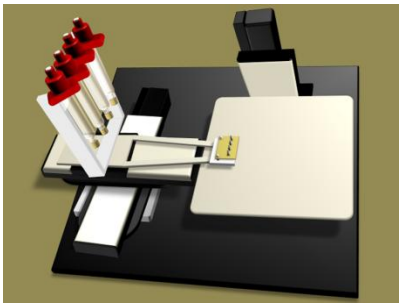
[DRAFT] NOT FOR DISTRIBUTION



TissueWorks®
Nexus

Software

User Manual

[DRAFT]

Brigham and Women's Hospital
Harvard Medical School
Tissue Imaging / Engineering Laboratory

Warnings and Important Check List

- Do not touch or apply force to the printer while the robot stage is moving.
- Before starting the printing sequence, make sure the operating condition of air pressure tank, valve, dispenser, tubing, and valve controller. Also check the all appropriate fluid lines and tubings in correct order.
- Before starting the software, make sure to turn on the power of robot controller (Newmark System). The robotic stages will not work properly if the power supply is off.
- Do not turn off the robot controller while the stage is in motion. Disruption of power during operation can result in damage to both the controller unit and the robotic components.
- Make sure to clean the dispensers before and after each use. Clogs are the primary cause for premature failure of the dispensing unit.

About the Icons

Warning and Attention

Important notes

Useful information

Disclaimer

Credits

Dr. Jong-Hwan Lee wrote the TissueWorks-Nexus, and Vivian K. Lee and Wonhye Lee composed the TissueWorks-Command Center. We acknowledge the efforts from Karl Edminster in the hardware design. Contributions from Philip Keegan, Jason Pinckney, Jason Debasitis, Francis Doyle, Samuel Polio, and Dr. Yeong-Bae Lee during the beta testing and upgrading the system are gratefully acknowledged.

Contents

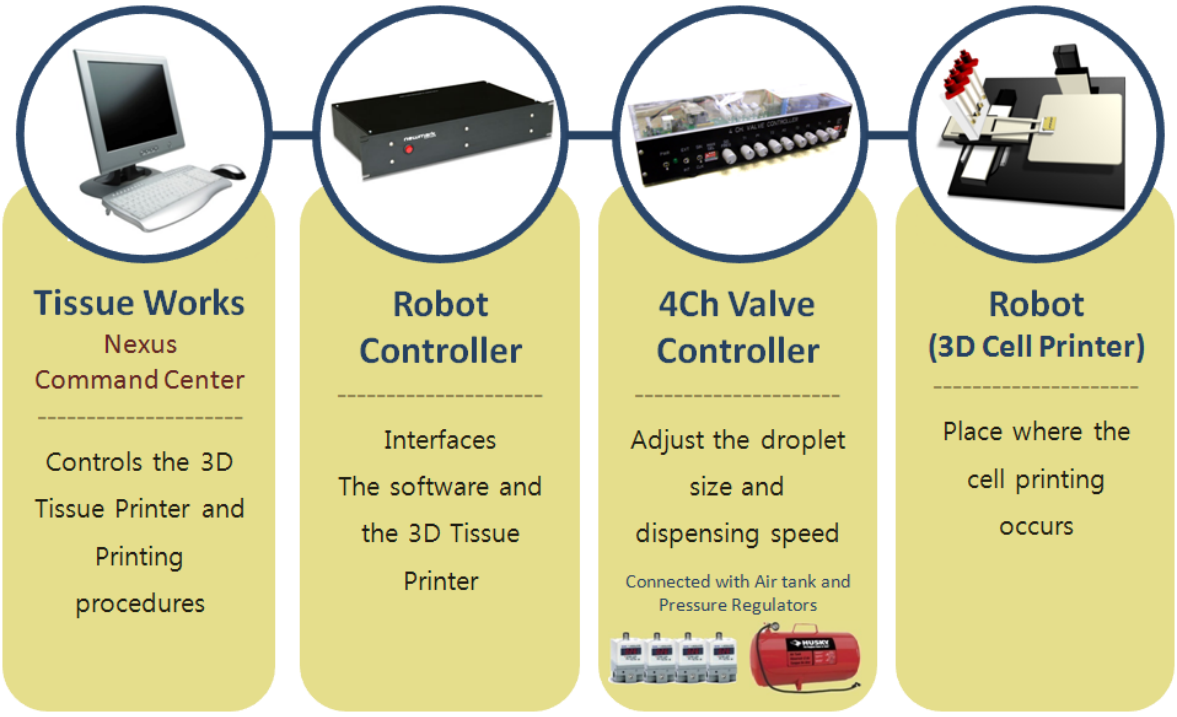
Introduction

The 'TissueWorks–Nexus' (TW-Nexus) is a MATLAB-based software that transforms custom-made 3D patterns into script files for the 3D Bio-Printer (3D BP). The resulting script files can be executed using either the 'TissueWorks–Command Center' (TW-CC) or DMC Terminal and the execution will be realized as the printing operation of 3D BP.

The 'TW-CC' is stand-alone software that supports various printing options as well as the calibration of the 3D BP.

The 'TW-Nexus' was developed based on the MATLAB v7.0 (Mathworks Inc., http://www.mathworks.com). In order to fully utilize the TW-Nexus, either version 7.0 or version 7.1 of MATLAB is required. Versions up to release 7.7.0.471 (R2008b) of MATLAB, TW-Nexus were tested, but some functions (e.g. zoom-in/out images) may not be supported in older versions. Windows XP and VISTA are supported. Computers with at least a Pentium IV (>2 GHz) processor and 1024 MB RAM are recommended.

Components of 3D Bio-Printer (3D BP)



Installation of TissueWorks–Nexus (TW-Nexus)

1. Double-click the 'TissueWorks_Nexus.zip' file in the installation CD.

2. Assign the target directory to unzip TW-Nexus.

3. The directory of 'TissueWorks_Nexus' will be generated under the target directory and the source codes will be saved inside the directory.

Please remember the full path of the 'TissueWorks_Nexus' directory.

Changing the MATLAB startup directory using the shortcut for the MATLAB program

To change the startup directory on Windows platforms using the shortcut,

1. Right-click the shortcut icon for MATLAB and select **Properties** from the context menu.

   The Properties dialog box for MATLAB opens to the **Shortcut** pane.

2. The **Target** field contains the full path to start MATLAB.

   By default, the startup directory is My Documents\MATLAB or Documents\MATLAB on Windows Vista platforms; for more information, see Startup Directory (Folder) on Windows Platforms.

   In the **Start in** field, specify the full path to the directory in which you want MATLAB to start, and click **OK**.

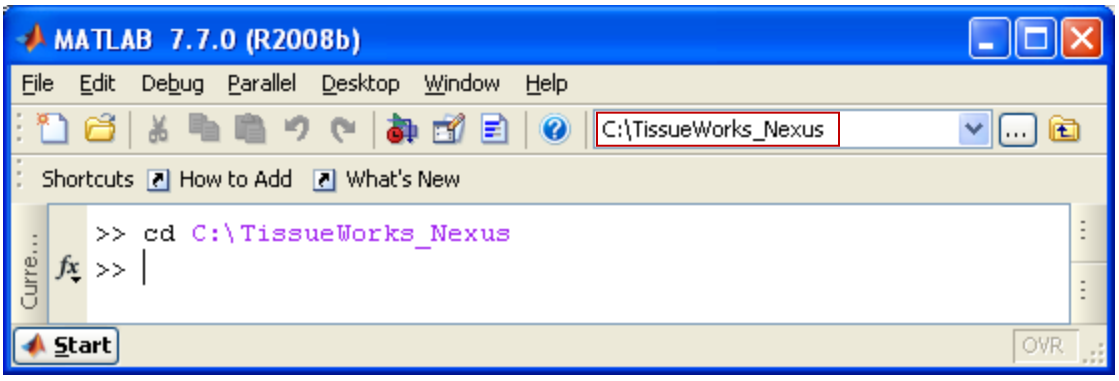
The next time you start MATLAB using that shortcut icon, the current directory will be the one you specified in step 2.

Ref. http://www.mathworks.com/access/helpdesk/help/techdoc/matlab_env/f8-10506.html

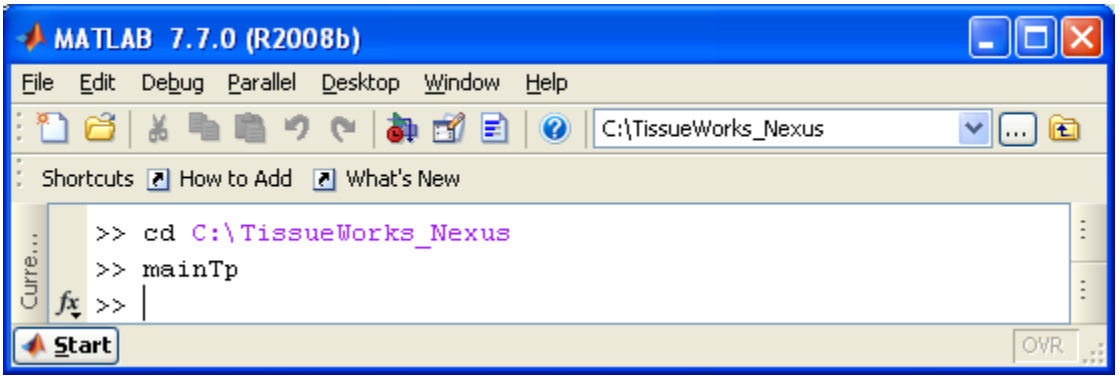
Start and End of TW-Nexus

1. Open MATLAB.

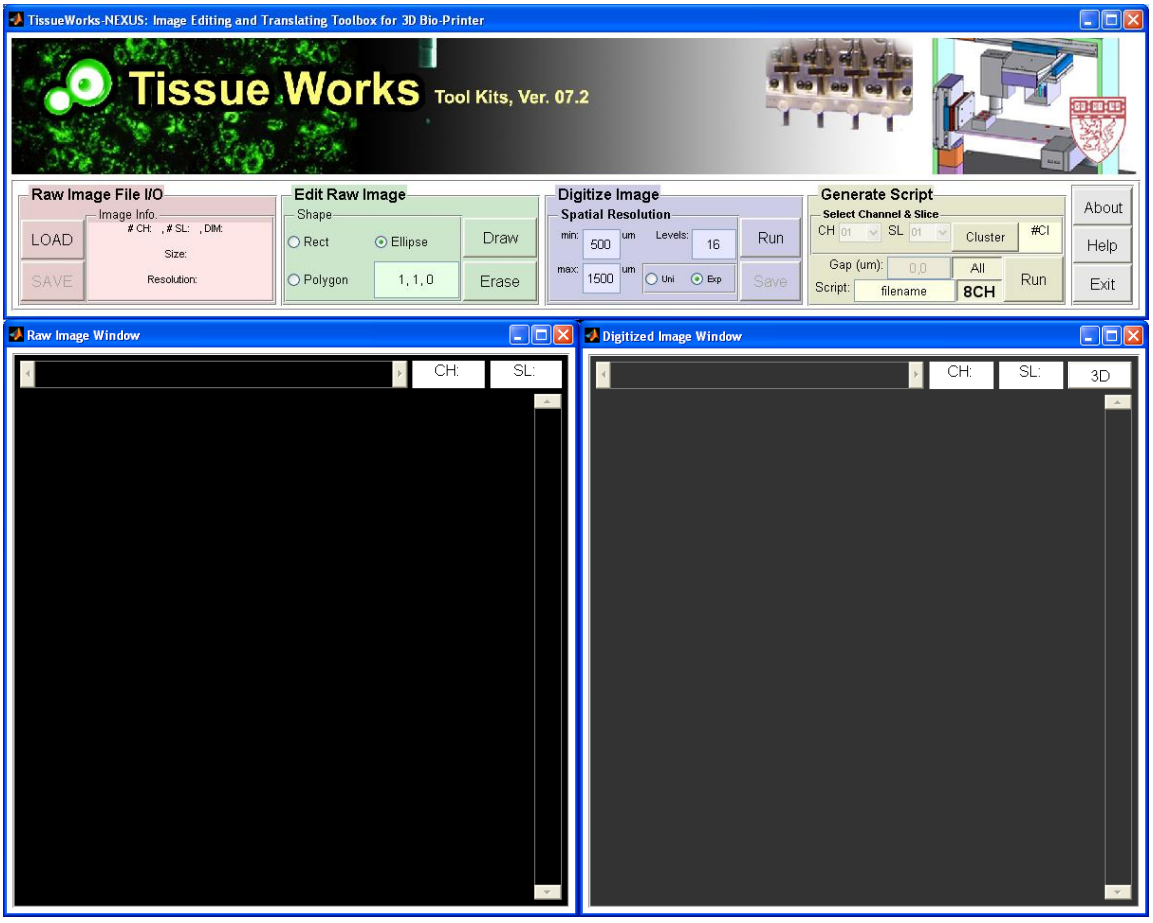
2. Change the current directory to the full directory of 'TW-Nexus'.



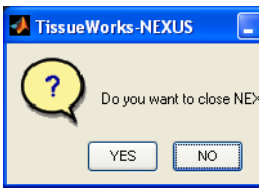
3. To start TW-Nexus, type ‘mainTp’ and press enter.



4. The three windows of TW-Nexus will be displayed as follows:



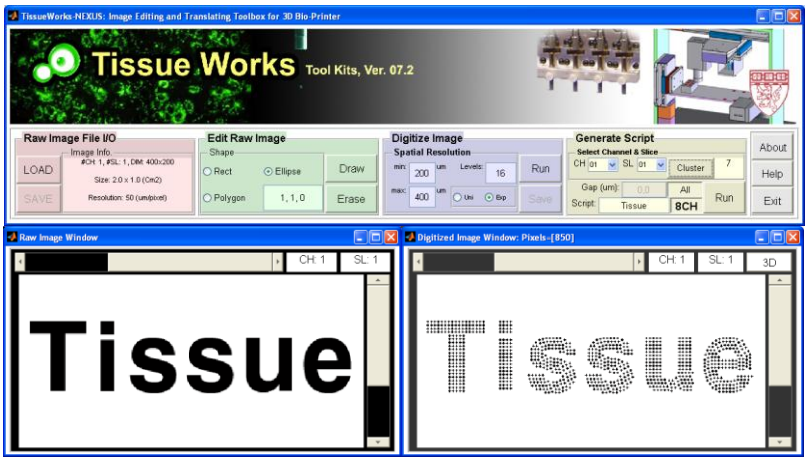
5. To close the TW-Nexus program, push the Exit button on the right side.



6. Push the YES button to confirm your choice.

Components of TW-Nexus

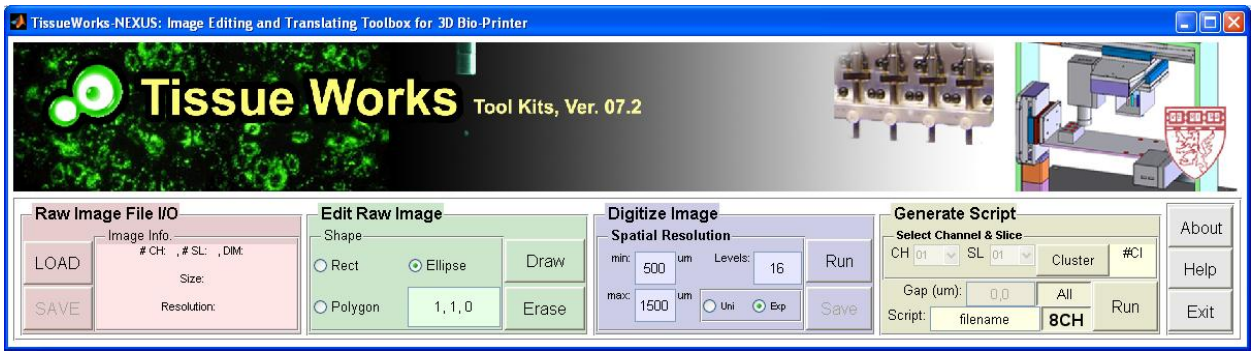
The TW-Nexus program consists of three windows. In the ‘Control Window’, the user can execute the implemented functions such as loading of raw bitmap image files, editing/digitizing of raw images, and generating scripts. In the ‘Raw Image Window’, the original raw image files of designed patterns are displayed. In the ‘Digitized Image Window’, the processed digitized images are displayed.



Control Window	
Raw Image Window	Digitized Image Window

■ Control Window

In the ‘Control Window’, the user can operate the implemented functions such as loading, editing, digitizing of raw bitmap images, and generating script files for 3D BP.



The Control Window consists of the following four parts:

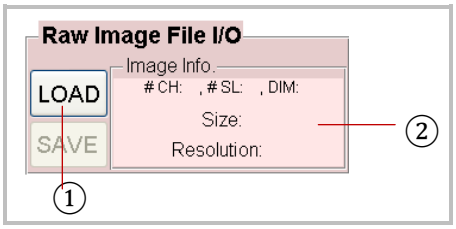
Raw Image File I/O: Load bitmap files and display the raw image patterns along with the information about the image(s) (e.g. number of channels/layers, size, and resolution).

Edit Raw Image: Add or Delete pre-defined patterns in the raw image (e.g. rectangular, elliptical, and polygonal shapes).

Digitize Image: Digitize the raw image patterns according to the desired options for digitization (e.g. minimum and maximum distances between droplets, number of levels, and a means for digitization)

Generate Scripts: Generate two script files with extension of .crd and .sen corresponding to the digitized image patterns. The resulting script files can be executed using 'TW-Command Center' (.crd files) or 'DMC Terminal' (.sen files) in order to automatically control 3D BP for printing of the designed 3D patterns.

□ Raw Image File I/O



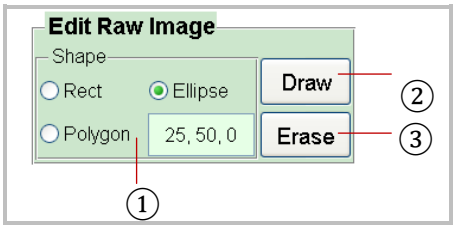
① Press the LOAD button to load image files. Select bitmap (BMP) raw image files of designed patterns

② Text box containing information about the image files. After loading the raw image files, the image's information is displayed, such as the number of channel, number of layer per channel, the X-Y dimensions, size, and resolution of each image.

Each file name of the bitmap raw image must include both the channel number and the layer/slice number as a postfix (i.e. 'filename_c#s#.bmp'). For example, a file named 'filename_c1s1.bmp' should be used for the bitmap image coresponding to the 1st channel and the 1st slice.

To load multiple bitmap files at the same time, the file names must be same except for the postfix (c#s#).

□ Edit Raw Image



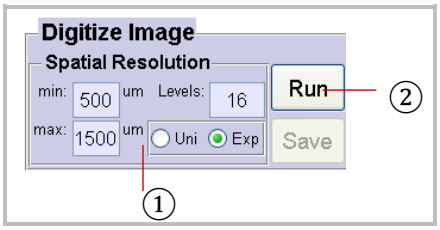
① Select a shape to be added. One of three shapes (rectangular, ellipse, & polygon) can be selected. The three numbers in the text box below 'Ellipse' denote the size (in millimeters) in the horizontal and vertical directions, and the angle of axis (ellipse only) and can be edited by the user (applicable for both rectangular and elliptical shapes).

② Push the DRAW button to draw a new shape. Add the selected shape onto the

loaded raw image pattern.

③ Push the ERASE button to erase the area within the pattern. Delete the area within the pattern using polygon-shaped area (patterns within the polygon-shaped area will be deleted).

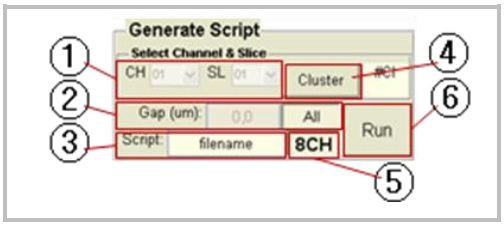
□ Digitize Image



① This section allows for the adjustment of the resolution of the digitized image. The user is able to adjust the minimum and maximum distance (mm) between printed points, the number of levels between the min and max distances, and the method of dividing the levels (uniform or exponential).

② Push the RUN button to begin digitization. The digitization is executed based on the adjusted parameters.

□ Generate Script



① This panel is designed to choose the channel and layer number to be translated into a script file. Pushing the RUN button generates script files for all loaded channels and layers.

② GAP (μm) adjusts the gap between the neighboring dispensers. The two numbers represent the distance of the adjacent dispensers in the horizontal and vertical (along the dispensers) axes, respectively. Note that this option is disabled and no longer needed since this function is implemented in TW-Command Center.

③ Type the script filename. The resulting script will be saved as the following filename:

filename_8CHc#s# e.g. filename_8CHc1s1

④ Push the CLUSTER button to begin clustering. Clustering ensures that the resulting script can be used to print the fastest path for the given pattern. The resulting number of clusters of the chosen image pattern will be shown in the text box. Check the resulting number of clusters.

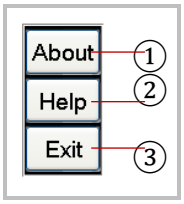
⑤ Toggle button to select 4 channel (4CH) or 8CH printer

⑥ Push the RUN button to generate script files for each loaded channel and layer.

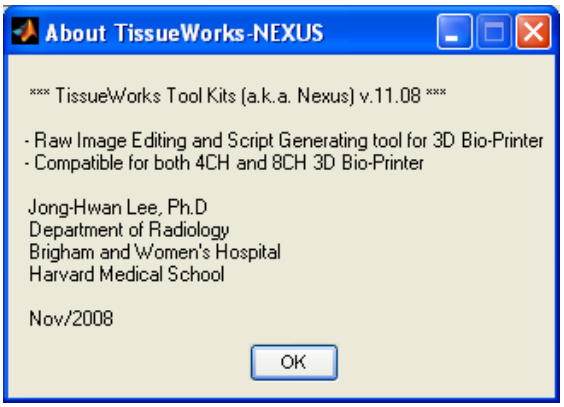
By pushing the RUN button, the digitized image patterns are automatically clustered

and the coordinates are subsequently generated into script files (.crd and .sen files).

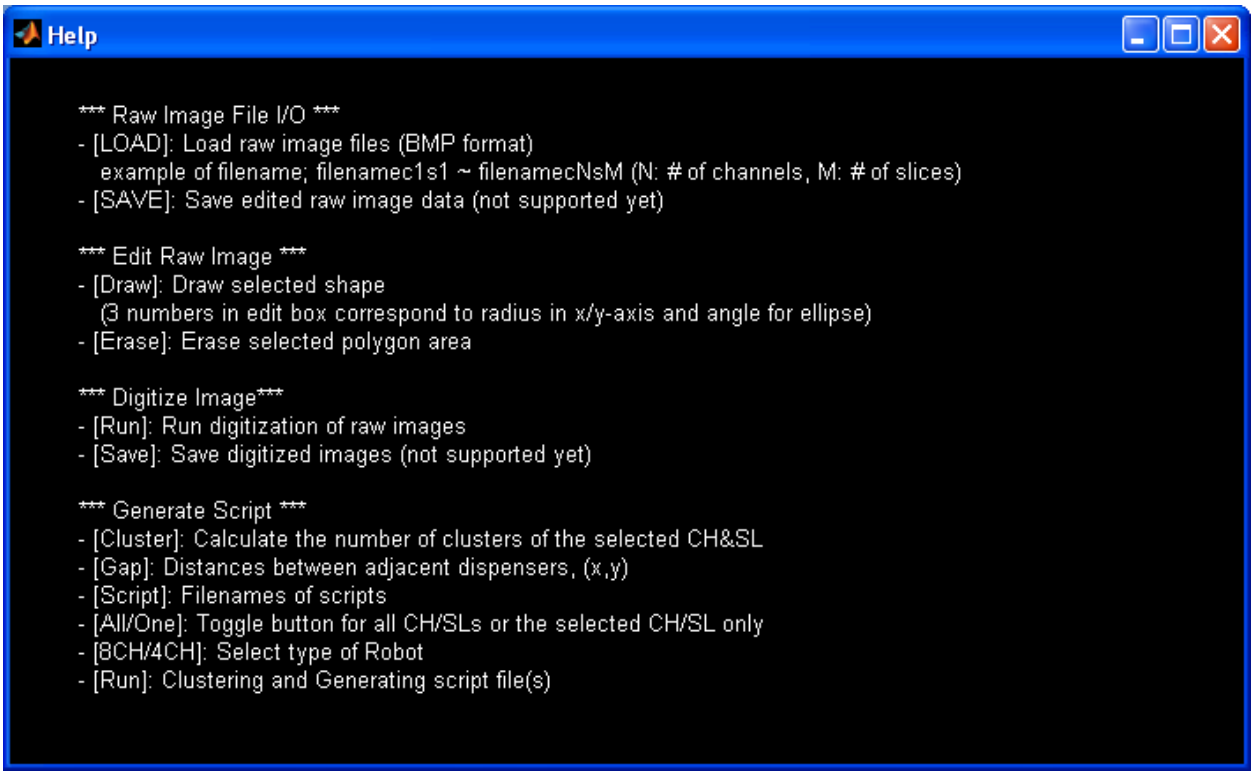
□ About/Help/Exit



①Push About to see a short description of the TW-Nexus.



②Push Help to see a brief guide for each of the panels.



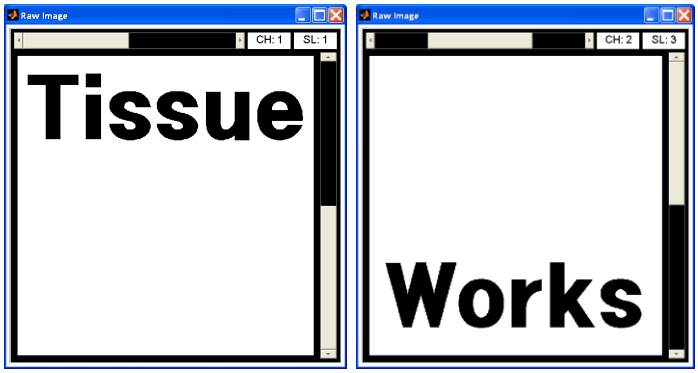
③Push Exit to terminate the TW-Nexus program.

Raw Image Window

In the ‘Raw Image Window’, the loaded original raw image patterns are displayed. The image pattern corresponding to a different channel and layer can be viewed using the horizontal and vertical scroll bars, respectively.

The pattern can be zoomed using the wheel of a computer mouse (forward: zoom-out; backward: zoom-in) and can be shifted using a right button of mouse (click-and-move; toggle: on/off). Note that the zoom function may not be work in MATLAB software other than v7.0 and v7.1.

[Examples of the loaded raw image patterns]



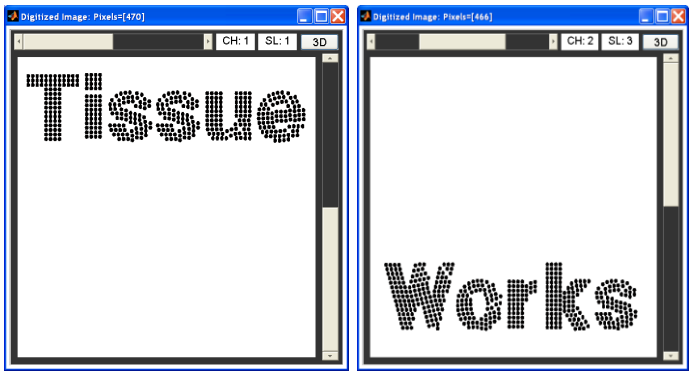
(Channel 1 & Layer 1) (Channel 2 & Layer 3)

Digitized Image Window

The digitized patterns from the original design patterns are displayed. In the digitized images, each dot represents a droplet to be dispensed. The digitized pattern corresponding to the different channel and layer numbers can also be displayed by using the top and right scroll bars.

The number on the top bar with title denotes the number of dots (droplets) within the selected digitized pattern. Again, the zoom feature of the pattern is available using the wheel of computer mouse (forward: zoom-out; backward: zoom-in) and moving the pattern is available using the right button click (toggle: on/off).

[Examples of the digitized patterns]



(Channel 1 & Layer 1) (Channel 2 & Layer 3)

Procedures

Preparing Bitmap (BMP) Raw Image Files

1. Using a graphics editor (e.g. Adobe Photoshop), draw desired patterns with a gray-scale mode.

2. Assign the spatial resolution of image to less than 600 dpi (dot-per-inch).

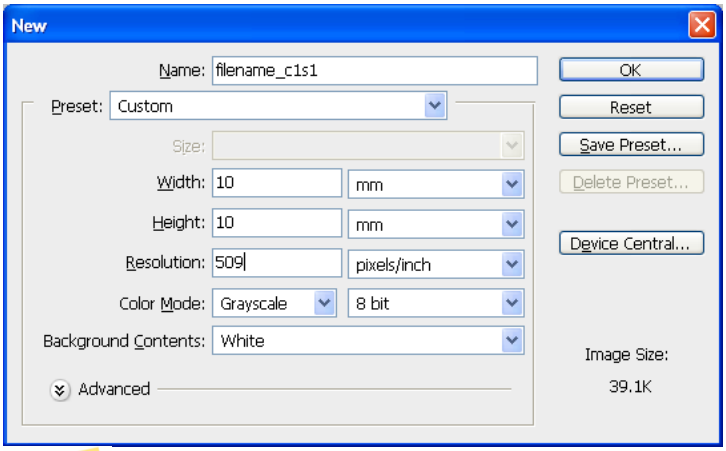
3. After drawing the patterns, save the pattern as a bitmap (BMP) image format file. The filename must contain the channel and layer numbers as a suffix: i.e.

filename_c[channel #]s[layer #].bmp e.g. filename_c1s1.bmp

When BMP raw image file(s) are prepared, the spatial resolution of the image file should be decided by considering both the desired minimum distance between droplets and the minimum movement limited by the 3D BP (50 µm). For example, when the desired minimum distance between droplets is 100 µm, the resolution of an original raw bitmap image must be higher than 254 dpi (=1 dot / 100 µm ÷ 25.4 mm/inch). Also, since the resolution of the 3D BP is lower than 508 dpi (=1 dot / 50 µm ÷ 25.4 mm/inch), a resolution lower than 600 dpi is recommended for the raw bitmap image file(s).

Calculate a minimum resolution into 'Dot per inch (DPI)
For inter-droplet distance of 100 µm,
Number of dots per inch = 1 inch / 100 µm + 1 = 25400 µm / 100 µm + 1 = 255
For inter-droplet distance of 50 µm,
Number of dots per inch = 1 inch / 50 µm + 1 = 25400 µm / 50 µm + 1 = 509
1 inch = 2.54 cm = 25. 4 mm = 25400 µm

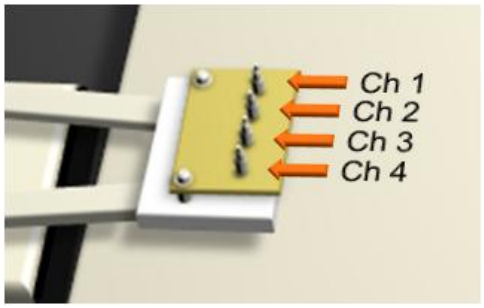
An example of the settings for a raw image using Photoshop (CS3 Extended, version 10.0)



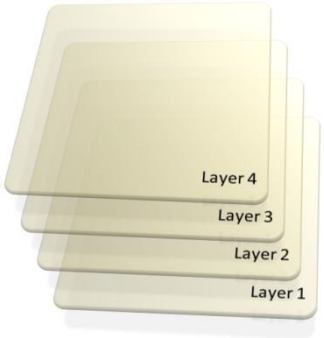
As the resolution of image increases, the computation time will also increase for processes such as the image loading, digitization, and clustering of the digitized image patterns.

The software recognizes only gray-scaled bitmap files (i.e. 8bit or integer value between 0 and 255 per pixel) as raw image patterns. The intensity values of each pixel are translated into the distance between neighboring droplets, and thus the density of the droplets can be represented from the gradient change of raw image patterns. The mapping between the gradient change of pixels and the density of droplets can be calibrated using the parameters available in the 'Digitize image' panel.

Nomenclature to identify the channels and the layer sequence.



(4 channels)



(4 layers)

■ Loading the Prepared Bitmap Files of the Designed Patterns

1. Click the LOAD button in the 'File I/O' panel of the 'Control Window'.

2. Once the window opens, select and open the prepared bitmap image file(s).

3. Once the images are loaded, the image pattern corresponding to channel 1 and layer 1 will be displayed on the ‘Raw Image Window’. Use the horizontal and vertical scroll bars to change the channel and layer number and check the patterns.

The raw image file(s) must be in bitmap format.

For the 3-D patterns consisting of multiple channels, the number of layers prepared for each channel should be same. Although there may be no pattern in a certain layer, a raw image file with the same size and resolution as other image files should be prepared and loaded. For example, if a certain 3-D design consisted with two-channel and two-layer has patterns only in layer 1 of channel 1 (filename_c1s1.bmp) and layer 2 of channel 2 (filename_c2s2.bmp), empty/white patterns (‘filename_c1s2.bmp’ & ‘filenmame_c2s1.bmp’) also need to be prepared and loaded together with ‘filename_c1s1.bmp’ and ‘filename_c2s2.bmp’.

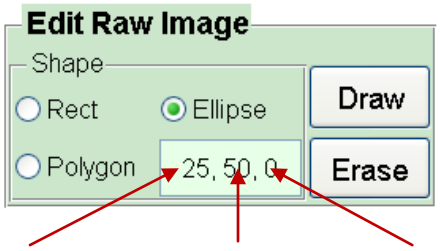
■ Editing the Loaded Images of Designed Patterns

□ Add New Patterns

1. Select the image to be edited by using the horizontal and vertical scroll bars of the ‘Raw Image’ window.

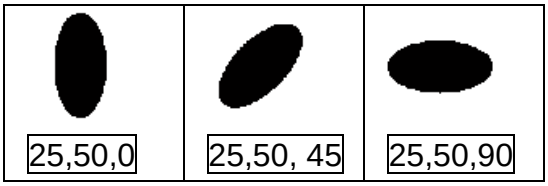
2. Select the desired pattern among the three available ones (rect., ellipse, & polygon) in the ‘Edit Raw Image’ panel of the ‘Control Window’.

3. For the rectangular or elliptical shape, the horizontal and vertical size can be edited by putting the values in the text box. Three numbers in the box correspond to the horizontal, vertical size (mm), and angle (°; elliptical shape only), respectively.



Horizontal size, vertical size, angle

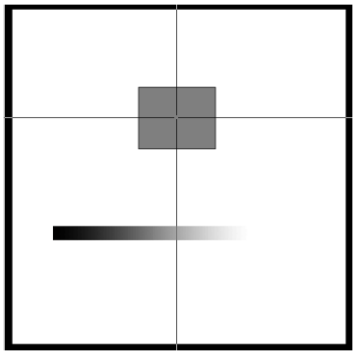
As an example, for the elliptical shape:



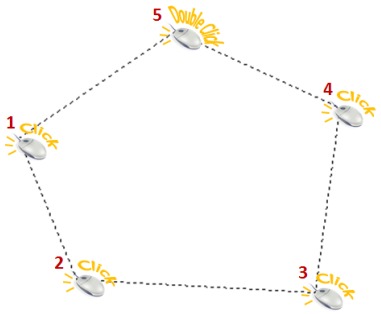
4. Once the shape of the desired pattern is selected, push the Draw button.

5. For the rectangular or elliptical shape, move the cursor onto the 'Raw Image Window' and click to the center of the desired location of the pattern. For the polygon shape, sequentially click the left-button of the mouse on the vertices of the desired polygon and double-click in the final vertex.

As an example, for the rectangular shape:



As an example, for the polygon shape:

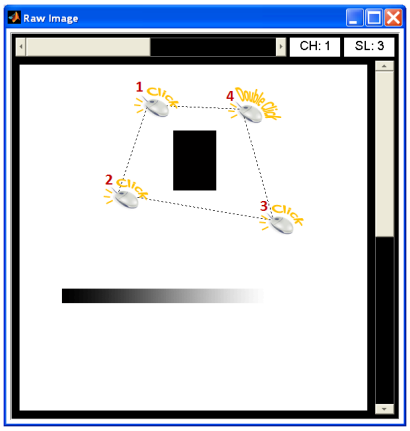


□ Delete Patterns

1. Select the image to be edited in the ‘Raw Image’ window.

2. Push the Erase button in the ‘Edit Raw Image’ panel of the ‘Control Window’.

3. In the ‘Raw Image’ window, select the polygon-shaped area, including the patterns to delete (i.e. click the left-button of the mouse and double-click for finalization).



Digitization of the Designed Patterns

1. After loading (& editing, if necessary) the raw designed patterns, they need to be digitized before the generation of the script files to operate the 3D BP. In the 'Digitize Image' panel, there are several parameters to be adjusted for the digitization process.

Min. / Max.: The minimum and maximum distances between the adjacent droplets to be printed. The gray-scaled gradation of the intensities of the raw image patterns are digitized (down-sampled) based on these values. In detail, the dark areas (i.e. low intensity) are translated into dense droplets at the minimum distance, whereas the areas of non-white, bright area (i.e. high intensities) are translated into sparse droplets at the maximum distance.

Levels: The number of steps between the minimum and maximum distances of neighboring droplets. For example, when the 'Levels' is 16, the gray scale intensities of the raw image patterns are digitized into 16 levels. Then, each intensity level is assigned to one of 16 distances between 'min' and 'max' distances. Note: the method for dividing the levels between min and max distances is based on the 'option for the digitization' described below.

Option for the digitization (Uni. / Exp.): There are two options for the digitization of gray-scale image intensities (i.e. distances of neighboring droplets). The option 'Uni' divides the min. and max. intensities/distances into the number of 'Levels' uniformly, whereas, the option 'Exp' divides them exponentially.

2. After confirming the options, push Run in the 'Digitize Image' panel.


The processing time of the digitization depends on the performance of the computer hardware and the raw image files (e.g. number of files (?), resolution, and size).


Please wait until the digitization is complete before continuing processing.

3. After digitization, the digitized patterns will be displayed in the 'Digitized Image' window with same number of channels and layers as the original raw images. In order to see the digitized patterns on the other channel/layer, use the horizontal and vertical scroll bars.

Generating Scripts

Once the digitization process is completed, the script file of each digitized pattern can be generated by following the procedure described below.

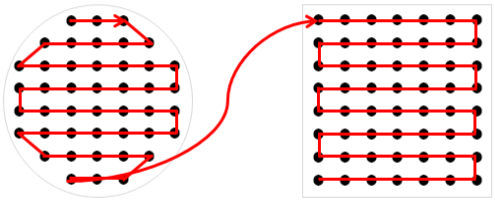
1. Select the channel (CH) and layer (SL) numbers in the 'Generate Script' panel of the 'Control Window'. The pattern in the selected image will be displayed in both the 'Raw Image Window' and 'Digitized Image Window'. The digitized pattern(s) will be used in generating script files for the 3D BP.

2. Run clustering by pushing the Cluster button. Once the clustering is finished, the resulting number of clusters will be displayed in the text box.

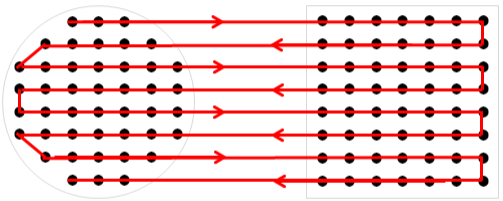
3. Type a filename for the resulting script file in the text box at the bottom.

4. Press the Run button to generate the scripts. Two different script files (file extension: .sen & .crd) with the typed filename will be generated inside the folder 'scpts' in the 'TissueWorks_Nexus' directory. Please make sure to correctly select the type of 3D BP (i.e. either 4CH or 8CH) using the toggle button.

The utility of the clustering process is illustrated in the following figure. With clustering, the two shapes (circle & rectangle) are regarded as separate patterns, and thus the 3D BP is prints two clustered patterns sequentially (i.e. firstly a circle, then a rectangle pattern). However, without the clustering, the circle and rectangle shapes are regarded as one pattern, and the 3D BP moves line-by-line across the two shapes. As we can see in the illustration, by adopting the clustering scheme, the total distance of movement of the 3D BP will be reduced and printing time will be saved.



With Clustering (two clusters)



Without Clustering (one cluster)

The first and second values in the 'Gap' denote the displacement (μm) between adjacent dispensers in horizontal and vertical axis, respectively. The calibration of gap is now implemented in TW-CC.

For the 8-CH 3D BP, the resulting filename of the script will be assigned as follows:

filename _8CHc channel #s layer# e.g. filename_8CHc1s1

Using the assigned filename, two types of script files will be generated: (1) a file with extension '.sen' to be executed using the built-in command, 'Download to Controller' in the DMC Terminal and (2) a file with extension '.crd' to be executed using the 'TW-CC'. It is recommend that the 'TW-CC' be used for executing the script file due to the limitations of the DMC Terminal (e.g. max. 80 lines), even though the results after using two programs are same.

 Description of the format of script files (sen & crd) is exemplified in the following table:

['.sen' file format]

Content	Description
#runTP	Label of the script
sx=31496.063;sy=sx/4;sv=4;SP sx*sv,sy*sv;	Movement speed of the 3D BP
PA sx*10.00,sy*10.00;BGXY;AMXY;	Move the BP to the origin of the printed pattern
nm=399;DA*[0];DM x[nm];DM y[nm];	Allocate the buffer for x-/y-locations

x[0]=sx*11.28;y[0]=sy*11.68;x[1]=sx*11.28;y[1]=sy*11.98;x[2]=sx*11.28;
y[2]=sy*12.28;x[3]=sx*11.28;y[3]=sy*12.57;
…
	Define the position (x, y) of droplets to be printed (unit: number of thread of x-/y-axis motor of 3D BP)

n=0; #loop PA [n],y[n]; BGXY;AMXY; SB1;WT100;CB1; n=n+1 IF n<nm JP#loop EN;	Loop of movement of 3D BP for the all of the defined locations of droplets

['.crd' file format]

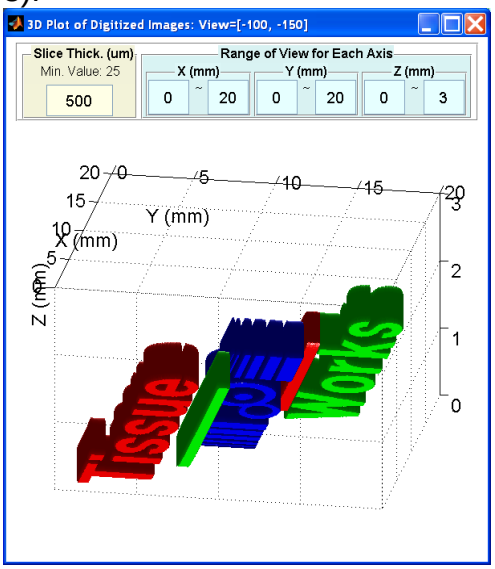
Content	Description
C:\DMCRobot\TP\design\ex001\c1s1.bmp	Filename of original bitmap raw image
1	Channel number
1	Layer number
20.000000 20.000000	Size of the image (x & y in mm)
10.000000 10.000000	Starting location of the printing (mm)
300 1000 16	Min/Max distances, & Levels during digitization
355118.110325 91929.133881 355118.110325 94291.338606 355118.110325 96653.543331 355118.110325 99015.748056 364566.929225 99015.748056 364566.929225 96653.543331 364566.929225 94291.338606 364566.929225 91929.133881 374015.748125 91929.133881 374015.748125 94291.338606 374015.748125 96653.543331 374015.748125 99015.748056 …	Position (x, y) of each droplet (unit: number of thread of x-/y-axis motor of 3D BP).

3D Rendering of the Designed Patterns

3D rendering is useful when the user wants to virtually represent the designed 3D patterns before the actual printing using 3D BP.

1. Push 3D on the top-right corner of the 'Digitized Image Window'.

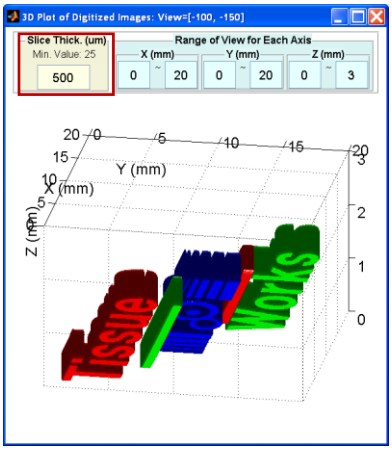
2. After the 3D rendering is finished, the 3D patterns will be displayed in a separate window as shown in the following figure (red: channel 1; green: channel 2; blue: channel 3).



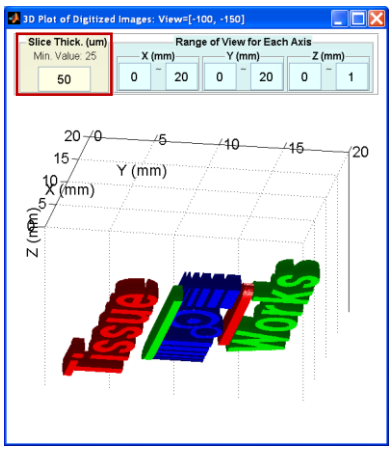
3. The various options for display (i.e. thickness of the pattern, range-of-view, angle-of-view) can be modified by typing the thickness/range and using arrow keys on a computer keyboard.

[Examples of modifying displaying options]

(1) Emulate the thickness of the patterns: type the desired thickness (μm) and press enter.

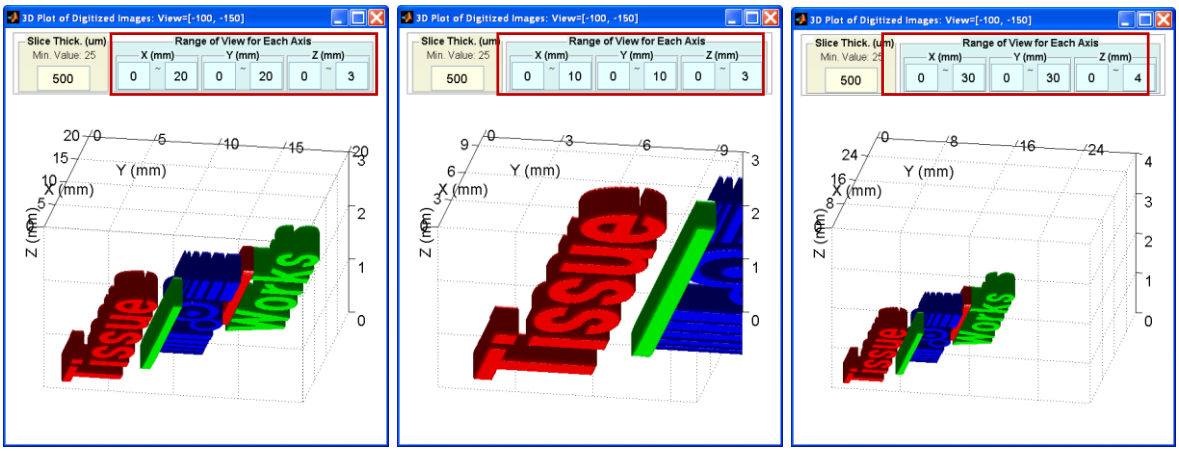


(thickness = 500 μm)



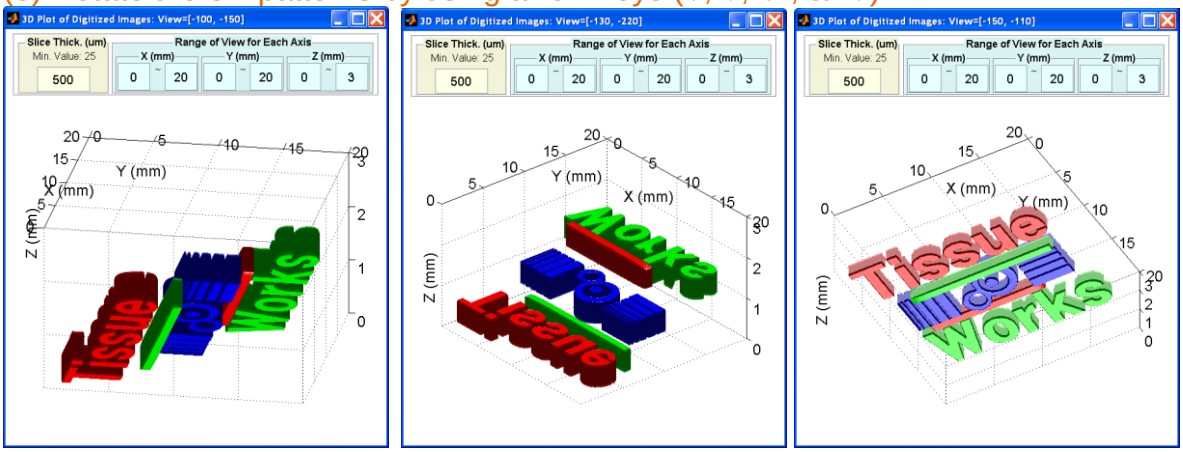
(thickness = 50 μm)

(2) Zoom-in/out by changing the range-of-view: type the desired range-of-view (mm) in each axis.



(Range-of-view by defaults) (zoom-in) (zoom-out)

(3) Rotate the 3D patterns by using arrow keys (↑, ↓, ←, & →)



The 3D rendering process requires large computational load. The computation time depends on the performance of computer hardware and number/resolution/size of the designed image patterns.

Wait until the 3D rendering process is finished before continuing processing.

The overlapped patterns from different channels within the same layer may cause an error during the 3D rendering.

Practice using Example Image Files

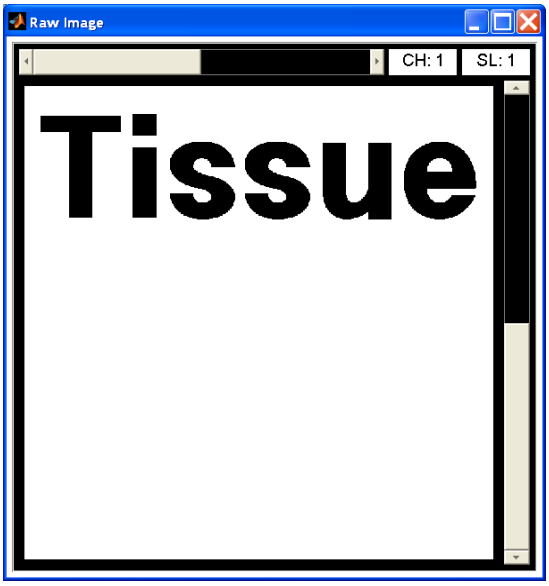
In this practice, the user will go though the crucial functions of the TW-Nexus using the provided example bitmap image files.

1. Start the TW-Nexus program by typing ‘mainTp’ in the console window of MATLAB.

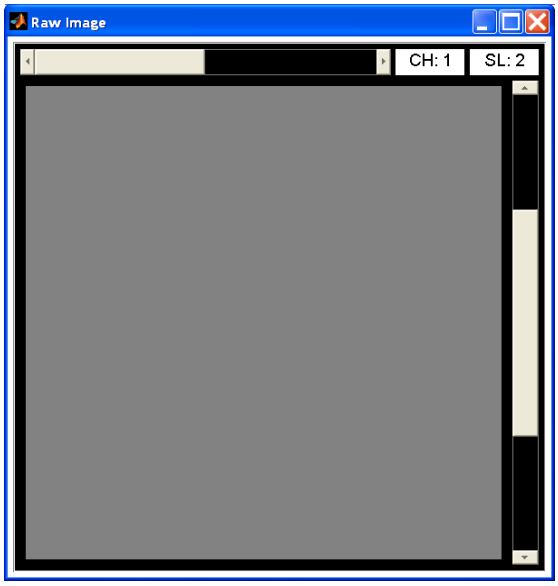
2. Push the LOAD button in the ‘Raw Image File I/O’ panel of the ‘Control Window’.

3. Once the popup window opens, select and open all 9 bitmap image files (c1s1.bmp ~ c3s3.bmp) inside the ‘example’ folder.

4. The raw image pattern of ‘c1s1.bmp’ will be displayed in the ‘Raw Image Window’. Check the other raw image patterns by moving the horizontal and vertical scroll bars. Note that, 5 out of 9 files contain designed patterns and the other 4 images have an empty pattern.

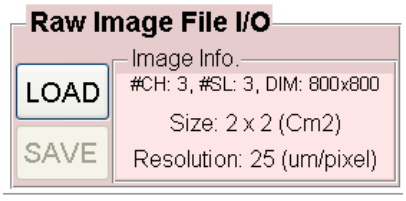


(channel 1 & layer 1)



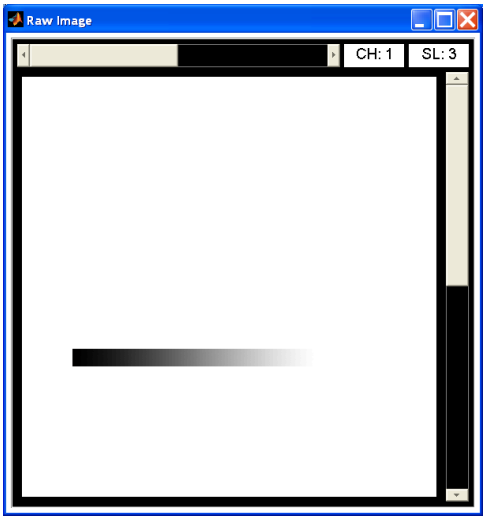
(channel 1 & layer 2)

5. Check the information of the designed image file shown in the ‘Image Info’ subpanel of the ‘Raw Image File I/O’ panel.



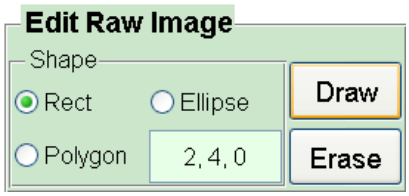
(number of channels=3; number of layers=3; number of pixels within an image=800x800; size of each image=2x2 mm^2; resolution of the image=25 μm/pixel)

6. Select the image of channel 1 and layer 3 in the ‘Raw Image Window’.

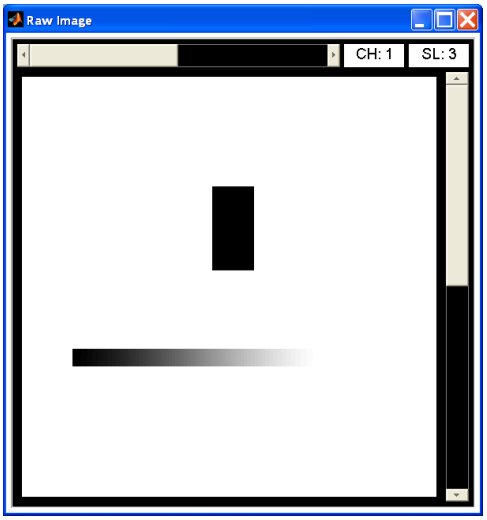


(Image of channel 1 and layer 3)

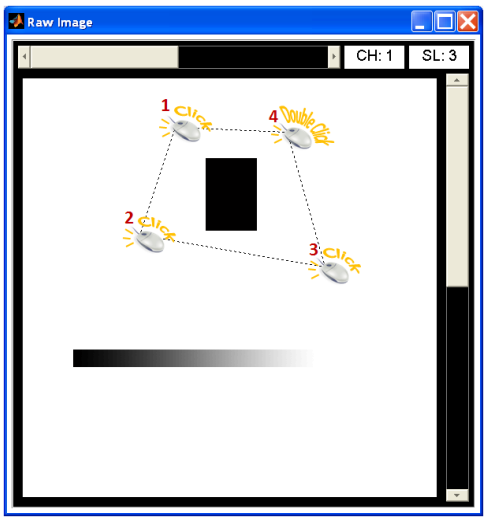
7. In the ‘Edit Raw Image’ panel, select the ‘Rect’ shape and type the size as (2, 4) in the text box.



8. Push Draw and move the mouse cursor on the center of the desired location within the ‘Raw Image Window’. Then left-click with the mouse. A rectangular pattern 2 x 4 mm^2 will be added in the image.

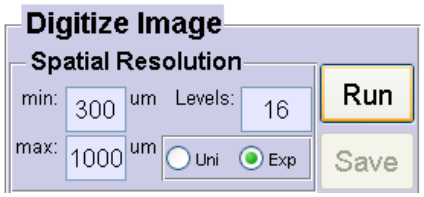


9. Push the Erase button and select a polygon-shaped area including the rectangular pattern that you want to erase.

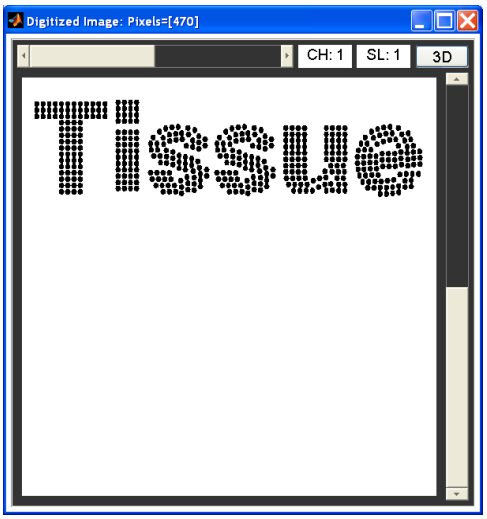


(Left-click the mouse on the vertices of a polygon-shaped area and double-click the button on the last vertex)

10. In the 'Digitize Image' panel, set the 'min' distance to 300 (μm), 'max' distance to 1000 (μm), 'Levels' to 16, and digitization option to 'Exp'. Then, press Run.



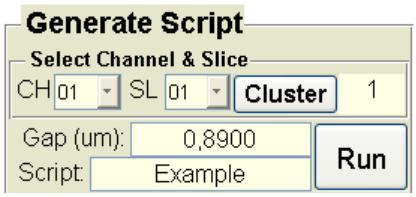
11. Once the digitization process is finished, the digitized pattern of channel 1/layer 1 will be displayed in the Digitized Image Window'. Check the digitized patterns by moving the horizontal and vertical scroll bars.



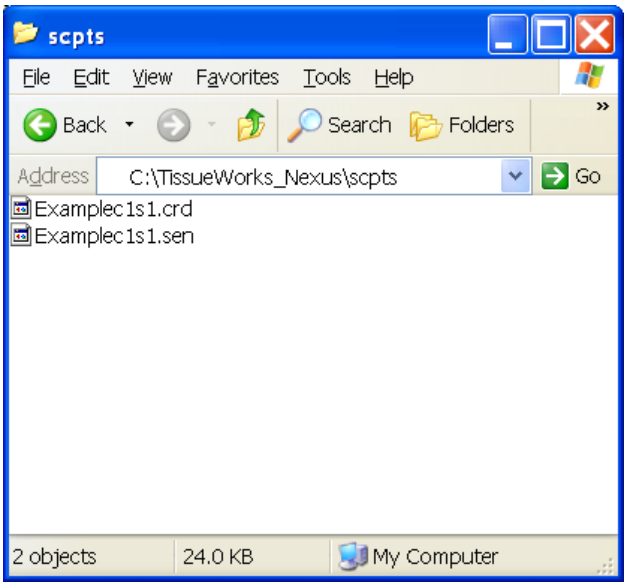
(Digitized pattern of channel 1/layer 1)

12. In the ‘Generate Script’ panel, select channel 1 and layer 1, and then press Cluster.

13. Confirm that the resulting number of clusters is 1. Change the script filename to ‘Example’ and change the type of 3D BP to ‘8CH’. Press Run.



14. Find the two resulting script files (‘Examplec1s1.crd’ & ‘Examplec1s1.sen’) in the ‘scpts’ folder in the ‘TissueWorks_Nexus’ folder.



15. Repeat the steps from 12 and 13 for the remaining four digitized image patterns.

Please select the toggle button to ‘ALL’, if you want to generate the script files corresponding to the all channels and layers by pushing the ‘RUN’ button once.

Note that no script file will be generated if the image contains no pattern.

16. Confirm that the 10 newly generated files (i.e. 5 crd & 5 sen files) are saved in the ‘scpts’ folder.