Surveyor Well Plate Scanning

Image Formats

There are options for different output of scanned images in the Surveyor software.

Surveyor by default generates scanned images in the Surveyor Workspace (.sws) format. This is a tiled image format comprising of:

- a folder on disk with the individual high resolution image tiles of the scan,
- low resolution thumbnail images, which are either individual.thb files or single .bin file,
- some metadata files e.g. tile position information,
- a single .sws file.

The software also allows export of scanned images into other mainstream formats such as BMP, Tiff, BigTiff, and these are saved as single file "mosaic images".

Single Field of View Acquisition

Some users are interested in acquiring a single camera image at each well location. Surveyor scan patterns can be configured to position the fields at spacing suitable for a particular well plate arrangement on the stage, as shown in Figure 1.

Scan: Sample Pattern Using JPEG Compression			
★ × Ø			
	`ቴ 🗖 🔍 🖑 👍 🖫 🖻		🗙 🌠 aA 🗟 🚍 🖼
8			
	Core Dettore Decention		
	Scan Pattern Fold of View Name: Sample Pattern Type: Well Plate Definition 0rigin: Number of fields in X: 6 Origin: Top-Left Step size X: 19030.00 1536 Well Plate If 353 Well Plate Image: Scanning: Image: Scanning: Image: Scanning: Image: Total fields: 24 Total area (sq. mm): 19.1367		
Screen Mag 3.69 X XY = [11.900, 0.000]	mm Tiles = 0 (0.0 MB) Pattern Fi	ields = 24 Turboscan (C	Cruise): 24.88 sec //

Figure 1 - Well plate scan pattern setup

When the final workspace is saved, the resulting output contains the individual tiled images at each well, and the text file XYZPositions.txt contains the XYZ position information for correlation purposes, as shown in Figure 2.

📓 C:\OlAutosave\single_fov\XYZPositions.txt - Notepad++												
<u>File Edit Search View Encoding Language Settings Macro Run Plugins Window ?</u>												
D 占	C 🖶 🗄 🖻 💫 🖧 👘 👘 ⊃ C # ½ ♀ ♀ 🖫 🔤 1 📰 🖉 🔊 ● 🗉 🕑 🔤											
🗎 XYZPositions.txt 🗵												
1	No,	х,	Υ,	Ζ,	R,	с,	Ch,	Zs,	wMicrons,	hMicrons,	wPixels,	, hPixels
2	1,	8511.8796,	7991.7846,	-495.30,	ο,	ο,	ο,	ο,	893.9106,	891.9950,	1600,	1600
3	2,	27541.8085,	8042.6564,	-495.25,	ο,	1,	ο,	ο,	893.9106,	891.9950,	1600,	1600
4	з,	46571.7708,	8083.5282,	-495.20,	ο,	2,	ο,	ο,	893.9106,	891.9950,	1600,	1600
5	4,	65601.7064,	8132.3999,	-495.25,	ο,	з,	ο,	ο,	893.9106,	891.9950,	1600,	1600
6	5,	8448.4366,	27024.6789,	-495.20,	1,	ο,	ο,	ο,	893.9106,	891.9950,	1600,	1600
7	6,	27478.3922,	27067.5507,	-495.15,	1,	1,	ο,	ο,	893.9106,	891.9950,	1600,	1600
8	7,	46508.3278,	27116.4224,	-495.20,	1,	2,	ο,	ο,	893.9106,	891.9950,	1600,	1600
9	8,	65538.2734,	27162.2942,	-495.10,	1,	з,	ο,	ο,	893.9106,	891.9950,	1600,	1600
10	9,	8385.0003,	46055.5731,	-495.20,	2,	ο,	ο,	ο,	893.9106,	891.9950,	1600,	1600
11	10,	27414.9626,	46096.4449,	-495.20,	2,	1,	ο,	ο,	893.9106,	891.9950,	1600,	1600
12	11,	46444.8915,	46147.3167,	-495.15,	2,	2,	ο,	ο,	893.9106,	891.9950,	1600,	1600
13	12,	65474.8438,	46191.1885,	-495.25,	2,	з,	ο,	ο,	893.9106,	891.9950,	1600,	1600
14												

Figure 2- XYZ data for well plate images

For focusing of samples there are a few options. Predictive Focus can be used, where a focus height map is setup by the user, or autofocusing can take place at each field or every *n*-th field of the scan. See Surveyor manual for details.

Single field of view acquisition in this manner has the benefit of not requiring the Navigator license option at additional expense.

Multiple Scan Pattern Acquisition

Additional functionality for well plate scanning is available using the Navigator mode of Surveyor, which is an extra license. Navigator mode allows for multiple scan pattern acquisition, within multiple workspace acquisition, bearing in mind that the workspace is a container for multiple scan patterns. Navigator mode will be necessary if the content of the wells do not fit within a single field of view at the desired magnification, or more flexibility is needed in file output.

Shown in Figure 3 is a 3x3 scan pattern positioned at the location of each of the wells. The Navigator dialog allows the user to position the first scan pattern over the first well, and then duplicate this at the correct spacing for the other wells.

Scan: A 1 Using JPEG Compression		
1 + × ₩ ∞ 1 + • • • • • • • • • • • • • • • • • •	≫	
	🗙 🕅 āĀ 😫 🚍 🖶	
	With Weild Plate A Weil Plate With Revision Instruction With NewScan-2 Instruction With A1 Instruction With A2 Instruction With A3 Instruction With Replicate Pattern Instruction Sat Well Plate Other Instruction Instruction <t< td=""><td></td></t<>	
	Jitem in Map I Ueselect patients when scanned Add Item Delete Item Image: Close Close	Ħ
Screen Mag 3.69 X XY = [17.734,	0.936] mm Tiles = 0 (0.0 MB) Pattern Fields X = 3, Y = 3, Total = 9 Turboscan(stepped): 6.70 se	

Figure 3 - Multiple scan pattern arrangement in Navigator dialog

Focusing can consist of a Predictive Focus map for each individual scan pattern, one single focus map for the entire stage region, or autofocusing every *n*-th field of the scan patterns.

It is also possible to configure circle-type scan patterns which are relevant to many well plate systems. This will scan the minimum number of rectangular fields of view for a given size of well, bearing in mind the camera field of view is always rectangular. See Figure 4 for an example.

Scan Pattern Properties		KA.	×	
Pattem Focusing Field of	View			
Name:	A 1			
Туре:	Circle		•	
Definition				
Sample diameter (mm):	4.00			
Coverage:	Full coverage	•		*
Origin:	Top-Left	•		
X step size (μm):	870.45	🔽 Auto-Stepsize		
Y step size (μm):	868.58	🔽 Overlap field edg	es	
Scanning:	33	42 📩		
Total fields:	25	Width (mm):	4.3757	
Total area (sq. mm):	19.9341	Height (mm):	4.3663	
	OK Ca	ncel Apply	Help	

Figure 4 - Circle scan patterns

Navigator Output Options

There is greater flexibility in the file output using the Navigator license. Saving of workspaces or mosaic images can take place automatically, therefore the system has the potential be unattended as the scan takes place.

Saving the workspace (.sws) will contain the contents of all of the scan patterns inside it, ie. all of the wells within an entire workspace.

The contents of each scan pattern can be output as individual mosaic images, ie. each well can be an individual image on disk.

These filenames are designated in workspace name\pattern name\pattern name.bmp arrangements, and can also contain time stamp information in the path to avoid overwriting previous scans using the same set of item names.

Navigator can also be combined with other Surveyor licenses such as Multichannel fluorescence scanning or Z-Stack scanning, and the filenames are adjusted with appropriate channel or stack designations. In this way, Navigator is a very powerful and flexible option to Surveyor. See Figure 5 for save options available in this dialog.

🔊 Navigator —	×						
Navigator AutoSave Options							
Save Workspace							
✓ Save thumbnails							
Clear tiles after save (can reduce memory requirements for large scan batches)							
Save XYZPosition file as: *.txt 💌 🔽 Include additional data							
Each workspace is saved in folder structure "root destination\workspace name\". Additional data = row, col, channel, stack, width, height of each tile.							
V Save Mosaic							
Image format: BigTiff (.tif) 💌 Scale by: 1 👘							
Save all channels							
🗖 Save all planes							
Clear tiles after save (can reduce memory requirements for large scan batches)							
Each mosaic is saved in folder structure "root destination\workspace name\pattern name\".							
Root Destination Folder							
Select: C:\DIAutoSave Browse							
Add time stamp to folder path							
Enable Navigator Mode Close							

Figure 5 - Navigator output options