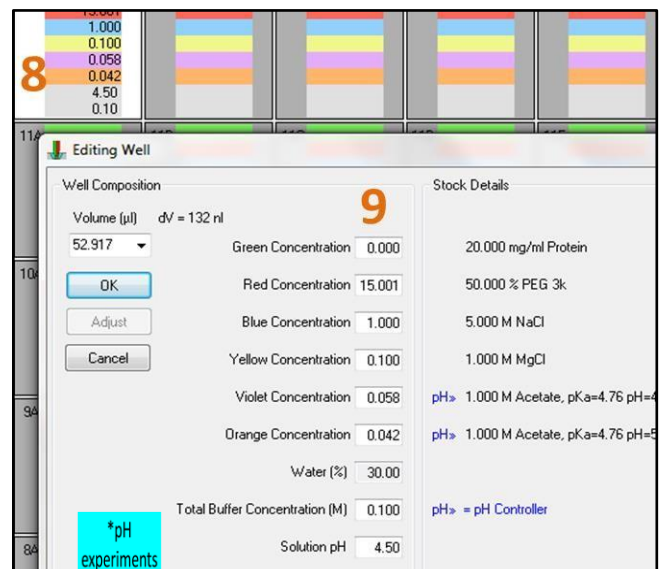
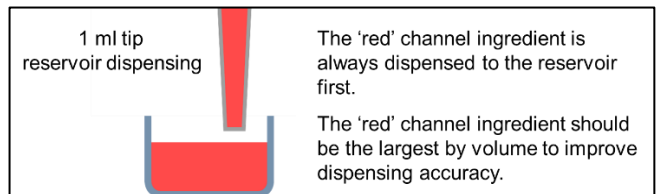
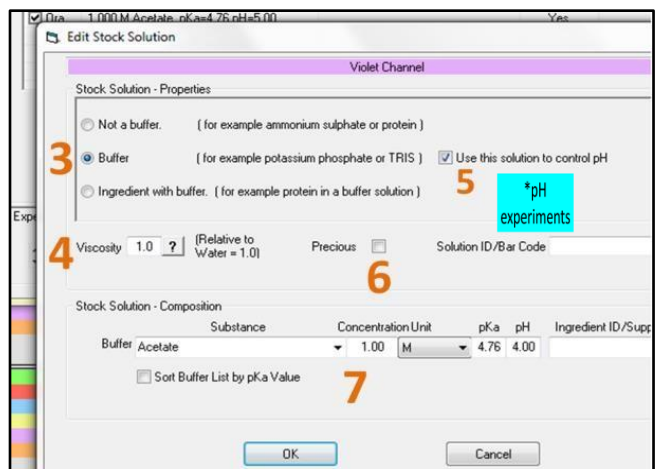
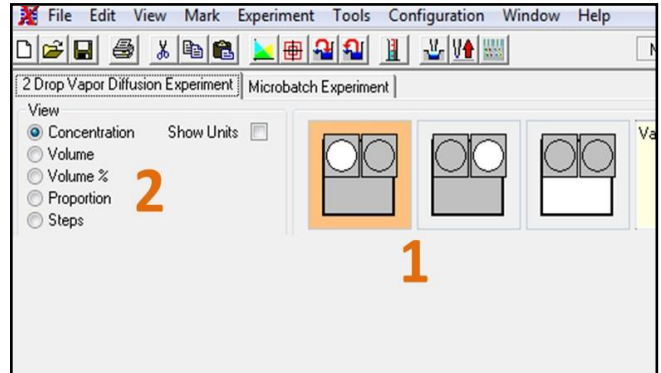


# Optimization – Experiment Design

1. Open **XStep.exe**
2. Select **File > New Project** to open the default template project
3. The spreadsheet design interface will appear:
  - On the left of the spreadsheet the first 6 ingredients are shown. (The seventh ingredient is always water and is not shown.)
  - The user can choose to edit the drops or reservoir (1)
    - Drops. Concentration calculated after dilution with protein
    - Reservoir. Concentration of reservoir solution.
  - The spreadsheet is normally viewed in Concentration mode. The user can also view well composition by: volume, percentage volume or number of motor steps. (2)
4. Specify experiment type. (Experiment -> Experiment type) There are 3 types of experiment:
  - **Microbatch under oil.** (Dispenses drops and covers with oil.)
  - **Vapour diffusion.** (Dispenses both the equilibration reservoir and the drop.)
  - **Stock Plate preparation.** (Dispenses just the reservoir or stock solution.)
5. Chose **Plate Type** (Experiment >Plate)
6. Specify **Stock Ingredients.** (Experiment > Stock Solutions)
  - Click an ingredient to edit it
  - For reservoir dispensing or stock plate filling the **RED** channel ingredient is always dispensed first by the 1 ml tip. To improve dispensing accuracy it is recommended to dispense the largest ingredient by volume first. **Ideally the volume of the first ingredient should be larger than 5µl.**
  - Specify ingredient type. Whether it is a buffer or not. (3)
  - Specify viscosity. Click [?] for examples. (4)
  - **For pH experiments:** select to use buffer as pH controller for drop or reservoir pH. (5)
  - Precious solutions are dispensed to the drop only. Precious solutions are loaded from PCR tubes(6)
  - Specify Ingredient name or choose buffer from the list. Specify the concentration (and pH for buffers). (7)
7. Edit wells
  - Select a well e.g. reservoir 12A. Right click and select edit. This opens the Edit Well window. (8)
  - Specify the concentration of each ingredient (9). **For pH experiments** specify the 'Total Buffer Concentration' then specify the final 'solution pH'



# Optimization – Experiment Design

## 8. Design 'Auto Design' experiments



- Define a well with the central condition for the optimization
- Right click the central condition and click 'Auto Design'
- Specify the variation of each ingredient (10) (%)
- Choose the Box Benken or Central Composite experiment design. Or create your own by choosing how many parameters to vary. (11)
- Click ok to generate the experiment. (12)

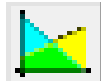
Variable	Center Value	Variation (%)
20.00 mg/ml Protein	0.000	Constant
50.00 % PEG 3k	15.001	±33.0%
5.00 M NaCl	1.000	±25.0%
1.00 M MgCl	0.100	±100.0%
1.00 M Acetate	0.056	From pH
1.00 M Acetate	0.042	From pH
Final Solution pH	4.500	±10.0%
Total Buffer Concentration	0.100	Constant
Volume [µl]	52.917	Constant

Current Design: 4D Central Composite

Wells: 1 (Center Points), 0 (1 parameter), 24 (2 parameters), 32 (3 parameters), 16 (4 parameters), 0 (5 parameters), 0 (6 parameters), 0 (7 parameters)

Total Wells: 25

## 9. Design 'Gradient' experiments



- Specify Maximum and minimum conditions for the gradient in opposite corners of a block of wells
- Drag a grid to select the block of wells (13)
- While the block is selected, click Interpolate. (Tools -> Interpolate)
- Specify whether the gradient ranges from top left to bottom right, or bottom left to top right. (14)
- Choose which ingredients to vary in which axis. (15)
- For pH experiments** choose an axis to vary 'Nett solution pH' in. This is required to create a **linear pH gradient** rather than a linear buffer concentration gradient. Buffer ingredients should then say 'Varying: Defined by pH'.
- Click interpolate to generate gradient. (16)

Ingredient	From	To	Change	Varying
20.00 mg/ml Protein	0.000	0.000	0.000	Constant
50.00 % PEG 3350	20.000	15.000	-5.000	X Only
4.00 M NaCl	1.000	1.000	0.000	Constant
1.00 M MgCl	0.100	0.100	0.000	Constant
1.00 M Acetate	0.000	0.100	0.100	Defined by pH
1.00 M Acetate	0.100	0.000	-0.100	Defined by pH
Nett Solution pH	5.00	4.00	-1.00	Y Only
Total Buffer Concentration	0.10	0.10	0.00	Constant
Solution Volume	999.998	999.998	0.000	Constant

Direction:  Bottom left to top right  Top left to bottom right

## 10. Generate drops from reservoirs. If the experiment was designed by editing the reservoir then it is possible to automatically generate the corresponding protein drop for vapour diffusion experiments. Click Generate drop solution from reservoirs. (17) The user will then be asked to specify the drop volume and proportion of protein in the drop

## 11. It is also possible to generate reservoir solutions from drops (18) if the experiment was designed in drop view.

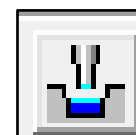
File Edit View Mark Experiment Tools Configuration Window Help

2 Drop Vapor Diffusion Experiment Microbatch Experiment

## 12. A yellow warning triangle will appear if the concentration of the well is too high. Either lower the well concentration or increase the concentration of the stock ingredient(s)

12A 0.000  
1.000  
0.000  
0.000  
0.000  
7.55  
0.00

## 13. Click the dispense button (icon shown right) to begin the experiment.



For 1 ml tip only reservoir dispensing select 'No' when asked by Front Panel.exe if you wish to use the microtip to assist with reservoir dispensing.