APR 04, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.4r3l27rrxg1y/v1

Protocol Citation: Christine Camacho, Hadeesha Piyadasa, Benjamin Oberlton, Alex Kong, Cameron Sowers, Sricharan Reddy Varra, Albert Tsai 2023. Tile/SED/Array Interface (TSAI) for Multiplexed Ion Beam Imaging (MIBI) run setup. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l27rrxg1y/v1

Protocol status: Working
We use this protocol and it's working

Created: Apr 03, 2023

Last Modified: Apr 04, 2023

PROTOCOL integer ID:
79943

Tile/SED/Array Interface (TSAI) for Multiplexed Ion Beam Imaging (MIBI) run setup

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ABSTRACT

The Multiplexed Ion Beam Imager (MIBI) is a next-generation mass spectrometry-based microscope which generates 40+ plex images of protein expression in histologic tissues, enabling detailed dissection of cellular phenotypes and histoarchitectural organization. A key bottleneck in its operation occurs when users select the physical locations on the tissue for imaging. As the scale and complexity of MIBI experiments have increased, the manufacturer-provided interface and third party tools have become increasingly unwieldy for imaging large tissue microarrays and tiled tissue areas. Thus, we have developed a web-based graphical interface layer for users to set imaging locations using intuitive mouse gestures such as drag-and-drop, click-and-drag, and polygon draw. Written according to web standards already built into modern web browsers, it requires no installation of external programs, extensions, or patches. Of interest to the hundreds of current MIBI users, our interface simplifies setup of complex experiments and has drastically reduced our setup time.

Keywords: Multiplexed Ion Beam Imaging, MIBI, MIBIscope, Tile/SED/Array Interface, TSAI

MIBIscope general guidelines

1 Keep the MIBIscope in "Standby" mode when not actively setting up a run. Keeping it in SED mode ablates (burns through) tissue as you navigate, so work quickly and efficiently.

2 Perform run setup (including FOV navigation) in "QC - 300µm" mode.

3 Perform detector calibration before imaging runs, at most once per day.

Load the MIBI slide and and create a template .json file

4 Log into MIBItracker in the internet browser.

5 In the “Slides” tab, accession a new slide and add a new section.

6 In the “Resources” tab, select or create a marker panel.

7 In the “Sections” tab, add the new section to the panel.

8 Log into MIBIcontrol in the internet browser.

9 Load the MIBI slide by clicking “Exchange Sample” and selecting the new slide.

10 Create a template FOV by clicking “Add FOV” and setting the frame dimensions, FOV size, dwell time, imaging mode, and section ID.

11 Export (download) the FOV list to a .json file.

12 Download the optical image to a .png file.

Optical image-stage motor coregistration

13 Open the TSAI web UI in the internet browser.

14 If coregistration has not been previously performed, the optical coregistration menu should automatically open. If it has been performed and is adequate, these steps do not need to be repeated.

15 Open the “Optical Coregistration” menu.

16 Click “Copy automatic registration code to clipboard”.

17 Open MIBIcontrol in the internet browser.

18 Type “Ctrl+Shift+J” to open the browser console, or right click on the page and click “Inspect”, then open the “Console” tab.

19 Paste the code into the console and press “Enter”.

20 Click the link generated in the console. This will load the coregistration into the TSAI web UI and save it as a cookie, so it persists and does not need to be repeated unless there is a change to the instrument hardware.

Tiled SED scan

21 Load the .png and .json files by dragging and dropping them onto the TSAI web UI.

22 Open the “SED Tiler” menu and click on a text box in the top row.

23 Click (± drag) on the optical image to select the top left corner for the SED scan.

24 Press the “D” key or click on a text box in the second row in the “SED Tiler” menu.

25 Click (± drag) on the optical image to select the bottom right corner for the SED scan.

26 In the “SED Tiler” menu click “Copy SED scan and shift correction code to clipboard”.

27 Open MIBIcontrol in the internet browser.

28 Paste the code into the console and press “Enter”.

29 Put the MIBI into SED mode on the “QC - 300µm” setting, move to an area you will *not* be acquiring in your run, and adjust the gain, focus, and stigmation.

30 Type “Shift+T” to start the tiled SED scan.

31 When finished, it should automatically save a new .png file of the tiled SED image. You may add characters to the beginning of the file name but do not modify any other part of the file name.

32 If the tiles in the SED are misaligned, contact Ionpath support to adjust the motor and imaging beam, or you may attempt manual software correction using the other keyboard controls.

33 If the tiled SED is adequate, press “Escape”.

34 Return to the TSAI web UI.

35 Drag and drop the SED .png file onto the TSAI web UI.

36 Click on the “SED” tab and adjust the zoom.

37 To adjust image brightness and contrast and/or drawing options such as line thickness and cursor size, use the “Slide Options” menu above the SED image.

Tissue microarray (TMA)

38 If setting FOVs for a grid of TMA spots, set the pattern of FOVs to be replicated by adjusting the columns and rows in the relevant tile of the “Tiles” column and checking/unchecking the boxes in the “Map”.

39 Click “TMA” to open the TMA options menu. Set the number of rows and columns in the TMA. If necessary, add a naming prefix and edit the starting row and column numbering.

40 On the SED image, click on the four corners of the TMA. Click and drag the circled corners to adjust the positioning of the crosshairs so they best match the TMA spots.

41 Click “Build TMA” from the TMA options menu.

42 Hover over each tile in the “Tiles” column to check its positioning. To adjust, click “Move”. Then click and drag on the slide image or press the keyboard arrow keys.

- Holding the “Shift” key and pressing arrow keys will move a farther distance.
- Holding the “Alt” (Windows) or “Opt” (Mac) key and pressing arrow keys will move a shorter distance.
- When “Move” is selected, pressing “A” will go to the previous tile and pressing “D” will go to the next tile.
- To remove the tile, uncheck the checkbox next to the tile name or click “Delete”.
- To adjust other tile settings, click the “☰” button to expand the settings menu if it is not visible.

Area/polygon tile

43 If setting FOVs to cover a contiguous area of tissue, click “Polygon” in the relevant tile of the “Tiles” column.

44 Click on the SED image to set the vertices/corners of the area to be tiled. Double-click to close the polygon and cover the area with FOVs.

45 Scroll to the bottom of the “Tiles” column and click the “☰” button in the new polygon tile to see the tile map.

46 Toggle individual tiles on or off by clicking on the tile map, or by clicking “Clicker” and clicking on the tiled FOVs in the SED image.

47 To toggle off multiple FOVs, click on “Eraser” and then click and drag on the tiled FOVs in the SED image.

48 To toggle on multiple FOVs, click on “Clicker” and then click and drag on the empty areas in the SED image covered by the tile map. You may need to increase the numbers of rows or columns, insert rows above using the “▲” button, or insert columns to the left using the “◄” button.

49 To adjust tile positioning, click “Move”. Then click and drag on the slide image or press the keyboard arrow keys.

- Holding the “Shift” key and pressing arrow keys will move a farther distance.
- Holding the “Alt” (Windows) or “Opt” (Mac) key and pressing arrow keys will move a shorter distance.

FOV navigation and adjustment

50 If SED tiling is misaligned, you may adjust exact FOV positioning in the MIBIcontrol SED mode with the aid of the below keyboard controls.

51 Open the “FOV Navigation/Adjustment” menu below the SED image.

52 Click “Copy FOV navigation code to clipboard”.

53 Open MIBIcontrol in the internet browser.

54 Put the MIBI into SED mode and adjust the gain, focus, and stigmation.

55 Type “Ctrl+Shift+J” to open the browser console, or right click on the page and click “Inspect”, then open the “Console” tab.

56 Paste the code into the console and press “Enter”.

57 The code will automatically navigate to the first FOV and the exact FOV positioning displayed in the MIBIcontrol SED image.

58 To adjust the SED magnification, press the “B” (50 µm), “N” (100 µm), “M”, (200 µm), “,” (400 µm), “.” (800 µm), or “/” (maximum) keys. The 400 µm, 800 µm, and maximum settings will also draw corners indicating the FOV for smaller magnifications.

59 To move the FOV, press the keyboard arrow keys. Save the position by pressing “W”.
- Holding the “Shift” key and pressing arrow keys will move a farther distance.
- Holding the “Alt” (Windows) or “Opt” (Mac) key and pressing arrow keys will move a shorter distance.
- Note that only R1C1 of any given tile can be moved.

60 To toggle an FOV on or off, press “T”.

61 To change the FOV size, press “2” (200 µm), “4” (400 µm), or “8” (800 µm). The raster dimensions will be scaled proportionately.

62 To save an image of the SED image and overlaid crosshair, press “S”.

To save a draft of the adjustments to a .txt file, press “X”.

63

64 When satisfied, press “D” to go to the next FOV, or “A” to go back to the previous FOV.

65 Repeat steps 59-64 for all FOVs.

66 When finished with all FOVs, press “X” or “Escape”, which will automatically save adjustments to a .txt file and attempt to copy them to the clipboard.

67 Return to the TSAI web UI.

68 Drag and drop the .txt file onto the TSAI web UI or paste the adjustments into the text box in the “FOV Navigation/Adjustment” menu.

69 Click “Adjust” to apply adjustments to the tiles in the “Tiles” column.

JSON file generation and import

70 Check the list of tiles and estimated run time under “Output” below the “Tiles” column.

71 To create a JSON file in the same order as the FOVs in the “Tiles” column, click “Create JSON file (sequential order)”.

72 To create a JSON file in random order, click one of the “Create JSON file (random order” buttons.
- “Different tiles mixed together” randomizes in a way that randomly jumps between different tiles.
- “Keeping tiles together” randomizes the order of tiles and within each tile, but only jumps to a new tile when the prior one is complete.

73 Save the resulting .json file to an appropriate location on the computer.

74 Return to MIBIcontrol.

75 Click “Import FOVs” and select the generated .json file.

Focusing and run start

76 For final focusing, move to an area that you will *not* be acquiring in your run, in order to avoid ablating areas that you will be acquiring.

77 Focus in your acquisition mode.

77.1 Set the imaging mode to that you will be using for acquisition (e.g., Coarse, Fine, Superfine).

77.2 Set lens 2 such that the center of the SED is in sharp focus, and the top and the bottom are equally blurred.

77.3 Set the stigmation angle and magnitude to correct residual blurring.

78 Check the current and adjust lens 1 as necessary.

78.1 In the "Target Selection" drop-down menu, select "Faraday Cup".

78.2 Set the mode to "Spot".

78.3 Set the lens 1 voltage such that current falls within the range indicated in the MIBIscope Quick Start Guide, e.g. 9.5 ± 0.2 nA for Coarse. Increasing lens 1 decreases the current. Decreasing lens 1 increases the current.

79 Click “Start run”.