue sampled from the donor block. From a single donor block, different histological types can be sampled. To define a project, you will have to define which Tissue types you will want to sample from each donor block. Example: tumour tissue, normal tissue, inflammatory tissue. To create a list of tissue types, choose from the menu File/New/List of tissue types or Ctrl +L.

- **Define a Tissue array -** 'Define Tissue Array' means create a new template of tissue array. To access the interface 'Define a Tissue Array', either choose from the menu File/New/Tissue Array or press CTRL+T.
  1. Fill in the name of the Tissue array, comments and author. Then click 'NEXT' on to go to the next step.
  2. Choose a template of recipient block which was created before. You can choose a file either from the list of block as shown here above or from the browser folder by clicking on  . The file has an extension .tmb. Once the block selected, the properties are displayed at the left side of the interface.
  3. Enter spot parameters. Choose a spot size (diameter) by clicking in the round button. Choose a spot spacing which is the space between two centres of neighbouring spots. This distance is in microns. The minimal distance is spot diameter + 100 µm. You can use the up and down cursors to change the spacing with 100 µm steps. Click on  to calculate the total of spots created. Click on  to close this window. The value displays on the left of the interface. You can modify your settings at any time to obtain more or less spots. Just click on  after having modified your settings. Click on 'NEXT' to continue.
  4. Select the numbering type. Click on  the button to define grids and sub-grids.
  5. Create grids and sub-grids by deleting complete lines and columns of spots. To do so, double-click on the first spot of each column and line, spots turn red; then click on  and  . The complete line and column are automatically selected and coloured in white. See Figure 1.
  5. You have now to number the grids. To do so, click on  . The number of the grid displays according the numbering mode. With the mouse, draw a rectangle around the first grid included all the spots of this grid. The drawing displays (see below). Click on  . Then draw the following grid and so on.
  6. Completion of the tissue array (Figure 2).

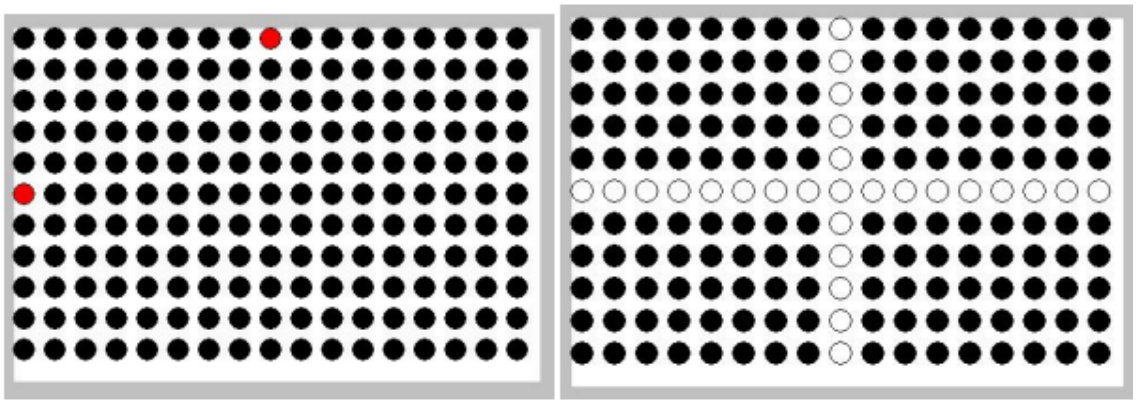

Figure 1 Example of deleting column and row in TMA map

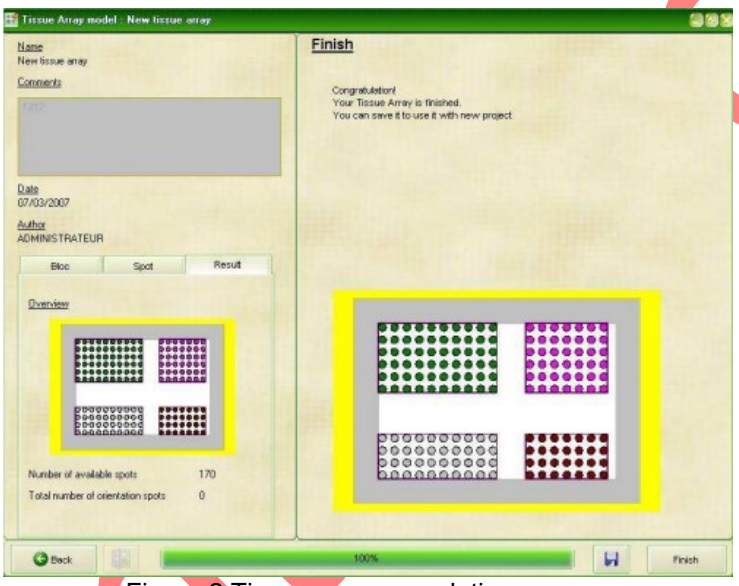

Figure 2 Tissue array completion

4.2. Defining a project in using TMADesigner_2

'Define a Project' consists of importing patients blocks data to fill into a template of tissue array that was created before. To access the interface 'Define a Project', you can either access through the menu File/New/ Tissue Array Project either by pressing on CTRL+P.

- Fill in the name of the Tissue array, comments and author.
- Before starting to define your project, you will need to:
  - Create the list of donor blocks you will use in your project
  - Define the tissue types you will be willing to sample from the donor blocks: tumour, normal tissue, etc…
  - Define the number of replicates for each donor block and Tissue types,
  - Calculate the estimated number of spots positions required.

  This will facilitate the choice of the tissue array template or even help you defining a new tissue array template for this project.
- Download the excel file which has the list of patients block details.
- Download a list of tissue types.
- Set the tissue types and replicates sampled for each block. First select the blocks from the list of blocks. Then, in the histological type table, click into the case Checks to choose the tissue type, then go to the next field to set the

number of replicates for this tissue. Do the same for all tissue types required. Then click on  button to apply settings.

- Choose the tissue array template from the list or from the folder browser. The image and the properties of the loaded tissue array template are displayed on the left of the interface (Figure 3).
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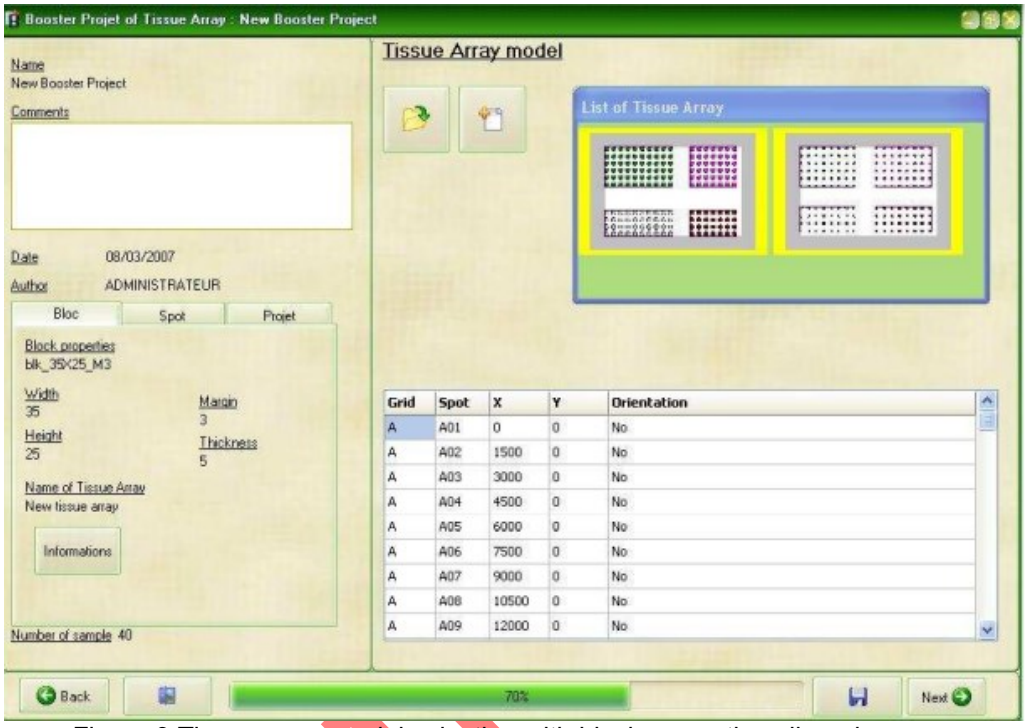


Figure 3 Tissue array model selection with block properties aligned

- Set the number of copy blocks by using the up and down cursors. Click on  to define names to recipients blocks.
- Choose a filling mode: in this step, all the spots will be transferred into the tissue array template chosen. Each line of the preceding table will be associated with a grid number and a spot number together with micron coordinates. Click 'NEXT' to continue.
- At the end of the filling procedure, the overview of the Tissue array displays. Then you can be saved in the Project folder. Each time a tissue array project is designed the software creates file and folder. Project files are organized as follows for TMADesigner®2 version 1.0.0.10 and superior.

4.3 TMA construction in MiniCore software.

- On starting MiniCoreControl software, MiniCore does initialize.
- Loading the Tissue Array Project which has been created in TMADesigner2 software in 'New tissue array'.
- Confirm the correct punch size.
- Setting the Recipient Blocks Position- Select the recipient and donor block positions by simply clicking on the carousel and choosing from the drop-down menu (Figure 4).

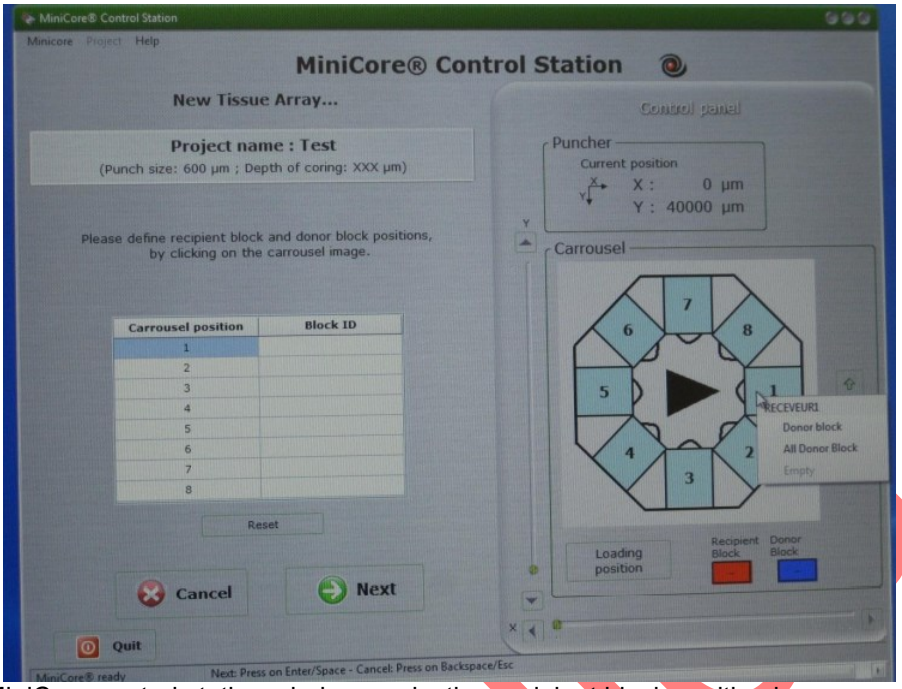


Figure 4 MiniCore control station window - selecting recipient block position in a carousel

- Set Zero Position in a recipient block (Figure 5) - TMADesigner® 2 calculates the exact position of each spot from a specific point called the zero position. This zero position is situated at the position of the first spot of your tissue array. You must first define the edge of the recipient block wax by moving the green and red cross hairs to the top and left edges of the wax.

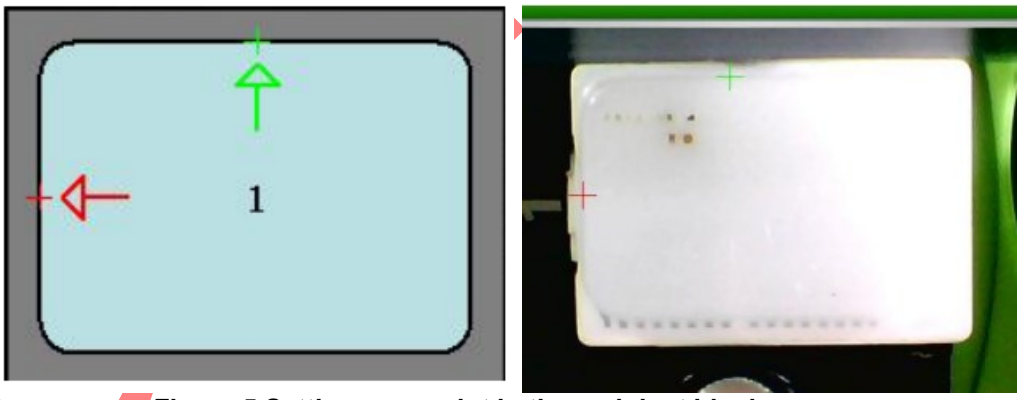


Figure 5 Setting zero point in the recipient block

- "Pre"coring into the recipient block - Before coring the recipient block ensure that the selector is set in position "Recipient ". Move the turret down until it stops. Press the button to rotate the puncher as shown in Figure 6. Then, release the turret so that it moves up. Move the selector in position "Transfer" to extract the paraffin core and remove. Click on "Next" or push the footswitch or use the keyboard to go to the next step

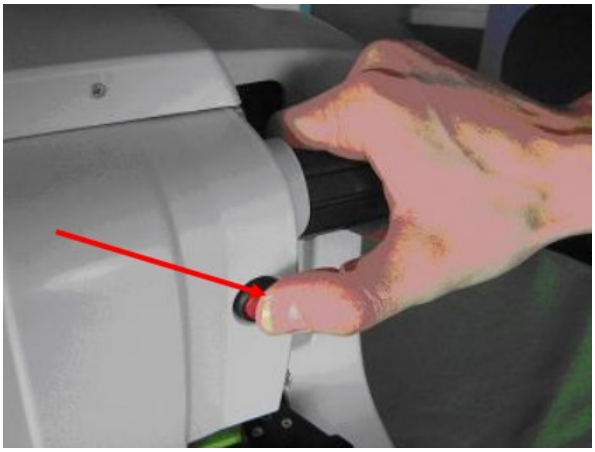


Figure 6 Pre-coring into the recipient block

- Selecting sampling points on the donor block (Figure 7) - Move the selector handle to “Donor” position. Click on Pointing to display the image of donor block. Add points of selection by clicking on image. All sampling positions for this block must be selected at this time. At the upper right corner, MiniCore Control software displays the number and the tissue type you must select on the current donor block

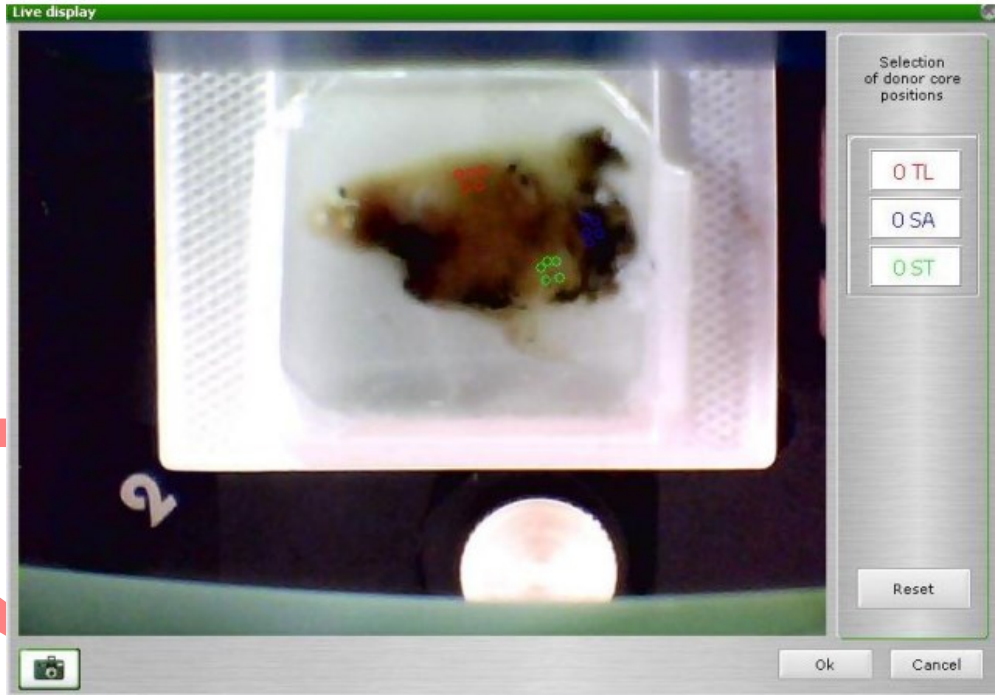


Figure 7 Selecting sampling points on donor block

- Sampling into donor block - Push the punch head down until you reach the stop position: The stop kit is then in contact with the donor block. Press the punch rotation button. Click on “Next” to move the punch to the pre cored hole in the recipient block

- Transfering the core into the recipient block - Push down the punch head until it stops. The punch must be is at level with the top of the recipient paraffin. Move the selector to position “Transfer” whilst holding the punch head down.
- Donor Core Transfer Validation (Figure 8) - If the core in progress (inside the red square) is properly transferred to the recipient block, Click on “Validation”. The MiniCore will move to the next position and MiniCoreControl software will display the information for the next Spot.

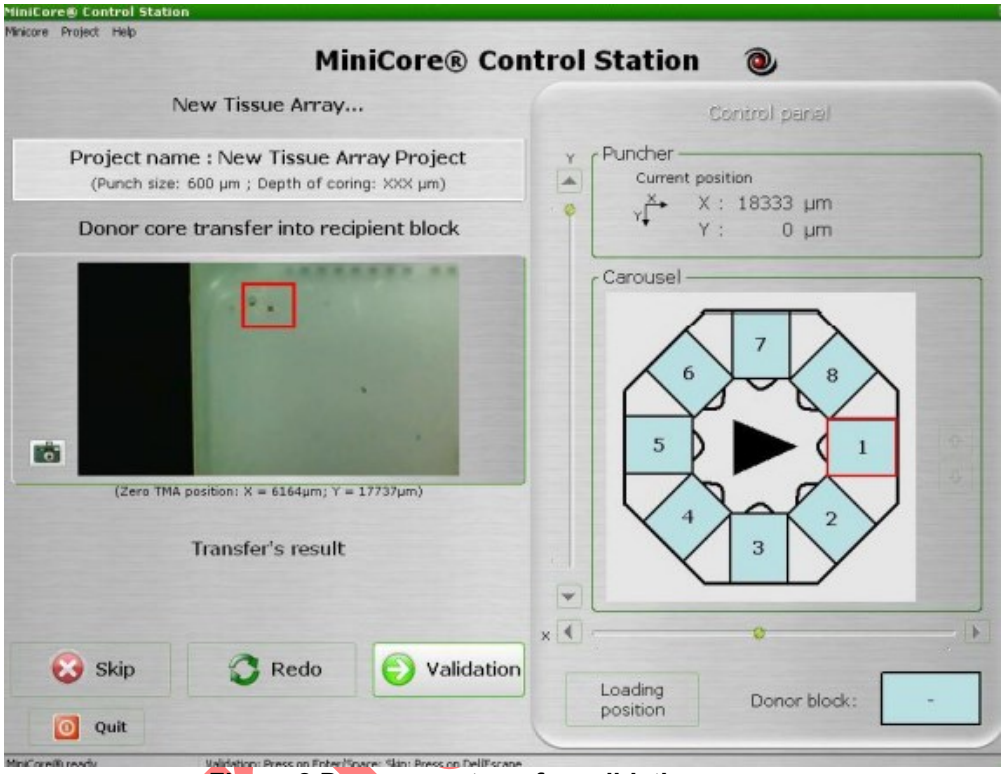


Figure 8 Donor core transfer validation

- End of tissue array - When reaching the last position of a tissue array, screen displays End of Tissue Array. Click on “FINISH” to validate the project. An excel file is generated in the MiniCore project folder. The excel file contains all of the data recorded during the array construction

4.4 Cleaning and maintenance

Clean the machine with a microtome brush to remove any residual paraffin debris that could block the movement of the carousel or punch head. Punches should be cleaned by removing from MiniCore and wiping the individual parts with a suitable solvent.

NEVER immerse punches or other parts in xylene or any other solvent.

NEVER remove the case of the MiniCore. There are no user serviceable parts inside.

5.0 Personal protection –
A Howie coat must be worn at all times.

6.0 Spillages –
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –
All staff should undergo training in this technique before performing the procedure.

8.0 Related documents –
SOPs: SASoM-EQUIP-109-Minicore Tissue microarrayer.

9.0 Approval and sign off –

Author:

Name: In Hwa Um

Position: Post Doctorate

Signature:  Date: 03/11/2021

Management Approval:

Name: Peter Mullen

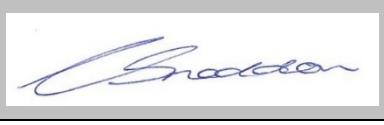
Position: SOP Administrator

Signature:  Date: 03/11/2021

QA release by:

Name: Claire Sneddon

Position: QA Manager

Signature:  Date: 04/11/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE