SIED
Significant Isotopic Enrichment Detection
Version 1.71

User's Guide

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Chapter 1 : General remarks

1. License

To publish a work in which SIED and/or one of its utilities is applied, you have to cite : Delaune, A., Poutrain, P., Gibouin, D., Gangwe-Nana, G., Jourdain, B., Norris, V., Ripoll, C., and Cabin-Flaman, A. (2013). SIED: a new tool to detect significant isotopic enrichments in NanoSIMS50 images. *In* 3rd NanoSIMS International Workshop. Luxembourg.

To publish a work explaining the calculation of SIED, you have to cite : Delaune, A., Cabin-Flaman, A., Legent, G., Gibouin, D., Smet-Nocca, C., Lefebvre, F., Benecke, A., Vasse, M., and Ripoll, C. (2013). 50nm-Scale Localization of Single Unmodified, Isotopically Enriched, Proteins in Cells. PLOS ONE *8*, e56559.

To publish or distribute a work based on this code, you have to cite the authors of the present work.

SIED : Delaune A, Ripoll C. and Cabin-Flaman A.
SIEDmaker and SIEDmaker4win : Delaune A. and Thibaud L.
WIN2SUN and WIN2SUN32 : Delaune A.

2. Scientific background

SIED, Significant Isotopic Enrichment Detection is a tool designed to help the analysis of Secondary Ion Mass Spectrometry (SIMS) isotopic images. In such isotopic analysis, at least 2 isotopes are imaged, i.e. one image represents the localization of one isotope and an other image represents the localization of another isotope, with the relative abundances of the two isotopes are assumed to be constant in “unenriched” samples. This can be exemplified by the analysis of $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$ for which the relative abundance of $^{12}C^{15}N^- = \frac{^{12}C^{15}N}{^{12}C^{15}N + ^{12}C^{14}N}$ is close to 0.0037.

SIMS analysis leads to the counting of secondary ions, i.e. a (small) fraction of the two isotopes from the sample, and incidentally to statistical fluctuations in the measure of the relative isotopic abundances. Depending on the total amount of detected secondary ions (counting statistics), the statistical fluctuations induce a “noise” that has to be compared to the measure. The less the counting statistics, the less the measure/noise ratio and incidentally that the more difficult is the detection of small enrichments in one of the two isotopes due to sample labeling. The aim of SIED is to solve this problem by comparing the measured isotopic abundances to the expected statistical fluctuations (that depends on the counting statistics) for each pixel of the images (Delaune et al., 2013a). For more details, the principle of SIED calculations has been published in (Delaune et

al., 2013). Briefly, as illustrated (illustration 1), SIED opens the configuration file (namely config.ini) that contains the analysis parameters. From these parameters, there are the name of the file corresponding to the image of the isotope designated as “label mass” ($^{12}C^{15}N$ in the previous example) and the name of the file corresponding to the image of the other isotope designated as “non-label mass” ($^{12}C^{14}N$ in the previous example). When SIED opens these files, it convert the images in arrays where each cell corresponds to a pixel of the original images. SIED then calculates the isotopic fraction for each pixel (and creates a new array) and then the mean isotopic fraction of the image. This value can be used as a reference isotopic fraction, i.e., the isotopic fraction that is considered as the real value of the sample and from which, pixels with significantly different value will be searched. Alternatively, the reference isotopic fraction can be entered by the user as a parameter. To estimate the stochastic distribution of the measured isotopic fraction around the reference isotopic fraction, the counting statistics is estimated. From the non-label mass array, SIED computes the lambda parameter for each pixel. For each pixel, the method consist in researching the value of lambda parameter that maximizes the sum of Poisson probabilities (probability to obtain the measured value with this lambda value) of the neighboring pixels. The extension of the neighboring is defined in the config.ini file as the rank of the matrix, i.e., the distance (in pixels) around the analyzed pixel. Clearly, a rank 0 means the analyzed pixel alone is taken into account, a rank 1 means the 9 pixels of the squared centered on the analyzed pixel are taken into account, rank 2 means the 25 pixels etc... The quadratic effect of the rank on the number of calculation explains why this step is time-consuming. Hopefully, SIED is multi-threaded. Once the array of lambda parameters is calculated, the pvalues, i.e. (in simplified terms) the probability that the measured isotopic fraction is not different than the reference, are computed. Once again, this step is time-consuming but accelerated by multi-threading. Finally, a statistical test is performed (using Bonferronni correction for multiple test) to assess whether each pixel is significant or not, and output files are saved.

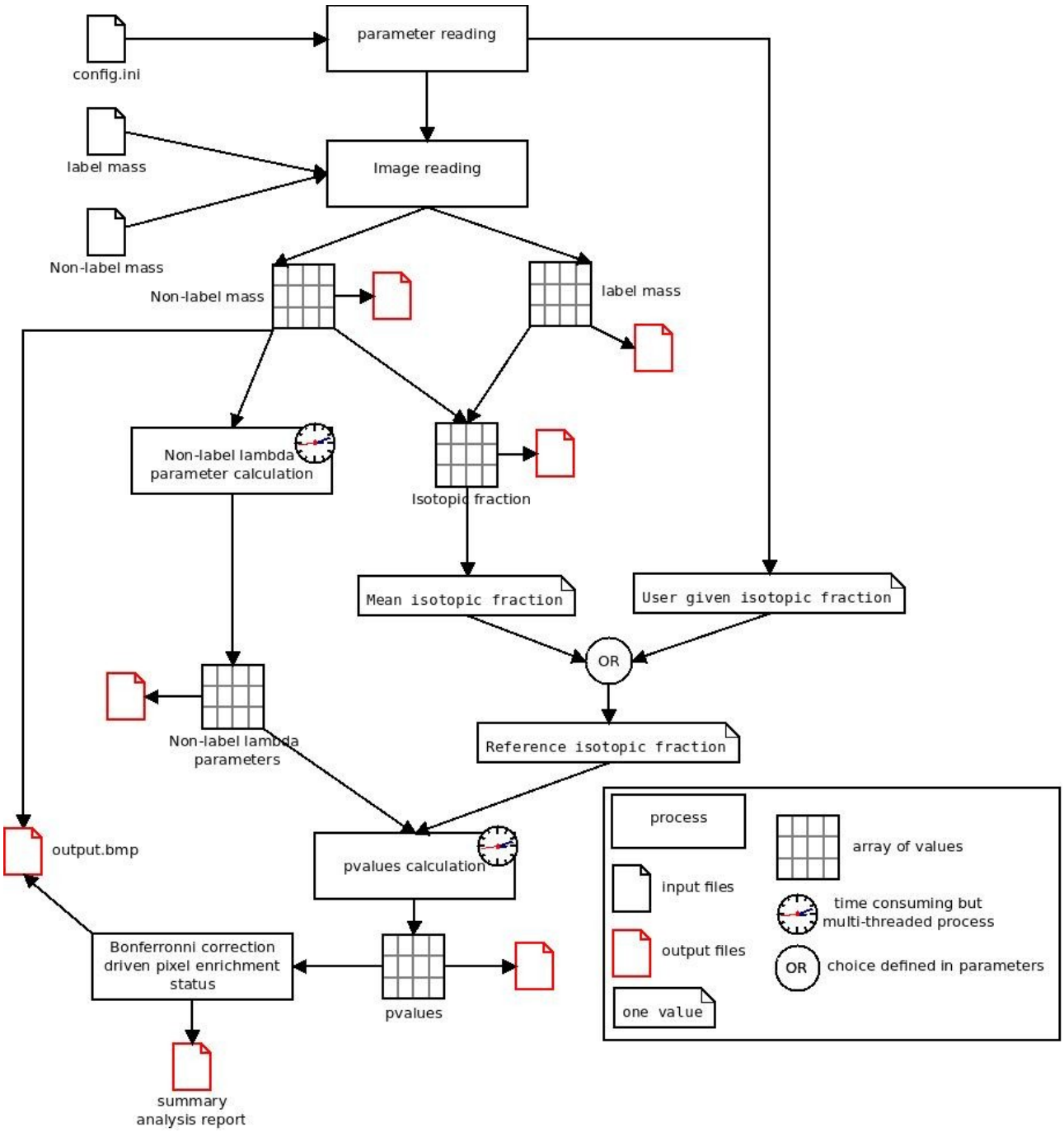


Illustration 1: Diagram of SIED analysis

By extension, in the case of isotopically-enriched samples in which a local variation in enrichment (local higher enrichment or local lower enrichment compared to the average enrichment of the sample) has to be assayed, a similar calculation can be done (two-tailed test).

In this manual, you will find the instructions to install and use SIED and its companion programs. Depending on the origin of the images you have to analyze (NanoSIMS50 images or other SIMS images) and depending on the operating system of your computer (Linux or Windows system), the procedure may change. This manual is an attempt to explain each step allowing any user, from novice to advanced user, to perform SIED analysis. However, some operations are supposed to be obvious and/or depend on your operating system (OS). Report to the manual of your OS for this basic features (copy files etc...). Hence, basic knowledge on navigation between directories on UNIX-like systems is required. A brief tutorial can be found on

http://ryanstutorials.net/linuxtutorial/

FINALLY, you'll need administrator permissions to perform some steps of the installation. If you don't, contact your system administrator.

3. Organization of analysis

The analysis of an image using SIED is decomposed in several steps. The first step is the data extraction and conversion (in a txt format that SIED can read) and the setting of analysis parameters. The second step is the SIED analysis and p-values computation. The third and final step is the visualization and interpretation of results. Each step (described in following chapters) can be performed either on a unique computer or on different computers. Example: The first and third steps can be performed on a classic office computer while the time consuming calculations of the second step can be performed on a calculation server or cluster (see illustration 2).

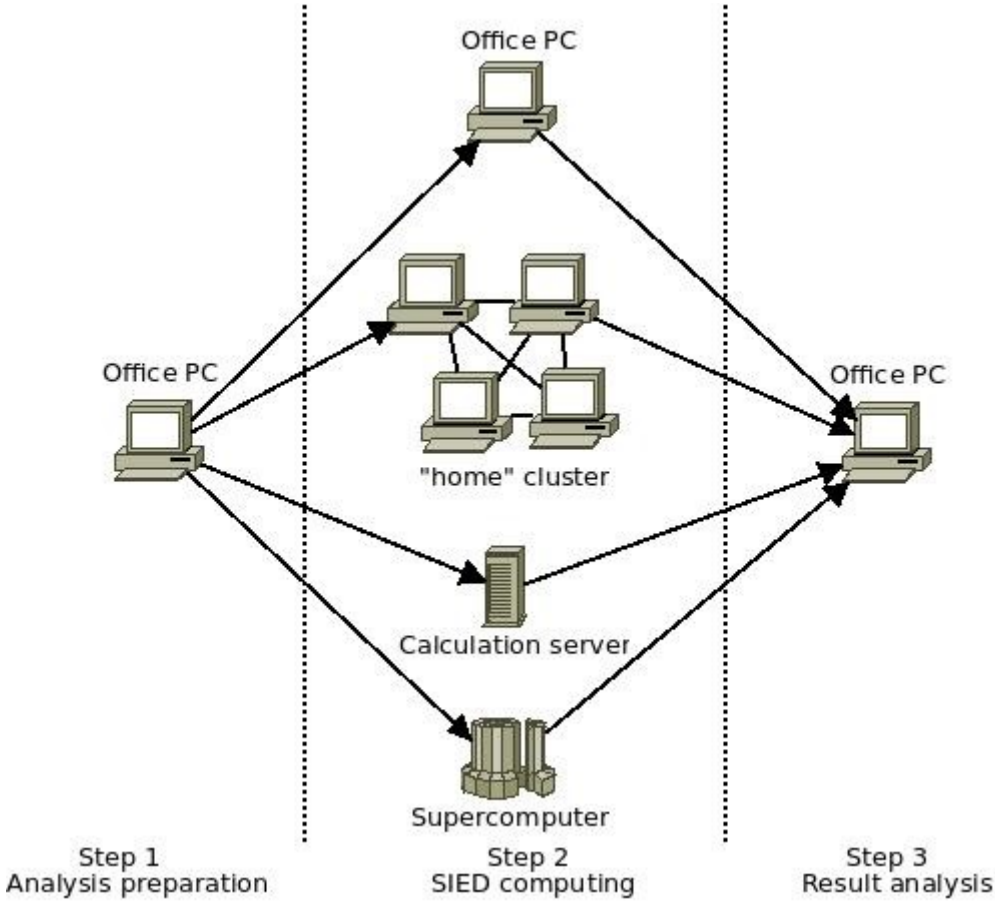


Illustration 2: example of multi-platform organized analysis

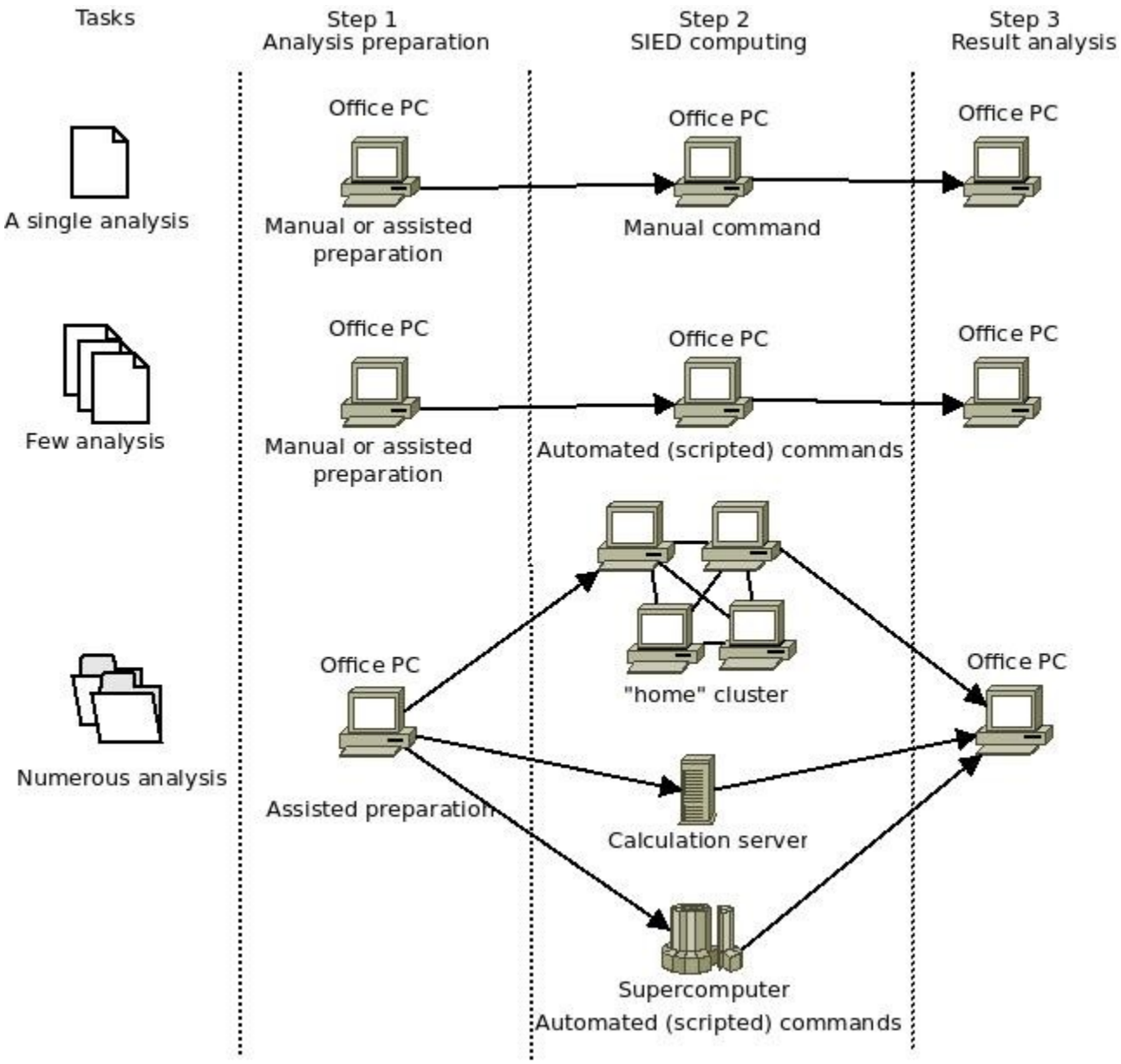


Illustration 3: Choice of the organization of analysis

Obviously, if such multi-platform analysis organization is chosen, files must be transferred between the computers and the user has to know how to reach these files. For ease of presentation, this manual will present instructions for each steps and then exemplifies the case where the three steps (at least the two first steps) are performed on the same computer. For the same reason, examples will be described with “default” working directories. Once you'll master this examples, it will be very easy (and recommended) to adapt your workflow (and the commands to type) to your actually desired organization. The choice of your organization (all steps with a single computer or use of a calculation server) strongly depends on your available hardware but also on the number of analysis you have to do. The illustration 3 shows some encountered situations and the most reasonable choices. (Please note that the proposed “choices” will make all sense only when you'll have read the following chapters).

SIED is delivered with various companion software to assist you during the “analysis preparation” step and offer the possibility to automate (at least in part) the “SIED calculation” step by generating a script (file that contains a list of commands the computer has to execute automatically). These companions are designed to open Cameca Nanosims50 files. If you have to analyze a lot of non-NanoSIMS50 images, I strongly recommend you to use the sources of these

companions as a scaffold to create new programs to assist your “analysis preparation” step. On request, we may help you in this effort. You are encouraged to communicate us such programs.

4. Files of the package

sied.c : Source of SIED. This program allows the detection of significant enrichments (increase in isotopic fraction) - or significant isotopic heterogeneities (isotopic fraction is significantly low or high) - compared to a reference isotopic fraction (e.g. the natural isotopic fraction or the measured isotopic fraction in a standard sample...).

siedmaker.c : (Linux x86 program) source of SIEDmaker. This program is a tool that allows the extraction of NanoSIMS50 image data from Cameca *.im* files and makes easier the edition of configuration files used by SIED program.

siedmaker_2_31.c : (Linux x86 program) source of the old 2.31 version SIEDmaker. This program is a tool that allows the extraction of NanoSIMS50 image data from 16-bit Cameca *.im* files and makes easier the edition of configuration files used by SIED program.

SIEDmaker4win.exe : (Windows program) This program is a tool that allows the extraction of NanoSIMS50 image data from Cameca *.im* files and makes easier the edition of configuration files used by SIED program.

SIEDmaker4win.c : (Windows program) source of SIEDmaker4win.

AD_4h_8_p1.zip : archive containing SIMS images used as example in this manual.

AD_TemNeg_13.im : Cameca NanoSIMS50 image file used as example in this manual. This file was generated with a NanoSIMS50 SUN station. Please check the version of the SIEDmaker you use to open this file.

GrilleSitest_D14_2.im : This file was generated with a NanoSIMS50 windows station.

WIN2SUN.exe and **convert_win2sun.bat** : (Windows program) This program allows conversion of new windows station Cameca NS50 to the older file format (SUN station).

w**in2sun.c** : Source of WIN2SUN. This program allows conversion of new windows station Cameca NS50 to the older file format (SUN station).

s**un2win.c** : Source of SUN2WIN. This program allows conversion of old 16-bit SUN station Cameca NS50 to the new 32-bit file format (Windows station).

Gpl.txt : text version of the gpl license.

SIED Usersguide.pdf : This manual.

Chapter 2 : Analysis preparation step

Introduction :

The “analysis preparation” step consist on the extraction and the conversion of the data to a format that can be read by SIED and the writing of a file containing the parameters of SIED analysis. Depending on the origin of your data and the operating system of your computer you have to adapt the way to do as illustrated in the following table.

Your OS / Data origin	GNU Linux	MS windows
Cameca NanoSIMS50 SUN station	SIEDmaker 2.31 or older Assisted data extraction Assisted parameter selection Automated script generation	SIEDmaker4win 0.95 or older Assisted data extraction Assisted parameter selection Automated script generation
Cameca NanoSIMS50 Windows station	SIEDmaker 2.32 or newer Assisted data extraction Assisted parameter selection Automated script generation	Conversion format Win2Sun SIEDmaker4win 0.95 or older Assisted data extraction Assisted parameter selection Automated script generation
Cameca IMS xF	SIEDmaker_IMS No longer developed Available on request	SIEDmaker_IMS4win No longer developed Available on request
Other	Manual data extraction Manual parameter selection Manual script writing Contact us for help in program writing and/or distribution in the package	

The following sections contain informations about the data format and the parameter setting for SIED analysis followed by the instructions (how to install and to use) concerning the companion software.

5. Image data format

SIED requires the images are in a text format. Each pixel value has to be a positive integer (no decimal separator). The pixels have to be separated by a tabulation. Even it is not necessary for SIED calculation, it is ***highly recommended*** that each line is separated by an “end of line” separator (line feed). The reason is to maintain compatibility with spreadsheets and “txt image” format of ImageJ.

6. Manual selection of parameters and edition of “config.ini” file

In addition to images to be analyzed, SIED requires parameters for the analysis. These parameters have to be stored in a file (traditionally named config.ini) that will be read by SIED.
Note 1: the config.ini file has to be located in the same directory than the images (in text format) to be analyzed.
Note 2: It is HIGHLY RECOMMENDED to use separate directories for separate analysis.

The analysis parameters can be manually edited in the config.ini file. However, to analyze a NanoSIMS50 image, it is strongly recommended to use SIED_maker (or SIEDmaker4win.exe) to generate this file (see sections 8 and 9).

Structure of the **config.ini** file :

Each line must contain one parameter (and only one).

```
Height (in pixel) of the image
Width (in pixel) of the image
Extension of the matrix
image of label mass
image of non-label mass
threshold of analysis
threshold of mean fraction (reference) calculation
image of additional mass
reference isotopic fraction
second class probability risk
Mode of the statistical test (One or Two-tailed)
prefix of output files
```

- Height (in pixel) of the image : number of lines of the SIMS images (e.g. 128, 256, 512...). Must be an integer number.
- Width (in pixel) of the image : number of rows of the SIMS images (e.g. 128, 256, 512...). Must be an integer number. Remark : the number of lines and rows are often identical the same
- Extension of the matrix : used for lambda parameters calculation. Accepted values 0 (no lambda parameter calculation, the number of counts of image “denominator” is used as lambda parameter), 1, 2, 3 (default recommended value) and 4. Note that increasing this value improves the lambda parameter determination but increases also the computation time and reduces the number of analyzed pixels (and the size of output files).

- Image of label mass : name of the file containing the count number of the tested mass. The file must be in the current directory and be at text format (see section 5.).
- Image of “Non-label mass” : name of the file containing the count number of the non-tested isotope. The calculated isotopic fraction for each pixel is *pixel image label / (pixel image label + pixel image non-label)*. The file must be in the current directory and be at text format (see section 5.).
- Threshold of analysis : Minimum value of a pixel in the denominator image to be analyzed. Allows to exclude pixels for which the counting statistics is too low. To avoid division by zero, the value must be at least equal to 1. This parameter must be an integer.
- Threshold of mean fraction calculation : minimum value of a pixel in the denominator image to be included in the calculation of mean isotopic fraction. Note that pixels below this value but above the threshold of analysis are analyzed. This parameter must be an integer equal or superior to zero.
- Image of additional mass : name of the file containing the count number of an additional mass. No calculation is performed on this image. This parameter is only used to display the value of a pixel on the summary output file. The file must be in the current directory and be at text format (see section 5.). If no additional image is required, enter the name of the numerator image or denominator image.
- Reference isotopic fraction : Isotopic fraction that is compared to the measured isotopic fraction for each pixel. Usually, set the reference isotopic fraction to the natural isotopic fraction of the “numerator” isotope, or the isotopic fraction measured in a standard sample. If this value is negative, the reference isotopic fraction will be the mean of measured isotopic fraction of pixels for which the counts on “denominator” image are above the threshold of mean fraction calculation. This parameter must be a positive or negative float.
- Second class probability risk : alpha risk of the second class positive pixels. It means, the probability to observe at least one positive pixel of second class for an unenriched sample. The alpha risk for first class pixels is 0.05. As described in (Delaune et al., 2013), an alpha risk of 0.05 induces a risk of false negative pixels. The use of a second class alpha risk allows to reduce the number of false negative. This value must be a positive float number. The recommended value is 0.1.
- Mode of the statistical test (One or Two-tailed) : Indicates whether the statistical test will be performed on a two-tailed or a one-tailed distribution. Values must be integers 0 (zero, for one-tailed distribution) or 1 (one, for two-tailed distribution). 0 is recommanded to study isotopic enrichments (only high isotopic fractions) while 1 is recommanded to study isotopic heterogeneity (both low and high isotopic fractions).
- Prefix of output files : prefix of the output files. Useful to identify the original image on the name of output files.

Example of **config.ini** file (Note that the text in italic at the right of the frame is NOT in the config file but is only the description and explanation of each line of the file) :

```
128                          height of the image (in pixel)
128                          width of image (in pixel) (here an image of 128x128 pixels)
3                            rank 3. output image files will be 122x122 pixels
AD_4h_8_p1_12C_15N_.txt      image of the mass 27
AD_4h_8_p1_12C_14N_.txt      image of the mass 26
600                          only pixels with 12C14N>=600 will be analyzed
0                            useless because reference isotopic fraction is set below
AD_4h_8_p1_16O_.txt          the value of 16O will be displayed for each positive pixels
0.0037                       the reference is the 15N natural isotopic fraction
0.1                          second class pixels will have an alpha risk <=0.1
0                            two-tailed test is OFF (One-tailed test will be performed)
AD_4h_8_p1                   prefix that reminds the name of sample
```

7. Writing a Script and data organization

When manually extracting, converting and setting the analysis parameters, you have to take great care of your file location. This is particularly critical if an image has to be analyzed using several sets of varying parameters. Please note that **SIED always overwrite existing files**. To avoid data loss it is **HIGHLY RECOMMENDED** to respect the rule “One analysis, one directory”. This rule is applied by the companion software (SIEDmaker and SIEDmaker4win) to assist the “analysis preparation” step. I recommend to create a directory for each plane of an image and a subfolder for each set of parameters as illustrated below (illustration 4).

A script is a text file containing all the instructions to sequentially and automatically execute multiple SIED analysis. The script file has to be localized on the parent directory of the “analysis directories” (see illustration 4). To fully understand what to put in your script file, you have to refer to SIED instruction (chapter SIED calculation, section Y Use of SIED) and I also suggest to read documentations about bash script. The next frame is an example of script corresponding to the analysis depicted on illustration 4. You can use it as model for your scripts.

```
#!/bin/bash                                                  header of script
DIR=$( cd "$( dirname "$ BASH_SOURCE[0]}" )" && pwd )        save the parent directory

cd $DIR                                                      go to parent directory
cd image1                                                    go to folder image1
cd parameter1                                                go to subfolder parameter1
mpiexec -np 8  SIED config.ini                               execute the SIED analysis
                                                             in this directory
cd $DIR                                                      …
cd image1                                                    repeat for each analysis
cd parameter2
mpiexec -np 8  SIED config.ini

cd $DIR
cd image2
mpiexec -np 8  SIED config.ini

cd $DIR
cd image3
mpiexec -np 8  SIED config.ini

exit                                                         end of the script
```

I suggest to save the file with the name “script.sh”. You can use another filename but remember to replace script.sh by your filename in further command lines.
Note 1: The words in bold in the frame have to be modified and adapted to your analysis. The name of directories (words after “cd ”) and the number of threads (number after “-np “) have to be changed (for the number of threads see chapter SIED calculation, section Y Use of SIED).
Note 2: the second line allow to save the path of the parent directory while the lines “cd $DIR” command to return to this parent directory.
Note 3: It is recommended to finish the script by the word “exit” but it also works without.
Note 4: the script has to be written using UNIX line endings and not Windows or Mac format. Do not use the microsoft notepad editor. I suggest the free editor “medit” (http://mooedit.sourceforge.net) with selecting “UNIX (LF)” “line endings” in the “Document” menu.

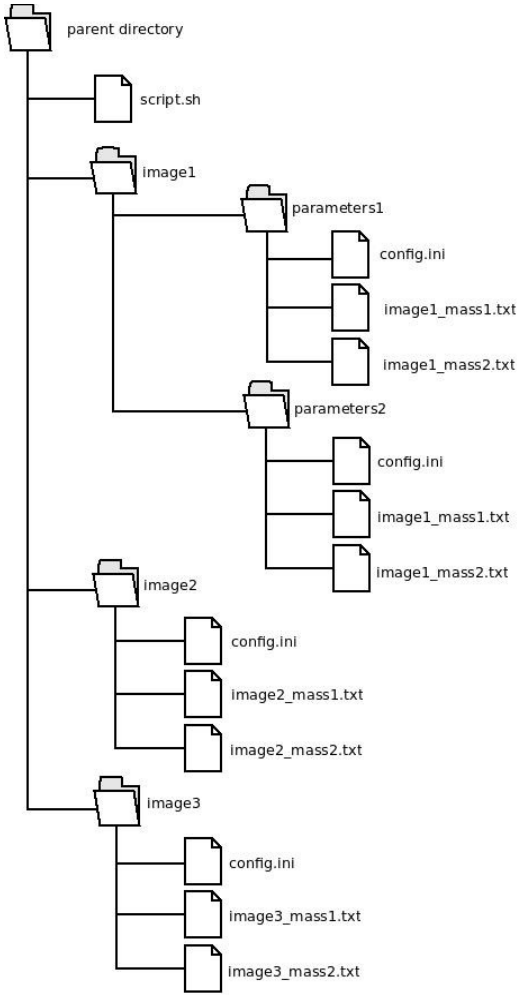


Illustration 4: Example of file and folder organization

EXECUTING A SCRIPT

To execute a script and perform the SIED analysis, you need a shell to interpret the script. On GNU Linux operating system you already have “bash” (Bourne Again Shell). On Windows systems, you need to execute the script on Cygwin (see Chapter SIED Calculation step, Section B/ Installation on Windows systems). Move to the parent directory containing your script.sh file and the folders of analysis and type :

```
bash script.sh
```

Note: to work, SIED has to be installed. See Chapter SIED Calculation step

8. SIEDmaker (GNU Linux user only)

This graphical tool allows to open cameca NanoSIMS50 *.im* file, extract the images, edit the configuration file for SIED and generate script for sequentially launch multiple analyses.

INSTALLATION :

SIEDmaker uses the Gimp Toolkit 2 (GTK+ 2) library. Download and install the latest version on official site http://www.gtk.org/ or install the package for your distribution. For apt using Linux distributions just type :

```
sudo apt-get install libgtk2.0-dev
```

go to the directory you stored siedmaker.c source file and compile it by typing (on a single line):

```
gcc `pkg-config gtk+-2.0 --cflags` siedmaker.c -o SIEDmaker `pkg-config gtk+-2.0 --libs` -lm
```

Note 1: Ignore the warnings at compilation. Normally, it will create the binary SIEDmaker.
Note 2: Do not confound the character ` with the character '.
Note 3: If you encounter problems with compilation, it is certainly due to your GTK library.

Install the program in your path by typing

```
sudo cp SIEDmaker /usr/bin
```

Now you can execute SIEDmaker by typing

```
SIEDmaker
```

Note : if you have to analyze old 16-bit SUN station, you would prefer to use SIEDmaker 2.31. You can compile it by typing :

```
gcc `pkg-config gtk+-2.0 --cflags` siedmaker_2_31.c -o SIEDmaker_SUN `pkg-config gtk+-2.0 --libs` -lm
```

A preferable alternative method is to convert the old format (16-bit) to the new one (32-bit) in order to use the new SIEDmaker version. To compile the converter type :

```
gcc -o SUN2WIN sun2win.c -lm
sudo cp SUN2WIN /usr/bin
```

USE OF SIEDmaker :

Proceed as follow :

Launch SIEDmaker by typing on the terminal :

```
SIEDmaker
```

It will open the graphical application.

Click on **Open** button. It opens a dialog box allowing to explore your files. Select a cameca *.im* file to open it. The box "**Analysis prefix**" will automatically be filled by the name of .im file.

Note 1: you have to verify that to "strange" characters are displayed in this box

Note 2: if your .im file comes from a converted windows format to a SUN format, the filename begins with SUN_. When opened with SIEDmaker, this prefix is automatically removed.

Select the plane of analysis on the box "**Active Plane**".

Note 1: after having selected a plane, this will activate the selection boxes "Label Mass", "Non-label mass", "Add. Mass" and the "reference mass" for plane summation. It will also set a default value of the "Shift max".

OPTIONNAL: perform plane summation. This feature automatically register the different plane images for lateral shift (rotation and non-solid transformation are not supported). Choose between "least square", "joint histogram" and "1D projection" (a home-made very quick method of registering) method of registering. Choose the "reference mass", i.e. the mass of the images used to register the planes. Click on the "Sum Planes" button to perform the summation process. This will require a lot of time depending on your compter, the number of planes, the raster and the chosen method. A progress bar will display the advancement each time a plane is registered. At the end of summation process, you can select in the box "Active plane" the line "plane_sum".

Remark : some unexpected results can occur with this feature. This has to be improved (and debugged) in future versions.

Select the image of the mass of the "label" you want to detect in the box "**Label mass**".

Select the mass image in the box "**Non-label mass**". The corresponding image will be displayed on the top left of the screen and cannot be moved. You may have to move the main window in order to continue.

Select the additional image file in the box "**Add. mass**".

Choose the extension of the matrix (rank) used for lambda determination by selecting between "**Matrix rank 0, 1, 2, 3 or 4**". It is highly recommended to keep the rank 3 as selected by default.

Select the threshold of analysis by moving the cursor "**Analysis threshold**". Red pixels (or yellow if they are also below the reference threshold) on the displayed "Non-label mass" image are pixels that will be excluded from analysis. You can finely tune the threshold value using the left and right arrows of your keyboard.

Select the threshold for mean isotopic fraction calculation by moving the cursor "**Reference threshold**". Green pixels (or yellow pixels if they are also below the analysis threshold) are excluded from this calculation. Alternatively, check the box "Set reference value" in order to manually enter the value of reference isotopic fraction. In this case, the cursor "Reference threshold" will be hidden.

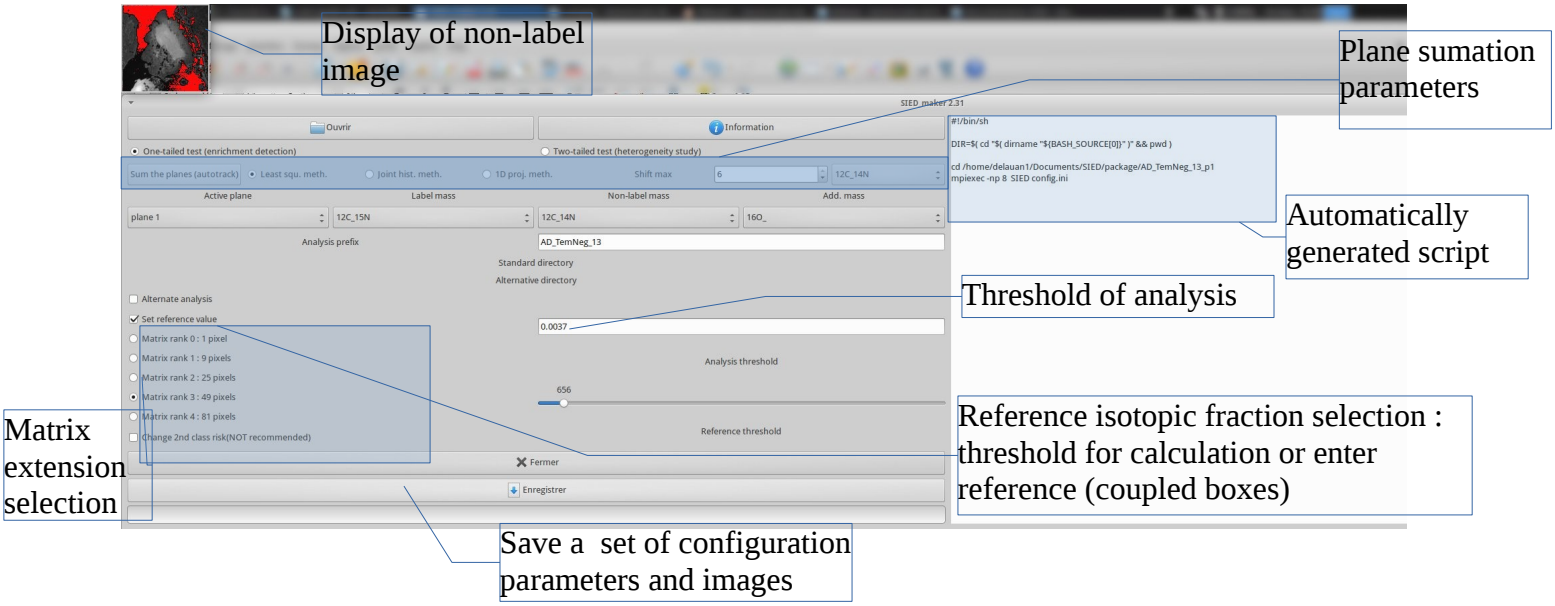
Click on "**Save**" button to save the image and **config.ini** files. Automatically, a folder named prefix_pX will be created where the files will be saved. By default, the prefix is the

name of the .im file and displayed in the box “Analysis prefix”. This prefix can be edited. The number X, following prefix_p, is the number of the selected plane in the box “Active plane”. In the right panel, an automated script is proposed. You can copy the text in a new text file (named for instance script.sh) you can execute (command line “bash script.sh”) to sequentially launch multiple SIED analyses.

Optionally, you can edit an alternative set of parameters by checking the box “**Alternate analysis**”. It opens a selection box in which the user has to choose the parameter that will differ between alternative analysis. The possible choices are “**Matrix rank**” to test an alternative extension matrix, “**Anal.thresh**” to test an alternative threshold of analysis, “**Ref.thresh**” to test another threshold for reference isotopic fraction calculation, “**Reference**” to test an alternative reference isotopic fraction or “**2ndclass_risk**” to test an alternative second class alpha risk. On the case you check this box, when saving the images, 2 subfolders will be created : standard folder for the analysis with the previously selected parameters, and an alternative folder with the same parameters except the alternative parameter you manually entered.
Note : It can be judicious to select a threshold for reference isotopic fraction calculation as standard configuration and select manually a reference isotopic fraction as alternative configuration.

Remark 1: If you check the box “**Change 2nd class risk (NOT recommended)**”, it will ask you to enter the second class pixel alpha risk (default value 0.1). **This is not recommended**. You'd better use the SIED calculated p-values with another multiple test correction (than Bonferronni) than to increase the 2nd class alpha risk.

Remark 2: The script generated can be manually edited in order to modify the number of threads, or change the path of files to be analyzed. Note the second line “DIR= $ ...” is useless in this generated script but is kept to ease the modification of the directory path when it's required (see section 7 writing a script).
Remark 3: After having saved a configuration, you can open a new image or change the plane of images to edit a new set of configurations. The generated script cumulates every saved configurations until SIEDmaker is closed.



When you have finished a session, you can copy the script and save it on a text file (suggested filename script.sh) on the parent directory that contains all the folders of analysis. To execute the script and incidentally do the SIED computation step, go to the parent

directory and type :

```
bash script.sh
```

Note: to work, SIED has to be installed. See Chapter SIED Calculation step

Note about Cameca *.im* file : SIED_maker v2.31 and older open only *.im* files created with the 16-bit SUN station. New format files (including windows station created files) have different offsets for informations and have to be converted to older file format. Alternatively, the open_im function on the source code of SIED_maker has to be modified. WARNING : the new versions of SIEDmaker (2.32 and newer) open 32-bit windows station cameca .im file and, incidentally, older files will need to be converted to new format … Check the version of SIEDmaker you have !

CONVERSION OF 32 bit WINDOWS STATION .im FILES :

If you have an old version of SIEDmaker (2.31 or older), to open Cameca NanoSIMS50 .im files that were generated with the Windows station, you have to convert them to the old format (the same generated by the SUN station). The present package contains the utility to do this : WIN2SUN

First, compile and install it by typing the following command on the directory containing the file win2sun.c :

```
gcc win2sun.c -o WIN2SUN -lm
sudo cp WIN2SUN /usr/bin
```

Then to use it, type in the directory containing your image.im file (format windows station) :

```
WIN2SUN image.im
```

It will create a file SUN_image.im you can open with SIEDmaker.

Note: To ensure compatibility, a reciprocal utility SUN2WIN is also distributed with SIEDmaker version 2.32 and newer to open old format files. To use it just type :

```
SUN2WIN image.im
```

9. SIEDmaker4win.exe (for windows user only)

SIEDmaker4win.exe is a graphical tool that allow the opening of a cameca NS50 image file (.im), its data extraction and the automated edition of config.ini file. It has been successfully tested on win2k, winXP, win vista, win7 and win10.

INSTALLATION:

There is no need to install in the sense you can execute the program from anywhere. To ease the use, you can copy the SIEDmaker4win.exe file in a folder of your choice and create a shortcut you can copy on your desktop and/or your start menu.

USE:

The illustration 5 shows the displayed window on start.

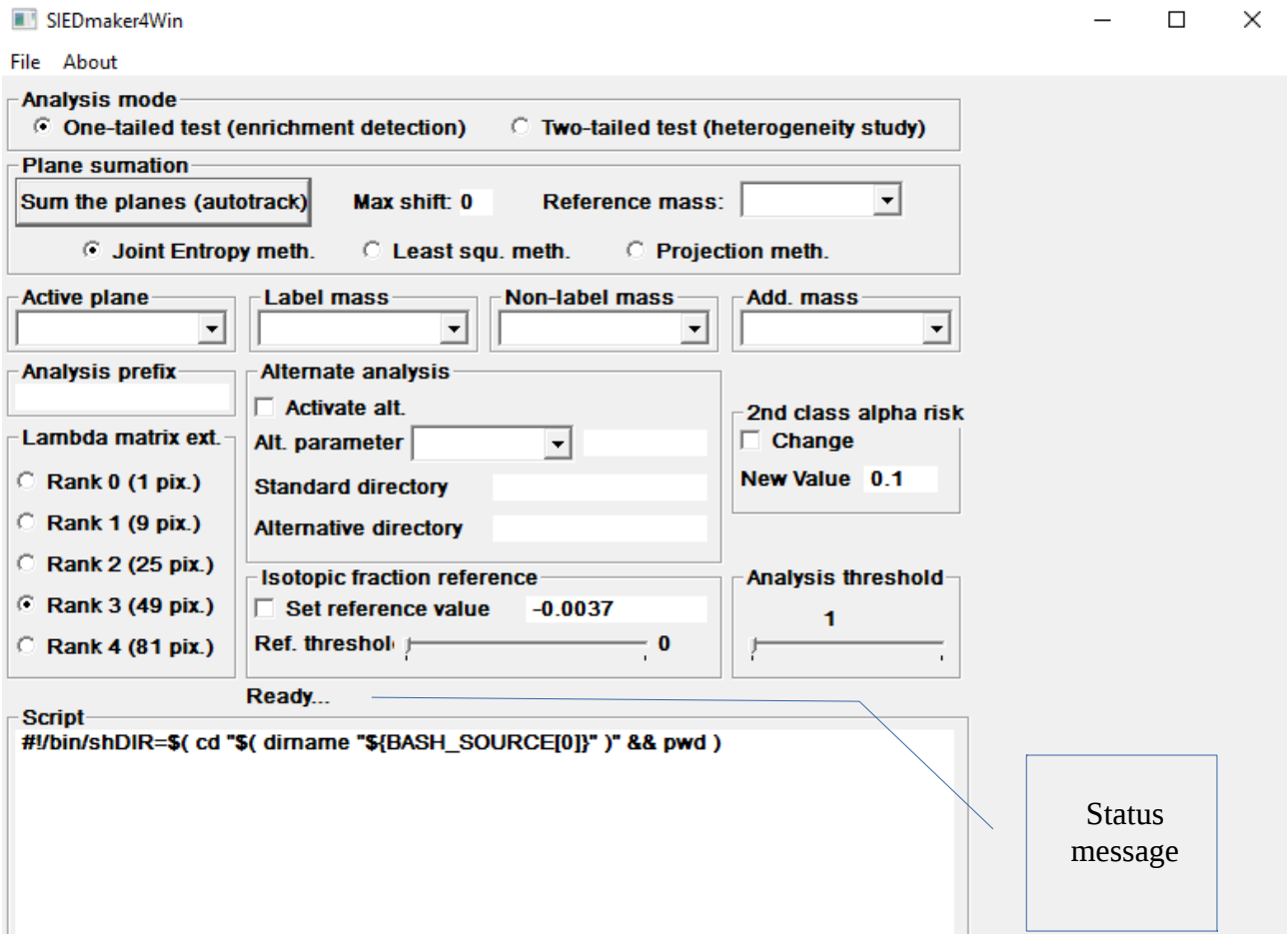


Illustration 5 SIEDmaker4win.exe on start

A/ Open a Cameca .im file

In the menu **File**, click on **Open...** A dialog box will open. Select a Cameca NS50 .im file and click on the open button. A status message will be displayed (see illustration 5) indicating the progression of the opening (ex : “Opening mass 2/5 plane 1 / 3 … Please wait”). When the image is loaded, the status message turns to “Ready...” and the field Analysis prefix is prefilled with the name of the opened .im file.

Note: SIEDmaker4win v0.94 and older can open cameca .im files that have been generated with the SUN station. To open newer windows station files, you have to convert them to SUN format with the tool WIN2SUN.exe (see below).

B/ (Optional) Do plane summation

For multiplane analysis, the addition of all the planes can be desired, especially when a high counting statistics is required. In such case, select first a “reference mass” in the combo box. The image of this reference mass will be used if an alignment between the planes is required. Note that even if no alignment is done, the selection of this “reference mass” is still required.

Then, you have to set the “max shift” value. The “max shift” is the maximum shift, in pixel, between all the planes. If 0 (zero) is keep, no alignment will be done. For higher values, an optimum alignment will be searched. Note that the resulting sum image will have the same dimension and is centered on the first plane (hence, the resulting image may be truncated to keep initial the dimension).

Choose between “least square”, “joint histogram” and “1D projection” (a home-made very quick method of registering) method of registering.

Finally, click on the “**Sum the planes (autotrack)**” button to compute the alignment. Depending on your computer, the dimension of the images, the max “shift value” and the number of planes, the phase can be quite long. During the computation, a status message is displayed (ex: “Computation of plane 3/14 … please wait”). When the computation is finished, the status message is replaced by “Sum completed. Ready...”.

Note: a file shift.txt is created in the directory of the .im file. This file contains an array of the shifts for each plane. This file has no utility except for the user who want to optimize the alignment.

C/ Choose the plane to be analyzed

Select the desired plane on the combo box “**Active plane**”. On clicking on the box, a list of planes will be shown. And the end of the list, a line “plane_sum” is always displayed.

Note 1: If you don't have perform the step B/ Plane summation, the plane_sum is empty. If you have performed, “plane_sum” will contain the sum images.

Note 2: all items of the list may not be displayed if there is a large number of planes. However, you can select any of them by pressing the down arrow key.

Note 3: After having selected an active plane, the combo boxes “Label mass”, Background mass” and “Add. Mass” will be filled.

D/ Select the mass of the isotope to be analyzed

Select the mass on the combo box “**Label mass**”

Note : Only 5 masses are shown in the list. If more masses are recorded (for example in a NS50L .im file), you can select them by pressing the down arrow key.

E/ Select the corresponding major isotopes

Select the mass on the combo box “**Non-label mass**”. On the right panel, the image of this mass will be displayed as illustrated (illustration 6).

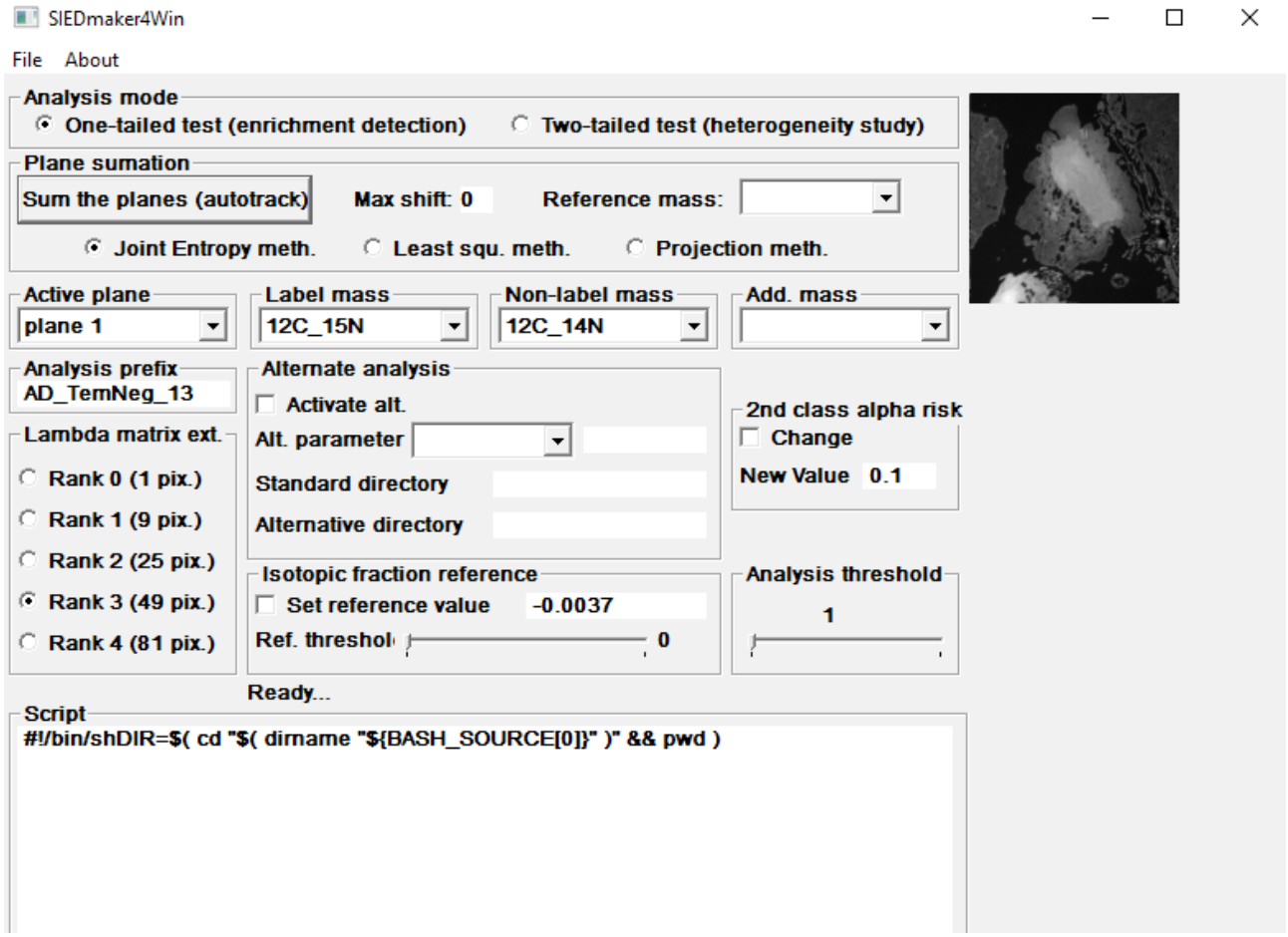


Illustration 6 Selection of "Non-label image" and its display

F/ (Optional) Select an additional mass

At your convenience, you can select an additional mass on the combo box "**Add. Mass**".

Note 1: that allows this mass to be extracted in a txt image format. Additionally, in the "summary" file of SIED analysis, the value of this mass will be prompted for each significantly enriched (based on the "label mass") pixel.

Note 2: No calculation will be performed on this mass

Note 3: If unselected, the "Background mass" will be used as additional mass as this parameter is required for SIED analysis.

G/ Parameter settings for SIED analysis

* **Analysis mode** : Select whether the statistical test has to be "one-tailed" (enrichment detection) or "two-tailed" (detection of either significantly low or high isotopic fraction, i.e. heterogeneity study).

Note: In this version, the analysis mode cannot be selected as an alternative parameter. When desired, you have to save the parameters using an analysis mode, and then save again with the other analysis mode after having changed the analysis prefix (in order to create another folder, as see below).

* **Analysis prefix** : name of the prefix of the output files and of the folder in which data will be saved. You can let the default text or change it at your convenience.

Note: you have to check that no odd character is automatically written in this field.

*__Lambda matrix ext.__ To determine the statistics, the lambda parameter of each pixel of the image is estimated by searching an optimal value using a square of 1 (rank 0), 9 (rank 1), 25 (rank 2), 49 (rank 3) or 81 (rank 4) pixels centered on the analyzed pixel. Briefly, on homogeneous sample, the more the number of pixels taken into account, the more precise is the estimation, but the more the computation time. On our samples, we estimated that the rank 3 is the best compromise and then, this is the default value. RECOMMENDED : let the default selection.

*__Isotopic fraction reference:__ allow to set the method, or directly the value, to set the reference isotopic fraction of SIED analysis. There are 2 ways:

The first way (and the default one) is to let SIED calculate the mean isotopic fraction. You can apply a threshold value on the non-label mass in order to limit the calculation to the sole pixels which have a high value (and are incidentally less noised). This is performed with the slider "**Ref. Threshold**" when the checkbox is unchecked and the value in the editable field is negative.

The second way is to enter the value of the reference of your choice(ex: the natural isotopic fraction). This is performed by checking the checkbox and entering a positive value in the editable field.

Note 1: When using the slider, the pixels with values below the threshold (not taken into account for calculation of the mean isotopic fraction) are displayed in green (or yellow if they also are below the analysis threshold as seen below)

Note 2: It can be advantageous to uncheck the box and to activate the alternate analysis with a given (ex the natural) reference isotopic fraction.

*__Analysis threshold__: allow to exclude pixels with too low statistics (and incidentally less reliable) from the SIED analysis. Use the slider to select the minimum non-label mass value for a pixel to be analyzed.

Note 1: When using the slider, the pixels with values below the threshold (not analyzed) are displayed in red (or yellow if they also are below the reference threshold as seen above)

Note 2: The minimum value is 1 in order to avoid division by zero during SIED analysis.

*__2nd class alpha risk__: (__NOT RECOMMENDED TO CHANGE__) In the case the statistical test is too conservative for your needs, you can increase the alpha risk (risk to obtain a false positive) by checking the box and enter a higher value in the field "**New value**".

Note: ***It is not recommended***. If the statistical test is too conservative for you, you'd better try implement another correction for multiple test (than the Bonferronni) with the computed pvalues.

*__Alternate analysis__: (OPTIONAL) in the case you have to test (and compare) two different parameters of analysis, you can check the box "**Activate alt.**" and select the parameter to change in the combo box "**Alt. Parameter**". You'll have to modify its value in the next field. Depending on the selected "**alt. Parameter**", two directory names are proposed (one for the standard parameter and another one for the alternate parameter).

Note 1: This functionality *is not recommended for novice*. If you are not sure, prepare two sets of analysis parameters on two independent session (i.e. save with one parameter, close SIEDmaker4win, copy the files (eventually, launch the SIED analysis) and restart a new selection with SIEDmaker4win).

Note 2: The standard directory is used for the parameter value set with the "normal" way to set the value. The alternative directory is used for the parameter value set in the field "Alt. Parameter". Don't hesitate to change the directory names to avoid confusion.

Note 3: the analysis files are no longer saved in the "Prefix name" folder but are saved on the two subfolders "standard" and "alternative".

H/ Save the analysis parameters

Click on the menu **File** and then **Save...** A status message is prompted showing progression. During the save process, a script is generated in the field "**Script**". When the images are saved, the status message is "**Images are saved.**". At this point, you can open a new image and prepare another SIED analysis.

Note: It is normal that the script displays strange characters (rectangles). This is due to the difference of end of line coding between Windows and Linux.

I / (**HIGHLY RECOMMENDED** but OPTIONNAL) Copy the script.

To avoid typing the command line to perform SIED analysis, you can select all the text in the field “Script” and copy it on the clipboard. Then paste it on the notepad and save the file (suggested filename script.sh) on the parent directory that contains the folder of analysis. The script is planed for the case where the SIED analysis will be performed on the default directory containing directly the subfolders that were created by SIEDmaker4win.exe. (ex: if you use cygwin to use SIED, the default directory is c:\cygwin\home\username\, then you have to copy the script.sh (or whatever the name used to save the script) and the folder created by SIEDmaker4win in c:\cygwin\home\username\)

Note: It is normal that the script displays strange characters (rectangles) or that every commands are in the same line. This is due to the difference of end of line encoding between windows and linux.

J/ (OPTIONAL, recommended ***only*** for users that are familiar with UNIX systems) Edit the script

You can edit the script to modify the number of threads (value after “-np”) or the path of analysis (for advanced users).

Note: Do not use the Microsoft notepad program to edit the script, this will cause errors when executing on cygwin or Linux. Edition is only possible with an editor you can parameter to use UNIX encoding rather than windows or Mac encoding. I suggest “medit” (http://mooedit.sourceforge.net) with selecting “UNIX (LF)” “line endings” in the “Document” menu.

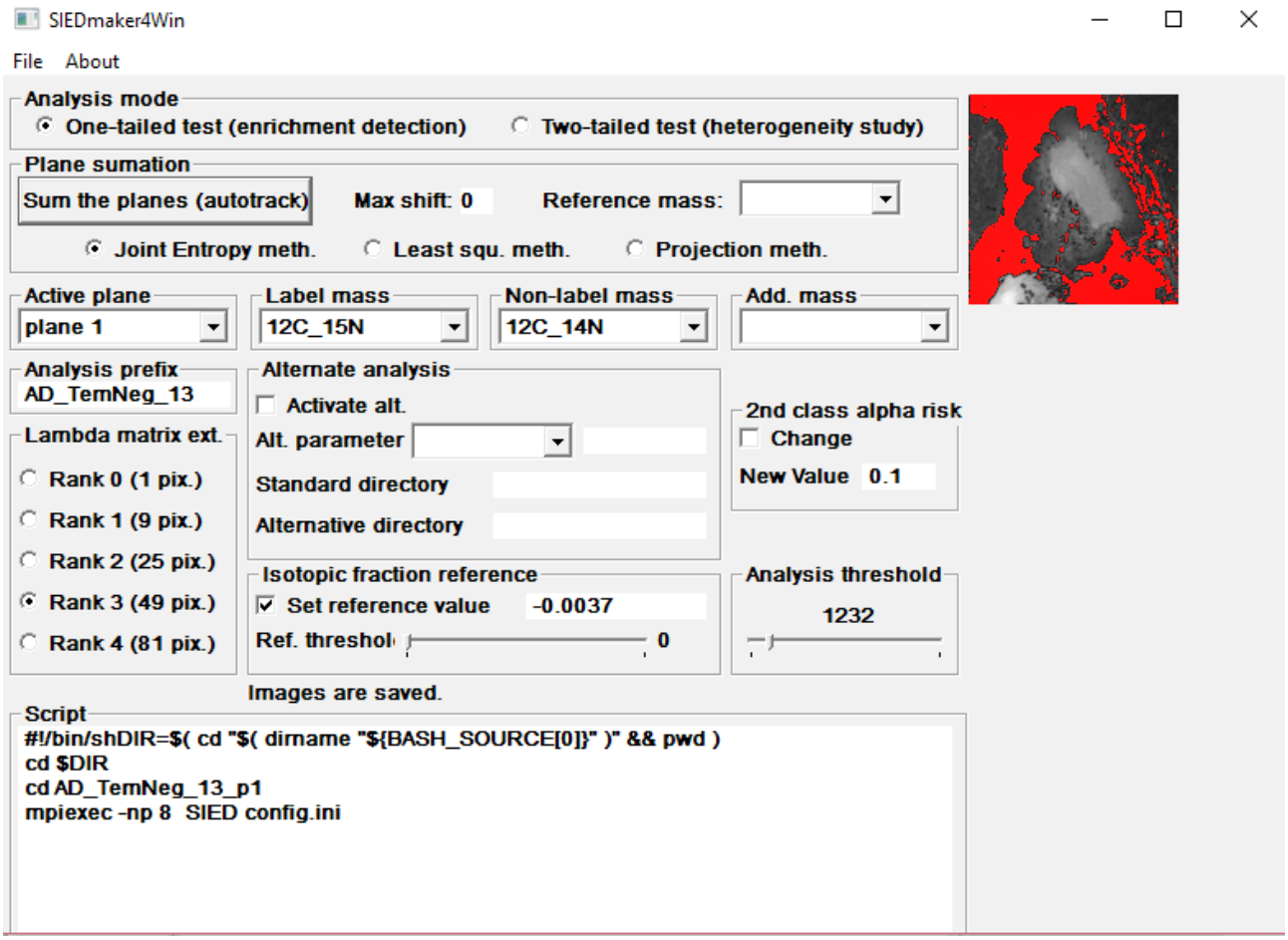


Illustration 7 Example of saved parameters

K/ Copy all the folders and script in the directory of analysis

For Cygwin users (windows) : move the created folders in your “home” folder of cygwin (default : c:\cygwin\home\username\ )

Example of Illustration 7:
You have copied the example NS50 file “AD_TemNeg_13.im” on your desktop. You have parametered an analysis of the first plane with SIEDmaker4win and saved your work. On your desktop, a folder named “AD_TemNeg_13_p1” has been created.
You have to copy this folder in c:\cygwin\home\username\
Then you have a “c:\cygwin\home\username\AD_TemNeg_13_p1\” directory containing 3 files

AD_TemNeg_13_p1_12C_15N.txt
AD_TemNeg_13_p1_12C_14N.txt
config.ini

You also have copied and saved the script in the file script.sh on the “c:\cygwin\home\username\” directory

L/ Execute the script : SIED analysis

Open Cygwin terminal (for SIED analysis under windows) and type :

```
bash script.sh
```

Note1: to work, SIED has to be installed. See Chapter SIED Calculation step.
Note2: Cygwin has also to be installed. See Chapter SIED Calculation step.

This will have for effect to automatically go to each directory containing images to analyze and launch SIED analysis.
Note 1: replace “script.sh” by the filename you used when saving the script (bash filename.sh)
Note 2: a script can be used to program tens of analysis.
Note 3: During the execution of the script, nearly all the CPU resources are used, slowing down your system.

CONVERSION OF WINDOWS .im FILES :

To open Cameca NanoSIMS50 .im files that were generated with the Windows station, you have to convert them to the old format (the same generated by the SUN station). The present package contains the utility to do this : WIN2SUN.exe and convert_win2sun.bat

There are several alternative ways depending on your permissions on your computer (numbered from the most to the less user-friendly) :

1- Copy WIN2SUN.exe in your windows directory (you'll need administrator permissions)

copy convert_win2sun.bat file on your desktop

Then, you can directly drag and drop the image.im files to the icon convert_win2sun on your desktop to convert the file.

2- If you don't have administrator permission to copy WIN2SUN.exe on your windows directory, you can copy it in the directory containing the files to convert

Copy convert_win2sun.bat file on your desktop

Then, you can directly drag and drop the image.im files to the icon convert_win2sun on your desktop to convert the file.

3- Copy WIN2SUN.exe on your windows directory, you can copy it in the directory containing the files to convert

in command line, go to the directory containing the files to convert and type

```
WIN2SUN.exe image.im
```

In any case, it will create a file SUN_image.im in the same directory than your original image.im file.

Note: To ensure compatibility, a reciprocal utility SUN2WIN will be distributed with the next SIEDmaker4win version that will open new windows format files.

Chapter 3 : SIED Calculation step

10. Requirements and installation

To reduce the execution time, SIED uses the Message Passing Interface (MPI) library. The probabilities calculations require also the GNU Scientific Library (GSL).

A/ Installation on GNU Linux systems :

a\ Install openMPI and GSL libraries. Depending on your distribution, compiled packages can be found.

OpenMPI : http://www.open-mpi.org for download and documentation

GSL : http://www.gnu.org/software/gsl/ for download and documentation

Remark : on Linux distribution that use apt-get (such as Debian, Ubuntu etc...) install the libraries by just typing :

```
sudo apt-get update
sudo apt-get install openmpi-bin libopenmpi-dev libgsl23 libgslcblas0
libgsl-dev
```

In the case you have to install the libraries from source, see directly the documentation of the library.

b\ Compile SIED

go to the SIED folder and type:

```
mpicc -o SIED sied.c -lgsl -lgslcblas -lm
```

c\ Install SIED

```
sudo cp SIED /usr/bin
```

B/ Installation on Windows systems :

To work properly, SIED requires the installation of openMPI and GNU Scientific libraries that are not natively supported on Windows systems. I present here, the most easy way I have found to install and use SIED. You have first to install Cygwin, that is an environment for Windows system giving Linux functionalities (including the required libraries for SIED).

Note: it is possible to use SIED without Cygwin, but it will not be documented here.

a\ Download the Cygwin installer (exists for 32 and 64 bit systems) at http://www.cygwin.com

b\ Execute the installer (Setup-x86.exe or setupx86_64.exe as far as I know) to install cygwin.

The internet installation is recommended. Select the mirror site of your choice (all proposed mirror sites will work but some are quicker depending on your geographical location).

Always choose the installation default parameters

Add the following packages when asked (Select Packages, See illustration 8) by clicking on the ↻ (the text “skip” will be replaced by the version number of the package to be installed. When selected to be installed, a package may appear as selected in various sections; don't worry, it will be installed only once).

List of packages to be installed :

devel → gcc-core
libs → libgsl-devel
libs → libopenmpi
libs → libopenmpi-devel

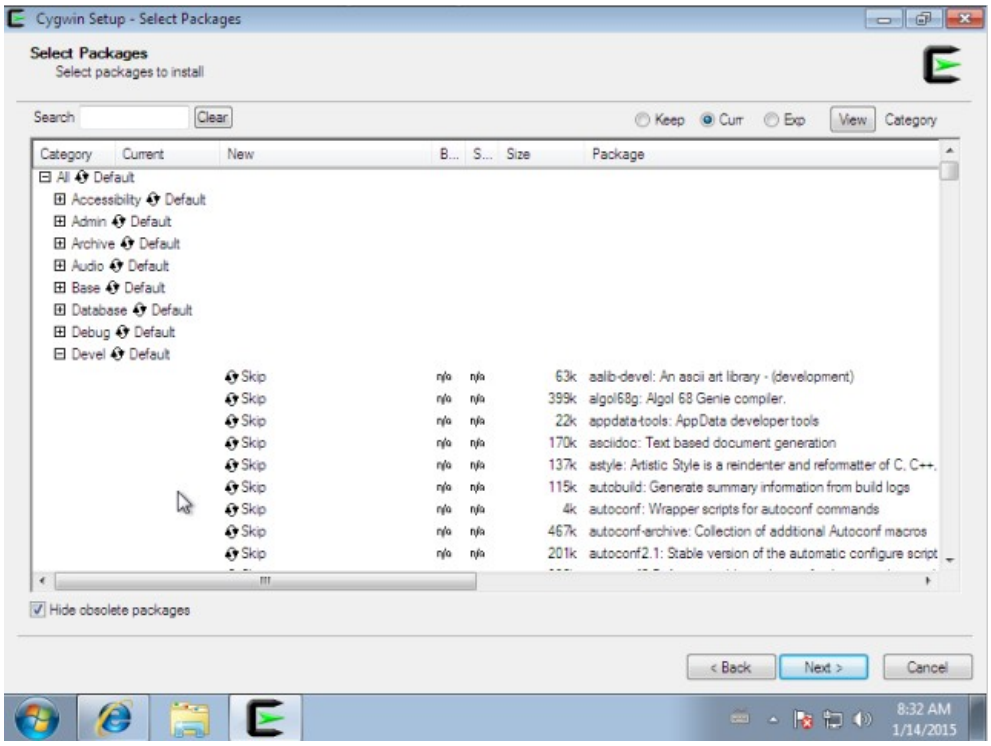


Illustration 8 Cygwin installation. Selection of the packages to install.

Then click on “next”, the packages will be downloaded (may take a while) and then installed.

After installation completed, launch Cygwin Terminal, in order to create (automatic on first start) your “home” folder. On opening, it will look like the following frame. A green line is prompted with “user”, your username (test in the example of illustration 9) and “computername”, the name of your computer (test-PC in the illustration 9). Note that a folder “c:\cygwin\home\user” is created, with “user” your username (“test” in the illustration 9) and that this is the default folder on start (it is designated as “~” character).

```
user @ computername ~
$
```

Note: It is recommended to use this directory as the parent directory for your SIED analysis.

After the $, you can type various commands. Here, a brief summary of essential commands :

ls	List the files and directories in the current directory
cd “folder”	Change directory to the directory named “folder”
cd ..	Change directory to the “upper” directory
cd	Change to the default directory (“c:\cygwin\home\user”)

Note for novices : the syntax is the same as for UNIX or Linux and is sensitive to lowercase and uppercase. The directory separator is the “/” character and not “\” (as it is for windows systems). Note also that you can use the “tab” key to perform auto completion of your command (helpful to avoid syntax mistakes).
For more details, see Cygwin documentation.

c\ copy SIED source (the file “sied.c” in lowercase) in the folder “c:\cygwin\home\user” (see illustration 9)

d\ Compile SIED by typing the command (see illustration 9):

```
mpicc -o SIED sied.c -lgsl -lgslcblas -lm
```

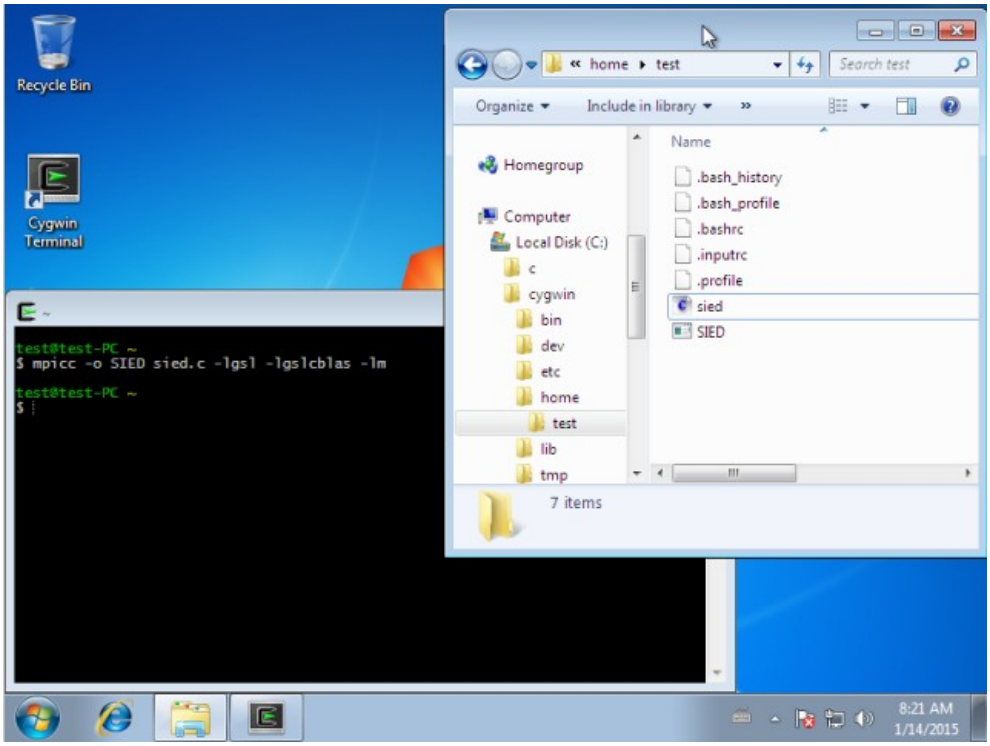


Illustration 9 SIED compilation. The source file sied.c has been copied in the c:\cygwin\home\test folder (test is the username here). Cygwin starts with this directory as default.

In absence of error prompt, this will create the executable SIED.exe (SIED in upper case)

application.

e\ Install SIED by typing :

```
cp SIED /usr/bin
```

Then, in absence of error prompt, SIED is installed on your system and can be used as explained in the following sections.

11. Use of SIED

There is two ways to use SIED. The first way, the most general and difficult, consists in executing SIED on manually extracted images. This is required to analyze non-NanoSIMS50 images. The second way consists in executing SIED in an automated manner after the extraction of NS50 images with SIEDmaker or SIEDmaker4win. In this section, the first way is explained. The second way is discussed and explained in SIEDmaker and SIEDmaker4win sections.

A/ General use

In the directory containing the text image files and configuration file (suggested name "**config.ini**", see section 6), type in command line :

```
mpiexec -np 8  SIED config.ini
```

This command will launch the analysis with the parameters contained in "**config.ini**" file with 8 threads. Adjust the number following "-np" with the number of desired threads (try first the number of cores of your processor). For more information about multithreading, see your MPI library documentation.

Note: During the execution of SIED, nearly all the CPU resources are used, slowing down your system.

An example of files to be analyzed is given in the archive AD_4h_8_p1.zip

*unzip the archive AD_4h_8_p1.zip in your /home/"user" directory. This will create the folder ../home/"user"/AD_4h_8_p1/

*On command line (terminal for Linux user or cygwin terminal for windows users), go to this directory and execute SIED analysis by typing (see illustration 10, on windows system)

```
cd
cd AD_4h_8_p1
mpiexec -np 2  SIED config.ini
```

Note: Replace the value "2" given just after "-np" by the number of threads you want to execute. By default, try the number of cores of your processor.

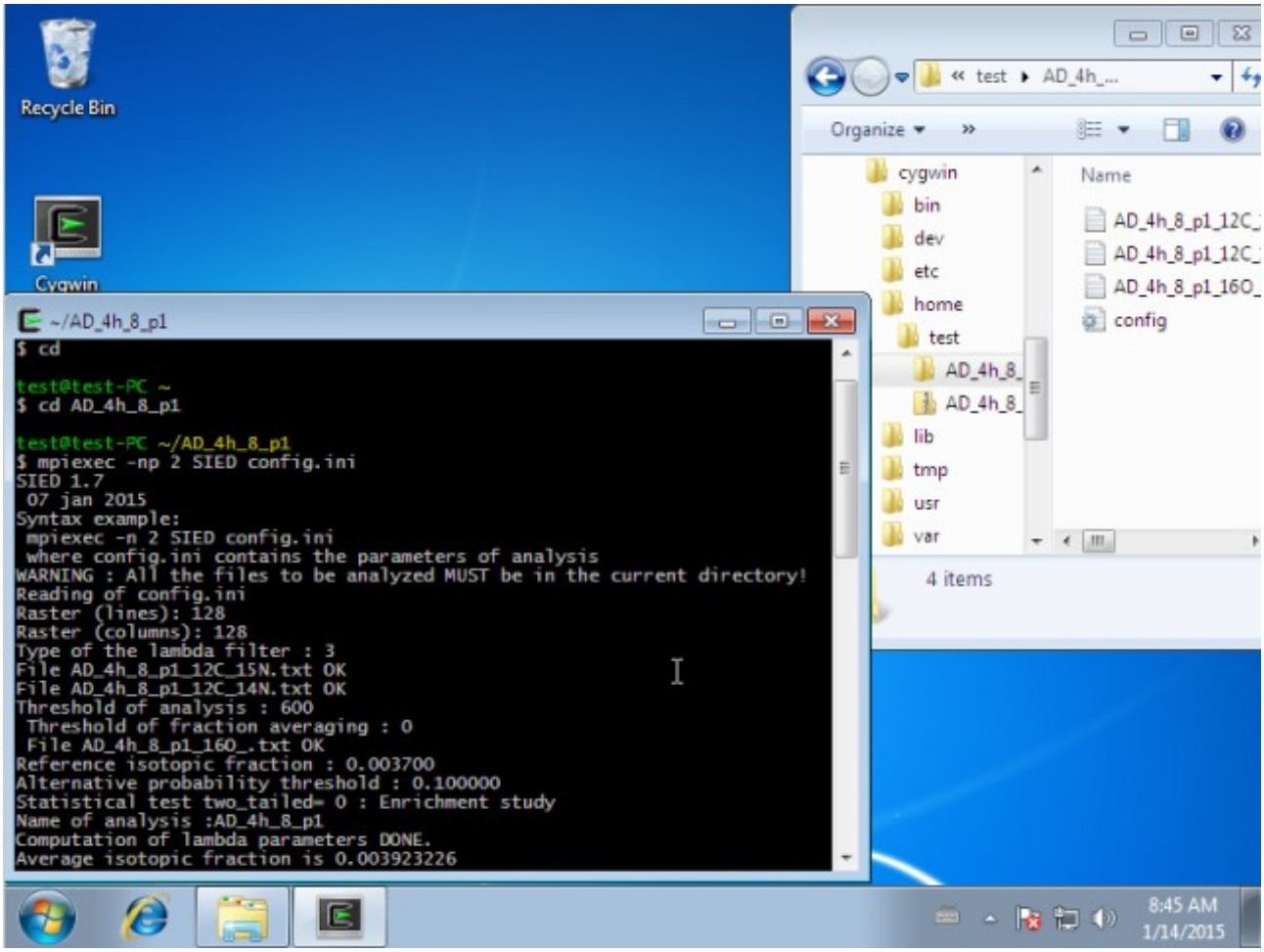


Illustration 10 Example of SIED analysis on cygwin.

Chapter 4 : Result analysis step

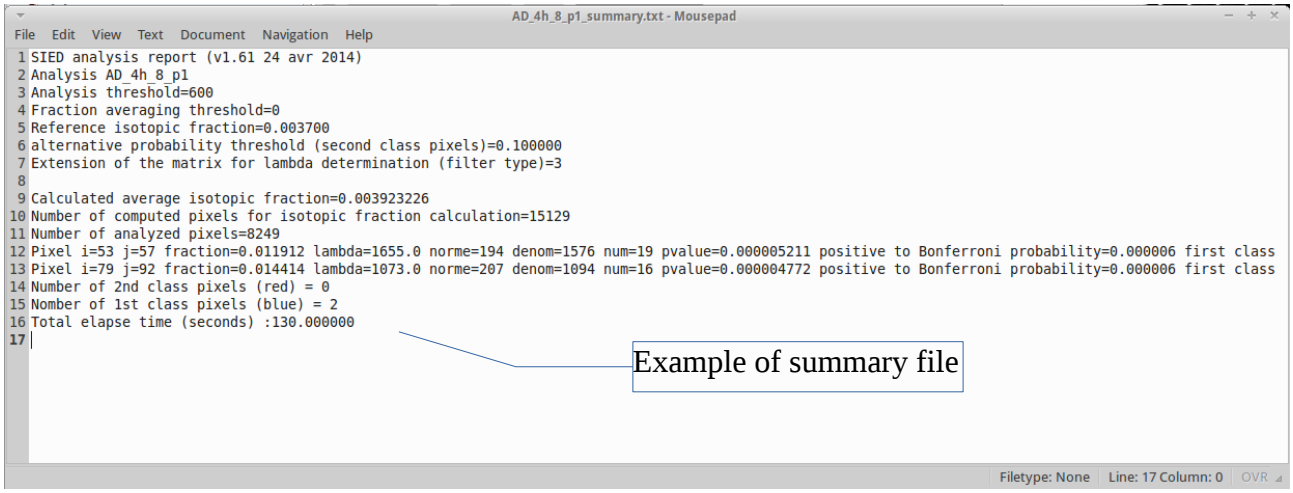
At the end of a SIED analysis, several output files are generated. This section explains how to read these output result files.

12. SIED output files

***prefix*_summary.txt** : is a text file containing informations about analysis parameters (8 first lines) and summarizes results (line 10 to end of file).
Note: do not open this file with Microsoft Notepad (End of line not compatible). Prefer the use of MS Wordpad to read this file. Alternatively, I suggest the free text editor "medit" (http://mooedit.sourceforge.net).

line 1 : version of SIED software
line 2 : name of the prefix
line 3 : type of analysis (One-tailed or two tailed)
line 4 : threshold of analysis
line 5 : threshold for reference isotopic fraction calculations
line 6 : reference isotopic fraction entered in config file
line 7 : second class pixels alpha risk
line 8 : extension of the matrix for lambda determination
line 10 : calculated mean isotopic fraction (from pixels above the corresponding threshold)
line 11 : number of pixels used to calculate the mean isotopic fraction in the image
line 12 : number of analyzed pixels (above analysis threshold, inside the raster-2*extension of the matrix square, and for which a lambda parameter has been successfully found). This number is the one used for Bonferronni correction.
lines 13 to XX : if significant enrichments are found, displays in the same line:
coordinates i (number of line) j (number of row),
isotopic fraction,
lambda parameter of the pixel,
"norme" the value of the additional image,
"denom" the value of denominator image,
"num" the value of numerator image,
"pvalue" the probability to measure an isotopic fraction as high as the really measured if the real isotopic fraction of the sample was the reference isotopic fraction; followed by the corrected pvalue threshold (risk alpha corrected with Bonferronni method) and the classification of the positive pixel (first class or second class).
lines XX to end : number of 2nd class pixels, number of 1st class pixels and time of computation (in seconds).

Note: The line numbering is not written in the file but can be displayed by some text editors as illustrated below.



The following output files are all text images files. They can be read using text editor (not very useful) or spreadsheets (Note: images with width larger than 256 pixels can induce a crash of some spreadsheets) to read the values and perform some calculations. They can also be imported with ImageJ (http://imagej.nih.gov/ij/ for documentation and download) to visualize them (File/import/Text image …).

***prefix*_crop_mass.txt** : images at the format text of the "mass" corresponding isotopes (numerator or denominator). The dimension of these images are truncated by twice the rank of the matrix.

***prefix*_positive_*info*.txt** : with "*info*" is the name of label mass, non-label mass or fraction : image txt containing respectively the counts of label mass, non-label mass or isotopic fraction of 1st and 2nd class pixels.

***prefix*_positive_class1_*info*.txt** : idem as previous, but only for 1st class pixels.

***prefix*_fraction.txt**: image txt of the isotopic fraction of analyzed pixels.

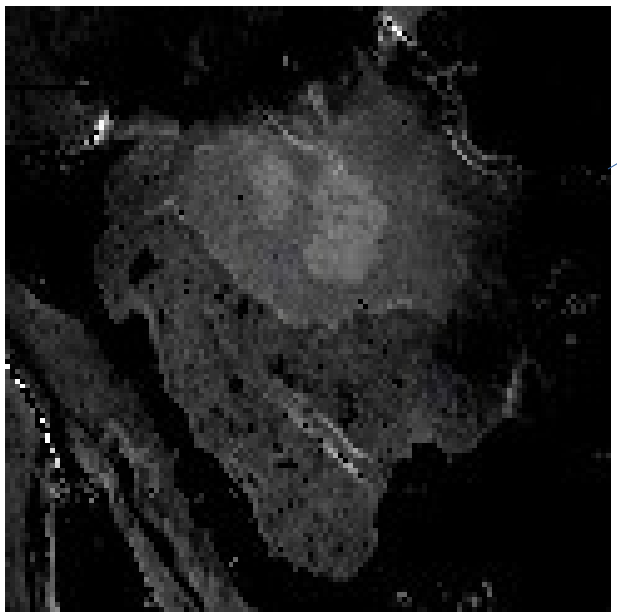
***prefix*_lambda.txt**: image txt of the lambda parameters (for non-label mass) that have been successfully determined.

Note: If required, you can easily obtain the image of lambda parameter of label-mass by multiplying prefix_lambda.txt image by the isotopic reference used during the analysis.

***prefix*_pvalue.txt** : image of the pvalues of analyzed pixels.

***prefix*_image_out.bmp** : image at the bitmap format showing the denominator analyzed pixels in gray levels, first class pixels in blue, second class pixels in red and unanalyzed pixels in black. For SIED2t output images, blue pixels are significantly below reference

isotopic fraction, red pixels are significantly above reference. Dark blue or red are 2nd class pixels and light blue or red pixels are 1st class pixels.



Example of image_out.bmp

13. Interpretation

This section is not an absolute rule to interpret your results. This is just a discussion about the pvalues and the questions “what is a positive pixel?” and “what is a negative pixel?”.

pvalue: the pvalue of a pixel is the probability to measure for this pixel an isotopic fraction at least as high as the measured one, given the fact that the real isotopic fraction of the sputtered volume corresponding to this pixel is the reference isotopic fraction. Clearly, in One-tailed mode, if the pvalue is “very low”, the measured isotopic fraction is probably not a measure of the reference isotopic fraction but a measure of “another” isotopic fraction (probably a higher isotopic fraction !). If the pvalue is not “very low”, the probability that the measured isotopic fraction is a simple fluctuation of the reference isotopic fraction cannot be neglected. In two-tailed mode, a “very low” pvalue can be interpreted as above. However, a “very high” pvalue means that the probability to measure for this pixel an isotopic fraction at least as low as the measured one became very low and this measure is certainly a measure of “another” isotopic fraction (probably a lower isotopic fraction !). Between “very low” and “very high” pvalues, the measured isotopic fractions are probably just simple fluctuations of the reference isotopic fraction.
Note: the pvalue does not take into account the localization of the pixel nor the isotopic fraction of the neighbor pixels. See “negative pixel” discussion.

First class pixel: a first class pixel is a pixel for which the pvalue is considered “very low” or “very high” using the Bonferronni correction for multiple tests. The alpha risk is below 0.05.

Second class pixel: a second class pixel is a pixel for which the pvalue is considered “low” or “high”. Using the Bonferronni correction for multiple tests, the corresponding alpha risk is below 0.10 (for default analysis parameters). Depending on your sample, your research thema etc... you have to choose is such a pixel is of interest or not.

Negative pixel: A pixel that is not declared as a first (or second) class pixel is not necessarily uninteresting. The case you use SIED in two-tailed to verify the isotopic homogeneity of a sample is a good example. Another case where the sole pvalue cannot help to conclude is when the difference between the reference isotopic fraction and real isotopic fraction of the sputtered volume is lower than the measure uncertainty. In such a case, one can accuse the lack of sensitivity of the technology or modify the analysis

conditions (increasing the counting statistics to reduce the uncertainties). Other solutions are to use another statistical test (Bonferronni is too conservative) or to try taking into account the neighboring measured isotopic fraction (to detect local heterogeneities for example). Obviously, many other solutions can be found !

Chapter 5 : Troubleshooting

14. Errors installing Cygwin

For windows user : (see p32-33, in chapter 3 : SIED Calculation step, part Installation on Windows systems).

Error message appears :

```
Error bash mpicc : command not found
```

after typing the command :

```
mpicc -o SIED sied.c -lgsl -lgslcblas -lm
```

This is due to an installation error of a package that is required to compile SIED.

SOLUTION :

Reinstall Cygwin with all the required packages (see list p32) and make sure that a version number of the package appears instead of “skip” after the package selection (click on “↻”).

15. Errors in installation on GNU Linux systems

For Linux user : (see p31, in chapter 3 : SIED Calculation step, part installation on GNU Linux systems).

Error message appears :

```
Command ‘mpicc’ not found but can be installed with :
        sudo apt install lam4-dev
        sudo apt install libopenmpi-dev
        sudo apt install mpich
```

This is due to an installation error of a library that is required to compile SIED.

SOLUTION :

Reinstall the required library :

- openmpi-bin
- libopenmpi-dev
- libgsl23
- libgslcblas0
- libgsl-dev

By the following command :

```
sudo apt-get update
sudo apt-get install openmpi-bin libopenmpi-dev libgsl23 libgslcblas0 libgsl-dev
```

16. Errors in using SIED

For all user : (see p35, in chapter 3 : SIED Calculation step, part Use of SIED).

Error message appears :

```
SIED 1.71
 28 jan 2015
Syntax example:
 mpiexec -n 2 SIED config.ini
 where config.ini contains the parameters of analysis
WARNING : All the files to be analyzed MUST be in the current directory!
Reading of config.ini
Raster (lines): 128
Raster (columns): 1
Type of the lambda filter : 6540336
Unvalid type of lambda filter. The default value = 3 (matrix 7x7) is used
File 0▒c Error while reading 0▒c
Verify your config file.
Program exit.
```

If we enter the command :

```
mpiexec -np 2 SIED config.ini
```

Without being directly in the folder containing the images in .txt format and the config file.

SOLUTION :

Use the “cd” command to be in the folder that contains the images in .txt format and the config file.

```
cd “name of the folder”
```

then the following command should work:

```
mpiexec -np 2 SIED config.ini
```

17. Error Linux command

Error message appears :

```
Could not open lock file – open (13 : permission denied)
```

SOLUTION :

You need to have admin permissions to do this action. Depending on your distrib, you may use “sudo” at the beginning of the command or switch to “su” mode. For more details, contact your

administrator and/or read http://ryanstutorials.net/linuxtutorial.

18. SIEDmaker

First problem : the SIEDmaker (with linux) window is large and can be larger than the screen. Therefore, it can not be closed with the cross at the right top.

SOLUTION :

There is a “close” button in the main window (at the bottom) to close the SIEDmaker window.

Second problem : The non-labeled image display is impossible to move and may hide the main window of SIEDmaker. It interferes with the completing of parameters.

SOLUTION :

The main SIEDmaker window can be moved. If you can’t, change the screen resolution.

Third problem : Special characters may appear in the name of the created file.
This is a problem of reading and writing of the file name.

SOLUTION :

Be careful of what is displayed in “prefix analysis” and delete special characters manually.

Fourth problem : Use SIEDmaker with Windows.
SIEDmaker has POSIX-specific folder creation commands. The directory is not equivalent with Windows.

SOLUTION :

Use SIEDmaker4win. Otherwise, you have to modify the source code of SIEDmaker to integrate the folder creation commands with Windows.

Fifth problem : the registration of image planes (after having clicked “SUM the planes”) takes a very long time.

SOLUTION :

If there is a lot of plane during a “summation plane” analysis, the shift max value has to be edited. Reducing it to a more reasonable value reduces the analysis time. Otherwise, the adjustment method can be changed by the projection method for example.

19. SIED analysis time

SIED analysis time is very long.

SOLUTION :

There are several ways to reduce analysis time :

- increase the number of cores used for analysis or try on another more powerful computer. The use of a cluster may also be a solution.
- increase threshold analysis. This reduces the number of pixels to analyze.

- Reducing the rank matrix saves time. By default, this is on the rank 3, but if you select rank 2, the number of pixels taken into account is divided by 2. However, be careful, this increases the risk of having a false positive.

20. Image .txt opening

.txt images created by the SIEDmaker or SIEDmaker4win software cannot be opened. Displays a table with numbers.

SOLUTION :

Use imageJ software. Select “import” then “text image” to open the generated .txt images.

Chapter 6 : Contact informations and references

21. Contact and informations

This software has been developed by Anthony Delaune, Camille Ripoll and Armelle Cabin-Flaman

The “companion” software SIEDmaker and SIEDmaker4win were developed by Anthony Delaune and Loïc Thibaud.

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15. References

Delaune, A., Poutrain, P., Gibouin, D., Gangwe-Nana, G., Jourdain, B., Norris, V., Ripoll, C., and Cabin-Flaman, A. (2013). SIED: a new tool to detect significant isotopic enrichments in NanoSIMS50 images. *In* 3rd NanoSIMS International Workshop. Luxembourg.

Delaune, A., Cabin-Flaman, A., Legent, G., Gibouin, D., Smet-Nocca, C., Lefebvre, F., Benecke, A., Vasse, M., and Ripoll, C. (2013). 50nm-Scale Localization of Single Unmodified, Isotopically Enriched, Proteins in Cells. PLOS ONE *8*, e56559.

16. Links

http://imagej.nih.gov/ij/ ImageJ: multi-platform image editor
http://mooedit.sourceforge.net medit: multi-platform text editor (compatible with UNIX line endings format)
http://www.cygwin.com Cygwin: Windows program giving Linux functionalities.

http://www.gtk.org/ GTK+2.0: Library required for SIEDmaker
http://www.open-mpi.org OpenMPI: library required for SIED
http://www.gnu.org/software/gsl/ GSL: library required for SIED

http://ryanstutorials.net/linuxtutorial/ Linux tutorial.
http://www.gnu.org/licenses/ GNU License of SIED and companion software

17. Acknowledgements

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