

Instructions for Opticon Analysis Program

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The program was written for Opticon Monitor 1.08 and Mac OS X. It may need modification for later editions of Opticon Monitor.

To work correctly, the reference wells and sample wells must be laid out in the same order on the left and right hand sides of the plate (NOT with mirror symmetry). Blank wells should not be highlighted. Only the left hand side (reference) wells should be labeled in the plate file.

1. Export the data file as a CSV (comma separated values) file.
Open the data file in Opticon (either the run has just finished or choose *File>>Open data file*). Click the *Quantitation* button on the toolbar. Use the pulldown menu *Quantitation>>Export>>CSV*. In the dialog box, choose to export all steps, with normalized data. Leave the boxes checked to include sample labels, protocol and analysis labels with the file.
2. Transfer the file, via the server, to your home directory.
From the finder, choose *Go>>Home* [shortcut is shift-function-H]. Drag the file into this directory. You may want to rename it with a shorter name, but do not forget the original name. You will also need to have a copy of **ff.txt** in this directory.
3. Open the Terminal program (in the Utilities folder of OS X).
4. Type this command:

```
awk -f ff.txt input.csv
```

where **input.csv** is the name of the data file.
5. Several moments later, the prompt should reappear in the get two output files, OP1.csv and OP2.csv appearing in your home directory. (You may need to click on the home directory window to see them appear.) You can close Terminal at this point.
6. Open a copy of the Excel template file **template.xlt** and rename it as a workbook with the original data file name (e.g. 20060718_104500.xls).
7. Open OP1.csv and paste the columns into the “annealing data” page of the excel file.
8. Open OP2.csv and paste the rows into the “Cot calculation” page of the excel file.
9. Examine the “annealing chart reference” page to see if the curves look OK.
10. Read the intercept of the annealing curves on the “annealing chart reading” page (don’t change the settings of the annealing chart reference page). The intercept chosen will depend on the quality of the data, the complexity of the samples and the duration of the experiment. In general, a higher intercept is better (e.g. .80) but often only .70 or lower is possible. The page is set up to display the .68-.72 range of the annealing curve as a default. Double click on the chart axes to change the x and y ranges to better read the curve. This brings up the Format Axis dialog box. Choose “scale” and change the minimum and maximum values to enlarge the region of interest.
11. Write down the intercept values for each sample using pen and paper. Enter them into the “Time” row of the “Cot calculation” worksheet. The Cot values (concentration x time) should be automatically calculated, and plotted on the “Cot histogram” worksheet.
12. Save the analysis file as a workbook with the same name you have been using (e.g. 20060718_104500.xls) and back it up to the server.