LA-iMageS v1.1.5

User manual

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1. Introduction

LA-iMageS is a software tool to evaluate the elemental distribution analysis in different samples. It is well defined in the literature that metals (e.g., Fe, Cu, Zn, Mn, Co, Mg, and others) and nonmetals, such as Se, S and P, can be found heterogeneously in biological or medical tissues. An evaluation of the precise localization of an element in biological, metallurgical, environmental, nanomaterial, among others samples is a challenging task in analytical chemistry, and different research areas such as biological, medical, chemical and others need to assess this information for helping the comprehension of pathological status, response to stress condition, among others. In this context, *LA-iMageS* was developed to combine the advantages of Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) acquisition with an easy way to build a multi-elemental image with the possibility of choosing the best internal standard. This software presents a simple interface, where the user can readily identify the main parameters in the ablation process and quickly generate the image. Additionally, 3D representation of samples provides the elemental distribution in each point of ablation, relating to the value of intensity obtained in the single acquisition. It is important to remark that the 3D visualization reflects the signal intensity of each analyte and not the real topology of the sample

This document is meant to serve as a guide for the practical use of *LA-iMageS*. It includes explanations of all of its functionalities in order to give a basic idea of usage.

2. Installation

To install *LA-iMageS*, a setup.exe file is provided which runs an easy step-by-step installation process. Make double click on setup.exe in order to launch the installation wizard (Figure 1).
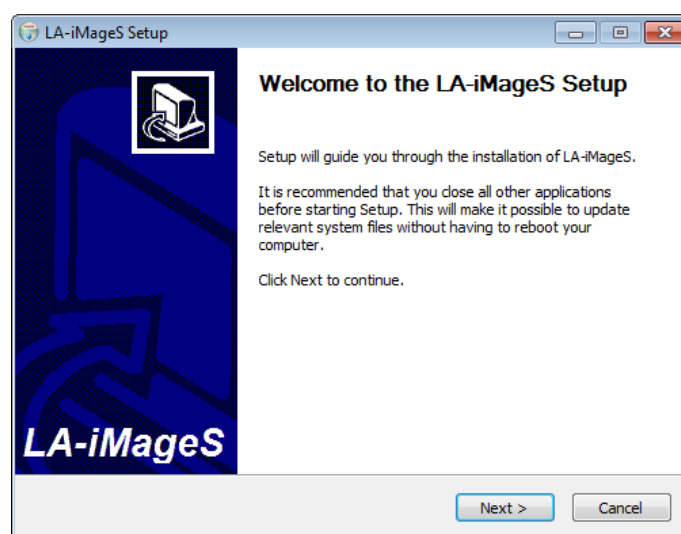

Figure 1. LA-iMageS installation wizard.

3. Main User Interface

The main user interface of *LA-iMageS* is organized into three main sections: the toolbar, the clipboard, and the analysis viewer. In the Figure 2, you can see the general aspect of *LA-iMageS* and the location of each section.
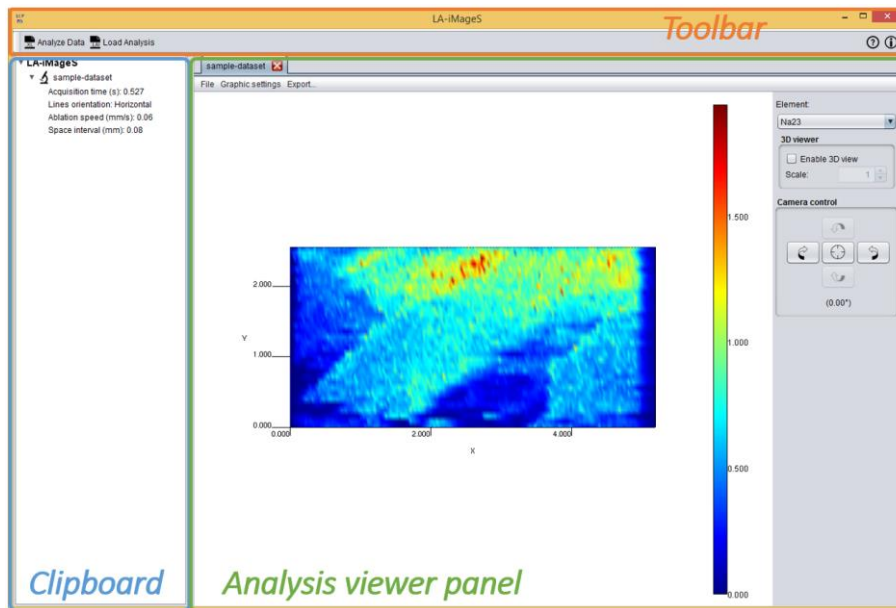
Figure 2. LA-iMageS main window.

Through the toolbar, you will be able to access the main functions of *LA-iMageS*, where the most important is the data analysis operation. On the clipboard tree, you will find a list with the data loaded. Finally, you will be able to explore your data through the analysis viewer.

4. Loading data

In this section, you will learn the dataset formats accepted by *LA-iMageS* and how to load them into the application.

4.1. Dataset Formats

LA-iMageS takes as input datasets generated by ICP-MS instrument control software and exported in the Perkin-Elmer Elan 'XL' format (*.xl).

4.1.1. XL Format

The Perkin-Elmer Elan 'XL' format (*.xl) is generated by control software of ICP-MS instruments from Perkin-Elmer such as Elan 6x00 or Elan DRC-e.

When using this format in *LA-iMageS*, each dataset should be placed in a folder containing the *.xl files corresponding to each data line taken by the ICP-MS instrument. Listing 1 shows an example of a valid XL file.

Listing 1. Example of a valid XL file.

```
Intensity Vs Time, Counts Per Second
Time in Seconds ,SeO96,SO48,C13
0.,0.,290.0050460878,1250.093757031


0.,0.,280.00470407903,1230.090780699


0.,0.,630.0238149002,1150.079355475
```

```
0.,0.,2010.2424352377,1230.090780699
```

The name of the folder will be used as the dataset's name. Each line file must contain a number that indicates the order in which each line has been acquired by the ICP-MS instrument. For example, if you have a dataset with 10 lines, a valid set of names can be: *line 1.xl*, *line 2.xl*, *line 3.xl*, *line 4.xl*, *line 5.xl*, *line 6.xl*, *line 7.xl*, *line 8.xl*, *line 9.xl* and *line 10.xl*.

Additionally, the dataset folder may contain two files: *parameters.conf* and *positions.txt*. Sections 4.1.1.1 and 4.1.1.2 presents this configuration files.

4.1.1.1. The parameters.conf file

The *parameters.conf* file is used to store the ICP-MS data acquisition parameters:

- **Standard**: the standard element in the dataset. The other elements will be normalized using this element as standard. Internal standard is used to normalize the results and to overcome the instrumental oscillation. Users must choose a specific element to be monitored, for example, a known matrix element in the sample or those intentionally added by user. The chosen element should minimize the variability of the ablation process, which can be caused by local differences in tissue thickness and different interaction between the laser and the sample surface. Thus, it will be possible to show that observed signal corresponds to an elemental concentration in a specific local.
- **Ablation speed**: speed set by the user in the laser operational mode as ***continuous firing***. This parameter relates to the spot size, which is determined by the laser beam diameter, and frequency, which corresponds to repetition rate of laser. Usually, a lower value of ablation speed than spot size is used.
- **Acquisition time**: refers to the time needed by the acquisition of one point for all elements monitored by the ICP-MS. This parameter is intrinsically linked to ICP-MS parameters, such as the number of isotopes monitored, sweeps, number of replicates and dwell (or residence) time. The acquisition time should not be higher than 1.0 s, since that the elemental distribution information would be lost.
- **Space interval**: is the distance among the center of two lines. The lowest space interval results in the highest image resolution.

This file is optional and it is used by the *Load Data* operation (see section 4.2) in order to automatically load the acquisition parameters. If this file is not present in the dataset's directory, user must explicitly introduce them. Listing 2 shows an example of a valid *parameters.conf* file.

Listing 2. parameters.conf file.

```
standard=C13
ablation.speed=0.06
acquisition.time=0.324
space.interval=0.12
```

4.1.1.2. The positions.txt file

The *positions.txt* file is used to store the physical position of each line in the instrument during the ablation process. Listing 3 shows an example of a valid *positions.txt* file.

Listing 3. positions.txt file.

```
LINE
16.5460,31.3610,4.4210
17.5140,31.3610,4.4210
LINE
16.1270,31.2410,4.4210
17.9880,31.2410,4.4110
LINE
15.9720,31.1210,4.4150
18.7480,31.1210,4.3550
LINE
15.6940,31.0010,4.4150
19.0860,31.0010,4.3250
```

This format consists in blocks that starts with by keyword LINE followed by at least two lines of three comma-separated numbers (X, Y, Z). The first and last lines of numbers represent the initial and final positions of the corresponding line. There must be the same number of LINE blocks and *.xl files.

This file is optional and it is used by the *LA-iMageS* software in order to read line's positions and determine whether they are horizontal or vertical: if each line has the same X position and Y initial and final positions are different, it means that it is vertical; otherwise, if each line has the same Y position and X initial and final positions are different, it means that it is horizontal. If this file is not present in the dataset's directory, then *La-iMageS* will consider that lines are horizontal and will automatically generate its positions based on the acquisition parameters.

4.2. Analyze data

The analysis execution is started by clicking on the *Analyze data* button of the toolbar, which will show you the *Analyze Data* dialog. As you can see in the Figure 3, this dialog requires you to introduce the ICP-Ms acquisition parameters (refer to section 4.1.1.1 for a deepest explanation of these parameters):

- **Data directory**: the directory where the dataset to be analyzed is stored.
- **Standard**: the standard element in the dataset.
- **Ablation speed**: speed set by the user in the laser operational mode as *continuous firing*.
- **Acquisition time**: refers to the time needed by the acquisition of one point for all elements monitored by the ICP-MS.
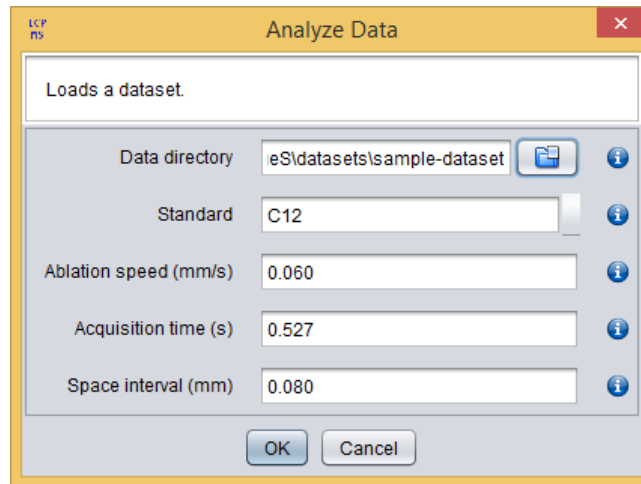- **Space interval**: is the distance among the center of two lines.

Figure 3. Analyze Data dialog.

If the *parameters.conf* file is present in the data directory, then the data acquisition parameters are automatically loaded. You will notice that loaded parameters are highlighted with a green background.

4.3. Load analysis

La-iMageS allows you to save your analysis in *.lai* files. The *Load Analysis* operation will show you the *Load Analysis* dialog (Figure 4), which requires you to select the *.lai* file with the analysis.
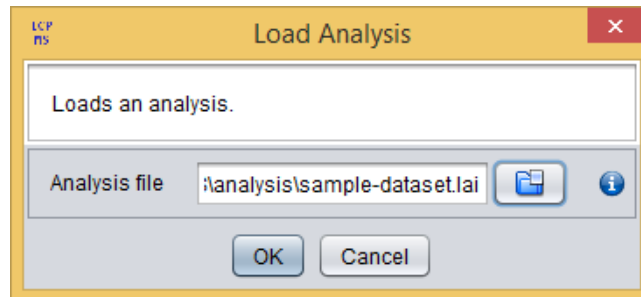

Figure 4. Load Analysis dialog.

5. Exploring data

Once the analysis execution finishes, a new analysis result will appear in the clipboard and in the *Analysis viewer* panel, as you can see in Figure 4. Although the dataset will be automatically showed in the *Analysis viewer* panel once it has been loaded, at any time you can click on an analysis dataset in the clipboard to access its view.
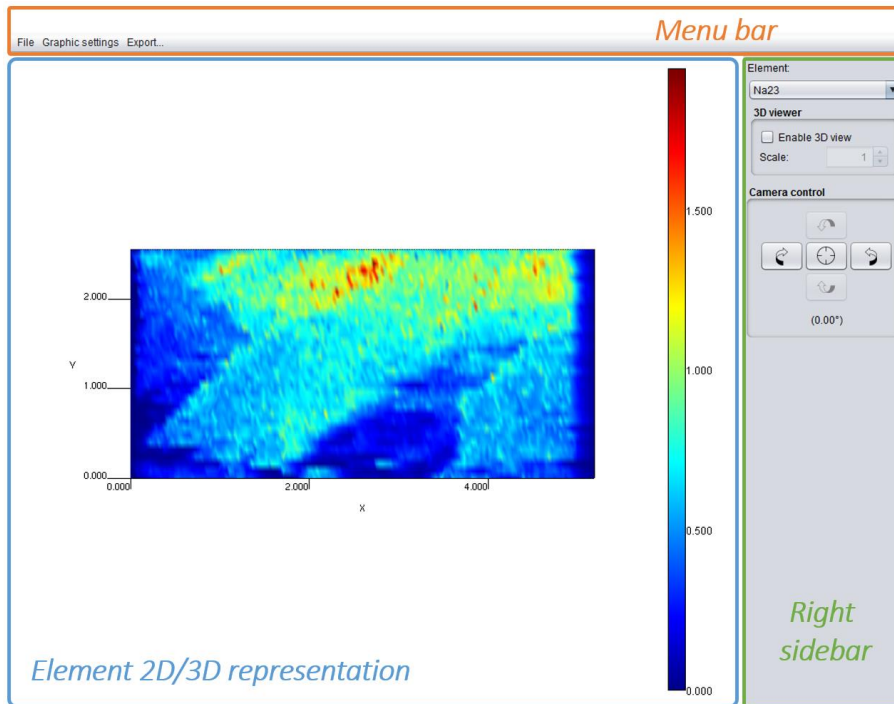
Figure 5. Analysis viewer panel.

5.1. The Analysis Viewer panel

The *Analysis Viewer* panel consists of a 2D/3D representation of the current element with a menu bar and a right sidebar with several configuration options, as shown in Figure 5. It is important to remark that the 3D visualization reflects the signal intensity of each analyte and not the real topology of the sample.

In Figure 6, the ion intensity profile of ^{23}Na$^+$ in a section of penile cancer tissue is shown. To obtain two-dimensional imaging of element distribution, the region of interest was systematically screened (line by line). The spot size of laser was 65 µm. The highest values of ^{23}Na$^+$ are presented in red and the lowest in blue. The change in ion intensity demonstrates an inhomogeneous element distribution in the line scan measured. The SLC8A1/NCX1 gene expression is associated with the sodium distribution in penile cancer [1], which is compatible with the function of this gene. Thus, an increased sodium distribution is presented in the top of the tissue, which corroborates with healthy status of the donor and to histopathological evaluation.
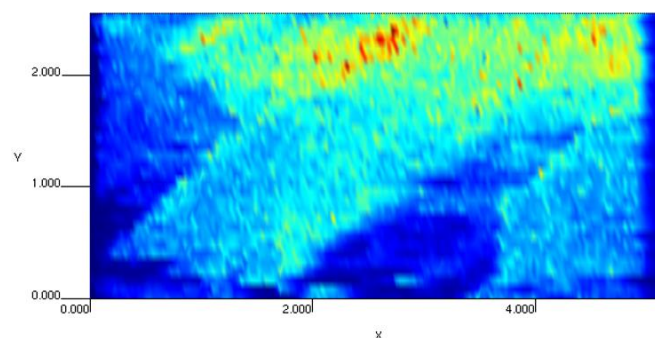

Figure 6. Element data viewer.

5.2. The right sidebar

The right sidebar (Figure 7) allows you to:

- Choose the element that is being currently rendered.
- Enable or disable the 3D view and set the scaling factor. Please, note that 3D visualization reflects the signal intensity of each analyte and not the real topology of the sample.
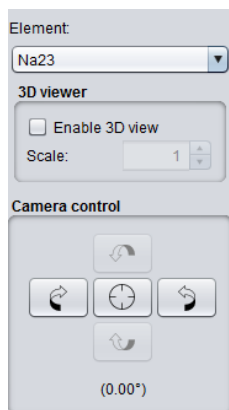- Control the camera position.

Figure 7. The right sidebar.

5.3. The File menu

The *File* menu contains the *Save Analysis* option that allows you to store the current analysis (i.e., dataset and current graphical configuration) in a *.lai* file. Analysis saved using this option can be loaded afterwards using *Load Analysis* operation (see section 4.3).

5.4. The Graphic Settings menu

The *Graphic Settings* menu (Figure 8) allows you to:

- Change the *Interpolation Level*. By default *None* is selected, which means that no interpolation is applied.
- Change the *Color Map* used to render the data.
- Change the *Range Mode*. This option allows you to change the color map range, which can be: *Element*, so that the element range is used; *Dataset*, so that the dataset range is used; and *Custom*, so that you can use a custom range.
- Show/hide the *Color Bar Legend*.
- Show/Hide the *Axes*.
- Show/Hide the *Axes tick lines*.
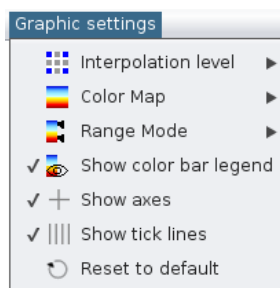- Reset configuration to default settings.

Figure 8. The Graphic Settings menu.

5.5. The Export menu

After exploring your data, you would usually want to use the results in other software applications, or you would just want to save them for further queries. To do so, *LA-iMageS* allows you to export the element data in two different formats: as CSV files or as PNG images, via the Export menu (Figure 9).
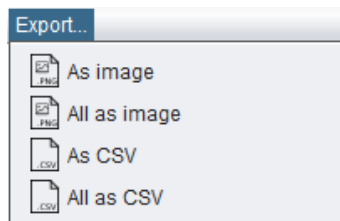

Figure 9. The Export menu.

5.5.1. Export as CSV

CSV (Comma Separated Values) is a general format to store tabular results in plain text files. The main advantage of this format is that many data analysis and manipulation software applications are capable to import data stored in this format.

The export menu offers you two options to export your data as CSV: *Export As CSV*, to export the data of the element that is being currently displayed, and *Export All as CSV*, to export the data of each element in the dataset into CSV files (one CSV per element).

By clicking the *Export As CSV/All as CSV* options, a new dialog (Figure 10) will appear allowing you to select:

- **File**:
    - o *Export As CSV*: the file where the data will be stored. By default, this file is *element.csv* in your home folder.
    - o *Export All as CSV*: the directory where the data will be stored. By default, this directory is your home folder.
- **Format**: the CSV format to export the quantification table. *LA-iMageS* includes two preconfigured formats for Excel and Open/LibreOffice applications. In case you need a specific CSV format suitable for other applications, you can select the *Custom* option, which allows you to configure the format as will be explained later.
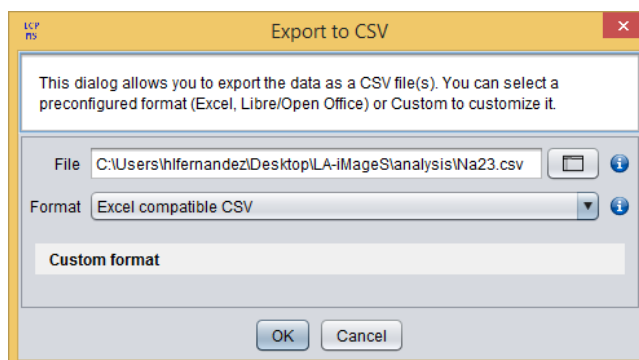

Figure 10. Export As CSV dialog.

As commented above, you can customize the CSV format by selecting the *Custom* format option. When selecting this option, a custom format panel will be shown in the dialog, as shown

in Figure 11. The options included in this panel will allow you to specify the following export parameters:

- **Quote headers**: if this option is selected, then headers will be quoted in the resulting CSV file.
- **Column separator**: the separator character/s between columns.
- **Decimal separator**: the character to use as decimal separator. Note that the decimal separator should be different from the column separator; otherwise, it may result in an unreadable CSV file.
- **Line break**: the type of line break depending in the target operating system.
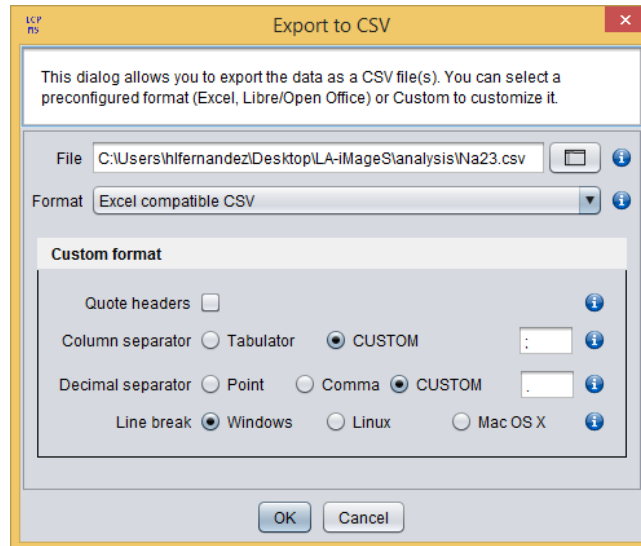

Figure 11. Export As CSV dialog showing the format customization.

5.5.2. Export as image

PNG (Portable Network Graphics) is a popular lossless format to store images. The export menu offers you two options to export your element view as PNG:

- *Export As PNG*: to export the view of the element that is being currently displayed. This option exports the image with the current graphical settings.

- *Export All as PNG*: to export the views of each element in the dataset into PNG files (one PNG per element). This option allows you to configure the graphical settings that will be applied to the exported images.

By clicking the *Export As PNG*, a new dialog (Figure 12) will appear allowing you to select:

- **File**: the file where the image will be stored. By default, this file is *element.png* in your home folder.
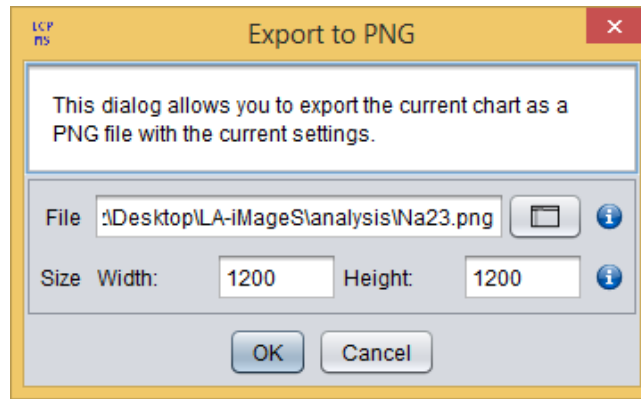- **Size**: *width* and *height* of the image.

Figure 12. Export As PNG dialog.

By clicking the *Export All as PNG*, a new dialog (Figure 13) will appear allowing you to select:

- **File**: the directory where the data will be stored. By default, this directory is your home folder.
- **Interpolation level**: the interpolation level used to generate the images.
- **Color map**: the color map used to generate the images.
- **3D view**: whether images are 3D or not.
- **Scale**: the scaling factor. Note that it only applies if 3D option is enabled.
- **View point**: the camera angle in degrees.
- **Show color bar legend**: whether color bar legend must be visible or not.
- **Show axes**: whether axes must be visible or not.
- **Show tick lines**: whether axes tick lines must be visible or not.
- **Range mode**: the color map range mode.
- **Range**: if *Custom* range mode is selected, the color map range.
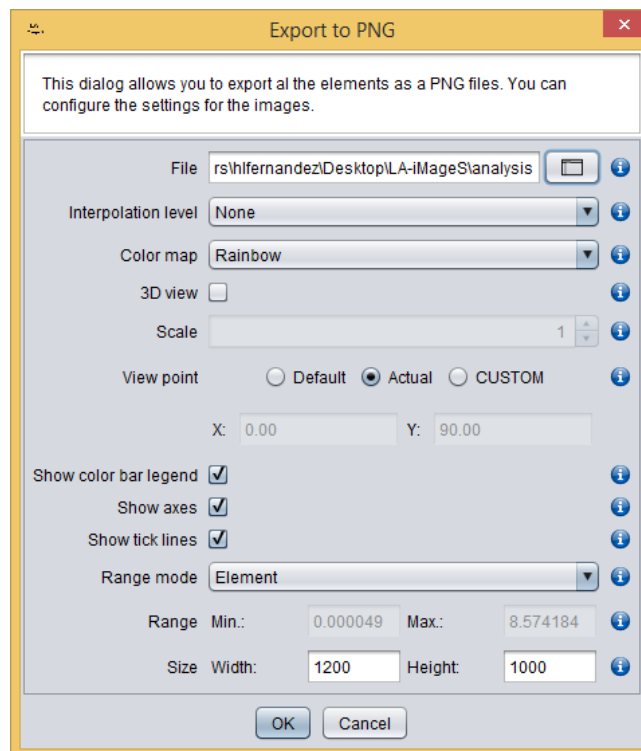- **Size**: *width* and *height* of the image.

Figure 13. Export All as PNG dialog.

6. About

LA-iMageS has been developed by the collaboration of:

- The Next Generation Computer Systems Group, **SING** (http://sing.ei.uvigo.es/).
- The **Bioscope** Research Group (http://www.bioscopegroup.org/).
- The **GEPAM** Research Group (http://gepam.iqm.unicamp.br/).

7. References

[1] J. J. Munoz, S. A. Drigo, M. C. Barros-Filho, F. A. Marchi, C. Scapulatempo-Neto, G. S. Pessoa, G. C. Guimaraes, J. C. S. Trindade Filho, A. Lopes, M. A. Z. Arruda and S. R. R. Rogatto, *J. Urol.*, 2014, 194, 245.