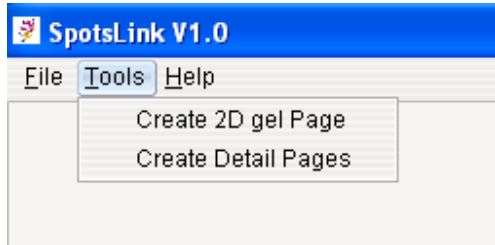


The use of SpotLink V1.0

SpotLink V1.0 is composed of two parts: create 2D gel page and create spot detail page:



1> create 2D gel page

To create 2D gel page, the software requires 3 input file.

The first input file contains spot position information which takes the following format: SpotNumber---positionX---positionY, where spotNumber is the spot number in integer, positionX is the x-coordinate (pI axis) and positionY is the y-coordinate (mass axis). “---” is tab delimitation. **Example:** file “SpotList4_7.txt” for soybean:

```
83      2142    148
106     2315    159
107     2358    161
122     2406    177
126     1779    179
135     2454    187
138     2315    186
143     734     193
```

.....

The second input file is the 2D gel picture in **bmp** format. The spot position information was acquired from this file. After the software loaded the file, it reads the picture width and height and calculates the size of the display picture.

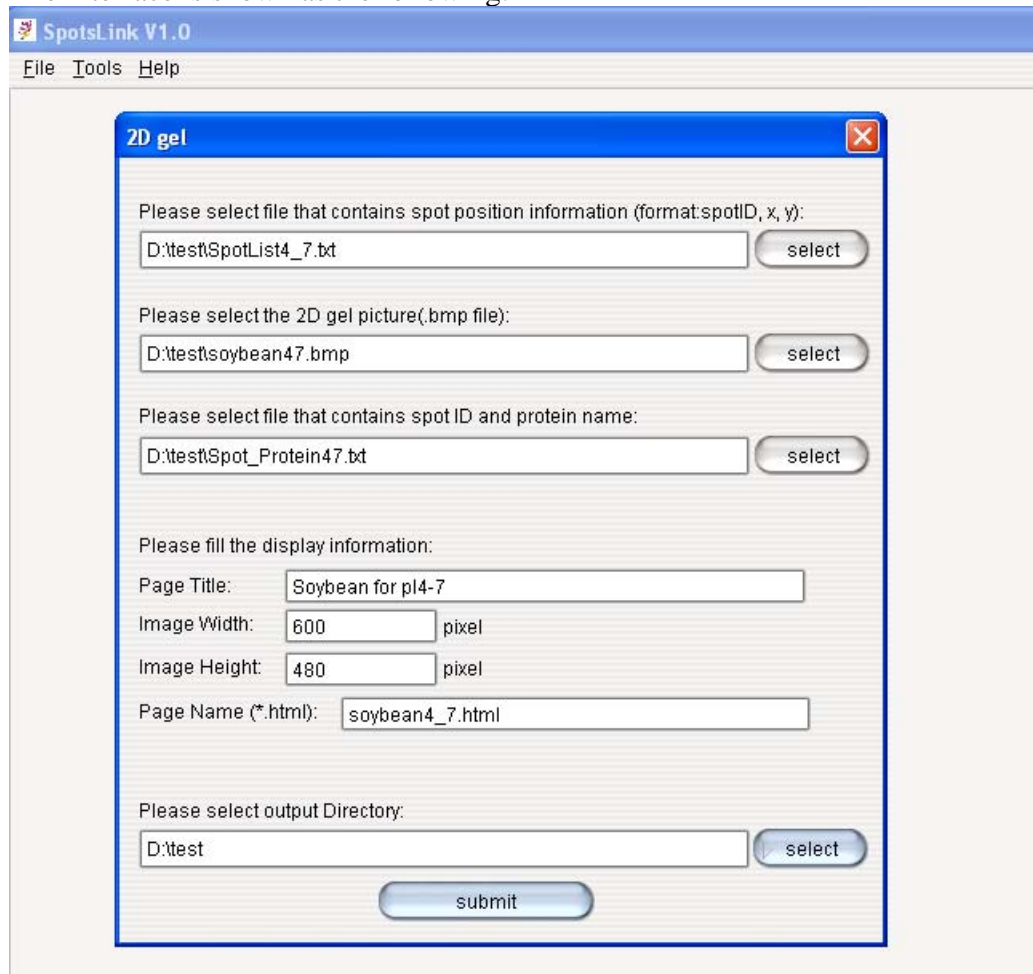
The third input file contains the information of spot number which takes the following format: SpotNumber---ProteinName, where spotNumber is the same with that in the first file and ProteinName is the corresponding protein to the spot.

Example: file “Spot_Protein47.txt” for soybean:

```
297    Methionine synthase [Glycine max]
300    Methionine synthase [Glycine max]
381    Succinate dehydrogenase (ubiquinone) flavoprotein subunit [Arabidopsis thaliana]
480    Acetohydroxy acid isomeroreductase [Medicago truncatula]
477    Aldehyde dehydrogenase [Medicago truncatula]
560    UDP-glucose/GDP-mannose dehydrogenase family, NAD binding domain, putati
550    tubulin B4 [Glycine max]
```

.....

The interface is shown as the following:



The screenshot shows the 'SpotsLink V1.0' application window. Inside, a '2D gel' dialog box is open. The dialog box has a blue title bar with a close button. It contains the following elements:

- Text: "Please select file that contains spot position information (format:spotID, x, y):"
Input field: "D:\test\SpotList4_7.bt"
Button: "select"
- Text: "Please select the 2D gel picture(.bmp file):"
Input field: "D:\test\soybean47.bmp"
Button: "select"
- Text: "Please select file that contains spot ID and protein name:"
Input field: "D:\test\Spot_Protein47.bt"
Button: "select"
- Text: "Please fill the display information:"
Page Title: "Soybean for pl4-7"
Image Width: "600" pixel
Image Height: "480" pixel
Page Name (*.html): "soybean4_7.html"
- Text: "Please select output Directory:"
Input field: "D:\test"
Button: "select"
- Button: "submit"

Users should select the 3 files first then fill some necessary information as well:

- Page Title: it will appear as the title of the web page.
- Image Width: the picture width the user wants the picture display with.
Default 600pixel, user can change it according to his/her monitor size
- Image Height: the picture height the user wants the picture display with.
Default 480pixel, user can change it according to his/her monitor size
- Page Name: the web page file will be saved as this name.

Finally, users should select an output directory to save the page.

After all is done, click "submit" button and view the result.

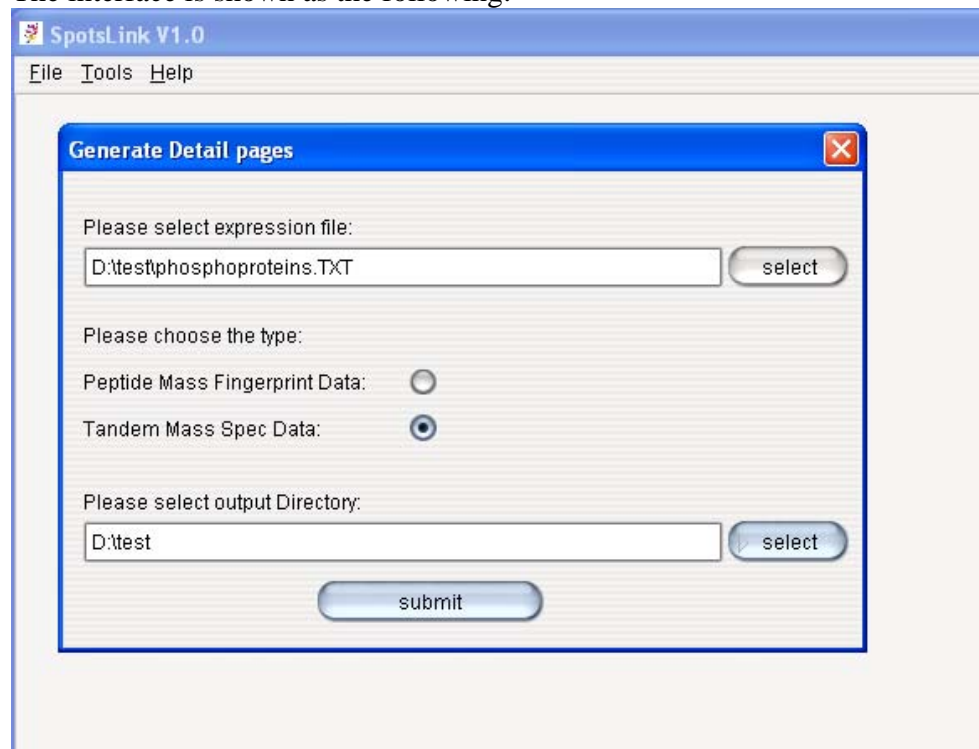
2> create spot detail page from a detail file

To create spot detail page, 1 file should be prepared. The file includes 11 column as following: SpotNumber---exp1---std1--- exp2---std2--- exp3---std3--- exp4---std4--- exp5---std5, where exp1-exp5 is the expression data for the protein in the successive 5 time points; std1-std5 is the corresponding standard deviation of the expression. **Example:** file “phosphoproteins.txt”:

340	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1056	0.0695
343	0.0000	0.0000	0.0000	0.0000	0.1353	0.0980	0.0000	0.0000	0.0000	0.0000
348	0.0000	0.0000	0.0000	0.0000	0.1482	0.0476	0.0000	0.0000	0.0000	0.0000
351	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3949	0.2325	0.3752	0.1722
353	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6325	0.4117	0.2025	0.1131
355	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2817	0.0760

.....

The interface is shown as the following:



The user need to specify the expression file first and then choose the data type. If the data is mass fingerprint data, the program will give the output pages name “MassSpotXXX.html”, where XXX is the spot number; if the data is Tandem Mass Spec Data, the name is “MSMSSpotXXX.html”.

Finally, the user need select an output directory and submit the task to save the page.

Notice: we are using free software “wgnuplot” to make the figures. "wgnuplot" runs well under current directory. So we need to specify the output directory as the root directory of the software and include “wgnuplot” in it.