

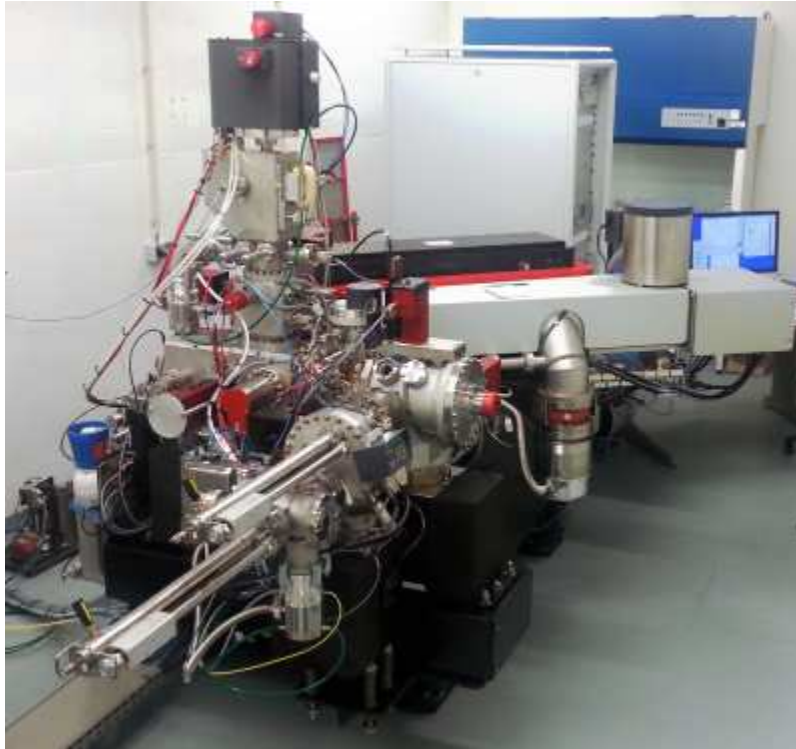


nanosims-wiki

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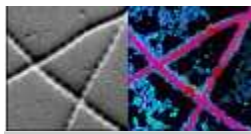
## NanoSIMS facility at Utrecht University

NanoSIMS 50L is one of the advanced instruments of the [National facility for high-resolution in situ isotope and element analysis](http://nanosims.geo.uu.nl/) [<http://nanosims.geo.uu.nl/>]. This website aims to give potential users a brief overview of the instrument, its capabilities and limits, as well as outline the procedures that they should follow in order to avail of measuring time, prepare their samples, conduct the measurements and analyse the data.



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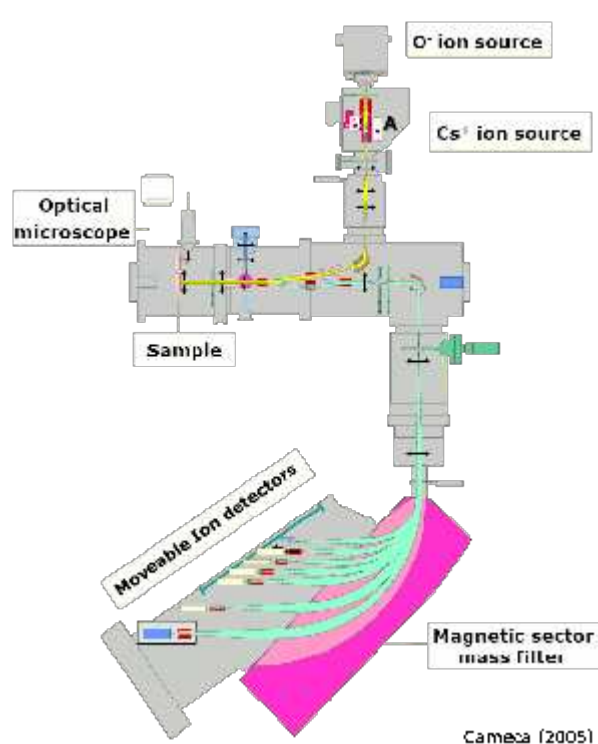
## nanosims-wiki

# Brief introduction to NanoSIMS

NanoSIMS is an analytical technique that allows analysis of **elemental and isotopic composition** of a solid sample at a **sub-micrometer spatial scale** (down to 50 nm). Although originally “NanoSIMS” was the name of an instrument that makes such an analysis possible (NanoSIMS 50 or 50L, produced by Cameca), it is now commonly used as synonym for the technique itself. An informative overview of the NanoSIMS technique, together with many examples of applications ranging from cell biology to microbial ecophysiology to cosmochemistry, can be found on the Cameca website [<http://www.cameca.com/instruments-for-research/sims.aspx>] or in a number of review articles, e.g., Lechene et al. (2006) [<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1781526/>], Wagner (2009) [<http://www.annualreviews.org/doi/abs/10.1146/annurev.micro.091208.073233>], Hoppe et al. (2013) [<http://onlinelibrary.wiley.com/doi/10.1111/j.1751-908X.2013.00239.x/abstract>] (download).

## Basic principle

NanoSIMS analysis uses a **finely focused beam of ions** (ion probe) to **erode the sample surface**. The **secondary ions** (atomic and molecular) that are produced in this process are then **mass analyzed**.



Although this basic principle is simple, the technical realization for achieving high sensitivity, mass and spatial resolution requires some very sophisticated technology. This technology is put together in an instrument called NanoSIMS 50 or 50L, produced by Cameca. Aspects of the instrument that are important from the perspective of the **sample preparation** and **measurements** performed at the Utrecht University are briefly **discussed below**.

## Things to remember about the measurement

### Versatile but destructive

Since any material will be eroded when hit by high-energy ions, NanoSIMS can be used to analyse practically **any kind of solid material**. On the other hand, because of this erosion, the technique is **destructive**. For example, when analysing mineral particles of about 100 nm in size, the particles will be eroded away in a few seconds. When analysing microbial cells, which are typically 1-2 microns in size, they will be eroded

away on the scale of minutes.

## High spatial resolution

Spatial resolution of **~50 nm** is achieved by using a **focused ion beam** that hits the sample surface perpendicularly to its surface. By **rastering** such a beam over the sample surface, high-spatial resolution images can be obtained. Clearly, the sample position must be highly stable to achieve images with accurate spatial information. Here, **temperature control** of the laboratory is essential to minimize distortions of the images, which is important especially when taking multiple planes of the same field of view.

## High mass resolution

The mass spectrometer used for the analysis has a very **high mass resolution** ( $M/dM > 5000$ ). This allows discrimination between ions of very close masses. For example, when analyzing organic material enriched in  $^{15}\text{N}$ , one must be able to distinguish the molecular ion  $^{12}\text{C}^{15}\text{N}^-$  (mass 27.000) from the molecular ions  $^{13}\text{C}^{14}\text{N}^-$  (mass 27.006) and  $^{12}\text{C}^{14}\text{N}^1\text{H}^-$  (mass 27.011), as these are all likely eroded simultaneously from the sample. To be able to do this, mass resolution ( $M/dM$ ) of better than  $27/0.006 = 4500$  is required.

## Simultaneous measurement of up to 7 masses

NanoSIMS 50/50L uses a **magnet to separate the masses**. According to the law of electromagnetism, namely the Lorentz magnetic force, the trajectory of a charged particle traveling in a magnetic field is curved, with the curvature proportional to the ratio between the particle's mass and charge ( $m/q$ ). After passing through the magnet, particles with different  $m/q$  ratios will emerge at different locations, where they can be detected by sensitive detectors. NanoSIMS 50L has 7 detectors, which makes it possible to study **co-localization of 7 masses** in the sample.

## Wide range of detected masses, but ...

In principle, atomic or molecular ions with **masses ranging from hydrogen to uranium and beyond can be detected** by NanoSIMS. However, there are some **restrictions** with respect to the simultaneous detection of multiple masses.

1. Because of the finite size of the magnet, the **minimum and maximum masses** that can be analyzed simultaneously **cannot differ by more than a factor of 22**. For example, masses ranging from 1 amu to 22 amu can be detected simultaneously, but if you want to detect sulphur (mass 32 amu), you cannot simultaneously detect hydrogen (mass 1 amu).
2. The detected elements need to be **ionized**. Because some elements are preferably ionized as **positive** ions while others as **negative** ions, it is possible to simultaneously detect only a combination of elements that ionize either as positive or negative ions.
3. Some elements are very **difficult to ionize**, and are therefore preferably detected as **molecular ions** (e.g. N is detected as  $\text{CN}^-$ ). This brings along problems with **interferences**. For example, when analyzing N as  $^{12}\text{C}^{14}\text{N}^-$  (mass 26.0??) from an organic material, molecular ions  $^{13}\text{C}^{13}\text{C}^-$  (mass 26.0??) and  $^{12}\text{C}^{13}\text{C}^1\text{H}^-$  (mass 26.0??) will also be eroded simultaneously from the sample. Therefore, if the sample is substantially enriched in  $^{13}\text{C}$ , it can cause difficulties in accurate detection of its nitrogen content or isotopic composition.
4. Due to the finite physical size of the detectors and the need to place them next to each other when detecting similar masses, there is a **limit** with respect to the **separation of masses that can be detected simultaneously**. An approximate formula for calculating the difference in masses that can be detected simultaneously is  $dM = \sqrt{M_{\text{max}} \times M} \times 0.017$ , where  $M_{\text{max}}$  is the maximum detected mass and  $M$  is the base mass for which one wants to simultaneously detected also the mass  $M+dM$ . For example, if the maximal detected mass is set to  $M_{\text{max}} = 127$  amu, and one wants to detect mass 12 amu ( $^{12}\text{C}$ ), one cannot detect masses that are closer to 12 amu than  $dM = 0.66$  amu. Since the distance between  $^{12}\text{C}$  and  $^{13}\text{C}$  is 1 amu, their simultaneous detection together with mass 127 is possible. (Note that  $dM$  in this paragraph should not be confused with the mass resolution mentioned above.)

## Probing ions: Cs+ vs. O-

Experiments showed that the **efficiency of emission** of ionized particles differs if the surface bombardment is done with different primary ions. The best efficiency is achieved by using **Cs<sup>+</sup> for detecting negative ions** and **O<sup>-</sup> for detecting positive ions**. Although both primary ion sources are available with the NanoSIMS 50/50L instrument, only one can be used at a time. The fact that **switching** to a different source is a **lengthy procedure** imposes some constraints on the experimental design and the subsequent NanoSIMS analysis.

## Complex ion optics

To achieve **maximum sensitivity** of the instrument, many components of its ion optics need to be **fine-tuned**. Since a number of these optimization steps need to be done frequently, even when moving from one field of view on the sample to another, the **total analysis** of a batch

of samples (replicates+treatments) from a given experiment **may take some time** (several days of continuous measurement time). Clearly, the experimental design should consider this very carefully.

## Things to remember when preparing samples

### Sample charging

Since the detection by NanoSIMS is achieved through bombardment with ions, net charge would be deposited onto the sample during the measurement if the charge was not removed. Since most of the analyzed samples will not be sufficiently conductive, samples should be **coated with a thin conductive layer** (e.g. Au, C, Au-Pd), which will **facilitate removal of the excess charge** and thus prevent problems associated with sample charging. For samples that are put on a support substrate (e.g. cells or tissues on a filter or a glass slide), the conductive layer deposition can be done **before or after** the sample deposition. A gold-coating instrument is available at Utrecht University.

### Flat sample surface

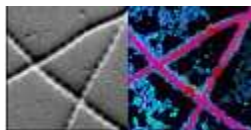
Experiments showed that the detected signal is **affected** by minute **changes in topography** of the sample surface. To minimize these artifacts, **samples** should be prepared **as flat as possible**. For geological samples, polishing with a 30-50 nm powder is advised. Polishing instruments are available at Utrecht University.

### Thin sample sections

As mentioned above, NanoSIMS is a **surface probing technique**, with the thickness of the probed surface in the range of a few nanometers. This is important to realize when analyzing thicker samples such as tissues; features seen in a microscope, obtained for example in a transmission or fluorescence mode, may not be “visible” to NanoSIMS if they are buried deeper in the sample. The only way to get to them is by eroding the layers of the sample above them. Although this can be, in principle, done by NanoSIMS itself, clearly this would not be the most efficient way to measure. Therefore, the sample should be prepared preferably in **thin sections**.

### Need for high vacuum

Because the **detection** of ionized particles eroded from the sample surface is based on their **acceleration** through a high electric potential, it is essential that the whole process occurs in a **high vacuum** ( $<10^{-7}$  Pa,  $<10^{-9}$  mbar) to avoid arcing. This means that the analyzed **samples must withstand such vacuum**. Specifically, they should not contain any volatile substances.



## Application for the NanoSIMS analysis at Utrecht University

The NanoSIMS facility at Utrecht University is **open to external users**. To avail of the analysis time, you need to write a **brief proposal**, which will be evaluated by a committee of scientists. Upon successful evaluation, you will be offered possible time slots for the measurement, as well as an estimate of the measurement duration and costs. After a mutual agreement has been reached, a more concrete planning can commence.

### The proposal should state:

1. Project **name** (few words) and **acronym** (max 8 characters long).
2. **Name, affiliation** and **contact details** (telephone, email) of the corresponding **scientist(s)** and of the project **PI(s)**.
3. **Type of analysis**: e.g., proof of concept for a research proposal, systematic data gathering (in one slot, or spread over several slots depending on the results), entirely exploratory.
4. Brief **introduction** to the topic of the project, including **scientific relevance** and current **state of the art** (max 300 words).
5. **Objectives** of the measurements:
  - a. what you expect to find out or prove (hypothesis) using NanoSIMS
  - b. why you need to do that using NanoSIMS
  - c. which elements/isotopes you want to measure
  - d. which measurement mode you wish to apply (imaging, line profile, depth profile, single spots)
  - e. which precision/accuracy (isotopic and spatial) you require
6. Which **complementary data** will be used with the NanoSIMS data?
  - a. available at present (include a list to support your application)
  - b. available in the future
7. Brief **sample description**:
  - a. basic character (e.g., washed cells on a filter, mixture of cells and sediment on a filter, resin-embedded mineral particles, tissue sections, etc.)
  - b. brief description of the sample preparation
  - c. **estimated number of samples** and individual **fields of interest** on the samples

Please use this [template](#) to complete your proposal. Save the file as PROJECT\_ACRONYM-Your\_Name-Date.doc and e-mail it to Dr. Lubos Polerecky.

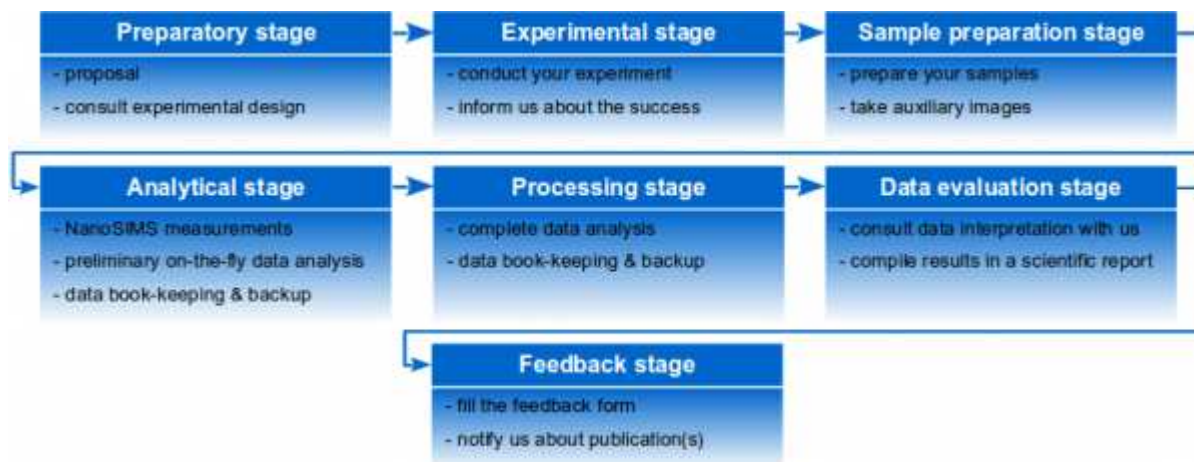
Do not hesitate to [contact us](#) if you have further questions.



## nanosims-wiki

# Flow of the NanoSIMS analysis

To allow optimisation of the flow of NanoSIMS measurements at our facility, we ask users to follow these generic steps.



## Early preparatory stage

1. Apply for NanoSIMS measurement time
  - Write a short proposal and send it to us for evaluation and comments
2. Consult us about the experimental design
  - what is possible to measure with NanoSIMS
  - which additional (possibly parallel) analyses would you like to perform on your samples
  - how should the samples be prepared
  - how many replicates will be realistic
  - when is the realistic time for the NanoSIMS analysis (check [schedule \[http://nanosims.geo.uu.nl/nanosims-wiki/html/nanosims\\_calendar.html\]](http://nanosims.geo.uu.nl/nanosims-wiki/html/nanosims_calendar.html))
  - how long will the analysis take
  - how much will it cost

## Experimental stage

1. Conduct the experiment
2. Inform us about the success of your experiment
  - to initiate more detailed scheduling
  - to modify scheduling

## Sample preparation stage

1. Prepare your samples
  - on filters, glass-slides, Si-wafers, embedded in a resin and polished, etc., adhering to these [basic guidelines](#)
  - if you plan to study specific minerals/elements, **prepare your own standards** (some standards are available at Utrecht University)
  - if necessary, deposit a thin conducting coating (e.g., Au) on the samples (can be done at Utrecht University)
2. Organize your samples
  - **make high-resolution images of your whole samples** (they can be any kind of images, e.g., microscope images, TEM/SEM images); these will be used for [quick navigation](#) through the sample while it is in the NanoSIMS instrument
  - note co-ordinates of easily visible “landmarks” on your samples (see [example](#))

- note co-ordinates of regions of interest that are to be analyzed with NanoSIMS
  - label your samples and regions of interest
  - compile a detailed measurement protocol (what should be measured and in which order of priority)
  - consult the detailed measurement protocol with us
  - familiarize yourself with this [spreadsheet](#), which you will be filling during the NanoSIMS measurements and analysis to help us keep track of what has been measured
3. Bring/Send to us the samples together with the annotated images

## Analytical stage

1. If possible, attend the NanoSIMS analysis
  - to guide the operator
  - to understand the effort involved
  - to make more detailed notes about the measurement
2. If personal attendance is not possible, stay connected to a “hot-line” 😊
  - in this case the operator will keep notes about the measurement
3. **Do preliminary processing/analysis of the generated data** (see [note below](#))
  - either “on-the-fly”, or at least at the end of each measuring day
  - with the [software](#) and computer provided, such analysis is possible as soon as the measurement of a particular region of interest has finished.
4. Store and backup your raw data, log files and other notes gathered during the measurements
  - on our server, to which you will have a password protected access
  - in your own storage space
5. **Link sample labels** from the measurement protocol generated by you with **filenames** assigned during the NanoSIMS measurement
  - remember, our system for keeping track of what has been measured may differ from yours, and there is nothing worse than a bunch of raw data files with no meaning assigned to them

## Data processing and analysis stage

1. Analyze **all** your raw data
  - the [Look@NanoSIMS](#) software that we provide helps you doing that in an easy and fast way
  - remember: even if a particular measurement did not seem to produce scientific value for you, it may have produced a useful know-how for us
2. Keep a backup of the processed and analyzed data
  - on your own storage space
  - on our server, to be used as a point of reference when specific questions about data analysis arise
3. Contact us if you have specific needs for data processing/analysis
  - we are quick to implement new features to the program

## Data evaluation stage

1. Consult the results and your interpretation of them with us
  - to validate that you have done the processing and analysis correctly
  - to ensure that you are not over-interpreting the data
2. Compile data in a scientific report

## Feedback stage

1. Fill this [feedback form](#), save it as PROJECT\_ACRONYM-Your\_Name-Feedback.doc, and e-mail it to us
  - constructive criticism and suggestions for improvement are always welcome
  - we ask you to do so within 3 months after the measurements were finished
2. Notify us when the scientific report was published - it will feel good for us too!

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## Note regarding data processing and analysis

We recommend that you familiarize yourself with the data processing and analysis software **before** your scheduled measurement time. After all, unless you explicitly want us to do the complete analysis for you, it will be you who will need to analyze all the data.

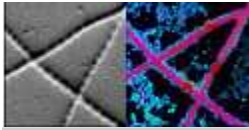
To learn about the software, schedule a day or two for a crash-course, or sign up to workshops that will be scheduled in regular intervals once a critical mass of users has been established.

The logic behind this recommendation is simple but powerful: doing preliminary analysis of the generated data “on-the-fly” will not only give you peace of mind that everything is working as you hoped for, but will also **enable better decision-making about the measurement priorities!** By looking at your samples with the unprecedented detail offered by NanoSIMS, you will very likely discover something completely unexpected. In such a situation, **being able to improvise based on a last-minute information is often as important as being systematic.**

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## nanosims-wiki

# Preparation of samples for NanoSIMS analysis

Sample preparation is a big part of the NanoSIMS analysis, and there is **no general rule** for how to do it best. Basically, it's like cooking — one needs to adhere to some basic guidelines, but the result will differ from cook to cook. Since many people have used NanoSIMS as one of the analytical methods, it is best to seek inspiration and useful tricks in published literature. A good starting point may be the list of papers compiled according to a given theme by Cameca [<http://www.cameca.com/literature/scientific-publications/nanosims.aspx>] or in a recent review by Hoppe et al. (2013) [<http://onlinelibrary.wiley.com/doi/10.1111/j.1751-908X.2013.00239.x/abstract>]. Samples prepared for other techniques such as TEM or SEM are usually **good also for the analysis by NanoSIMS**, so seeking expertise from people in this area may also be very useful.

## Sample dimensions

An important **constraint** on the samples for NanoSIMS is their **size**. Presently, our sample holders support **discs with a diameter of 0.5 cm, 1 cm, 0.5 in and 1 in**. Note that the outermost ring of about 0.5 mm thickness is unusable due to coverage by the holder rim. The **height** of the sample can be in the range **0.1-1 mm** (e.g. a glass disk or a filter) or in the range **4-5 mm** (e.g., a resin-embedded sample).



## Basic guidelines of sample preparation

As touched upon in the [introduction to NanoSIMS](#), the samples need to be

- **dry** - this means they should contain minimal amounts of volatile compounds such as water or oils, and allow reaching the desired vacuum in the analytical chamber of  $<1 \times 10^{-7}$  Pa (see the note on [resin-embedded samples](#) below)
- **flat** - remember, small surface irregularities may look like mountains under NanoSIMS; we recommend a final polishing step using 30-50 nm X particles; polishing facility is available at Utrecht University
- **thin** - this is important when you want to combine the NanoSIMS images with other imagery, such as TEM or fluorescence imaging
- **conductive** - if the sample itself is not conductive, a thin conductive layer needs to be deposited on top of it, preferably just before placing the sample in the NanoSIMS instrument; facility for Au deposition is available at Utrecht University

Additionally, they should be

- **able to withstand high electrical fields**
- **compact** - that is, not contain any “loose pieces” that could detach if the sample is placed in a vacuum; to ensure this, we use dry nitrogen gas to blow away any such pieces before we put the sample into our vacuum chamber

For **microbial cells in a suspension, filtration** on a standard polycarbonate filter (0.2 micron pore-size; see for example here [http://www.millipore.com/catalogue/module/c153#2]) followed by **drying** and **deposition** of a thin Au-film works well. Deposition on a glass slide (its size must be compatible with the sample holders; see for example here [http://www.marienfeld-superior.com/index.php/cover-glasses/articles/cover-glasses-thickness-no-1-circular.html]) also works well. For **microbial cells in sediments, separation** of the cells from the sediment should be tried first, followed by deposition on a filter or a glass slide. In both cases, when preparing cells from water samples that contain dissolved minerals, **washing with MilliQ** before deposition on the filter/glass slide is advisable to minimize the formation of microcrystals during the drying procedure.

For samples such as **tissues, sediments, or soils**, where preservation of the spatial organization of the constituents is important, **embedding in a resin or wax** can be done.

When studying elements/compounds that **diffuse easily** within cells or are **soluble**, steps used for chemical fixation of the cells, which are known to remove the soluble compounds or redistribute the diffusible compounds, should be kept at minimum. **Shock-freezing** followed by **controlled thawing** has been shown to lead good results (REF).

For both resin/wax-embedded and shock-frozen biological samples, the sample should be **cut in thin sections** using a microtome. For resin-embedded geological samples, **polishing** is necessary.

## Resin-embedded samples

The resins-embedded samples need to be between **4 mm and 5 mm high**.

A number of people use **Araldite (502 26 g)** as the resin of choice.

The resin will contain traces of volatile compounds that will outgas when the sample is exposed to the vacuum. To be able to reach the desired vacuum of  $<1 \times 10^{-7}$  Pa in the analytical chamber, this outgassing rate should be kept at minimum. This can be helped by casting the sample for example in a **metallic ring** that is **gas-impermeable** and also **non-magnetic**. Such rings (diameter 1 cm, height 0.5 cm) can be purchased from Cameca. Additionally, **polishing** of both front and back sample surfaces will help minimize the total surface area through which outgassing occurs.

We ask you to deliver the resin-embedded samples at least **a week prior to the scheduled measurement**, so that we can start the degassing process early enough.

## Marking

**Fields of view** analyzed by NanoSIMS will be **very small** (typically from 1 to 100 microns), which makes it difficult and often time-consuming to locate them on a sample of several tens of square-mm in size. We ask you to **make high-resolution images of your samples** (the entire sample, or at least a major part of it) and **mark in these images the fields of view** that you wish to analyze by NanoSIMS (a low-resolution example of a clearly annotated image is shown here). These images should be **submitted together with the samples**. This will significantly enhance the efficiency of the NanoSIMS analysis, as we will be able to measure straight away the fields of interest rather than spend time locating them first. Note that if you do not provide these kinds of marked images, we will analyze only randomly selected areas.

A possible approach for marking is to use a **laser dissection microscope** to mark fields of interest by ablating material around them with a strong focused laser beam. Although quite sophisticated, this technique does not always give satisfactory results, as the marks are often not clearly visible by the NanoSIMS CCD camera.

A simpler approach is to use **navigation based on co-ordinates**. First, take a high-resolution image of the **entire sample**, which can be done by stitching together microscope images of the sample obtained at a lower-magnification but with a high pixel resolution camera. Then, **mark co-ordinates** of 3 prominent "landmarks" as well as of the regions of interest. Since viewing of the sample in the NanoSIMS chamber is aided by a CCD camera, the three landmarks will be easily located. Subsequently, real positions of the regions of interest in the NanoSIMS can be easily found using the noted co-ordinates and some simple algebra. A spread-sheet implementing this calculation is available here.

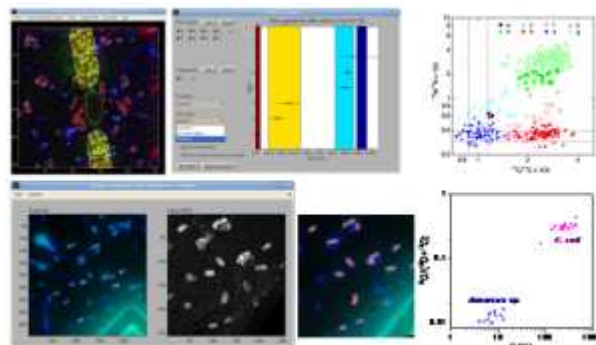


**nanosims-wiki**

## Look@NanoSIMS

**Look@NanoSIMS** is a Matlab-based freeware program for the analysis of NanoSIMS data. It is an alternative to other programs developed for that purpose, such as WinImage, Open\_MIMS or L'Image.

This program is provided on a computer in the NanoSIMS laboratory, and we recommend it for most of the analyses of your NanoSIMS data. You can download and use it for free.



## Features

- display of planes for detected masses
- drift-corrected accumulation of planes
- manual and semi-automated definition of ROIs (regions of interest)
  - definition of ROIs based on an external image (e.g. FISH or TEM) is also possible
- ROI classification (manual & automated)
- quantification of elemental and isotopic composition
  - images
  - lateral profiles
  - depth profiles (in ROIs)
  - histograms
  - scatter plots
- RGB composition
- statistical comparison of ROIs and ROI classes from one or multiple datasets
- dead-time and QSA corrections
- logs of additional features can be viewed [here](http://magnum.mpi-bremen.de/~lpolerec/LANS/changes) [http://magnum.mpi-bremen.de/~lpolerec/LANS/changes]

## Download

- latest stable version [http://magnum.mpi-bremen.de/~lpolerec/LANS/program/] of the program.
- manual and article [http://magnum.mpi-bremen.de/~lpolerec/LANS/manual/]
- poster & slides [http://magnum.mpi-bremen.de/~lpolerec/LANS/presentations/]
- example datasets [http://magnum.mpi-bremen.de/~lpolerec/LANS/data/]
- you can also join a dedicated **Look@NanoSIMS group at Dropbox** to access the most up-to-date version of the program. Just let us know your e-mail address and we will send you a Dropbox-invitation to the folder.

## Citation

L. Polerecky, B. Adam, J. Milucka, N. Musat, T. Vagner, M. M. M. Kuypers (2012). Look@NanoSIMS – a tool for the analysis of nanoSIMS data in environmental microbiology. *Environmental Microbiology* 14 (4): 1009–1023 (doi:10.1111/j.1462-2920.2011.02681.x)

## Contact

E-mail us if you experience problems or if you would like to have new features added to the program.  
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## Report a bug

Although the program has been tested extensively, there may still be some bugs introduced when new features are added. When **reporting a bug**, please **include the following auxiliary files**:

- a screenshot of your Look@nanoSIMS Matlab session
- text of the error-message
- the input file (\*.im) for which the error occurred
- the zipped folder that contains all the output (e.g. the files xyalign.mat, cells.mat, cells.dat, prefs.mat) that you have generated with the data until the error occurred.

Please realize that the error needs to be first reproduced before it can be fixed.

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