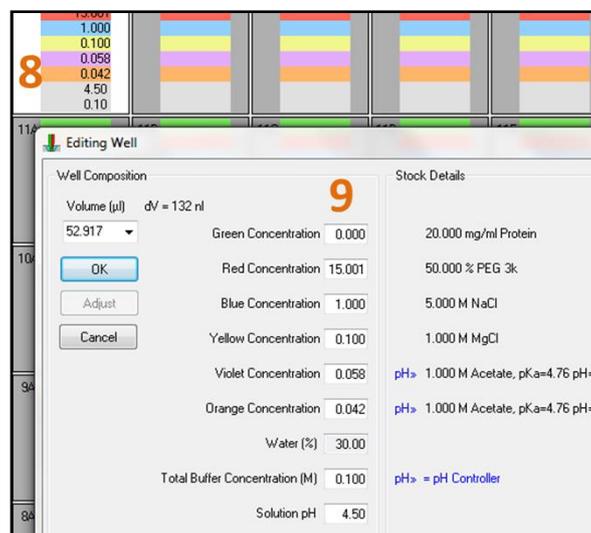
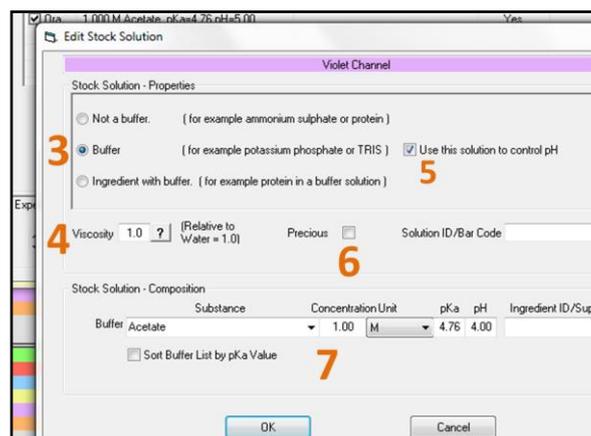
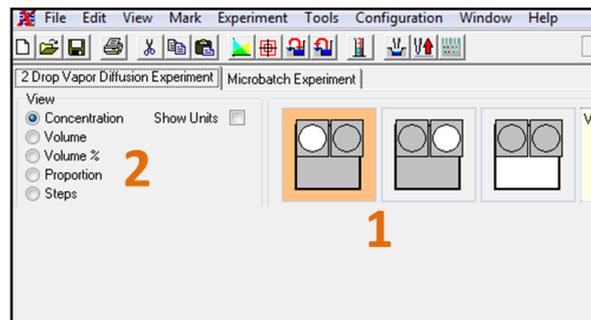


Optimization – XStep Quick Start

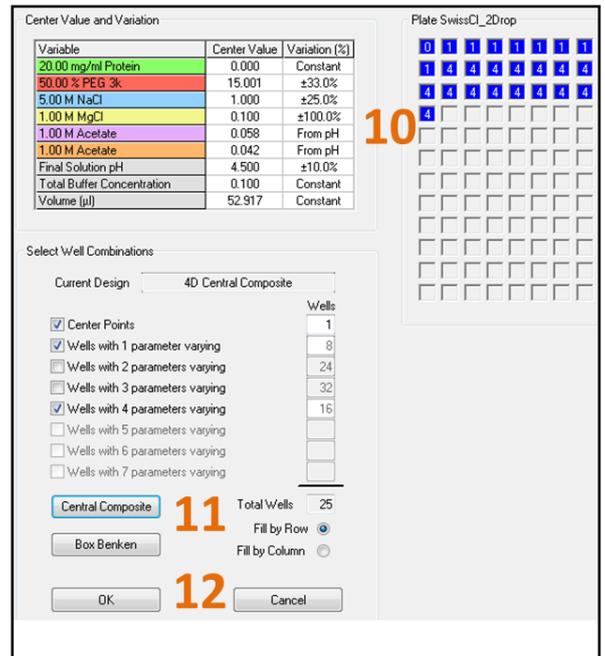
Experiment design

- Open **XStep** by double clicking the desktop shortcut:
 - Create a new Project. (File -> New Project.)
 - The spreadsheet design interface will open.
 - 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)
- 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.
- Choose Plate type. (Experiment -> Plate)
E.g. MRC Maxi, Douglas Vapour batch
- Specify Ingredients for the experiment. Experiment -> Stock solutions
 - Clicking on ingredients allows them to be edited
 - Specify ingredient type. Whether it is a buffer or not. (3)
 - Viscosity. Set to 1.0 for non-viscous solutions. E.g. Set to 20.0 for 50% PEG 3K. (4)
 - Use buffer to control pH (buffer type only.) Select to use buffer as pH controller for drop or reservoir pH. (5)
 - Precious. If a solution is precious it is dispensed to just the drop and not the reservoir. (6)
 - Ingredient name, concentration and pH. (7)
- To create a multivariate **auto design** experiment:
 - Select a well e.g. reservoir 12A. Right click and select edit. This opens the **Edit Well** window. (8)
 - Specify the central condition for the optimization. E.g. the hit condition (9) when done click ok.



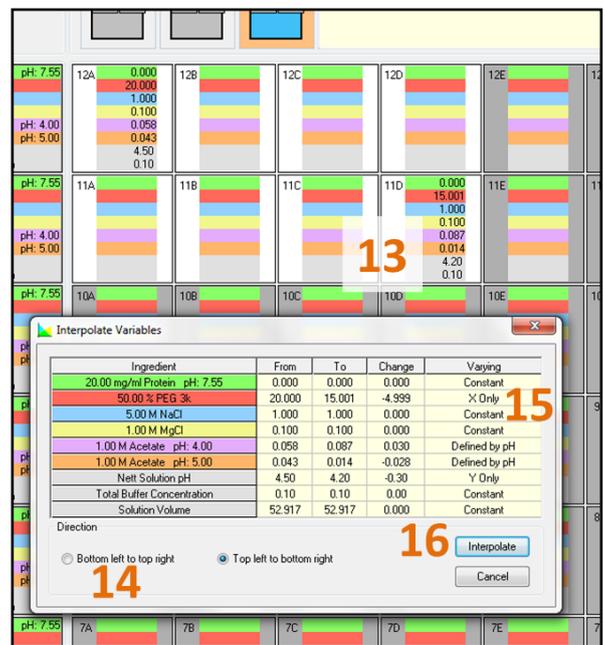
Optimization – XStep Quick Start

- c. Right click the well and select **auto design**.
- d. Specify the variation of each ingredient (10)
- e. Choose Box Benken or Central composite experiment designs. Or choose how many parameters to vary. (11)
- f. Click ok to generate the experiment. (12)



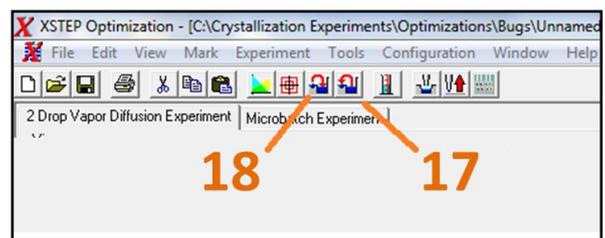
7. To design a **gradient** optimization experiment:

- a. Specify Maximum and minimum conditions for the gradient in opposite corners of a block of wells.
- b. Drag a grid over the block of wells (13)
- c. Click **Interpolate**. (Tools -> Interpolate)
- d. Specify whether the gradient ranges from **top left to bottom right**, or **bottom left to top right**. (14)
- e. Choose which ingredients to vary in which axis. (15)
- f. Click interplotate to generate gradient. (16)



8. 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

9. It is also possible to **Generate reservoir solutions from drops** (18) if the experiment was designed in drop view.



10. Click the **dispense** button to begin the experiment:

