### Install CIMAGE and ReAdW:

1. Install R (<http://cran.r-project.org/>)
2. Install gcc-c++ (**sudo zypper install gcc-c++**)
3. Install “texinfo” for “texi2dvi” (**sudo zypper install texinfo**)
4. Install netCDF (<http://www.unidata.ucar.edu/downloads/netcdf/index.jsp>) for dependency (compile with “-fPIC” option, i.e., **export CFLAGS=-fPIC**)
   1. ./configure --prefix=/usr/local/bin
   2. make
   3. make install
5. Install zlib (<http://zlib.net/>) for dependency
6. Install XCMS (<http://www.bioconductor.org/packages/release/bioc/html/xcms.html>) in R
7. Install LIMMA (<http://bioinf.wehi.edu.au/limma/>) in R
8. Install pdftk (**sudo zypper install pdftk**)
9. Unpack the cimage\_public.tar to a preferred location “your\_folder”
10. Define an environment variable CIMAGE\_PATH pointing to the “your\_folder” ( **export CIMAGE\_PATH=your\_folder** or **setenv CIMAGE\_PATH your\_folder**)
11. Install ReAdW on your mass-spec computer (winXP) for mzXML conversion:
    1. Install cygwin (<http://www.cygwin.com/>) on your winXP computer
    2. Copy the ReAdW-4.2.1 folder (within the ReAdW folder) to the C drive root directory
    3. Copy the zlib1.dll to the winXP system folder “C:\WINDOWS\SYSTEM”
    4. Copy the run\_readw script into the Cygwin local bin folder “C:\cygwin\usr\local\bin”
    5. Open a cygwin window and go to your folder with the RAW files and type “run\_readw raw\_file\_names” (supporting wild-card characters, such as” myRAW\*” for all files with their names starting with “myRAW”). If you do not give a file name, all RAW files in the current folder will be converted.

### Run CIMAGE to analyze data

1. make a folder such as "my\_experiment"  
  
2. make a folder "dta" in the "my\_experiment" folder  
  
3. copy the converted "**TAG**\_0?.mzXML" files to the "my\_experiment" folder  
  
4. copy the searched DTASelect-filter.txt files into the "dta" folder and make sure that they have names like "DTASelect-filter\_**TAG**\_light/.txt" or "DTASelect-filter\_**TAG**\_heavy.txt", in which **"TAG"** is the one you use to name those mzXML files.  
  
5. go into the "dta" folder and run the "cimage" program by typing:  
  
*cimage     your-cimage-params-file****TAG***.   
  
**Edit your cimage.params file to point to the right light/heavy chemical composition files.**

Template cimage.params file and common light/heavy chemical composition files can be found in the CIMGAE installation folder:

Default – SILAC

IA – isoTOP-ABPP with IA-alkyne probe

N15 – N15 metoblic labeling  
  
Light/heavy tables are strictly tab-delimited text files, so it is better to use EXCEL to import, edit and then export it. Again, substitute TAG with your chosen name.  
  
6. If it runs fine, it will generate a "output" folder in which there will be a pdf file of chromatograph, a "to\_excel" text file for your editing in execel and a folder of "PNG" containing all individual graphic files.  
  
7. move to "my\_experiment" folder and generate a html version of the result by typing:  
  
*cimage\_combine     [by\_protein]     output\_rt\_10\_sn\_2.5.to\_excel.txt     dta*   
  
and this will generate a **combine\_dta.html** and a raw text file **combine\_dta.txt**. Skip the **by\_protein** option if you would like to by default group identified peptides by their sequence instead of their parent proteins. There are more flexible ways to combine multiple results folders into one master list, and discuss with me if you would like to try additional options.  
  
8. To compare ratios obtained by multiple different cimage runs, you can run **cimage\_compare** program by typing:   
  
*cimage\_compare     [by\_protein]     file1     column1     outname1     file2     column2     outname2 ...*   
  
in which "file1" and "file2" are full names (with paths) of the two combined\_dta.txt files to be compared, "column1" and "column2" are names of ratio columns in each combined\_dta.txt file, and "outname1" and "outname2" are names of ratios columns when they are output into a tab-delimited text file side by side that you can import to EXCEL for further analysis.   
  
for example, if you like to compare protein silac ratios (column "mr.set\_1" in combined\_dta.txt files) obtained from two experiments "exp1" and "exp2", the command to run would be:  
  
*cimage\_compare     [by\_protein]     /your-folder/exp1/combined\_dta.txt     set\_1     my\_exp1     /your-folder/exp2/combined\_dta.txt     set\_1     my\_exp2*