Method Manager 4 : VVP Data In

The VVP pipettor can also accept instruction using variables. Either with a CSV file or method variable. **This will overwrite any instructions sent to the pipettor statically through the "Pipettor" tab.** The structure of the data is comma delimited, then semi-colon delimited.

The comma separates different channel commands, while the semicolon separates different parameters of a channel command. The CSV file is meant to be a human readable form of the method variable. Consequently the CSV file is a little more complicated than the method variable which is one line of 96 commands to the pipettor that is prefaced by the VVP input command (i.e., "VI;12;8", "VMDI;12;8", etc).

All input command files have a special code and the dimensions of the pipette tool stored in the first cell in the array. For input files the code and pipettor dimensions are validated at run-time to confirm the code matches the method command and the dimensions match the pipettor head. If the code is not correct for the method command or the pipettor dimensions do not match the hardware then an error is generated. For output commands the first cell contains the file code, an error flag, and then pipette head dimensions are recorded for tracking purposes.

File codes are as follows

Input file for Aspirate, Dispense, Mix, or LLD: "VI"

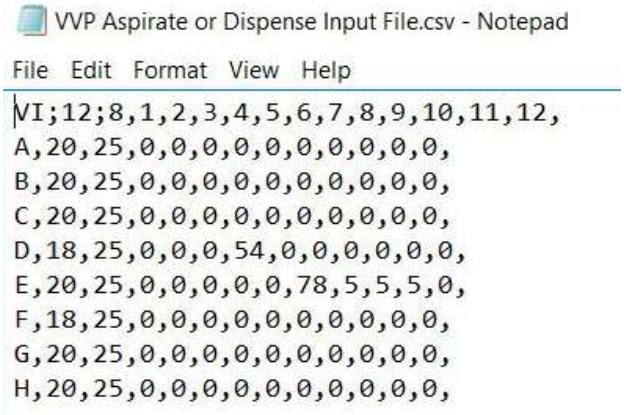
Input file for MultiDispense: "VMDI"

These full structure of these commands take in account the size of the head (i.e. 96 channel head). The structure is: VVP command input; column size; row size. So for a regular aspirate or dispense on a 96 channel head this instruction would be: "VI;12;8".

The CSV file is meant to be read in excel so that the channels on the head map to the cells that are on the screen:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
	VI;12;8	1	2	3	4	5	6	7	8	9	10	11	12	
2	A	20	25	0	0	0	0	0	0	0	0	0	0	
3	B	20	25	0	0	0	0	0	0	0	0	0	0	
4	C	20	25	0	0	0	0	0	0	0	0	0	0	
5	D	18	25	0	0	0	54	0	0	0	0	0	0	
6	E	20	25	0	0	0	0	0	78	5	5	5	0	
7	F	18	25	0	0	0	0	0	0	0	0	0	0	
8	G	20	25	0	0	0	0	0	0	0	0	0	0	
9	H	20	25	0	0	0	0	0	0	0	0	0	0	
10														

Raw Text Version of above Excel file:



Note: Use MS notepad or other basic text editors to edit the file to prevent extraneous characters to be saved with the file.

When MM4 parses a CSV it ignores the cells that act as place holders for the column and row names. It reads the A1 cell to get the type of VVP command to execute then reads cells B2:M9. All of excel column A and excel row 1 are ignored (with the exception of A1) as these are meant to be for the user to read the column and row of the plate.

The **method variable** version of this VVP command looks like this:

Basically a comma separated, row major (A01,A02, A03, etc.) oriented version of the command with no human readable information.

Format for individual channel instructions: <Destination well>;<Volume>;<lead air gap>;<trailing air gap>;<VVP residual volume>

<Destination Well> → only used in Multi-Dispense not Aspirate

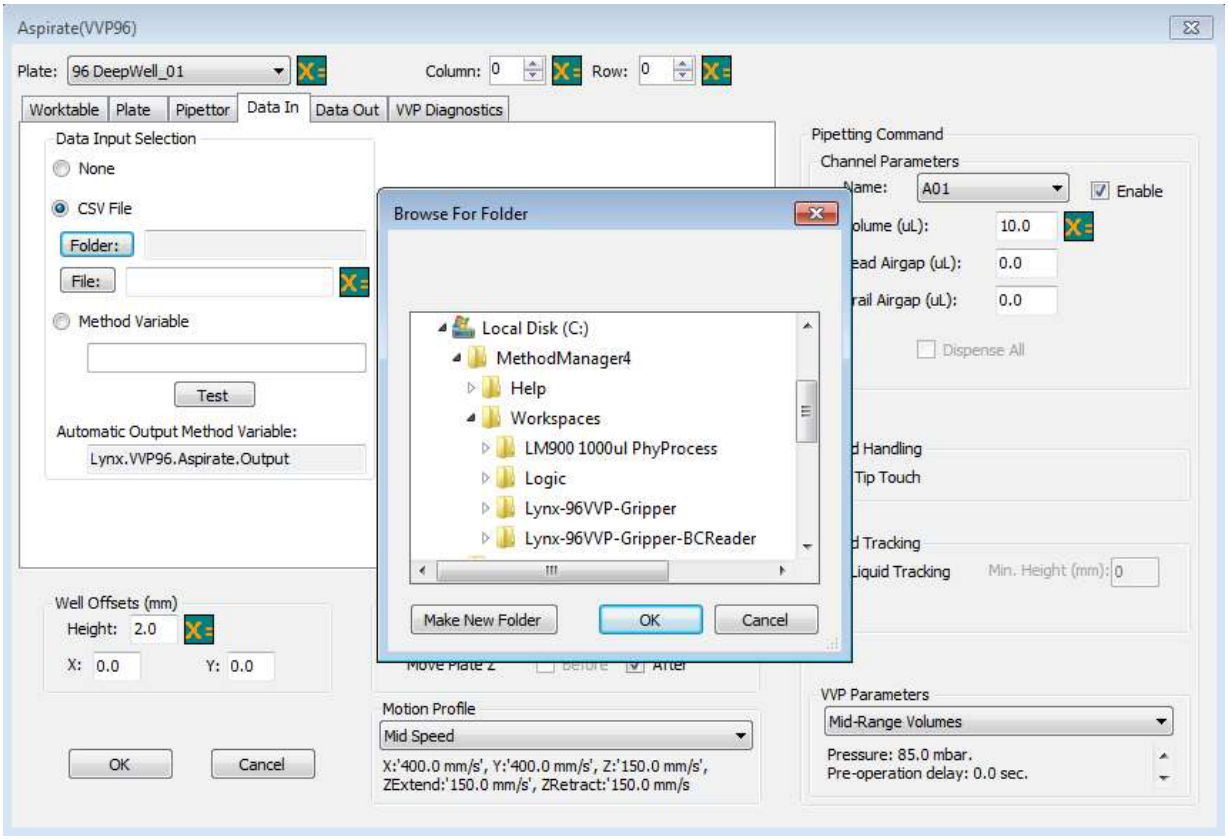
<Volume> → always required. Units in ul.

<Lead air gap or Blowout> → Optional, default = 0ul

<Trailing air gap> → Optional, default = 0ul

<VVP residual Volume> → Optional, default = from internal tables

Data Input Selection: Folder browsing is available to attach a CSV file from a particular folder location. File choice may also be a variable name and is programmed by the “X=” next to the File button.

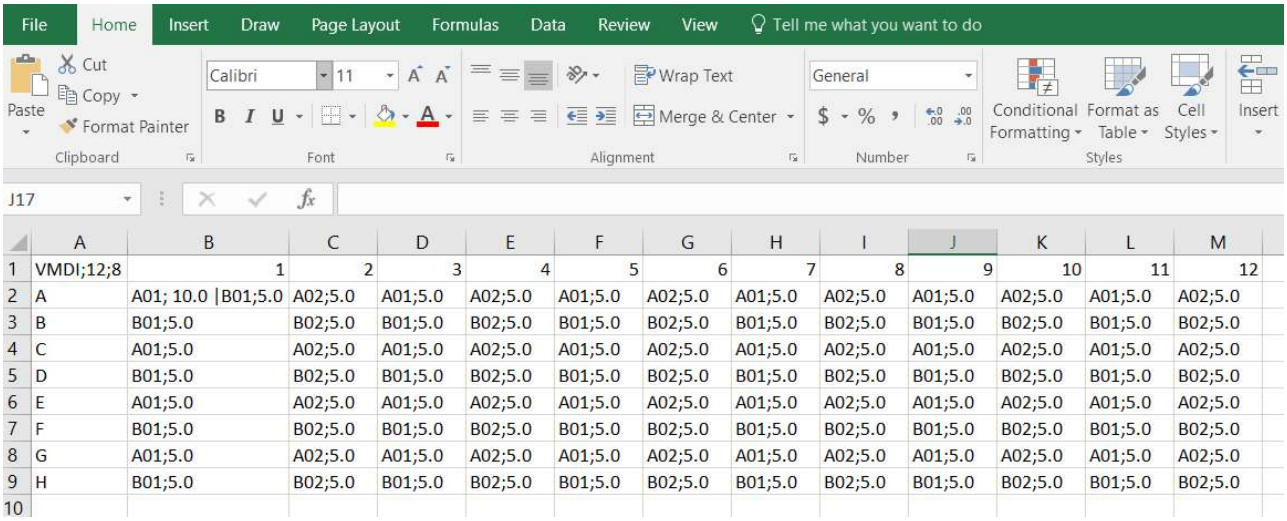


Input files do not contain any commands for positioning the head over plates or labware but output files do contain tracking information indicating which channels operated on which wells. An exception to this is the MultiDispense file which maps the contents of the channels to destination wells on a single plate, but these files do not specify the actual destination plate.

Because the files are intended to be viewed and edited in spreadsheet software the format allows for row and column annotation to appear in the file which is ignored when the file is parsed in MethodManager4. The data for each channel is separated by a comma and the parameters for the individual channel (volume, lead air gap, trail air gap) are separated by semicolons. In the case of the MultiDispense command where a single channel can have multiple destinations the destinations are separated by the pipe character ('|'). Apart from the first cell in the file the rest of the top row and left column are ignored and are intended to hold row and column annotation to make the file easy to read in spreadsheet software

Multi Dispense

The Multi Dispense allows the user to dispense multiple designated channels on the head to different wells in the destination plate. The Multi Dispense is always performed above the plate because the tips would crash into the labware when the head is in offset positions. There is also the danger of contamination of wells that would have unwanted tips in them. For example, if the head is instructed to dispense volume from the A1 tip into the H12 well, the head will need to offset 11 columns to the right and 7 rows down (if all the channels were lined up with their respective wells before moving). Since the tips aren't submerged in the liquid the user needs to use higher pressure profiles to get all the liquid out of the tip (>100mBars).



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	VMDI;12;8	1	2	3	4	5	6	7	8	9	10	11	12
2	A	A01; 10.0 |B01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0
3	B	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0
4	C	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0
5	D	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0
6	E	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0
7	F	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0
8	G	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0	A01;5.0	A02;5.0
9	H	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0	B01;5.0	B02;5.0
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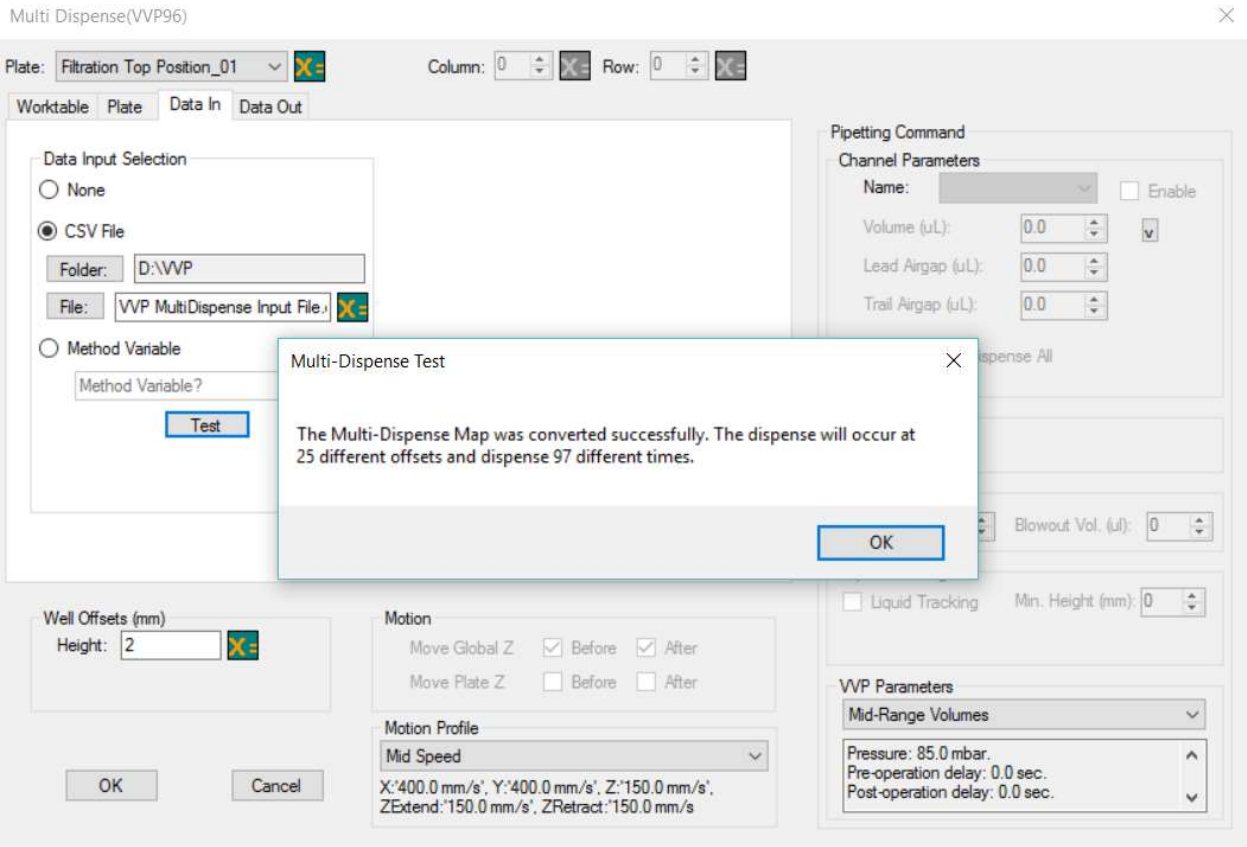
The instructions for the Multi-Dispense need to be in a standard comma delimited CSV (NOT Macintosh) format. Using Excel is the best way to visually see it for a user because each cell can be mapped to a channel on the head. These commands can also be conveyed through method variables, which is discussed at the end of this section. See picture below:

Note: this is a comma delimited file which is being shown in an Excel format. The raw text would show commas between each cell.

The VVP command for multi-dispense is VMDI;12;8 which is in cell A1. In the file it will be the first element on the first line. The rest of Row 1 and Column1 of the excel sheet is ignored by MM4, so we put human readable data like the Row letter and Column number of the channel that will be given the dispense instruction so its easy for the user to see which channels are getting what instructions.

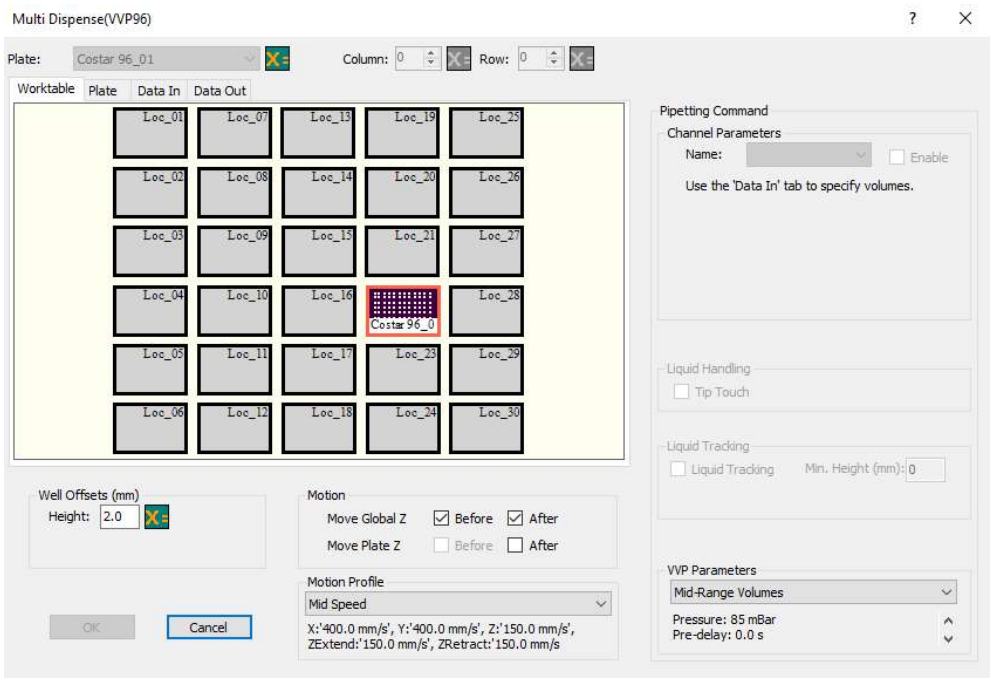
The instructions for Multi Dispense are formatted as follows: **WellName;Volume**. So B01;20 tells the pipettor to dispense 5ul into well B01 from the channel it was called from. In the example above, if you look at Channel H01 (cell 9B in the excel file) you can see that this channel is going to pipette into well B01.

If one channel is going to multiple destinations then use a '|' delimiter to send multiple instructions: **WellName;Volume | WellName;Volume | and so on.** For example this instruction: A01;15|B01;12|C03;15 would dispense to three destinations: A01, B01 and C03 using 15ul, 12ul and 15ul respectively. Channel A1 in the above picture is an example of multi-destination, multi-dispensing.



MM4 parses the entire file or method variables and figures out how much to offset the head and also maximizes the number of dispenses per offset position. You can test this file and see MM4 calculate the number of moves and dispenses it will need and will move in the most efficient manner by minimizing the number of offsets and maximizing the number of dispenses per offset. To do this go into the “Data In” tab on the multi-dispense step then select the CSV file which contains your pipetting instructions. Click test and it will tell you how many offset positions the head will go into and how many dispense operations will occur. The example in the above picture was used below:

Like all other VVP commands the user can also use a method variable to convey the Multi-dispense instructions. The method variable is slightly different in that it is just one long line of comma delimited instructions. This contrasts with a CSV file which contains 9 lines since they are split by new lines. The CSV also has extra human readable information.



Note:The height parameter specifies the distance from the plate clearance the command will be executed at.

Multi Dispensing to Multiple Destination Plates

As of **MM4 1.4.7545** or later build, Multi-Dispense VMDI input supports multi-destination CSV worklist where a **':'** delimiter (colon) is used to define a destination plate name on the work table with following format:

PlateName:WellName;Volume|Platename:WellName;Volume|and so on.

As the <Blowout> and <Trailing air gap> are optional after Volume field.

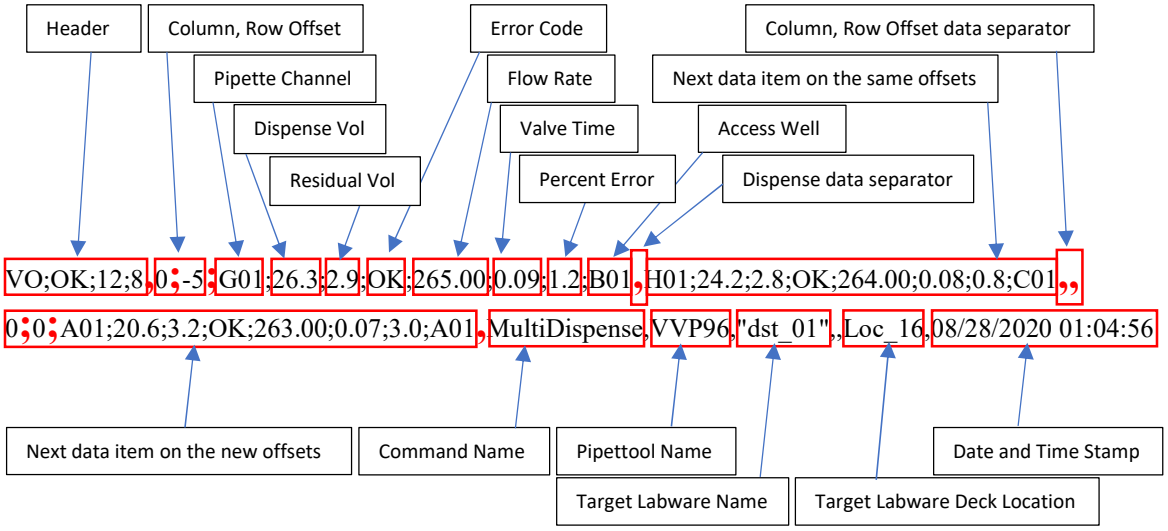
Example:

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	VMDI;12;8	1	2	3	4	5	6	7	8	9	10	11	12
2	A	dst_01:A01;20|A02;25|dst_02:A01;30|dst_03:A01;25											
3	B	dst_02:A01;21											
4	C	dst_01:A01;22											
5	D	dst_02:A01;23											
6	E	dst_01:B01;24											
7	F	dst_02:B01;25											
8	G	dst_01:B01;26											
9	H	dst_02:B01;27											

*Note:**dst_01**, **dst_02**, and **dst_03** are target destination plates for this Multi Dispense VMDI.*

The output result of Multi Dispense to multiple destination plates will be store together in "**Lynx.VVP96.MultiDispense.Output**" variable with ***Newline*** (CR + LF) separating outputs from each target destination plate.

Output Fields Explanation:



Examples:

Single-Plate Mult-Dispense Output

VO;OK;12;8,0;-5;G01;26.3;2.9;OK;265.00;0.09;1.2;B01,,0;-3;E01;24.2;2.8;OK;264.00;0.08;0.8;B01,,0;-2;C01;22.3;2.9;OK;262.00;0.07;1.4;A01,,0;0;A01;20.6;3.2;OK;263.00;0.07;3.0;A01,,1;0;A01;20.6;3.2;OK;263.00;0.07;3.0;A02,MultiDispense,VVP96,"dst_01",,Loc_16,08/28/2020 01:04:56

Multi-Plate (3 plates) Mult-Dispense Output

VO;OK;12;8,0;-5;G01;26.3;2.9;OK;265.00;0.09;1.2;B01,,0;-3;E01;24.2;2.8;OK;264.00;0.08;0.8;B01,,0;-2;C01;22.3;2.9;OK;262.00;0.07;1.4;A01,,0;0;A01;20.6;3.2;OK;263.00;0.07;3.0;A01,,1;0;A01;20.6;3.2;OK;263.00;0.07;3.0;A02,MultiDispense,VVP96,"dst_01",,Loc_16,08/28/2020 01:04:56
VO;OK;12;8,0;-6;H01;27.3;2.9;OK;265.00;0.09;1.1;B01,,0;-4;F01;25.4;3.0;OK;254.00;0.09;1.6;B01,,0;-3;D01;23.4;3.0;OK;255.00;0.08;1.7;A01,,0;-1;B01;21.1;2.7;OK;266.00;0.07;0.5;A01,,0;0;A01;30.3;2.9;OK;263.00;0.10;1.0;A01,MultiDispense,VVP96,"dst_02",,Loc_22,08/28/2020 01:05:11
VO;OK;12;8,0;0;A01;25.3;2.9;OK;266.00;0.08;1.2;A01,MultiDispense,VVP96,"dst_03",,Loc_10,08/28/2020 01:05:18

Multi Dispensing to Different Types of Labware

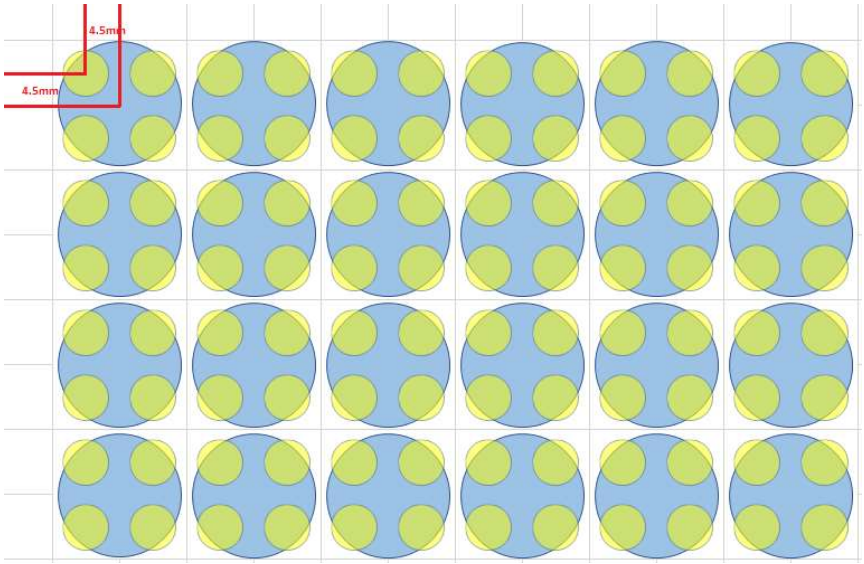
MM4 calculates variation of column and row offsets (permutations) only from the pipette tool's A01 and the target's labware A01. So, in standard use case where the pipette tool has 9mm column and row spacing and the target labware also has 9mm column and row spacing, all pipette tool channels are guarantee access to all target labware wells. (see examples below)

However, if the target labware column and row spacing are not 9mm such as 18mm, some of the tool's channel will not be able to access any well due to column and row offsets are increment by 18mm. In the example below, tool's channels on the even column numbers as well as rows B, D, F, and H have no well access on any available column or row offsets.

In some cases, depending on well shape as well as well column and row spacing, shifting the pipette tool A01 starting position in relation to target labware's A01 might help the pipette tool gain access to all wells. For instance, with the 24 well 18mm well column and row spacing, we can simply shift the pipette tool starting position to the left and up using formula below:

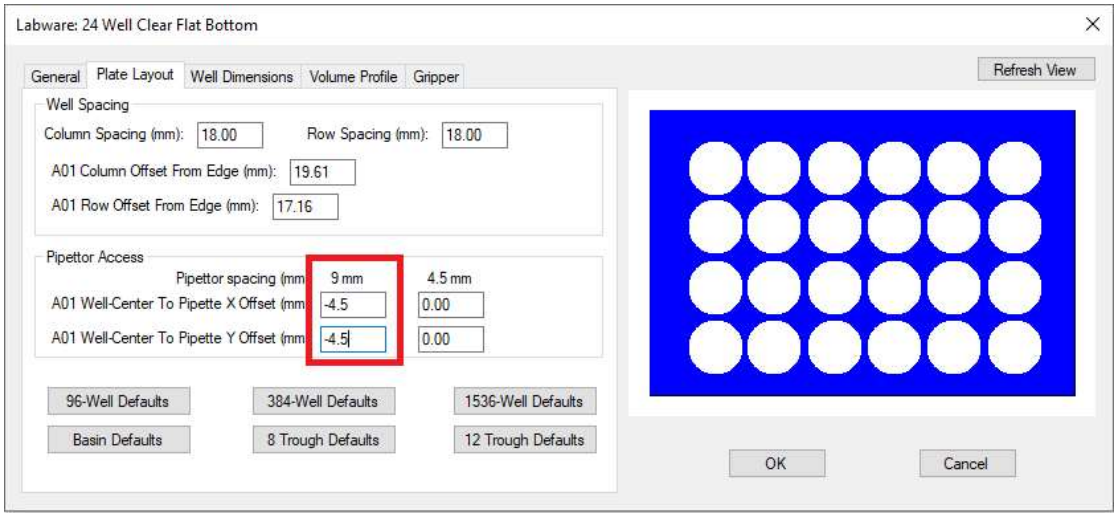
Shift Left = -1 x ((well column spacing/2) – (pipette column spacing/2))
Shift Up = -1 x ((well row spacing/2) – (pipette row spacing/2))

Using 18mm well column and row spacing and 9mm pipette tool's column and row spacing, the amount to shift to the left and up are both -4.5mm respectively. All pipette tool channels gain access to all well with these -4.5mm offsets.

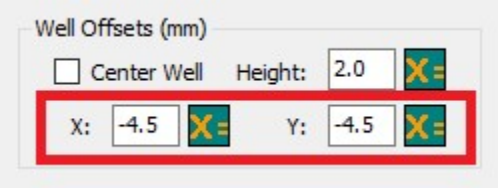


There are two ways to apply X and Y shift offsets:

1. Modifying labware Pipette Access A01 Well-Center to Pipette Offsets



2. Applying In-Well X and Y Offsets in the Multi-Dispense command

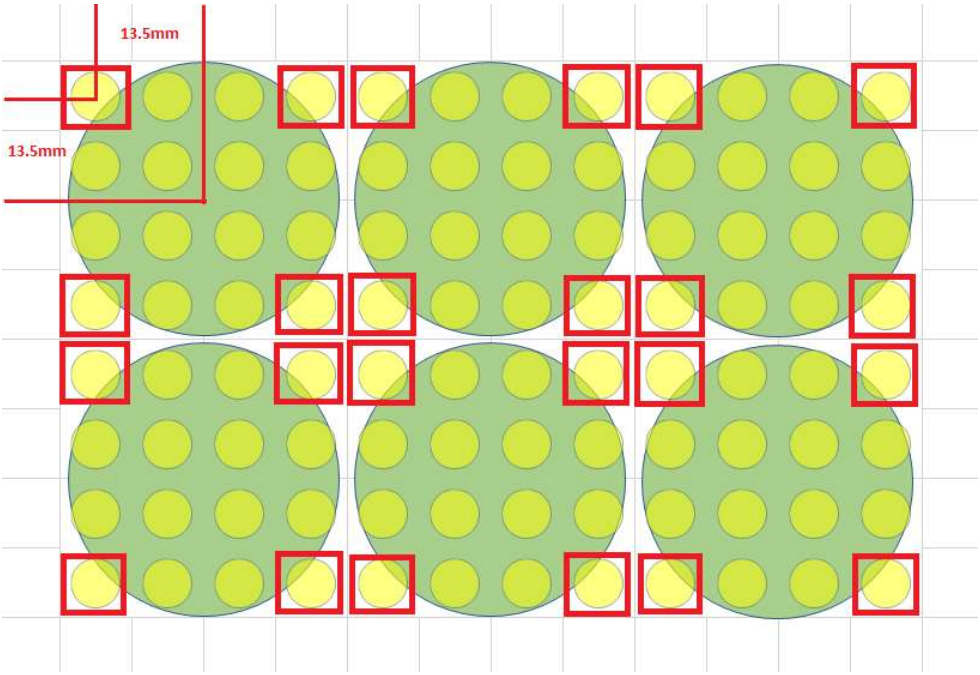


Key benefits of using X and Y offsets with larger well are:

- Dispense multiple channels to the target wells simultaneously
- Minimize number of permutations

However, there are a few disadvantages and limitations as following:

- In the case where well are very large and round well shape, applying the offsets from formula above are not likely to solve the problem due to the well shape. For example, 6 well plate with 36mm well column and row spacing, the amount to shift calculated from the formula is -13.5mm, there will be channels on the pipette tool that do not have access to wells as seen below:



- Dispensing will not be performed at the well-center location
- May not work properly on labware with irregular well column and row spacing

As of MM4 1.4.7958 or later, an additional option has been added to the Multi-Dispense command called "Center Well" locating the Well Offsets section. When enabled (checked), X and Y offsets will also be parts of permutation calculations to make sure that all pipette tool

channels have access to all wells on the target labware regardless column and row spacing of both pipette tool or target labware.

Key benefits of using the Center Well options are:

- Guarantee access of all pipette tool's channels on any well on target labware
- Dispensing at the center of well
- Works on any tool configuration
- Works on any plate configuration

One main disadvantage when using the Center Well enabled comparing to using X and Y offsets method is that this method could be slower due to the number of permutations calculated to make sure all pipette tool's channels aligned on each target well center.

The Multi-Dispense command with Center Well option enabled also provided slightly different fields on the **Lynx.VVP96.MultiDispense.Output** with additional X and Y offsets fields. The permutation will consist of 4 fields instead of 2 fields as following:

Column Offset; Row Offset; XOffset (mm); YOffset (mm)

Below is an example of Multi-Dispense output with Center Well option enabled:

VO;OK;12;8
,**0;0;0;0**;A01;220.4;2.9;OK;296.00;0.73;0.2;A01,A05;220.5;3.0;OK;286.00;0.76;0.2;A03,A07;220.4;2.9;OK;283.00;0.77;0.2;A04,A09;220.3;2.8;OK;290.00;0.75;0.1;A05,**„0;2;-9;0**;A02;220.5;3.0;OK;313.00;0.69;0.2;C01,C06;220.3;2.8;OK;295.00;0.74;0.1;D03,**„0;3;0;0**;A03;220.4;2.9;OK;298.00;0.73;0.2;D02,**„-1;1;9;0**;A04;220.5;3.0;OK;306.00;0.71;0.2;B02,**„0;0;-9;-9**;B10;220.3;2.8;OK;301.00;0.72;0.1;A05,F06;220.2;2.8;OK;286.00;0.76;0.1;C03,F10;220.4;2.9;OK;287.00;0.76;0.2;C05,**„0;1;0;-9**;B11;220.4;2.9;OK;299.00;0.73;0.2;B06,**„-1;2;9;-9**;B12;220.4;2.9;OK;301.00;0.72;0.2;C06,**„0;-1;0;0**;C01;220.4;2.9;OK;299.00;0.73;0.2;A01,C05;220.3;2.8;OK;292.00;0.74;0.1;A03,E01;220.2;2.8;OK;300.00;0.72;0.1;B01,E05;220.3;2.8;OK;289.00;0.75;0.1;B03,**„0;1;-9;0**;C02;220.4;2.9;OK;300.00;0.73;0.2;C01,E02;220.4;2.9;OK;303.00;0.72;0.2;D01,**„0;2;0;0**;C03;220.5;3.0;OK;300.00;0.73;0.2;D02,**„0;1;0;0**;C11;220.4;2.9;OK;305.00;0.71;0.2;C06,E11;220.5;3.0;OK;302.00;0.72;0.2;D06,**„0;-2;0;9**;D01;220.3;2.8;OK;306.00;0.71;0.1;A01,D05;220.2;2.8;OK;296.00;0.73;0.1;A03,**„0;0;-9;9**;D02;220.4;2.9;OK;297.00;0.73;0.2;C01,**„0;1;0;9**;D03;220.4;2.9;OK;304.00;0.72;0.2;D02,D11;220.3;2.9;OK;301.00;0.72;0.1;D06,**„-1;-1;9;9**;D04;220.4;2.9;OK;294.00;0.74;0.2;B02,D08;220.3;2.8;OK;286.00;0.76;0.1;B04,H08;220.3;2.8;OK;299.00;0.73;0.1;D04,H12;220.3;2.8;OK;307.00;0.71;0.1;D06,**„0;1;-9;9**;D06;220.4;2.9;OK;300.00;0.73;0.2;D03,**„0;-1;-9;0**;G06;220.4;2.9;OK;297.00;0.73;0.2;C03,**„0;-3;0;9**;H01;220.4;2.9;OK;305.00;0.71;0.2;B01,H05;220.4;2.9;OK;300.00;0.72;0.2;B03,**„0;-4;0;9**;H03;220.4;2.9;OK;314.00;0.69;0.2;A02,H11;220.4;2.9;OK;305.00;0.71;0.2;A06,**„0;-2;-9;9**;H06;220.4;2.9;OK;304.00;0.72;0.2;C03,
MultiDispense,VVP96,"Destination",,Loc_10,10/16/2021 09:49:22