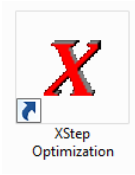
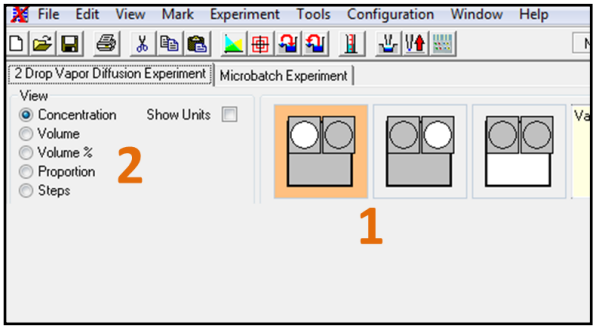
Experiment design



1. Open **XStep** by double clicking the desktop shortcut:

2. 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)**



3. Specify experiment type. (Experiment -> Experiment type)
There are 3 types of experiment:

a. **Microbatch under oil**.
Dispenses drops and covers with oil.

b. **Vapour diffusion**.
Dispenses both the equilibration reservoir and the drop.

c. **Stock Plate preparation**.
Dispenses just the reservoir or stock solution.

4. Choose Plate type. (Experiment -> Plate)
E.g. MRC Maxi, Douglas Vapour batch

5. Specify Ingredients for the experiment. Experiment -> Stock solutions

a. Clicking on ingredients allows them to be edited

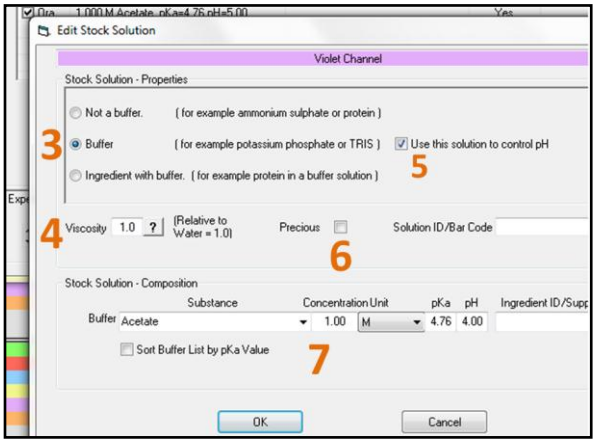
b. Specify ingredient type. Whether it is a buffer or not. **(3)**

c. Viscosity. Set to 1.0 for non-viscous solutions. E.g. Set to 20.0 for 50% PEG 3K. **(4)**

d. Use buffer to control pH (buffer type only.) Select to use buffer as pH controller for drop or reservoir pH. **(5)**

e. Precious. If a solution is precious it is dispensed to just the drop and not the reservoir. **(6)**

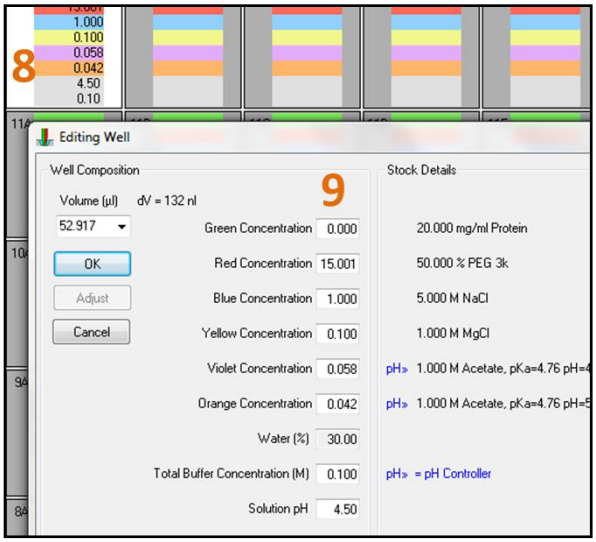
f. Ingredient name, concentration and pH. **(7)**



6. To create a multivariate **auto design** experiment:

a. Select a well e.g. reservoir 12A. Right click and select edit. This opens the **Edit Well** window. **(8)**

b. Specify the central condition for the optimization. E.g. the hit condition **(9)** when done click ok.

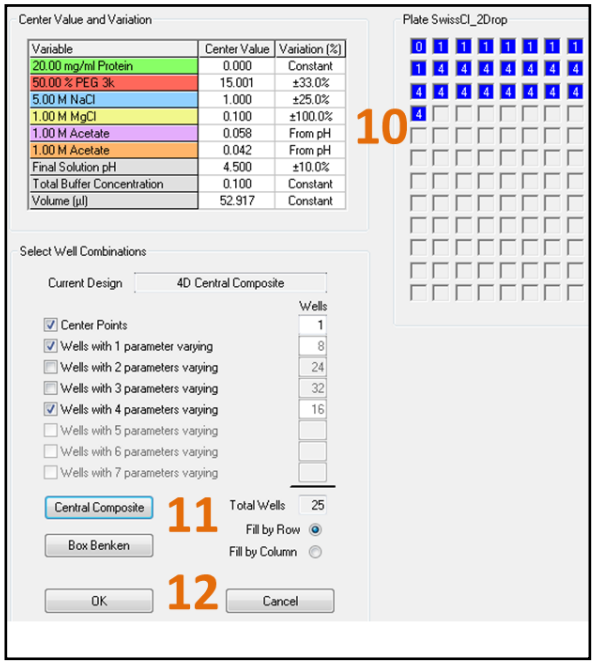


c. Right click the well and select **auto design.**

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.

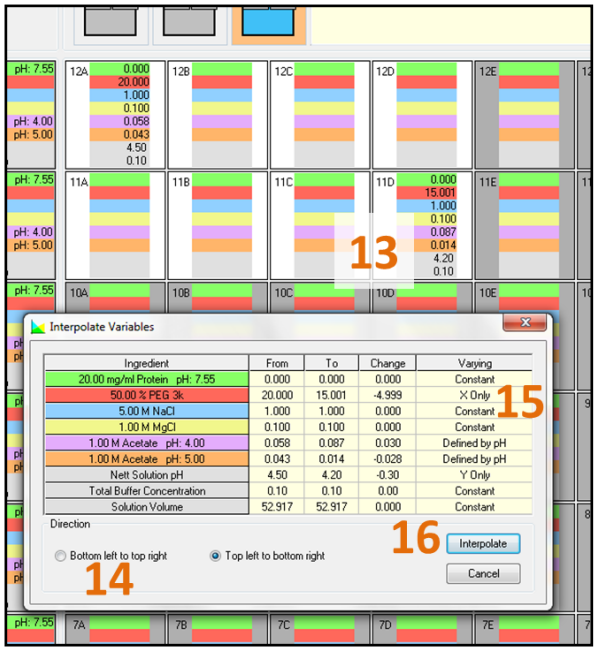
b. Drag a grid over the block of wells **(13)**

c. Click **Interpolate**. (Tools -> Interpolate)

d. Specify whether the gradient ranges from **top left to bottom right**, or **bottom left to top right.** **(14)**

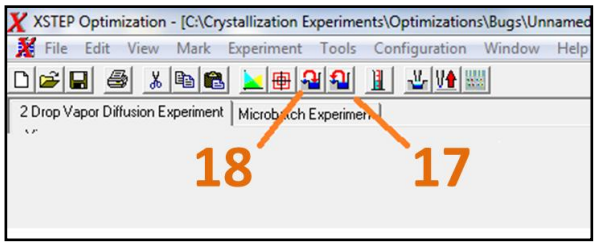
e. Choose which ingredients to vary in which axis. **(15)**

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: