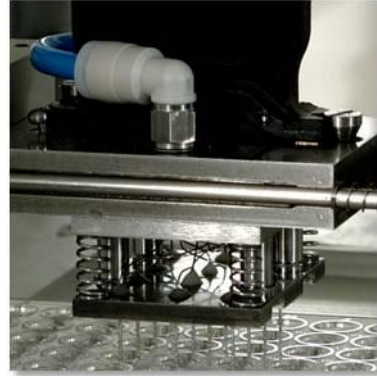
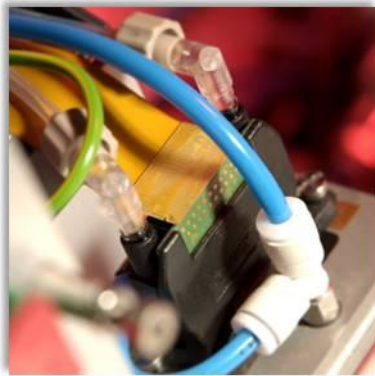




Command Centre™ for Marathon v1.4



User Manual

November 2013

Contents

Command Centre™ for Marathon v1.4 user manual.....	6
1. Introduction	6
2. Getting Started.....	7
2.1 Installing Command Centre™ v1.4	7
2.2 Running Command Centre™ v1.4	7
3. Menus	9
3.1 File menu.....	9
3.1.1 New Print Run	9
3.1.2 Load Print Run.....	10
3.1.3 Save Print Run	10
3.1.4 Repeat Last Print Run.....	10
3.1.5 Merge Samples and GAL Files.....	10
3.1.6 Generate Microplate Map File.....	12
3.1.7 Recent File.....	15
3.1.8 Exit.....	15
3.2 Options Menu	16
3.2.1 Initialise Instrument.....	16
3.2.2 Set Nozzles In Use	17
3.2.3 User Preferences.....	19
3.2.4 'K' Test Procedure	21
3.2.5 Empty Waste Bath	23
3.2.6 Fill/Empty System Buffer Bottles	23
3.2.7 JetSpyder Purge	24
3.2.8 Load/Unload Microplates and Slides	24
3.2.9 Printhead Purge	25
3.2.10 Print Test Slide	25

3.2.11 Wipe Nozzle Plate	27
3.2.12 Shutdown Instrument	27
3.3 View Menu	27
3.4 Help Menu.....	27
3.4.1 Activate Command Centre™ For Marathon v1.4	28
3.4.2 Command Centre™ for Marathon v1.4 help.....	28
3.4.3 About Command Centre™ for Marathon v1.4	28
3.5 Service Engineer menu.....	30
4. Print run Settings - Tabs	31
4.1 Source Microplate Properties Tab	32
4.1.1 Selecting microplate type and quantity	32
4.1.2 Microplates filled (vertically / horizontally).....	33
4.1.2 Partially filled microplates	35
4.1.3 Additional cleaning routine.....	35
4.2 JetSpyder Properties Tab	39
4.2.1 JetSpyder selection	39
4.2.2 Activating/de-activating printhead nozzles	40
4.2.3 Sample (nozzle) suppression.....	41
4.2.4 Reuse available space when using suppressed nozzles	41
4.3 Slide Properties Tab	43
4.3.1 Setting slide quantity	44
4.3.2 Adjusting print margins.....	44
4.3.3 Mini-arrays	45
4.4 Spot Properties Tab.....	46
4.4.1 Setting number of drops per spot.....	47
4.4.2 Setting printing pitch - horizontal; vertical; between sample sets	48
4.4.3 Selecting the number of repeats	48

4.4.4	Layout options I - rectangular versus hexagonal printing.....	48
4.4.5	Layout options II - Single pass versus Multi-pass repeats.....	49
4.4.6	Layout options III - Centring spot layouts within mini-arrays.....	50
4.4.7	Layout options IV – Blank Rows	51
4.5	Overview Tab	53
4.6	Start Tab.....	54
4.6.1	Print run options	54
4.6.2	Output file types	55
4.6.3	Input files	55
4.6.4	Time Estimator	56
4.7	target Microplate Properties Tab.....	57
4.7.1	Setting output plate quantity.....	58
4.7.2	Adjusting mini-array margins.....	58
4.8	Multiple print runs	59
4.8.1	Setting global parameters.....	60
4.8.2	Selecting/deselecting subruns	60
4.8.3	defining individual parameters	61
5.	Fault Recovery.....	62
6.	Tutorial.....	65
6.1	Designing a mini-array print run on slides	65
6.1.1	Creating a new print run	66
6.1.2	Defining the number of samples to be printed & the microplate type	67
6.1.3	Defining print margins & printing areas; number of slides to print.....	68
6.1.4	Defining spot size & pitch; number of repeats; spacing; array layout.....	70
6.1.5	Visualising the proposed layout.....	71
6.1.6	Suppressing samples; activating & deactivating printhead nozzles	72

6.1.7 Choosing location for saving output files; file naming; input file processing; choosing printing mode; Estimating Print Run Time; starting the print run.....	73
6.2 Designing a multiple print run	75
6.2.1 Creating a new print run	75
6.2.2 Microplate and trays settings	75
6.2.3 Setting number of sets of 12 samples.....	77
6.2.4 Setting Jetspyder type and nozzle in use	78
6.2.5 Selecting CSV/GAL file location.....	78
6.2.6 Selecting/deselecting subruns	79
6.2.7 Defining sub-run parameters.....	81

1. INTRODUCTION

This guide is provided to help you understand how to use Command Centre™ Software v1.4 for your Marathon Inkjet Microarrayer. The Command Centre™ Software v1.4 user manual includes information about the menus and commands within the software, and gives you an introduction to the different tabs with which you will interact when setting up a print run, with explanations of how to use them. Additionally at the back you will find a tutorial section which provides you with step-by-step instructions for creating print run protocols for printing microarrays on glass slides, and also on two-part microplates.

2. GETTING STARTED

This section provides all of the information required to get started on running the Arrayjet microarray printing instrument as quickly as possible.

2.1 INSTALLING COMMAND CENTRE™ V1.4

Microarrayers which are operating with an earlier version of Command Centre™, and are under warranty or service contract are eligible for a free upgrade to Command Centre™ v1.4. Nominated primary contacts or designated system owners will have received a communication from Arrayjet [1]. If you have not received any of this information, please contact Arrayjet.

In the event that the microarrayer is not within the warranty period or under a service contract it is still possible to purchase upgrades to Command Centre™. Please contact Arrayjet for further information.

Please note: if you are neither in possession of a microarrayer under warranty or service contract, and have not purchased an upgrade to Command Centre™, but have received either the executable from a source other than Arrayjet, or the instructions to download the executable from a source other than Arrayjet, the microarrayer in question should not be upgraded. To continue with the upgrade constitutes theft of Arrayjet software, and in the event that Arrayjet discovers the theft action will be taken against the perpetrator and any other party in any way involved.

Once the new executable has been downloaded, the software is ready to be installed.

Upon installation the software requires to be licensed. Please enter the licence key, supplied by Arrayjet, when prompted to do so.

2.2 RUNNING COMMAND CENTRE™ V1.4

The installation of Command Centre™ will leave a shortcut to the executable on the desktop.

- **Double-click the icon** - this will start a new session of Command Centre™;
- Select **File->New Print run->Slides** to create a new print run protocol on microarray slides;
- Select **File->Load Print run->Slides** to load an existing print run protocol for microarray slides;
- Select **File->New Print run->Microplates** to create a new print run protocol on microplates;
- Select **File->Load Print run->Microplates** to load an existing print run protocol for microplates.
- Select **File->New Print run->Multiple Print Run (slides only)** to create a new multiple print run protocol on microarray slides;

[1] Installation of new Command Centre™ v1.4 may require new settings under the Service Engineer menu. In the event that your microarrayer is operating with an earlier version of Command Centre (pre v1.3) and you are eligible for the free upgrade, installation will be performed by a Service Engineer as part of the next preventative maintenance visit. For more information, please contact Arrayjet.

- Select **File->Load Print run->Multiple Print Run** (slides only) to load an existing multiple print run protocol on microarray slides.

Choosing to create a new print run protocol will open the main Command Centre™ window, with which the user interacts to define the various parameters of the print run protocol, including microplate type, JetSpyder™ type, slide and spot properties and start settings.

The default properties for a new slide run allow you to start a run just after selecting the microplate type and, if different, the JetSpyder™ type, these being the only critical details for a trial run. If no type of JetSpyder™ was previously selected the High Capacity JetSpyder™ will be selected by default, otherwise the previous option is retained. All nozzles are activated at first (before user deactivating them) and the reuse of space on external samples suppression is set to on, which enforces wraparound printing mode for repeats. By default 2 full plates of samples are printed once in a single printable area with 2mm margins and drops are spaced at 0.2mm from each other.

Choosing to load an existing print run will open a window on the desktop which enables the user to explore the hard drive of the control PC for the appropriate print run protocol.

Command Centre™ is designed to be backward compatible, therefore runs defined in previous versions of software will be correctly interpreted. By loading an old file you will notice that the option “Do not reuse space allocated for suppressed external nozzles” is ticked, as the reuse of space taken by suppressed nozzles is a new option which should not change the layout originally defined.

3. MENUS

The main window in Command Centre™ software contains a set of menus displayed along its uppermost left hand edge. Each of these menus will be addressed in turn, with the Service Engineer menu covered last.

3.1 FILE MENU

The file menu contains the following menu items:

- New Print run
- Load Print run
- Save Print run
- Repeat last print run
- Merge Samples and GAL Files
- Generate Microplate Map File
- Recent files
- Exit

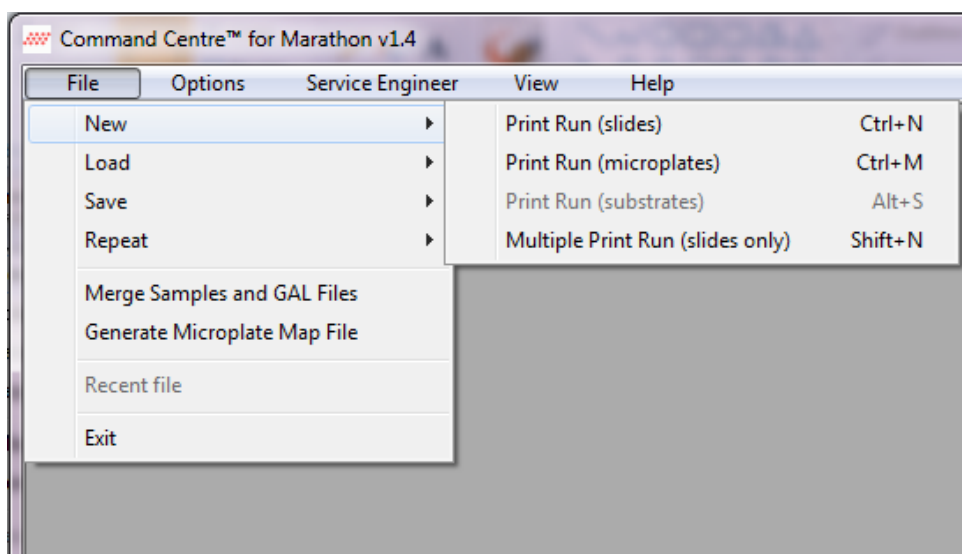


Figure 3-1 The File menu

3.1.1 NEW PRINT RUN

When designing a new print run protocol the user will select this option. A further option is then immediately available - to design the new print run:

- on microarray slides: **File>New>Print Run (Slides)**
- on special two-part plates: **File>New>Print Run (Microplates)**
- or Multiple Print Runs: **File>New>Multiple Print Run (Slides only)[2]**

[2] Command Centre™ for Marathon v1.4 also offers additional substrate printing. Please contact Arrayjet for further information.

3.1.2 LOAD PRINT RUN

When choosing to perform a previously created and saved print run protocol the user will select this option. A further option is then immediately available:

- to load a print run which prints microarrays on slides: **File>Load>Print Run (Slides)**
- on special two-part plates: **File>Load>Print Run (Microplates)**
- or Multiple Print Runs: **File>Load>Print Run>Multiple Print Run (Slides only)**

3.1.3 SAVE PRINT RUN

When choosing to save a print run protocol which has been defined during the current session of Command Centre™ the user will select this option. A further option is then available, to save the currently defined print run which prints microarrays:

- on slides: **File>Save>print run (Slides)**
- on special two-part plates: **File>Save>print run (Microplates)**
- or Multiple Print Runs: **File>Save>print run>Multiple Print Run (Slides only)**



NOTE: It is only possible to save a like-for-like print run from within its own definition, i.e. it is **not** possible to define a print run on slides and then save it as a print run on microplates. The same is true when defining/saving a Multiple Print Run (Slides only).

3.1.4 REPEAT LAST PRINT RUN

This command enables the user to repeat the last print run performed during the current session of Command Centre™, this option being lost on exiting the software.

Note: when starting a new session of Command Centre™ this option is not immediately available to the user, a new run needs to be executed for the repeat to become available.

3.1.5 MERGE SAMPLES AND GAL FILES

The GAL/CSV³ files are output files, by default generated during a print run or a simulation run, unless deliberately disabled. The user has the option to postpone the naming of samples associated to each feature in a generated GAL/CSV file to a later time, if no input file is selected before starting the run. This command enables the user to combine existing GAL/CSV files with an input file which contains information relating to the biological content of probe samples printed on the microarray:

³ CSV is an acronym for Comma Separated Variable file, and refers to a type of flat text file. GAL is an acronym for GenePix Array List file, and is another form of flat file not dissimilar to CSV files. More information concerning GAL files can be found at: http://www.moleculardevices.com/Documents/general-documents/mkt-appnotes/microarray-appnotes/GenePix_Pro_AppNote_Making_GAL_FILES_rev_B.pdf

File>Merge Samples and GAL Files

The input file provided by the user must be in flat text file format with variables separated by a comma (CSV) or tab (tab delimited), or other delimiter (e.g. semi-colon). This is effectively a table with a number of rows and columns, each of which containing mapping information concerning the microplates and the samples stored in them. Each row should include information about a location in a given microplate and the sample which was associated to this particular well, for example:

1A2,antibody A

The example line stands for plate 1, well on row A and column 2, delimiter (in this case comma) and text describing the biological sample.

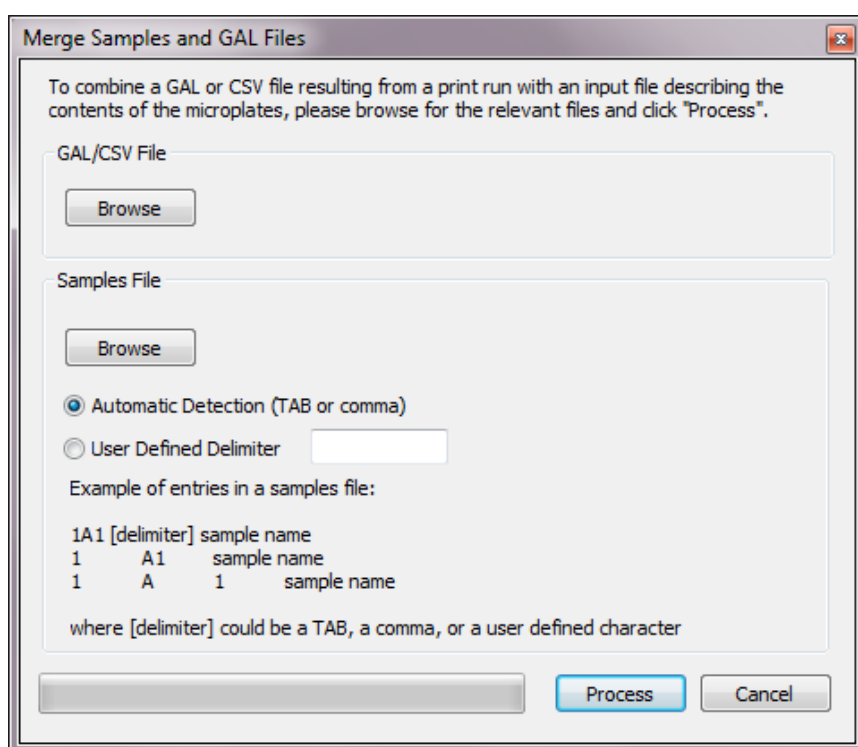


Figure 3-2 Selecting Merge Samples and GAL files Option from the file Menu

This command creates a new CSV/GAL output file now containing the sample name/well descriptions, and preserves the file name, by appending “(samples mapped)”.

On opening the **Input File Processing** window, the user selects the relevant GAL/CSV output file by clicking the **Browse** button in the **GAL/CSV Output File** group box. GAL/CSV files are generated automatically while printing, if the option to generate them was preserved ticked as default. The default Output file location is under C:/Program Files/Arrayjet/Marathon Software/Output Files/ and it is named after the date and time of the run’s start (following the format DDMMYY_hhmm).

The relevant sample name/well description information is provided by selecting an input file using the **Browse** button in the **Samples File** group box.

To choose the type of delimiter, either **Automatic Detection Mode** (default) or **User-Defined Delimiter** is selected via the appropriate radio buttons. The number of columns used for the microplate ID is automatically detected where the delimiter is a TAB. However, for a comma or user defined character the well id must be separated from the sample's description by only one special character (comma or user-defined delimiter). Examples are provided in the dialog box **Figure 3-2**.

Once all the parameters have been defined, the user clicks the **Process** button to continue and the new output file is created and a success message informs about the new file's location.

3.1.6 GENERATE MICROPLATE MAP FILE

The JetSpyder™ is specially designed to evenly aspirate samples from the microplates and distribute them linearly within the print head. In order to facilitate the well plate filling for a specific layout, this feature takes as input the linear distribution of samples to be printed in the array and maps them to the microplate's wells.

The option is available under **File -> Generate Microplate Map File**. This will open a dialog window (**Figure 3-3**) which allows users to select the array layout samples file, the microplate type and filling mode, as well as the JetSpyder™ to be used (by default the last used JetSpyder™ will be selected).

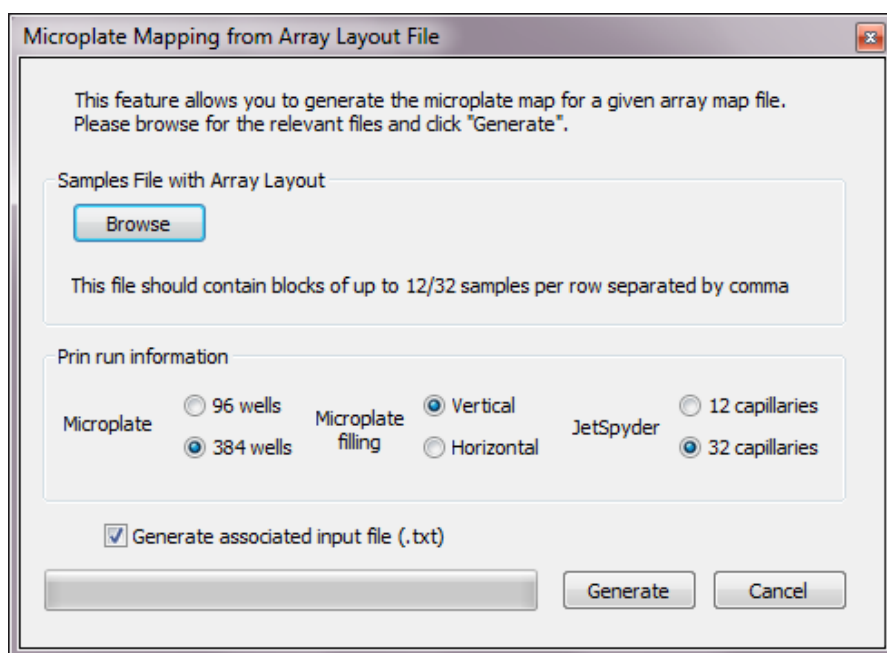


Figure 3-3 Selecting Generate Microplate Map File Option from the file Menu

The array layout file should be a comma separated values (.csv) file with samples provided in the order they are printed. As a Marathon instrument can print sets of up to 12 or 32 samples (depending on the JetSpyder™ type and on the samples suppressed), the print run should be designed first to have a good understanding on the array's structure and the blocks to be printed from one aspiration. The block from the top right corner is to be printed first, followed by the ones to its left up to the left margin, then continuing onto the next row again from right to left.

The samples file with the array layout should reflect this structure by providing in order the groups of samples printed together. Each row should be a set of up to 12 or 32 samples separated by comma, showing the order which the group of samples should follow on the slide. Each pickup of samples (excluding reaspirations of the same samples) should have an associated row of samples in this file, all consisting of the same number of samples. Please note that repeats or mini-arrays should not have entries in this file, as they all come from the same aspiration.

For clarification an example is provided below. Take a simple layout of 48 samples to be printed, as shown in **(Figure 3-4)**. The associated samples.csv file was built by taking every set of 12 samples from the top right corner to the left, then moving to the next row and writing down for each of these blocks the names of the samples to be printed, just the way they should appear on the slide.

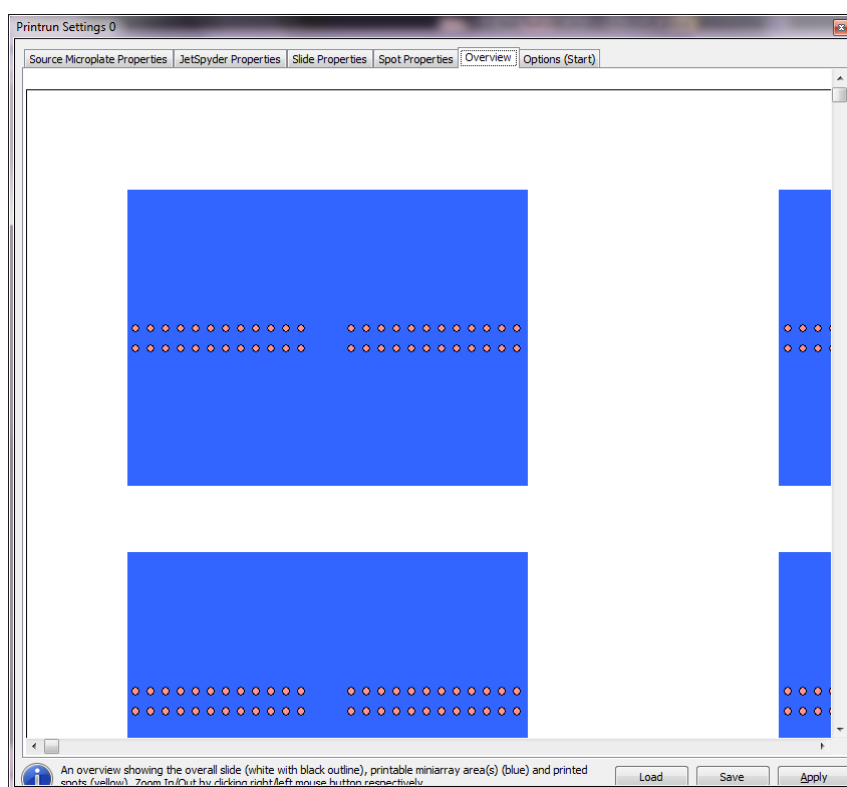


Figure 3-4 Layout Samples File

Microsoft Excel could be used for writing the array layout samples file, but this should be saved as Other Formats -> Comma Delimited (.csv) file. For the layout in **Figure 3-4**, a good example for the samples file is shown in **Figure 3-5**. The names of the samples have been chosen to be very short in order to allow integration with the layout view (**Figure 3-6**).

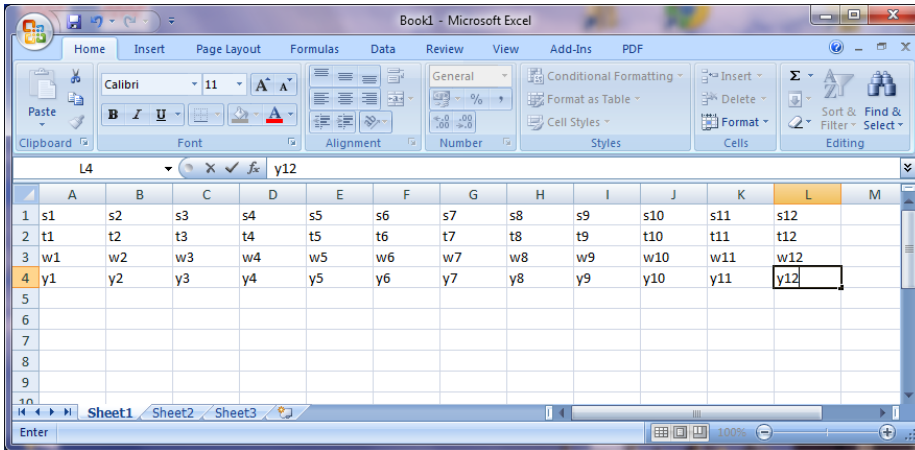


Figure 3-5 Example of samples .csv file generation

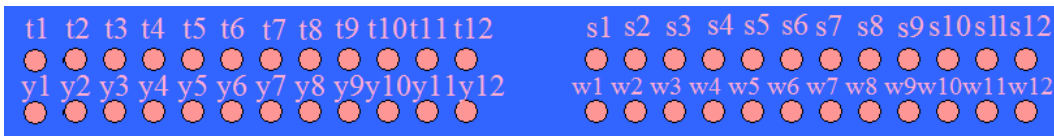


Figure 3-6 Integrated layout with samples names

For the generation of microplate filling map, the array layout file is required in the “Sample File with Array Layout” section and can be selected by clicking the Browse button. After selecting the JetSpyder™ type, the microplate type and filling mode used, the microplate map file is generated in the same folder as the samples file, preserving the name to which it appends “wellplate map”. The resulting file is a Comma Delimiter (.csv) file that can be opened with Excel for a tabular view. For this particular samples example, with a 12 JetSpyder™ and 384 wells microplate filled vertically, the output file is shown in **Figure 3-7**.

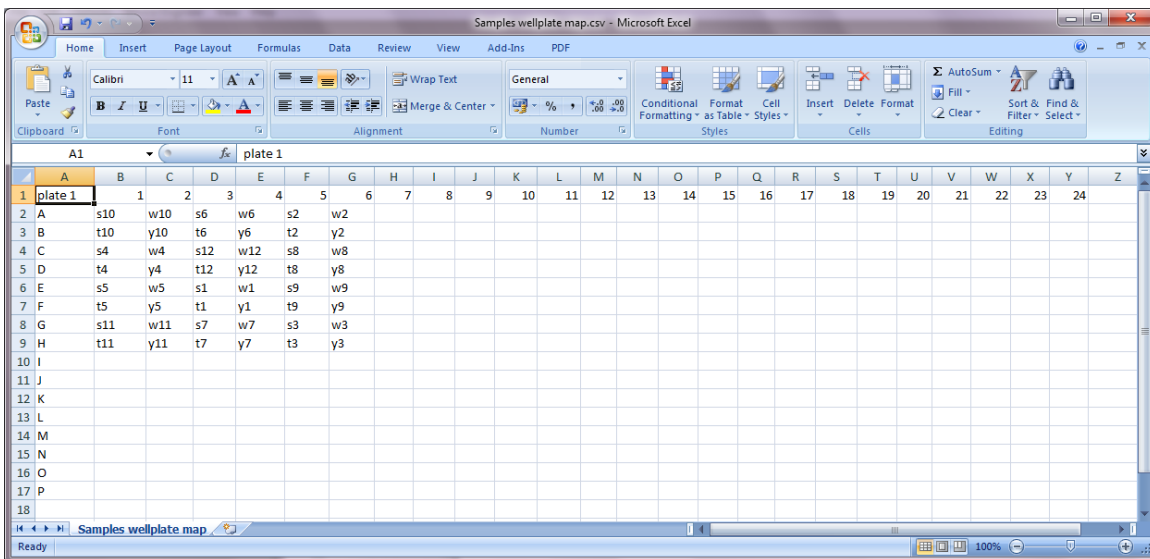


Figure 3-7 Microplate samples mapping

The last option in the Microplate Mapping dialog (**Figure 3-3**) is the automatic generation of an input file. This is needed in the generation of a complete GAL file, which includes the name of the sample associated to each feature. By default the option is ticked as usually for any new layout, users will require an input file as well. However, if the input file is already available, this option can be disabled and the file will not be generated. The input file is generated in the same folder as the array layout file, using the same starting name followed by "input file.txt".

On clicking the Generate button, the file(s) are created and a success message advises about the location of the newly generated files.

3.1.7 RECENT FILE

A list of recently used files which have been used in connection with the current session or in previous sessions of Command Centre™ is shown under the File menu.

3.1.8 EXIT

This command will exit the current session of Command Centre™.

3.2 OPTIONS MENU

The Options menu contains the commands for the Marathon Inkjet Microarrayer to perform a number of basic maintenance tasks. On starting a new session of Command Centre™ the only menu items immediately available to the user are the following:

- Initialise Instrument
- Set Nozzles in Use
- User Preferences

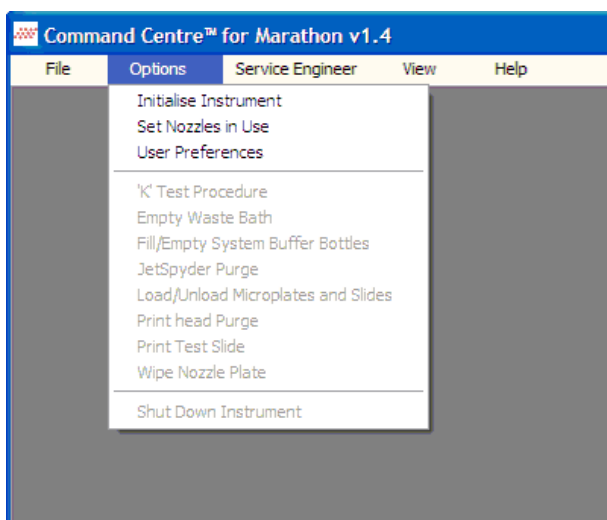


Figure 3-8 The Options menu

3.2.1 INITIALISE INSTRUMENT

During initialisation all axes are moved to the home position, and the linear motions and the syringe pump of the microarrayer are calibrated. The vacuum pumps are also activated.

Once initialisation has been successfully completed, the instrument is ready to perform a print run. The user can also perform basic housekeeping tasks, as required (under the **Options** menu) in order to get the best performance from the Marathon Inkjet Microarrayer.

To preserve the life of the vacuum pumps this new version of Command Centre™ embeds a timer which, after initialisation, counts for 30 minutes before the pumps are turned off. The timer is restarted by any mouse click activity, counting only periods of inactivity, in order not to interfere with any operations called by the user. At the end of the 30 minutes of inactivity, a dialog box (**Figure 3-9**) signals the automatic shut down.

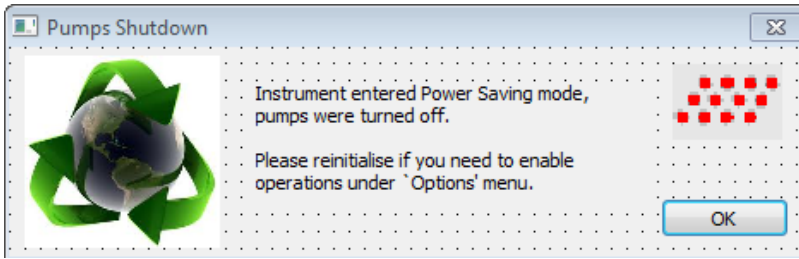


Figure 3-9 Power Saving Mode

3.2.2 SET NOZZLES IN USE

This command brings up a window which shows all the nozzles used by the printhead for the selected JetSpyder™. This is shown as a grid pattern and also as an annotated diagram of the test slide image, showing which spots in the test slide pattern are printed with which nozzles, in order to assist the user with fault diagnosis in nozzle performance.

There is also a pull-down for selecting the JetSpyder™ to be used for the print run: JetSpyder™ 12 High Capacity (**Figure 3-10**), JetSpyder™ 12 Low Volume (**Figure 3-11**), or JetSpyder™ 32 (**Figure 3-12**). The nozzles in the grid pattern are coloured **green** for **active** and **red** for **inactive**. To activate or deactivate a given nozzle the user simply **left clicks** on the nozzle in question with the mouse, and the nozzle will change colour between red and green.

The user is also able to switch on/off the nozzles in the **JetSpyder Properties tab** and the changes made in this tab will be reflected in the **Set Nozzles in Use** window under the **Options** menu.

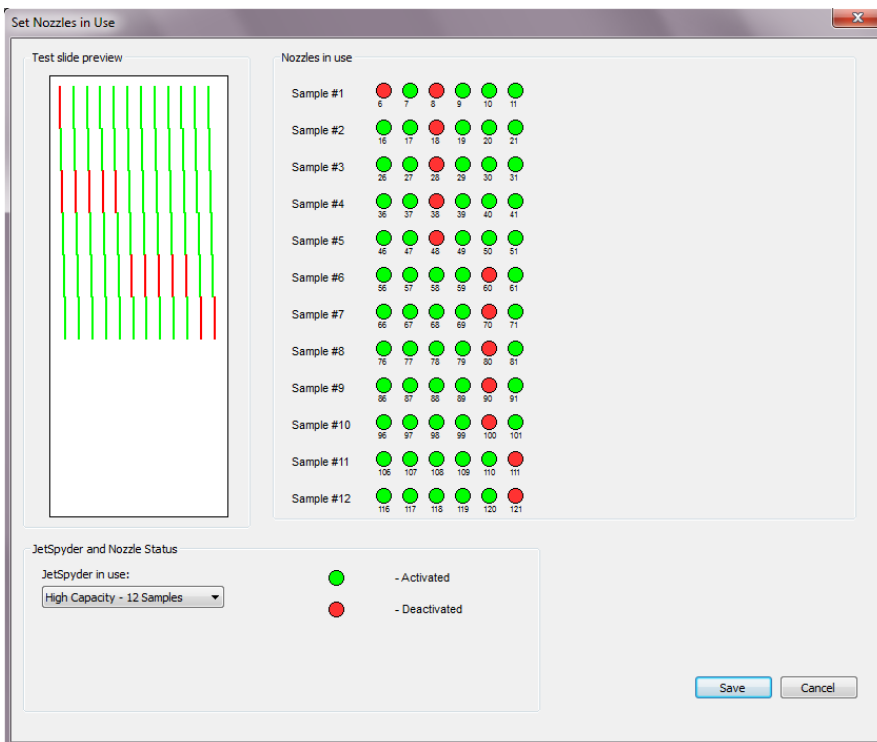


Figure 3-10 Nozzles in use for a 12 JetSpyder™ High Capacity

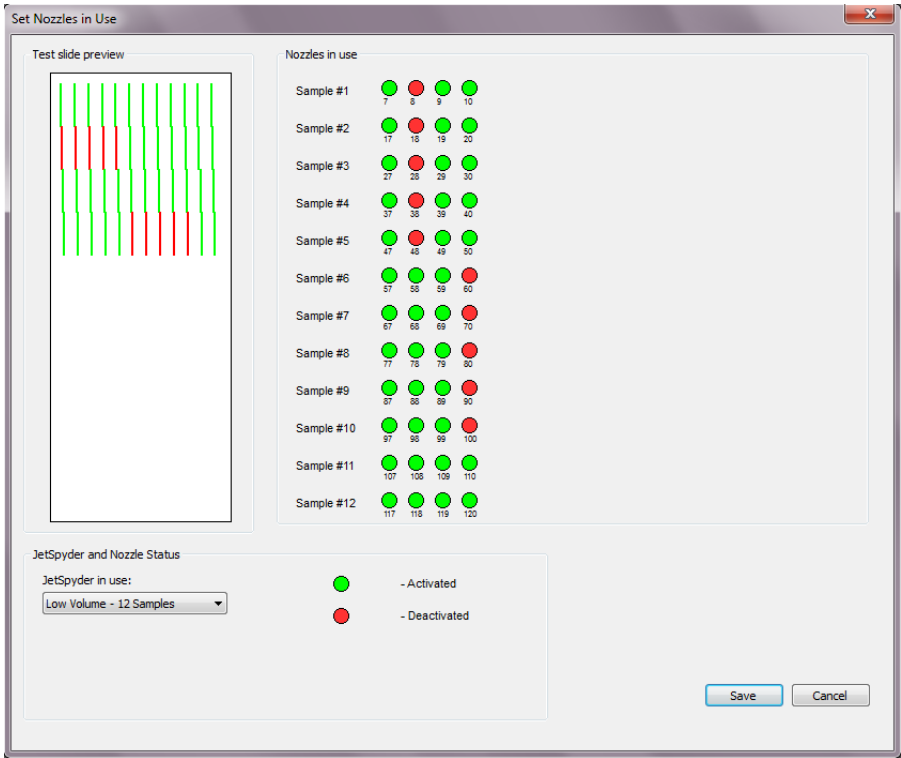


Figure 3-11 Nozzles in use for a 12 JetSpyder™ Low Volume

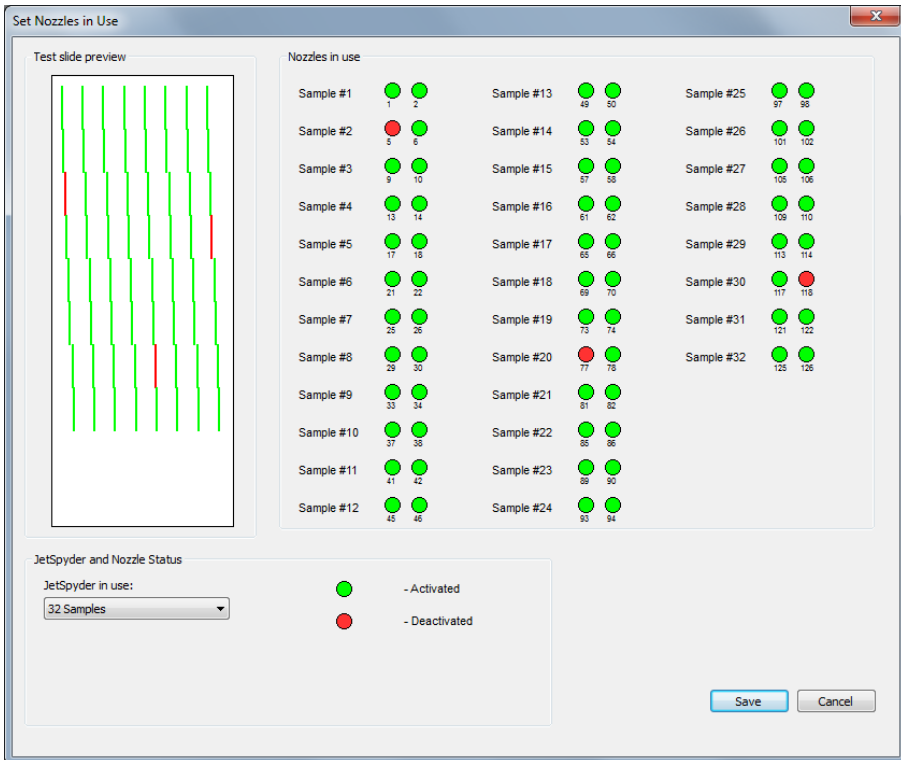


Figure 3-12 Nozzles in use for a 32 JetSpyder™

3.2.3 USER PREFERENCES

Command Centre™ for Marathon allows the user to modify a number of settings by accessing the **User Preferences** window.

In the **Printing Preferences** tab, the user can adjust the number of printhead/JetSpyder™ purges; number of printhead/JetSpyder purges before redraw; maximum drops per nozzle; automatic pressure logging and the efficiency factor which affects the voltage used in the printing of samples from printhead.

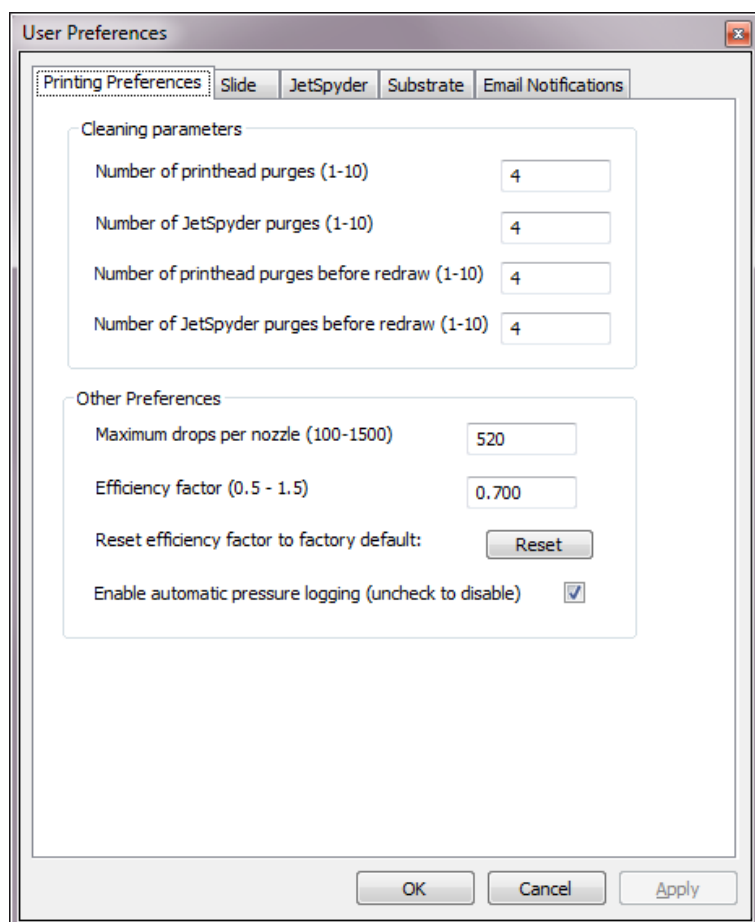


Figure 3-13 The User Preferences dialog box

Whenever the number of drops per spot printed is high and one aspiration does not suffice for all the slides to be printed, redraws from the same wells are required. However, before a redraw, intensive cleaning is not necessarily required as there is no danger of carry over therefore allowing a lower number of purges to be performed before a redraw would improve the time taken for the print run and reduce the amount of system buffer used without affecting the quality of the print.

On instruments equipped with a pressure transducer, the automatic pressure logging feature measures and records the stiffness at the start and end of a print run, as well as during every aspiration. Please be aware that the pressure measured during an aspiration will return a negative value, as the liquid is subjected to a negative pressure during the suck operation, as opposed to a

positive pressure when purging. A normal value for the pressure read during an aspiration is between -15kPa and -30kPa. Above -15kPa may indicate that air has been aspirated, while below -30kPa may be indicative of a higher viscosity sample.

The pressure measured at the start and end of the print run (K Test) should ideally be above 200kPa.

If desired, the user can reset the current values of efficiency factor and slide length/width to that of the factory-set defaults contained in the machine parameters file.

The **Slide** tab allows the modification of slide's dimensions: width and length. By default these are set to 25.0mm and 75.0mm, respectively.

JetSpyder™ tab allows the user to increase the aspiration volumes for each type of JetSpyder. By default these are set to standard.

Email Notifications tab shown in **Figure 3-14**, allows you to enable alerts on print finished, buffer refill / waste empty or well-plates reload requests, as well as other issues that might interrupt the printing.

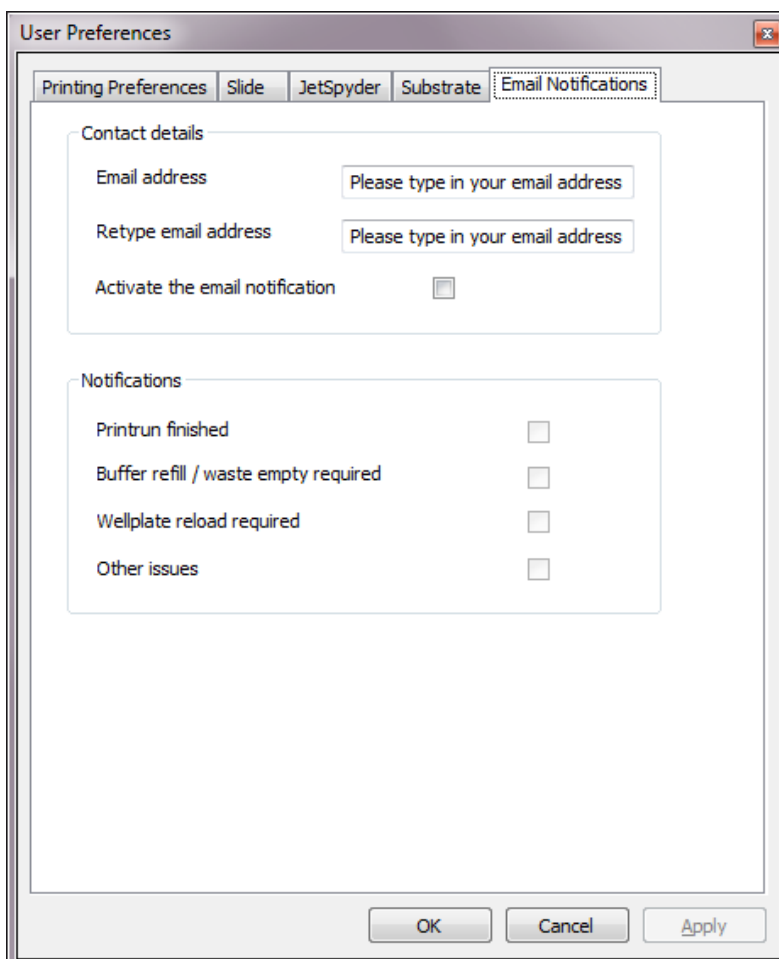


Figure 3-14 Email Notifications

If the instrument is fitted with a waste level sensor, the second type of notification will be sent in two cases: if clean buffer is empty or if waste bottle is exceeding the maximum limit. For no sensor fitted, these types of notification will be sent only if the small buffer reservoir cannot be filled in a given interval of time, mostly caused by clean bottle being empty. In this latter case, it is very important that the user empties the waste bottle when filling the clean bottle, to avoid overflow.

Other issues notification informs the user electronically about any sort of run interruptions (unless this is caused by a power cut), offering some guidance on how to recover the run after investigating and preferably remediating the problem's source. Fault recovery is automatically launched on a software restart after any unfinished run which passed the initialisation stage.

A valid email address must be entered and the **Notifications** group must have at least one option selected when **Activate the email notification** is ticked. The email can be deleted only by disabling **Activate the email notification**, otherwise it will be remembered for next time.

Another type of notification, which is seamlessly provided, is the automatic signalling of issues to support@arrayjet.co.uk, with the user's consensus, when replying yes to the question "Would you like to send this to Arrayjet?". This makes it easier to inform Arrayjet should there be a problem with the instrument. It also allows automatic attachment of debugging information, as the log file is read and embedded within the email's body.

Please note that the Email Notification feature requires the computer connected to the instrument to be networked, with good internet connection and no restrictions from the network setup when the notification is being sent.

3.2.4 'K' TEST PROCEDURE

This command will perform a K-test, which is carried out to determine if the hydraulic system is appropriately pressurised and to validate that no air is present in the printhead. The latest production models of the Arrayjet Marathon series of Inkjet Microarrays come equipped with a suitable pressure transducer in order to enable measurement of hydraulic pressure (K).

On older instruments, this capability is enabled by the fitting of an Arrayjet-approved pressure transducer and a suitably recent version of Command Centre™ for Marathon. For more information, please contact Arrayjet.

To perform a K-test, the user must first select the task from the Options menu.

By selecting:

Options>Perform 'K' Test Procedure

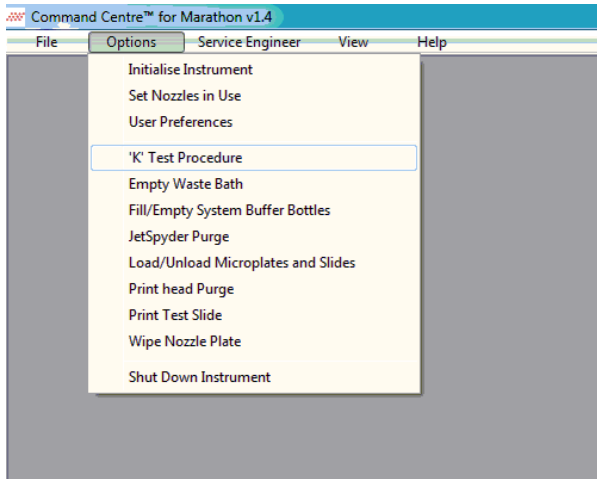


Figure 3-15 Selecting K-test from the Options menu.

This will initiate the sequence for performing a K-test. The user will then be presented with the option of performing the K-test with a **12 sample JetSpyder** or a **32 sample JetSpyder** in the **K-test Operation** pop-up box (**Figure 3-16**)

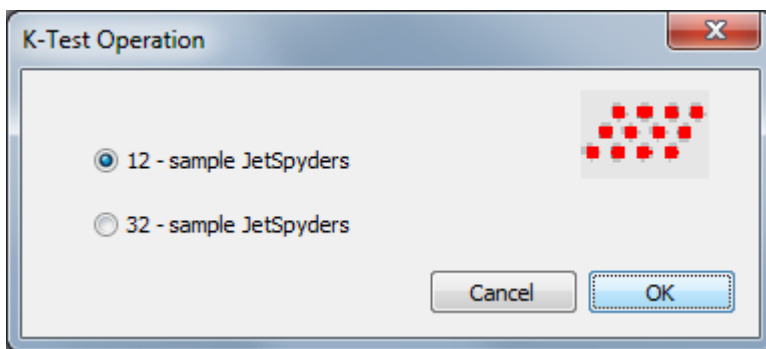


Figure 3-16 The K-Test Operation pop-up box

Once the user has made their selection and clicked **OK**, the instrument will proceed with the K-test procedure. Having measured the K of the instrument, the software will report the result (**Figure 3-17**) and then after final operations it will inform the user that the K-test has been successfully completed (**Figure 3-18**).

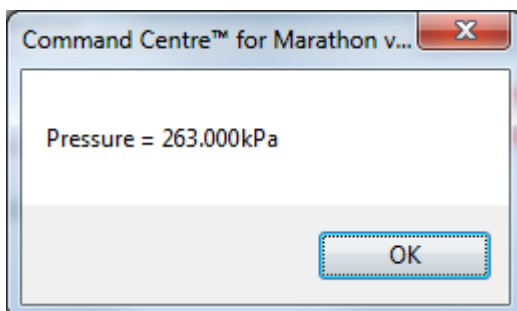


Figure 3-17 Pressure value pop-up box

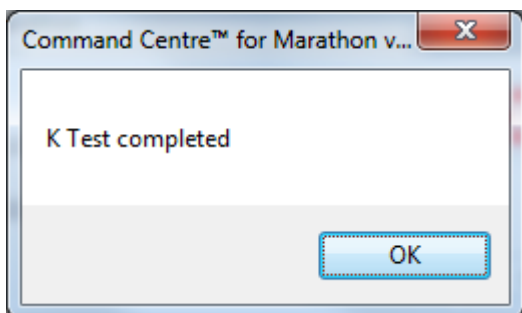


Figure 3-18 K Test completed pop-up box

3.2.5 EMPTY WASTE BATH

The waste bath is the container with a wiper above which the printhead purges into during its cleaning routines. This command will evacuate any liquid from the waste bath of the Marathon Inkjet Microarrayer. The user must click the button marked **Empty Bath** which appears in a pop-up dialog box in the main window in order to perform this task (**Figure 3-19**). A status bar in the pop-up informs the user of the progress of this task.



Figure 3-19 The Empty Waste Bath box

3.2.6 FILL/EMPTY SYSTEM BUFFER BOTTLES

This command deactivates the vacuum within the microarrayer so that the user can open both of the system buffer bottles. A dialog box with required operations will pop up in the user interface (**Figure 3-20**). Once the required operations have been performed, the user must click the button marked **OK** to proceed with the re-initialisation.



The user must empty the waste bottle every time the clean buffer bottle is filled, in order to prevent overflow of the waste bottle. Please use the marked reference on the bottle for the maximum level of clean buffer (if none is provided, please allow a few centimetres

to remain empty from the top of the bottle).



When re-filling the system with clean buffer, **under no circumstances** must the user tamper with the small liquid reservoir bottle, which is mounted inside the unit; only the clean buffer bottle (usually placed underneath the instrument) must be used when re-filling the system with clean system buffer.

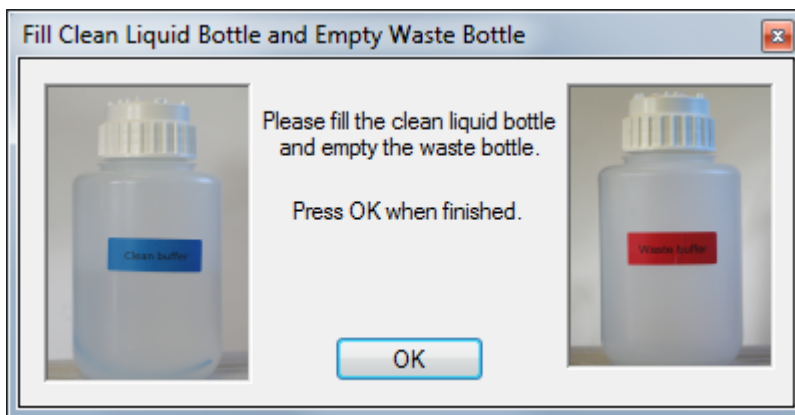


Figure 3-20 Fill Clean Liquid Bottle and Empty Waste Bottle box

3.2.7 JETSPYDER PURGE

This command enables the user to clean the JetSpyder™ by purging system buffer through it when the JetSpyder is parked in its cleaning station. A dialog box with a button marked **Purge JetSpyder** will appear in the main window (Figure 3-21), and the user must click this button to commence the JetSpyder purge. A status bar in the pop-up informs the user of the progress of the task.

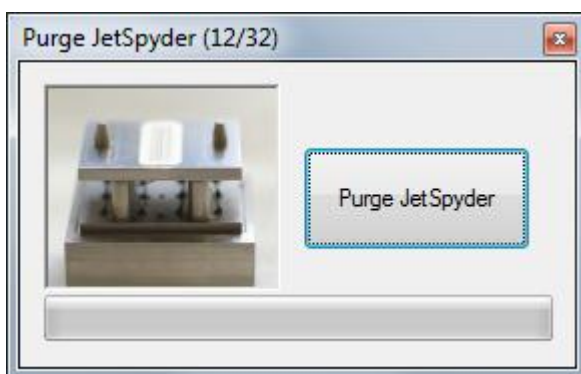


Figure 3-21 The Purge JetSpyder (12/32) box

3.2.8 LOAD/UNLOAD MICROPLATES AND SLIDES

This command enables the user to open the main doors of the microarrayer for the purposes of loading or unloading microplates containing the probe samples which are to be printed or have been printed on the microarray slides, as well as loading or unloading the printable microarray slides/part

plates. A pop-up with a button marked **Load or Unload Slides/Plates** will appear in the main window (**Figure 3-22**). The user must click on this button before proceeding to open the doors of the microarrayer. Once clicked, this pop-up is replaced by a second pop-up with instructions and a button marked **OK**. The user must click OK on completion of the instructions in the pop-up, after closing the instrument's door and pressing the green start button located on the upper-left hand corner of the frame of the instrument.

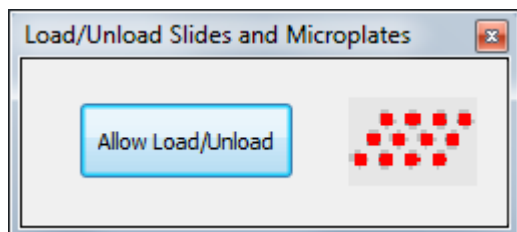


Figure 3-22 Load or unload plates and slides box

3.2.9 PRINTHEAD PURGE

This command enables the user to perform a printhead clean by purging system buffer through it. A dialog box with two buttons, one marked **Purge 1x** and one marked **Purge 4x**, will appear in the main window (**Figure 3-23**), and the user must click on one of these buttons to commence the printhead purge(s). The status bar at the bottom of this window informs user of the progress of the task given.

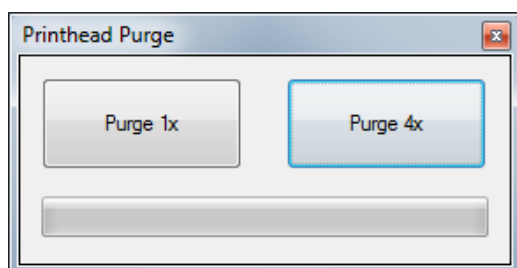


Figure 3-23 The Printhead Purge box

3.2.10 PRINT TEST SLIDE

The user can print a test slide to determine which of the nozzles are printing correctly or otherwise at any time. If there is no clean slide in the test-slide position, the **Load test slide** button must be first clicked on the **Test Slide** pop-up (**Figure 3-24**). This will allow the user to open the instrument cover and insert a blank test slide in the appropriate position on the instrument: this is a unique position clearly separate from the slide trays, and is found on the left-hand side of the unit, close to the waste bath - the place where the printhead purges. The user must then close the door; press the green start button located in the front panel of the instrument.

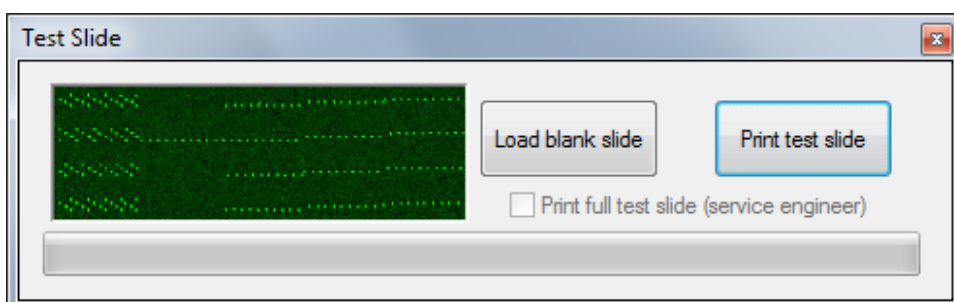


Figure 3-24 The Test Slide control panel

By clicking on the **Print test slide** button, the microarrayer will print a hexagonal pattern followed by a staircase on the slide loaded. When completed, an alert box (**Figure 3-25**) will remind the user to remove the printed test slide, then **Close the instrument doors and press the green start button** (located on the upper-left hand corner of the frame of the instrument) before pressing OK.

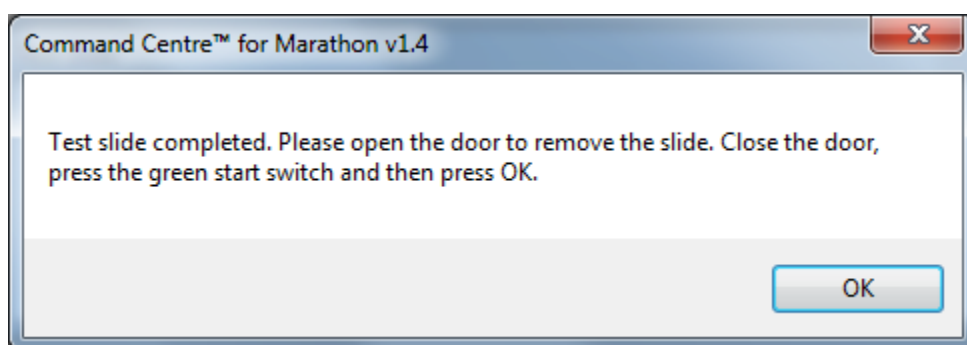


Figure 3-25 Test slide alert window

The nozzles printed on a test slide are a subset of the printhead's nozzles, which consist only of the relevant nozzles for the selected JetSpyder™. The test slides for a JetSpyder™ with 12 capillaries create 12 staircases across the slide, one for each group of nozzles associated to one sample. These consist of four or six steps (lines) depending on whether it is a Low Volume (**Figure 3-11**) or a High Capacity (**Figure 3-10**) JetSpyder™. Each line shown is printed by a single nozzle which makes it easy to spot blocked nozzles causing missing lines.

The 32 JetSpyder™ aspirates 32 samples at once, each into two nozzles. It was not feasible to place 32 staircases of two steps across the slide, therefore a staircase comprises of four samples which are groups of two. The test slide consists of 8 staircases, each of 8 steps printed by nozzles associated to four samples (**Figure 3-12**).

For a service engineer it is also possible to print a full test slide (independent of JetSpyder™ selected) showing all the nozzles of the printhead. This is useful during initial setup and commissioning of the instrument.

Please refer to section **3.2.2 Set Nozzles in Use** for images of what each testslide looks like.

3.2.11 WIPE NOZZLE PLATE

This command performs an additional wipe of the printhead nozzle plate. On call, a pop-up box with a button marked **Wipe nozzle plate** (Figure 3-26) will appear on top of the main window. In order for the microarrayer to execute the command, the user must click this button. A status bar in the pop-up informs the user of the progress of the task. On completion of the wipe, the pop-up will close.



Figure 3-26 The Wipe Nozzle Plate box

3.2.12 SHUTDOWN INSTRUMENT

This command performs a controlled shutdown of the microarrayer. An alert box will appear in the main window asking the user to confirm the shutdown command (Figure 3-27). The user must click the **Yes** button in order to perform the shutdown. Once the shutdown has been completed, the user can switch off the microarrayer at the main power switch.

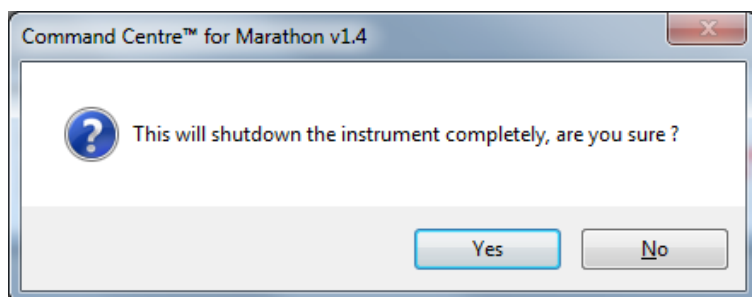


Figure 3-27 The shutdown instrument alert box

3.3 VIEW MENU

The view menu contains only one item - the status bar. This can be checked or unchecked: default setting is **checked**.

3.4 HELP MENU

The help menu (Figure 3-28) contains three items:

- Activate Command Centre™ for Marathon v1.4
- Command Centre™ for Marathon v1.4 Help
- About Command Centre™ for Marathon v1.4

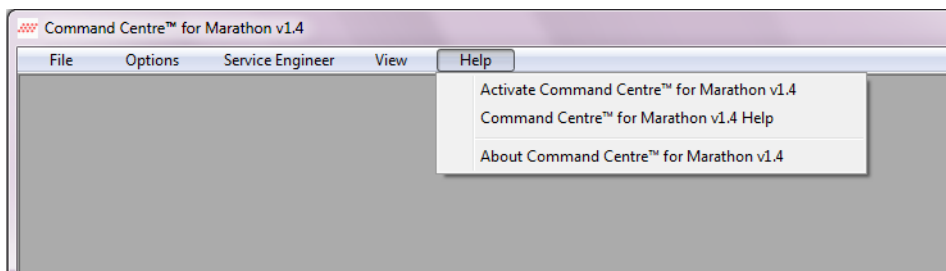


Figure 3-28 The Help menu

3.4.1 ACTIVATE COMMAND CENTRE™ FOR MARATHON v1.4

Within 30 days of installation of a new version of Command Centre™ software the user will need to input a license key to enable continued use of the software. When the activate command has been executed, a dialog box will pop-up in the main window (**Figure 3-29**). The box contains a space into which the license key must be entered. Once the key has been entered, the user must click the button labelled **Register**. On entering of the correct key, software registration will complete successfully and the software needs to be restarted for its full functionality to be enabled.



Figure 3-29 Marathon Software Activation box

3.4.2 COMMAND CENTRE™ FOR MARATHON v1.4 HELP

Selecting this menu item will open an embedded version of this User Manual. This contains information about Command Centre™ for Marathon, including operating instructions.

3.4.3 ABOUT COMMAND CENTRE™ FOR MARATHON v1.4

This menu item, when selected, launches a small pop-up window which provides information concerning the software version installed, and also displays the number of sample sets printed since

the instrument was installed, and since the last service. The window can be dismissed by pressing the **Esc** key.

3.5 SERVICE ENGINEER MENU

Service engineers may on occasion need to modify the settings of the instrument parameters and/or control from low-level equipment such as the syringe, drive motors, cage assemblies, printhead drive electronics and the PLC inputs and outputs. These options are not available to end users who are not trained service engineers, and are password-protected in a secure area of Command Centre™ for Marathon software.

To access the Service Engineer menu the user is required to enter a password (**Figure 3-30**)

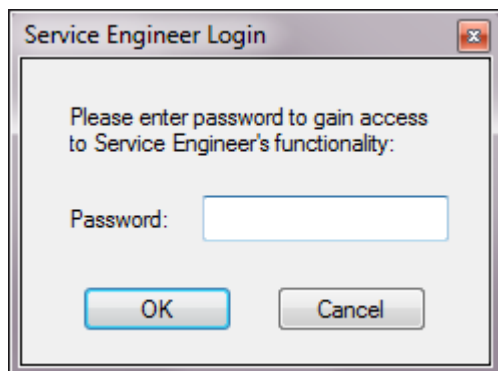


Figure 3-30 The Service Engineer Login box

The Service engineer can control all the operational components of the microarrayer through Command Centre™ for Marathon. They shall perform various maintenance tasks and system checks where and when required in order to ensure that the instrument remains operational.

4. PRINT RUN SETTINGS - TABS

When designing a new print run protocol (command: **File>New Print Run>Slides**) a new window opens within the Command Centre™ main window (**Figure 4-1**). The window consists of a number of tabs, and within each tab the user can adjust various parameters of the print run to be performed according to their requirements. The various tabs, listed in order, are:

- Source Microplate Properties
- JetSpyder Properties
- Slide Properties
- Spot Properties
- Overview
- Options (Start)

Each tab is explored in the following pages, together with the user-definable parameters. Note: the example new print run protocol created in this section of the User Manual is for microarrays printed on slides; printing microarrays on two-part microplates is covered in Section 4.7 – **Target Microplate Properties** tab.

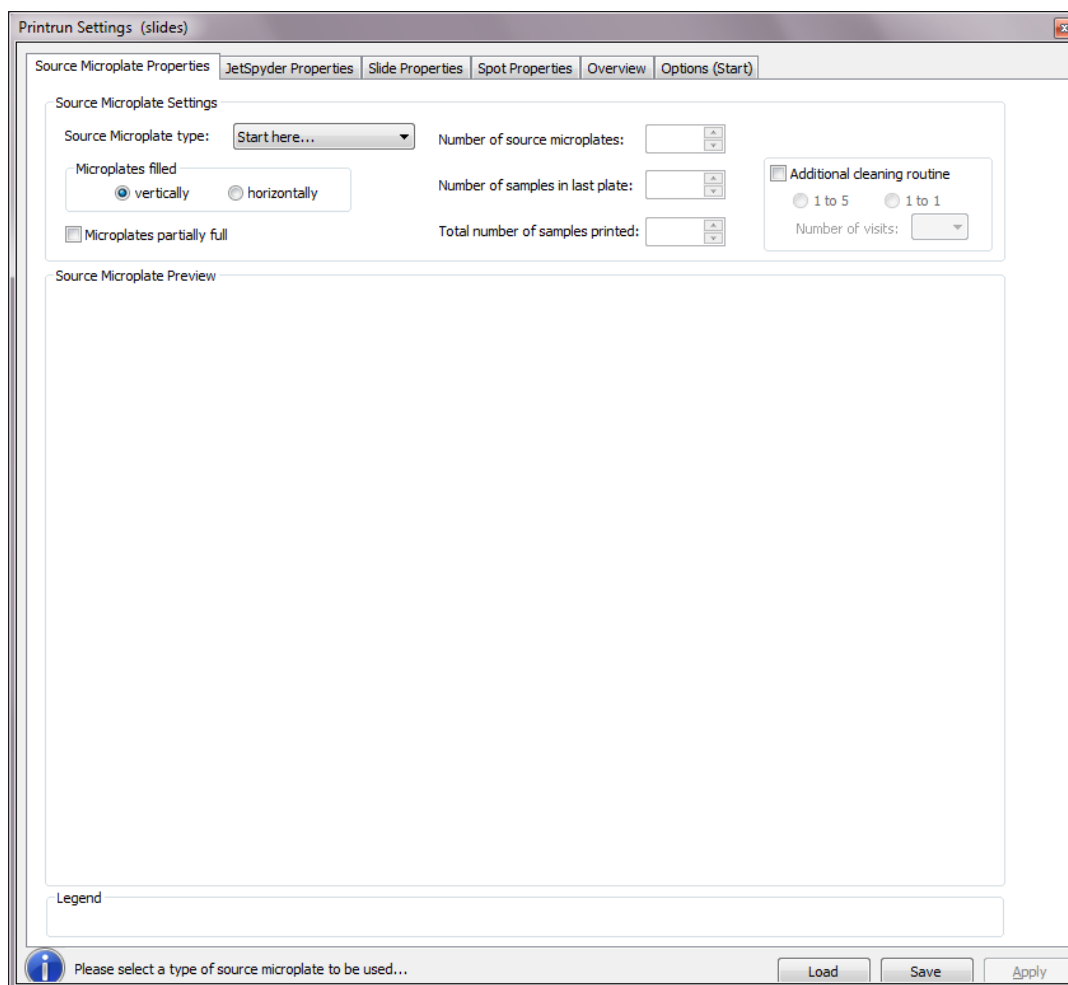


Figure 4-1 The Print run Settings (slides) window

4.1 SOURCE MICROPLATE PROPERTIES TAB

The **Source Microplate Properties** tab is the first tab which the user can and must interact with when designing a new print run protocol. In this tab the user must provide information concerning the type and number of microplates, and how they have been filled, thereby providing information concerning how many probe samples are to be printed during the print run.

4.1.1 SELECTING MICROPLATE TYPE AND QUANTITY

Source Microplate type is selected from a pull-down list in the **Source Microplate Settings** group box in the upper left hand corner of the **Source Microplate Properties** tab (**Figure 4-2**). The options available to the user are **Standard 384 (well)** or **Standard 96 (well)**, which correspond to microarray-specific microplate types which the microarrayer has been calibrated for. These microplates are available for purchase - please contact Arrayjet for more details.

Note: The **Source Microplate type** must be selected before the user can proceed further with the set-up of a print run.

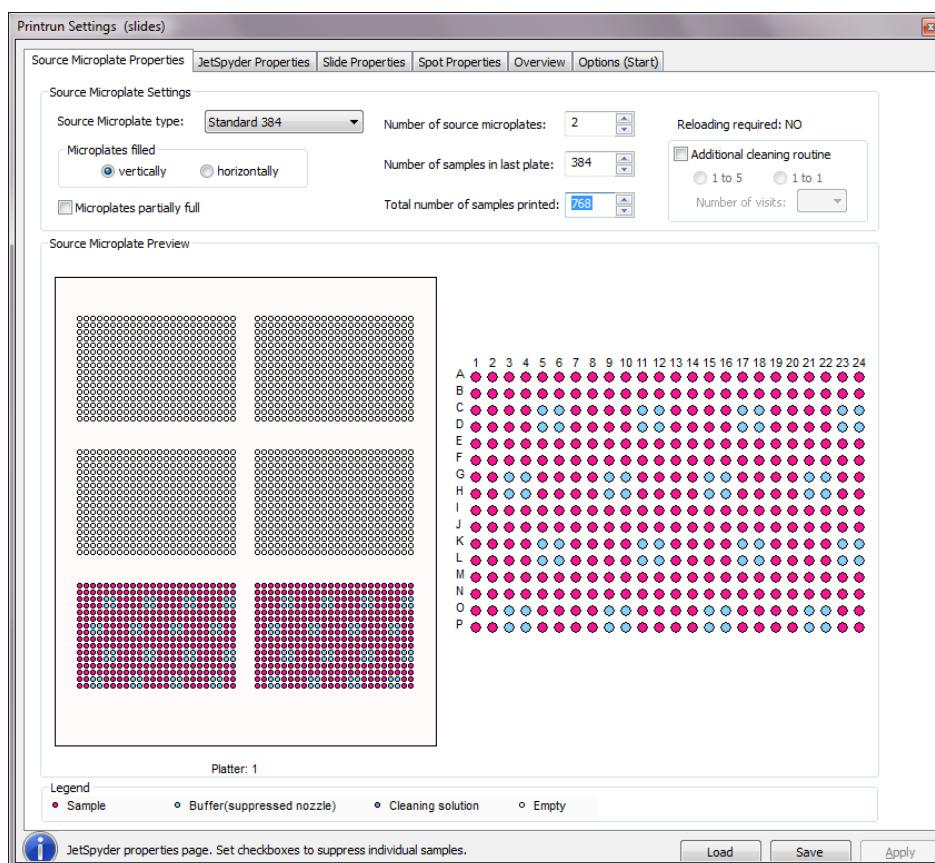


Figure 4-2 The Source Microplate Properties tab

Once the microplate type has been selected, the lower left part of the **Source Microplate Properties** tab view is then filled with the view of six microplates, with the appropriate number of wells in each plate – 96 or 384. The view of the microplates changes in real time with the inputs of the user - as

sample or microplate numbers increase or decrease, the changes are reflected in the **Source Microplate Preview** box. This includes changes between full or partially full plates (see 4.1.2), and whether or not the additional cleaning routine is selected (see 4.1.3).

The microplate's overview on the right hand side of **Source Microplate Properties** is available for less than 7 plates of samples and cleaning solution. It brings a useful enlarged representation of the first plate which helps determining how plates should be filled particularly in cases where suppressed nozzles are used (as shown in **Figure 4-2**). Please note that all plates follow the same pattern, except for the last one which might be a subset of this.

Number of source microplates is entered by the user, either typed as text, or via the spin control provided in the **Source Microplate Settings** group box in the upper side of the **Source Microplate Properties** tab. As this number is increased, to a maximum of 96 microplates, the figures shown in the remaining two edit boxes, **Number of samples in last plate** and **Total number of samples printed**, located beneath the **Number of source microplates** edit box, also adjust in real time. It is therefore possible for the user to choose how they prefer to provide information about the number of samples to be printed - any of those 3 options is available to them. The Arrayjet microarrays have a maximum limit for samples printed depending on the type of instrument and whether a reload is performed by the user. The four possible cases are summarised in the following table. The maximum number of samples which may be printed in a single print run on a **Marathon Inkjet Microarrayer** is **36,864**, which corresponds to **96x384-well microplate**. In contrast the **Super-Marathon** and **Ultra-Marathon II Inkjet Microarrayers**, as well as having an increased walk-away capacity over the standard Marathon (6 microplates) of **48 in total**, can both be re-loaded with a further 144 microplates for a total capacity of **192 microplates per print run or 73,728 samples in total**.

Microarrayer	Walk-away Capacity	Reload Capacity
Standard Marathon / Ultra-Marathon I	6 plates (max* 2,304 samples)	96 plates (max* 36,864 samples)
Super-Marathon / Ultra-Marathon II	48 plates (max 18,432 samples)	192 plates (max* 73,728 samples)

*the maximum number of samples are calculated considering the use of 384-well microplates

4.1.2 MICROPLATES FILLED (VERTICALLY / HORIZONTALLY)

The source microplates may either be filled vertically (see **Figure 4-3**) or horizontally (see **Figure 4-4**). All the microplates within a print run must be filled in the same manner.

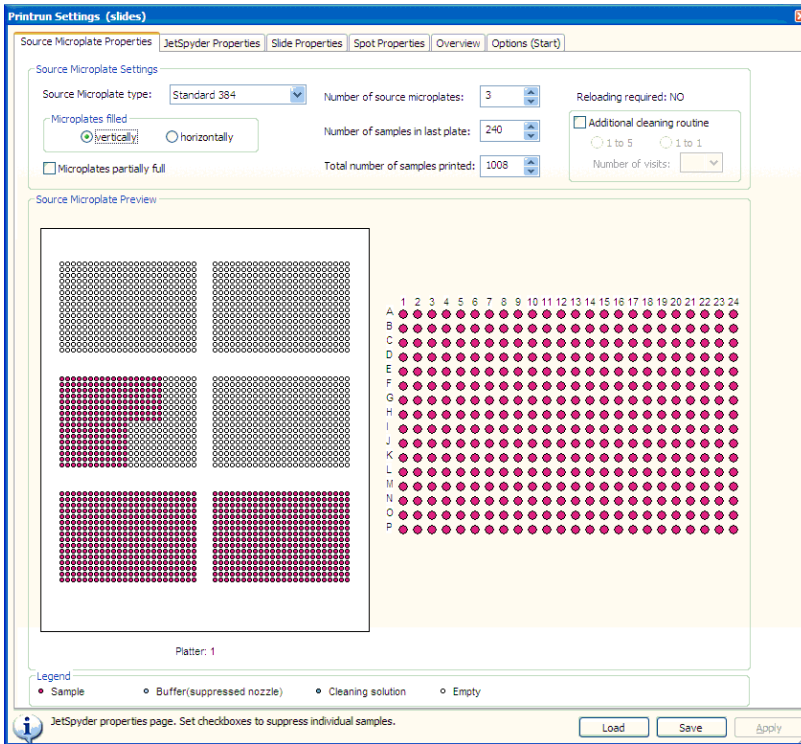


Figure 4-3 Microplates filled vertically

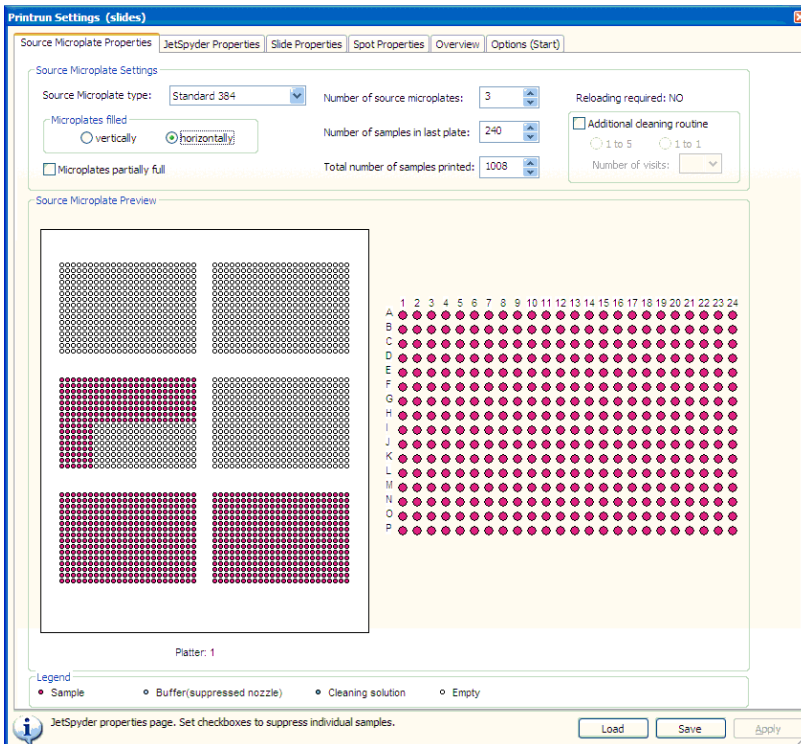


Figure 4-4 Microplates filled horizontally

4.1.2 PARTIALLY FILLED MICROPLATES

If the user requires probe samples from more than one microplate, but does not require each of the microplates to be printed in their entirety, the option is available to treat all plates as partially filled.

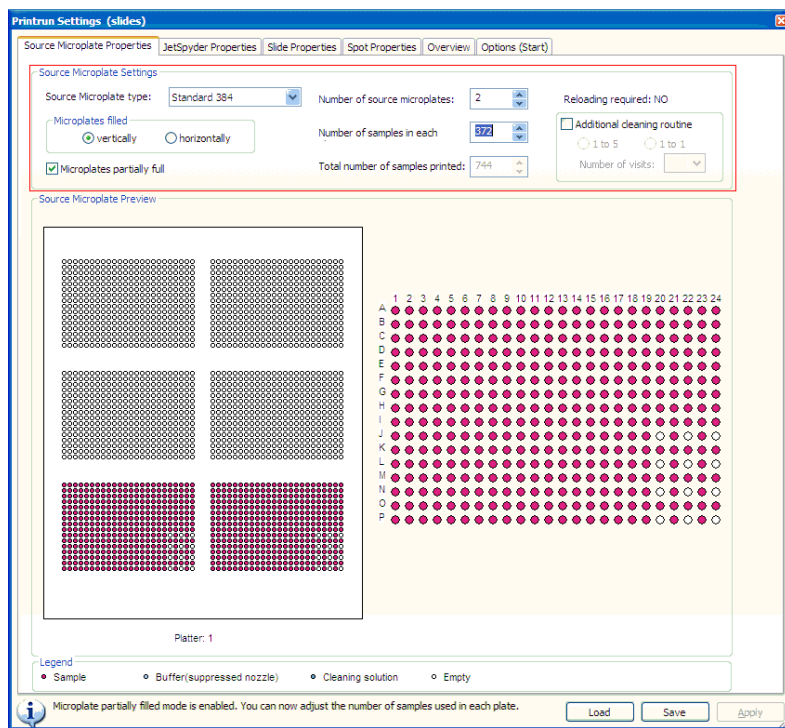


Figure 4-5 The Source Microplate Properties tab showing microplates partially filled in the preview box

When inputting data or adjusting the number of samples, the number of red and white boxes in each plate shown in the **Source Microplate Preview** box, which represent wells containing and not containing samples respectively, changes in real time. Furthermore, the changes accurately reflect the sampling schema employed by the JetSpyder™ during a real print run.

4.1.3 ADDITIONAL CLEANING ROUTINE

Sometimes the user may require a microarray to be produced using a sample type which is particularly adhesive. Other times it may be that the user requires additional stringency with respect to the acceptable level of carryover contamination in the production of microarrays. For such print runs Arrayjet has developed and implemented an additional cleaning routine, which is achieved by substituting either one of the sample plate positions with a cleaning plate containing a suitable solvent (1 to 5) or a dedicated cleaning plate for each sample plate (1 to 1). If the user wants to implement the additional cleaning routine, they must check the **Additional cleaning routine** box and select either the “1 to 5” or “1 to 1” option (**Figures 4-6 and 4-7**).

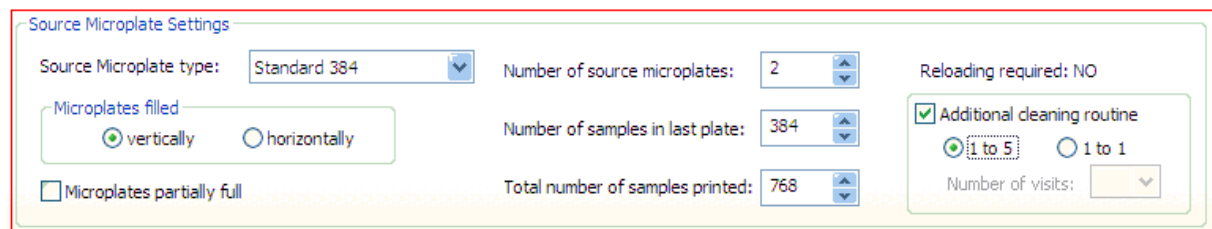
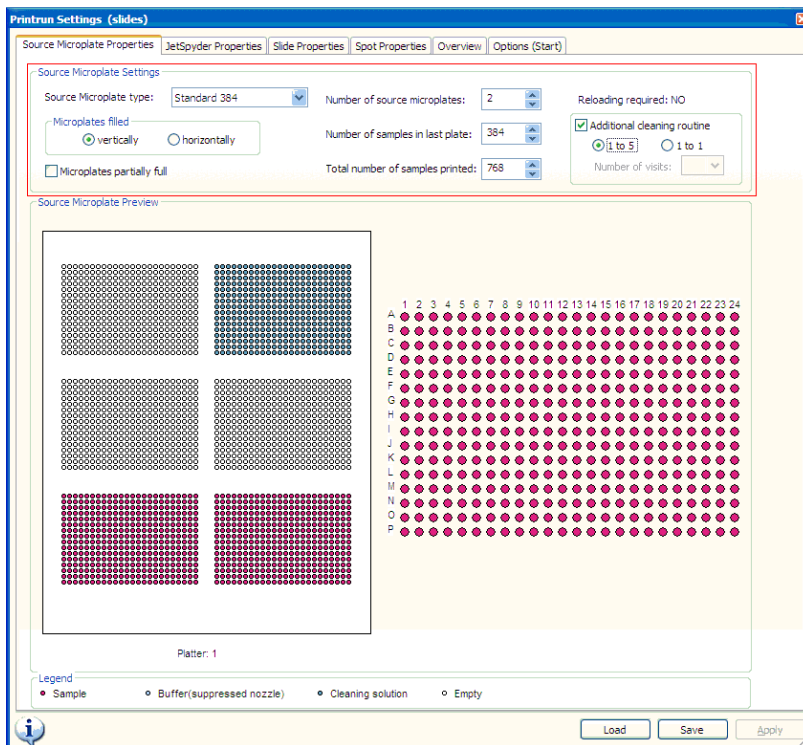


Figure 4-6 The Source Microplate Properties tab showing the additional cleaning plate 1 to 5 in the preview box

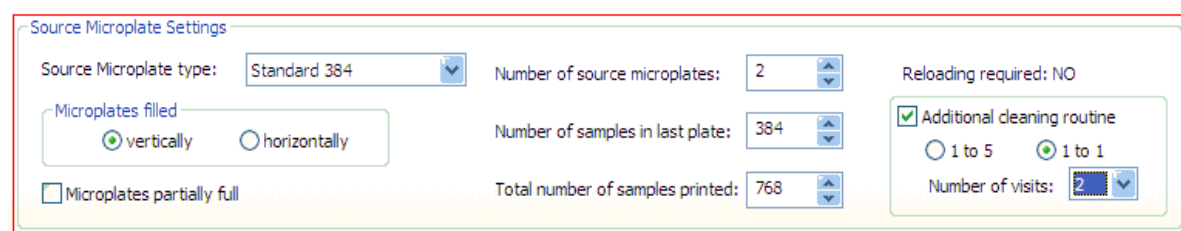
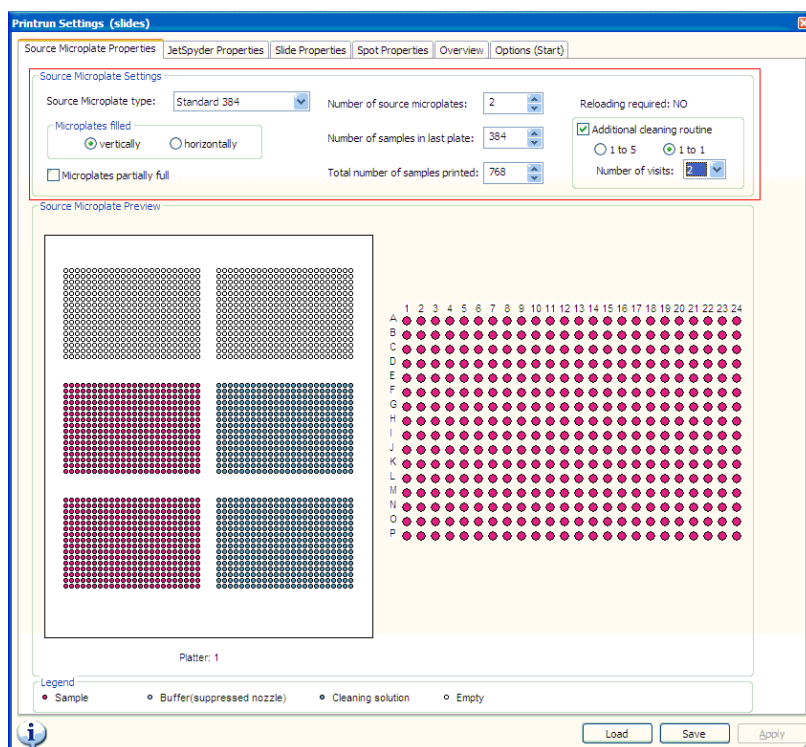


Figure 4-7 The Source Microplate Properties tab showing the additional cleaning plate 1 to 1 in the preview box

The microplate view in the **Source Microplate Preview** box will instruct the user in which position to place the cleaning plate/s, and the well-colouring schema will instruct the user in the quantity of wells to fill, and which wells to fill, though this corresponds exactly with the sample plate/s⁶. For information concerning suitable solvents for cleaning the printhead and JetSpyder, please contact Arrayjet.

If a delidder is enabled then no lids should be placed on the cleaning plates as it is not so critical if the cleaning solution is subject to evaporation. **Lids must not be placed on the cleaning plates.**

Particularly viscose or “sticky” samples could need special cleaning procedures, requiring a user-adjustable number of visits to a cleaning plate after printing each set of samples. Therefore, the “Source Microplate Properties” tab under a “New print run” setup allows the user to decide upon the number of visits to the cleaning plate.

⁶ When the additional cleaning routine is selected the maximum number of plates available (with reloading) is limited to the total of both the source and the cleaning plates.

As the screenshot in **Figure 4-7** shows, Command Centre™ for Marathon allows the selection of a number between 1 and 5, available when “Additional cleaning routine” with “1 to 1” option is selected. The “1 to 1” option suggests that for each microplate of samples there should be placed an associated cleaning plate, as displayed on the “Source Microplate preview”.

Note that when the “1 to 5” cleaning plate option is selected it is only permissible to have a single visit to the cleaning plate for each sample printed.

4.2 JETSPYDER PROPERTIES TAB

The **JetSpyder Properties** tab is the second tab into which the user must input data when designing a new print run protocol. This tab is used to inform the operating system which JetSpyder™ is in use, and also the number of printhead nozzles in use. Also, this tab provides test slide preview information.

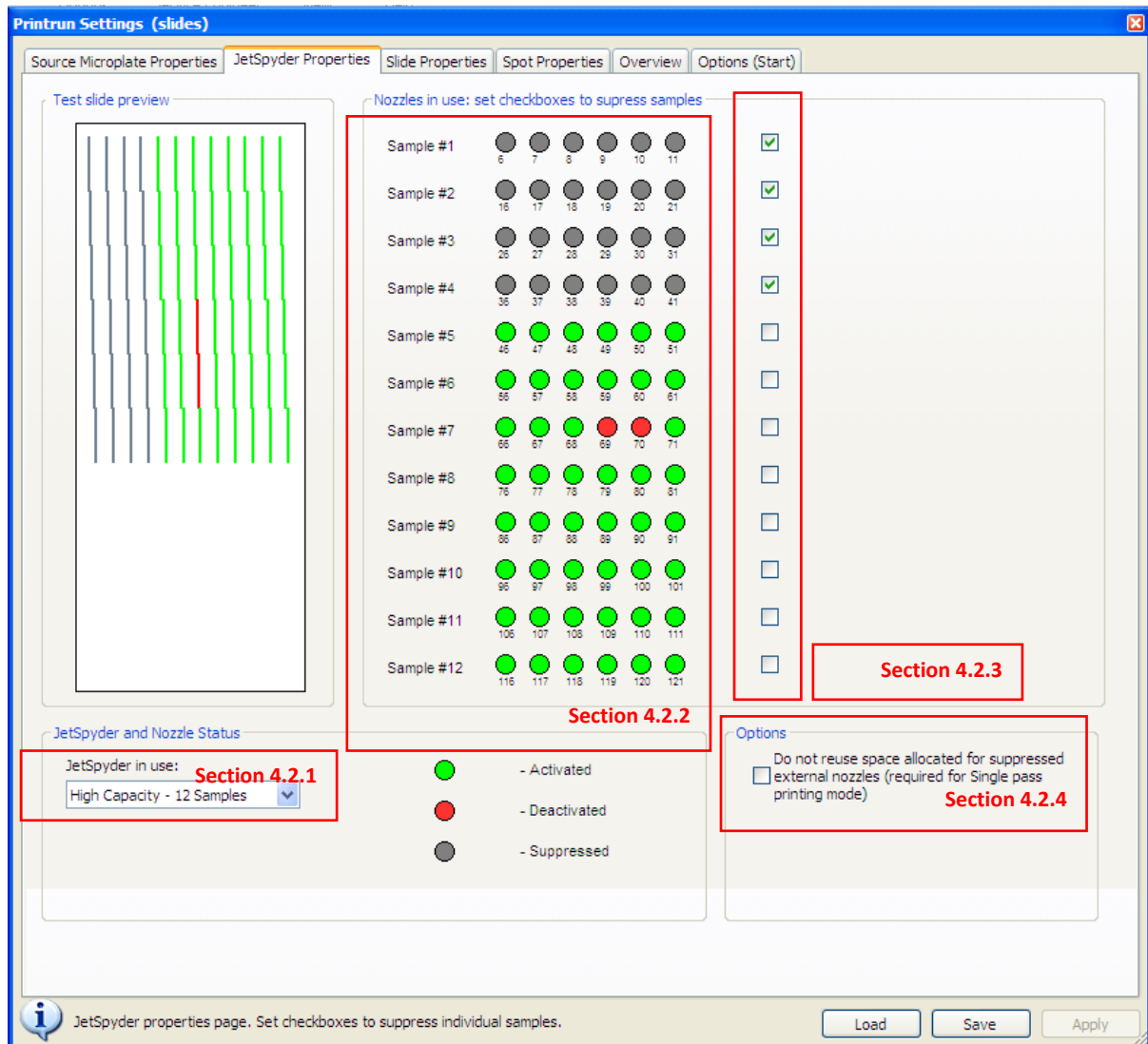


Figure 4-8 The JetSpyder Properties tab

4.2.1 JETSPYDER SELECTION

To switch between JetSpyder™ types, use the drop-down menu located on the **JetSpyder and Nozzle status** box in the bottom left corner of the **JetSpyder Properties** tab. The selected

JetSpyder™ type must match the actual JetSpyder™ used in the instrument. A maximum of two JetSpyders can be concurrently loaded within an instrument (a 32 type and a 12 High capacity or 12 Low volume).

4.2.2 ACTIVATING/DE-ACTIVATING PRINTHEAD NOZZLES

In this section of the **JetSpyder Properties** tab the user is able to toggle the status of individual nozzles. The user can inform the operating system which nozzles are to be considered **activated**, and therefore available for use, or **deactivated**, and therefore not available for use, during a given print run. By moving the cursor to the intended nozzle and **clicking the left mouse button** the user can toggle nozzles between **activated (green)** and **deactivated (red)** states. The activated and deactivated nozzles are represented on the **Test slide preview** in **green** and **red** respectively. As an illustration, if nozzles 69 and 70 were deactivated they would be shown in **red** and the **Test slide preview** updated accordingly (**Figure 4-9**).

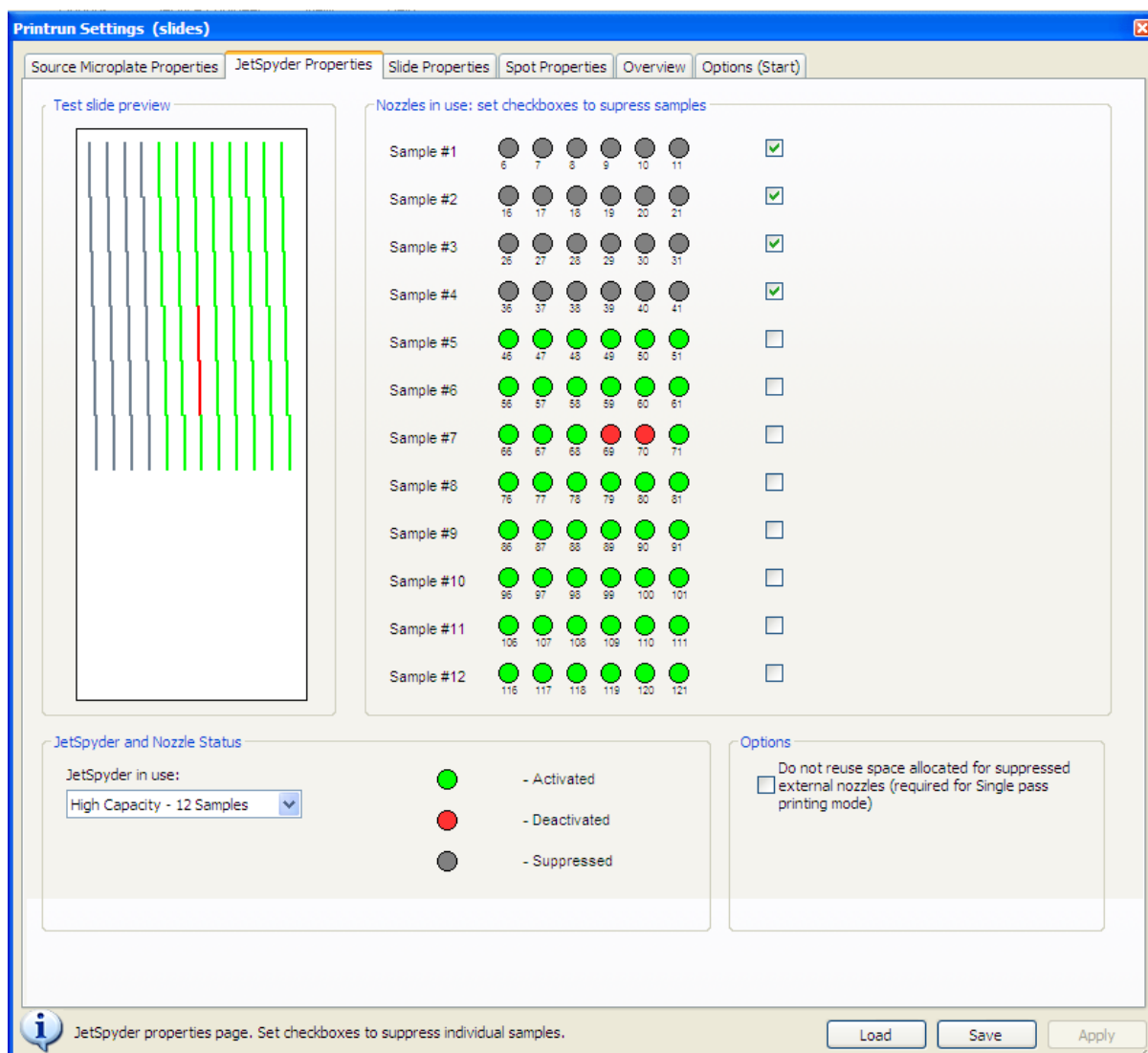


Figure 4-9 The JetSpyder Properties tab-activating and deactivating printhead nozzles

4.2.3 SAMPLE (NOZZLE) SUPPRESSION

Command Centre™ for Marathon v1.4 includes a sample suppression function in the **JetSpyder Properties** tab in the **Nozzles in use: set checkboxes to suppress samples** box. The default printing schema of Marathon Inkjet Microarrays is that probe samples are printed in horizontal lines in groups of 12 or 32 depending on the JetSpyder™ in use. In the event the user requires a microarray to be printed in which the number of samples printed horizontally is less than 12 or 32, the user simply **checks the boxes** pertaining to the nozzle groups to be suppressed, i.e. from which samples are not to be printed: samples will only be printed from nozzles groups which are left unchecked.



Sample suppression is **not a global setting**, in that it applies only to a given print run, and not to any other print run. The **default** setting for sample suppression is that **no samples are suppressed**, so when generating a new print run, unless otherwise specified by the user, no samples/nozzles are suppressed

Sample suppression does not interfere with nozzle activation/inactivation within the GUI, and the colour-coding chosen to illustrate active, inactive and suppressed nozzles is designed to avoid confusion in this regard.



Although given nozzles are prevented from printing samples when suppressed, **nozzles must still be primed with liquid**, since aspiration is controlled via a single syringe pump and all nozzles aspirate samples, albeit different samples, simultaneously.

The user must understand which wells in the microtitre sample plates need to be filled with a substitute sample. The sample substitute needs to closely resemble the sample in viscosity and surface tension, and so it is recommended that neat printing buffer is used.



The microarrayer, when processing a print run in which samples are suppressed, will either **leave space for them** in the horizontal plane during printing **OR allow the space to be used** for the next set of samples depending on the setting within the JetSpyder Properties tab (see **Section 4.2.4**).

It is important to understand which samples will be printed, where within the microarray and which samples to suppress to obtain the desired result. The Overview tab helps with a visual representation of the spots printed and inner suppressed samples introduce gaps by doubling the pitch between its two adjacent spots.

4.2.4 REUSE AVAILABLE SPACE WHEN USING SUPPRESSED NOZZLES

The Marathon was initially designed to print linear blocks of 12 or 32 spots, depending on the type of JetSpyder™ in use. This remains the optimum case for printing and there was no restriction in printing less than that in one pass, just that the space taken by the missing samples was reserved, which was not always desirable.

This version of Command Centre™ allows reuse of space for suppressed samples which boosts the usefulness of this important feature.

Figure 4-10 shows an example of layout with 16 spots which was obtained by printing 8 samples out of 12 in two passes. By setting the spacing between sets of samples and between spots to the same value, the output is a linear block of 16 samples, which looks exactly like using a higher sized JetSpyder™.

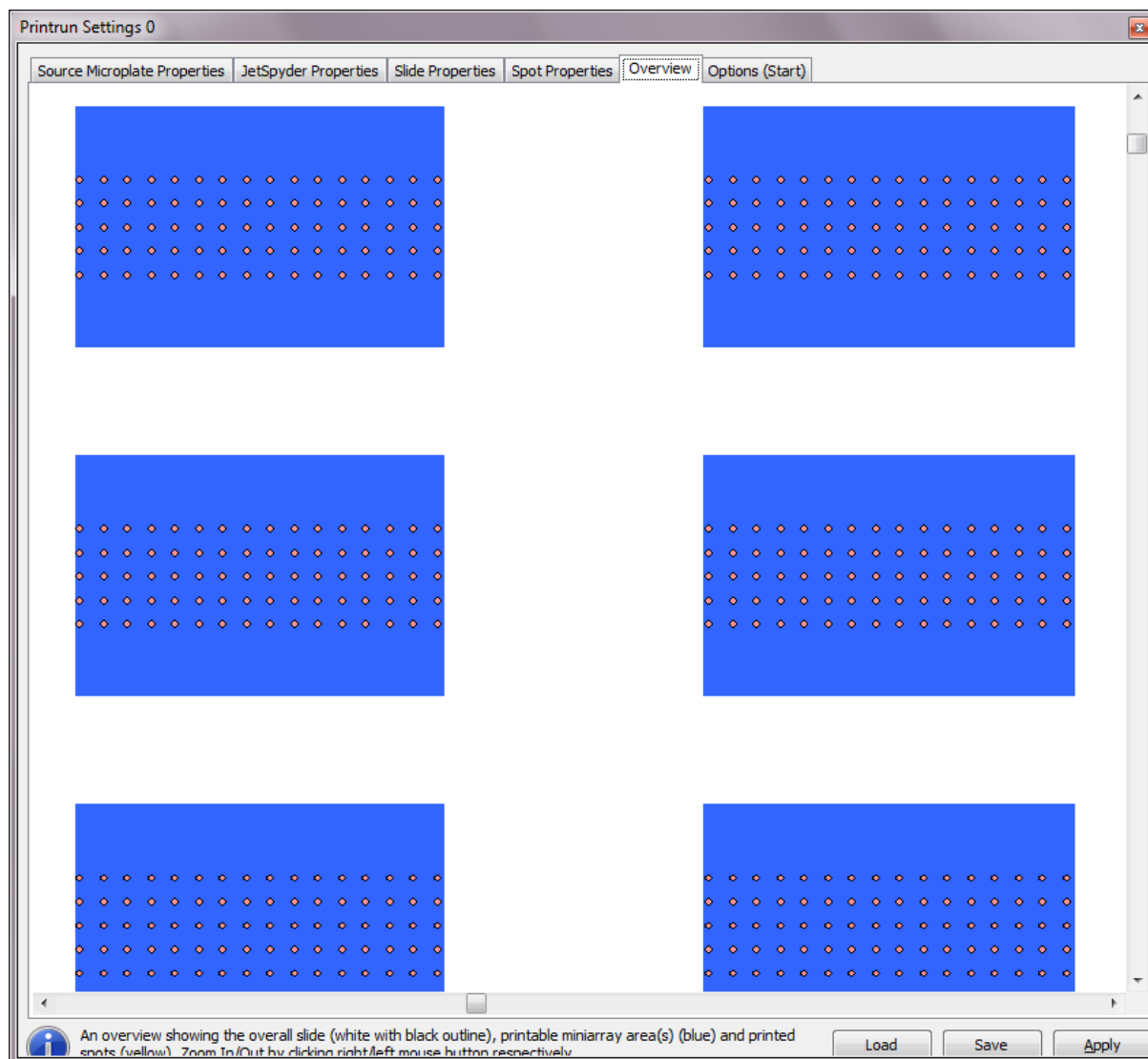


Figure 4-10 Layout 16 linear blocks

This layout was obtained by suppressing the last 4 sets of nozzles, associated to the final 4 samples. The "JetSpyder Properties" tab still allows the user to opt for no reuse of space which is there for backward compatibility. There may be previous layouts designed to accommodate the no reuse of space occupied by external suppressed samples and these files can be loaded into the new software, which will automatically tick that option.

Please note that, as the message of this option mentions, the single pass printing mode (for repeat sets of samples) is not available when the reuse of space for external suppressed nozzles is selected. Wraparound mode is imposed to be able to print repeat sets of samples immediately after the current set ends, as technically the two sets of samples overlap.

4.3 SLIDE PROPERTIES TAB

The **Slide Properties** tab is the third tab into which the user must input data when designing a new print run protocol. This tab is used to setup the number of slides on which microarrays are to be printed, of the print margins required, and whether or not mini-arrays are to be printed and if so, the layout in terms of the number and size of the mini-arrays, and their spatial locations on the slides.

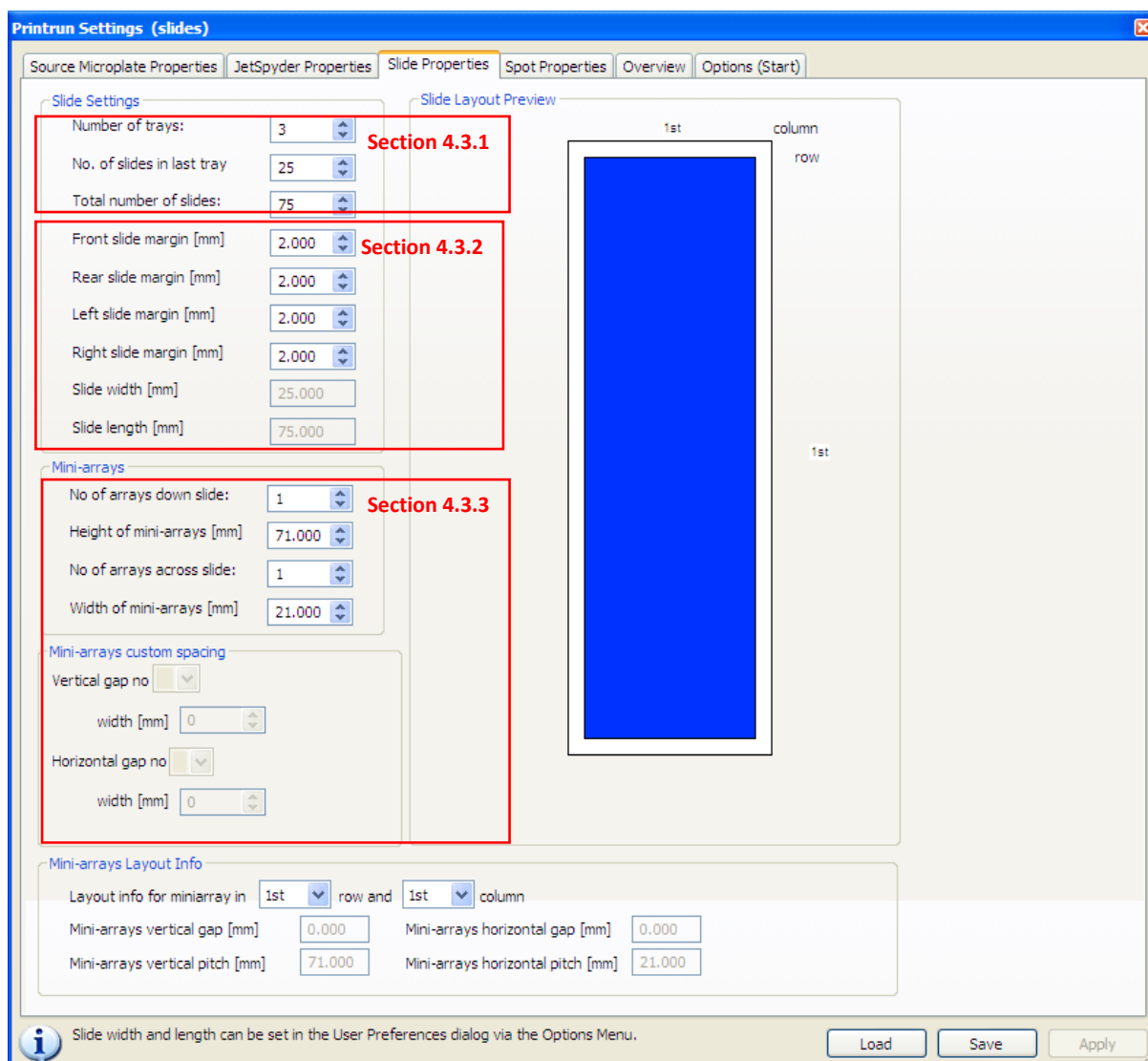


Figure 4-11 The Slide Properties tab

The **Slide Properties** tab provides a graphical overview of an individual slide and its printable areas, which can be modified using standard spin controls and edit boxes (**Figure 4-11**). Within the overall

outline of the slide (shown in white) the printable array area(s) are represented by blue squares. Up to 100 slides, held on 4 trays of 25 slides each, may be printed in a single unattended print run on a Marathon or Super-Marathon Inkjet Microarrayer; in contrast the Ultra-Marathon and Ultra-Marathon II Inkjet Microarrayers can print microarrays on up to 1,000 slides in a single, unattended print run – the slide tray handling systems in the Ultra-Marathon and Ultra-Marathon II have a capacity of up to 40 slide trays.

It is possible to change the printable area (margins, widths and lengths) using the spin controls in increments of plus or minus 0.1 mm. Larger parameter changes can be effected by directly entering values in the edit boxes but only if these values are possible: it would not be possible, for example, for a user to select a larger print area than the area of the slide itself. The slide length and width are shown but cannot be modified on this tab: the default slide dimensions can be set in the **User Preferences** window (Section 3.3.3).

4.3.1 SETTING SLIDE QUANTITY

The user must provide information concerning the number of slides to be printed. This is performed by inputting information into the edit boxes in the **Slide Settings** group box, found in the upper left corner of the **Slide Properties** tab. Here the user may, either by directly inputting numbers into the edit boxes or using the spin controls provided, select a number of slides between 1 and 100 (between 1 and 1000 for an Ultra-Marathon or Ultra-Marathon II). This can be achieved by either entering the **Total number of slides** (between 1 and 100, between 1 and 1000 for an Ultra-Marathon or Ultra-Marathon II), or by inputting information into the box marked **Number of trays** (between 1 and 4, between 1 and 40 for an Ultra-Marathon or Ultra-Marathon II), and then inputting information into the box marked **No. slides in last tray** (between 1 and 25).

4.3.2 ADJUSTING PRINT MARGINS

Print margins define the area in which the microarray or mini-arrays are to be printed. By use of the edit boxes and spin controls within the **Slide Settings** group box the user can define the margins within which the required microarray layout is to be printed. The margins shown on the diagram of the single slide in the **Slide Layout Preview** window will change in real time with the adjustments made by the user, so the user can make and visualise fine adjustments to the margins simultaneously.

The default setting for all margins is 2 millimetres (mm). It is not possible however for the user to define a print margin which is too small to print the number of samples they have selected in the **Microplate Properties** tab. Print margins are not to be confused with slide dimensions, which are edited in the **User Preferences** window.

4.3.3 MINI-ARRAYS

It is possible for the user to divide the area in which the microarray is to be printed into multiple mini-arrays. The user can set the number of mini-arrays to be printed horizontally and vertically, e.g. 2 across x 8 down (a popular combination) to give 16 mini-arrays. An example mini-array layout is shown in **Figure 4-12**.

The user can set the dimensions of the mini-arrays using the spin controls and edit boxes provided in the **Mini-arrays** group box: mini-arrays cannot be larger than the print margins allow, and cannot be too small for the number of samples the user has selected to print in the **Microplate Properties** tab.

Command Centre™ assumes that the mini-arrays are to be spaced evenly within the area defined by the print margins selected by the user. However using the mini-arrays custom spacing the user can define both the horizontal and vertical gaps between each of the mini-arrays. This is represented in the slide layout preview and the gaps and pitches of each mini-array can be displayed within the mini-array layout info as shown in **Figure 4-13**.

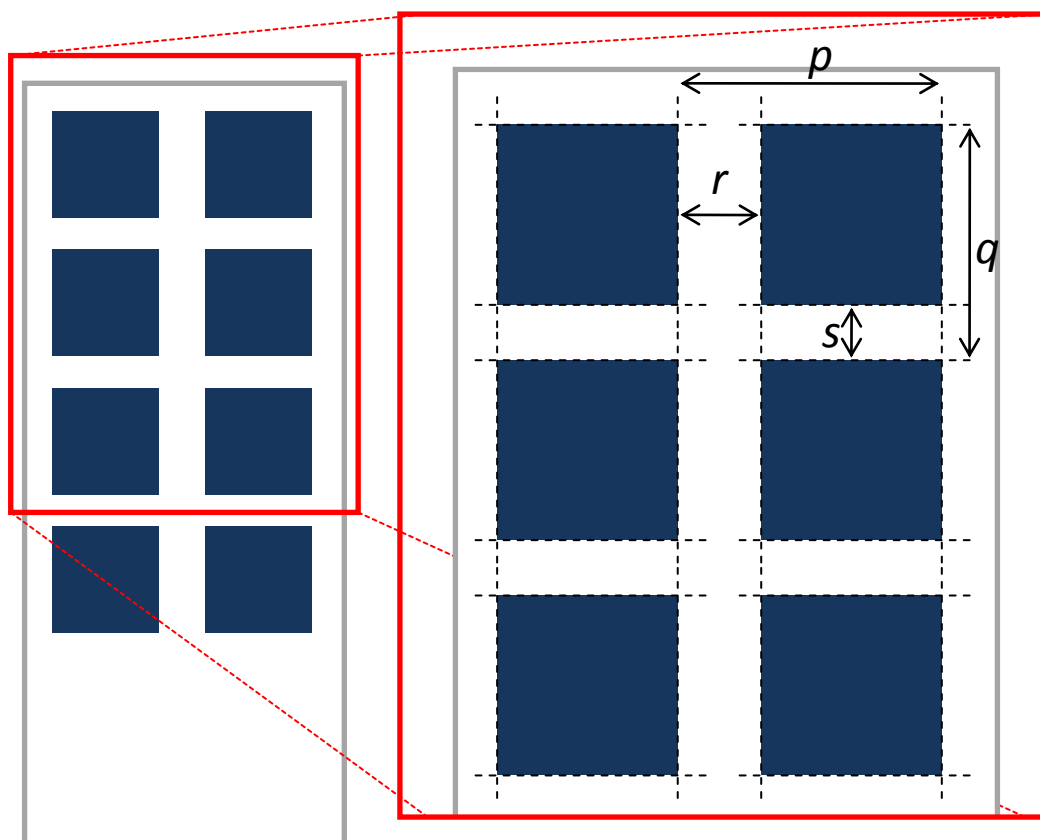


Figure 4-12 Mini-array pitches and gaps – vertical and horizontal -not directly editable by the user:

- Horizontal pitch p
- Vertical pitch q
- Horizontal gap r
- Vertical gap s

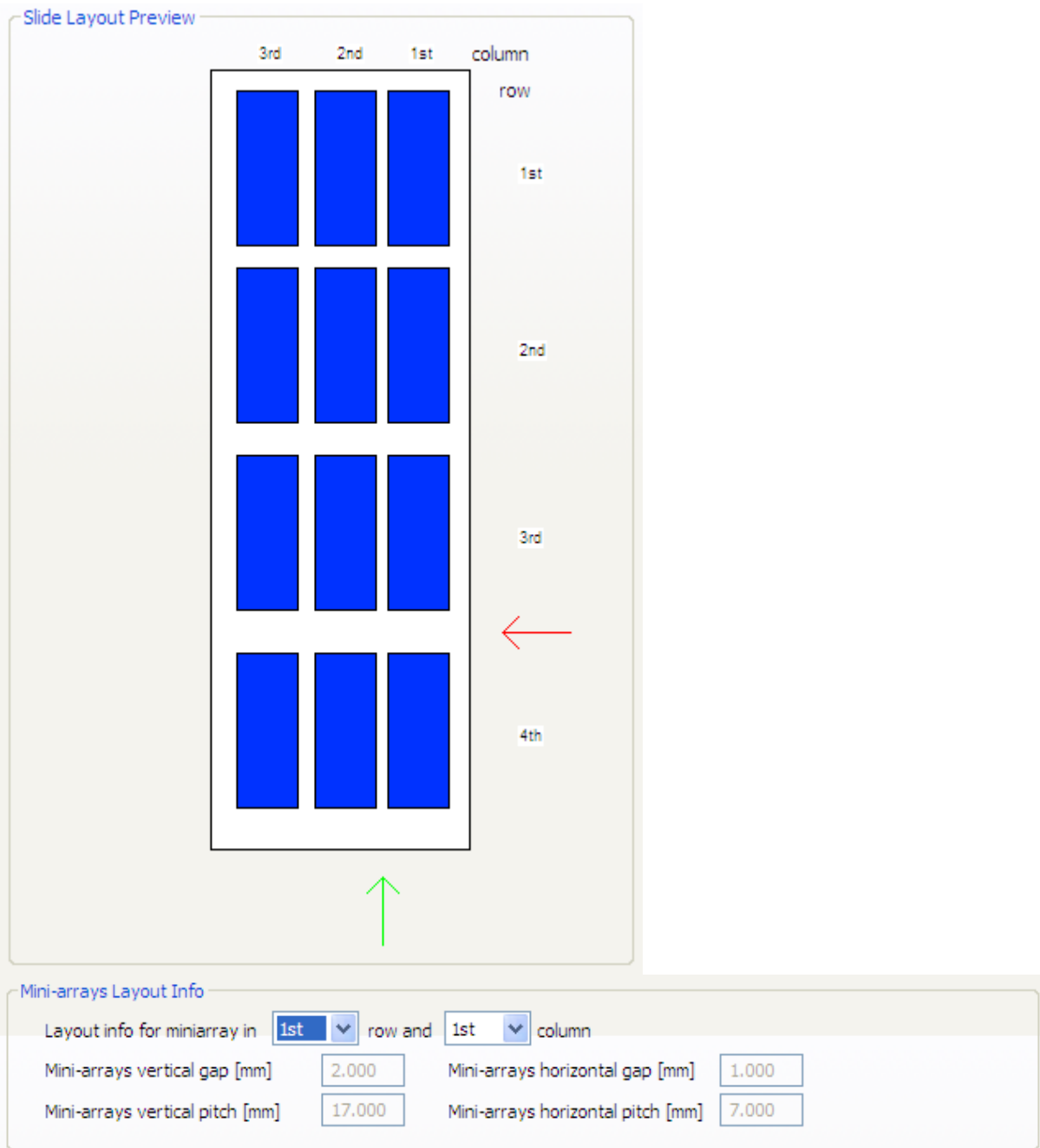


Figure 4-13 Custom mini-array pitches and gaps

4.4 SPOT PROPERTIES TAB

The **Spot Properties** tab is the fourth tab into which the user must input data when designing a new print run protocol. This tab is used to inform the operating system of the number of drops per spot, number of repeats, the spot margins required, and the layout options.

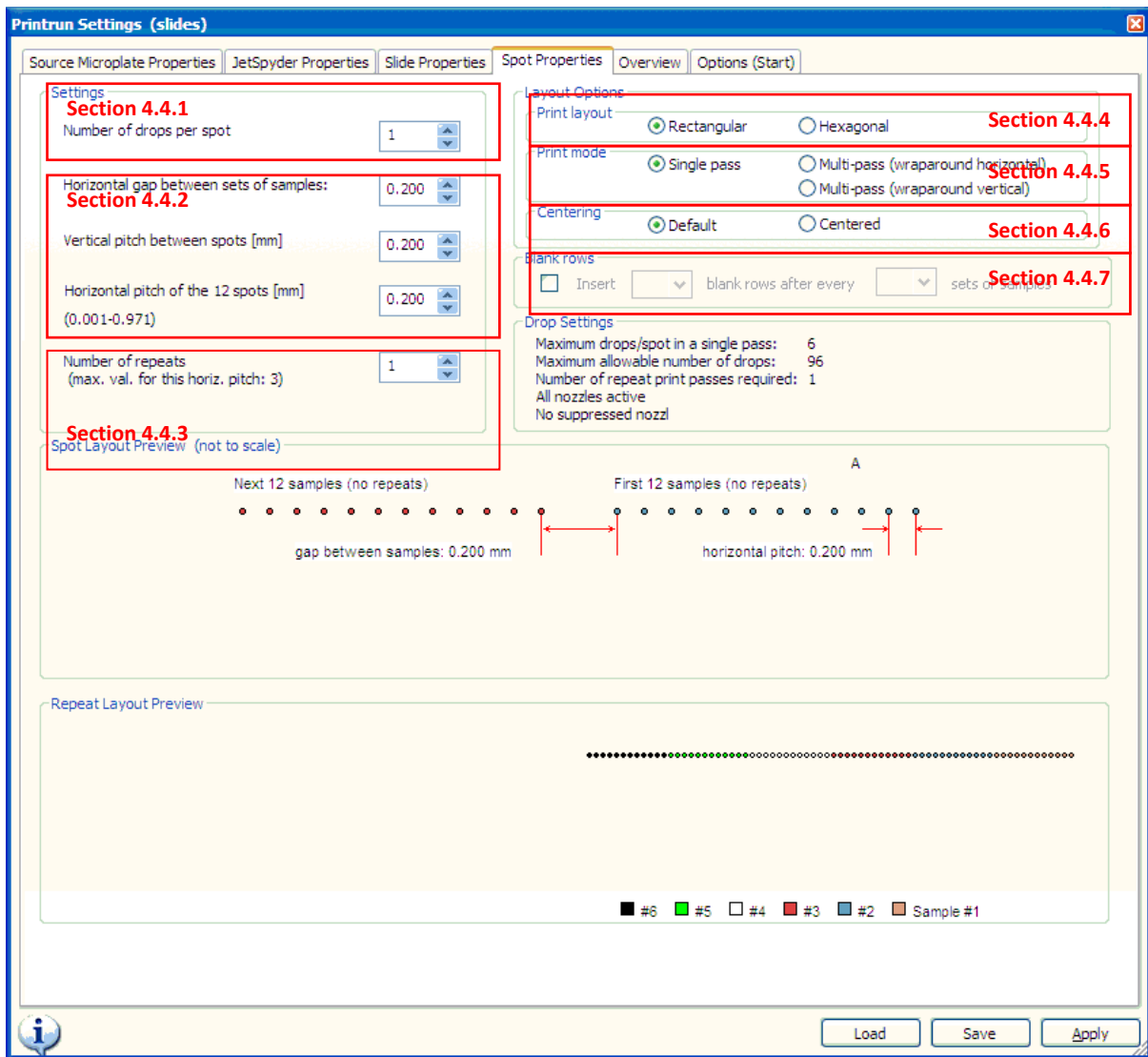


Figure 4-14 The Spot Properties tab

The parameters defined in the **Spot Properties** tab (**Figure 4-14**) dictate the layout within each array. The software assumes default pitch and gap parameters based on an assumed spot diameter of 100 microns.

4.4.1 SETTING NUMBER OF DROPS PER SPOT

The user must provide information concerning the number of drops per spot to be printed. In the **Settings** group box in the upper left corner of the **Spot Properties** tab it is possible to, either by directly inputting a number into the edit box or using the spin controls provided, select a number of drops per spot between 1 and 100⁸.

⁸ The print head will continue to make passes over the slides to print numbers of drops per spot in excess of the number of available nozzles per sample. The maximum number of drops (up to 100) shall be a multiple of the maximum number of passes. For more information, please contact Arrayjet.

4.4.2 SETTING PRINTING PITCH - HORIZONTAL; VERTICAL; BETWEEN SAMPLE SETS

The user must set the horizontal and vertical spot pitch (centre-to-centre distance between spots) using the edit/spin controls in the **Settings** group box. When creating a new print run their default values are set to 0.2mm.



All values of pitch, or centre-to-centre distance, between spots are shown in millimetres.



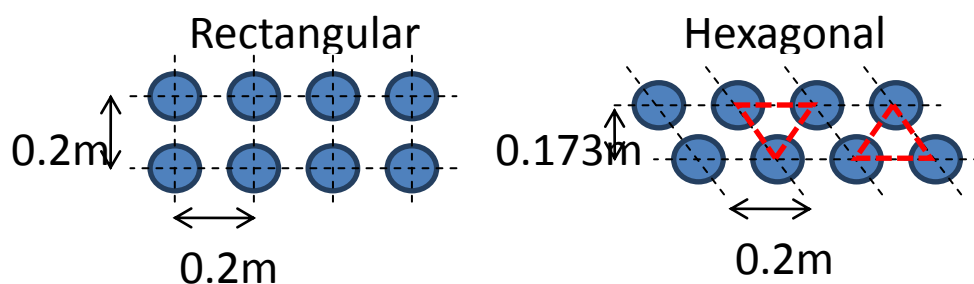
Whereas **pitch** is the centre-to-centre distance between individual spots, **gap** is the horizontal spacing, centre-to-centre, between the last spot of one set of samples and the first spot of the next set of samples. It can be set to the same value as the horizontal pitch

4.4.3 SELECTING THE NUMBER OF REPEATS

The user must set the number of repeats using the edit/spin controls in the **Settings** group box. The maximum number of repeats allowed depends on the mode selected: repeats may be printed in a single pass or in multiple passes of the printhead (single pass versus multi-pass wraparound repeats). See **section 4.4.5** for more detailed information.

4.4.4 LAYOUT OPTIONS I - RECTANGULAR VERSUS HEXAGONAL PRINTING

Selecting the most appropriate horizontal and vertical pitches and gaps for a print run allows the user to place spots at high density while ensuring that adjacent spots do not merge on the array (**Figure 4-15**)



In Hexagonal Grid, each triplet of spots forms an  equilateral triangle

Figure 4-15 Hexagonal grid allows spots to be packed together more densely in vertical pitch.

The optimum spot pitch for a particular print run will vary depending on the diameter of the sample spots printed. Spot diameter varies depending on the sample type, print buffer chemistry, surface chemistry and the number of drops printed per spot.

Spots may be packed more tightly together by selecting to use a hexagonal printing layout; the vertical pitch is automatically reduced so the spot-spot pitch in the diagonal and horizontal directions are the same (**Figure 4-15**). This will increase the spot density, over rectangular printing, by approximately 15%.



In **hexagonal grid** printing the vertical pitch is automatically calculated by taking the horizontal pitch defined by the user and multiplying it by $\sqrt{3}/2$; the user is only free to select the horizontal pitch.

To choose between rectangular and hexagonal spot layouts, click the appropriate radio button in the **Layout Options** group box. The default layout when creating a new print run is **Rectangular**.

When setting a rectangular array layout, the user first selects values for horizontal pitch and vertical pitch between spots (Section 4.4.2).

When setting a hexagonal array layout the user first selects a value for horizontal pitch and then clicks the button for **Hexagonal** printing. The software will automatically calculate the correct vertical pitch in relation to the horizontal pitch selected.

4.4.5 LAYOUT OPTIONS II - SINGLE PASS VERSUS MULTI-PASS REPEATS



Please note that Single Pass mode can be enabled by selecting **Do not reuse space allocated for suppressed external nozzles (required for Single Pass printing mode)** from the **JetSpyder Properties** tab, followed by selecting **Single Pass** from the **Print mode** block in **Spot Properties** tab. This is a special, very efficient mode of printing which allows spotting more repeats in one pass, but imposes the spacing between repeats to a fix value depending on the spot pitch.

When printing in **single pass** mode the user is able to print up to 4 repeats of a set of 12 or 32 samples in one pass of the printhead on every slide or within a single mini-array on every slide⁹. In this mode the gap between repeats is determined by the horizontal pitch and is not changeable by the user. In contrast when printing in **multi-pass** mode, each of the repeats are printed in a separate pass of the printhead which takes a little longer but offers the advantage of more efficient use of space, selectable gap between repeats and increases the numbers of repeats allowed to 999 (space permitted). The maximum number of repeats that can be printed in a single pass depends on the horizontal pitch and the print margins defined by the user. Command Centre™ will show the maximum number of repeats available for a particular horizontal pitch within a given set of print margins (see **Figure 4-16**).

⁹ Mini-arrays are all identical to each other.

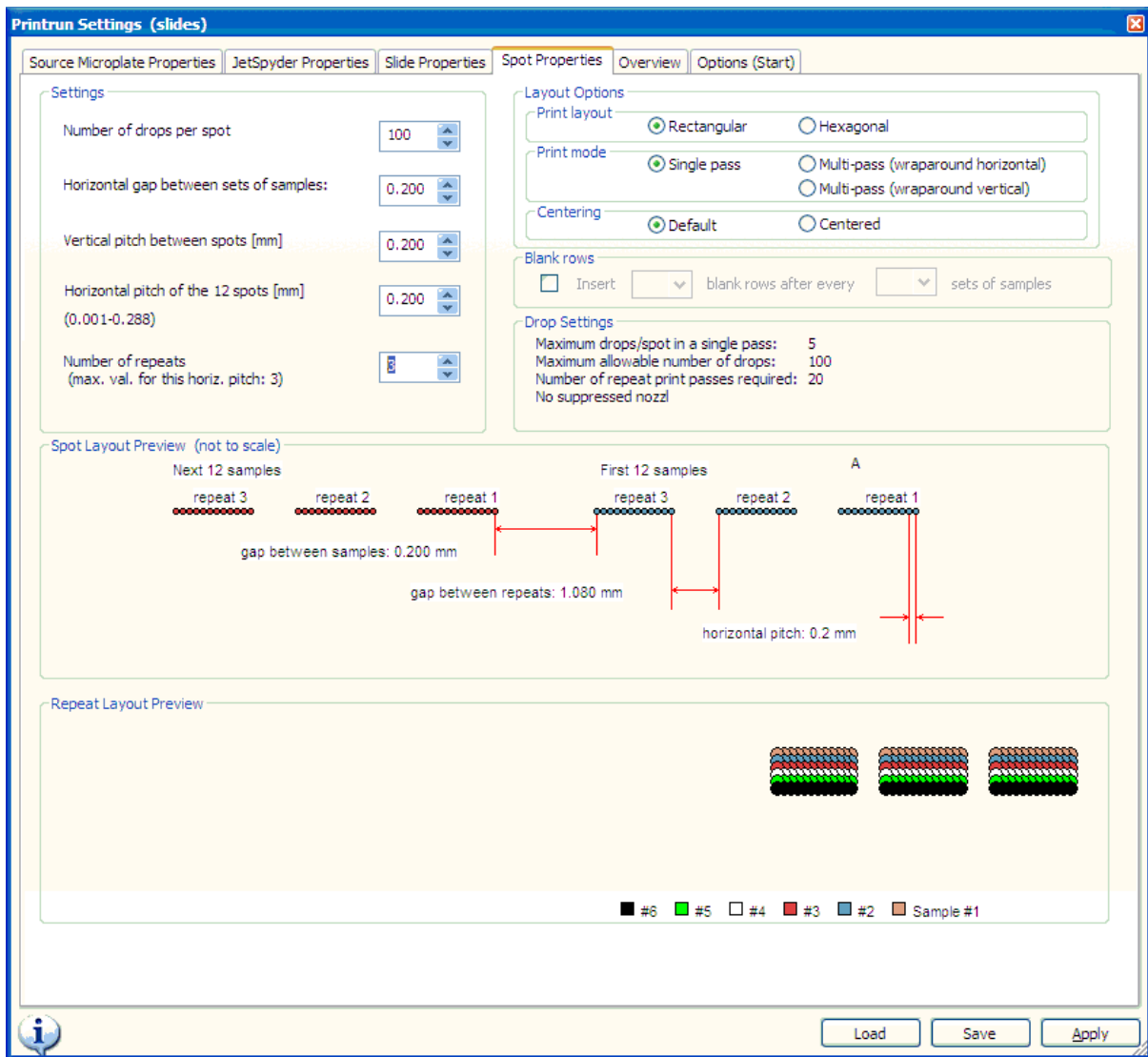


Figure 4-16 Number of repeats

Selecting the most appropriate mode of printing repeats for a given print run allows the user to place spots at higher speed or to maximise the use of space, depending on their requirements.

In multi-pass repeats the user has the choice to place the repeats either horizontally or vertically. By selecting wraparound horizontal all the repeats are placed next to each other starting from the right most position working leftwards, wrapping onto a new row if necessary. In wraparound vertical all the repeats shall be placed directly below each other starting from the topmost position working downwards, within the slide.

4.4.6 LAYOUT OPTIONS III - CENTRING SPOT LAYOUTS WITHIN MINI-ARRAYS

Although once defined the mini-array target area dimensions are fixed, the positioning of the actual spots to be printed within a mini-array can be adjusted by the user such that spot layout is centred

within the mini-array target area, and not positioned in the top-right-corner as was previously the case (**Figure 4-17**).

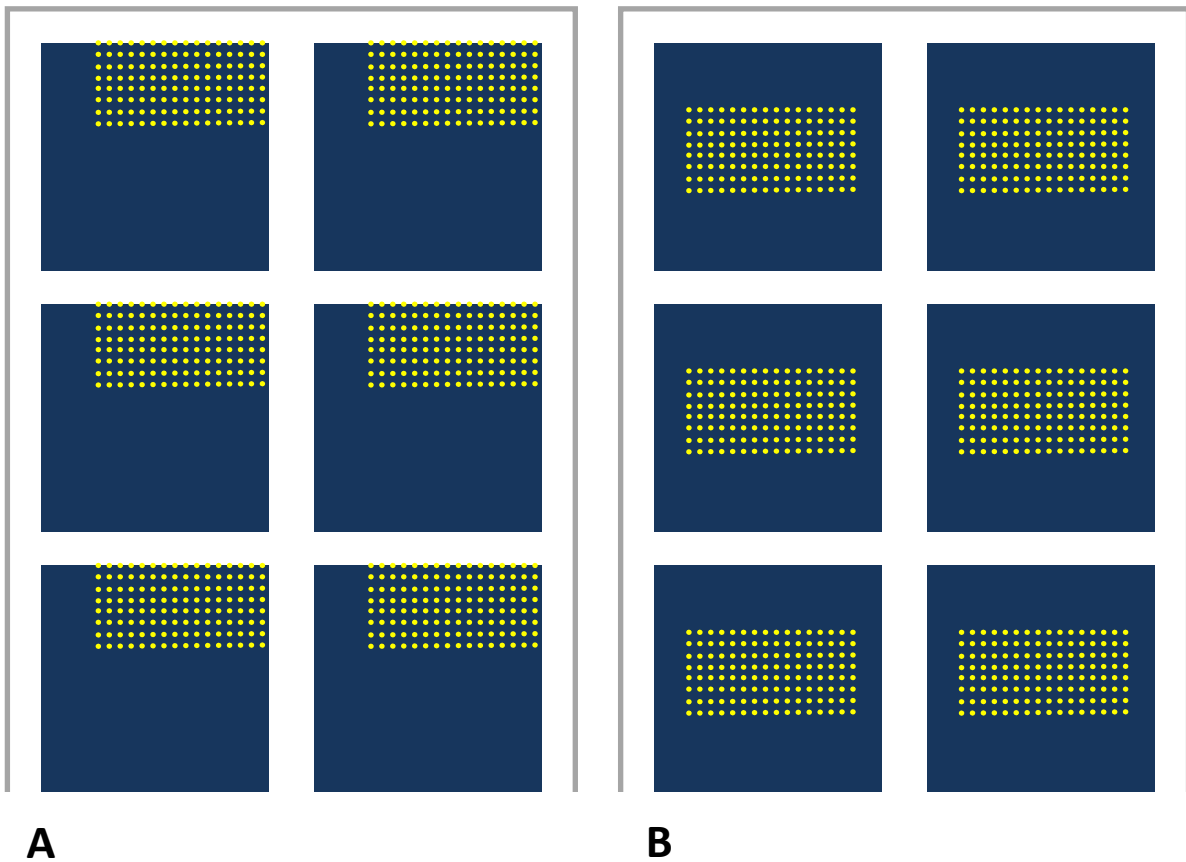


Figure 4-17 Spot layout oriented to top right corner (A) versus centred spot layout (B) within mini-array target areas.

4.4.7 LAYOUT OPTIONS IV – BLANK ROWS

In order to split the print layout into regularly spaced blocks there is a new feature that allows a number of blank rows (1 to 5) to be inserted after every user defined number of printed sets of samples. **Figure 4-18** shows an example of inserting 2 blank rows every 20 sets of samples and the resulting print layout is shown on the overview shown in **Figure 4-19**.

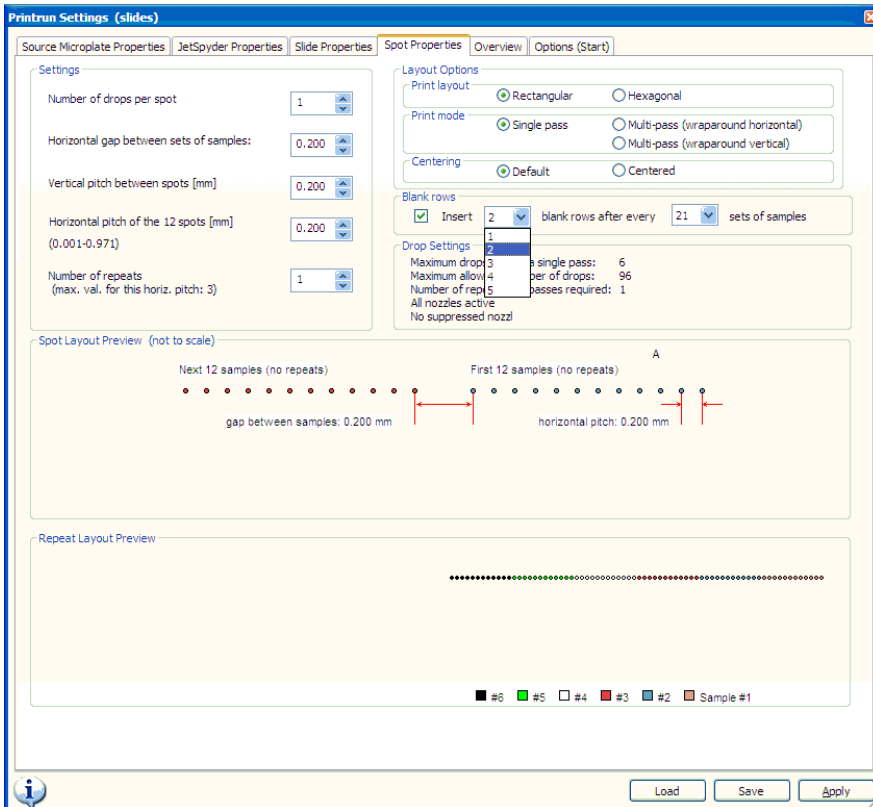


Figure 4-18 Enabling Blank Rows

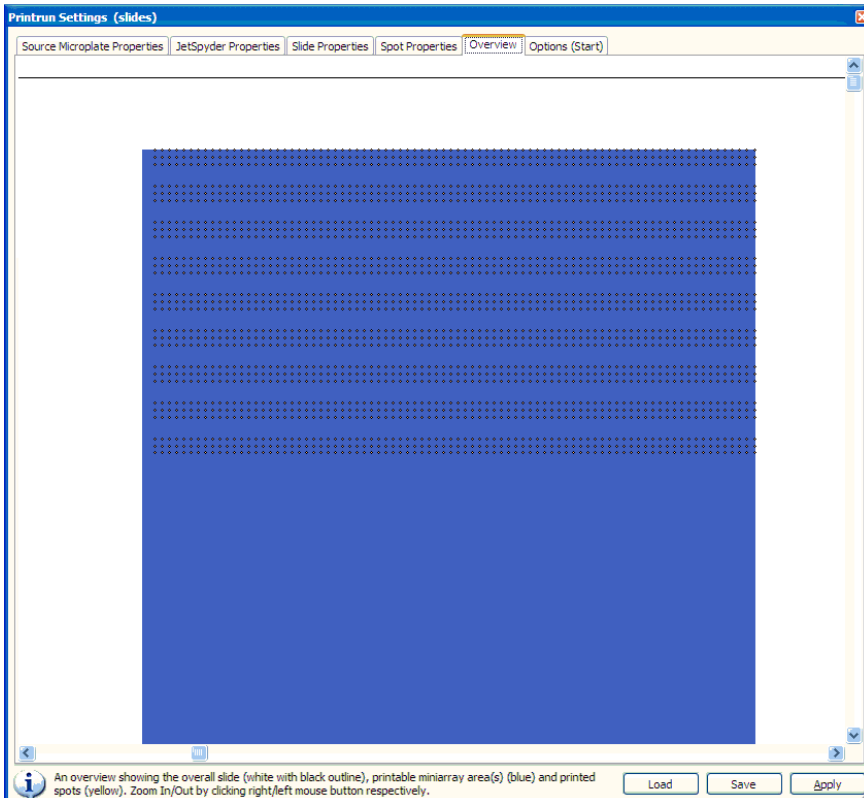


Figure 4-19 Print overview showing blank rows

4.5 OVERVIEW TAB

The **Overview** tab is the fifth tab when designing a new print run protocol. Within the **Overview** tab (**Figure 4-20**) the user will see an overview of both the printable areas (blue) and the spots to be printed (yellow). It is possible to zoom into or out of the overview by clicking right and left mouse buttons respectively.

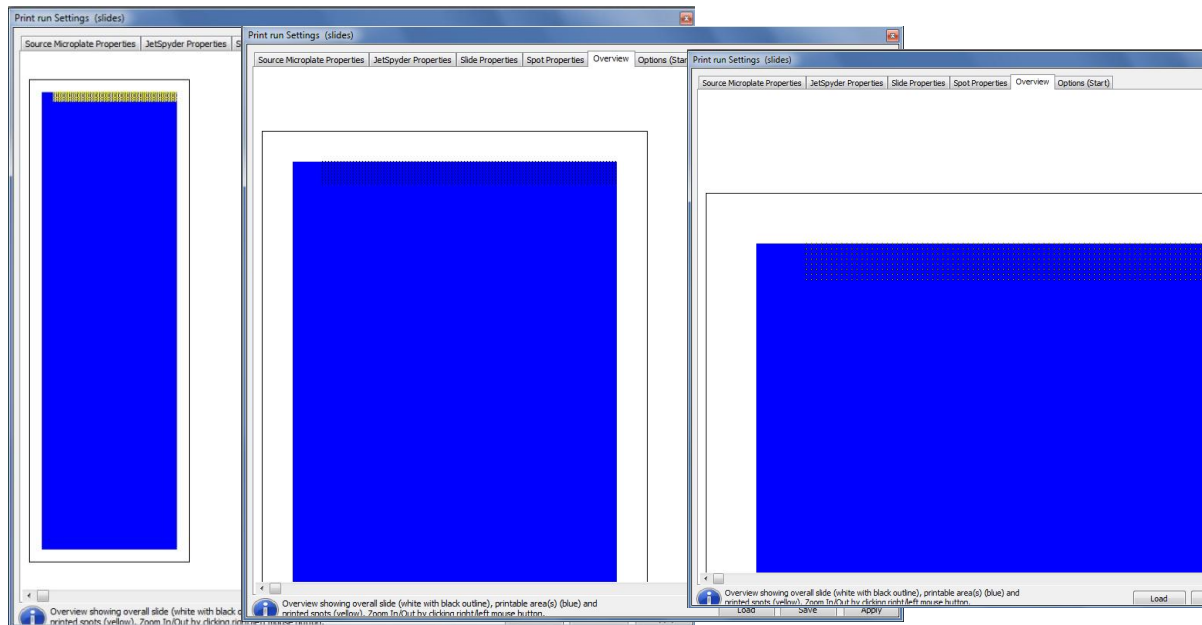


Figure 4-20 The Overview tab

4.6 START TAB

The **Start** tab is the sixth and final tab into which the user must input data when designing a new print run protocol. This tab is used to instruct the operating system to start the print run and provides the various print run options (section 4.6.1) and output file types, as well as enabling the user to select an input file to bring over probe sample annotations and merge them with the output file of the print run (section 4.6.2).

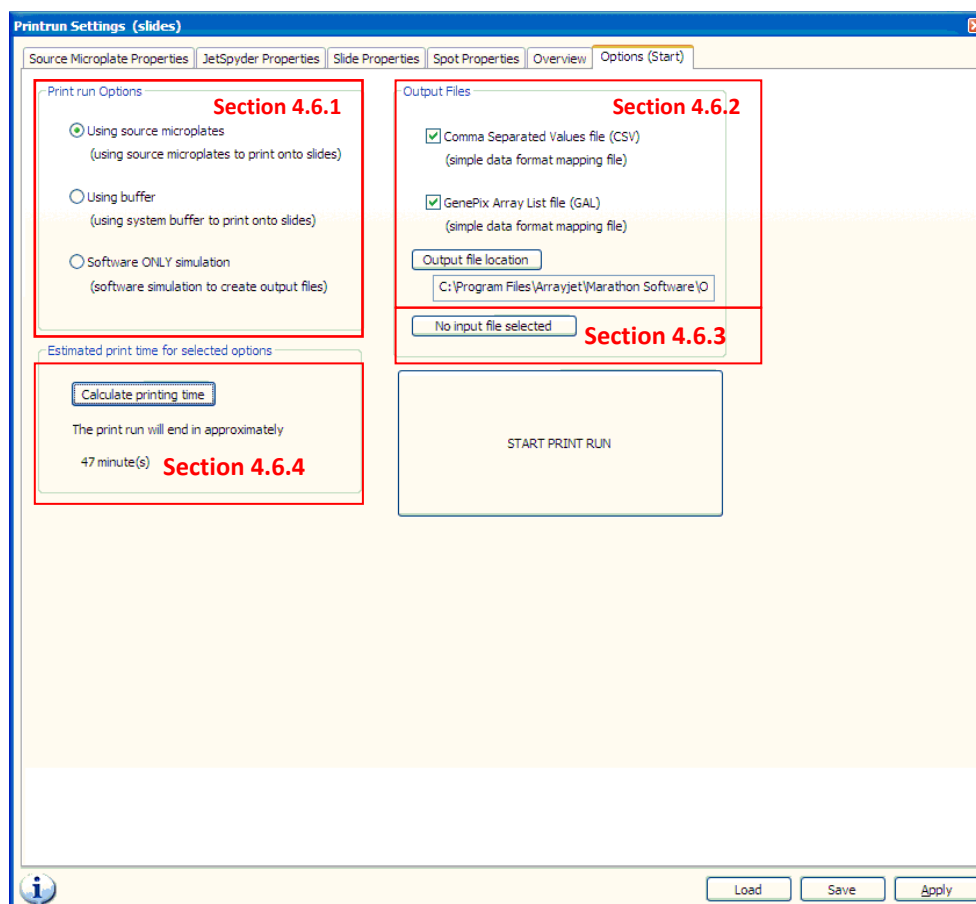


Figure 4-21 The Start tab

4.6.1 PRINT RUN OPTIONS

A print run protocol may be run in one of three different modes:

- **Microplate** – The system will print probe samples from microplates according to the parameters set in the print run protocol;
- **Buffer** – The system will print only system buffer according to the parameters set in the print run protocol. This mode is used for protocol testing and does not create an output file;

- **Simulation** – The system simulates the test run protocol; no actual printing takes place but the relevant output file is still produced according to the user-defined parameters. This mode may be used to confirm that input/output files and tracking are operating according to user requirements.

It is possible to toggle between the different printing modes using the radio buttons in the **Print run Options** group box.

4.6.2 OUTPUT FILE TYPES

The user’s choice of output files may be made from Comma Separated Variable (CSV) and/or Genepix Array List (GAL) formats by selecting the appropriate checkboxes in the **Output Files** group box. These output files can be transferred to a specific file location using **Save as** button also found in the **Output Files** group box. The output CSV/GAL files are generated once the print run is complete.¹⁰ The currently set output file location is displayed below the Output file location selection button.

4.6.3 INPUT FILES

It is possible for the user to select an input file for use with the print run prior to starting a print run. By clicking on the **No input file selected** button in the **Output Files** group box in the **Start** tab, the user activates the **Load Input File Options** pop-up (**Figure 4-22**) which invites them to browse for an input file, by clicking the **Browse** button in the **Load Input File** box.

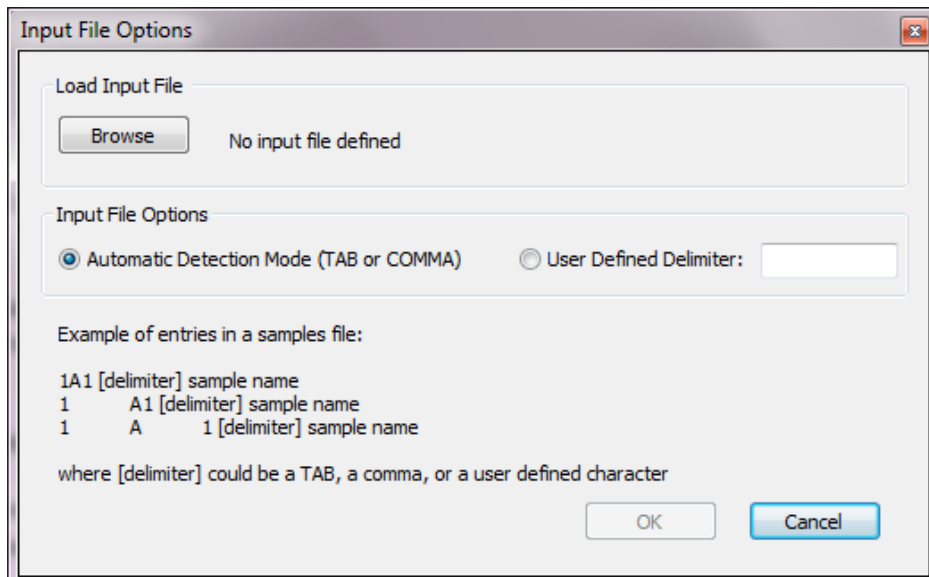


Figure 4-22 Input File Options pop-up

¹⁰ Buffer print runs do not generate output files

Before doing this however the user must inform the software of the delimiter type in the input file they intend to use for the print run. There are two options available, both of which can be found in the **Input File Options** pop-up, within the **Input File Options** box: **Automatic Detection Mode (TAB or COMMA)** and **User Defined Delimiter**. If the user requires a specific, non-standard delimiter, such as a semi-colon, the correct choice is **User Defined Delimiter**, by clicking the appropriate radio button and entering the delimiter of choice as text in the box. If the user does not require a non-standard delimiter then the correct choice is **Automatic Detection Mode**. The software automatically detects how many columns in the input file are used for the Microplate ID if TAB separated values are used. This may be from 1 to 3 as shown in **Figure 4-23**.

Option	Input File Column 1	Input File Column 2	Input File Column 3
1 Column ID	1A1		
2 Column ID	1	A1	
3 Column ID	1	A	1

Figure 4-23 Column use in input files for microplate identification: 1, 2 AND 3 column ID options

The user must select then select the appropriate input file using the **Browse** button.



Input file processing is not a smart process; that is, if the wrong file is selected, there is no error message or other warning to alert the user to the mistake. The output file however may be corrupted as a result.

4.6.4 TIME ESTIMATOR

This feature is extremely useful in that it will provide an estimation of the print time required for the given run. This is particularly useful for planning the print start and finish times of the microarrayer.

Note that the time given is only an estimate and the actual printrun time may vary slightly.

4.7 TARGET MICROPLATE PROPERTIES TAB

When designing a new print run protocol to print onto target microplates (command: **File>New Print run>microplates**) a new window opens within the Command Centre™ main window (**Figure 4-24**), with a new tab in place of the **Slide Properties** tab – **Target Microplate Properties**:

- Source Microplate Properties
- JetSpyder Properties
- *Target Microplate Properties*
- Spot Properties
- Overview
- Options (Start)

Source Microplate Properties; Spot Properties; Overview¹¹; JetSpyder™ Properties; and Start tabs are the same as when designing a new print run protocol for slides (Sections 4.1, 4.2, 4.4, 4.5 and 4.6). There are fewer parameters to set in the **Target Microplate Properties** tab compared to the **Slide Properties** tab.

The **Target Microplate Properties** tab is the third tab into which the user must input data when designing a new print run protocol for printing onto microplates. This tab is used to inform the operating system of the number of target plates on which microarrays are to be printed and the layout in terms of the print margins required.

The **Target Microplate Properties** tab provides a graphical overview of the target microplates and their printable areas using standard spin controls and edit boxes (**Figure 4-24**). The printable array area(s) are represented by blue squares and the number of mini-arrays that can be printed is fixed at 96 (12 across, 8 down). The Marathon can hold 4 trays each containing 5 target microplates, for a maximum of 20 microplates.

¹¹ The overview tab is not strictly the same as for a slide based print run, as it is adapted to the target microplate layout. The principle is the same, however; it shows an overview of the printable areas, spots printed and enables the user to zoom in/out of the view by clicking right/left mouse button.

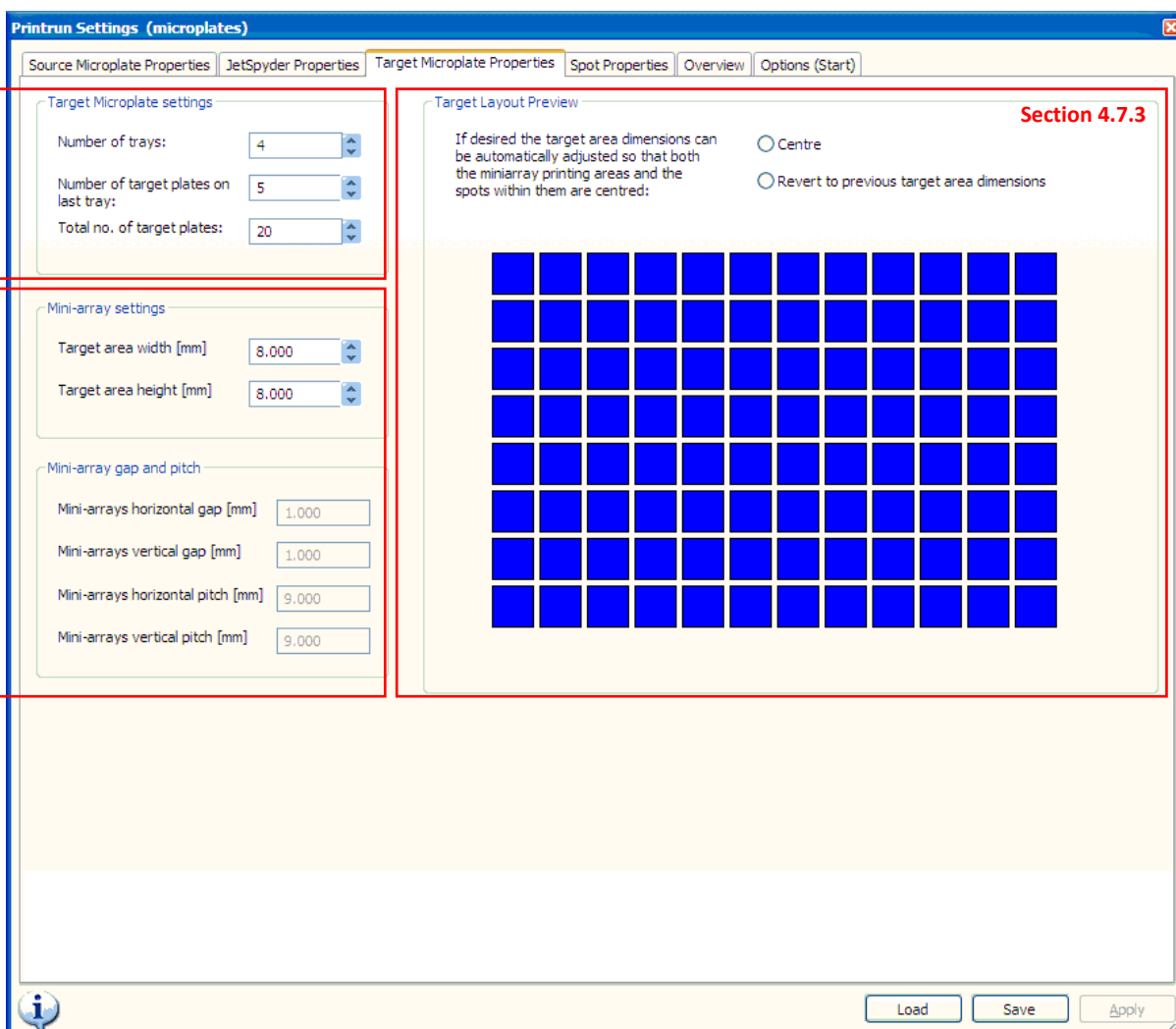


Figure 4-24 The Target Microplate Properties tab

4.7.1 SETTING OUTPUT PLATE QUANTITY

Instead of printing up to 100 slides as with slide-based printing, the user can enter up to 20 output plates using either the spin control or edit box provided. The same information may also be entered by selecting the number of trays and the number of target plates on the last tray.

4.7.2 ADJUSTING MINI-ARRAY MARGINS

Each target plate consists of 96 individual printable areas (mini-arrays), whose target areas can be adjusted up to a maximum of 8.0 mm in length by 8.0 mm in width using the spin controls or edit boxes provided. When increasing or decreasing the target area widths and lengths, the mini-array gaps and pitches are re-calculated automatically so that the printing areas stay centred within the target areas. For more information on mini-array gaps and pitches, see section 4.3.3.

4.8 MULTIPLE PRINT RUNS

This capability enables users to define different print run parameters for different sub-print-runs, or subruns, and print them in the same, uninterrupted print run. A subrun is defined as a print run within a print run, in which a discrete subset of samples is printed to a discrete subset of slides. The objective of the multiple print run capability is to enable the user to leave the microarrayer running for as long as possible, maximising production capacity, whilst performing a number of wholly different print runs: the user can therefore print one set of slides from one group of microplates with one set of parameters (margins; pitches; drops per spot; mini-array layout and so on), complete the print run, and then immediately, and without further intervention, start another print run from another group of microplates onto another group of slides with an entirely different set of parameters. This process can be repeated until all of the slides on the bed have been printed and/or all of the microplate capacity of the unit, with or without manual re-loading of microplates, has been used. Importantly, subrun definitions are limited to whole microplates – it is not possible to finish one subrun within a microplate and start the following subrun within the same microplate. Furthermore, this capability is not yet available for the Ultra-Marathon and Ultra-Marathon II Inkjet Microarrayers, and is only available for the Marathon and Super-Marathon.

When designing a new print run protocol to create multiple print runs simultaneously (command: **File>New Print run>Multiple Print Runs (slides only)**) a new window opens within the Command Centre™ (**Figure 4-25**).

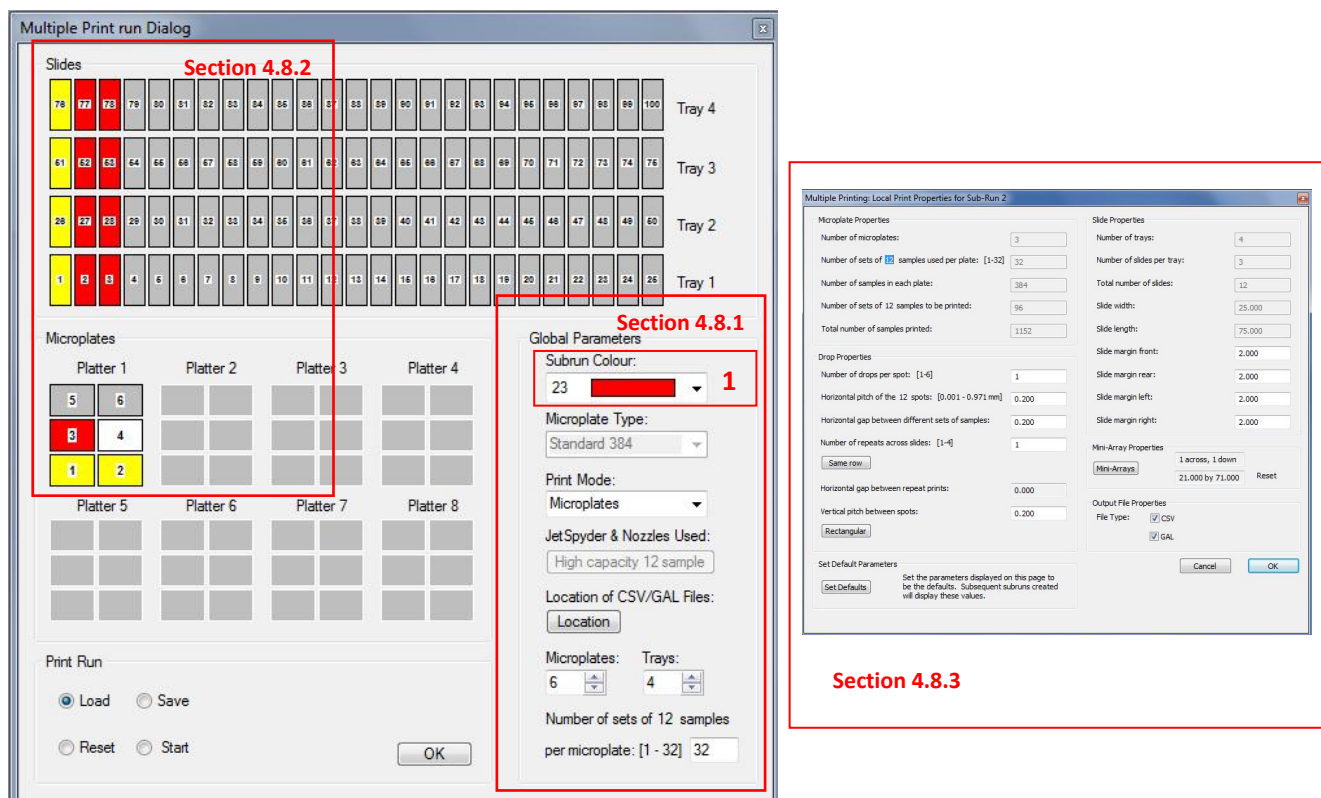


Figure 4-25 The Multiple Print run Dialog window

The **Multiple Print run Dialog** window provides a graphical overview of the position of the groups of microplates and slides, differentiated by colour, on the bed of the microarrayer. The user must input parameters for each subrun individually, defining the printing parameters and microarray layout¹².

4.8.1 SETTING GLOBAL PARAMETERS

The process of creating a multiple print run on microarray slides is similar to that described in section 4.3, **Slide Properties** tab, though there is no graphical interface for this or any other function when programming in the **Multiple Print run Dialog** window. When creating a new print run for slides, none of the parameters in the other tabs can be set until the user has first selected the **Microplate type**, using the drop-down menu shown in the Global Parameters section (**Figure 4-25**, section 4.8): in the case of multiple print runs, global parameters are *those parameters which apply to all of the subruns defined within a multiple print run protocol*.



Global parameters apply equally to each individual subrun defined within a multiple print run protocol.

After defining the Microplate type, the user may select the Print mode, JetSpyder™ type, the save location of CSV/GAL files, number of microplate trays and number of sets of samples. All of these global parameters settings can be found under the print run section:

Action	Section
Selecting Microplate type	4.1.1
Selecting Print Mode	4.6.1
Selecting JetSpyder type	4.2.1
GAL file save location	4.6.2
Number of Microplates and trays	4.1.1
Number of sets of 12 samples per microplate	4.1.1

4.8.2 SELECTING/DESELECTING SUBRUNS

Subrun colour is selected by using the drop-down menu shown in the Global Parameters section (**Figure 4-25; 1**). The number of microplates, and the platters on which they are loaded, and the number of slides, and the trays on which they are loaded, is selected by making mouse clicks in the appropriate areas on the **Multiple Print run Dialog** window: clicking on a microplate when a colour has been selected will add it to the microplates to be used in a particular subrun definition; the same is true for microarray slides. An example is shown in **Figure 4-25** where the first 2 microplates and the first 4 slides have been selected and given the colour yellow. The user may then define further subruns by repeating the operation, choosing new colours and selecting the appropriate microplates

¹² Command Centre™ for Marathon v 1.4 shows individual parameters for each cluster but not a graphical representation. The parameter setting functionality is the same as for single print run for slides as has been shown on the table below.

and slides. One microplate has been coloured in red as an example in **Figure 4-25**. Subruns may be deselected by choosing the first colour (grey) and then clicking the microplates to be deselected.



It is necessary at the outset to define the total number of microplates required for all of the subruns and then select the number of microplates for each subrun individually.

4.8.3 DEFINING INDIVIDUAL PARAMETERS

Every subrun has individual parameters that can be changed. Double-clicking on the slides to be used within a particular subrun opens a new window in which the user can define the print run parameters for that subrun. The number of microplates, number of slide trays, and the number of slides per tray has been defined previously by the user in the **Multiple Print run Dialog** window. Slide properties and drop properties have been described in detail in the sections 4.3 and 4.4. The following table lists the sub-sections where this information can be found.

Action	Section
Setting slide quantity	4.3.1
Adjusting print margins	4.3.2
Setting Mini-arrays	4.3.3
Setting number of drops per spot	4.4.1
Setting printing pitch	4.4.2
Selecting number of repeats	4.4.3
Layout options I (Hexagonal-rectangular)	4.4.4
Layout options II (single-wraparound)	4.4.5

5. FAULT RECOVERY

In the event that a print run does not successfully complete then there shall be the opportunity to perform a fault recovery on that run when the software is next started.

This allows the printing to resume from where it faulted, preserving the print layout. At most one slide, if the fault occurred during printing, may be corrupted.

Command Centre™ for Marathon allows a run to be recovered from most faults, from power cuts to slides outside their mounting slot impeding the print-head movement. When the software is restarted after a run which did not end properly, a dialog window (**Figure 5-1**) will ask if recovery is needed.

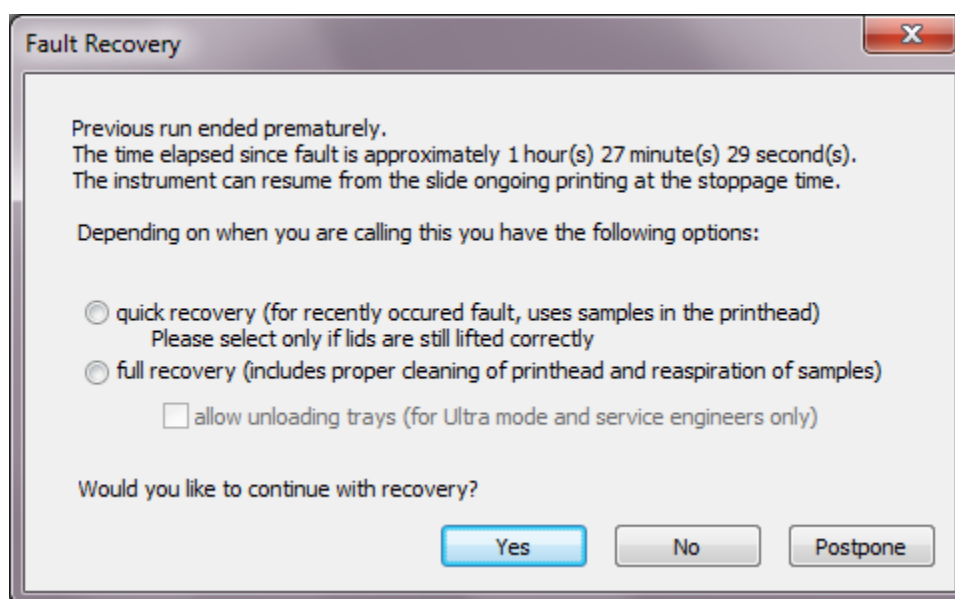


Figure 5-1 Fault recovery options

From the moment the fault occurs, the instrument stops its operations, waiting for further instructions. Meanwhile, the physical properties of aspirated samples in the print-head could be affected by evaporation. Depending on the interval of time since the fault occurred, and on samples properties as well as temperature and humidity, a run could be recovered by using the remaining sample in the print head (quick recovery). If a prolonged period of time has passed and there is doubt on the freshness of the samples, then if there is enough samples left in the microplate for another aspiration, the run can be recovered after proper cleaning of print-head and reaspiring the current sample from the microplate (full recovery).

If the fault requires service engineer investigation or simply if the user wants to check some parameters such as stiffness (K Test) or nozzles printing (test slide) before launching the fault recovery, the Postpone button allows this. However, this disables the quick recovery as the initialisation will purge the sample from the print-head.

For Ultra Marathons, Postpone followed by initialisation from the Options menu is not indicated and the help of a Service Engineer is recommended if fault investigation needs to be carried out without affecting the interrupted run. Fault recovery routine resumes the run from the same cages position of when the fault occurred and initialisation sends both cage 1 and 2 fully up.

Moreover, for Ultra Marathon, if the cause of fault cannot be removed without trays being unloaded, the option “Allow unload” is enabled on full recovery selection. However, just because the stoppage could occur in the middle of a cage operation and this recovery starts with a slide platen zeroing which requires no trays on the platen or in between the platen and the first tray position, low-level cage control might be required. For this reason this special mode asks for log in as a service engineer to ensure the person calling it has been trained to perform these operations (**Figure 5-2**).

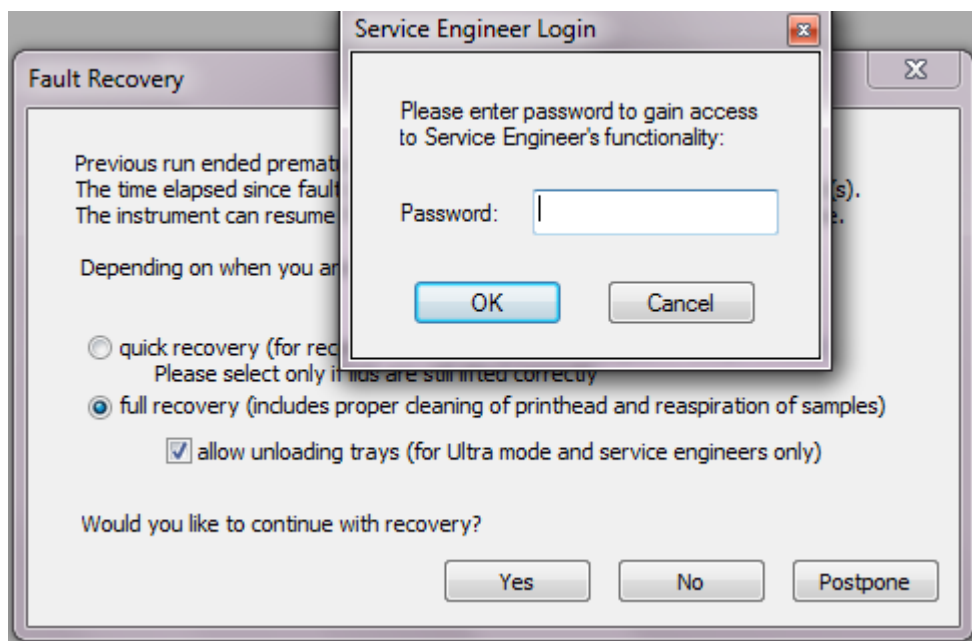


Figure 5-2 Ultra Marathon – tray unloading recovery

Recovery runs initialise the instrument, then clean and reaspirate only if full recovery was chosen. Printing is then resumed from the same tray and slide, same miniarray, repeat and pass which were undergoing printing when the fault occurred. Therefore, any printing corruption is reduced to at most one affected slide which will only have a few extra drops in the set of 12 or 32 samples being printed at the time of the fault.

Please note that recovery is available for printing onto slides, only when samples come from microplates (faulty buffer runs cannot be recovered). The generation of GAL and CSV files is continued in the recovered run, to accurately reflect at any time the samples printed on all slides. At the end of a successfully recovered run, the GAL and CSV files will look the same as if the run had been successful first time.

The logging of information from a recovered run will also be appended to the previous logfile to give a complete log of the current run. This file also records the recovery information as well as the name of the instrument.

6. TUTORIAL

In this section, the reader is taken through the actions required to create a print run protocol. This particular tutorial takes the reader through the process of creating a print run protocol on glass slides in which the printing area is divided into a number of mini-arrays.

6.1 DESIGNING A MINI-ARRAY PRINT RUN ON SLIDES

There are a number of actions the user must take in order to create a print run protocol for printing mini-arrays on microarray slides. The table below summarises the actions in a series of simple steps, and also provides a reference to the appropriate section in the manual in the event that more information is required.

Action	Tab/menu item	Manual reference
1. Creating a new print run	File->New Print Run ->slides	Section 3.1.1
2. Defining the number of samples to be printed & the microplate type	Source microplate properties	Section 4.1
3. Suppressing samples; activating & deactivating printhead nozzles	JetSpyder properties	Section 4.2
4. Defining print margins & printing areas; number of slides to print	Slide properties	Section 4.3
5. Defining spot size & pitch; number of repeats; spacing; array layout	Spot properties	Section 4.4
6. Visualising the proposed layout	Overview	Section 4.5
7. Choosing location for saving output files; file naming; input file processing; choosing printing mode; estimating print run time; starting the print run	Start	Section 4.6

In the example presented in this tutorial, the number of samples to be printed is **384**, and the mini-array layout on which the probe samples will be printed is a **sixteen pad** mini-array layout, **two columns by eight rows** (Figure 6-1)

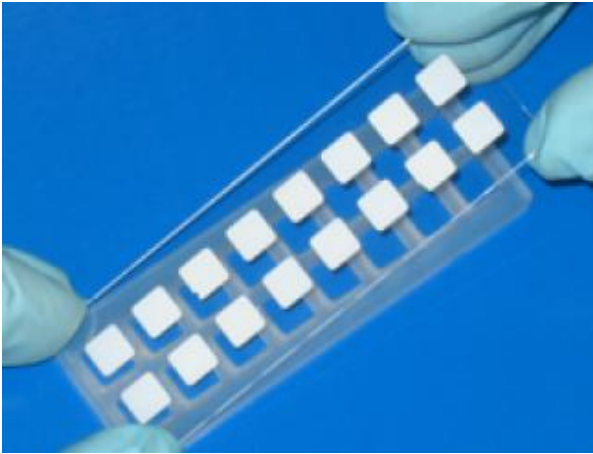


Figure 6-1 16 pad mini-array format.



Sixteen pad mini-array slides, such as the one shown in Figure 6-1, are available from Arrayjet. Please contact us for more information.

6.1.1 CREATING A NEW PRINT RUN

- Double-click the **Command Centre™** icon on the **desktop** to start a **new session of Command Centre™**
- Navigate to the **File** menu in the main **Command Centre™** window
- With the left mouse button, click **File>New>Print run (slides)** as shown in **Figure 6-2**.

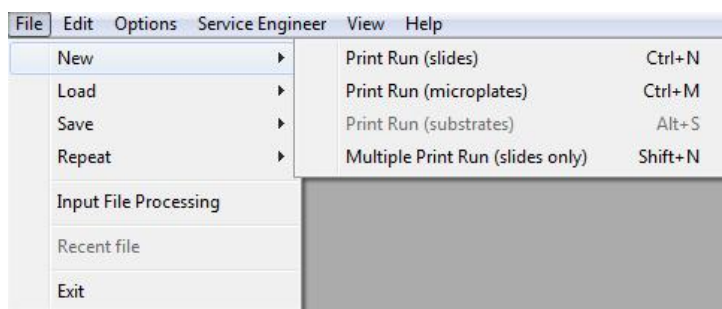


Figure 6-2 The File menu

6.1.2 DEFINING THE NUMBER OF SAMPLES TO BE PRINTED & THE MICROPLATE TYPE



The Marathon Inkjet Microarrayer is calibrated prior to shipment with a specific Arrayjet approved microtitre plate model in both 96-well and 384-well variants; use of an alternative model may result in damage to the JetSpyder™ or poor aspirating and printing performance. Please contact Arrayjet for more information;



It is essential to place the variant of microplate on the Marathon Inkjet Microarrayer which is correct for the print run being created/loaded: placement of the wrong microplate type (96-well versus 384-well) will result in damage to the JetSpyder™ and possibly to the microarrayer; the user will be liable for any and all damage which results from such an error.

- Navigate to the **Source Microplate Properties** tab
- Choose between a **96-well or 384-well microplate type** as shown in **Figure 6-3**.

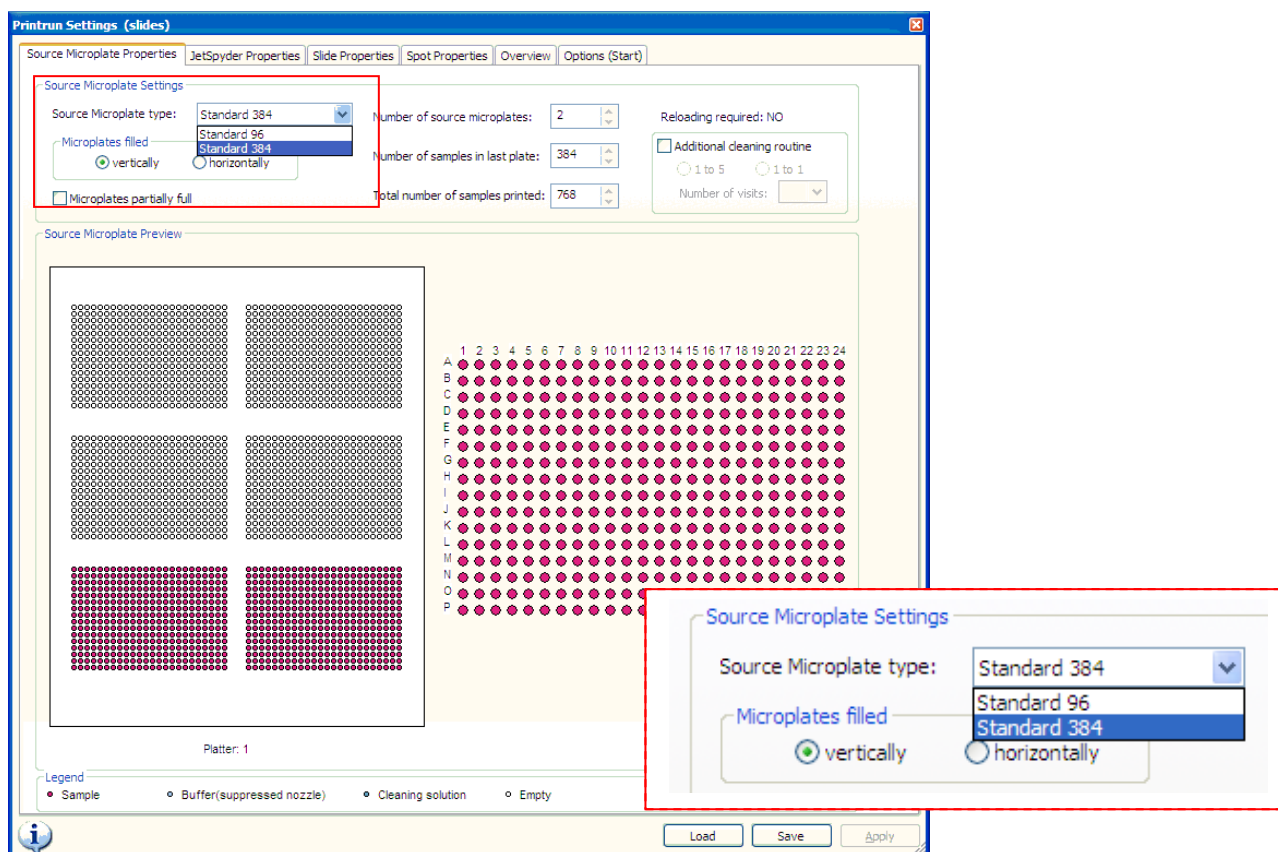


Figure 6-3 The Source Microplate Properties tab

6.1.3 DEFINING PRINT MARGINS & PRINTING AREAS; NUMBER OF SLIDES TO PRINT

- Navigate to the **Slide Properties** tab
- Enter the number of slides using the edit or the spin controls as shown in **Figure 6-4**
- Enter the slide margins using the edit or the spin controls as shown in **Figure 6-4**
- Enter the number of mini-arrays down and across as shown in **Figure 6-5**
- Make further refinements to the overall width and height of the mini-arrays (**Figure 6-6**).

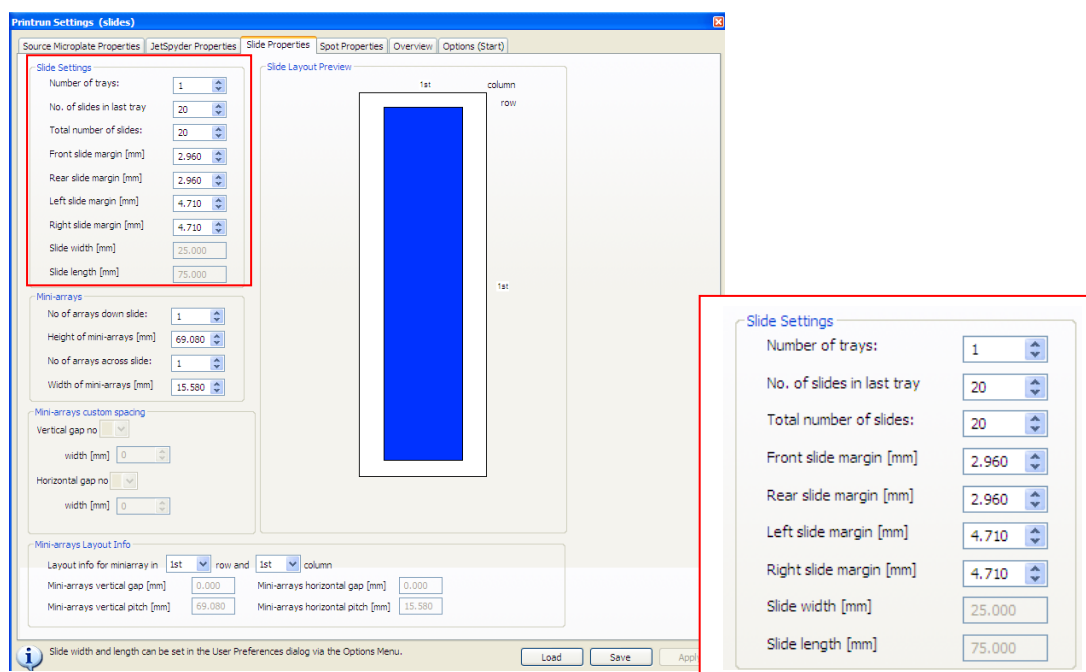


Figure 6-4 The Slide Properties tab-Setting slide margins



When two or more arrays (mini-arrays) are required per slide, setting the mini-array parameters from the **Mini-arrays** group box determines the number of mini-arrays across and down the slide, as well as the height/width of individual mini-arrays;



The **Slide Properties** tab shows the preview of only one slide: all of the slides to be printed within a given print run will be printed as exact replicates of the previewed slide in terms of the printing margins and layout chosen;



When modifying the print margins and layouts in the **Slide Properties** tab it will **not be possible** to set a print area which is **too small** to print the number of samples the user has defined, though the user may further reduce or increase the print margins once they have defined the **spot pitch** and **number of drops per spot**, in the **Spot Properties** tab, should that influence the dimensions of the printing area.

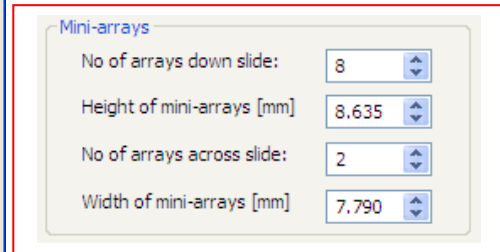
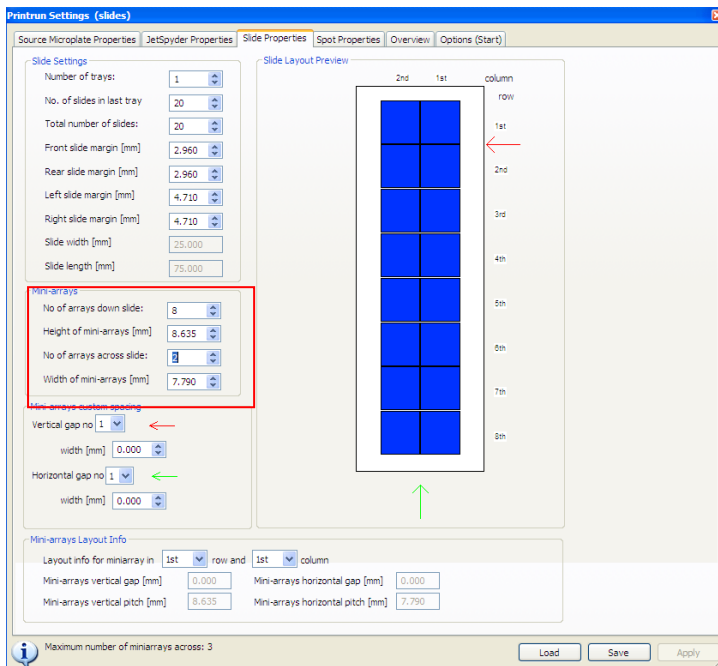




Figure 6-5 The Slide Properties tab-Setting number of mini-arrays

 The software will automatically calculate the width and height of each mini-array by assuming no space between arrays (Figure 6-5);

 If there is no space between mini-arrays, a thin white line is shown to indicate the boundaries between the mini-arrays (Figure 6-5).

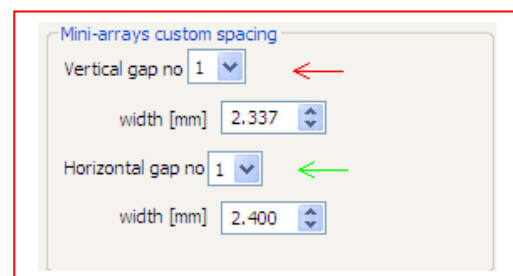
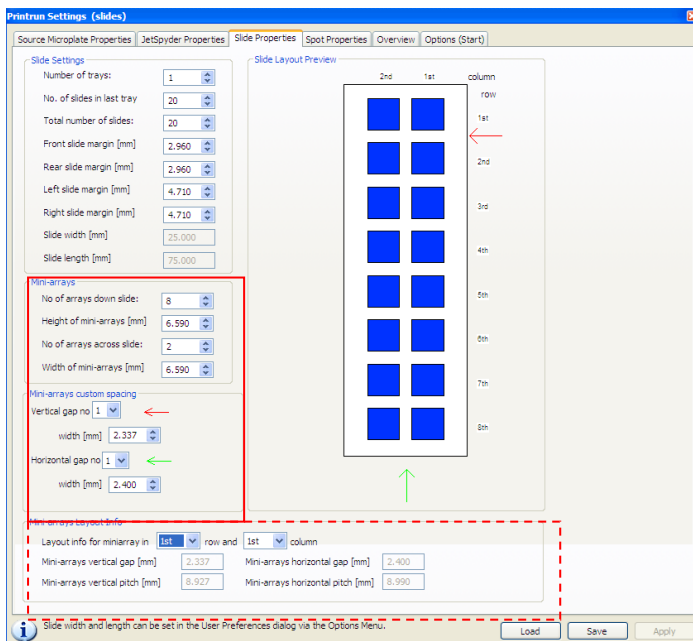


Figure 6-6 The Slide Properties tab-Setting the height and width of the mini-arrays



The software automatically calculates the resulting horizontal and vertical gaps between each mini-array, which are evenly spaced within the overall print margins defined for the slide;



The horizontal and vertical pitches between each array are displayed in the **Mini-arrays Layout Info** group box;



In this example, the mini-array height and width have been further reduced to 6.590 mm;



When the mini-array height and width are adjusted, the mini-array printable areas are automatically centred within the available print margins originally defined by the user.

6.1.4 DEFINING SPOT SIZE & PITCH; NUMBER OF REPEATS; SPACING; ARRAY LAYOUT

- Navigate to the **Spot Properties** tab
- To print bigger spots, **increase** the number of drops per spot in the **Settings** group box (**Figure 6-7**):

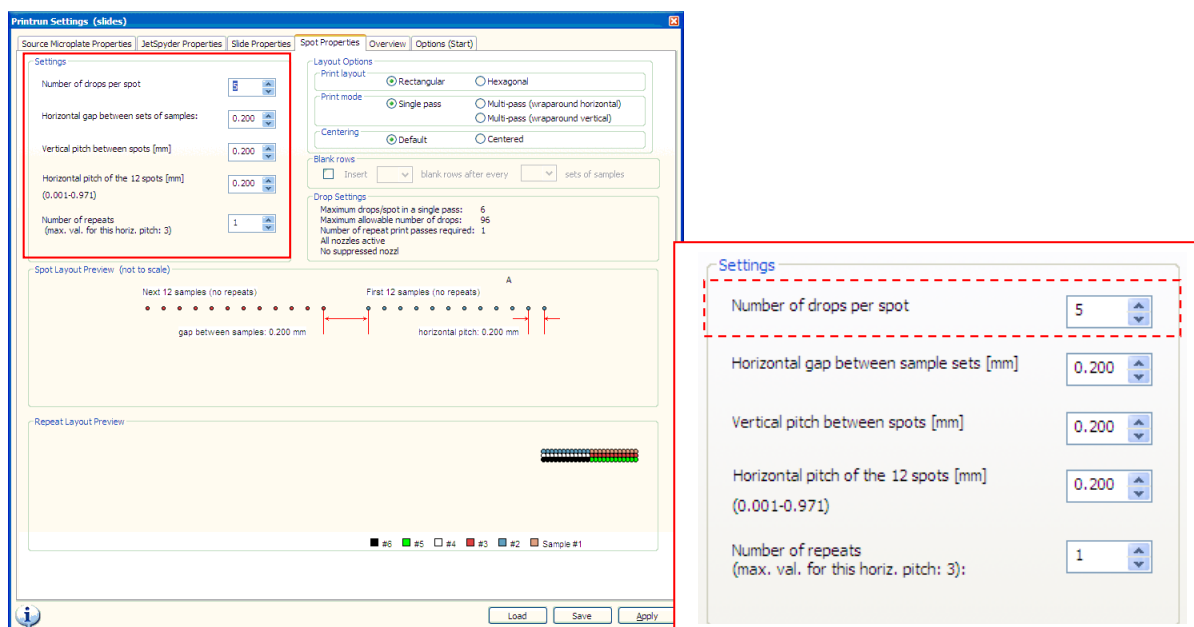


Figure 6-7 Increasing the number of drops per spot in the Spot Properties tab

- Centre the spots by clicking the **Centred** radio button in the **Layout Options** group box (**Figure 6-8**)

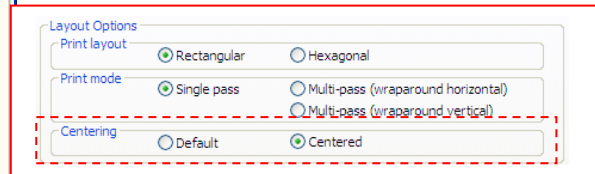
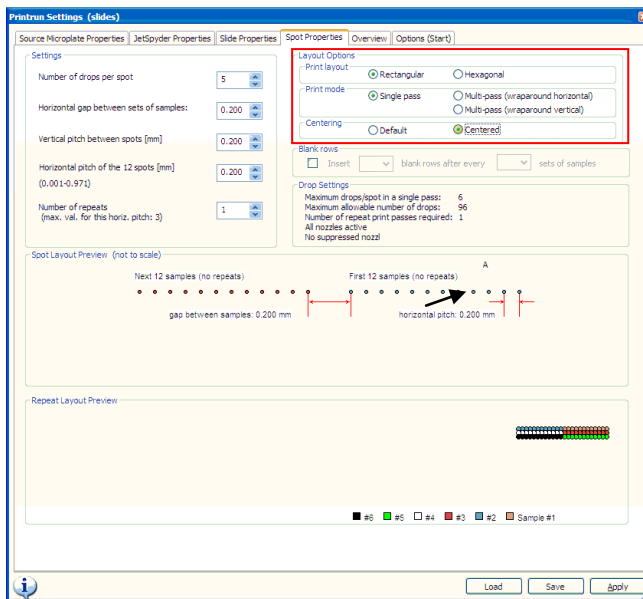


Figure 6-8 Centring mini-array spots in the Spot Properties tab

6.1.5 VISUALISING THE PROPOSED LAYOUT

- Navigate to the **Overview** tab (Figure 6-9)

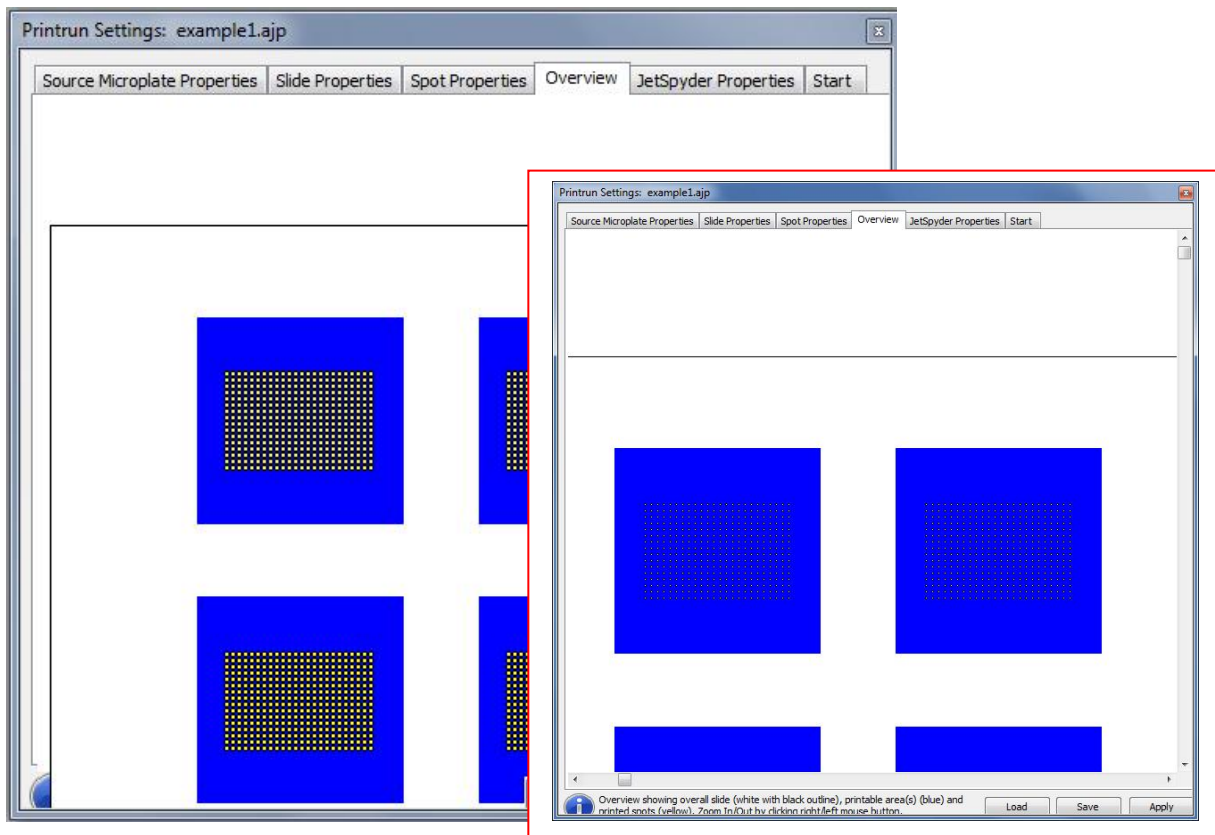


Figure 6-9 The Overview tab-5 drops per spot printing; centred mini-arrays shown in zoomed-in view



The Overview tab shows a preview of the layout of the microarrays as they will be printed by the microarrayer with the parameters defined by the user; a single slide is shown;



The user can zoom in and out of the preview by clicking on the right and left mouse buttons respectively.

The **Overview** tab, as previously stated, enables the user to visualise the microarrays to be printed as they will be printed on the selected substrates, with the appropriate number of features, bearing in mind the number of probe samples to be printed, and the number of features to be printed per probe sample. In the example shown in **Figure 6-9**, a number of mini-arrays are to be printed with the microarrays themselves neatly centred within the mini-array area, as shown by the features.

6.1.6 SUPPRESSING SAMPLES; ACTIVATING & DEACTIVATING PRINTHEAD NOZZLES



Meaningful interaction with the **JetSpyder Properties** tab requires the printing of a **Test Slide** in order to assess the performance of the printhead nozzles. If a **Test Slide** has not been printed prior to this point it is advisable to print one (see **Section 4.2.2**)

- Navigate to the **JetSpyder Properties** tab;
- Review the **Test Slide**;
- By selecting the appropriate area on the **Test Slide** preview or selecting the nozzle number in the **Nozzles in use** section, within the **JetSpyder Properties** tab, deactivate any nozzles which are not performing well (**Figure 6-10**);
- Alternatively, perform a series of purges and cleaning steps and re-try the **Test Slide** (see **Section 4.2.2**);
- If sample suppression is required for the desired array layout, suppress samples by checking the **Print Sample** checkbox; samples left unchecked will be printed as normal;
- Preview the desired print layout in the **Overview** tab (see **Section 4.5**) prior to starting the print run.

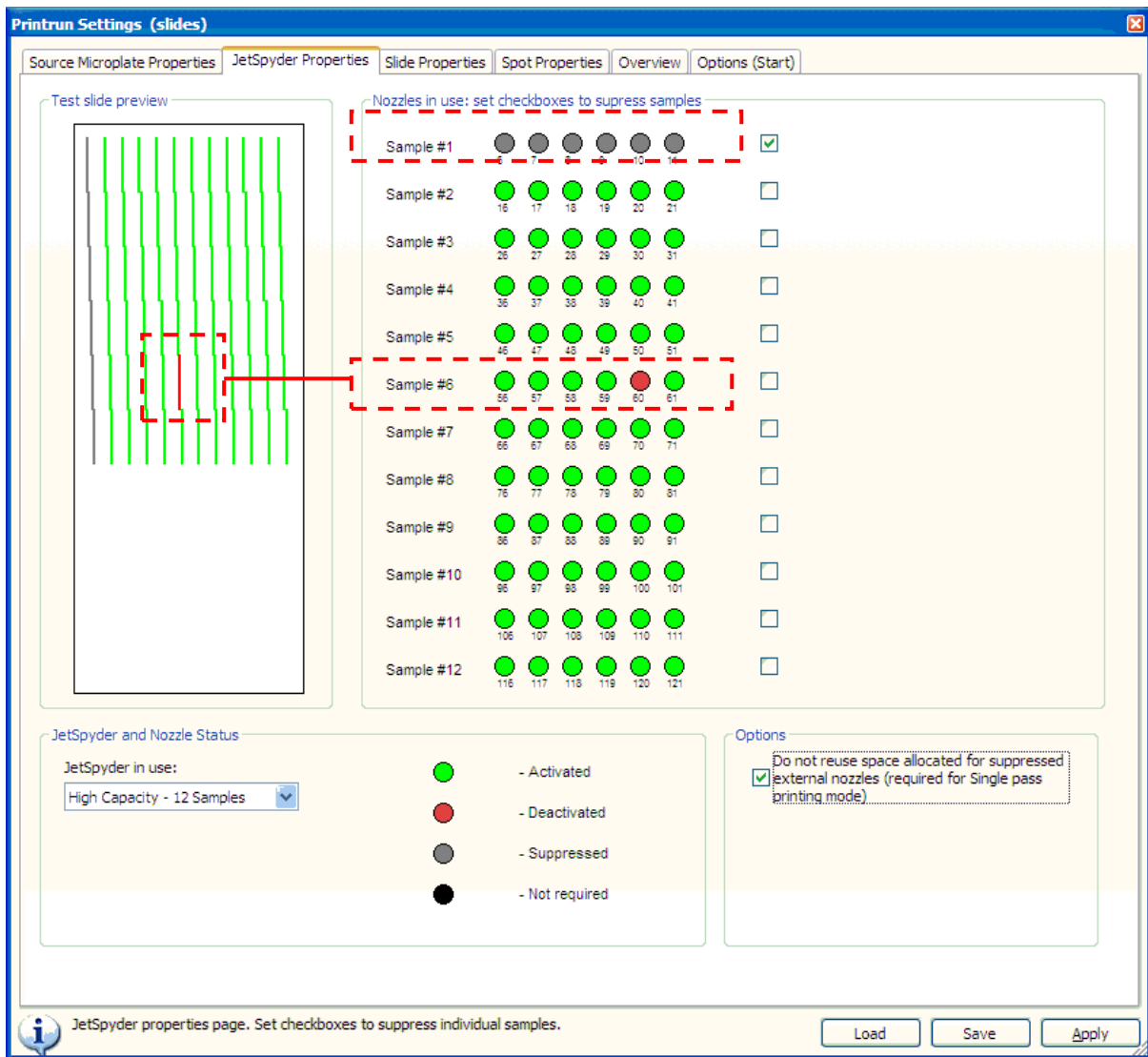


Figure 6-10 JetSpyder Properties tab - Test slide preview with nozzle #60 turned off; Sample #1 suppressed.



It is important to fully understand the plate/well – slide mapping schema employed by Arrayjet inkjet microarrays in order to select the appropriate samples for suppression yet obtain the desired microarray layout on each slide. For details please contact Arrayjet.

6.1.7 CHOOSING LOCATION FOR SAVING OUTPUT FILES; FILE NAMING; INPUT FILE PROCESSING; CHOOSING PRINTING MODE; ESTIMATING PRINT RUN TIME; STARTING THE PRINT RUN

- Navigate to the **Start** tab (**Figure 6-11**)
- Choose a mode of operation in the **Printron Options** box – i.e. how the run is to be performed by selecting the appropriate radio button next to the option of choice:-

- Microplate - by aspirating actual probe samples from microplates;
 - Buffer - not printing actual probe samples from microplates, but by printing system buffer;
 - Simulation - purely to generate an output file.
- Choose which output file format is required in the **Output Files** box by checking or unchecking the appropriate option:-
- CSV – Comma Separated Variable file;
 - GAL – Genepix Array List file
- Estimate the time to be taken for the printrun by clicking **Calculate printing time**.
- Start the print run by clicking the **START PRINT RUN** button!

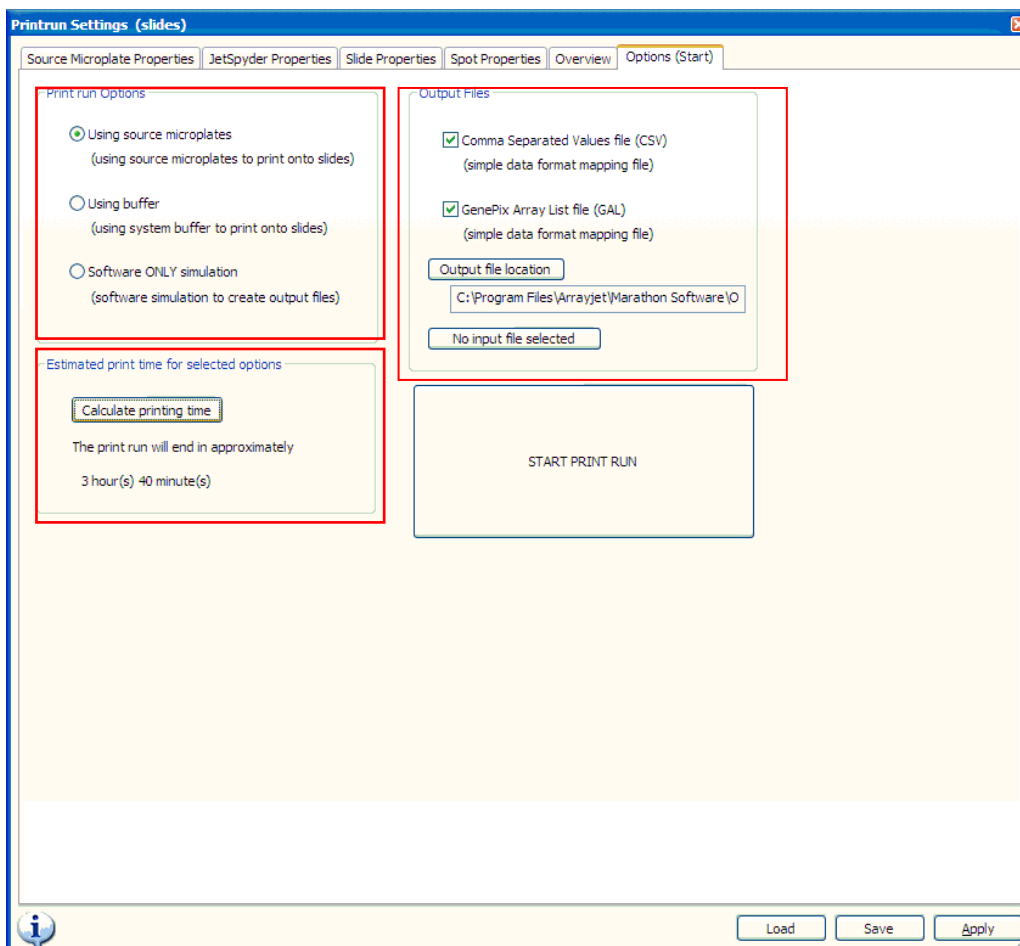


Figure 6-11 The Start tab

6.2 DESIGNING A MULTIPLE PRINT RUN

6.2.1 CREATING A NEW PRINT RUN

- Double-click the **Command Centre™** icon on the **desktop** to start a **new session of Command Centre™**.
- Navigate to the **File** menu in the main **Command Centre™** window.
- With the left mouse button, click **File>New>Multiple Print Run (slides only)** as shown in **Figure 6-12**.

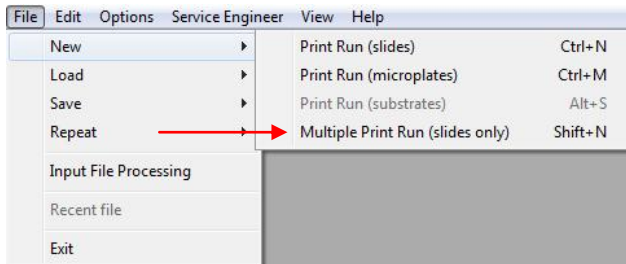


Figure 6-12 The File menu

6.2.2 MICROPLATE AND TRAYS SETTINGS

- **Choose between a 96-well or 384-well microplate type** as shown in **Figure 6-13**

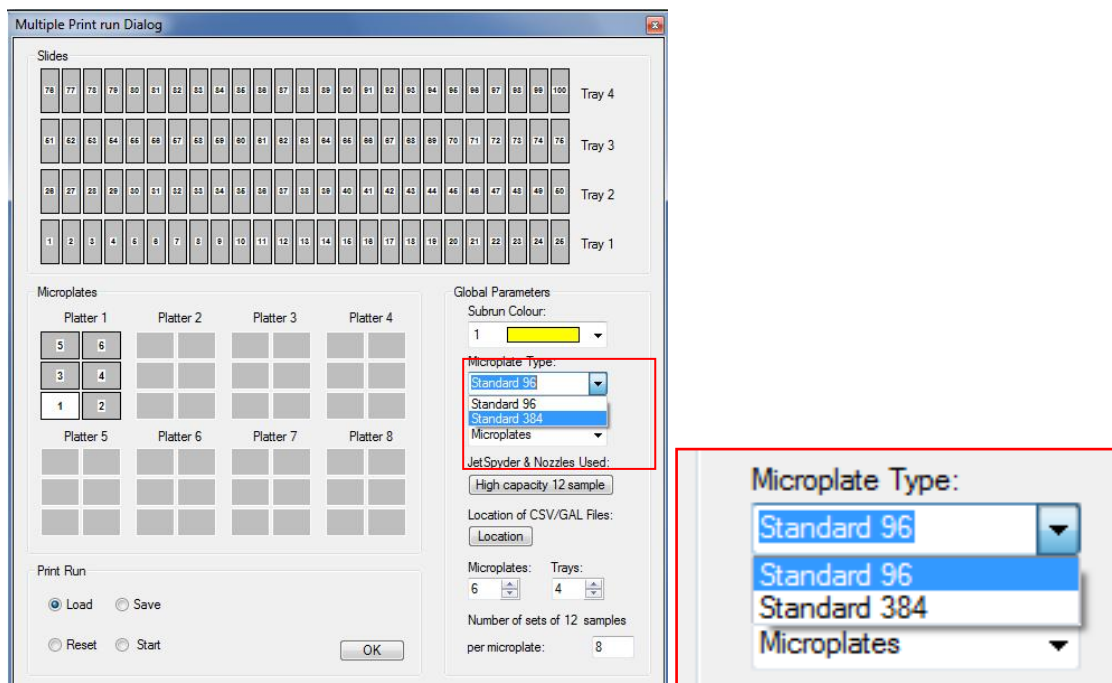


Figure 6-13 Selecting Microplate Type

- Set up the number of microplates and trays as shown in **Figure 6-14**.

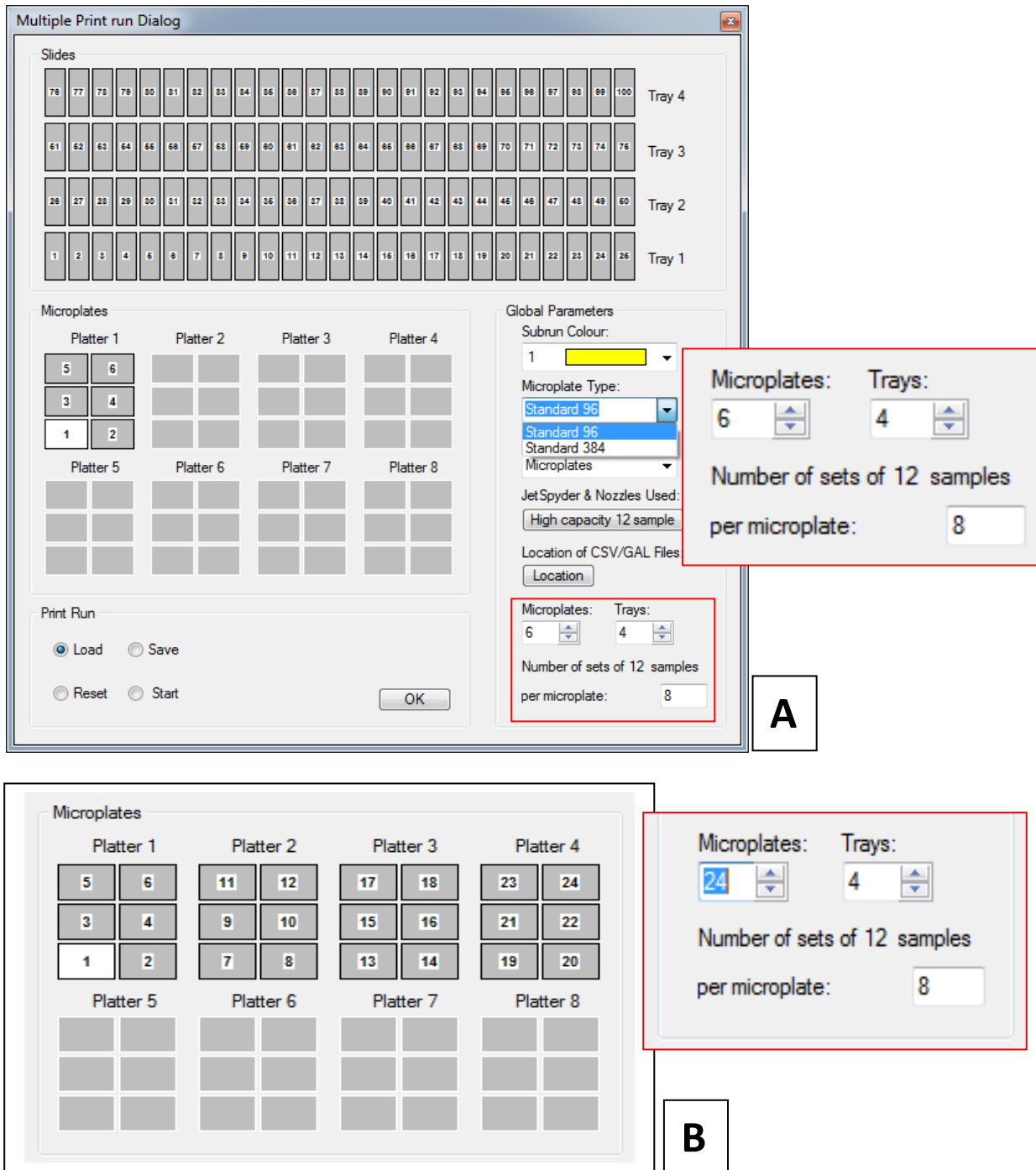


Figure 6-14 Selecting microplate and tray numbers causes a change in their graphical representations accordingly – 6-14A shows 6 microplates selected, whereas 6-14B shows 24.

6.2.3 SETTING NUMBER OF SETS OF 12 SAMPLES

- Set the number of sets of 12 samples to have in each microplate (**Figure 6-15**).

Depending on microplate type choice there is a maximum number of sets of 12 samples: For 384 well-plates the maximum number is 32 and for 96 well-plates the maximum number is 8¹³.



For help with selecting the number of sample sets per microplate, see Section 4.1.1

Microplates: 24 Trays: 4
Number of sets of 12 samples
per microplate: 8

A

Microplates: 24 Trays: 4
Number of sets of 32 samples
per microplate: [1 - 12] 12

B

Figure 6-15 Setting the number of sample sets per microplate – 96-well microplate with 12 Sample JetSpyder (A) and 384-well microplate with 32 Sample JetSpyder (B).

¹³ Graphical microplate representation section 4.1.1 will help the user to set up the numbers of sets of 12 samples accordingly.

6.2.4 SETTING JETSPYDER TYPE AND NOZZLE IN USE

- Select the JetSpyder type as shown in Figure 6-16;

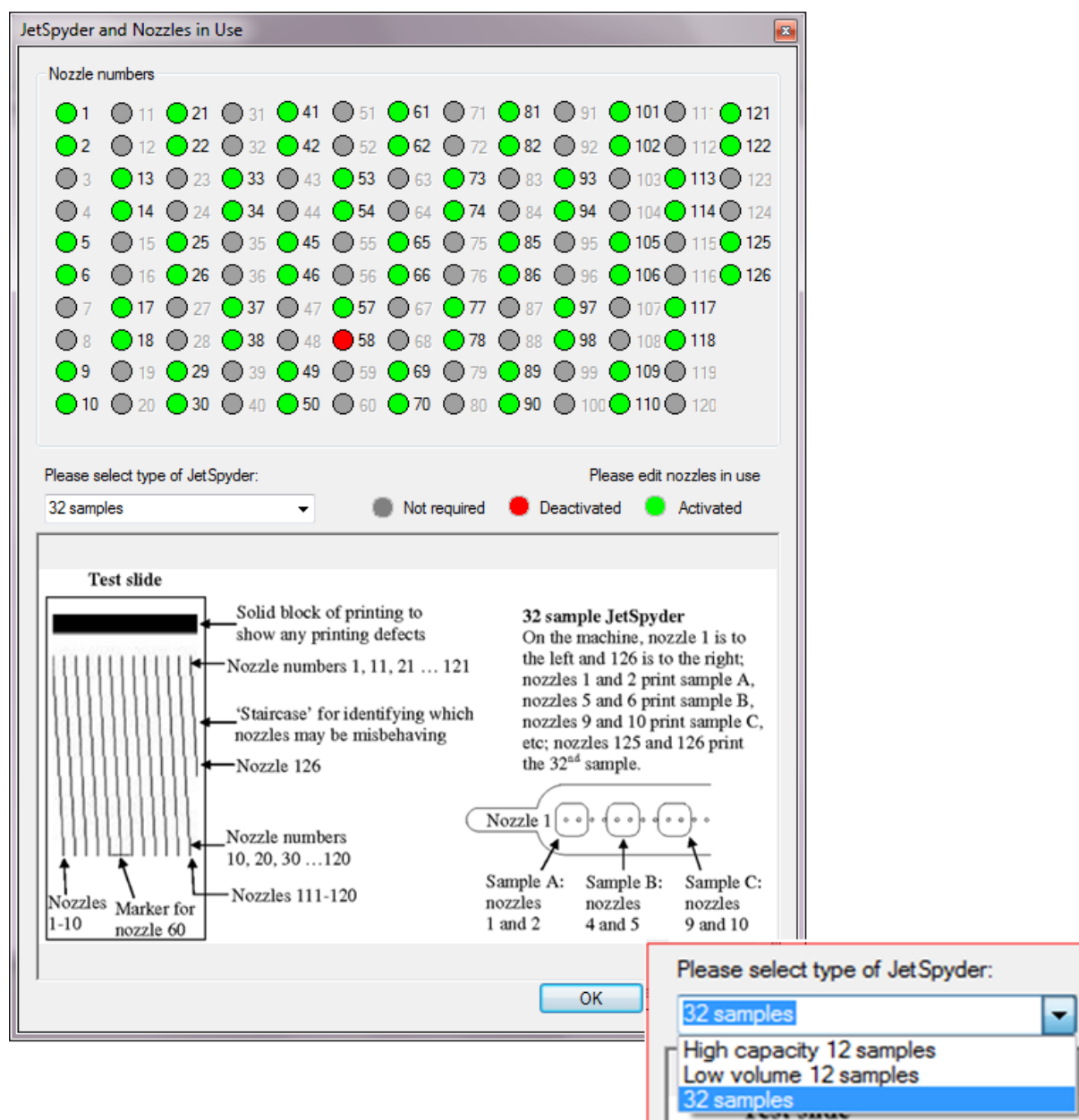


Figure 6-16 JetSpyder and Nozzles in Use window

- Review the **Test Slide**
- By selecting the appropriate nozzle on the **Nozzles in Use** window deactivate any nozzles which are not performing well (**Figure 6-16**); In this case nozzle 58.

6.2.5 SELECTING CSV/GAL FILE LOCATION

- Choose a location for the CSV/GAL file that will be generated from the print run (**Figure 6-17**)
- Click **Okay** when you have selected a location, and this will return you to the main window.

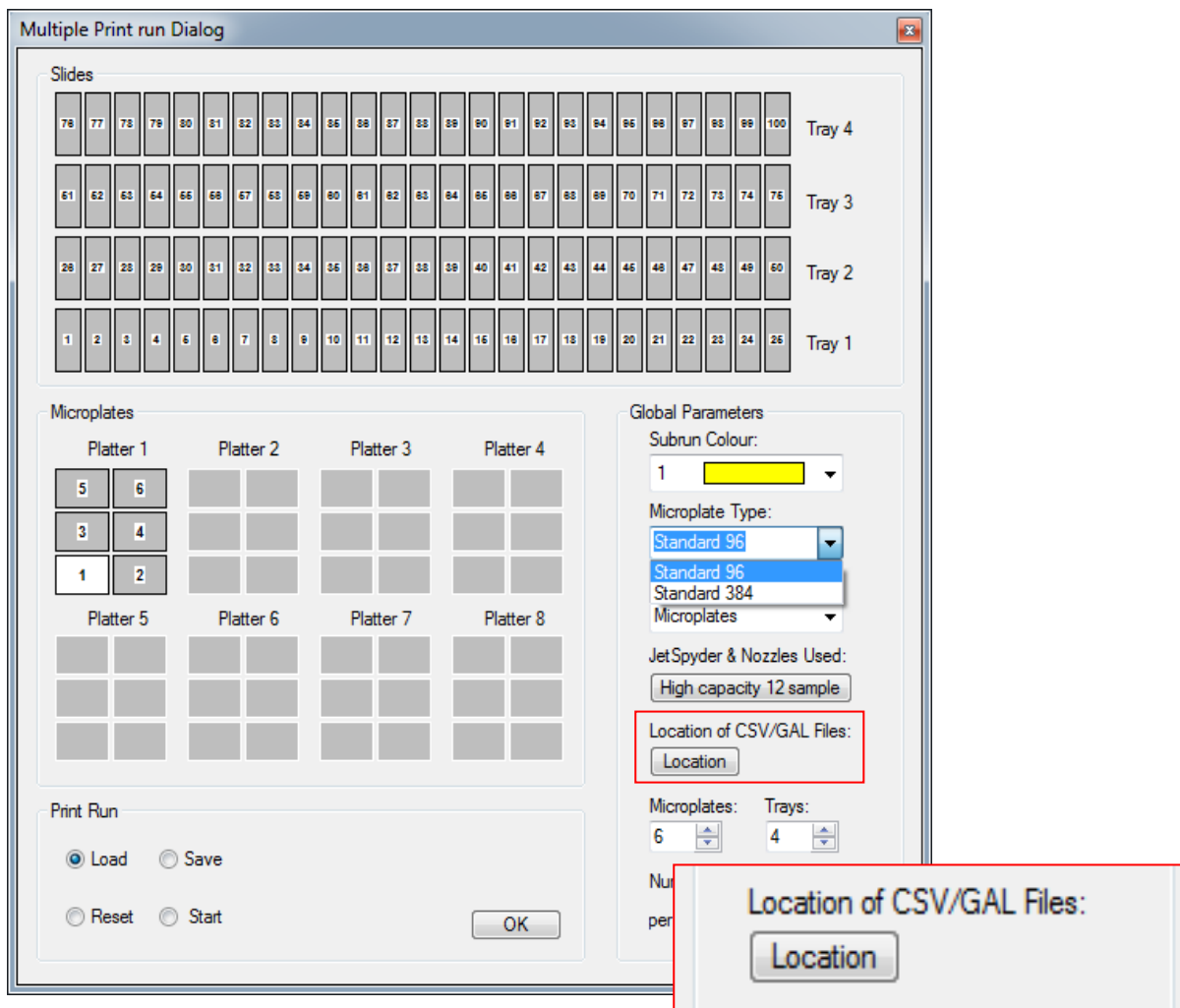


Figure 6-17 Setting CSV/GAL file location

6.2.6 SELECTING/DESELECTING SUBRUNS

- Once you have decided how many subruns you wish to program, select the colour you want for the first subrun and allocate microwell plates and slides to that subrun (Figure 6-18) – in the example shown in Figure 6-18, microplates¹⁴ and slides¹⁵ have been selected accordingly by clicking where required and using the colour yellow;
- When you have selected all of the required plates and slides for the first subrun, choose another colour and repeat the process – in the example shown in Figure 6-18 the colour purple has been selected.
- In order to deselect subruns, choose first colour on the list (grey 0) and click the appropriate microplates and slides to remove the subrun surplus to requirements.

¹⁴ Only whole microplates can be assigned to a subrun. Microplates cannot be shared between subruns.

¹⁵ Up to 4 trays can be selected. All selected trays shall have identical printing. Subruns are printed left to right across all selected trays.

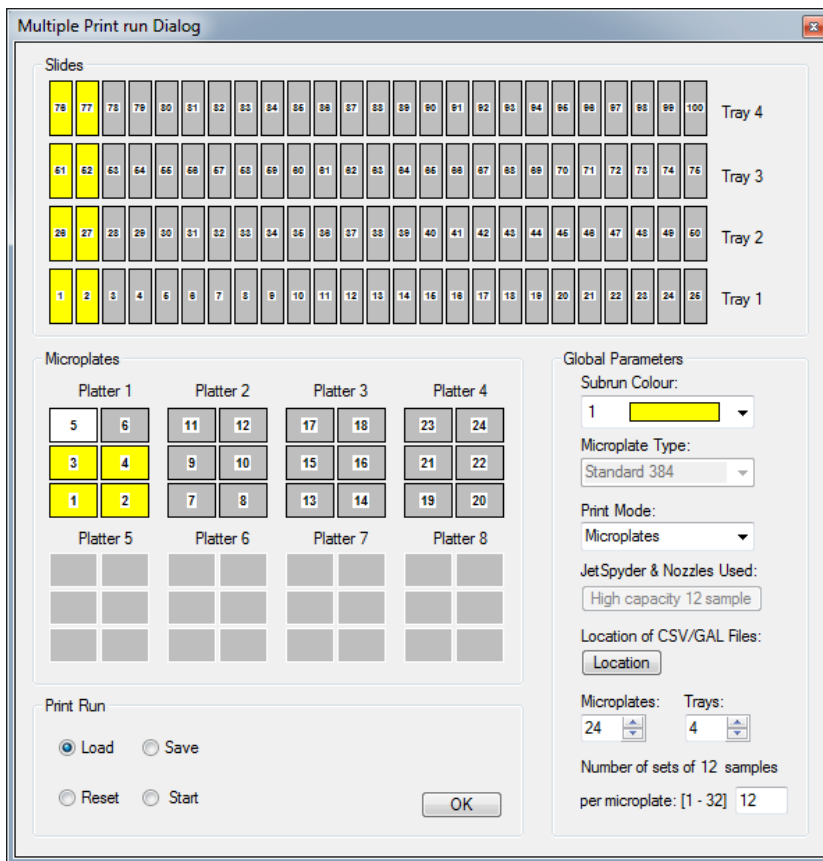
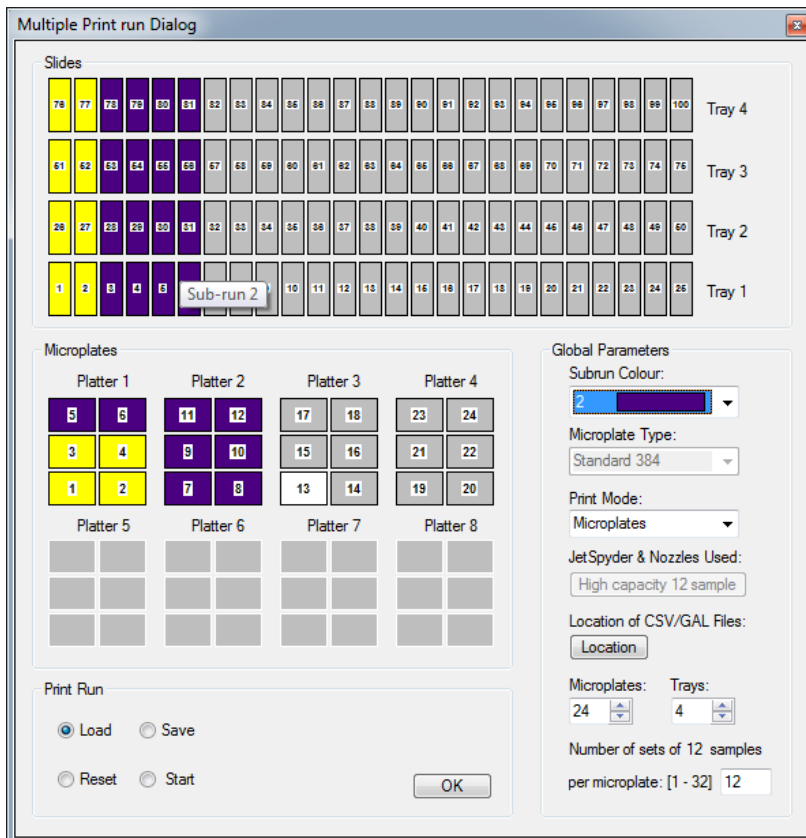


Figure 6-18 Creating a subrun: first subrun selected marked in yellow; second in purple



6.2.7 DEFINING SUB-RUN PARAMETERS

- Double click on the sub-run microplate or slide image and a new window will open

The screenshot shows a dialog box titled "Multiple Printing: Local Print Properties for Sub-Run 1". It is organized into several sections:

- Microplate Properties:** Number of microplates: 1; Number of sets of 12 samples used per plate: [1-32] 32; Number of samples in each plate: 384; Number of sets of 12 samples to be printed: 32; Total number of samples printed: 384.
- Drop Properties:** Number of drops per spot: [1-6] 1; Horizontal pitch of the 12 spots: [0.001 - 0.971 mm] 0.200; Horizontal gap between different sets of samples: 0.200; Number of repeats across slides: [1-4] 1; Buttons: Same row; Horizontal gap between repeat prints: 0.000; Vertical pitch between spots: 0.200; Button: Rectangular.
- Slide Properties:** Number of trays: 4; Number of slides per tray: 1; Total number of slides: 4; Slide width: 25.000; Slide length: 75.000; Slide margin front: 2.000; Slide margin rear: 2.000; Slide margin left: 2.000; Slide margin right: 2.000.
- Mini-Array Properties:** 1 across, 1 down; Button: Mini-Arrays; 21.000 by 71.000; Button: Reset.
- Output File Properties:** File Type: CSV, GAL.
- Set Default Parameters:** Button: Set Defaults; Text: Set the parameters displayed on this page to be the defaults. Subsequent subruns created will display these values.

Buttons: Cancel, OK.

Figure 6-19 Multiple printing sub-run parameters

- Define drop properties: For guidance use section 6.1.4 tutorial
 - Define slide margins and number of mini-arrays: For guidance use section 6.1.3 tutorial
 - Define Output File Properties: File naming; input file processing; choosing printing mode; starting the print run. For guidance follow section 6.1.6 tutorial
- Repeat the same operation for all the sub-runs created;



Subrun parameters **must be defined in order** -the parameters for subrun 1 must be defined first, followed by subrun 2, until all subrun parameters have been defined.

- Once the parameters are set up, start a print run by selecting the **Start** radio button and then clicking **OK**.

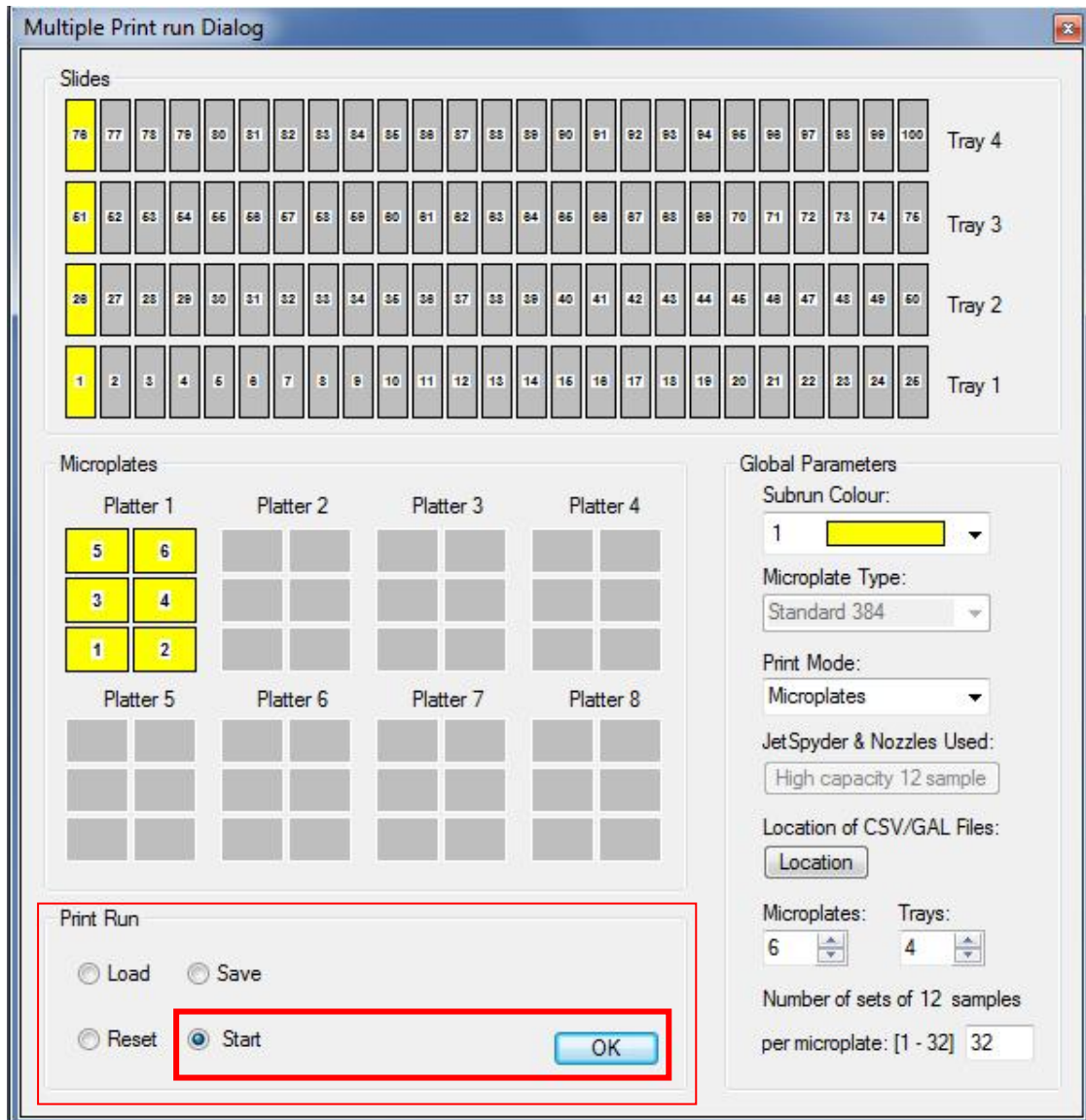


Figure 6-20 Start a multiple print run



In the event that microplates or trays have not been set up properly an error message will pop up. An example is shown in Figure 6-21

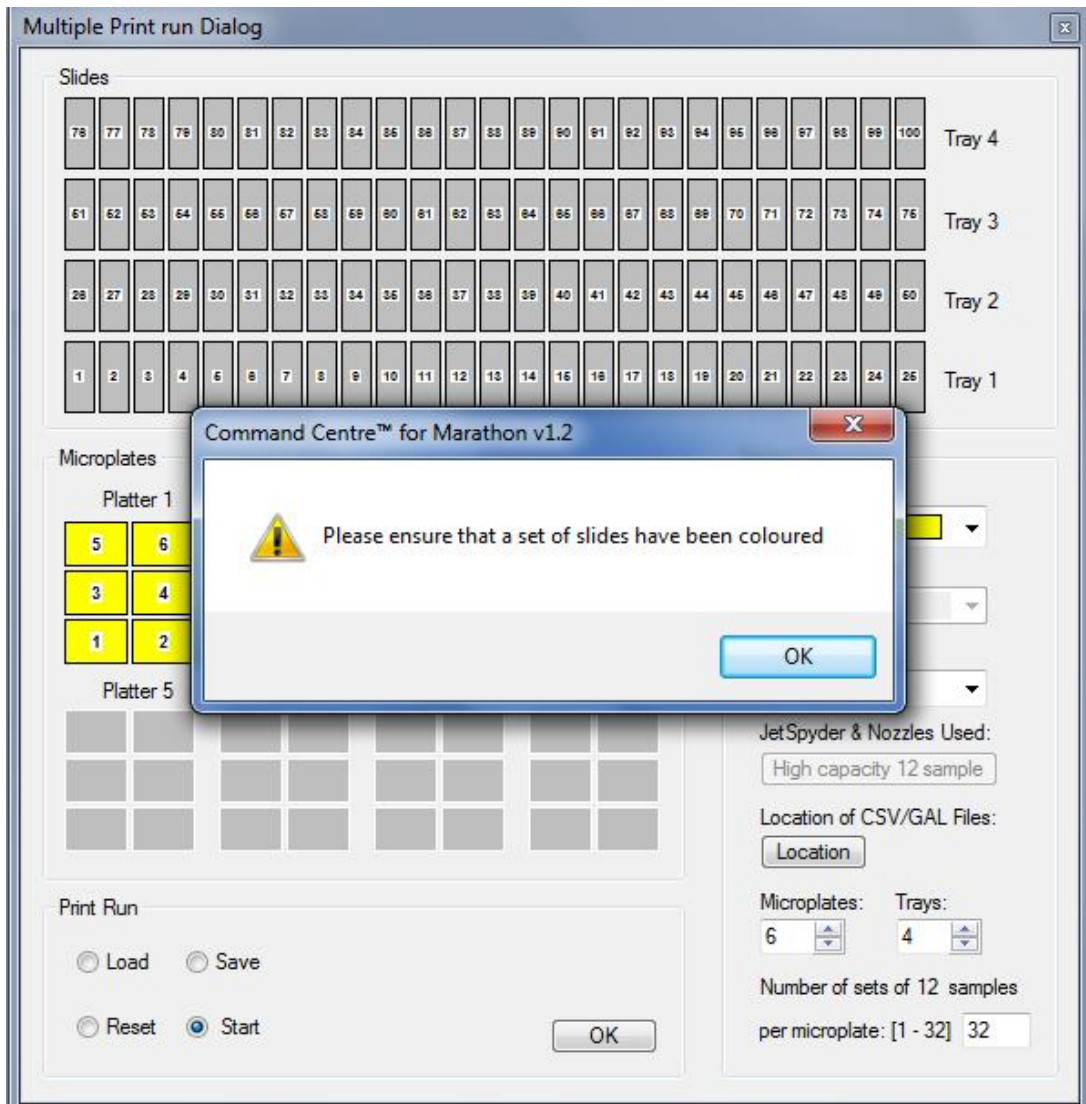


Figure 6-21 Multiple Print Run – error message: slides not coloured.

By following these tutorials the user should have been able to define both single and multiple print runs on microarray slides, and single print runs on microplates. In the event that further help or assistance is required, please contact Arrayjet via our technical support email:

support@arrayjet.co.uk

We value your feedback: please contact us and tell us what you liked, and didn't like about this user manual. We strive to constantly improve our products and services and your feedback is valuable to us and to other users of our technology and products. Please take two minutes of your time and let us know what you thought. You can do this by placing a call with your local representative or by sending an email to:

mail@arrayjet.co.uk

NOTES:

Arrayjet Ltd.
Midlothian Innovation Centre,
Pentlandfield,
Roslin, Midlothian
EH25 9RE, UK

T: +44(0) 131 440 5220
F: +44(0) 131 448 1500
E: mail@arrayjet.co.uk
W: www.arrayjet.co.uk

 **ARRAYJET**
powered by inkjet technology™