XStep Quick Start – Designing experiments   Douglas Instruments
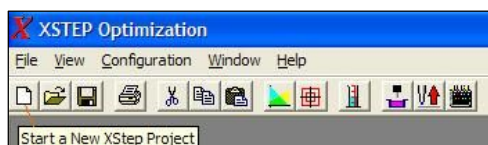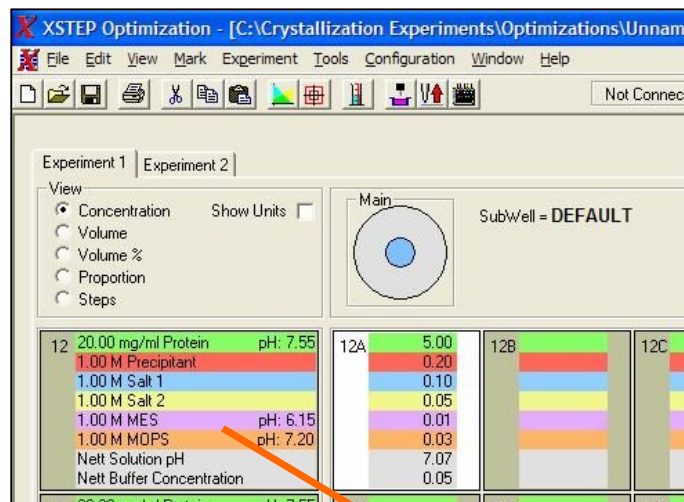
Step-by-Step Instructions

1. Create a new Project:

2. Layout of Spreadsheet

The spreadsheet displays a matrix of cells. Each cell represents a well and shows the composition of a droplet or crystallization trial. An XStep file represents a crystallization *project*, which may contain several spreadsheets - each spreadsheet corresponds to a plate or *experiment*.

On the left of the spreadsheet the first 6 (4 for Oryx6) ingredients of droplets are shown. These are typically protein, precipitant, additives, and buffers. The last channel always contains water, and it is not shown.

The spreadsheet is normally viewed in **Concentration Mode,** which shows concentrations and pHs. However, you can, if you choose, show the volume of each ingredient in a well, the percentage volume of each ingredient, or the number of steps that the dispensing syringes move. These alternative viewing modes can also be found on the **View** menu. Each *experiment* in the *project* maintains its own view mode.

3. Editing Ingredients

Double clicking on the ingredients at the left of the spreadsheet allows them to be edited.
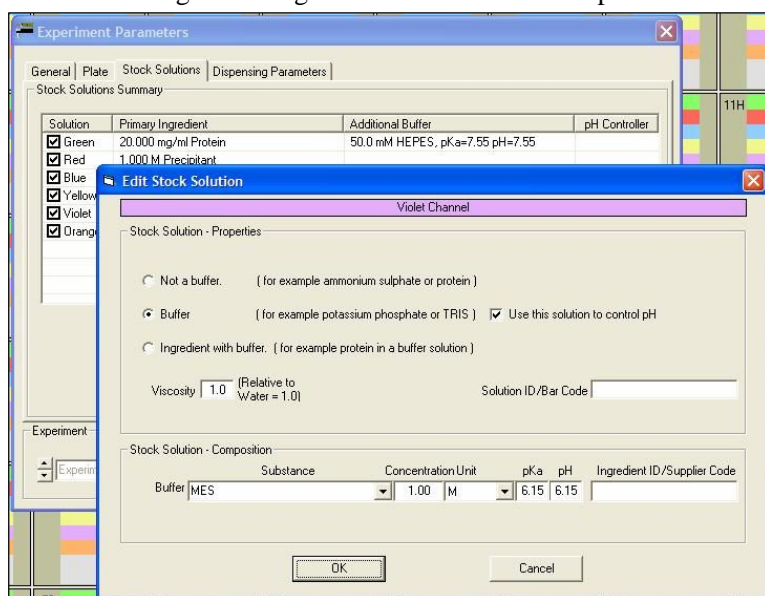
1. Click the specific ingredient to be edited.
2. Specify if this ingredient is or contains a buffer.
3. Enter the unit and name of the ingredient. You must specify well solution concentrations using the same unit as you used for the ingredient.
4. If there is a buffer present, enter the pKa of the buffer solution, followed by its pH.
5. If you are using viscous solutions such as PEG or MPD, enter the viscosity in the **Viscosity** box. Typical PEG solutions are around 40.
6. Enter or scan the solution bar code if available.
7. If the ingredient is a buffer and you want to use it to control pH check the 'Use this solution to control pH' box
8. Click on **OK**.

4. Editing Values in Cells
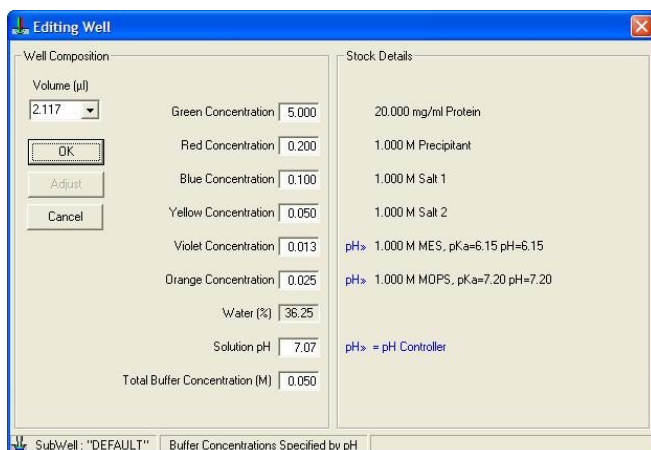
1. Double clicking on a cell allows its values to be edited.

2. The white channel on the **Oryx** chassis is conventionally used for water to obtain the concentrations specified in the other channels. The amount of water added from the white channel is not shown on the spreadsheet due to lack of space. However, the amount can be seen when you are editing a well.

3. It is also possible to edit multiple wells using this dialogue: drag a block with the cursor and right click, then select Edit from the menu. Values that are blank vary over the range selected. This option is useful to set certain values constant over a large block or to change the volume without affecting the concentrations.

Errors or corrections are indicated by a cell with a symbol adjacent to the affected channel. While editing a cell the nearest possible solution can be achieved by clicking on the adjust button. In all cases, however, you will get what you see – the affected cells have already been corrected and can be used safely.

5. Interpolate

If you enter values in opposite corners of a block of wells, you can then automatically fill in all the intervening wells by interpolation.
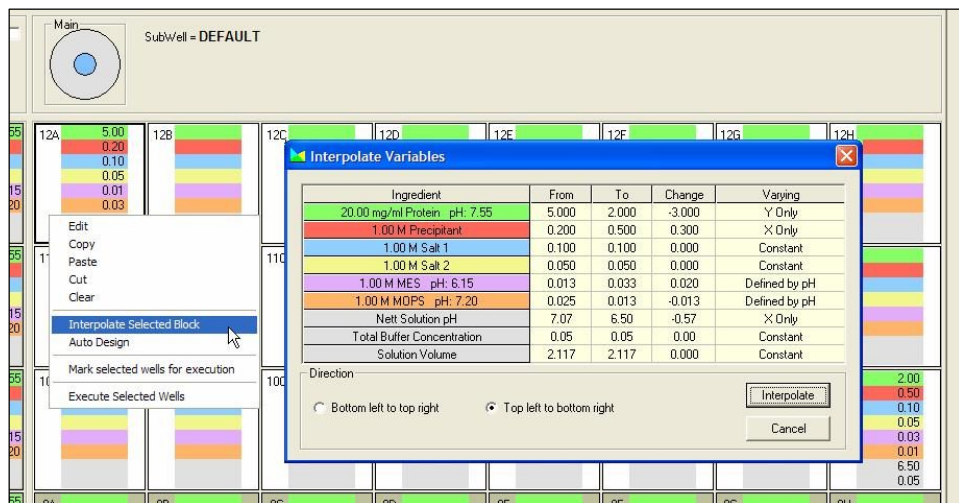

The principle of Interpolate is to provide 1-D or 2-D gradients of ingredient concentration. Typically, vary one ingredient in the Y direction (maybe Protein or Precipitant) and another in the X direction (e.g. Precipitant or Buffer/Additive) - this gives a 2-D interpolation.

It is also possible to do a big 1-D interpolation - using the **X then Y** variation (or the **Y then X**) in the **Interpolate** dialog. This means that the ingredient changes continuously in each well going from a minimum value in one corner to a maximum value in the other. (i.e. the sequence is like reading a book.)

1. Enter values into the wells at opposite corners of the block to be interpolated. (Note that this block need only be a part of the spreadsheet)
2. Select the block to be interpolated by dragging the mouse over it.
3. Right-click on the numbers of one of the selected wells, and click on **Interpolate Selected Block**.
4. Click on the down arrow in each **Varying** box and choose whether you would like to vary the corresponding ingredient in the X direction (**X Only**) or the Y direction (**Y Only**).
5. Select **Bottom Left to Top Right**, or **Top Right to Bottom Left**.
6. Select **Interpolate**.

6. Autodesign

This is generally the most effective method of optimization, since it uses "multivariate" designs, where all of the important variables are changed in each experimental run. This allows you to place points evenly around a

central point in a multi-dimensional experimental space. The central point is your best guess for optimal crystallization.
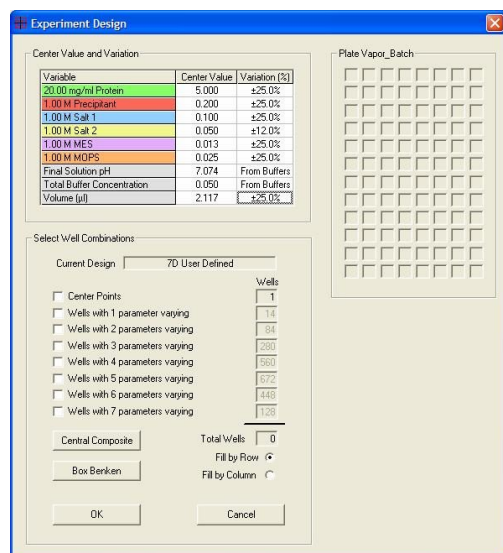
1. Move the cursor to the well around which you would like to base your experimental design.
2. Right-click on this well and select **Autodesign**.
3. For each variable, edit the **Variation(%)** value. This value might be anything from 100% (e.g. when you want to know whether an ingredient is necessary or not), to 5% (e.g. near the end of crystal optimization) or 0% (Constant).
4. Select the number of **Center Points** that you would like. (Textbooks often recommend several center points so that you have an idea of the experimental error of the system.)
5. Adjust the boxes for **Wells with 1, 2 ... 7 Parameters varying** until you have the number of wells that you want. In all cases the resulting wells will be positioned reasonably evenly in all directions around the center point. Alternatively, select the well-known **Central Composite** or **Box-Behnken** designs.
6. Click on **OK** to generate the experiment automatically.

7. Executing a Spreadsheet One Well at a Time

A spreadsheet can obviously be executed by selecting *Execute Experiment.* It may sometimes be very useful to execute part of an experiment, or to execute an experiment one well at a time. This can most easily be achieved by right-clicking on a well or wells and selecting *Execute Selected Wells.* This can, for example, be used to find the precipitation point of a protein using a particular precipitant. For example, a linear gradient can be set up across the whole plate varying only precipitant. A well near the center of the gradient is first dispensed, and precipitation looked for. The precipitation point is now searched for by dispensing one well at a time using a "binary chop" procedure. Eventually the precipitation point is found, using the minimum of protein. This is explained in more detail in Acta Crystallographica D. 50 (1994), pp 441-442.