macromizer™

User Manual

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1. Before you begin

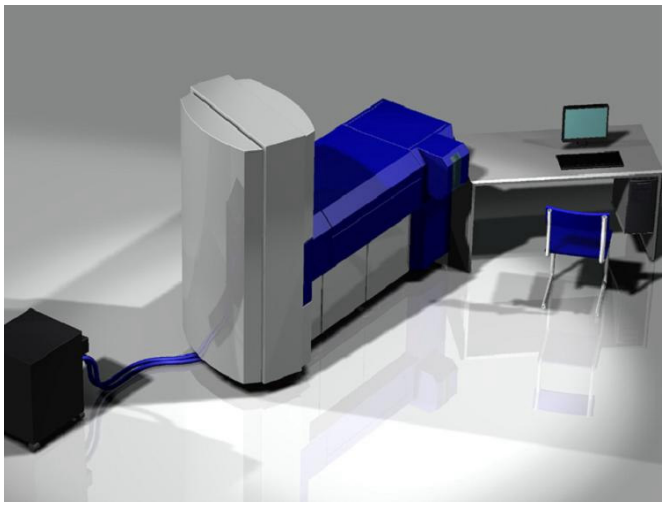
1.1. Introduction to system

The macromizer™ is a high mass MALDI-TOF spectrometer that, based on a cryo detector, is able to detect molecules with masses up to the megaDalton region.

The macromizer™ system consists of two parts. The first is a mass spectrometer instrument, which is a highly complex combination of mechanics, electronics and software. Second is the user interface, which is a standard PC used for control of the machine and for analyzing measurement results.

The macromizer™ is an enclosed integrated instrument, that needs electrical power and eventually cooling water for normal operation. All functions are controlled by the user interface, and only the sample plate has to be placed and removed from the machine manually.

The user interface (GUI) is connected to the macromizer™ through a standard Ethernet interface, and, if the network is fast enough, can be placed remotely from the instrument using the normal network. All normal functions are performed from the user interface.



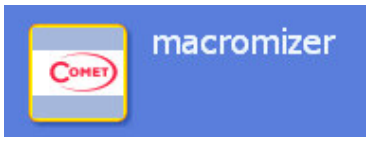
2. Starting up

2.1. Powering up

The powering up of macromizer™ is fairly complex and is normally done by a Comet service engineer.

The client PC is normally directly connected to the macromizer™ by a network cable to the LAN connector, therefore this PC should not be far away from macromizer™. It is possible to connect more clients to the macromizer™ by user network, however, only one client at a time can control the macromizer™.

It is possible that the client PC is shutdown and you have to start it the user PC by pressing the blue button and boot like a normal PC.



On the login screen select the macromizer™ user and login with the password "macromizer".

2.2. Starting the software

After powering up the macromizer™ the software can be started.

In the macromizer™ system several separate software components are running, and most of them are started automatically when the machine is powered up. The only software component that needs to be started is the application client, also called the GUI (Graphical User Interface). This software runs on the macromizer™ client PC.

The GUI software consist of three applications: Preparation, System Control and Data Analyzer. Because the GUI software is divided into more than one application, it is possible to run the applications from more than one computer, allowing several activities to be performed at the same time.

The Preparation application is started by clicking the following icon on the desktop:


Preparation.lnk

This application is used for defining and describing the sample plate contents. Each sample spot can be described with substance, matrix and with a comment. New sample plates can be defined here, and the sequence for automatic measurements is also generated in this application.

The system control application is started by clicking the following icon on the desktop:


System Control.lnk

This application is used for performing acquisitions, optimizing and setting up the acquisition methods and analyzing the acquired spectrums. In this application the basic controls of the macromizer™ can be found. These functions are used mainly by manual machine operation and service.

The data analyzer application is started by clicking the following icon on the desktop:


DataAnalyzer.lnk

This application is the stand alone version of the data analyzer and can also be found in the system control application. Acquired spectra can be analyzed in parallel while performing new measurements.

2.3. User levels and passwords

In the macromizer™ application software (combined acquisition and analyzer) three levels of user access can be setup (two different passwords):

Basis user:	Has access to the automated batch and data analyzer panel without restrictions
Super user:	Has access as the basis user and additional access to the acquisition panel
System administrator:	Has access as the basis user and additional access to the scheduling and system status panels

The passwords are setup in the configuration tab in the system status panel.

When one of the passwords is enabled, a change to one of the restricted panels will generate a request for the respective password. If the password is not entered correctly access to the requested panel is not granted.

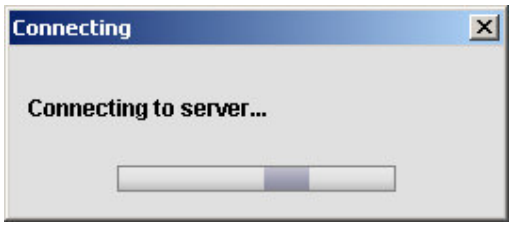
Please note, that when passwords are enabled the startup panel needs to be configured to a panel that is not restricted (setup of the startup panel is done in the configuration tab in the system status panel).

2.4. Machine save mode

When the macromizer™ is left in a running state (with the system control running) and is unused for a specific time, the macromizer™ will enter a mode called 'Machine Save Mode'. In this state all critical and power consuming components are shut down or turned off, and the cryostat valve and system control connection are closed.

In the 'Machine Save' state the following popup is shown:

To reconnect the system control and set the macromizer™ in an operational state, click 'Reconnect' and the following popup will be shown for several seconds while the connection is created:



All components are again returned to the operational level. Only the cryostat valve is still kept closed until a new acquisition is started (a popup will notify the user).

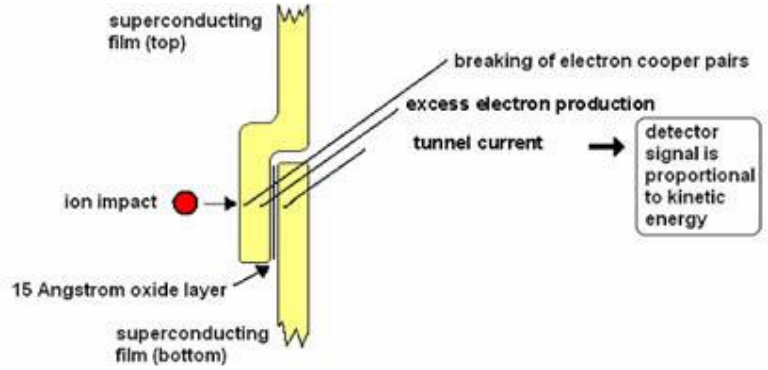
3. What is special for macromizer™

3.1. First high mass MALDI-TOF based on new cryogenic detection system

COMET AG of Switzerland is proud to introduce you to macromizer™, the first commercially available cryodetector mass spectrometer. macromizer™ combines the established features of MALDI-TOF (mass accuracy, sensitivity, reliability and throughput) with the unrivalled sensitivity of truly revolutionary detector technology.

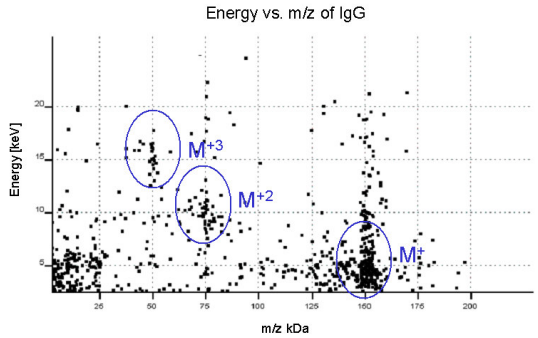
3.2. How Cryodetectors work

The detector is simply a junction between two superconducting films, separated by an ultra-thin oxide layer. The detector temperature is hold in its superconductive stage which is near the absolute zero because electrons exist in their superconductive stage as Cooper pairs. Impinging particles gene-rate phonons (lattice vibrations), which in turn break Cooper pairs into free electrons. Such electrons tunnel through the insulating oxide layer and thus cause a measurable current over the junction. The current is directly proportional to the (kinetic) energy depo-sited by the original impact. As the phonons diffuse, the Cooper pairs are restored and current ceases to flow. Clearly, every impact transfers energy to the junction and gene-rates a current, hence the detector is 100 % efficient. The kinetic energy of an accele-rated ion is the same regardless of its mass, therefore the current produced by the detector is also independent of its mass.



3.3. Energy resolution & charge discrimination

Another unique feature of cryodetector MS is that the energy of an ion is measured as well as its mass, giving macromizer™ data a 3rd dimension. Classically, in TOF experiments, ions with the same charge have the same kinetic energy, while doubly charged ions have twice the kinetic energy. macromizer™ uses an energy scatterplot to display ion energy against mass/charge ratio. An energy filtering tool allows selective display of different charge states. No other instrument offers this capability.



Scatter plot showing energy vs. mass of IgG at 150kDa. The singly charged ion energy is centered

3.4. Generating spectra from scatter plots

Conventional MALDI-TOF mass spectrometers generate their spectra directly from their detector signal output after analogue to digital conversion with high sampling rates, typically in the GHz range.

Cryodetector signal processing works differently: Each individual ion impinging on one of the detector elements generates a characteristic ‘event detection signal’. This signal is analyzed and the m/z value from the TOF information as well as the energy of the impinging ion event is determined. This evaluation is the basis of the scatter plot showing m/z versus energy. Ion events are either single ions hitting one detector element at a time or it can be two or several ions hitting the detector at the same time. The latter is called ‘pile ups’ and will generate a point in the scatter plot at the corresponding m/z position with doubly or multiple energy compared to the single ion event.

The scatter plot is the raw data of a macromizer™ measurement. It contains the information of all detector events in terms of TOF (converted into m/z) and energy. In order to convert that into a “conventional” TOF spectrum macromizer™ calculates how many ions did hit the detector at a given m/z value. In order to do that the operator provides a m/z interval or bin size (binary interval). The spectrum (m/z versus intensity) represents the number of detector events in the corresponding bin (m/z interval). If the bin size is chosen small, the spectrum has a fine structure; if the bin size is chosen larger the spectrum is smoother. The chosen bin size has therefore the meaning of a smoothing parameter and should be chosen accordingly.

(see figures 1+2)

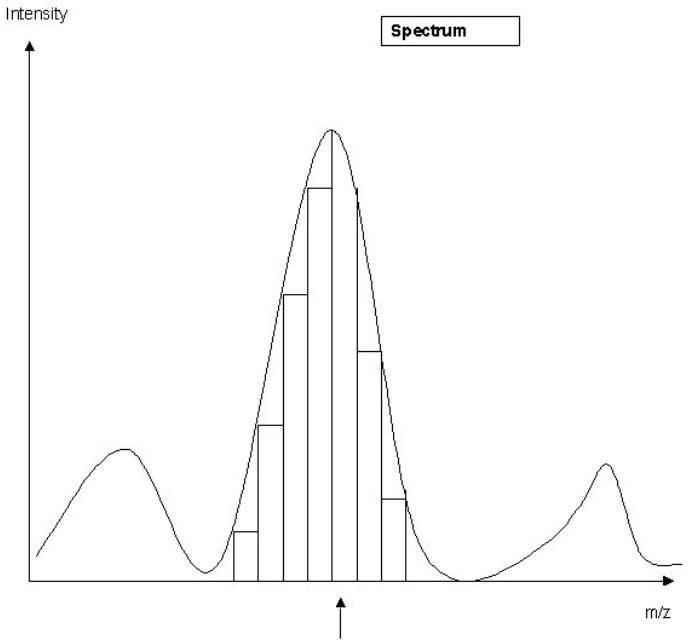


figure 1

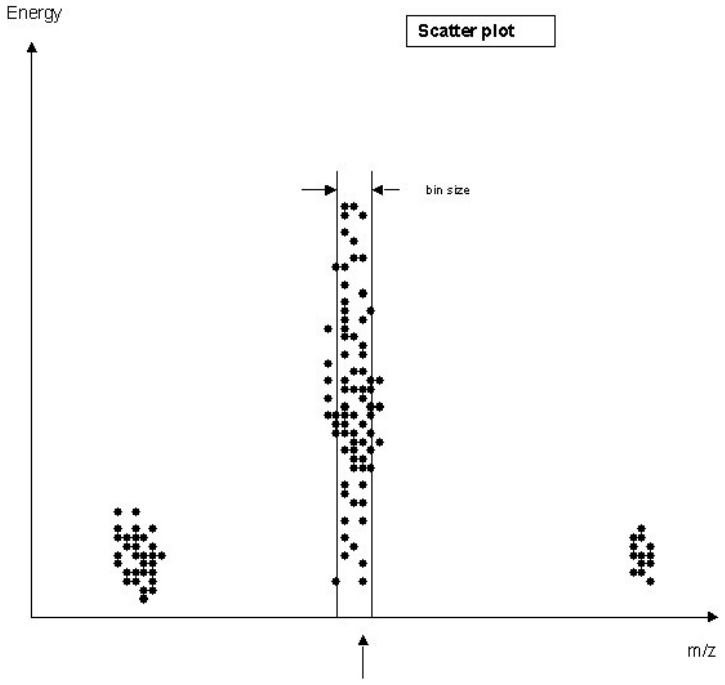


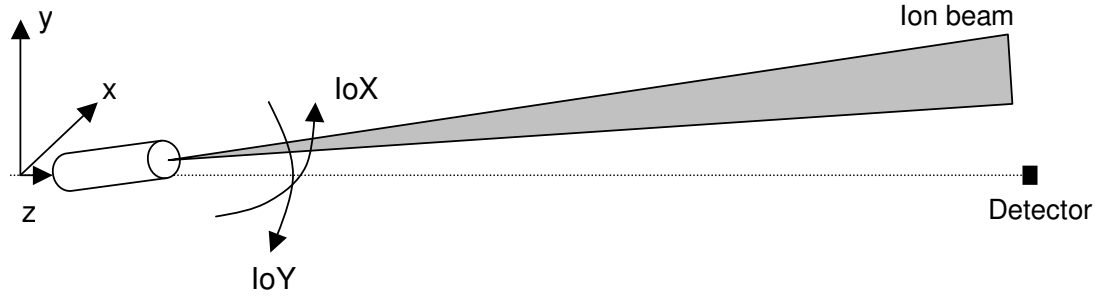
figure 2

3.5. Taking pile ups in the weighted histogram into account

In order to account for the pile ups (several ions hitting one detector element at the same time) the operator can activate the 'weighted histogram' function. If that is chosen, the energy information is taken into account to determine the intensity spectrum. That means that a pile up consisting of three ions of the same mass that hit the same detector element at the same time and thereby showing a 3 times higher energy compared to the single ion, will be accounted as 3 ions in the intensity versus m/z spectrum whereas without the weighted histogram function it would only be accounted as one ion in the spectrum.

3.6. Ion optics and adjustment

Compared to conventional detectors with a diameter of typically 20-40 mm, cryodetectors of macromizer™ are relatively small and have a diameter of around 1 mm. The instrument is therefore designed to focus and adjust the ion beam precisely on the small detector. The focussing is done with standard means of special ion optics design using optimized Einzel Lense design. The horizontal and vertical beam adjustment is done by the capability of tilting the ion optics around its x,y axis(see figure below). In order to find the optimum x,y angel of the ions optics, macromizer™ has a special ion optics test (IOT), as described in chapter *5.3.7.5* Ion Optics Test mode*, page 54.*



4. Sample preparation

MALDI-TOF MS or the explanation of a long abbreviation:

M matrix
A assisted
L laser
D desorption
I ionization
T time
O of
F flight
M mass
S spectrometry

In MALDI analysis ions are produced from solid matrix - analyte mixtures. Hillenkamp and Karas[1] as well as Tanaka and coworkers[2] improved a previously to laser desorption (LD) adapted method by doping small organic molecules (matrix) with a heavier molecule (analyte) and desorbing them

with the help of a pulsed laser beam. The matrix molecules dilute and isolate analyte molecules and serve as mediators for energy absorption.
Desorbed ions are accelerated with the help of an electric field and separated by flighttime in a field free flight tube. After leaving macromizer's™ flight tube the ions are detected in the cryostate with the help of super conducting tunneling junctions (STJ's).

To achieve signals at the detector from the sample under investigation one has to find a good combination of sample and matrix, to make sure that desorption and ionization of the molecules present are as good as possible.

4.1. Matrix requirements

characteristics for molecules to serve as matrix materials to be used with an UV - Laser are the following:

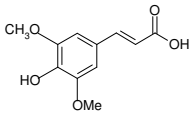
- Strong UV – Adsorption: the molar extinction coefficient (ε_λ) should be in the range between ε_λ ~10^3 to 10^5 L cm^{-1} (λ is usually around 330 nm).
- Vacuum stability: the matrix must not evaporate in the source.
- Miscibility: in ideal case matrix and analyte are soluble in the same solvent. After evaporation of the solvent homogeneous dispersion of the sample molecules in the matrix favours good ionisation yields.
- Chemical compatibility (of matrix and sample, as well as cationizing agent if one is necessary): Compatibility should prevent chemical reactions taking place on the target immediately after mixing of the components. The matrix must not have functional groups that can alter structure or molecular weight of the analyte and must thus be inert.
- Induced ionization: Usually MALDI produces pseudo – molecular ions $[M+X]^+$. Thus a source for the cations $[X^+]$ is needed. The matrix can be the source of Protons $[H^+]$. The other possibility for ionisation is cation attachment e.g. of $[Na^+]$, $[K^+]$, $[Ag^+]$, $[Cu^+]$. These cations can be added by suitable reagents (such as $NaOCOF_3$) or natural contamination ("sodium is everywhere") can be utilised. In some special cases also formation of radical cations is to be observed from the molecule under investigation by ejection of an electron, most probably caused by photochemical effects and assisted by electron transfer matrices.

Actually liquid and solid matrices are in use, depending on the preparation protocol, demand and characteristics of the sample.

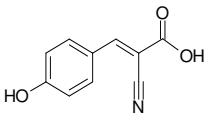
For some samples, such as oligonucleotides, which deprotonate easily, measurement in negative ion mode is to be preferred.

In the scheme in the next paragraph selected solid matrices are depicted. The picture contains the correct name, the structure and short names for the most often used matrices, as well as the mostly investigated samples with these materials.

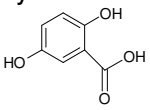
4.2. Matrix materials



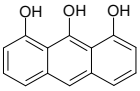
3,5-Dimethoxy-4-hydroxycinnamic acid
SA, Sinapinic acid
Used for Peptides, proteins and dendrimers



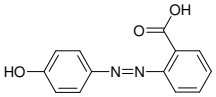
α-Cyano-4-hydroxycinnamic acid
α, α**-Cyano, CHCA**
Used for peptides and small proteins, carbohydrates



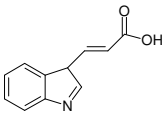
2,5-Dihydroxybenzoic acid
DHB, Gentisic acid
Used for Organic molecules, polar polymers and carbohydrates



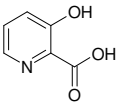
1,8,9-Trihydroxyanthracen
DIT, Dithranol
Used for Non-polar polymers, carbohydrates



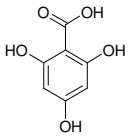
2-(4-Hydroxyphenylazo)benzoic acid
HABA
Used for polymers, peptides and proteins



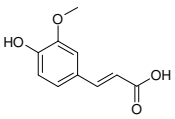
trans-3-(3-Indolyl)acrylic acid
IAA, beta-indole acrylic acid
Used for polymers



3-Hydroxypyridine-2-carboxylic acid
HPA, 3-Hydroxy picolinic acid
Used for oligonucleotides



2,4,6-Trihydroxy acetophenone
THAP
Used for oligonucleotides, carbohydrates, dendrimers



Trans-4-Hydroxy-3-methoxycinnamic acid
Ferulic acid
Used for high mass proteins

Concerning matrix selection for a given sample, only a short summary can be given here. There exist a lot of other molecules which can be used as matrix materials under special circumstances, such as 2-Mercaptobenzothiazole (for some peptides, proteins) or glycerol as well as 4-nitroaniline as liquid matrix materials or succinic acid, which is in use in combination with IR lasers.

4.3. Matrix selection and sample pretreatment

Choice of the appropriate matrix is one of the most critical tasks for successful MALDI experiments. Mostly, if no experiences concerning suitable method of preparation are available in the literature for a given analyte, sample preparation is a process of 'trial and error'.
Similar 'chemical properties' (likeness) of analyte and matrix are favourable, but they are no prerequisites, especially in the analysis of polymers.

Another parameter concerning sample preparation and consequently the quality of the final mass spectrum is the matrix to analyte ratio. The amount of matrix should be sufficiently high to preclude entanglement of the molecules and / or formation of regions of microcristallinity. Practically, the matrix to analyte ratio is typically in the range from 10^3 : 1 to 10^5 : 1. For polymer samples with a higher molecular mass (>15 kDa) a higher ratio is used (10^8 : 1) sometimes.

The exact value depends on the molecular mass and structure of the analyte, the number of different species within a mixture, laser wavelength and fluence but also on the purity of the sample. Especially high concentration of salts (source of sodium ions), detergents or buffers (for stabilizing proteins or oligucleotides in solution) can cause trouble.

For some biological samples removing or adding of buffers can be necessary. Especially if high salt concentrations are to be expected due to the origin of the sample as well as manufacturing / separation conditions special treatment of the sample or the readily prepared spot on the sample plate can be necessary.

Usual matrix concentrations are in the range from **10 – 20 mg/mL solvent**. Mostly used solvents are organic liquids such as ethanol, acetonitrile, methanol, acetone or tetrahydrofurane and mixtures containing trifluoroacetic acid (TFA) such as (50% acetonitrile and 50% of ultrapure water containing 0,1 % trifluoroacetic acid or 70 % acetonitrile and 30% of the TFA-solution).
Ideally sample and matrix are dissolved in the same solvent to avoid sample disintegration (segregation) during solvent evaporation.
Cleaning up matrix materials can be done by recristallization (dissolve solid and precipitate by adding liquid which does not favor dissolution of the substance).

4.4. Sample preparation methods

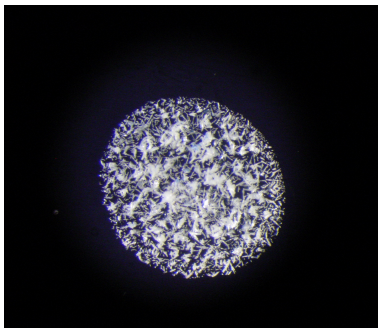
Sample preparation is crucial for the quality (especially regarding resolution and signal to noise ratios, but also sensitivity) of the MALDI spectra. Poor sample preparation will yield low resolution, poor reproducibility and reduced sensitivity. The most popular techniques for sample preparation are 'dried droplet' and variations of it such as 'overlayer or fast cristallisation'-preparation.

The goal of sample preparation is a thin uniform layer of crystals containing analyte and matrix. For practical instructions in sample preparation see next chapter.

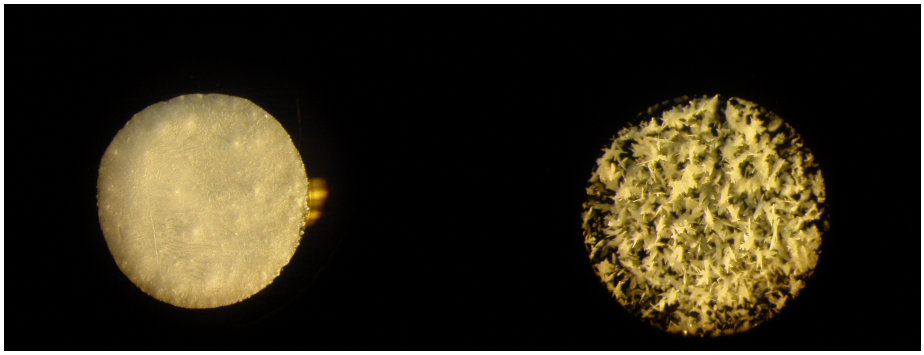
4.4.1. 'Dried droplet' - technique

This is perhaps the oldest method used for sample preparation, mentioned first time by Hillenkamp and coworkers in 1987 and is still used. A matrix solution is mixed with an analyte solution (c = 1 –

10 mg/mL). The solution of a cationizing agent (c = 1 – 10 mg/mL) can be added when necessary. The solutions can be mixed in various ratios, e.g. 10 : 1 : 1 and between 0,5 µL and 2 µL of this mixture are deposited on the MALDI target and air dried. After complete evaporation of the solvent, the target is loaded into the mass spectrometer. Reproducibility will be increased, when matrix / sample crystals are homogeneously dispersed, as can be seen in the picture below.

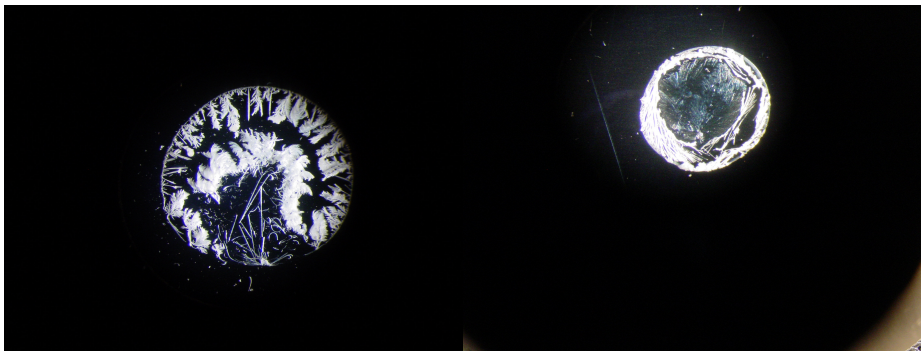


Washing of the sample surfaces with ultrapure water may also increase the quality of the spectra. Contaminants such as salts or buffers have the tendency to enrich at the surface and can in some cases be removed by washing. The picture below on the left hand side was taken after direct mixture of the sample with the matrix and drying under air. The picture below on the right was taken after depositing 2 µL of ultrapure water on top of the same spot for 10 seconds, removing it afterwards with the pipette and drying of the spot. Desalting can also be achieved by using micropipette tips packed with C_{18}, C_8 or C_4 material (for a small summary of suppliers see end of the chapter), depending on the analyte under investigation.



Dissolving the sample in a solvent compatible with the solvent of the matrix solution prevents precipitation / segregation of either the matrix or the analyte. The resulting spots are more homogenous. Depending on the matrix, the prepared sample can be stored in a vacuum chamber for some hours.

A common problem of the method is phase separation during crystallization (segregation due to different solvents of sample and matrix solutions, picture below on the left) as well as enrichment of the analyte preferred in big matrix crystals around the rim of the dried sample spot (picture below on the right). Rims are often pronounced in DHB- and dithranol - preparations.



Especially in cases when cations are needed for successful ionisation, i.e. for synthetic polymers or carbohydrates, separated crystallization of matrix and analyte provide big problems. Maragoni effects, which are responsible for mass transport resulting from movements induced by differences of the surface tension (e.g. from different evaporation rates of two solvents), can be used to explain segregation. Hydrophilic components of the sample are deposited preferentially at the periphery of the drop. During the MALDI measurement the laser often has to be moved above the sample surface on the look for so called “sweet spots” with proper matrix to analyte ratio. Segregation can be prevented by enhanced drying speed, resulting in more homogenous films / crystals. On the other hand separated crystallisation can also be an advantage, because frequently contaminants crystallize in the middle of the dried spot and the ionisation yield is increased.

4.4.2. Vacuum drying

The vacuum – assisted drying crystallization is a variation of the dried-droplet method. The final matrix - analyte drop is rapidly dried under vacuum. The homogeneity is increased as the size of the crystals is reduced, and reduced segregation of matrix and analyte is common. Hardware requirements are low (vacuum chamber), and drying time is reduced significantly, which is of interest for solvents with low volatility or laboratories having great numbers of samples. On the other hand there is no guarantee for better homogeneity and increased alkali cation adduct signal intensity is possible. Washing of the samplesurface with ultrapure water is helpful if the matrix used does not dissolve in water. Sometimes drying the analyte – matrix spot with warm air is reported to have comparable effects on the surface homogeneity because less time is available for extensively crystallization effects.

4.4.3. Polycrystalline thin films/ crushed crystal

This method produces a uniform layer of very small crystals. Based on “dried-droplet” preparation a layer of matrix solution is applied to the MALDI target. After air-drying, the resulting crystals are crushed by pressing with a clean glass slide or laboratory film and the excess pieces of the matrix are brushed away with a tissue or blown off by an inertgas stream. The remaining crystals serve as activated seed for the matrix – analyte mixture in a less volatile solvent, which is applied to the surface of the target afterwards and allowed to dry. The resulting film is usually very thin and homogeneous and can be washed before being completely dry, because most matrix materials are not immediately soluble in water and the film does not wash off easily.

4.4.4. Seeded films (fast-evaporation technique, overlayer technique)

This sample preparation method is again a modification of the “dried droplet” – technique.
The technique is a direct replacement of the “dried droplet” sample preparation with the goal of increased reproducibility, better resolution and higher mass accuracy. First a thin matrix layer in a volatile solvent is deposited onto the MALDI target and allowed to air dry. Then a drop of the

analyte solution is placed on top of the matrix crystals, using a solvent, which allows partial the matrix to be redissolved. If for this second step a matrix / analyte solution (with concentrations and mixing ratios in the range for the 'dried droplet' technique) is used, the method is called 'Overlayer' technique. Otherwise the name of 'fast-evaporation' is correct. The undissolved matrix crystals work as seeding sites and thus smaller cocrystals from matrix and analyte are produced. Washing of the resulting preparation is possible after complete drying of the solvent. Correlation between signal intensity and analyte concentration is enhanced. Problems can arise from complex mixtures, for such samples better results can be achieved by 'dried droplet'. Tuning the mixing ratios of preformed matrix layers and added analyte (matrix)-solutions can improve signal intensity, reproducibility and mass accuracy. 'Sweet-spot hunting' is no longer necessary because virtually the complete sample surface is of equal composition and fluctuations are low. On the other hand very volatile matrix-solvents create problems during the preparation of the MALDI sample due to decreased surface tension and thus wide and uncontrollable spreading of the matrix film.

4.4.5. Electrospray

Sample deposition by electrospray provides improvement of the homogeneity of the sample. Matrix – analyte solutions can be sprayed from a conventional electrospray device but with different parameters if compared to typical ESI conditions. Best results were obtained if the spray results in a completely dried polymer / matrix mixture arriving at the target surface, because then the produced microcrystals are distributed very evenly on the target. Cationisation agents can be included into the sample solution only in small concentrations, otherwise the electrospray will not work in stable state. On the other hand, segregation is reduced compared to e.g. 'dried droplet' preparation. The resulting sample is more resistant to laser irradiation, more shots can be taken from the same spot. Electrospray sample deposition provides a possibility for interfacing the eluents from Capillary Electrophoreses (CE) or Liquid Chromatography and making them accessible to MALDI MS. Handicaps of the method are the need for additional equipment and training as well as longer preparation times.

4.4.6. Spin coating

The idea to use spin coating for sample preparation was published by Perera et al.[3] Advantages of the method are improved homogeneity, reproducibility and ionization yields of the samples, but the technique is not applicable if conventional MALDI targets with a big number of sample wells are used. Mixing of different samples cannot be avoided.

4.4.7. Matrix-precoated targets

This method was developed for coupling of HPLC or capillary electrophoresis systems to MALDI – TOF MS. A heated capillary nebulizer is used to deposit the eluent on a MALDI target, which is already covered with a thin matrix film. One of the big advantages of the method is that a single drop of undiluted sample can be added to a precoated matrix spot, which can be analysed immediately after sample drying. Other methods use hydrophilised target surfaces to prepare smaller spots, thus needing less sample. A disadvantage of the latter example is the possibility of reduced sensitivity.

4.4.8. Liquid matrix MALDI

Up to now MALDI MS is primarily based on the laser desorption of a solid matrix - analyte cocrystal. Disadvantages of preparation methods are described above. Progress in preparation techniques is obvious but not overwhelming. Looking for better preparation, the possibility to use a liquid matrix is an imaginable alternative. One of the best arguments to use liquid matrices is

enhanced lifetime of sample spots. Besides that often better reproducibility is achieved because of the absence of sweet spots due to the 'self-healing' by diffusion of the sample surface immediately after the laser pulse.

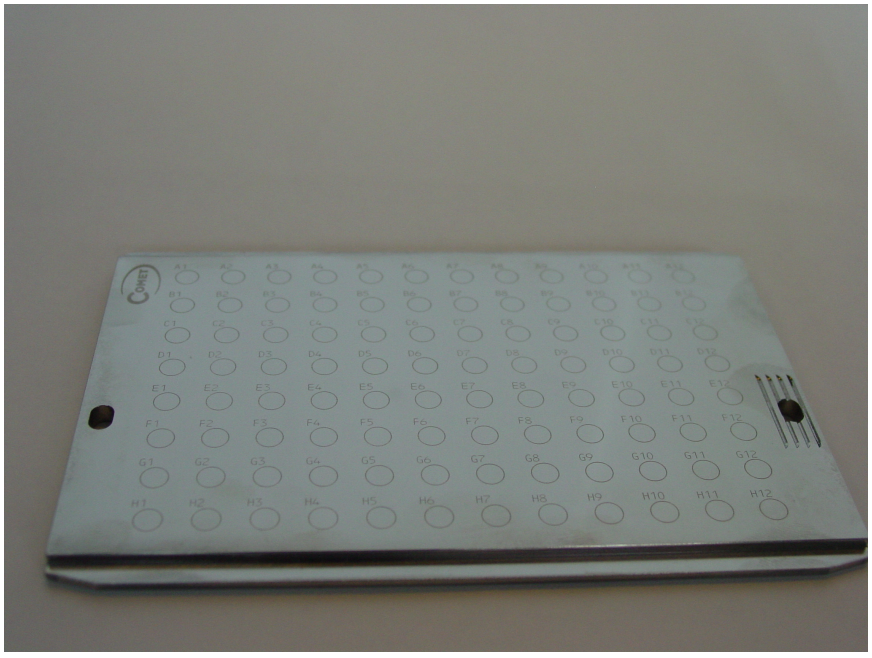
Different types of liquid matrices can be distinguished. There are chemical liquid matrices such as o-nitrophenyl octyl ether which work comparable to solid matrix materials, absorbing the laser energy and aiding the ionisation of the analyte, for example polystyrene. Another possibility is to use a particle-doped liquid matrix, like first published by Tanaka et al.[2] who mixed ultrafine cobalt powder (needed to absorb the laser energy) and low volatile glycerol for providing charges for the ionisation (probably by protonation / deprotonation mechanisms) to ionise proteins as well as polymers. Another possibility to achieve ionisation with liquid matrices is doping a liquid non absorbing medium (such as m-nitrobenzyl alcohol or 1,2,4-butanetriol) with a highly energy-absorbing organic compound (very often the solid matrix molecules or organic dyes) and to use it in polymer analysis. Problems arising from liquid matrix materials are the lack of suitable liquid phase chromophores absorbing appreciably at the N_2 - wavelength, reduced vacuum stability and a limited mass range (up to 25 kDa). Sample preparation can be very tricky, particularly if the sample holder is mounted vertically in the MALDI source. Samples can detach from the holder, if viscosity and surface tension are not appropriately adjusted. Perhaps one of the most essential drawbacks is decreased resolution.

Advantages of the method are improved sample stability and better miscibility with apolar samples. Besides that the decay of signal intensity as known from solid matrices is far less distinct and the thermalisation period (the time from starting the laser until more or less reproducible spectra can be obtained) of the liquid matrix is shorter.

4.5. Sample preparation on the target

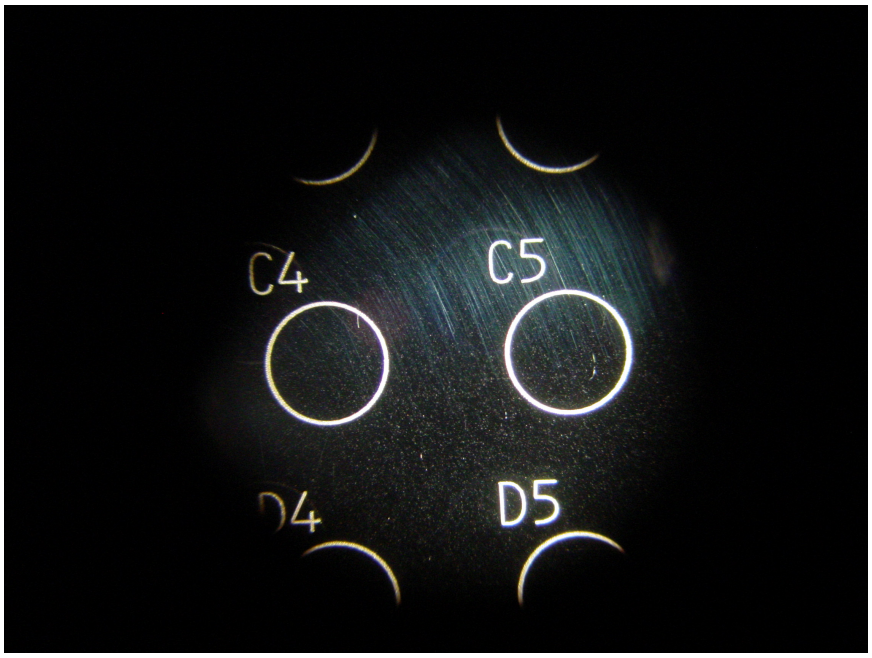
As mentioned above matrix materials are best dissolved in the same solvent as the sample. The (standard) sample preparation method described below is based on the overlayer technique with a seeding layer of matrix applied and dried before adding sample / matrix solution.

The medium to prepare the sample is macromizer's™ 96 well target which is depicted below.



Each well on the target is named separately in rows and lines to help you identifying your sample under investigation. A small section showing two wells and parts of the next two is depicted below. By using the 'Preparation Function' of the software *(described in chapter 4.6 Starting the preparation application, page 26)* it is possible mark each well on the target with the analyte, used matrix and further information around the preparation.

Make fresh matrix solutions every 2 days to avoid (partial) fragmentation of the matrix solution and thus degradation of the spectra quality. Store matrix solutions in the dark. Do not cool saturated matrix solutions to avoid matrix precipitation.



4.5.1. Proteins and peptides

Dissolve 1 mg sample in ultrapure (deionised) water or 0.1 %TFA in ultrapure water.
Dissolve **CHCA or SA** in 50%Acetonitrile and 50% of 0.1% TFA in ultrapure water at a concentration of 10 mg/mL. Vortex tube until solid is completely dissolved, which may take some time.

Apply 1 µl of matrix solution on the spot and let it dry.
Mix 1 µL of sample with 1 to 10 µl of matrix solution in the lid of an eppendorf tube and apply between 0.5 and 5 µL of the resulting mixture on top of the dry matrix spot. The mixing ration depends on the mass of the protein under investigation. If the measured signal intensity from the spot is low, dilution of the sample solution with ultrapure water (1 + 9 to 1 + 99), especially of low mass proteins, may help to reach the best ratio of sample to matrix (as mentioned above).

4.5.2. Oligonucleotides

Dissolve Oligonucleotides to a concentration of maximum 100 µM in ultrapure water.
Dissolve hydroxypicolinic acid (**HPA**) in 50% ultrapure water and 50% acetonitrile. Sometimes addition of diammonium citrate (0.1M) to the matrix solution helps in stabilizing the analyte and in ionizing). For smaller analytes trihydroxyacetophenone (**THAP**) dissolved in acetonitrile or acetonitrile / ultrapure water will give good spectra.

Prepare spot as described above with a bottom layer of matrix solution and apply matrix/analyte mixture on top of the dried spot and let it dry on air.

If oligonucleotides are not cleaned / separated by HPLC before MS measurement, desalting helps in increasing signal: noise ratio.

4.5.3. Polymers

Especially in polymer analysis matrix selection is crucial for success or failure of the analysis. One parameter for matrix selection is the polarity of the polymer under investigation. Polar polymers will give better spectra from polar matrices (with the help of alkali - cations such as sodium and potassium), apolar polymers will give better spectra from apolar matrix materials (in some cases addition of silver - or copper – ions helps).

Dissolve Polymer to a concentration of 1 – 3 mg/mL (depending on the mass range and the solubility of the polymer, as well as the number of sideproducts). Cleaner samples can be measured in lower concentrations.

Choose matrix : For polar polymers such as polyethyleneglycols (PEG) and similar samples Dihydroxybenzoic acid (**DHB** in 50% acetonitrile and 50% ultrapure water) or sinapinic acid (**SA**, also in acetonitrile / ultrapure water) will probably give best results.

Apolar aromatic polymers such as polystyrenes (PS) and conjugated aromatic materials will prefer apolar matrices such as dithranol (**DIT**), trans-3-indoleacrylic acid (**IAA**) or (2-(4-hydroxyphenylazo)benzoic acid) (**HABA**). Use organic solvents such as THF, acetone or ethanol to dissolve apolar matrix materials.

As most polymers do not accept protons from the matrix easily, ionization is often achieved by addition of a cationizing agent. Metal salts are used in most cases and the anion is supposed to be inert under the conditions used. Preferred salts are metals salts of the trifluoro acetic acid, which are very well soluble in most solvents. Use cationizing agents at a concentration of 1 – 2 mg/mL in the solvent also used for the matrix solution to avoid segregation.

Apply a drop of matrix solution to the target spot and let it dry. Mix analyte, matrix and cationizing agent at a ratio of 1 : 1 : 1 in the lid of a small tube and apply 0.5 µL to 2 µL on the dried matrix spot. Variation of the mixing ratio (especially increasing the matrix volume) may improve the quality of the spectra dramatically.

4.5.4. Oligosaccharides

Dissolve analyte at a concentration of 1 – 3 mg/mL in ultrapure water or other polar solvents. Use DHB solution as described above and follow instructions of preparing polar polymers without adding cationizing agents.

Appendix: Some Suppliers of micropipette tips
PolyLC, Inc. (Columbia, MD)
polylc@aol.com

Millipore Corp. (Bedford, MA)
http://www.millipore.com

Nest Group, Inc. (Southborough, MA)
http://www.nestgrp.com

Harvard Bioscience (Holliston, MA)
http://www.harvardbioscience.com

Princeton Separations (Adelphia, NJ)
http://www.prinsep.com

Proxeon (Odense, DK)
http://www.proxeon.com

Varian, Inc. (Palo Alto, CA)
http://www.varian.com

Michrom BioRescources, Inc. (Auborn, CA)
http://www.michrom.com

Glygen Corp. (Columbia, MD)
http://www.glygen.com

Geno Technology, Inc. (St.Louis, MO)
http://www.genotech@genotech.com

Pierce Chemical Co. (Rockford, IL)
http://www.piercenet.com

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[1] Michael Karas, D. Bachmann, Ute Bahr, Franz Hillenkamp, *International Journal of Mass Spectrometry and Ion Processes*, 1987, Vol 78, 53 – 68

[2] Koichi Tanaka, Hiroaki Waki, Yutaka Ido, Satoshi Akita, Yoshikazu Yoshida, Tamio Yoshida, *Rapid Communications in Mass Spectrometry*, 1988, Vol 2, 151 - 153

[3] Indral K. Perera, John Perkins, Stamatina Kantartzoglu, *Rapid Communications in Mass Spectrometry*, 1995, Vol 9, 180 - 187

4.6. Starting the preparation application

The preparation application is a stand-alone application that is started by clicking the following icon on the desktop:


Preparation.lnk

This application is designed to be used when the sample plate is prepared, and can be used independently from the acquisition and analyze applications. This independence allows the applicator to be installed on a computer that is placed in the laboratory close to the sample plate preparation working area.

4.7. The preparation application and its use

The preparation application serves two purposes: description of the sample plate with the samples on the plate and definition of the automation protocol for acquiring acquisitions without user interaction.

4.7.1. Sample plate description

The description of a sample plate is based on a physical plate that is identified by a sample plate name (the bar code). This name is the identification of the plate and will be shown in the first selection list (select sample plate) when loading a sample plate into the machine.

For this plate the geometrical information (the number of spots) is entered (by selecting from the list of possible plates), and the name of the actual measurement is given. Whenever a plate is cleaned for reuse the plate is ‘cleared’ and a new measurement for the plate is defined. The physical information stays unchanged for the plate. An old measurement will not be deleted by ‘clearing’ the plate and the acquisitions can always be found using the Data Analyzer application.

When the measurement is defined, each spot can be described by ‘Spot Description’, ‘Sample Name’ and ‘Matrix Name’. This information can be seen in the acquisition application when the plate is loaded into the machine. With this informations it is easier to find the right spot on the plate and to know what method to use for the acquisition.

4.7.2. Automation protocol

When using the macromizer™ in automated mode, the automation protocol which is the sequence of acquisitions and the associated methods is set up.

A list can be generated that shows the prepared acquisitions in sequential order, where each line represents an acquisition. The list can be seen in the acquisition application in the automation panel when the sample plate is loaded into the machine.

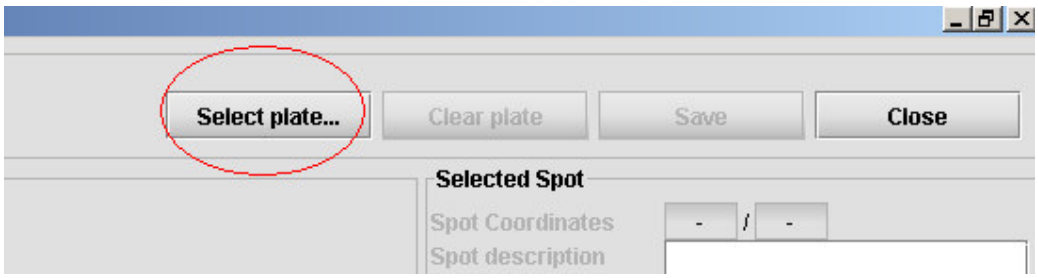
For each acquisition all information (name, path, description, spot position) and methods (acquisition profile, laser position sequence and calibration profile) are entered and saved with the plate and will determine how the acquisition is performed.

If the plate has already been used, and if new entries are needed for further acquisitions, it is possible to see the acquisitions already performed on the plate in the list. It is also possible to see how many laser shots have been made in each acquisitions. In this way it is possible to determine if a spot is burned out or if it can be used again.

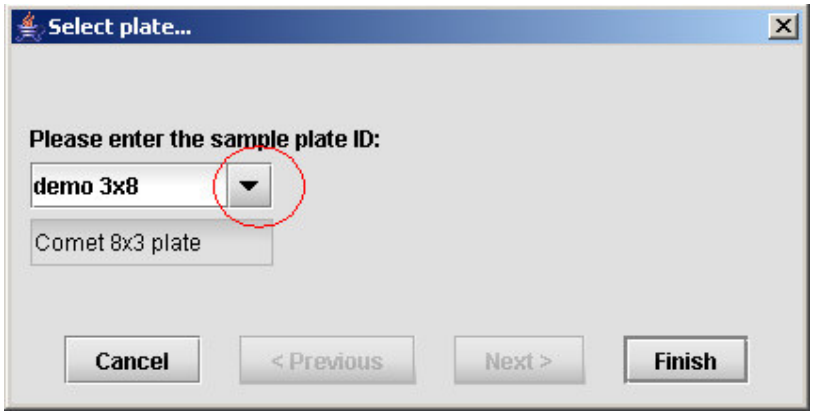
4.8. Selecting a sample plate

The first activity to be performed in the preparation application is to select an existing sample plate or to create a new plate.

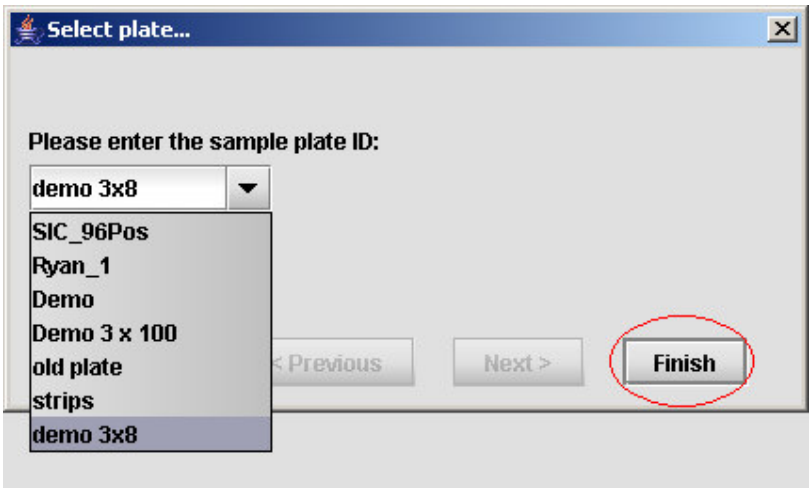
The selection of an existing sample plate is done by clicking the 'Select plate' button.



After clicking "Select Plate" the following popup will appear:

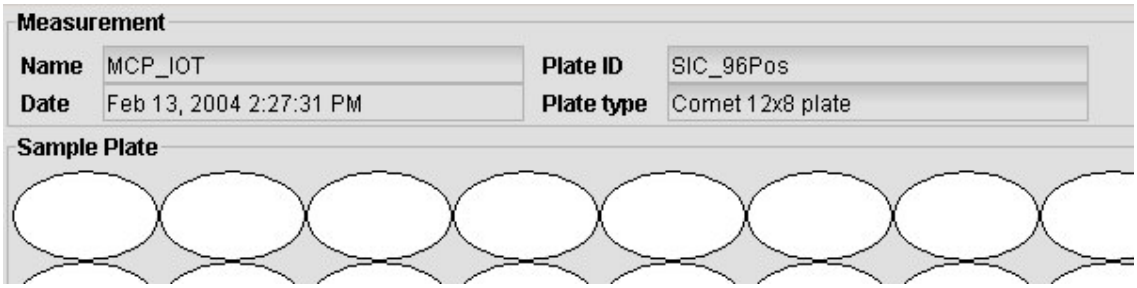


In this popup a list of already defined sample plates can be opened by clicking the arrow down to the right of the plate ID. The sample plate format is shown in the field under the plate ID.



After selecting a plate the plate information is loaded into the application by clicking the 'Finish' button.

After loading the plate information the plate data is shown in the upper area of the panel.



The field labeled 'Name' contains the name given to the physical plate (this is equivalent to the bar code for a plate). When a new physical plate is set up, this name is entered and cannot be changed.

The field labeled 'Pate ID' contains the identification of the current plate configuration (is equivalent to the measurement). This name is renewed when the plate is cleared (cleaned) for a new sample preparation.

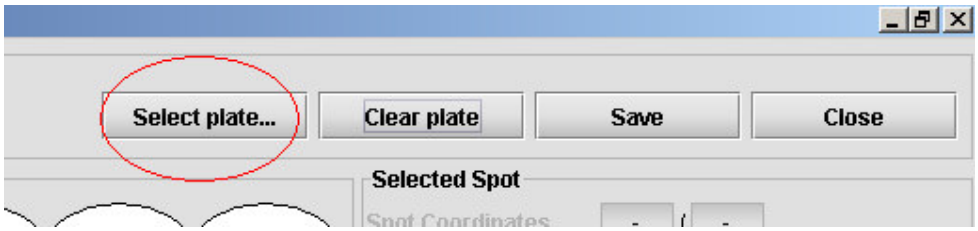
The field labeled 'Date' states the date and time when the plate configuration was created.

The field labeled 'Plate Type' shows the physical layout of the current plate (cannot be changed).

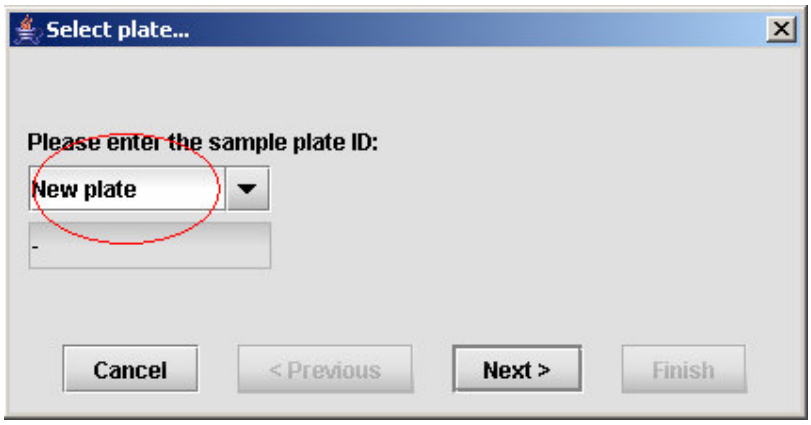
4.9. Creating a new sample plate

Before a sample plate can be used in the macromizer™ it needs to be defined in the database; otherwise it will not be selectable in the sample plate list when loading the plate into the machine.

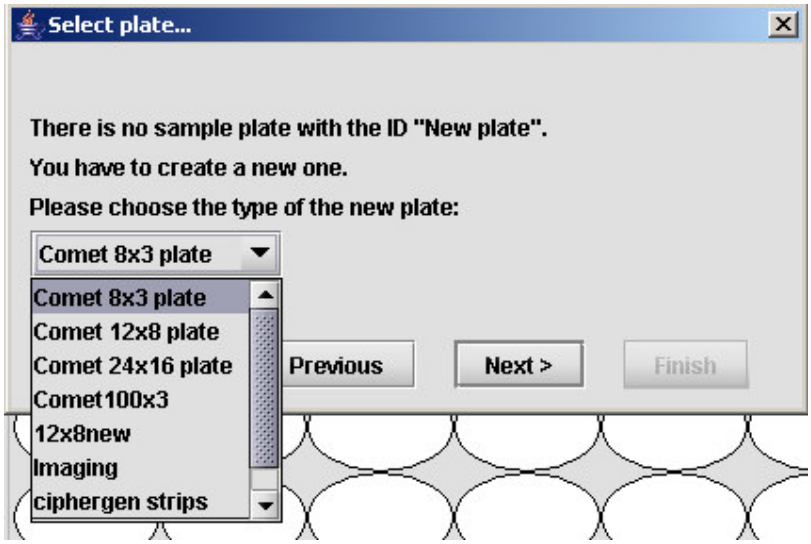
A new plate is defined by clicking 'Select plate' in the upper part of the panel.



The popup for selecting an existing plate is then shown and, instead of selecting a plate from the list, enter a new name in the ‘sample plate ID’ field.

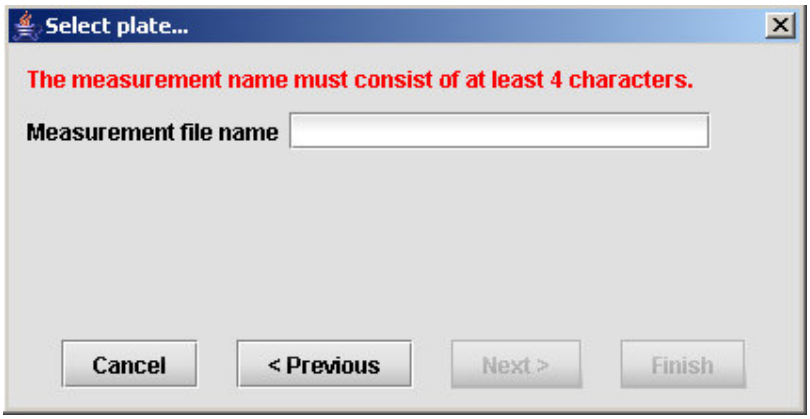


After entering a new name (that does not already exist) the button labeled ‘Next>’ is activated. By clicking ‘Next>’ the physical sample plate layout can be selected.



If the physical sample layout is not found in the list, a new layout can be created (see next section).

By clicking the ‘Next>’ button the popup for entering the measurement name is shown.



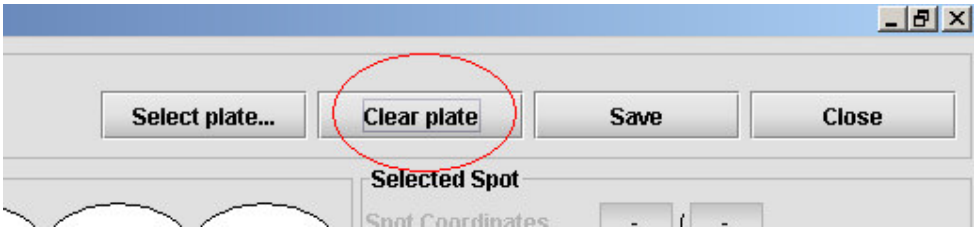
After entering at least 4 characters, the 'Finish' button is activated.

By clicking 'Finish' the sample plate is created and the sample plate information can now be entered.

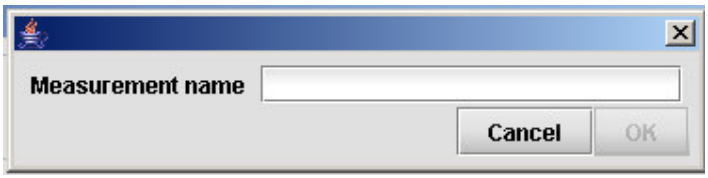
At any point in the sample plate creation process, the creation can be canceled by clicking the 'Cancel' button (the plate will then not be created), and any step can be redone by clicking the '<Previous' button.

4.10. Clearing a sample plate

When the sample pate is cleaned for a new sample preparation, a new name is given to the plate (the name seen in the data analyzer when loading measurements). This is done by clicking the 'Clear plate' button.



After clicking ‘Clear plate’ the following popup is shown:



Here the new measurement name is entered, and the measurements done on this plate will be saved under this measurement name.

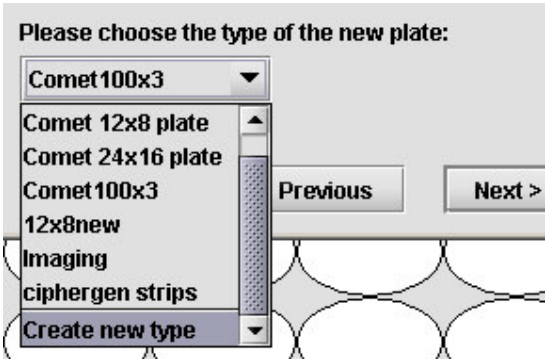
The old measurement is not deleted by clearing the plate. It is still in the database for selecting from the data analyzer. However it is not possible to perform further acquisitions for the old measurement.

By clicking ‘Cancel’ the plate is left unchanged and by clicking ‘OK‘ the old plate is cleared and the new plate is created.

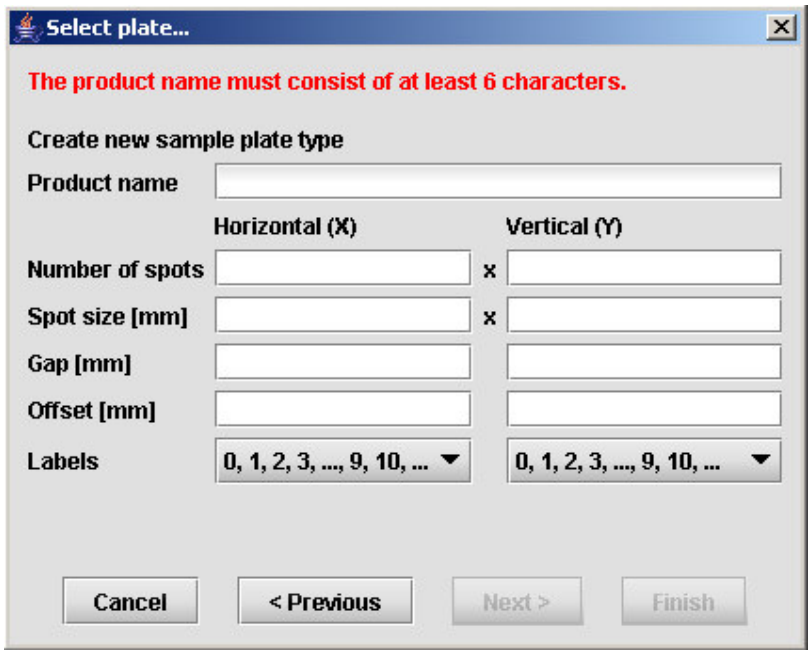
4.11. Creating a new sample plate type

When creating a new sample plate, the physical plate information must to be selected.

If the information for the new plate is not found in the drop down list, a new sample plate type can be created by selecting the menu item ‘Create new type’ in the plate type list. After the selection is made, click the button labeled ‘Next>’.



The name and the physical sample plate information is entered in the following popup.



The name of the plate type is entered in the field labeled ‘Product Name’.

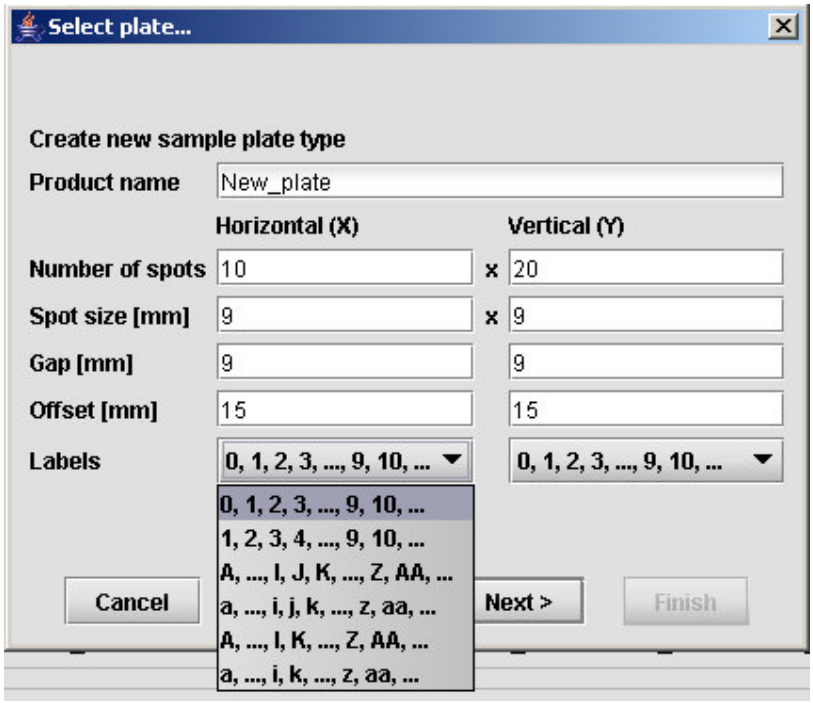
The number of horizontal and vertical spots is entered in the fields labeled ‘Number of spots’.

The horizontal and vertical spot size in millimeter is entered in the fields labeled ‘Spot size’.

The gap between the horizontal and vertical spots in millimeter is entered in the fields labeled ‘Gap’. Please note that if the spots have a size of 9 mm and are placed without gaps between them, the value for ‘Gap’ must be 9 mm.

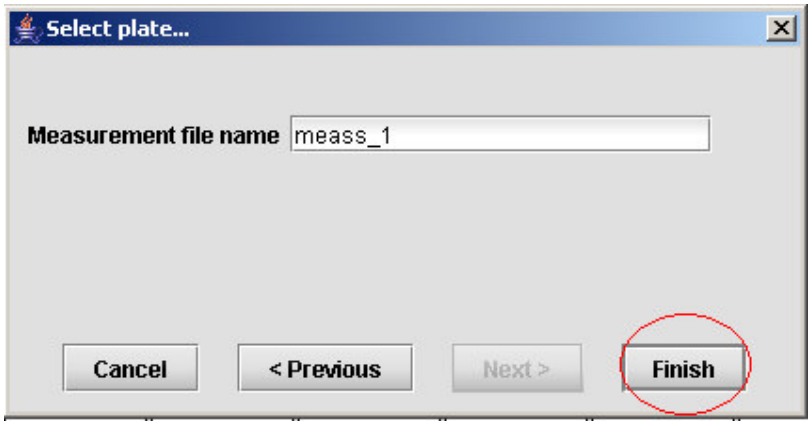
The horizontal and vertical starting point of the first spot (measured from the corner of the spot, not the center) on the plate (lower left corner) is entered in millimeters in the fields labeled ‘Offset’.

The labeling of the horizontal and vertical spots is selected from the two selection lists in the fields titled ‘Labels’.



Possible selections for labels are numbers (from 0 or from 1) , capital or lowercase letters, with or without the letter ‘j’.

After entering all information, the ‘Next>’ button is activated. By clicking ‘Next>’ the sample plate type is saved in the database (and cannot be changed or deleted) and the popup for entering the measurement name is shown.



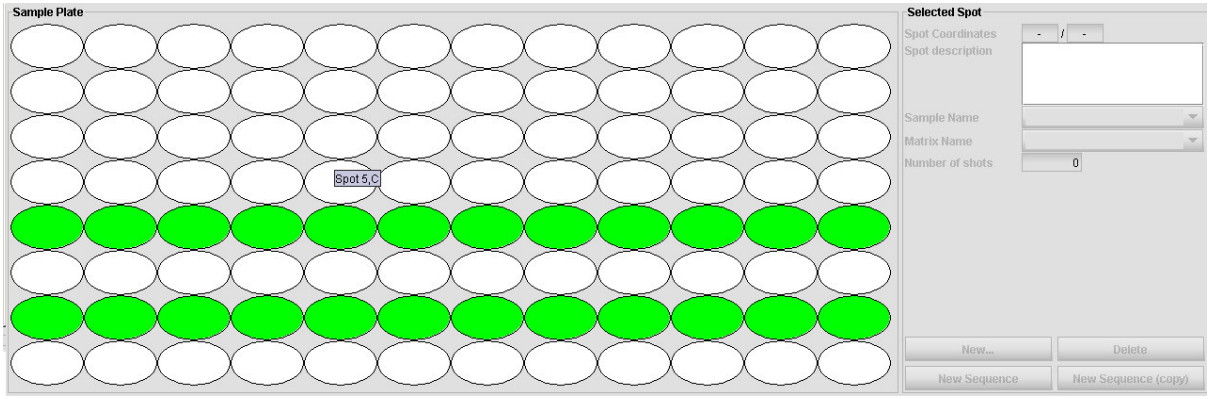
By clicking ‘Finish’ the sample plate is created and the sample plate information can now be entered.

4.12. Creating, copying and deleting spot information

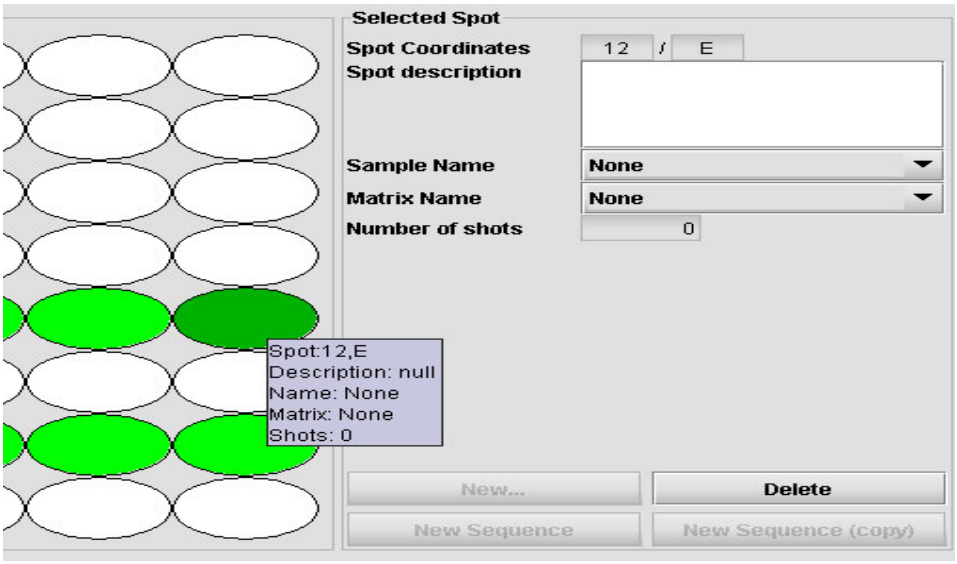
In the preparation application the individual spot information is defined. This information is used when performing an acquisition to identify what substance is on the spot, the matrix used, the number of laser shots performed on the spot, as well as other information.

The spot array is shown, in the central portion of the preparation panel, and each spot is identified by its spot coordinates. By placing the mouse curser on a spot, the spot position will appear.

A green spot color indicates that information for the spot exists, and a white spot color indicates no information for the spot exists



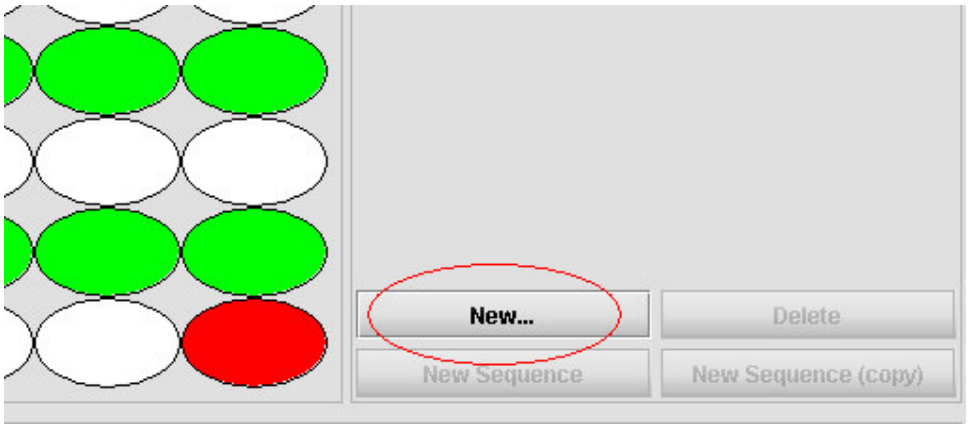
When information for a spot exists, the spot becomes a dark green color when selected. By placing the mouse cursor on the spot the spot coordinates and the other information for the spot is shown.



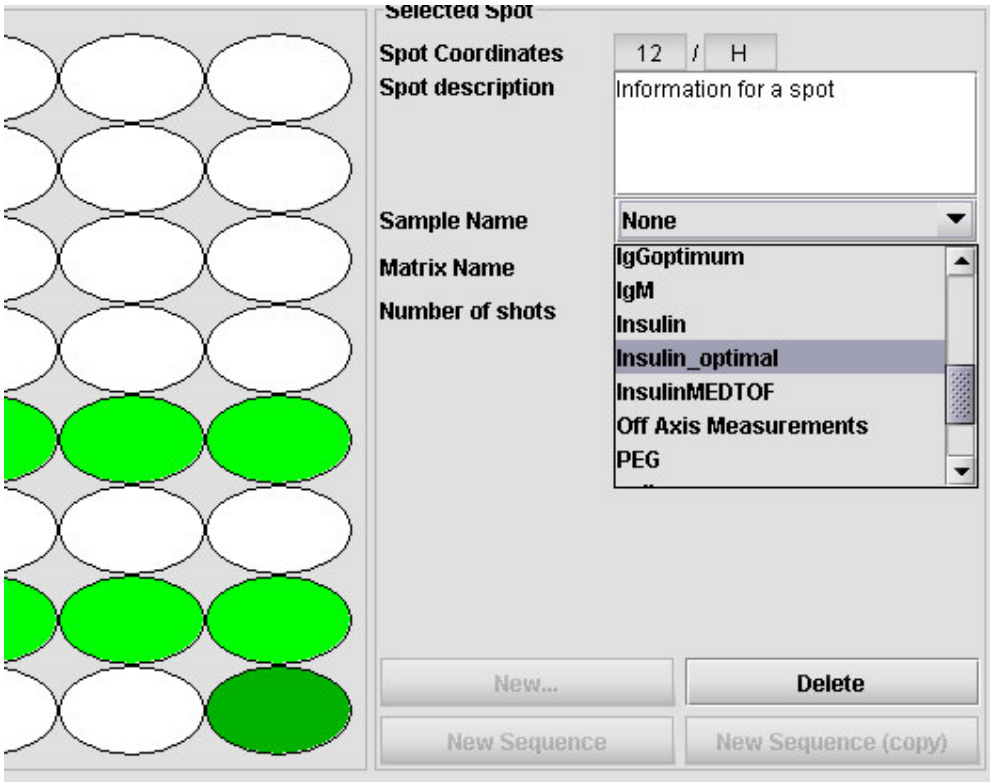
4.12.1. Creating new information for one spot

To create information for a spot that does not have any information, select the empty (white) spot by clicking on that spot in the spot array. If a spot selected that already have information, the existing information can be changed.

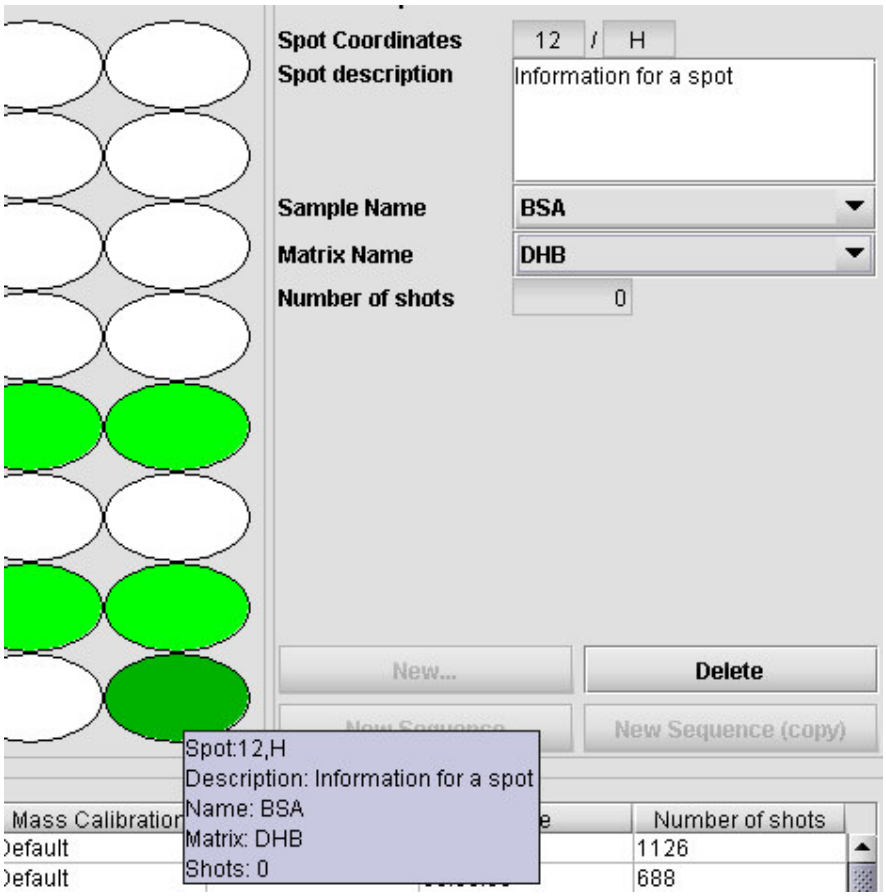
By clicking an empty spot, the spot color turns red. By clicking the 'New' button the spot information can be created.



It is possible to enter the spot description, select the sample name and matrix from the lists and see to the number of shots (if a used spot is selected). The procedure also applies when editing existing spot information.



After the spot information is entered of edited, it can be seen by clicking the spot or by placing the cursor on the spot.



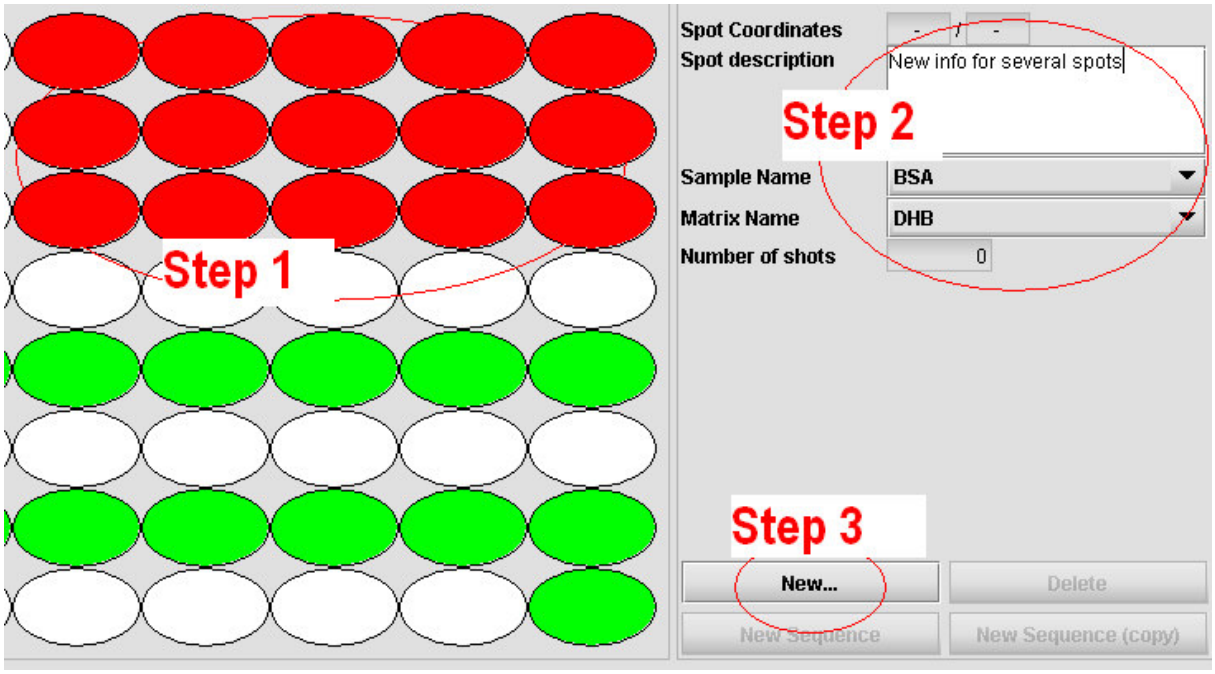
4.12.2. Creating spot information for multiple spots

When the spot information applies for a region of spots, this information can be entered for all spots in one operation.

First, select the upper left spot of the region with the left mouse button and then select the lower right spot of the region with the right mouse button (Step 1). All spots in the selected region are colored red.

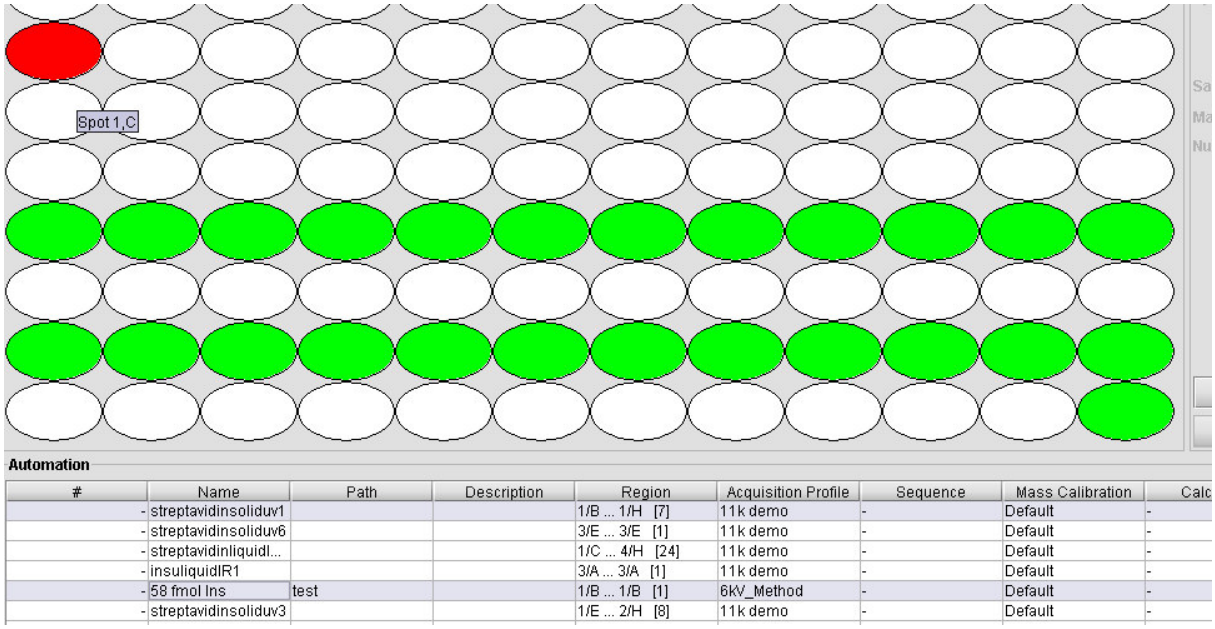
If all spots in the region were empty the ‘New’ button is activated and the spot information can be entered in the information fields (Step 2). If one of the spots already has spot information, the ‘Delete’ button is activated, and all spot information can be deleted for the region.

Finally, after entering all information for the region, the ‘New’ button is clicked and the information is generated for all selected spots.

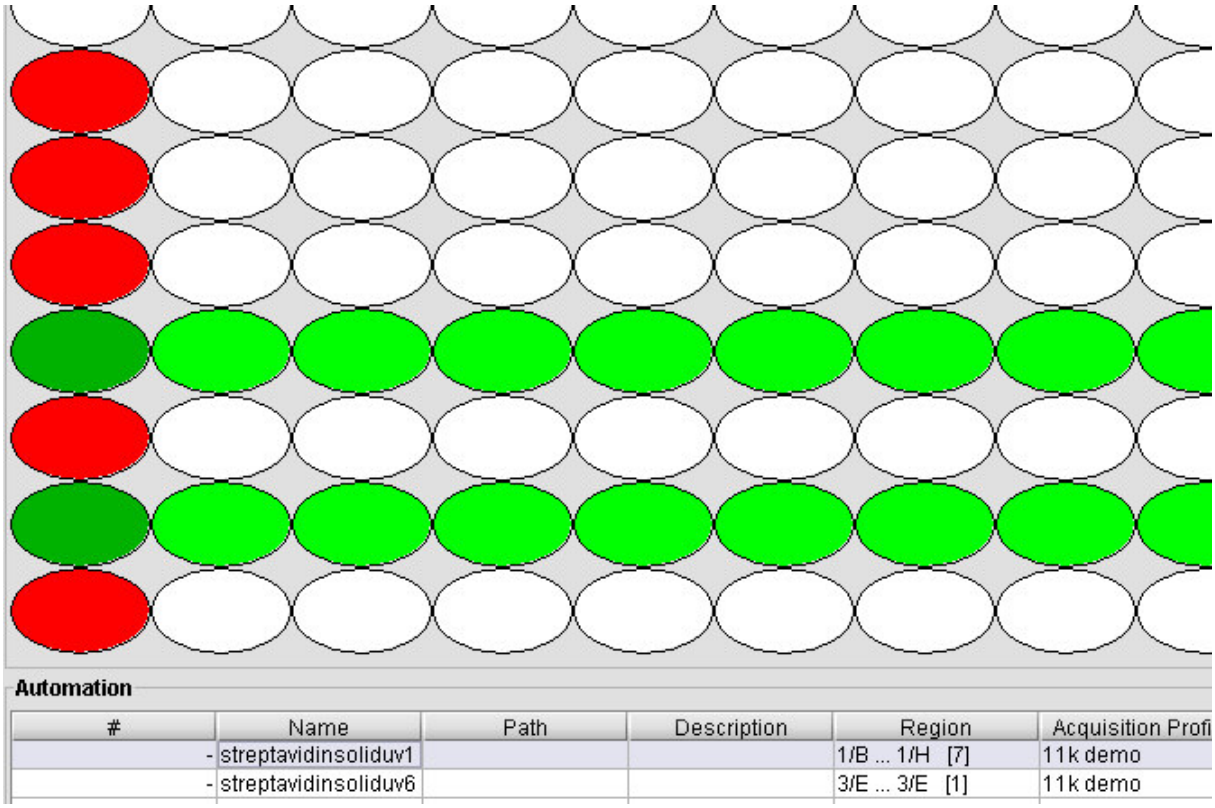


4.12.3. Viewing spot and acquisition information

When selecting a spot or a region of spots where an acquisition already exists for one or more of these spots, the related acquisition (submeasurement) will be highlighted in the list in the lower part of the preparation panel. This feature makes it easy to see which acquisitions have been performed on a specific spot.



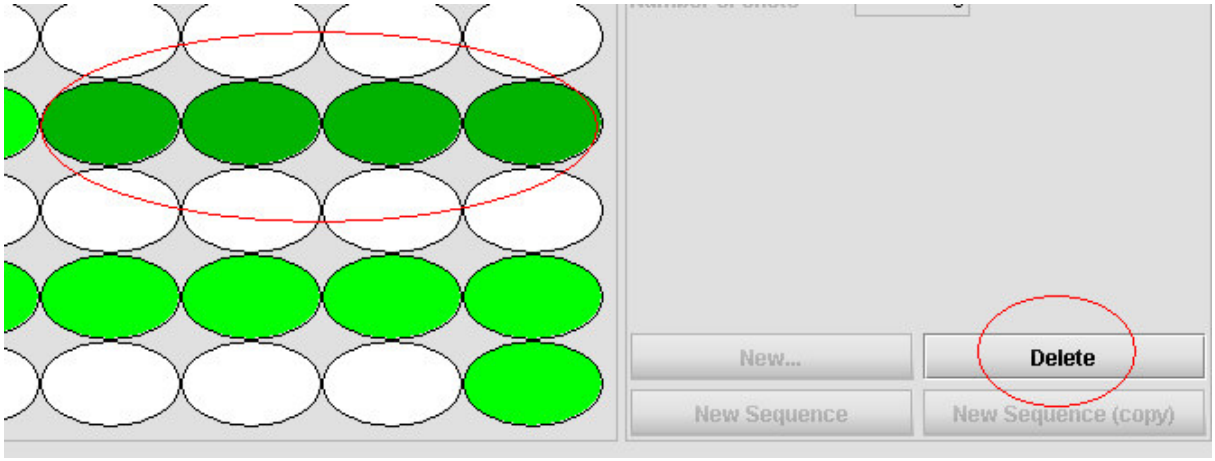
If a submeasurement is selected in the list, the related spot or region of spots is highlighted in the spot array. This feature makes it easy to see where on the sample plate the acquisition has been performed.



4.12.4. Deleting spot information

Deleting spot information is done by selecting a spot that contains information and clicking the 'Delete' button.

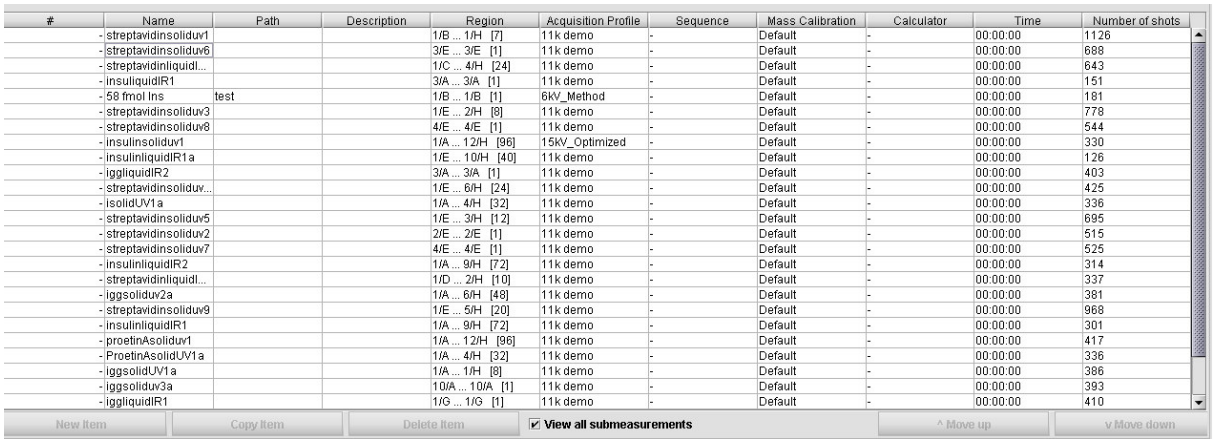
A region of spots can also be selected (selecting upper left corner of the region with left mouse button and lower right corner with right mouse button), and if one of the spots in the region contains spot information, the 'Delete' button will be activated. By clicking the 'Delete' button the spot information for all spots in the selected region is deleted.



4.13. Creating, copying and deleting automation sequences

When the macromizer™ is to be used in automatic batch mode, the sequence of acquisitions to be done is defined in this application.

The list in the lower part of the preparation panel shows by default the list of already performed acquisitions on the sample plate. It is seen here what name the submeasurements have, the methods used when acquiring the data, the spot position on the plate and the number of shots in each submeasurement.



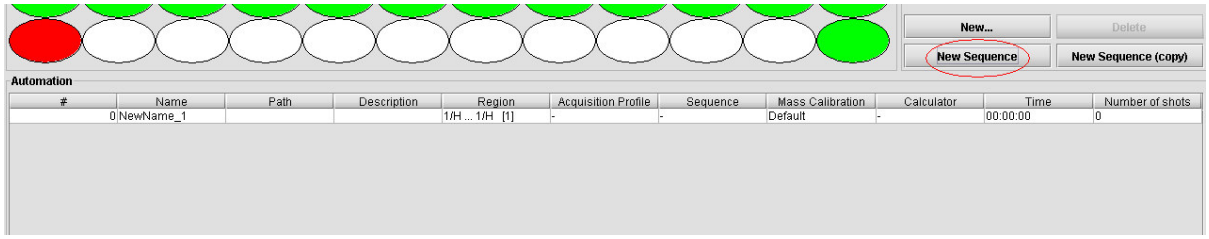
#	Name	Path	Description	Region	Acquisition Profile	Sequence	Mass Calibration	Calculator	Time	Number of shots
-	streptavidinsoliduv1			1/B ... 1/H [7]	11k demo	-	Default	-	00:00:00	1126
-	streptavidinsoliduv6			3/E ... 3/E [1]	11k demo	-	Default	-	00:00:00	688
-	streptavidinliquidI...			1/C ... 4/H [24]	11k demo	-	Default	-	00:00:00	643
-	insuliquidIR1			3/A ... 3/A [1]	11k demo	-	Default	-	00:00:00	151
-	58 fmol Ins	test		1/B ... 1/B [1]	6kV_Method	-	Default	-	00:00:00	181
-	streptavidinsoliduv3			1/E ... 2/H [8]	11k demo	-	Default	-	00:00:00	778
-	streptavidinsoliduv8			4/E ... 4/E [1]	11k demo	-	Default	-	00:00:00	544
-	insulinsoliduv1			1/A ... 12/H [96]	15kV_Optimized	-	Default	-	00:00:00	330
-	insulinliquidIR1a			1/E ... 10/H [40]	11k demo	-	Default	-	00:00:00	126
-	iggliquidIR2			3/A ... 3/A [1]	11k demo	-	Default	-	00:00:00	403
-	streptavidinsoliduv...			1/E ... 6/H [24]	11k demo	-	Default	-	00:00:00	425
-	isolidUV1a			1/A ... 4/H [32]	11k demo	-	Default	-	00:00:00	336
-	streptavidinsoliduv5			1/E ... 3/H [12]	11k demo	-	Default	-	00:00:00	695
-	streptavidinsoliduv2			2/E ... 2/E [1]	11k demo	-	Default	-	00:00:00	515
-	streptavidinsoliduv7			4/E ... 4/E [1]	11k demo	-	Default	-	00:00:00	525
-	insulinliquidIR2			1/A ... 9/H [72]	11k demo	-	Default	-	00:00:00	314
-	streptavidinliquidI...			1/D ... 2/H [10]	11k demo	-	Default	-	00:00:00	337
-	iggsoliduv2a			1/A ... 6/H [48]	11k demo	-	Default	-	00:00:00	381
-	streptavidinsoliduv9			1/E ... 5/H [20]	11k demo	-	Default	-	00:00:00	968
-	insulinliquidIR1			1/A ... 9/H [72]	11k demo	-	Default	-	00:00:00	301
-	proetinAsoliduv1			1/A ... 12/H [96]	11k demo	-	Default	-	00:00:00	417
-	ProetinAsolidUV1a			1/A ... 4/H [32]	11k demo	-	Default	-	00:00:00	336
-	iggsolidUV1a			1/A ... 1/H [8]	11k demo	-	Default	-	00:00:00	386
-	iggsoliduv3a			10/A ... 10/A [1]	11k demo	-	Default	-	00:00:00	393
-	iggliquidIR1			1/G ... 1/G [1]	11k demo	-	Default	-	00:00:00	410

New Item | Copy Item | Delete Item | View all submeasurements | ^ Move up | v Move down

By clicking the checkbox under the list, the list of already performed acquisitions is removed, and the definition of the automation sequence can start. If any submeasurements were prepared for automated acquisition and have not yet been run, they will be shown in the list.

4.13.1. Creating new automation sequence entries

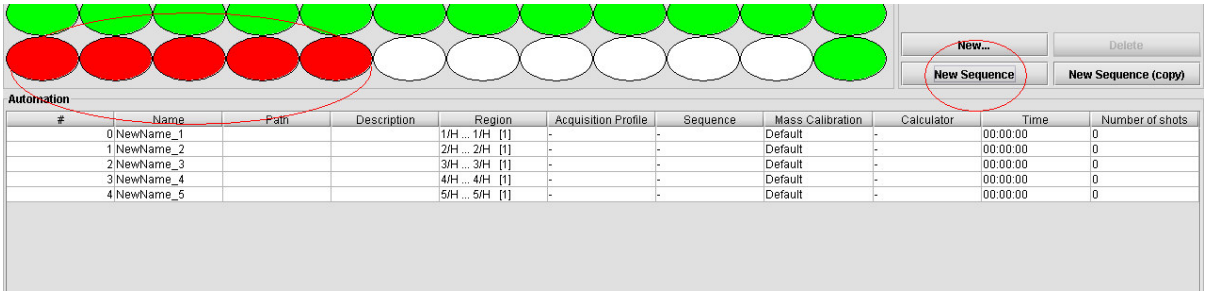
The creation of an automation sequence entry is done by selecting a spot and then clicking the 'New Sequence' button.



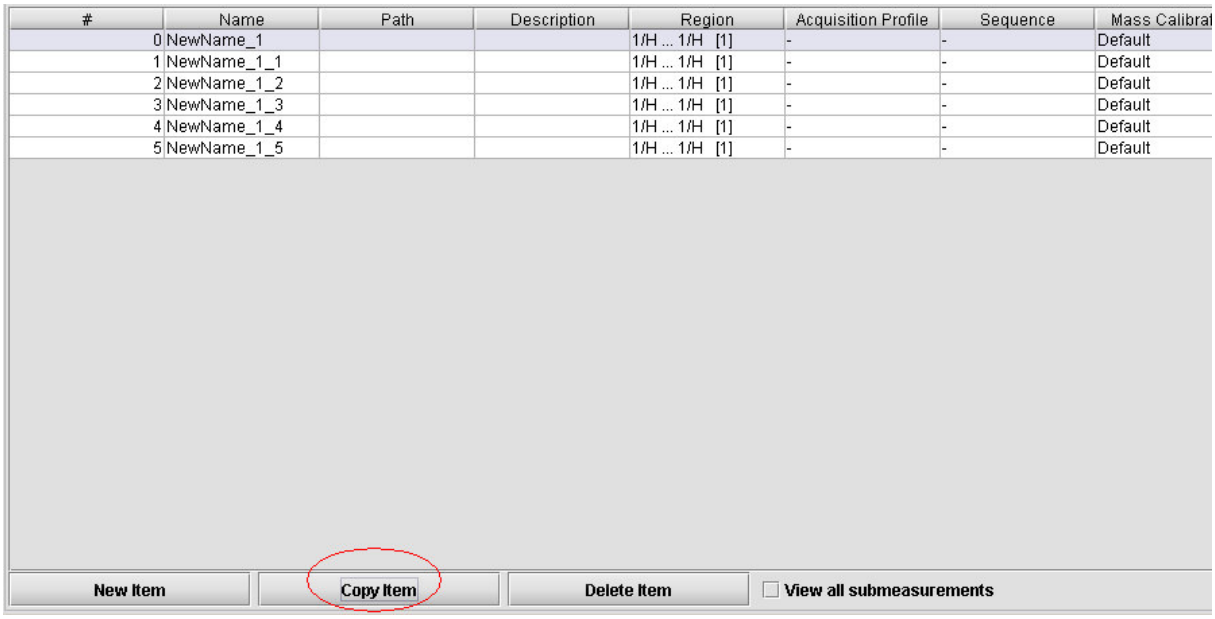
A new line in the list will then appear, where the spot region is set to the selected spot and a default name is given.

To generate a new sequence without selecting a spot, click the 'New Item' button under the list. This new sequence will contain no settings for the acquisition.

Additionally a region of spots can be selected and, by clicking ‘New Sequence’, one line for each spot in the region will be generated, with the spot region already set and with different default names.



By selecting one of the sequence lines and clicking the ‘Copy Item’ button a copy of the selected item is generated, adding an index to the default name.



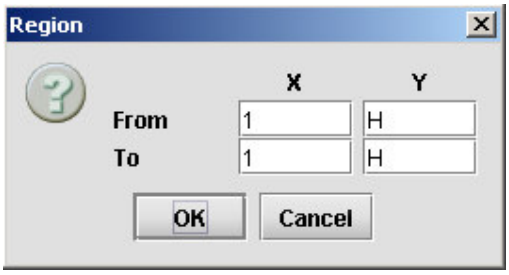
4.13.2. Customizing an automation sequence

By selecting one of the sequence lines, each item of the sequence can be set up. By double clicking the field labeled ‘Name’ in the selected line, a popup appears, where the name of the submeasurement can be entered or changed.

By double clicking the field labeled ‘Path’ a popup appears where the file path for the submeasurement can be entered or changed.

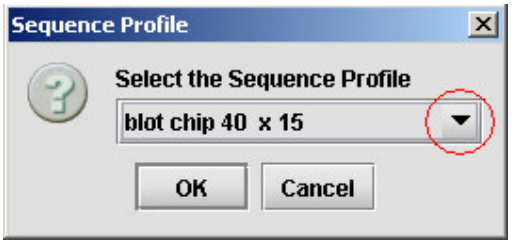
By double clicking the field labeled ‘Description’ a popup appear where the description for the submeasurement can be entered or changed.

By double clicking the field labeled ‘Region’ a popup appears where the spot or spot region for the submeasurement can be entered or changed.

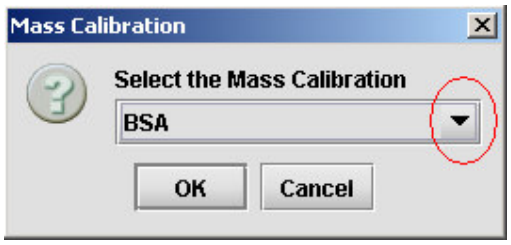


By double clicking the field labeled ‘Acquisition Profile’ a popup appears where the acquisition profile or method for the submeasurement can be selected. The methods for selection need to be prepared in the acquisition application beforehand.

By double clicking the field labeled ‘Sequence Profile’ a popup appears where the acquisition sequence profile for the submeasurement can be selected. The sequences for selection need to be prepared in the acquisition application beforehand. If no selection is made, the sequence from the acquisition method is used during the measurement.



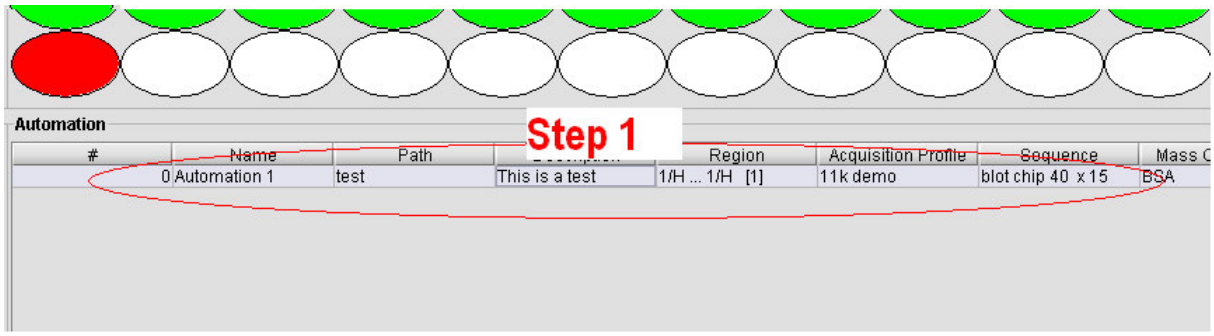
By double clicking the field labeled ‘Mass Calibration’ a popup appears where the mass calibration profile for the submeasurement can be selected. The mass calibration profiles for the selection need to be prepared in the data analyzer application beforehand. If no calibration profile is selected, the default calibration is used.



4.13.3. Copying an automation sequence

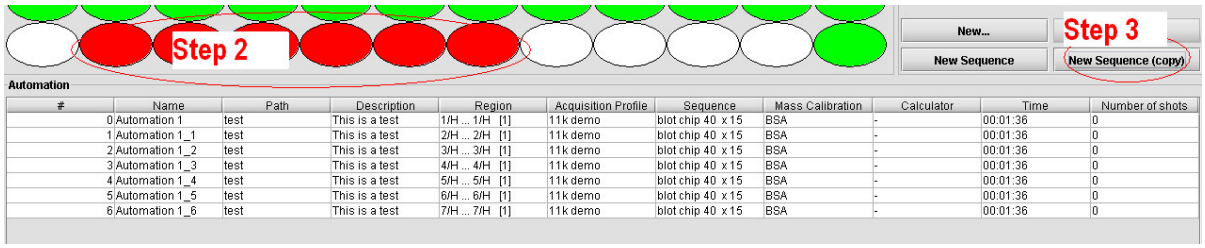
After an automation sequence is defined it can be copied to other spot positions that are to be run with the same acquisition parameters without having to do any manual changes to the sequence information.

First, select the automation sequence that is to be copied (Step 1).



Select the region of spots in the spot array by clicking left mouse button on upper left corner of the spot region, then click the right mouse button on the lower right corner of the region (Step 2).

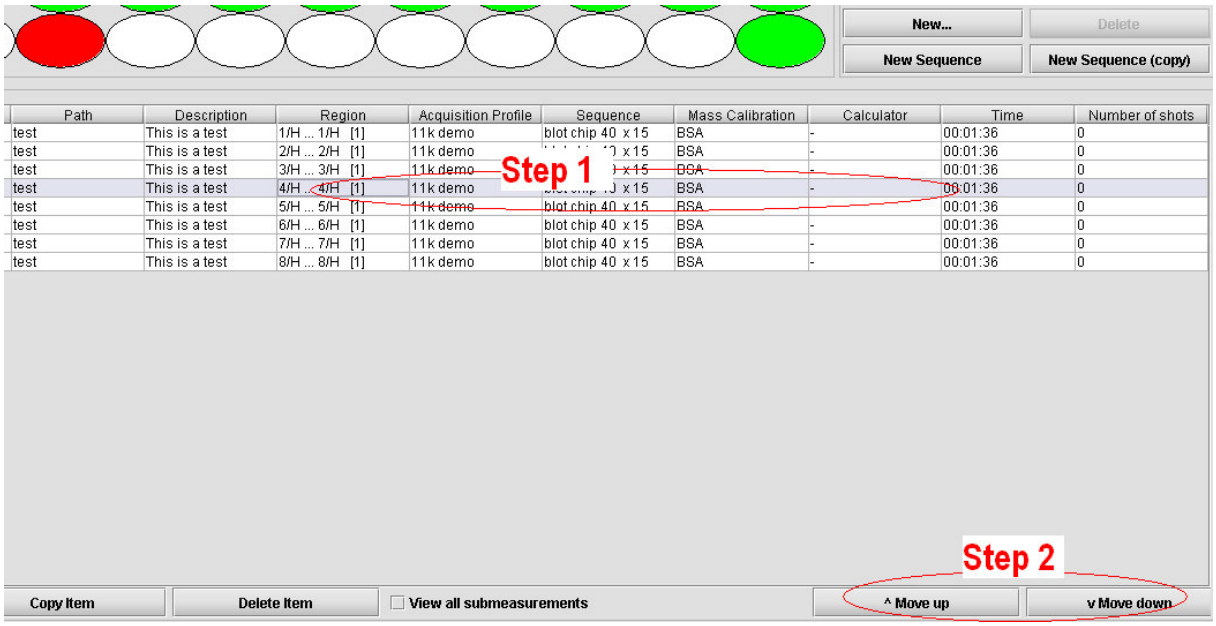
Finally, click the 'New Sequence (Copy)' button (Step 3) and a number of automation sequences will be generated, where the name is extended with an index and the spot region is adapted for the selected spots.



4.13.4. Changing the acquisition sequence order

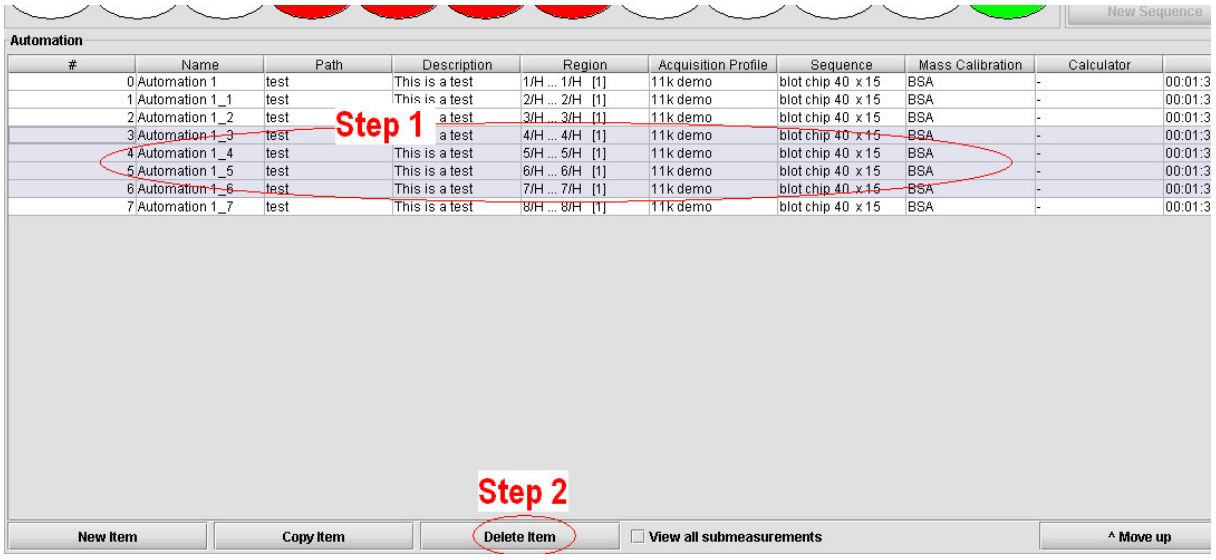
When an automation sequence is defined, the first field in the list will show the order in which each acquisition sequence is run. To change this order, select one or more of the sequences. The selected sequences will then be highlighted (Step 1). Using shift or ctrl and a mouse click

Press 'Move up' or 'Move down' to reposition the sequence(s) earlier or later in the automated sequence (Step 2).



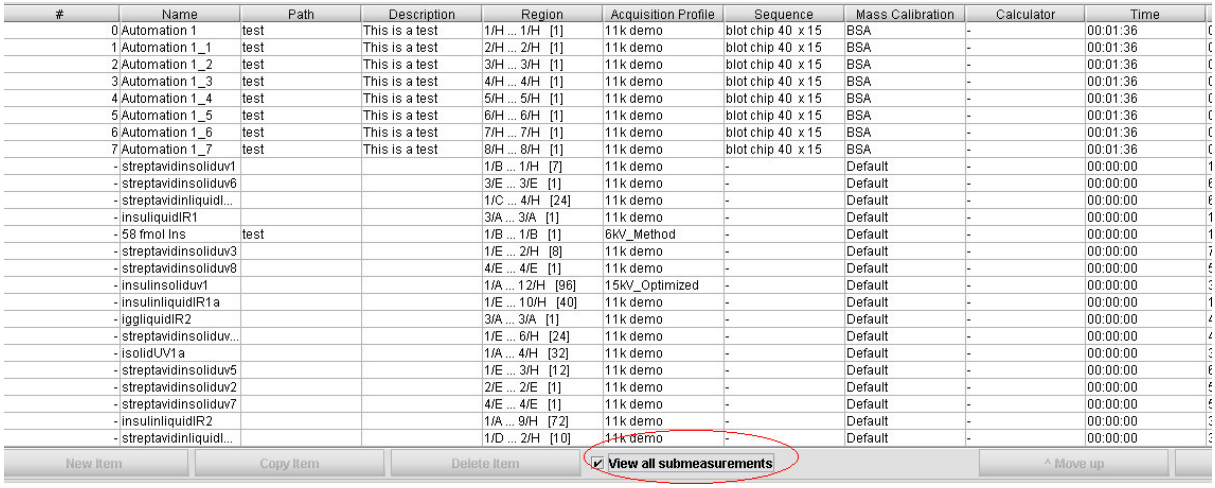
4.13.5. Deleting an automation sequence

To delete a sequence in the list, select one or more automation sequences (Step 1) and click the 'Delete Item' button (Step 2). The selected sequences will then be removed. Please note, there is no 'undo' function.



4.13.6. Complete sample plate overview

After defining the required automation sequences, the complete list of planned acquisitions and already performed acquisitions can be seen by clicking the checkbox ‘View all submeasurements’.

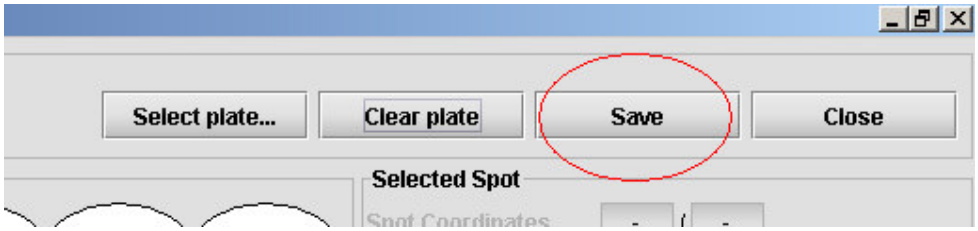


#	Name	Path	Description	Region	Acquisition Profile	Sequence	Mass Calibration	Calculator	Time
0	Automation 1	test	This is a test	1/H ... 1/H [1]	11k demo	blot chip 40 x 15	BSA	-	00:01:36
1	Automation 1_1	test	This is a test	2/H ... 2/H [1]	11k demo	blot chip 40 x 15	BSA	-	00:01:36
2	Automation 1_2	test	This is a test	3/H ... 3/H [1]	11k demo	blot chip 40 x 15	BSA	-	00:01:36
3	Automation 1_3	test	This is a test	4/H ... 4/H [1]	11k demo	blot chip 40 x 15	BSA	-	00:01:36
4	Automation 1_4	test	This is a test	5/H ... 5/H [1]	11k demo	blot chip 40 x 15	BSA	-	00:01:36
5	Automation 1_5	test	This is a test	6/H ... 6/H [1]	11k demo	blot chip 40 x 15	BSA	-	00:01:36
6	Automation 1_6	test	This is a test	7/H ... 7/H [1]	11k demo	blot chip 40 x 15	BSA	-	00:01:36
7	Automation 1_7	test	This is a test	8/H ... 8/H [1]	11k demo	blot chip 40 x 15	BSA	-	00:01:36
-	streptavidinsoliduv1			1/B ... 1/H [7]	11k demo	-	Default	-	00:00:00
-	streptavidinsoliduv6			3/E ... 3/E [1]	11k demo	-	Default	-	00:00:00
-	streptavidinliquidI...			1/C ... 4/H [24]	11k demo	-	Default	-	00:00:00
-	insuliquidIR1			3/A ... 3/A [1]	11k demo	-	Default	-	00:00:00
-	58 fmol Ins	test		1/B ... 1/B [1]	6kV_Method	-	Default	-	00:00:00
-	streptavidinsoliduv3			1/E ... 2/H [8]	11k demo	-	Default	-	00:00:00
-	streptavidinsoliduv8			4/E ... 4/E [1]	11k demo	-	Default	-	00:00:00
-	insulinsoliduv1			1/A ... 12/H [96]	15kV_Optimized	-	Default	-	00:00:00
-	insulinliquidIR1a			1/E ... 10/H [40]	11k demo	-	Default	-	00:00:00
-	iggliquidIR2			3/A ... 3/A [1]	11k demo	-	Default	-	00:00:00
-	streptavidinsoliduv...			1/E ... 6/H [24]	11k demo	-	Default	-	00:00:00
-	isolidUV1a			1/A ... 4/H [32]	11k demo	-	Default	-	00:00:00
-	streptavidinsoliduv5			1/E ... 3/H [12]	11k demo	-	Default	-	00:00:00
-	streptavidinsoliduv2			2/E ... 2/E [1]	11k demo	-	Default	-	00:00:00
-	streptavidinsoliduv7			4/E ... 4/E [1]	11k demo	-	Default	-	00:00:00
-	insulinliquidIR2			1/A ... 9/H [72]	11k demo	-	Default	-	00:00:00
-	streptavidinliquidI...			1/D ... 2/H [10]	11k demo	-	Default	-	00:00:00

New Item | Copy Item | Delete Item | View all submeasurements | ^ Move up

4.14. Saving sample plate information and automation sequences

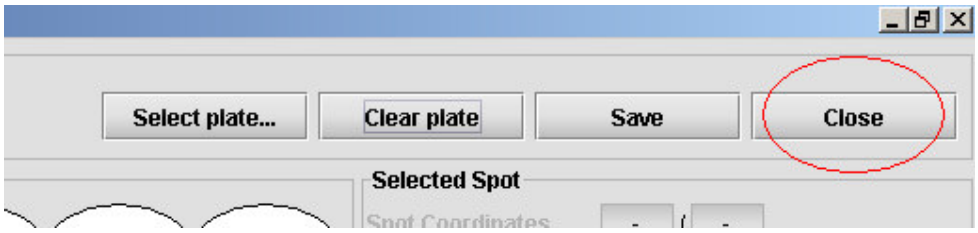
To save the sample plate information and the automated sequences, simply click the ‘Save’ button in the upper part of the panel.



The sample plate data is then saved in the database.

4.15. Closing preparation

The preparation application is closed by clicking the ‘Close’ button, NOT by clicking the ‘X’ in the upper right corner.



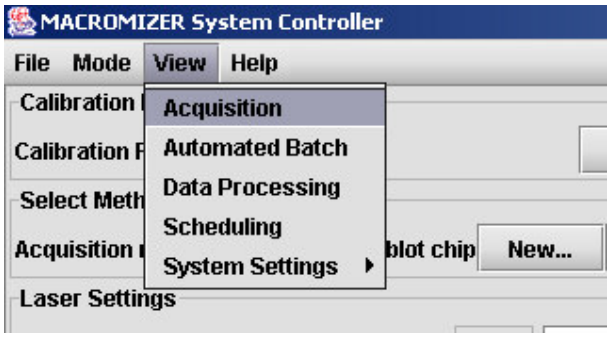
If the sample plate setup has been changed and has not been saved, a popup will warn the user. The popup will give the option to save or discard the changes or to cancel the closing.

5. Using and creating acquisition methods

5.1. Selecting the acquisition panel

The system controller application can be configured individually by the user. It is possible to select the initial window after a start up of the system and to enable password access restriction for the different menus.

If the acquisition panel is not visible, this panel can be selected through the pull down menu at the top left on the system control window. Select 'View' and in the pull down 'Acquisition'.



If a password for this panel is enabled, enter the correct password in the popup dialog.

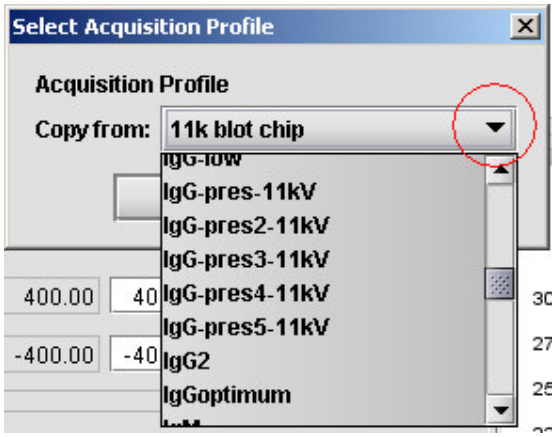
If the acquisition panel is the startup panel, and if the password is enabled, the password dialog will be shown before the application panel.

5.2. Selecting a predefined measurement method

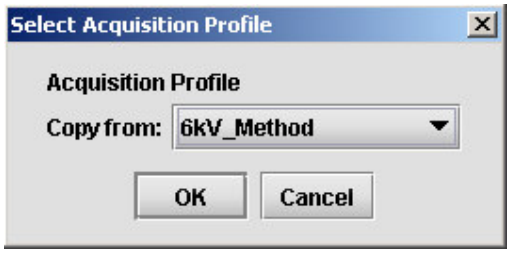
To simplify the setup of the macromizer™, acquisition methods can be stored in the database, allowing them to be selected at any time for a specific setup of the machine.

These predefined acquisition methods can be selected by clicking the 'New' button in the 'Select Method' area.

By clicking 'New', the ‘Select Acquisition Profile’ and the complete list of all stored methods can be found in the pull down menu.



Selecting one of the methods closes the list, showing the selected method in the field.

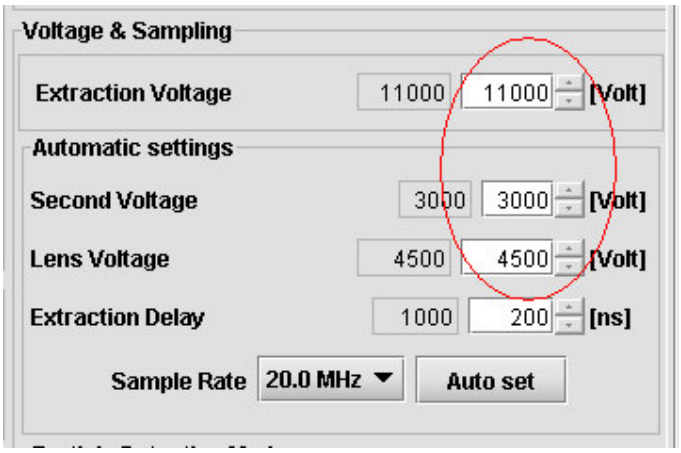


By accepting the related method, all the parameters are changed to the stored values. Canceling the operation leaves the macromizer™ settings unchanged.

5.3. Setting up a measurement method

5.3.1. Extraction voltage settings

The extraction voltage, the second voltage and the lens voltage can be entered (in Volts) in the area titled 'Voltage & Sampling'.



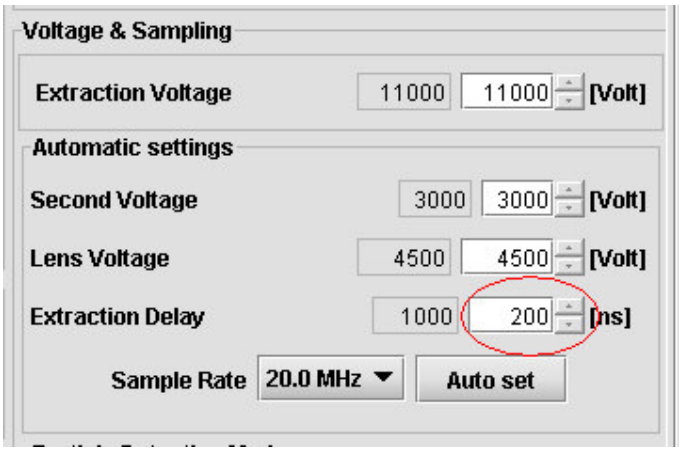
Each voltage can be set separately in the appropriate box, and the software automatically prevent the entered values from exceeding its maximum settings. The maximal voltage settings are summarized in the table below. As it can be seen, it is possible to use negative voltages for the acquisition in order to record negatively charged ions. It is important to notice that for this method the second voltage stays positive as it represents only a difference of potential and not the absolute voltage.

On the left of the entered value, the display shows the measured voltage which is applied. Any changes to the voltage takes several seconds before reaching the target value and there can be a slight difference between the target and the measured voltage.

Voltage	Minimum value	Maximum value
Extraction Voltage	-20kV	+20kV
Second Voltage	0	+3kV
Lens Voltage	-15kV	+15kV

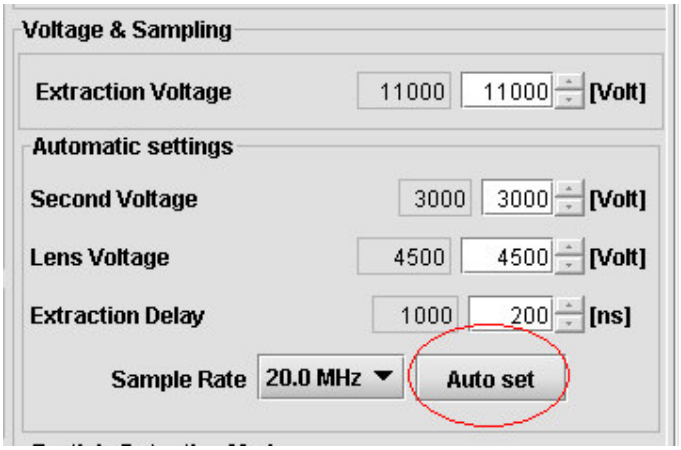
5.3.2. Delayed extraction setting

The delayed extraction time can also be set in the 'Voltage & Sampling' panel. The value is used to optimise the resolution and depends on the extraction voltage, the second voltage and the mass of the molecule. To obtain optimal spectra, the delay time must increase with the increasing size of the molecule. The range of the delay time is from 200 ns up to 10,000 ns.



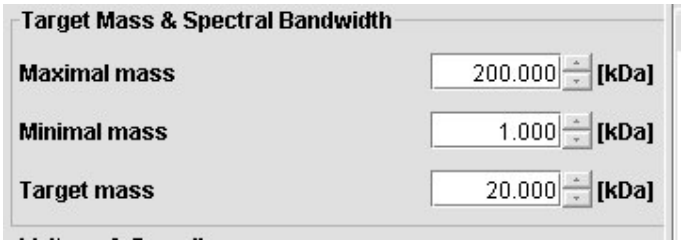
5.3.3. Auto setting

For a given extraction voltage, the 'Auto set' button changes the residual parameters automatically to the optimal factory settings. The extraction delay time is determined to obtain best results around the target mass (see next paragraph).



5.3.4. Min, max and target mass

The mass range for the acquisition can be defined in the area 'Target Mass & Spectral Bandwidth'.

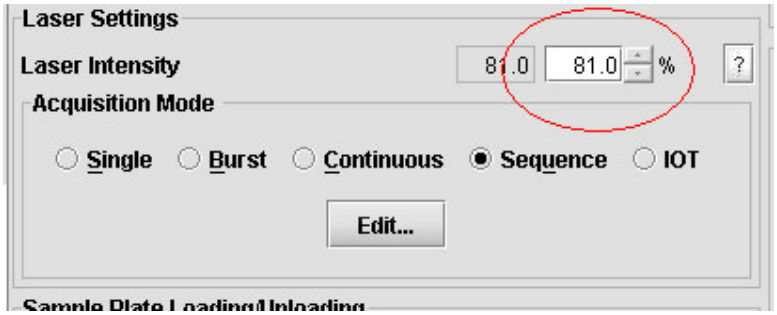


The minimum and maximum mass, entered in kiloDalton, define the mass range of the acquisition. The two plots are scaled to this range.

The target mass is used to obtain best mass accuracy around its value when working with the default calibration (please refer to the user's manual for further explanation).

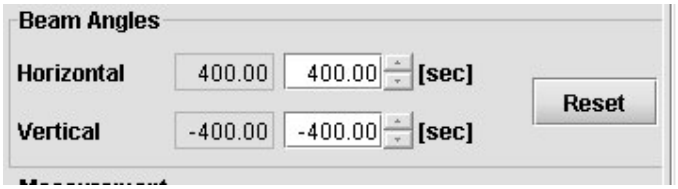
5.3.5. Laser intensity setting

The laser intensity is set in the area labeled 'Laser Settings' (in percent of full laser power).



5.3.6. Ion optics parameter setting

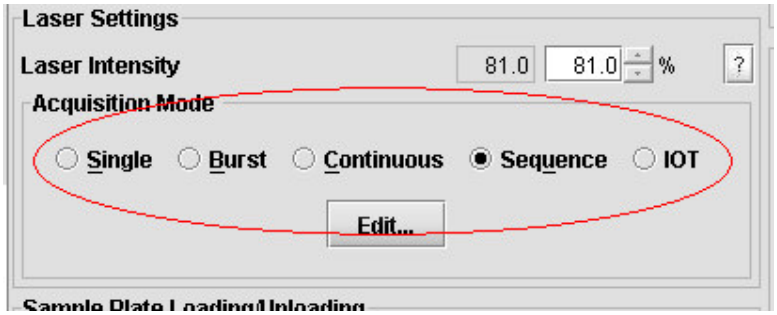
The design of the macromizer™ ion optic offers the possibility to mechanically rotate the ion extractor around its axes, both horizontally and vertically. This rotation, which allows the adjustment of the ion beam angles, is essential to the direction of the ion beam on the detector.



For adjustment of the ion optics, please refer to IOT procedure in *chapter 5.6 Ion optics test, page 67.*

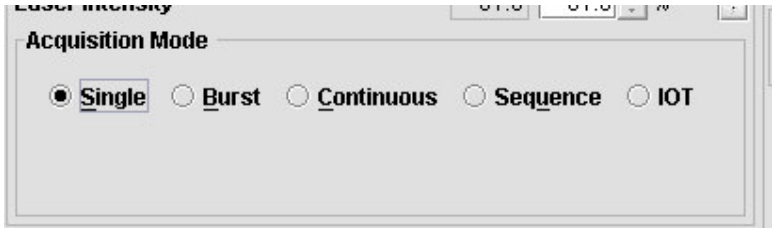
5.3.7. Acquisition mode setting

Five possible settings are available for acquisition. When selected, each type of acquisition shows its own papameters, which are explained in the following paragraphs.



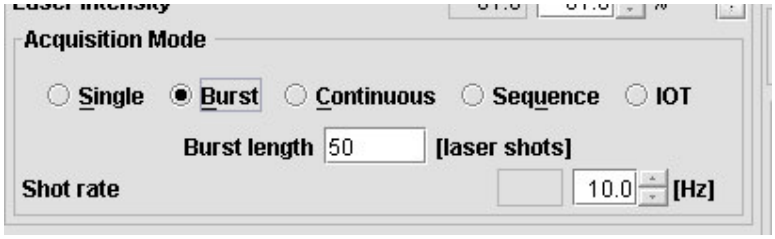
5.3.7.1. Single shot mode

In the single shot mode, one laser shot is generated at the selected spot position when the acquisition is started.



5.3.7.2. Burst mode

When selecting 'Burst' in the 'Acquisition Mode' area, two new fields appear: 'Burst length' and 'Shot rate'.



The burst length value determines the number of laser shots that occur from the first shot of the acquisition to the time the acquisition automatically ends. In the 'Measurement' area, the bar will show, in percent, how far the acquisition has progressed. All laser shots will be generated on the selected spot position. It is possible to change the spot position during the acquisition.

The shot rate value determines at which maximum frequency the laser should be fired. The field to the left of the entry field shows the actual shot frequency (this number can be less than the maximum depending on the mass range and the number of events).

5.3.7.3. Continuous mode

Continuous mode is selected by clicking 'Continuous' in the 'Acquisition Mode' area. One field appears where the laser shot rate can be entered.

In this mode the acquisition will continue with the maximum shot rate entered at the selected spot position until it is terminated manually by clicking the 'Stop' button in the 'Measurement' area. As in the Burst mode, it's possible to change the spot position during the acquisition

5.3.7.4. Sequence mode

The sequence mode is used to create a pattern for the laser shots to follow on the sample plate during acquisition (this pattern can be selected from the database and new sequences can be saved in the database).

The 'Edit' button will open a dialog window where the sequence can be selected, defined and saved. Refer to 'Setting up a sequence' chapter.

5.3.7.5. Ion Optics Test mode

Ion Optics Test mode is selected by clicking 'IOT' in the 'Acquisition Mode' area and is used to optimize the beam angles. This mode is not used for normal acquisitions.

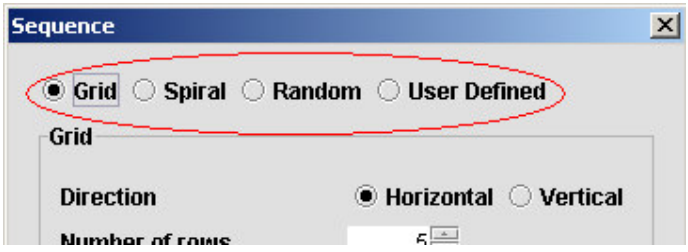
The 'Edit' button will open a dialog window where the settings for performing an ion optics test can be entered. The 'Chart' button will show the graphical output generated from a completed ion optics test.

5.4. Setting up a sequence

When a number of spots are to be analyzed with the same movement pattern of the laser, if the automated batch is being used or if a specific pattern is desired for a spot then, the acquisition mode is selected in the acquisition panel.

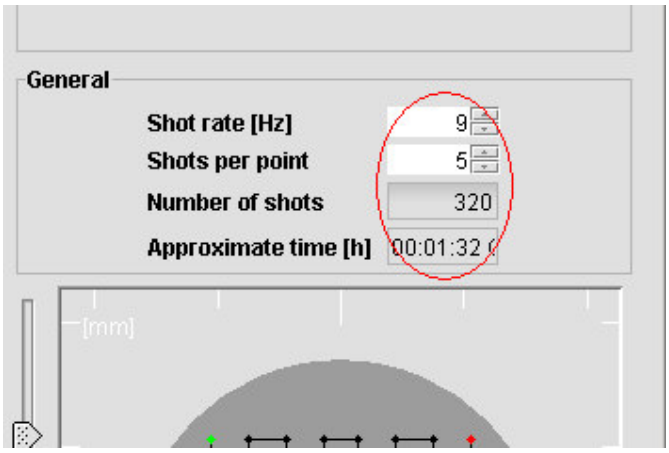
By selecting the 'Sequence' option it is than possible to select the sequence editor for defining a new acquisition sequence (laser movement pattern). The sequence can either be saved in the database for later use, saved for use in the preparation application when preparing an automated batch, or used directly in an acquisition.

After clicking the 'Edit' button, the sequence editor popup will appear. In the upper part of the popup it is possible to select from four different modes of generating sequences: Grid, Spiral, Random or User Defined.



In the ‘General’ selection, located in the central part of this popup, the shot rate and shots per point are entered.

From this information the number of shots in the sequence are calculated and the approximate acquisition time is estimated.

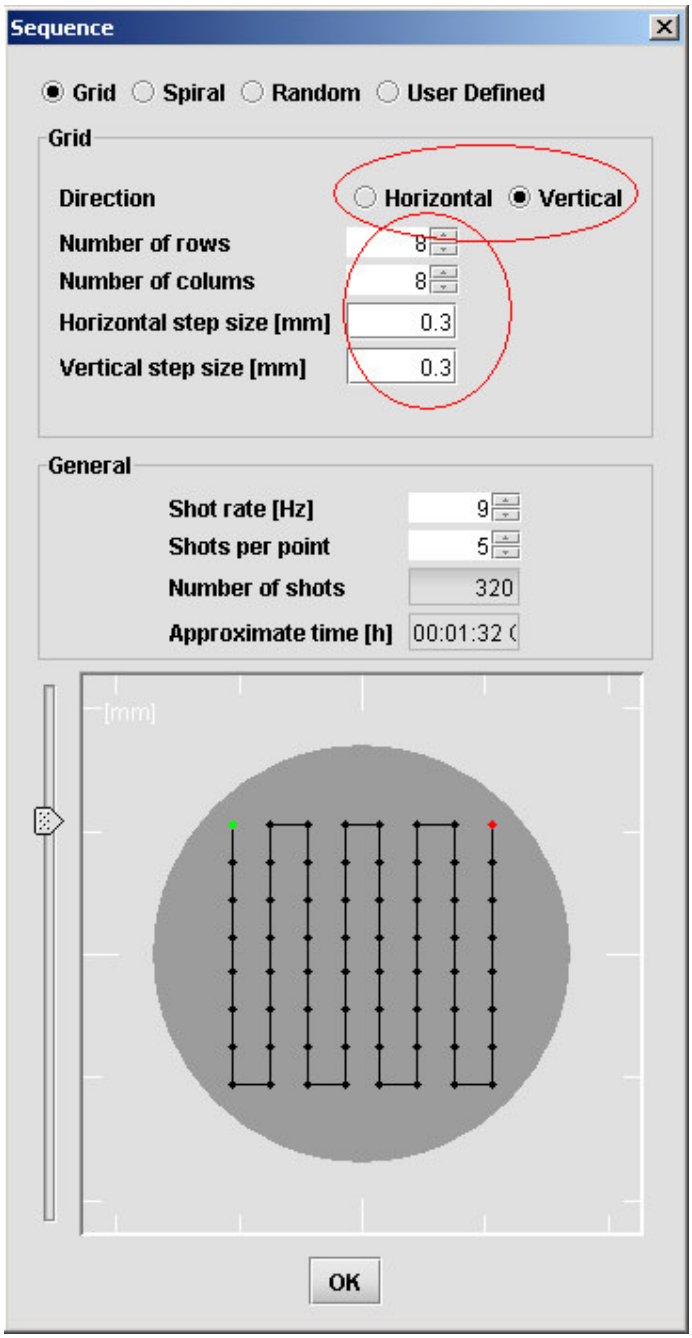


5.4.1. Grid sequence

When selecting the ‘Grid’ sequence option it is possible to generate sequences that will raster the spot in either a horizontal or vertical direction. The number of rows and columns can be set and the horizontal and vertical step size can be entered. From this information the grid sequence is generated and shown in the graphical sequence display located in the lower part of the sequence editor.

The green point in the graphical sequence display indicates the starting point of the sequence and the red point indicates the ending point.

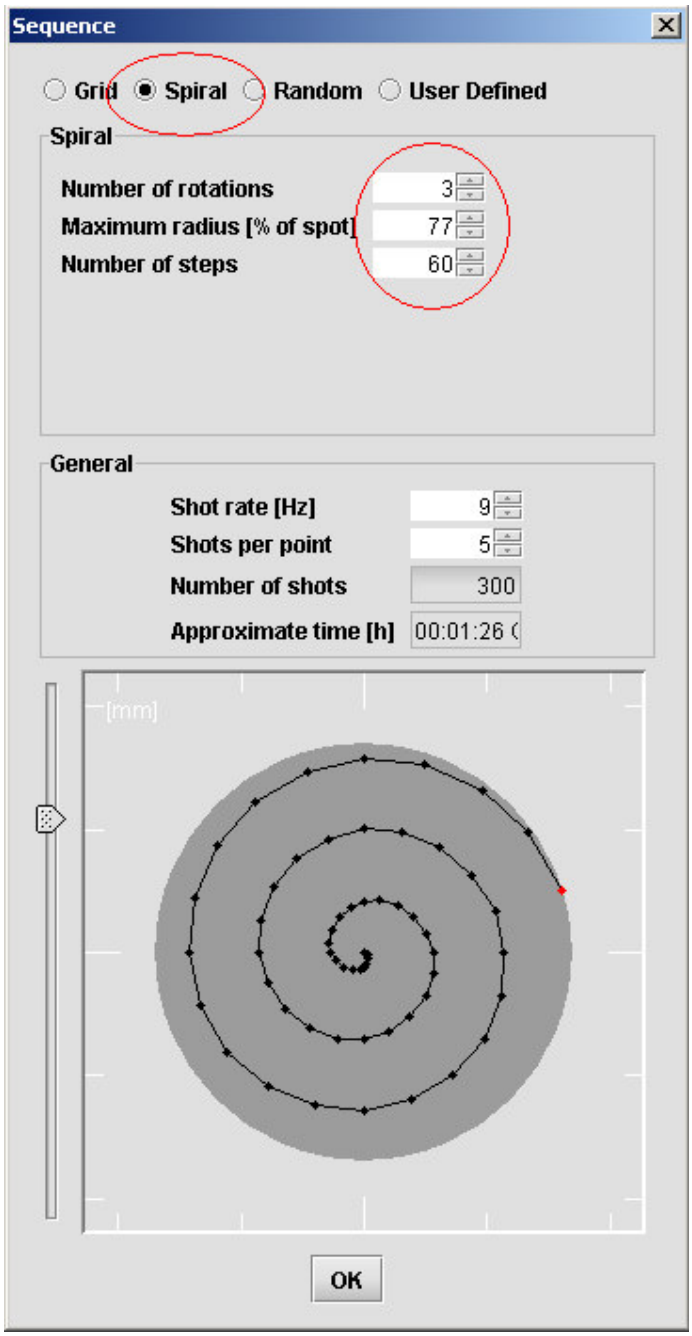
The sliding bar to the left of the graphical sequence is used to scale the ‘spot’ shown as the dark gray circle, which indicates how the sequence will fit the sample spot.



5.4.2. Spiral sequence

When selecting the 'Spiral' sequence option it is possible to generate sequences that will analyze the spot using a spiral movement which starts at the center and ends at the edge of the spot. The number of rotations to be performed, the spot size (entered as a percent value of the spot size) and the total number of steps can be entered.

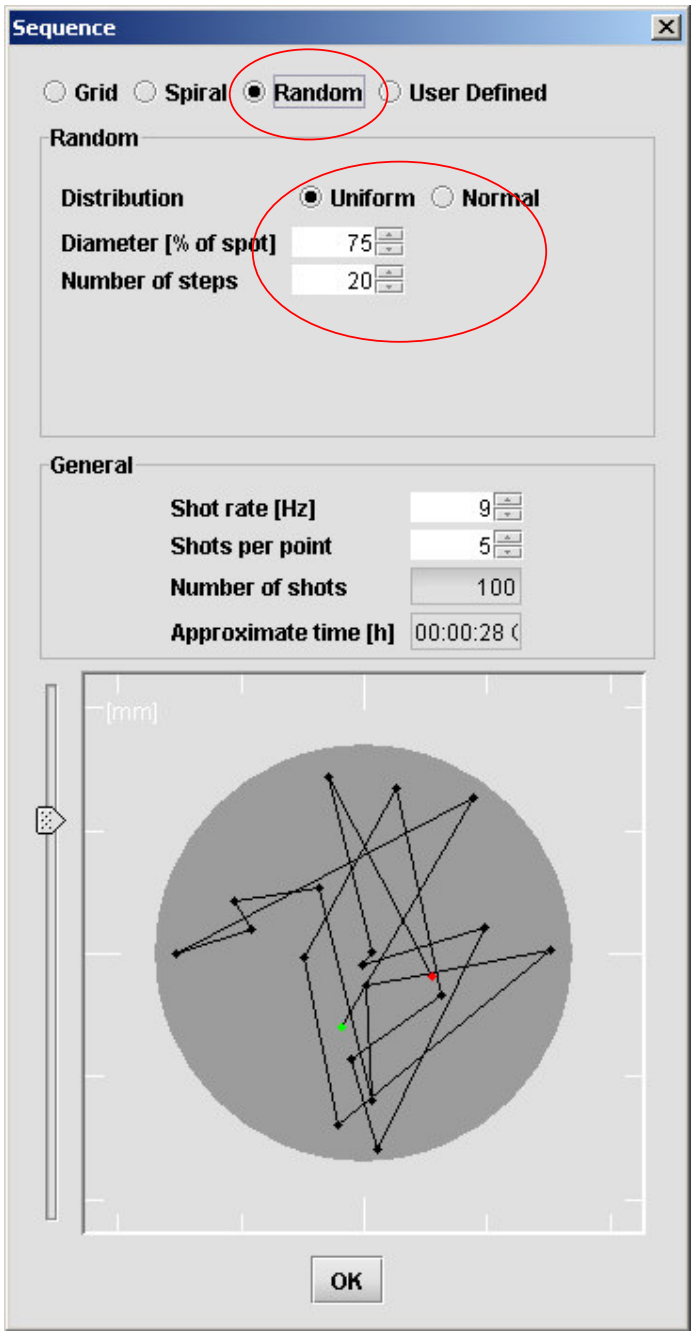
The resulting spiral sequence can be seen in the graphical sequence area.



5.4.3. Random sequence

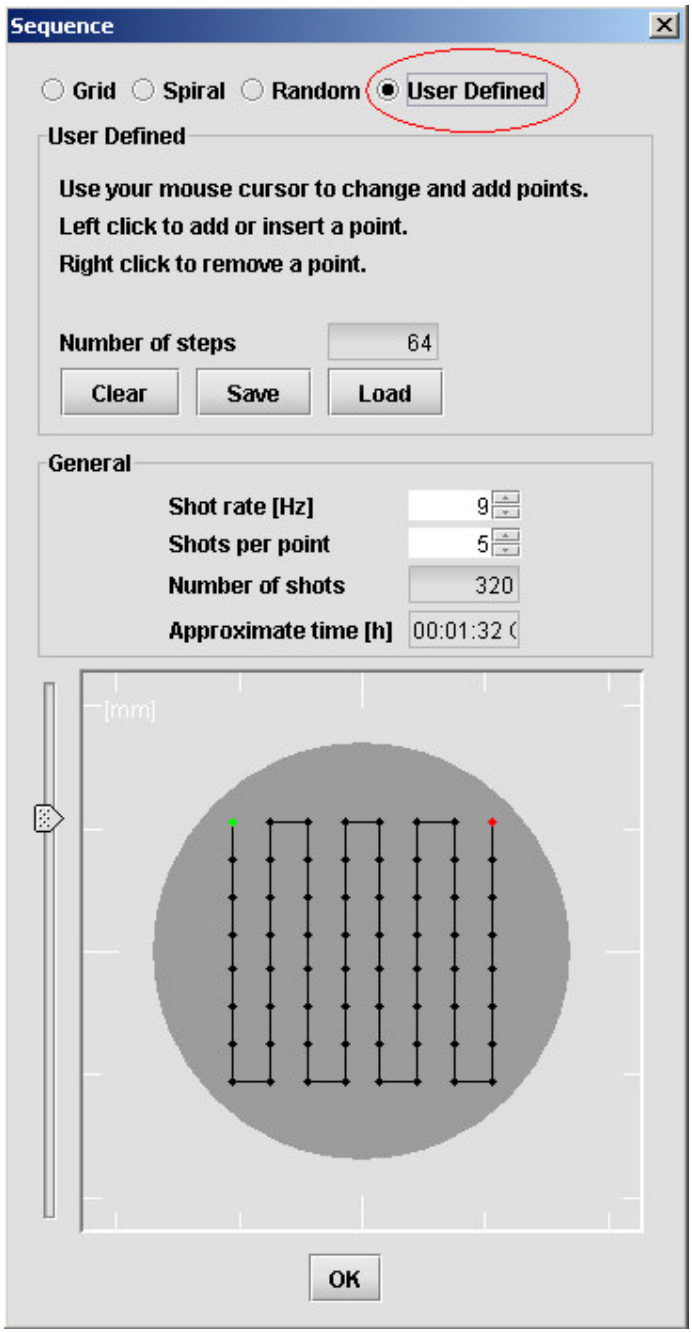
When selecting the ‘Random’ sequence option it is possible to generate randomized sequences, that will analyze the spot in a non structured pattern. It is possible to select from two different distributions of the sequence points:,Uniform distribution, where the points are placed uniformly on the complete spot, or Normal, where the points are placed in a two-dimentional normal distribution around the center of the spot.

The resulting random sequence can be seen in the graphical sequence area. Whenever the Random sequence option is selected a new random sequence is generated.

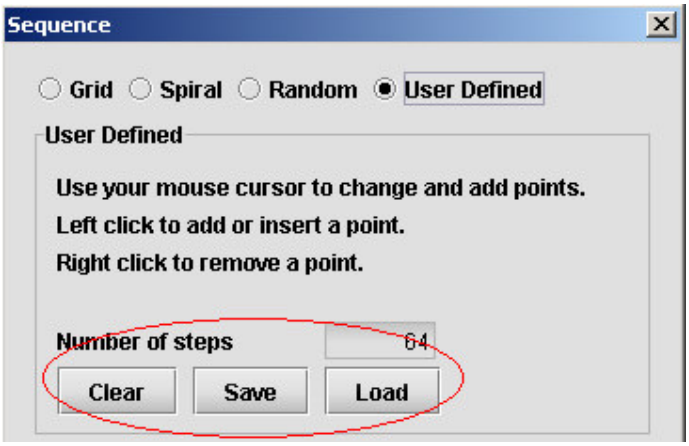


5.4.4. User Defined sequence

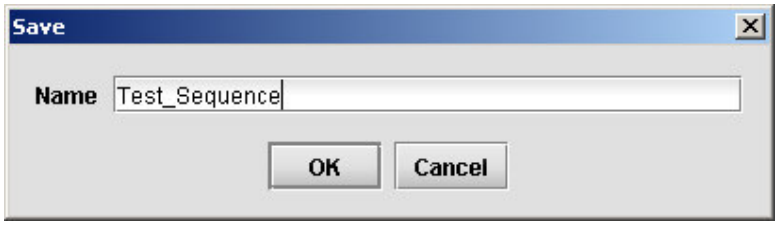
It is possible to generate a user defined sequence by selecting the sequence option ‘User Defined’. When this option is selectec, the sequence generated in the last selected option is kept and can now be changed by the user.



In the ‘User defined’ section it is possible to clear the sequence by clicking ‘Clear’. It is also possible to save the sequence in the database for later use or for use in the automated batch mode (selectable in the preparation application). It is possible to load a previously saved sequence from the database.

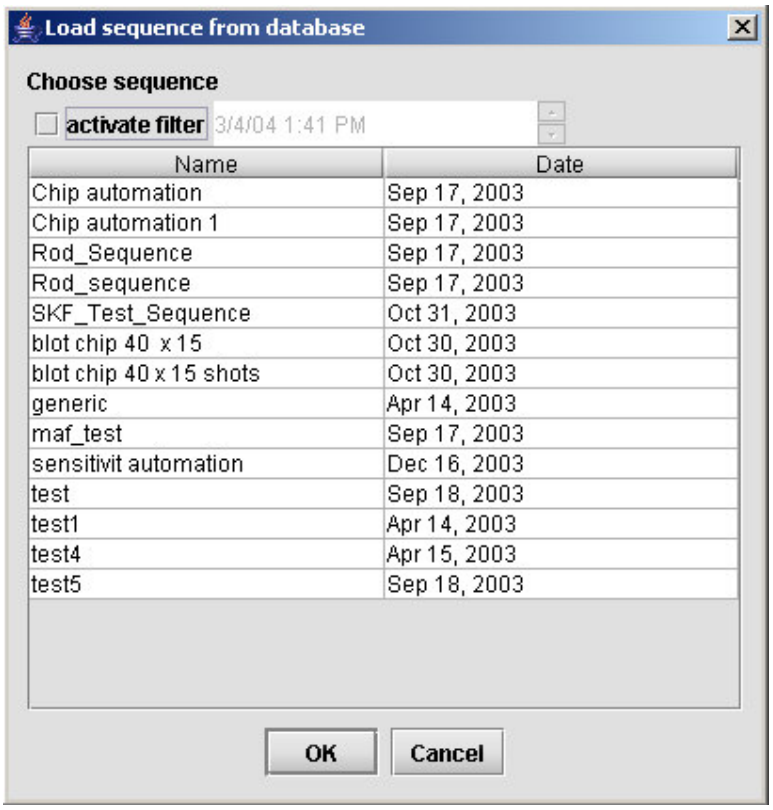


After clicking the ‘Save’ button the following popup will appear:

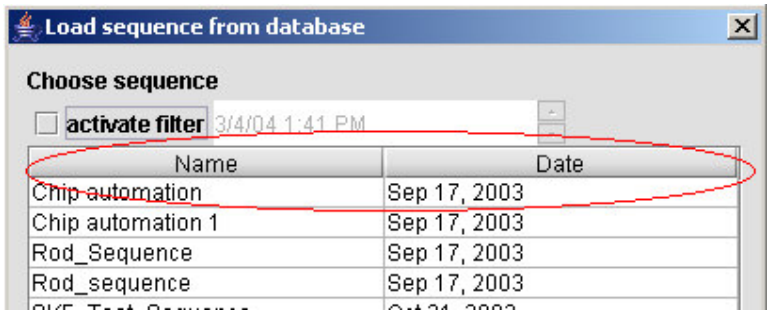


Here the name of the sequence is entered, and by clicking ‘OK’ it is saved in the database.

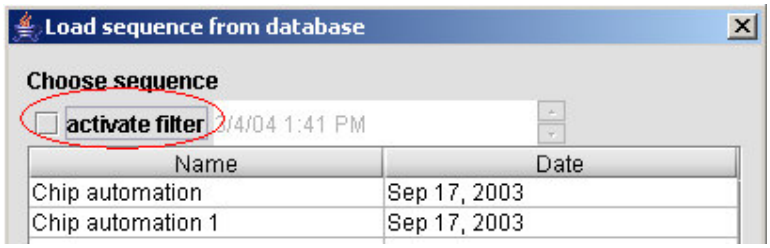
By clicking the 'Load' button the popup shown below will appear. Here all the saved sequences are shown in the list together with their date of creation.



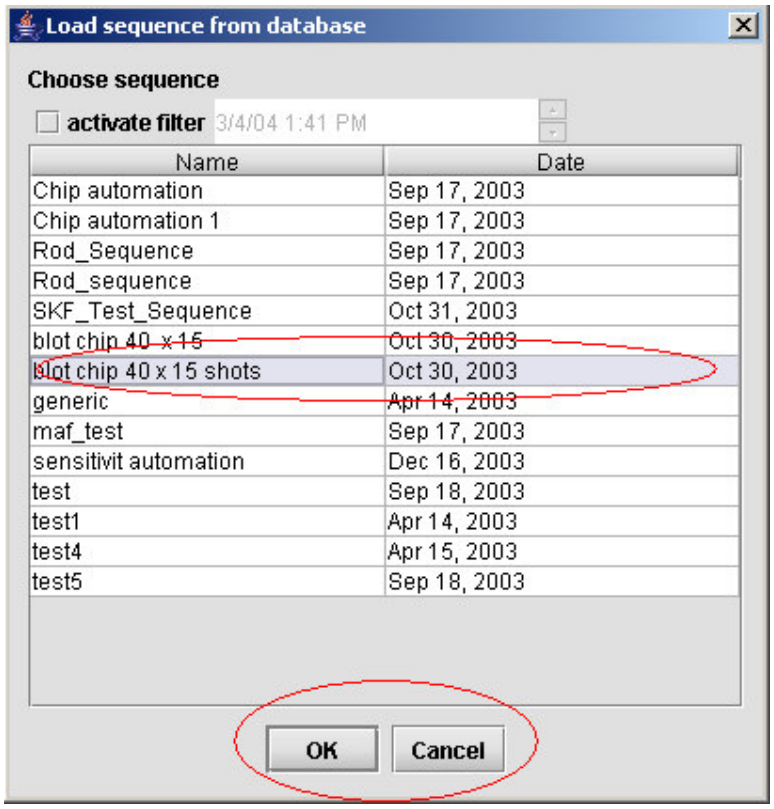
If the list of sequences is long, the bars on the top of the list (labeled 'Name', 'Date') can be clicked, thereby sorting the sequences in either name or date order (normal or reverse).



The 'Activate filter' option allows the list to be filtered by the date specified in the entry box.



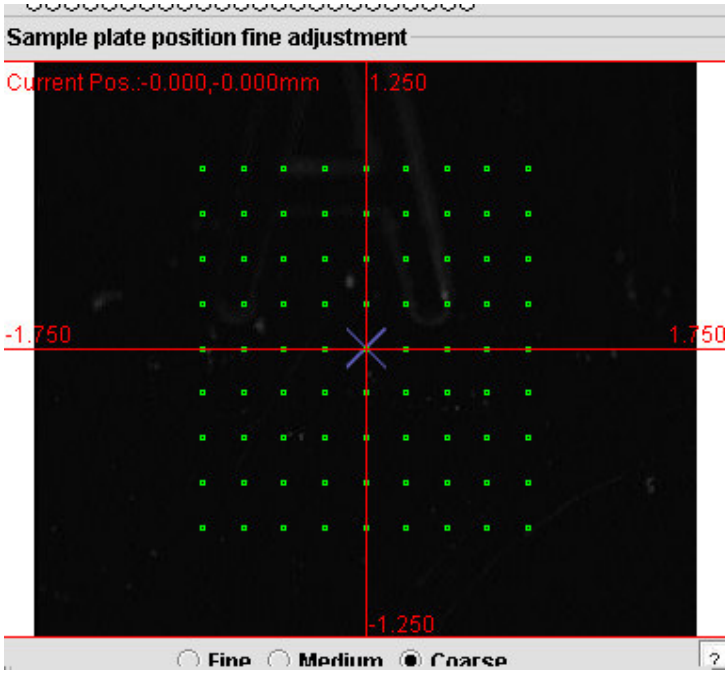
By selecting one of the sequences in the list and clicking ‘OK’ the sequence is loaded into the sequence editor.



When the definition of a sequence is completed, click the ‘OK’ button of the bottom of the sequence editor to start.

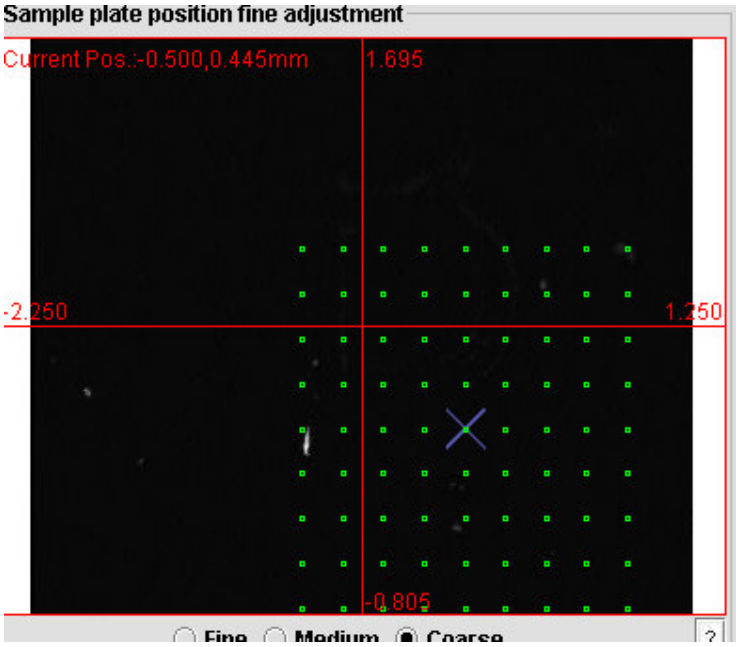
5.4.5. Placing the sequence center

After completing the sequence setup, the sample spot picture in the area ‘Sample plate position fine adjustment’ will display the actual positions on the spot with green boxes. Each of the boxes represent a sequence position, and the laser will hit the spot at these positions.

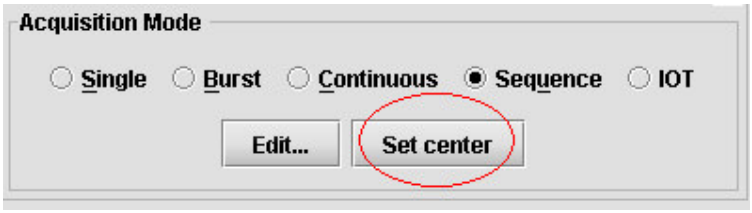


If the sample is not positioned at the center of the spot, it is possible to move the sequence to cover the complete sample.

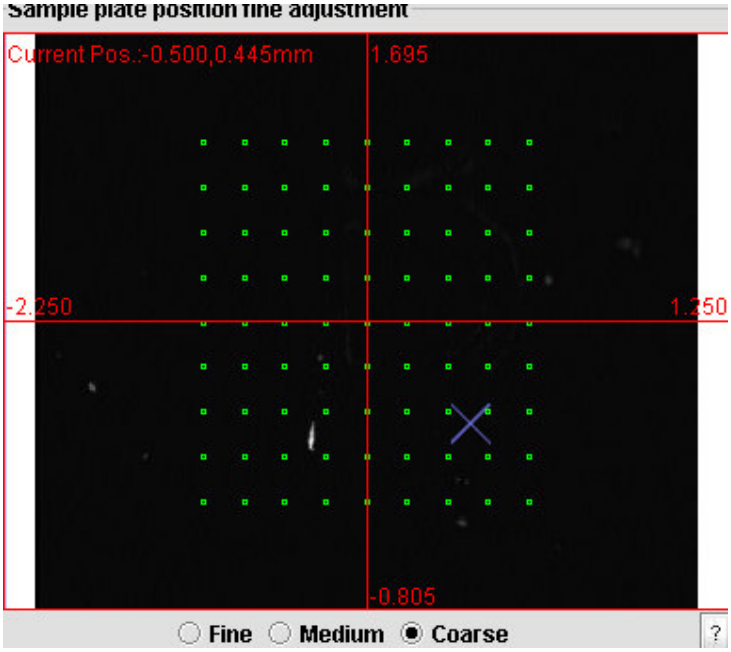
First, move the sample plate position so that the red cross is in the middle of the sample.



Then click the ‘Set Center’ button in the Acquisition mode area.



The sequence is now centered around the new position and is thereby centered on the sample.



5.5. How to optimize the acquisition parameters

The objective of the following paragraph is to find the best acquisition parameters for the instrument in order to increase the signal intensity and the resolution. It is important to mention that changing a sample will also change the optimal parameters and the optimization process should be redone. The obtained parameters should be saved as a new acquisition method for the sample. *(see chapter 5.9. Storing a measurement method, page 74)*

Before starting the optimization process, the extraction voltage must be defined. The extraction voltage can be raised up to 20 kV and this value should be used for general acquisitions. For high resolution acquisition of small proteins, best results are obtained by setting the extraction voltage to 6 kV. The increase in resolution for small molecules at low extraction voltage is due to the inherent long time resolution of the cryodetector[1].

[1] The resolution (R) is defined as $R=M/\Delta M=T/0.5*\Delta T$ where M is the mass and T the flight time of the ions. The R can be increased either by a higher T or a lower ΔT. ΔT contains velocity focusing of the ions as well as the detector time resolution. If the detector time resolution is the predominant contribution, which is the case for small molecules, the only way to increase the R is by a higher flight time T and thus the extraction voltage has to be lowered.

In function of the extraction voltage, the following acquisition parameters can be optimized shown in the first colon of the following table. The second colon shows their major impact on either signal intensity or on resolution.

Parameter	Main impact on
Second voltage	Resolution
Delay time	Resolution
Einzel lens voltage	Signal Intensity
Ion optics angle	Signal Intensity
Laser intensity	Signal Intensity, Resolution

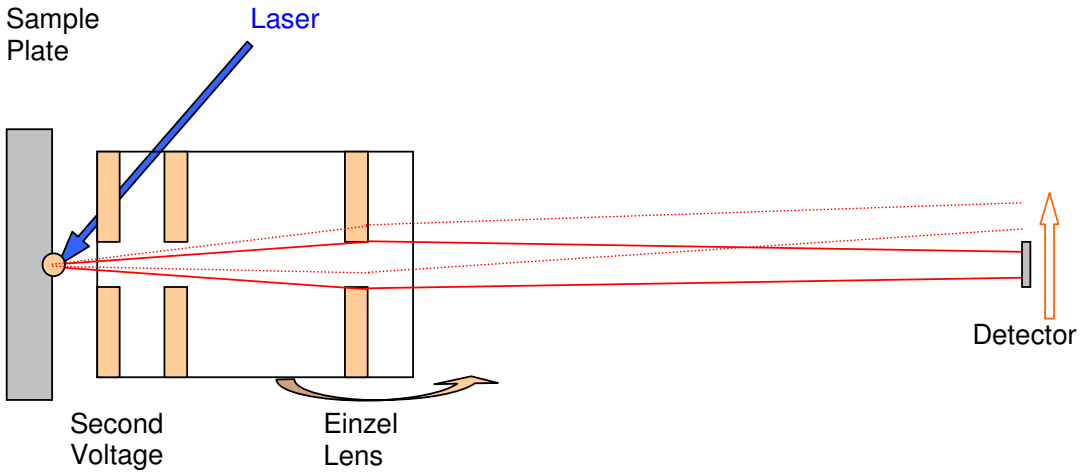
In the following paragraphs, a separation between the optimization of the resolution and the signal intensity will be made. The optimization of the signal intensity is treated in the paragraph *5.6. Ion optics test, page 67,* where the important parameters are the ion optics angles and the einzel lens voltage. The second voltage and delay time are involved in the optimization of the resolution and will be discussed in the *chapter 5.8. Optimizing the resolution, page 72.* The laser intensity will be discussed separately as it impacts the resolution as well as the signal intensity.

The general strategy of optimization is the following:

1. Insert a sample plate with a sample to analyze of interest into macromizer™
2. Choose an initial set of acquisition parameters
    a. Load an existing acquisition method
    b. Fix arbitrary parameters
3. Optimize the signal intensity using the ion optic test
    a. Find the optimal ion optics angles
    b. Optimize the einzel lens voltage
4. Optimize the resolution
    a. Find the optimal delayed time
    b. Find the optimal second voltage
5. Optimize the laser intensity

5.6. Ion optics test

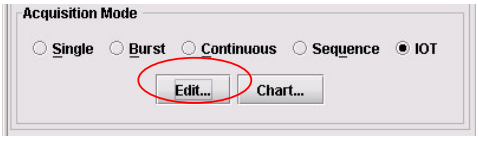
macromizer™ has a special sample stage with a turnable extraction system. The following figure shows a schematic view of the ion canon which can be tilted in two directions, horizontal and vertical around its rotation point lying on the surface of the sample plate. The angles are defined parallel to the flight axe and turning the ion canon will move the ion beam on the surface of the detector.



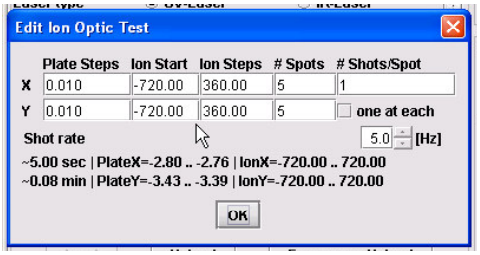
The software permits to set the beam angles from -720” up to 720”. Setting a value for the angles will turn the extraction system to this value and according to this the ion beam will change its angle. The beam angles can be considered as optimal when the ion beam overlaps completely the detector.
The ion beam is divergent after the first extraction region and must be focused on the detector in order to get a higher signal. This is achieved by the einzel lens and its voltage is optimal when the ion beam is focused on the detector.
The ion optics test permits to achieve these optimization by scanning the angles and measuring the intensity.

5.6.1. General steps of performing an ion optic test

- adjust the mass range around the target mass with minimal and maximal mass *(see chapter 5.3.4. Min, max and target mass, page 51)*
- choose a medium high laser intensity
- choose IOT under Acquisition Method

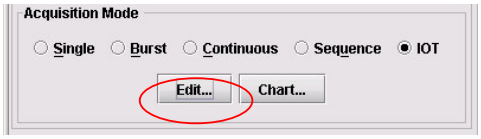


- select the edit button to modify the ion optic test parameters
- Definition of the Ion Optic Test parameters:
  - X: is the horizontal rotation of the ion optic



- Y: is the vertical rotation of the ion optic
- plate steps: moving of the sample plate in X-, resp. Y-direction between each angles
- Ion Start: starting angle of the test
- Ion Steps: increase of angles
- # Spots: number of increments into this direction
- # Shots/Spot: number of laser shots on each angle

- complete the parameters for the IOT and quit the popup windows
- choose a fresh sample spot
- start the test as a normal acquisition
- the result can be visualized in a new windows by the ‘chart’ button



It is important to consider that a sample spot can be burnt out before the test has finished and therefore the result can be influenced. Thus the number of total shots on the same spot should be smaller than the maximal shots for the given matrix. In the case of a sinapinic acid matrix, the upper limits lies around 50 shots.

5.6.2. Finding the optimal beam angles

The Ion Optic Test (IOT) can be divided into two major methods, a 2D and a 1D scan. The 2D scan is a 2 dimensional scan where both directions are changed during the test.

The advantage of the 2D scan is that the region of the optimal angle can be found rapidly but only approximative. The reason is the limitation of shots before the sample spot is burnt out. Therefore the total number of shots must be low and only a rough grid of angles can be scanned. This induces a rather high statistical fluctuation of the detected ions and a high error on the resulting optimum.

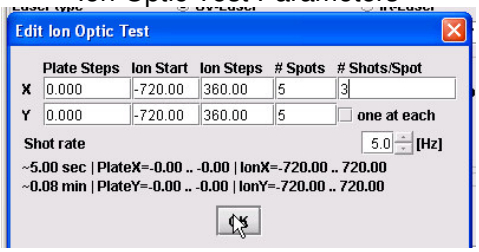
The 1D scan is a 1 dimensional scan where one direction is fixed and the second one is scanned. The advantage of the 1D scan is that an accurate position of optimal angles can be found. The reason is that a smaller spacement of the angles can be used for the scan and a better interpolation of the resulting signal gives a better ion beam profile. But the process is more time consuming as both direction have to be scanned separately.

When the optimal angles are not known at all, it is best to start with a 2D scan and then refine the search of the optimal angles with 1D scans in both direction.

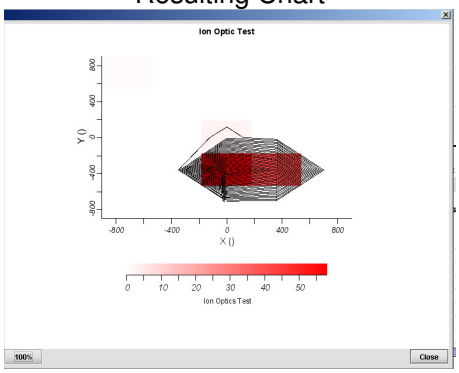
2D scan:

Typical parameters for the 2D scan are shown in following example:

Ion Optic Test Parameters



Resulting Chart



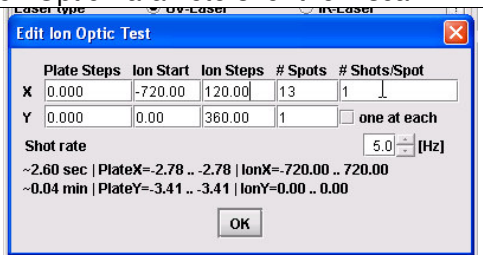
The scan has 5 steps in X and Y direction which gives a grid of 25 different angles. On each of these pair of values a 3 shot acquisition will be measured. The right side shows the corresponding chart and the optimal angles are around 180''/-360'' with a precision of +-180''.

1D scan:

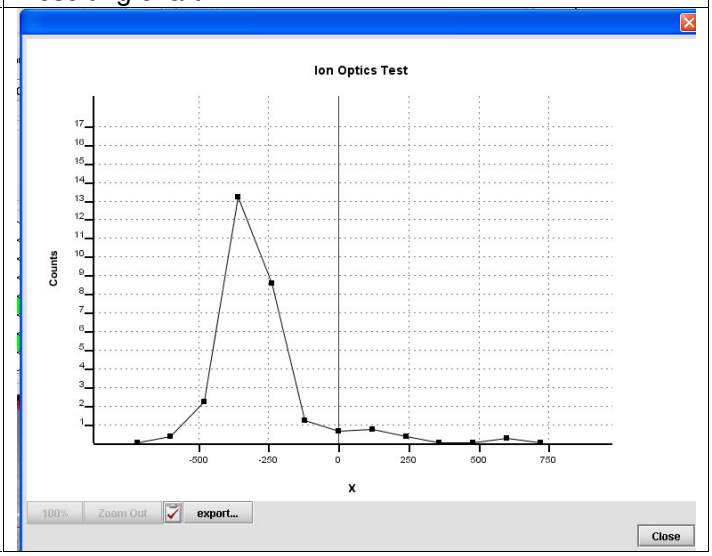
The 1D scan can be done in either in x or in y direction. To start with a scan, one direction is fixed near its optimum. In the following example, the y-angle is initially fixed at 0'' and the X-scan is achieved by 13 steps of 120'' and 1 shot per spot.

The scan can now be started as a normal acquisition. The visualization of the x-scan is given by chart button and the following graph shows up in a new windows.

Ion Optic Parameters for the x-scan

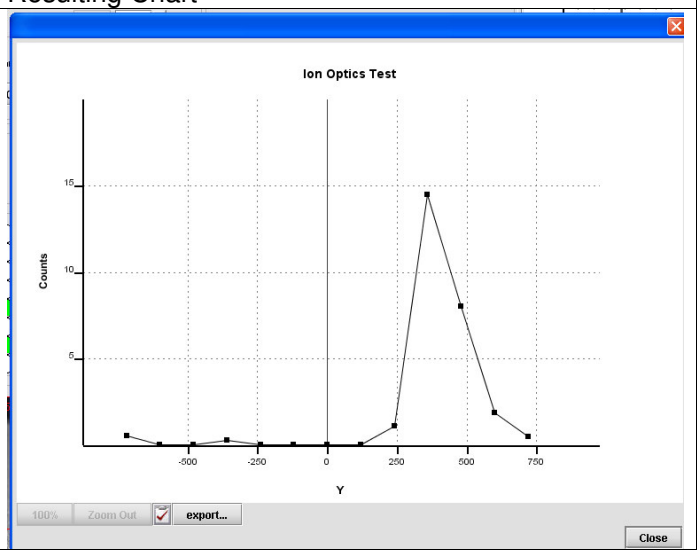


Resulting chart



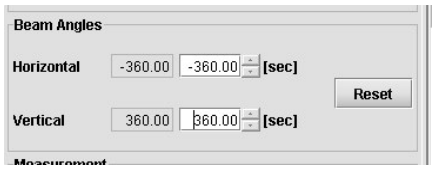
A maximum can be distinguished at -360'' and this angle is the optimal angle in the X-direction. For this value, the corresponding optima must be found in the Y-direction. The ion optic parameters for y-scan must be changed to match the new condition and the ion start angle in x-direction is now fixed to the previously found value of -360''. The number of spots in x-direction is set to 1. In the y-direction, the scanning parameters similar to x-scan are inserted.

The scan can be started and the result is shown on the following chart.

Ion Optic Parameters for y-scan	Resulting Chart
	

The popup window shows now the y-scan and its maximum is at 360''. This value is now used for the optimal y angle.

As the optimal angles have been found now, they can be inserted in the beam angles for further acquisition.



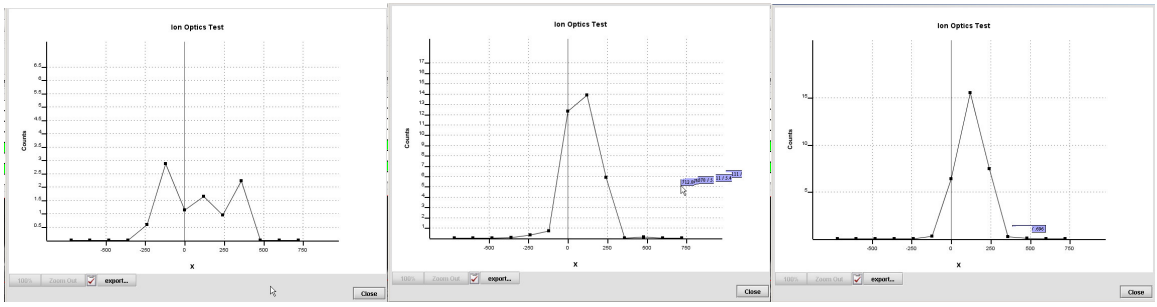
5.6.3. Optimizing the einzel lens voltage

The optimization of the einzel lens voltage is strongly related to the ion optic test and its optimization of the beam angles. The reason is that the profile obtained by a 1D scan corresponds in a first order to a cut through the ion beam. The criterion of an optimal einzel lens voltage is the minimization of the ion beam width without any loss in signal intensity.

Optimization procedure:

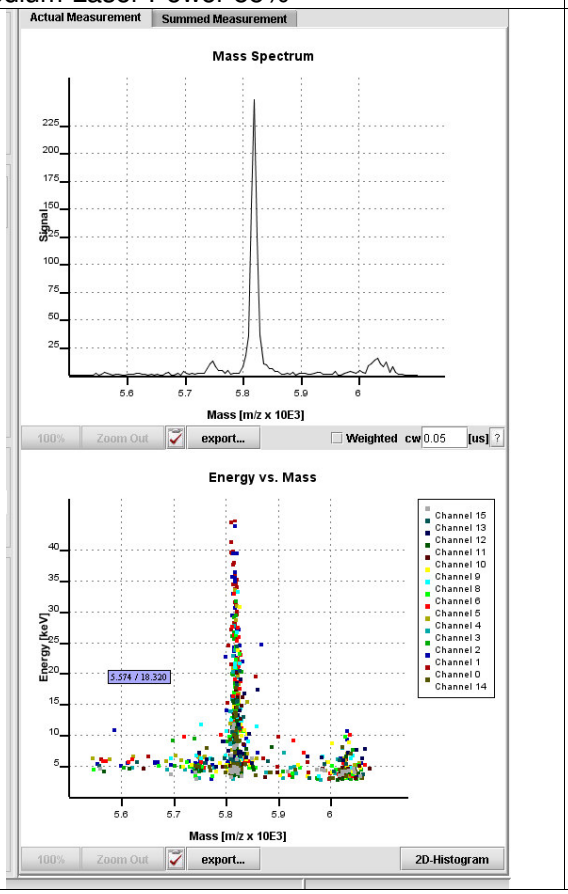
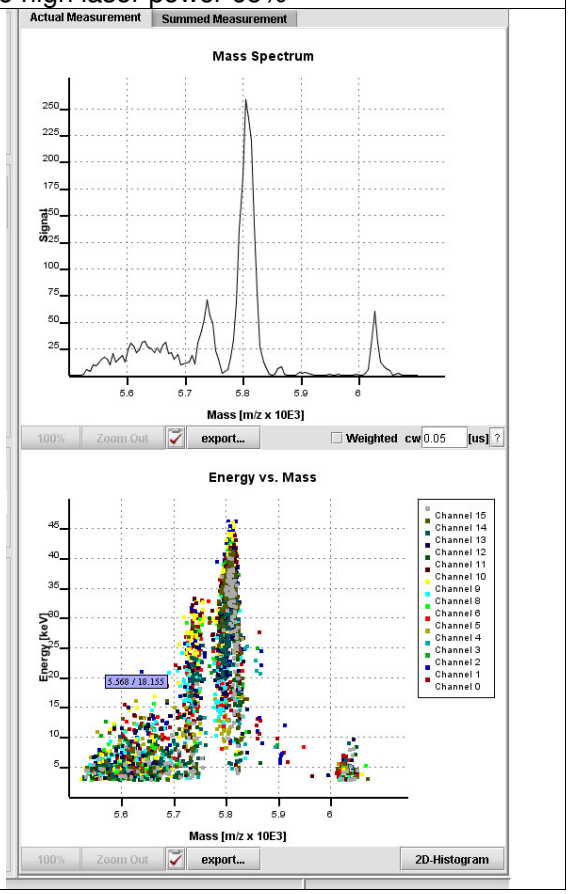
1. Choose an einzel lens voltage
   Best initial guess is to start with the half of the Extraction Voltage and then increase the einzel lens voltage
2. Perform the 1D scan as describe before for both direction always on a fresh sample spot
3. Determine the width of each profile
4. Increase the einzel lens voltage by steps of 100 V
5. Redo 2-4 until a minimum width of the profile has been found

The following three charts show the behaviour of ion beam profile when increasing the einzel lens voltage from left to right.



5.7. Optimizing the laser intensity

The laser intensity will influence strongly the acquisition. A too high laser power broadens the signal peak and therefore a drastically decrease in resolution is observed. If the laser power is decreased under a threshold, the MALDI process becomes inefficient and the molecules can't be ionized by the process any longer. The optimal laser intensity depends also on the matrix and on the sample. In general the laser intensity must be increased from α-cyano-4-hydroxinamic acid to sinapinic acid. The decrease in resolution in function of the laser power is shown on the following figure where the laser intensity is increased from 55% up to 65% for a insulin sample in a SA matrix.

Medium Laser Power 55%	Too high laser power 65%
	

Optimization procedure of the laser intensity is:

- choose a medium laser intensity (~50%)
- acquire a spectrum
- if no signal can be seen on the Mass Spectrum, increase the laser intensity by 5%
- if too many events are shown on the Energy Plot and the peaks are too broaden, decrease the laser intensity by 5%

The fine adjustment of the laser intensity:

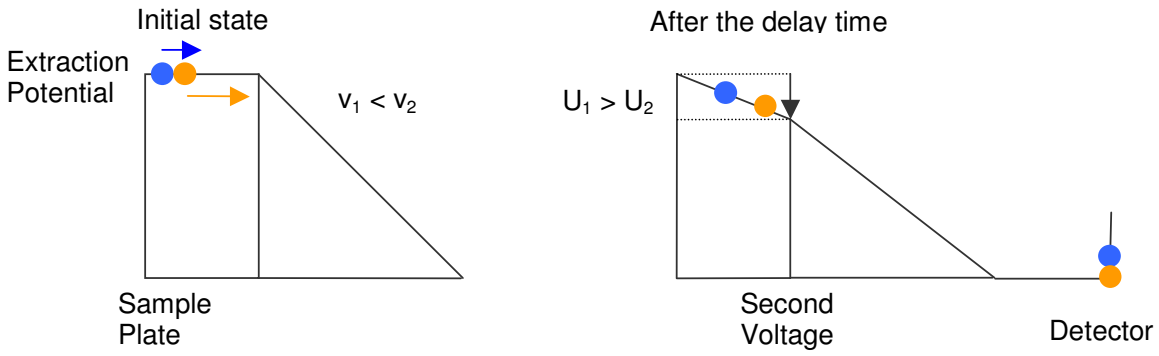
- change laser intensity by 1% and acquire a spectrum on a fresh spot
- determine the FWHM of a main peak in the Data Analyzer window *(see chapter 8. Analyzing data, page 96)*
- decrease the laser intensity for a smaller FWHM and a smaller signal
- increase the laser intensity for a higher signal

Repeat these steps until obtaining a moderate resolution and an acceptable signal.

5.8. Optimizing the resolution

The resolution can be improved by the delayed extraction of the ions. To obtain a high resolution in the MALDI process, the initial velocity distribution must be compensated. In a continuous extraction this initial distribution will broaden the ion peak considerably. A solution to this problem is to apply a high voltage pulse to the ions after a certain delay time. This method known as the velocity or energy focusing[2] compensates the initial velocity distribution of the ions and results in the increase of the resolution.

A simple illustration of this effect is shown on the following figure. The molecules ionized through the MALDI process fly first through a field free space. This is achieved by setting the same extraction voltage on the sample plate and the first extraction lens (called second voltage). So the ions drift in the field free space and will be separately in function of their initial velocity. After the delay time, a potential is applied on the second voltage to accelerate the ions. The slower ions will gain more energy as they are still nearer to the sample plate than the faster ones. Using the right combination between the switch time and the applied potential, all ions will reach the detector at the same time.



The two parameters, extraction delay and the second voltage, are interactive and for each delay time, there exist an optimal second voltage for a given mass.

A general strategy for optimizing the resolution is:

- Fix an initial set of acquisition parameters with optimal beam angles and laser intensity

[2] see W.C.Wiley and I.H.McLaren, Rev. Sci. Instr., 1955, **12**, 1150-57

- Global optimization of the delayed extraction time (scanning from 200 ns up to 9000 ns depending on the mass to optimize)
- Optimization of the second voltage
- Optimization of the einzel lens voltage for intensity
- Fine tuning of the delayed extraction time
- Adjusting laser power

There is an inherent 200 ns delay in the system and the delay time value must be higher than this value. The minimal increment of the delay time is of 12.5 ns and the applied delay time is shown beside the entered delay time.

Before starting the optimization of the resolution, an initial set of parameters must be used. A possible starting point is to use a working acquisition method *(see chapter 6.7. Selecting a method, page 83)* or starting with a complete new set of parameters. In the table below two sets of parameters are given.

Optimized Mass	HV1	HV2	HVE	Delayed Time
12 kDa	20 kV	3 kV	12 kV	750 ns
66 kDa	20 kV	3 kV	10.5 kV	1500 ns
5.8 kDa	6 kV	1.5 kV	3.2 kV	300 ns

Prior any optimization of the resolution, the optimal beam angle must be found *(see chapter 5.6. Ion optics test, page 67).*

The first step of the optimization is to find the correct delayed time. The procedure is the following:

- Choose a fresh spot
- Make a 100 shot burst acquisition
- Go to the Data Analyzer and determine the FWHM of the desired mass *(see chapter 8.10. Picking peaks manually and automatically, page 108)*
- Go back to acquisition and increase or decrease the delay time by step of 100 ns
- Restart

Continue these steps until you find a minima for the FWHM of the given mass.

After optimization of the delay time, the second voltage should be adjusted.

- Increase or decrease the second voltage by step of 50V
- Make again a 100 shot burst acquisition
- Determine the FWHM as before
- Restart until a minima of the FWHM is found

With the change of these parameters, the beam angles and the einzel lens must be rechecked for their optimum value.

- Optimize beam angles *(see chapter 5.6.2, Finding the optimal beam angles, page 68)*
- Optimize einzel lens voltage *(see chapter 5.6.3. Optimizing the einzel lens voltage, page 70)*

After the adjustments of the angles and einzel lens voltage, the delayed time can now be fine tuned.

- Choose a fresh spot
- Make a 100 shot burst acquisition

- Determine the FWHM of the desired mass *(see chapter 8.10. Picking peaks manually and automatically, page 108)*
- Go back to acquisition and increase or decrease the delay time
- Restart until a minimal FWHM is found

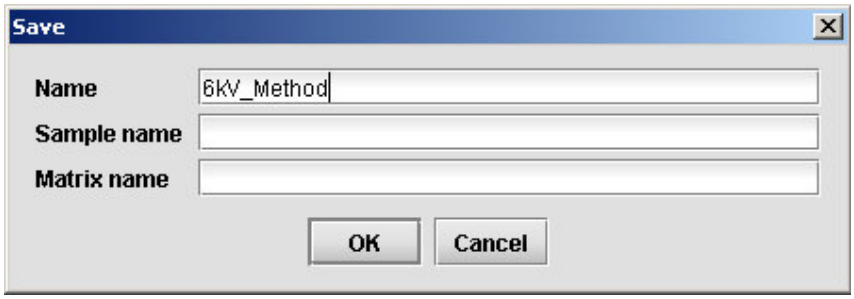
For small mass molecules the delay time can be changed by steps of 25 ns. For high mass molecules (>50 kDa) the influence of the delayed time is less marked and steps of 100 ns are sufficient.

5.9. Storing a measurement method

When the acquisition method is optimized, it can be saved in the database by clicking 'Save' in the 'Select Method' area.

After clicking 'Save' the save popup will appear. The name that is entered by the user, defines the acquisition method file name under which the measurement method can be recovered. The sample name and matrix name can be left blank or used for additional information about the method.

The name can be entered in the appropriate blank. By clicking 'OK' the method is saved, or by clicking 'Cancel' the save operation is canceled.

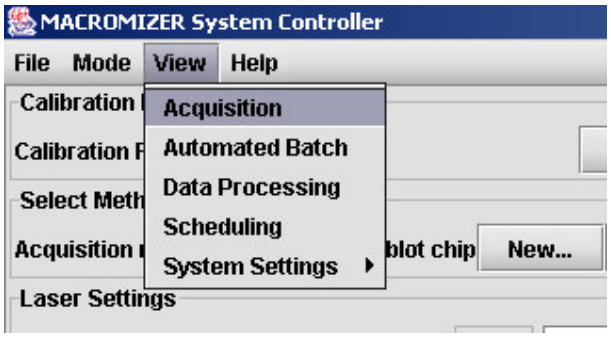


6. Acquiring spectra

6.1. Selecting the acquisition panel

The system controller application can be configured individually by the user. It is possible to select the initial window after a start up of the system and to enable password access restriction for the different menus.

If the acquisition panel is not visible, this panel can be selected through the pull down menu at the top left on the system control window. Select 'View' and in the pull down 'Acquisition'.



If a password for this panel is enabled, enter the correct password in the popup dialog.

If the acquisition panel is the startup panel, and if the password is enabled, the password dialog will be shown before the application panel.

6.2. Loading a sample plate in the macromizer™

After a sample plate is prepared, it is loaded into the macromizer™ by placing it in the sample plate load slot.

Once the plate is positioned in the macromizer™, click the 'Load' button in the 'Sample Plate Loading/Unloading' area.

After the 'Load' button is clicked a confirmation popup is shown. Click 'OK' to continue the sample load or 'Cancel' to cancel the loading.

While the sample plate is being loaded a popup with a moving bar is shown to indicate that the machine is busy.

A sample plate load takes about 40 seconds. If any problems occur during the loading a popup describing the problem and requesting an emergency unload will appear. If the emergency unload is accepted, the plate will be transported outside the macromizer™.

After a successful sample plate load a sample plate barcode must be selected. A selection can be made from a list of possible barcodes that have been defined in the preparation application *(See chaper 4.7. The preparation application and its use, page 26)*.

Select the sample plate barcode from the list and click 'OK'. A popup for the selection of the acquisition method will be shown. A predefined method can be selected from the list.

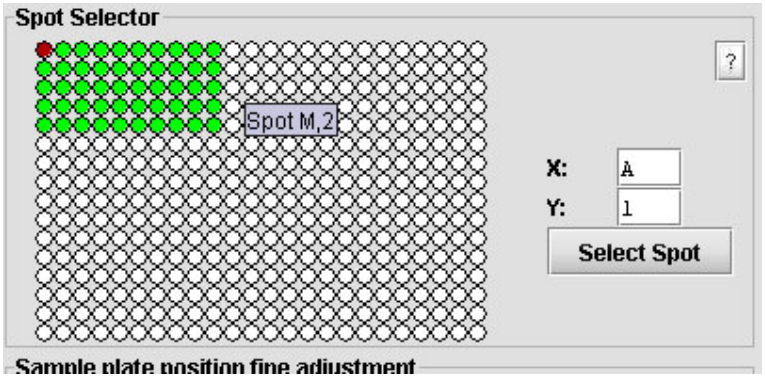
By clicking 'OK' the macromizer™ will be setup for performing acquisitions with the selected method.

6.3. Selecting a sample plate position

Before starting an acquisition, the sample plate must be moved to the desired position. There are two methods for moving the sample plate: using the 'Spot selector' or the 'Sample plate position fine adjustment'.

With the 'Spot Selector', the sample plate is represented by spots corresponding to the center of the real spot position. This representation can be defined by the user for each sample plate and is chosen when a new sample plate is loaded.

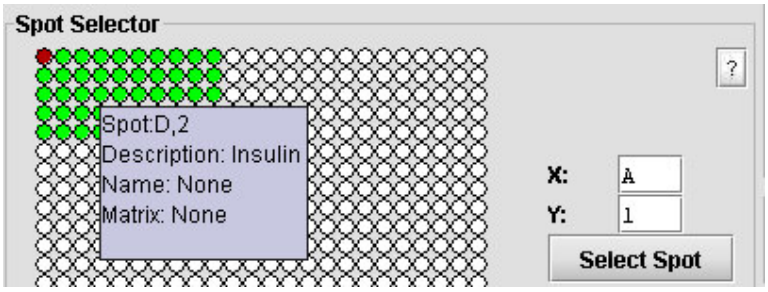
Selection of the spot position is made in the 'Spot Selector', and is done either by left-clicking the desired spot with the mouse on the graphical spot selection area (on the left), or by typing the X and Y positions of the desired spot in the two fields and clicking the 'Select Spot' button (on the right).



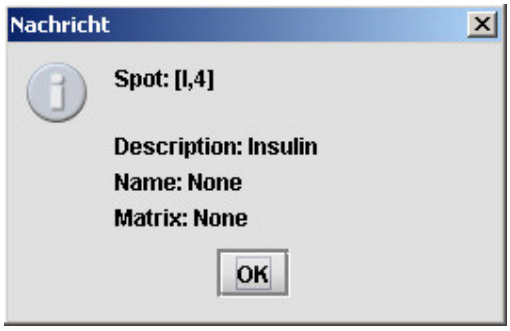
In the graphical spot selection area the present spot position is marked with a red colored spot, and the X and Y positions are shown in the respective fields.

A green colored spot indicates that during the sample plate preparation a description of the spot substance has been entered.

When the curser is placed on one of the spots in the graphical spot selector (without clicking) a blue colored box appears. The blue box shows the spot position and, if the spot is green in color, the spot description.

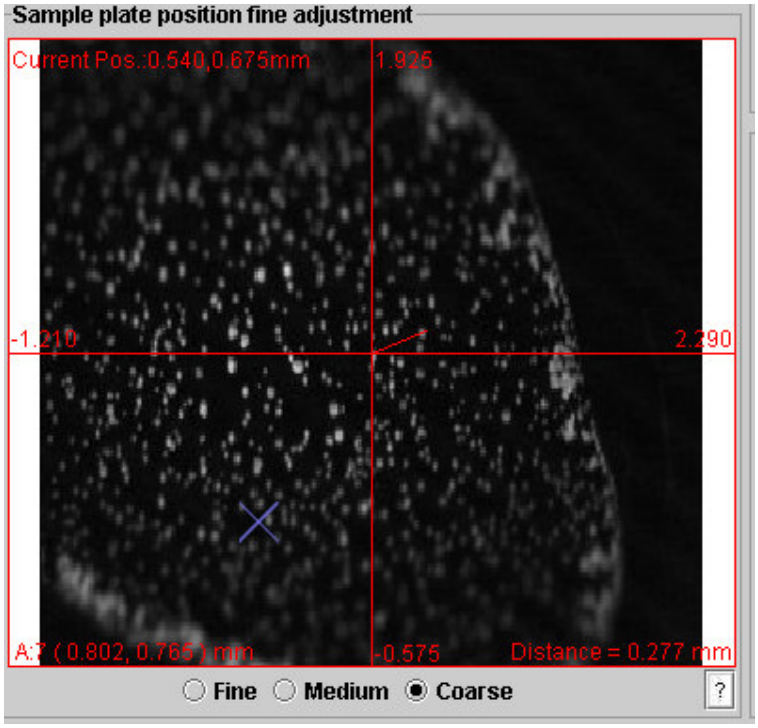


By right-clicking the mouse on a spot in the graphical spot selector, a popup is shown with the selected spot information.



After selecting a spot, the fine position adjustment can be done in the 'Sample plate position fine adjustment' area. A digital live image of the sample plate is shown for the selected spot area.

The center of the image (shown by the two red crossing lines) corresponds to the position on the sample. After clicking the right mouse button, the center of the spot is selected (this position is indicated in the image by the blue X). A target position can easily be reached by clicking on the picture with the left mouse button.



Moving the curser into the image area will show a red line originating from the center of the image and following the curser. In the lower right corner the distance from the center to the curser position is shown (in mm).

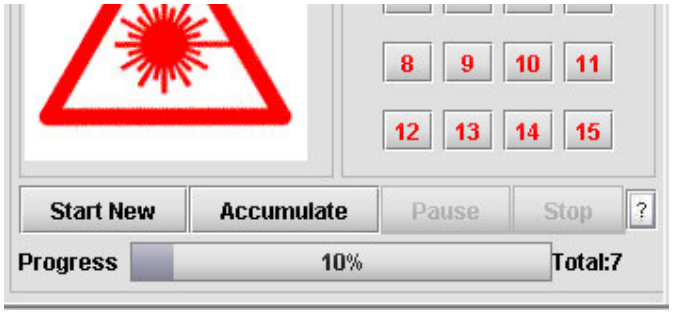
At the top of the image the current position within the spot is shown in mm. At the lower left the spot coordinates and the current curser position are shown. The position at the edges of the spot image are shown on the X and Y axis.

Below the image three zoom factors are available: 'Coarse' is full zoom, 'Fine' is the maximum zoom and 'Middle' is medium zoom. When zooming the image, a more precise spot position can be chosen.

6.4. Starting an acquisition

To start an acquisition the macromizer™ needs to be prepared. A sample plate must be loaded, and the acquisition method and a spot position selected.

When all parameters are set, click 'Start new' to clear the actual measurement and start the acquisition. If events are to be added to the actual spectrum, click 'Accumulate' to continue the acquisition without clearing the actual acquisition.

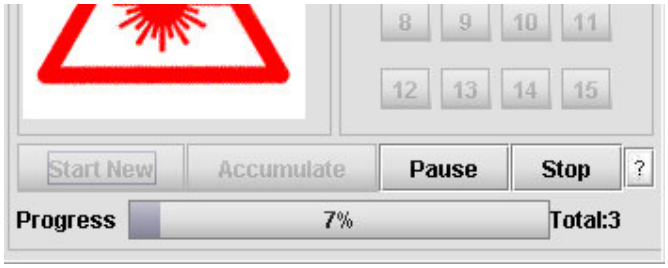


The macromizer™ is designed with a 'machine saver' feature, that sets the macromizer™ in a "sleep" condition when it has been left unused for a specific time. In this state, the Cryo Valve is closed to keep the cryostat in a safe state. By starting an acquisition the following popup will be shown:

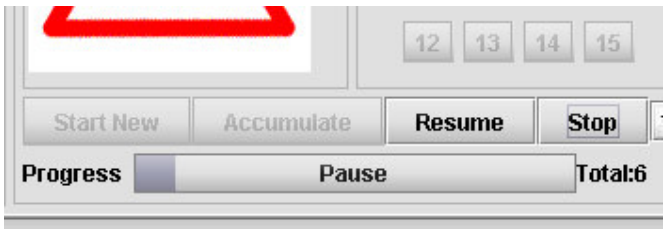
Click 'OK' to open the valve or click 'Cancel' to leave it closed. In the closed state no events will be detected during the acquisition.

When the acquisition is running the progress bar will show what percent of the prepared acquisition has been made.

While the acquisition is running the 'Pause' and 'Stop' buttons are activated.



When the 'Pause' button is clicked it will change into a 'Resume' button and the progress bar will show 'Pause'. By clicking 'Resume' the acquisition will continue.



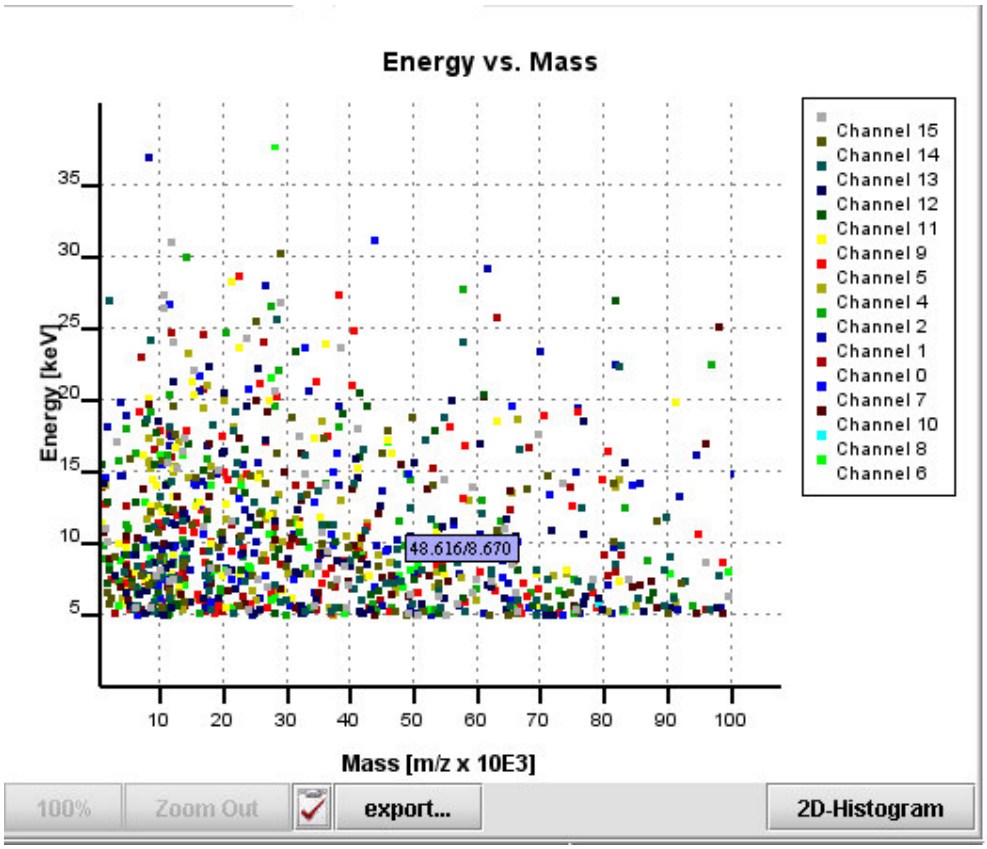
It is possible to click 'Stop' at any time during the acquisition process to terminate the running acquisition.

6.5. Observing signals during measurements

It is possible to observe what events are detected by the macromizer™ while an acquisition is running.

Each event is represented by a colored dot and is shown in the Energy vs. Mass scatter plot.

The ‘Mass’ scale is determined by the settings in the 'Target Mass and Spectral Bandwidth' area and scaling of the ‘Energy’ axis is done automatically when the acquisition is paused or stopped.

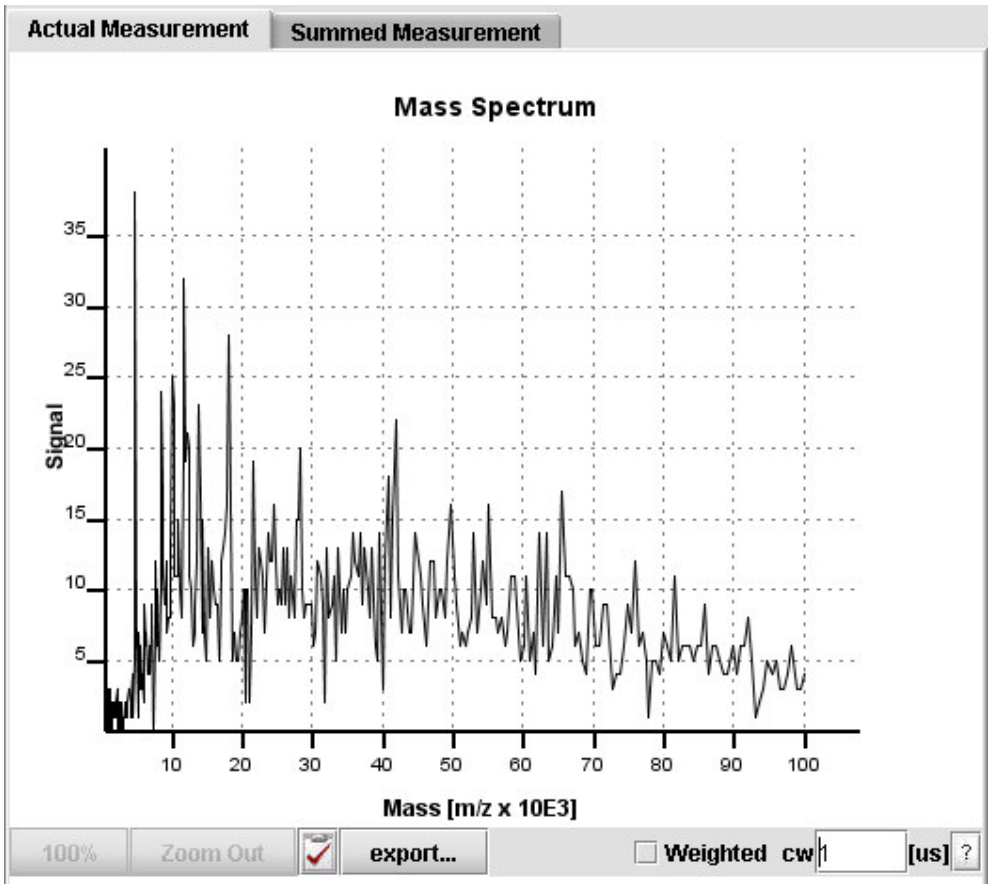


Each color represents one of the 16 channels on the detector and is indicated by the legend to the right. By placing the curser on the scatter plot, the mass and energy position is shown in the blue box.

Zooming in the scatter plot is done by holding the left mouse button activated and dragging a box to cover the area you wish to enlarge. By releasing the button a zoom to the selected area is done. Zoom to full is done by clicking the '100%' button, while zooming out in steps is done by clicking the 'Zoom Out' button.

Mass spectrum is generated at the same time that the events are shown in the scatter plot. The mass spectrum is a calculated histogram and the appearance depends on the setting of the ’Class width’ and the 'Weighted’ option.

The ‘Mass’ scale is determined by settings in the 'Target Mass and Spectral Bandwidth' area. Scaling of the ‘Signal’ axis is done automatically when the height of the spectrum exceeds the scale.



Two spectra can be generated for one acquisition: the actual and the summed. For a description of these two spectra please read further in this chapter.

Zooming in the mass spectrum is done by holding the left mouse button activated and dragging a box to cover the area you wish to enlarge. By releasing the button a zoom to the selected area is done. Zoom to full is done by clicking the '100%' button, while zooming out in steps is done by clicking the 'Zoom Out' button.

Export and print is possible for both graphs and will be discussed later in this chapter.

6.6. Changing acquisition parameters during a measurement

If changing of method parameters is required during an acquisition, the System Control offers two possibilities: selecting a new method or changing the parameters directly.

If the required parameter setting is already available in a predefined method, this method can be selected in the 'Select Method' area.

By clicking the 'New..' button a popup is shown with a list of possible methods. Select the required method from the list and click 'OK'. The macromizer™ parameters will be setup for new acquisitions. Click 'Cancel' to keep the previous settings.

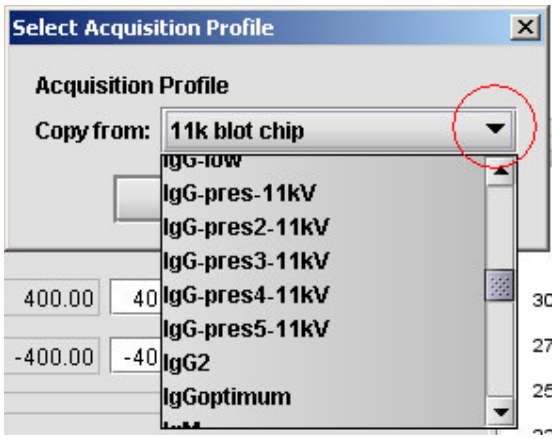
If acquisition parameters are changed manually, please refer to the chapter 'Using and creating acquisition methods' on how to set parameters and how to save a method.

6.7. Selecting a method

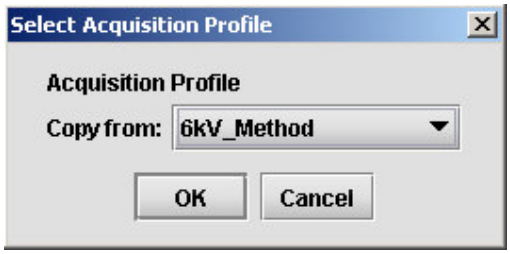
To simplify the setup of the macromizer™, acquisition methods can be stored in the database, allowing them to be selected at any time for a specific setup of the machine.

These predefined acquisition methods can be selected by clicking the 'New' button in the 'Select Method' area.

By clicking 'New', the 'Select Acquisition Profile' and the complete list of all stored methods can be found in the pull down menu.



Selecting one of the methods closes the list and shows the selected method in the field.



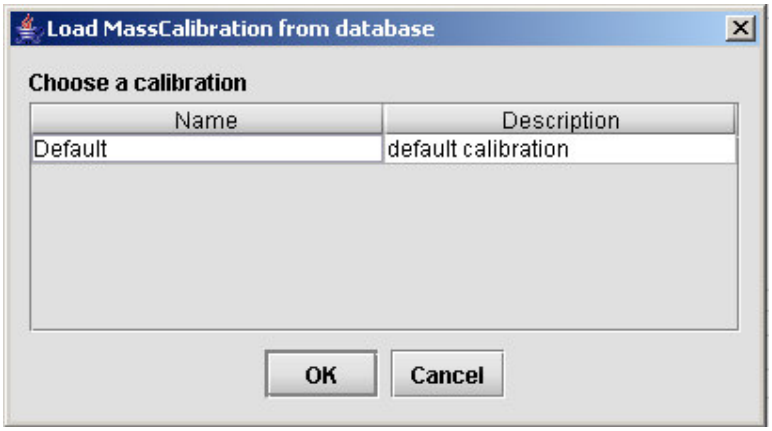
By accepting the related method all the parameters are changed to the stored values.
Canceling the operation leaves the macromizer™ settings unchanged.

6.8. Selection a calibration method

To simplify the setup of the macromizer™, calibration methods can be stored in the database, allowing them to be selected at any time for a specific setup of the machine.

These predefined calibration methods can be selected by clicking the ‘Select..’ button in the 'Calibration Data Selector' area.

By clicking ‘Select..’, the ‘Load MassCalibration from database’ dialog, along with a complete list of the calibrations, appears.



By selecting a calibration in the table and clicking the ‘OK’ button the new calibration method will be applied to the current acquisition. Canceling the operation leaves the macromizer™ settings unchanged.

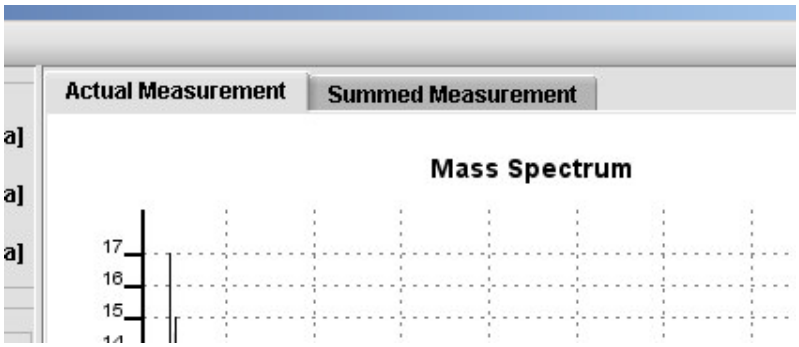
6.9. Actual and summed spectrum

Acquiring spectra can be done in either one or two steps.

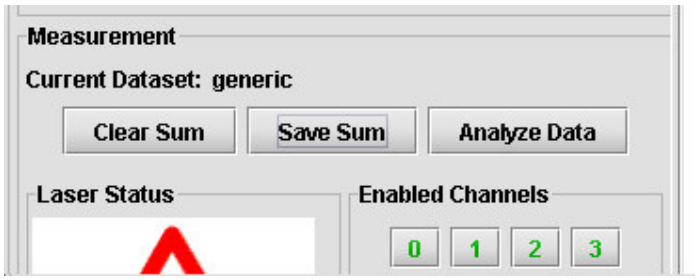
In the one step method, the acquisition is started as described in chapter ‘Starting an acquisition’. After a completed measurement is run, the spectrum is either transferred to the Data Analyzer for online analysis by clicking 'Analyze Data', or it is stored in the database by clicking 'Save Actual'. In this way, all events from the acquisition are saved in the spectrum.

In the two step method, the acquisition is performed as described previously, but the spectrum is not saved in the database directly. Instead, the spectrum is transferred to the summed spectrum by clicking 'Add To Sum', where several spectrums can be stored. This option makes it possible to select the optimal spectra and add these to the summed spectrum, filtering away bad or unusable spectra.

To view, analyze, and save the summed spectrum, select the tab 'Summed Measurement' above the Mass Spectrum histogram. To get back to the actual spectrum, select the tab 'Actual Measurement'.



The summed spectrum can then be transferred to the Data Analyzer for online analyzing by clicking 'Analyze Data' or can be stored in the database by clicking 'Save Sum'. This allows that only the selected events will be saved in the spectrum.



The two methods can also be used in combination. The user may analyze each actual spectrum online, adding them to the summed if desired, or analyze the summed spectrum online and then save.

By transferring the actual spectrum to the summed spectrum, the actual spectrum is automatically cleared. By clicking 'Clear Sum' the summed spectrum is cleared.

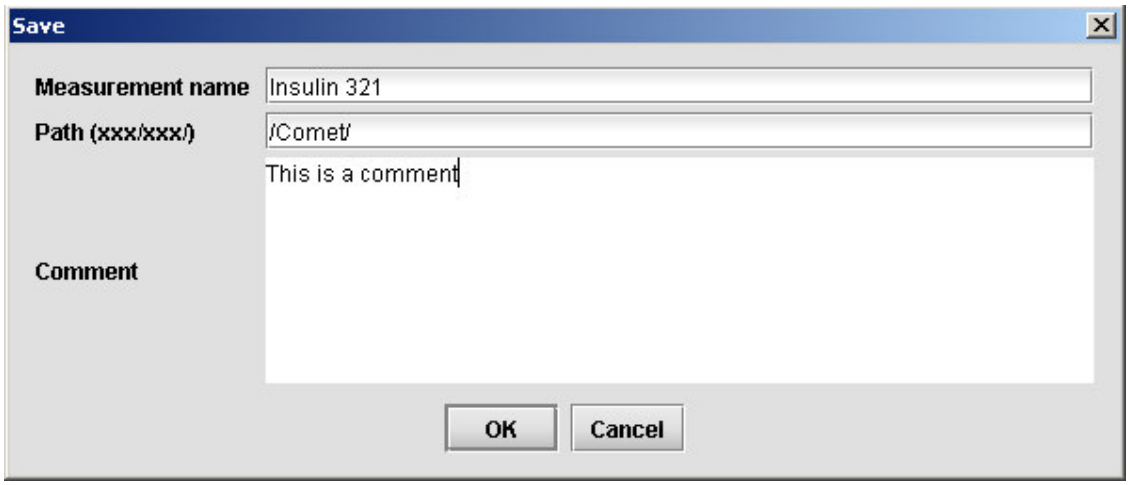
6.10. Saving spectra data

When an acquisition is completed, the spectrum can be saved in the macromizer™ database. This is done by clicking 'Save Actual' for the actual spectrum or 'Save Sum' for the summed spectrum.

When clicking the save button, the save dialog pops up. In this dialog the measurement name has to be entered (the 'filename').

It is also possible to enter a Path. This path will be used as the 'file placement' similar to a file system structure when retrieving the measurement in the Data Analyzer. If the Path is left empty, the measurement can be found in the Data Analyzer under the date (YYMMDD).

A Comment can be added to describe the measurement, and several lines of any text are permitted. This text will be visible in the Data Analyzer when printing the spectrum and scatter plot from the Data Analyzer (for details please refer to *chapter 8 Analyzing data, page 96*).

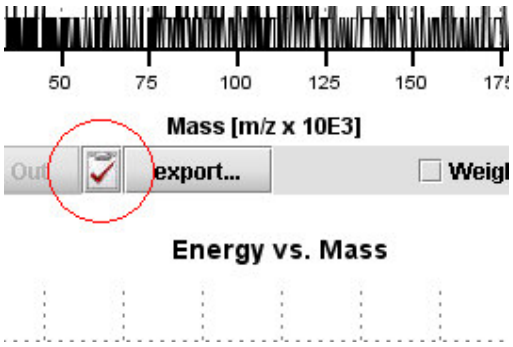


By clicking 'OK' the measurement is saved in the database. By clicking 'Cancel' the measurement is not saved.

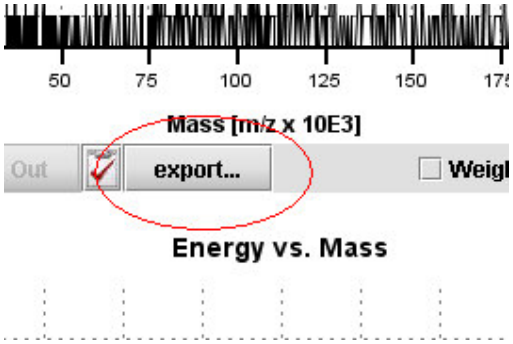
6.11. Exporting and printing spectra data

In the acquisition panel there are, for both the mass spectrum and for the scatter plot, two possibilities for transferring the graphics to another tool.

The first possibility is transferring the graphic to the Windows clipboard, where it can be pasted into standard office tools such as Microsoft Word. By clicking the icon indicated below, the graph above will be stored in the clipboard (no popup or dialogs are shown).

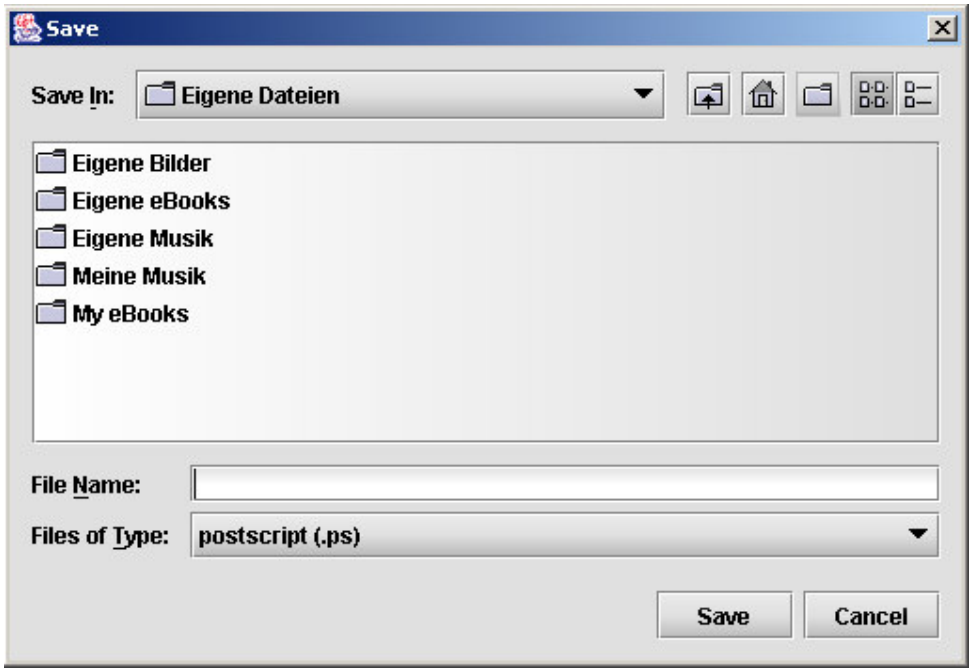


The second possibility is exporting the graphic to a file. By clicking the icon indicated below, the export dialog is shown.

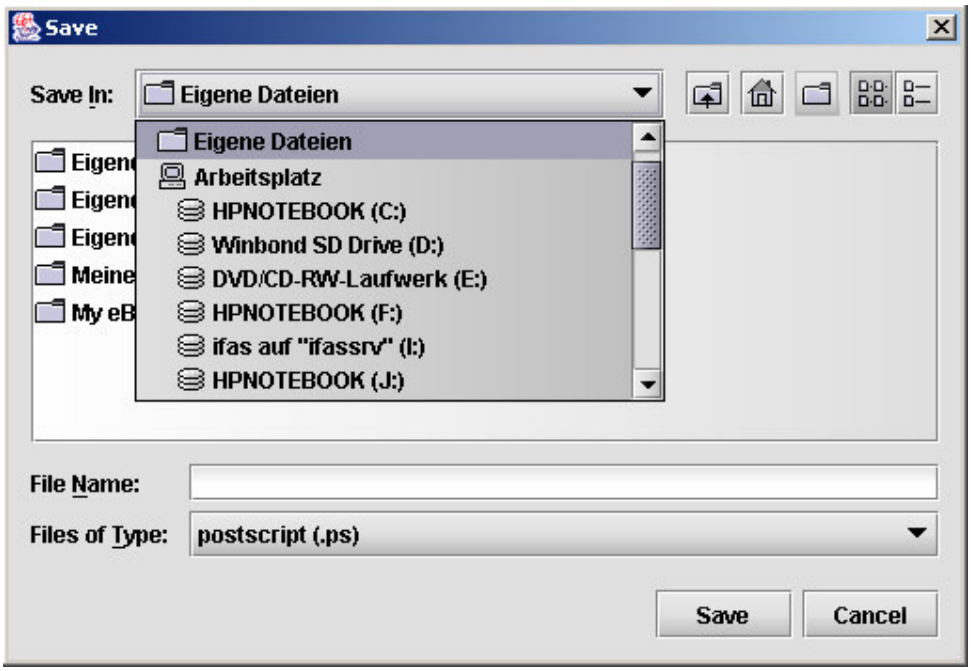


In the export dialog the file path, filename and format for the exported graphic must be selected. After the selections are made, click 'Save' to store the graphics in the file or click 'Cancel' to cancel the operation.

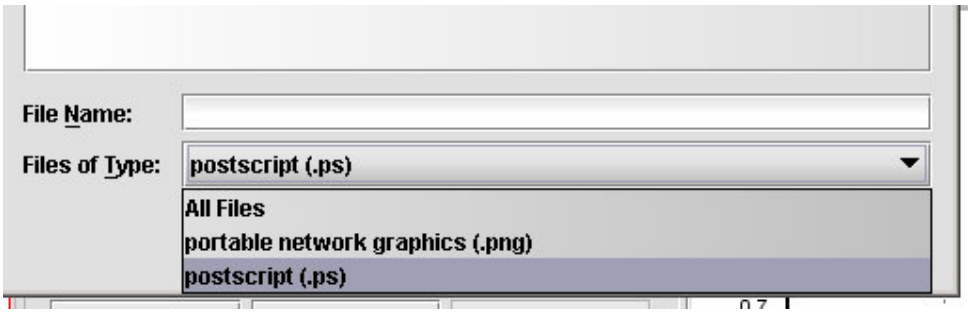
The filename is entered in the text box 'File Name'.



The path is selected in the pull down box 'Save in'.



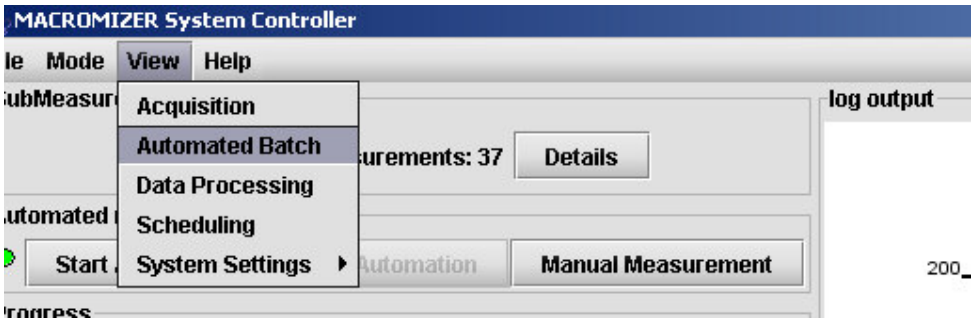
The file format is selected in the 'Data Format' pull down. Possible file formats are ‘.png’ (format to import in most text or graphics tools) or ‘.ps’ which is the standard PostScript format (Import format for Illustrater, print format for most printers).



7. Automated Batch

7.1. Selecting Automated Batch

The automated batch panel is selected by entering the 'View' menu in the upper menu bar and choosing item 'Automated Batch'.

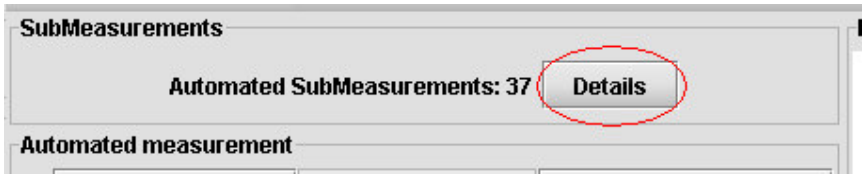


7.2. Submeasurement details

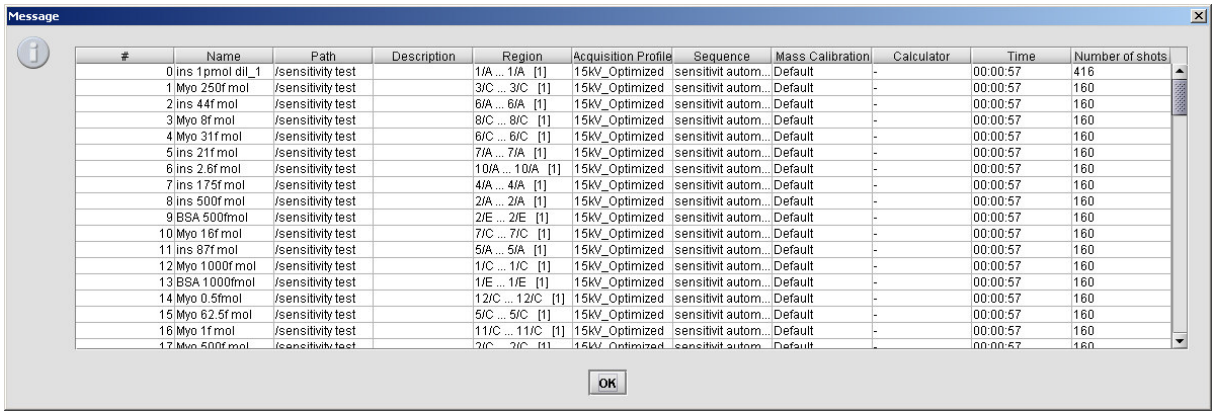
The measurements to be run are defined in the preparation application. Here a list of acquisitions are defined with all needed parameters and profiles.

The number of prepared measurements are shown in the automated batch panel.

By clicking the 'Details' button a list of the prepared measurements can be seen.



In the list of prepared measurements the planned acquisitions are shown in chronological order with all parameters and profiles entered in the preparation application.



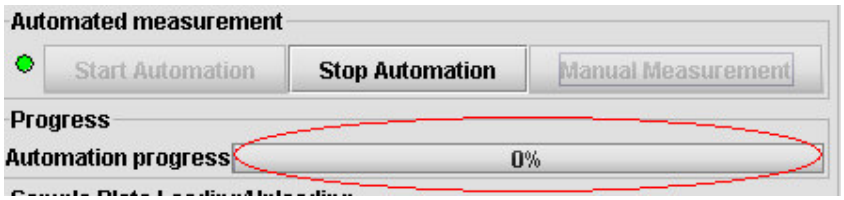
#	Name	Path	Description	Region	Acquisition Profile	Sequence	Mass Calibration	Calculator	Time	Number of shots
0	ins 1 pmol dil_1	/sensitivity test		1/A ... 1/A [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	416
1	Myo 250f mol	/sensitivity test		3/C ... 3/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
2	ins 44f mol	/sensitivity test		6/A ... 6/A [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
3	Myo 8f mol	/sensitivity test		8/C ... 8/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
4	Myo 31f mol	/sensitivity test		6/C ... 6/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
5	ins 21f mol	/sensitivity test		7/A ... 7/A [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
6	ins 2.6f mol	/sensitivity test		10/A ... 10/A [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
7	ins 175f mol	/sensitivity test		4/A ... 4/A [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
8	ins 500f mol	/sensitivity test		2/A ... 2/A [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
9	BSA 500fmol	/sensitivity test		2/E ... 2/E [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
10	Myo 16f mol	/sensitivity test		7/C ... 7/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
11	ins 87f mol	/sensitivity test		5/A ... 5/A [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
12	Myo 1000f mol	/sensitivity test		1/C ... 1/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
13	BSA 1000fmol	/sensitivity test		1/E ... 1/E [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
14	Myo 0.5fmol	/sensitivity test		12/C ... 12/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
15	Myo 62.5f mol	/sensitivity test		5/C ... 5/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
16	Myo 1f mol	/sensitivity test		11/C ... 11/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160
17	Myo 500f mol	/sensitivity test		3/C ... 3/C [1]	15kV_Optimized	sensitivit autom...	Default	-	00:00:57	160

The information cannot be changed here.

7.3. Starting and stopping automated measurements

The automated batch sequence is started by clicking the ‘Start Automation’ button in the menu area titled ‘Automated Measurement’.

While the acquisitions are running the ‘Automation Progress’ bar will show what percentage of the automated sequence has been completed. Additionally the logging information will continuously give information about the progress of the acquisitions.

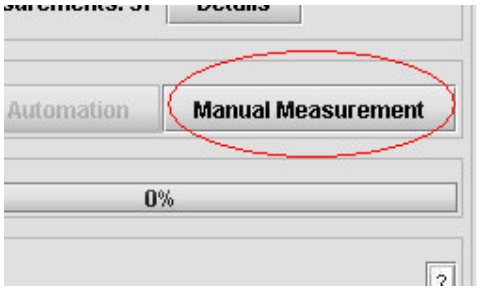


The automated sequence is stopped by clicking the ‘Stop Automation’ button. When the automation is restarted the acquisitions will start at the first measurement in the list.

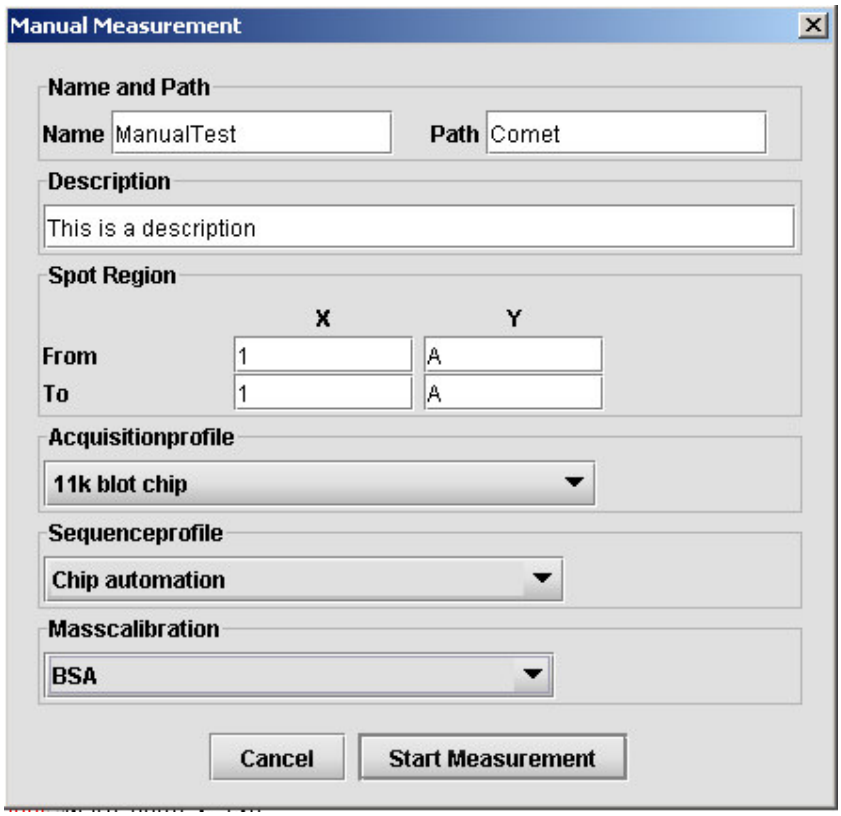
Whenever a measurement in the automated sequence has been completed (acquisition sequence complete and data saved in the database), it is removed from the list.

7.4. Setting up a measurement manually

Although all activities for the automated batch sequence are defined in the preparation application, it is possible to start an acquisition manually from the automated batch panel. This is done by clicking the 'Manual Measurement' button.



After selecting 'Manual Measurement', a popup is shown where all parameters for an acquisition can be entered. It is similar to the information as is entered when defining an entry in the automated batch sequence table.



After setting the parameters and selecting the profiles needed for the manual measurement, the acquisition is started by clicking the 'Start Measurement' button. The progress and result of the manual measurement is seen in the logging information.

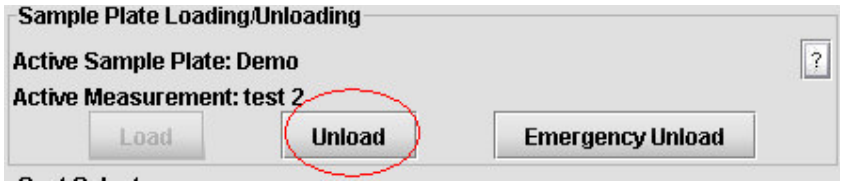
7.5. Sample plate load an unload

After a sample plate and the automated batch sequence are prepared, the sample plate is loaded into the macromizer™ by placing it in the sample plate load slot and the 'Load' button, which is located in the 'Sample Plate Loading/Unloading' area, is clicked.

After the 'Load' button is clicked the same sequence of activities are performed as described in the section 'Loading sample plate in the macromizer™' in the 'Acquiring spectra' chapter.

Please note that by loading the sample plate, the popup for selecting the acquisition method is shown. The selected method in this popup is overwritten by the method set for the actual batch sequence at the time of starting the automated batch sequence.

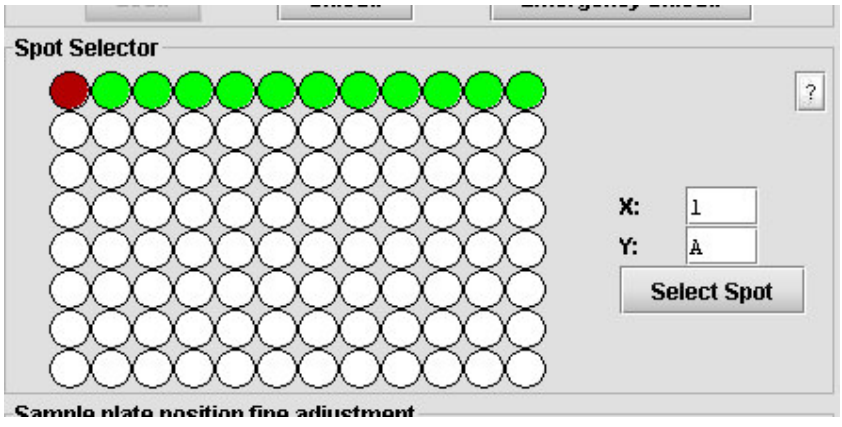
Unloading of the sample plate is done by clicking the 'Unload' button.



7.6. Selecting a spot

Selection of the sample plate spot is done automatically while the automated sequence is running.

It is also possible to manually select a spot in the 'Spot Selector' menu area, when the automation sequence is not running.



For more information on selecting spots and navigating in the camera image, please see the description in *chapter 6.3 Selecting a sample plate position, page 77.*

7.7. Export mass spectrum

In the automated batch panel there are two possibilities for transferring the mass spectrum graphics to another Software program/package.

The first possibility is transferring the graphic to the Windows clipboard, where it can be pasted into standard Software packages such as Microsoft Word. By clicking the icon indicated below, the graph above will be stored in the clipboard (no popup or dialogs are shown).

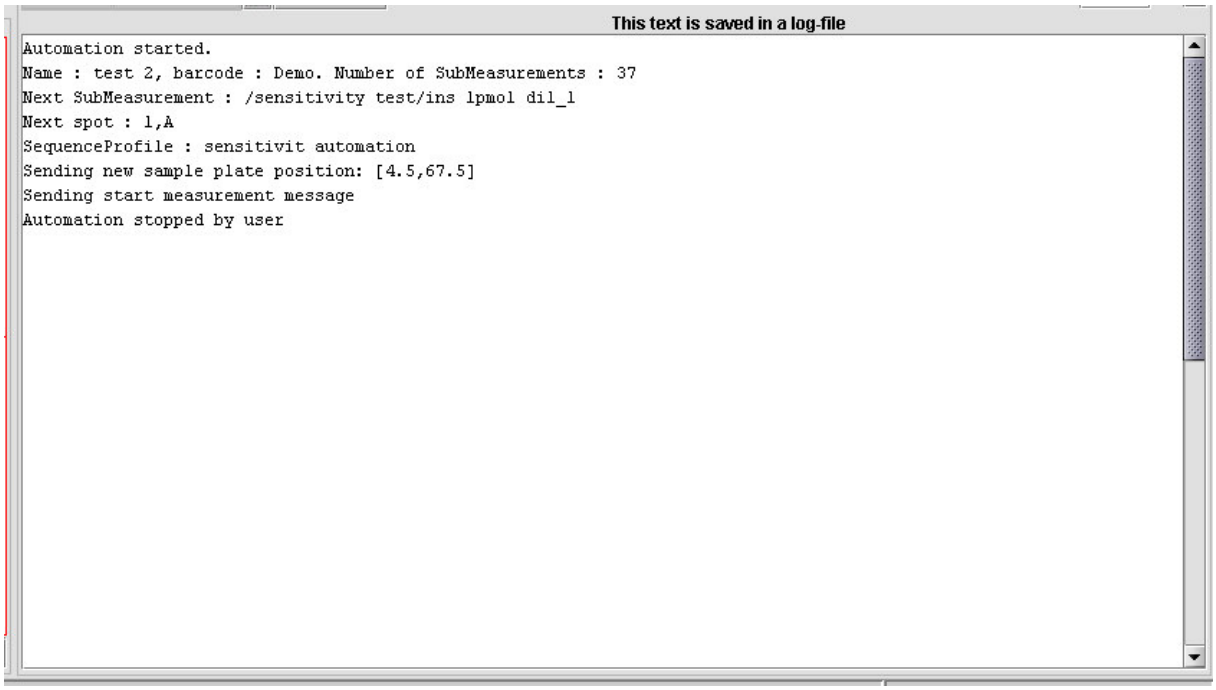
The second possibility is exporting the graphic to a file. By clicking the icon indicated below, the export dialog is shown.

In the export dialog the file path, filename and format for the exported graphic must be selected. After the selections are made, click ‘Save’ to store the graphics in the file or click ‘Cancel’ to cancel the operation.

For more detailed information regarding the export dialog please refer to the exporting and printing spectra in the section Acquiring spectra.

7.8. Logging information

While an automated batch sequence is running, the logging window will show the status and progress of the batch.



Additionally this information is being written to a text file (placed in the log-file directory) with the name '<date><time><sample plate name>.log'

After a completed unattended run, this file can be consulted for verifying the result.

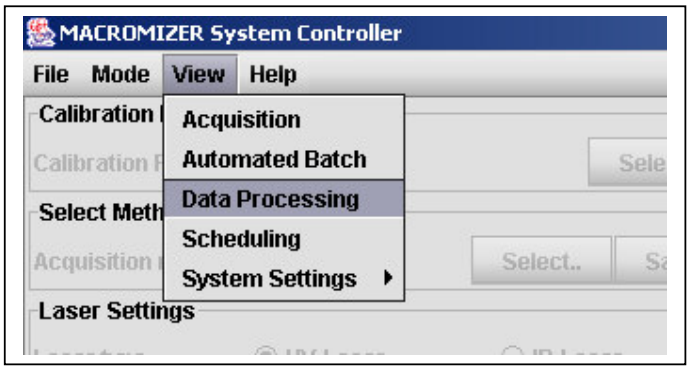
8. Analyzing data

8.1. Selecting the data analyzer panel

The data analyzer panel can be accessed either through the system control application as an embedded part or as stand-alone through the data analyzer application.

8.1.1. Embedded data analyzer panel

Start the system control as described in *chapter 2.2 Starting the software, page 9* and select in the 'View' menu 'Data Processing'.

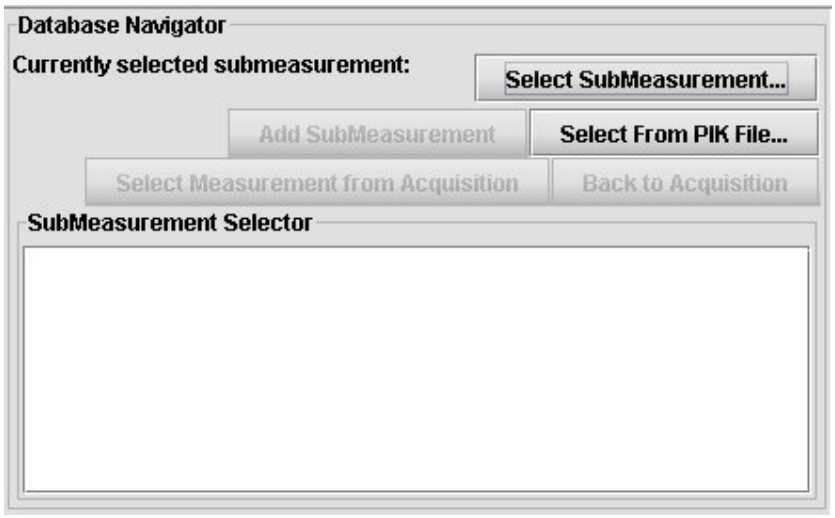


8.1.2. Stand alone data analyzer panel

To start the stand-alone data analyzer panel, double click the Data Analyzer icon.

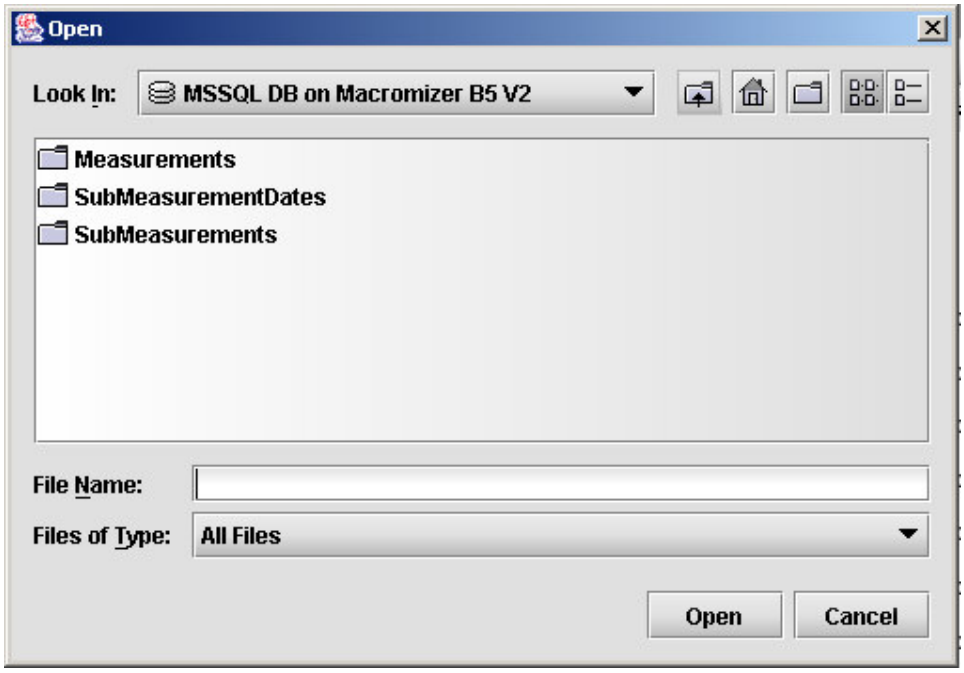
8.2. Loading a spectrum

This chapter describes the functions that are located in the bordered area labeled 'Database Navigator' in the upper left corner.



8.2.1. Loading one spectrum ('Select SubMeasurement')

After pushing the button 'Select SubMeasurement...', a file chooser will display three directories. The directory 'Measurements' contains the spectra filed first by measurement, then by their paths. The directory 'SubMeasurementDates' contains the spectra filed by date, then by their paths. Lastly, the directory 'SubMeasurements' contains the spectra only filed by their paths (the spectra which have no specific path are filed by date). The three directories are different ways of accessing the spectra, but the content is always the same.



After navigating to the appropriate spectrum and double clicking the name or pressing the ’Open’ button, the spectrum will begin to load. A loading dialog is shown until the spectrum is fully loaded. Several controls on the data analyzer panel will become enabled. The name of the active spectrum is shown just below the label ’Currently selected SubMeasurement’. The filename will also appear in the bordered area labeled ’SubMeasurement Selector’. The function of this area will be summarized in the section ‘Switching between loaded spectra’.

8.2.2. Loading multiple spectra (‘Add SubMeasurement’)

After having loaded a spectrum by means of the 'Select SubMeasurement' button, it is possible to add further spectra, which will overlay the ones previously loaded. The different spectra are not mixed in any way, but serve as a source of comparison. Pressing the 'Add SubMeasurement' button will bring up the same file chooser as the one clicking the 'Select SubMeasurement' button. The section "Loading one spectrum" contains further details. When the application has finished loading the chosen spectrum, there will be an additional spectrum label in the area called 'SubMeasurement Selector'.

8.2.3. Switching between loaded spectra (‘SubMeasurement Selector’)

If there are multiple spectra loaded, it is possible to switch between them by selecting the appropriate spectrum name from the list in the area called 'SubMeasurement Selector'. Below the label 'Currently selected SubMeasurement' the currently active SubMeasurement is shown.

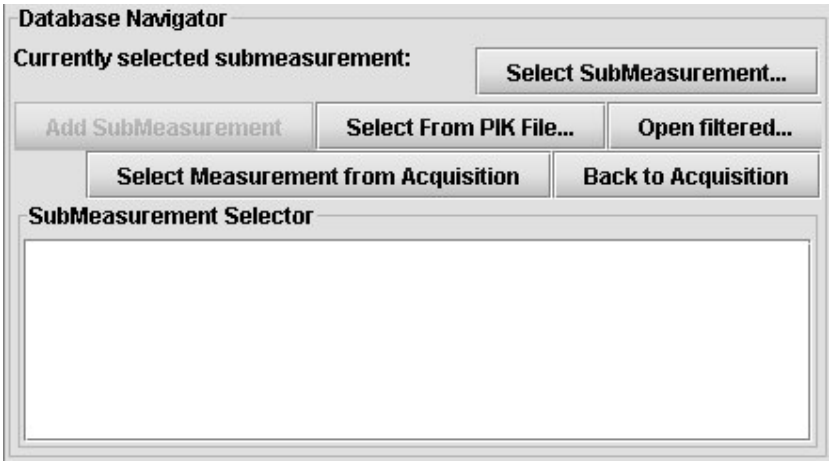
8.2.4. Interacting with the SystemControl (‘Select SubMeasurement from Acquisition’, ‘Back to Acquisition’)

If the data analyzer panel is accessed through the System Control, the buttons 'Select SubMeasurement from Acquisition' and 'Back to Acquisition' are enabled. If not, these button have no use and are disabled.

When the button 'Select SubMeasurement from Acquisition' is pressed the acquired data from the System Control is loaded without saving it first in the database.

Pressing the button 'Back to Acquisition' switches to the System Control panel. It is a substitute of the menu item 'Acquisition'.

8.3. Loading a spectrum from the acquisition panel



If the data analyzer panel is accessed through the System Control, the buttons 'Select Measurement from Acquisition' and 'Back to Acquisition' are enabled. If not, these button have no use and are disabled.

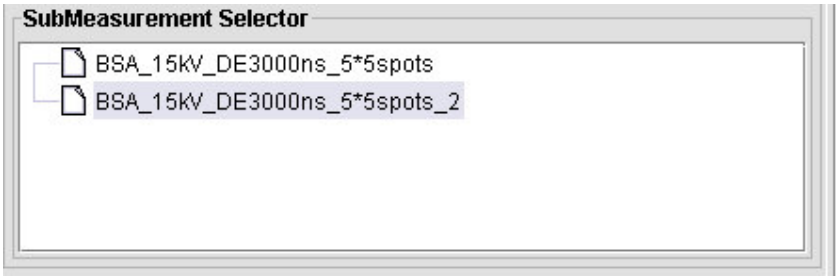
When the button 'Select SubMeasurement from Acquisition' is pressed the acquired data from the System Control is loaded without saving it first in the database.

Pressing the 'Back to Acquisition' button switches to the System Control panel. It is a substitute of the menu item 'Acquisition'.

8.4. Loading more than one spectrum

After having loaded a spectrum by means of the 'Select SubMeasurement' button, it is possible to add further spectra which will overlay the ones previously loaded. The different spectra are not mixed in any way, but serve as a source of comparison. Pressing the 'Select SubMeasurement' button will bring up the same file chooser as the one reached by clicking the 'Select SubMeasurement' button. The section 'Loading one spectrum' contains further details. When the application has finished loading the chosen spectrum there will be an additional spectrum label in the area called 'SubMeasurement Selector'.

8.4.1. Switching between loaded spectra ("SubMeasurement Selector")



If there are multiple spectra loaded, it is possible to switch between them by selecting the appropriate spectrum name from the list in the area called ‘SubMeasurement Selector’. The currently active SubMeasurement is shown below the label ‘Currently selected SubMeasurement’.

8.5. Generation of the mass spectrum

This chapter explains in detail the calculation and visualisation of the histogram.

8.5.1. Overview

The binning of the data is always done with the TOF values. The mass conversion is done after the binning. The histogram has two different visual forms. The mass dependent histogram and the running histogram. While the mass dependent histogram is used to show a wide mass range, the running histogram is used for data processing and for showing data with a narrow range. Furthermore the weighting of the events is explained. There is a normal weighting where each event has a weight of one and there is a energy dependent event weighting.

8.5.2. Mass dependent histogram

The reason why the mass dependent histogram was introduced, is the performance of the chart. Having a huge number of bins slows down noticeably the rendering of the histogram.

8.5.2.1. Calculation

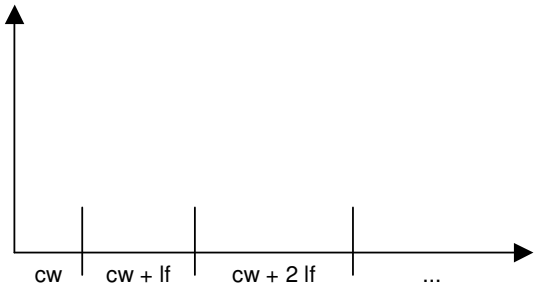
The class width is obtained as follows

$$cw_n = cw_{\min} + n \cdot lf$$

where cw_{min} and *lf* are free parameters, which can be set in the properties or directly in the application and *n* describes the number of the bin. cw_{min} is the minimal class width with which the histogram starts at low tof. For each following bin the class width grows by a factor *lf*.

Example:

$$cw_0 = cw_{\min}$$
$$cw_1 = cw_0 + lf = cw_{\min} + lf$$
$$cw_2 = cw_1 + lf = cw_{\min} + 2 \cdot lf$$
$$cw_3 = cw_2 + lf = cw_{\min} + 3 \cdot lf$$



The formula to find n from a given class width cw_n:

$$n = (cw_n - cw_{\min})/lf \quad .$$

To calculate the tof of the nth bin the following formula can be used:

$$tof_n = n \cdot cw_{\min} + n \cdot (n-1)/2 \cdot lf$$

.

And to obtain n from a given tof:

$$n = \sqrt{(2 \cdot cw_{\min}/lf - 1)^2 + 2 \cdot tof_n/lf} + 0.5 - cw_{\min}/lf$$

.

8.5.2.2. Where to set the class width

1. In the 'System Setting' menu the menu item 'Configuration' allows to change property values. The parameter name of cw_{min} is SYS_MCW_P. The unit is seconds. The parameter name of *lf* is SYS_LFCW_P, and the unit is as well seconds.
2. In the data analyzer panel pressing the button 'set classwidth' will show a dialog with two spinboxes called 'min. class width' and 'linear factor', where 'min. class width' equals to cw_{min} and 'linear factor' to *lf*.

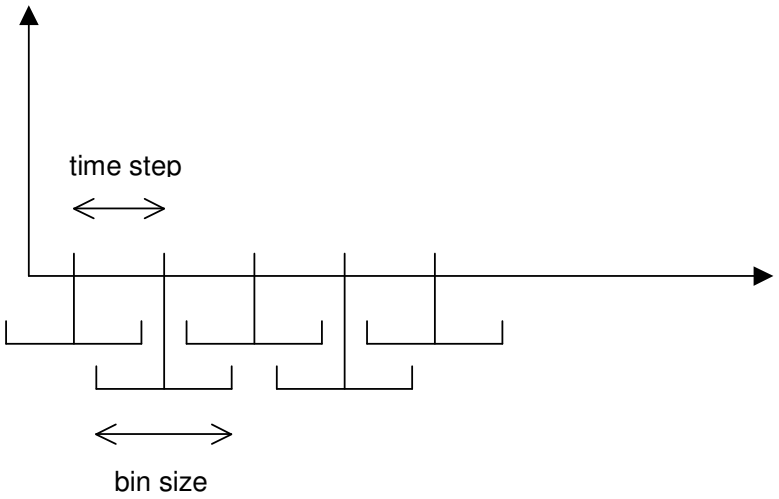
8.5.2.3. Example values

A set of possible values is show in the table.

SYS_MCW_P	SYS_LFCW_P	Comment
0.1e-8	0.1e-8	The default values

8.5.3. Running histogram

The speciality about the running histogram is the overlapping bins. This was introduced due to the small amount of events. Though they are highly significant it is not possible to calculate a correct histogram with insufficient events. The sum of the heights of each bin is therefore not the sum of all events used. The *time step* defines the step between the positions of the histogram points. The *bin size* defines the range which will be used to count the events. As can be seen in the drawing the bins do overlap. At the edges the of the running histogram the data is sloping down to zero. This region has a width of a half bin on each side and is not suitable for data processing.



The time step is calculated automatically. For peak picking it is set to a minimal value of 20e-9s.

8.5.3.1. Where to set the bin size

1. In the ‘System Settings’ menu the menu item ‘Configuration’ allows to change property values. The parameter name of the bin size is SYS_RUCW_P, the unit is seconds.
2. In the data analyzer panel pressing the button ‘set classwidth’ will show a dialog with a spinbox called ‘running class width’ meaning the ‘bin size’. The input is in micro seconds for convenience.

8.5.3.2. Example values

SYS_RUCW_P	Comment
360e-9s	The default value

8.5.4. Criteria

When the time step is bigger than the bin size, the mass dependent histogram is chosen. Otherwise it is the running histogram.

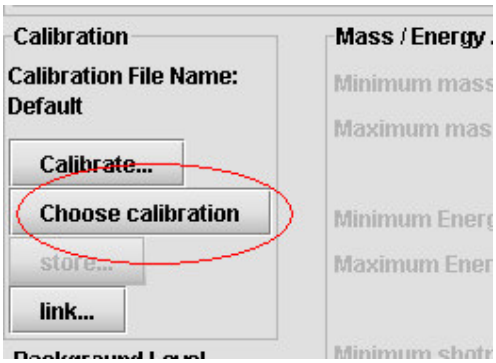
8.5.5. Weighting

There are two possibilities to weigh the events. The first and default is a uniform weighting. Each event counts as one. The second is energy weighted, the energy is summed. Like that an event with high energy is weighted more than one whit low energy.

8.6. Selecting a calibration file

After loading a new acquisition into the Data Analyzer, the spectrum is calibrated with the calibration file chosen by the acquisition process. If no calibration file was chosen, the default calibration is automatically applied.

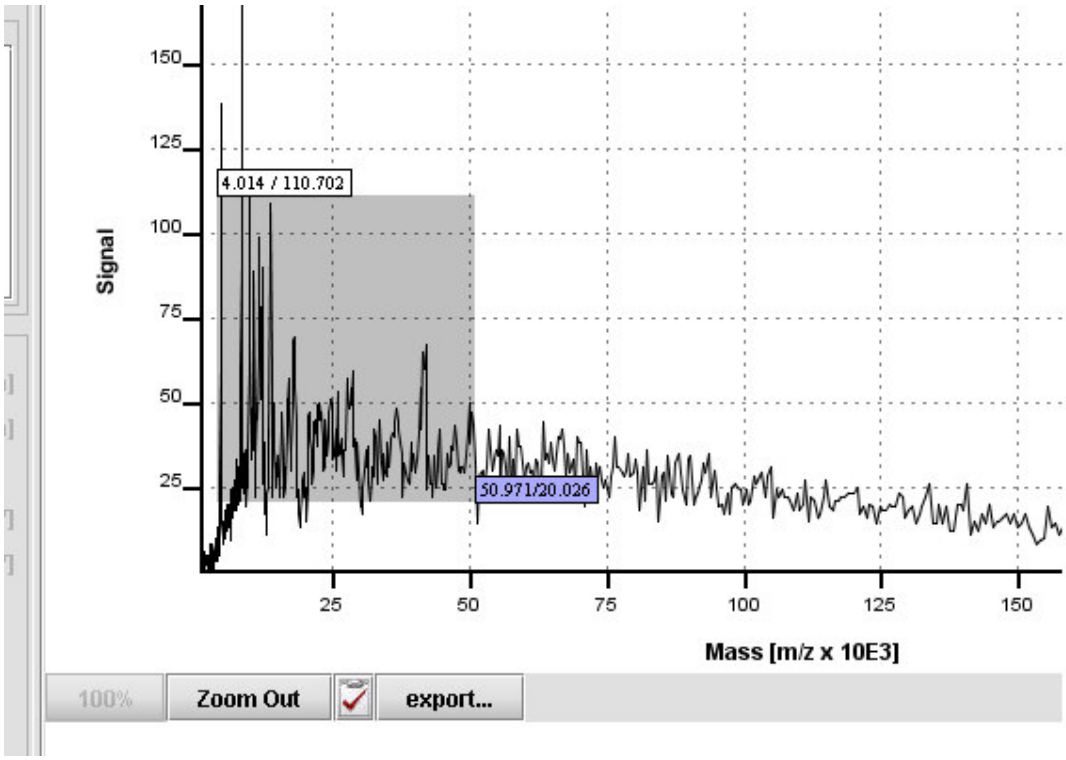
In the 'Calibration' area of the Data Analyzer panel it is possible to choose another calibration file for the spectrum. For the description on how to create a new calibration file please refer to *chapter 9. Using and creating calibration methods, page 130.*



For steps on choosing a new calibration file, please refer to *chapter 9. Using and creating calibration methods, page 130.*

8.7. Zooming

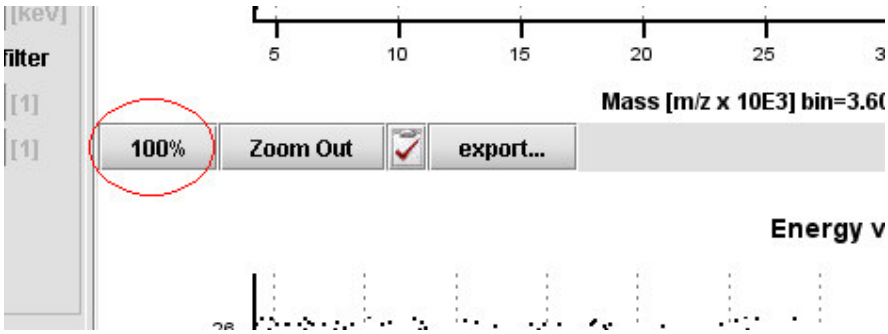
Zooming the graphs in the Data Analyzer is possible for both the spectrum and the scatter plot by using the mouse.



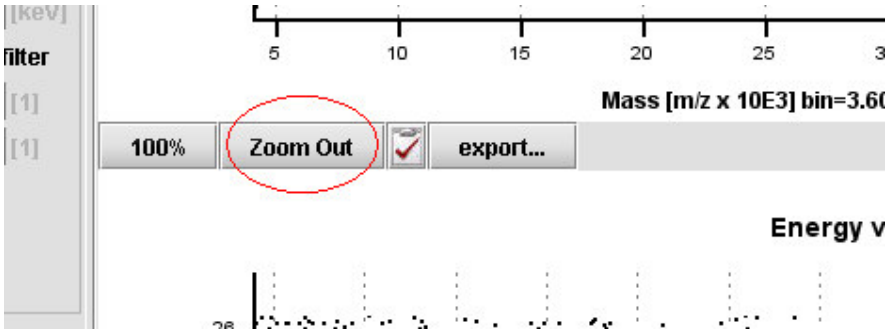
Zooming in the mass spectrum is done by holding the left mouse button activated and dragging a box. While dragging the box both the start and end points are shown.

In releasing the button a zoom to the selected mass area is done, but no zoom in the signal level occurs. Instead, the signal axis is rescaled to show the full spectrum for the selected mass area.

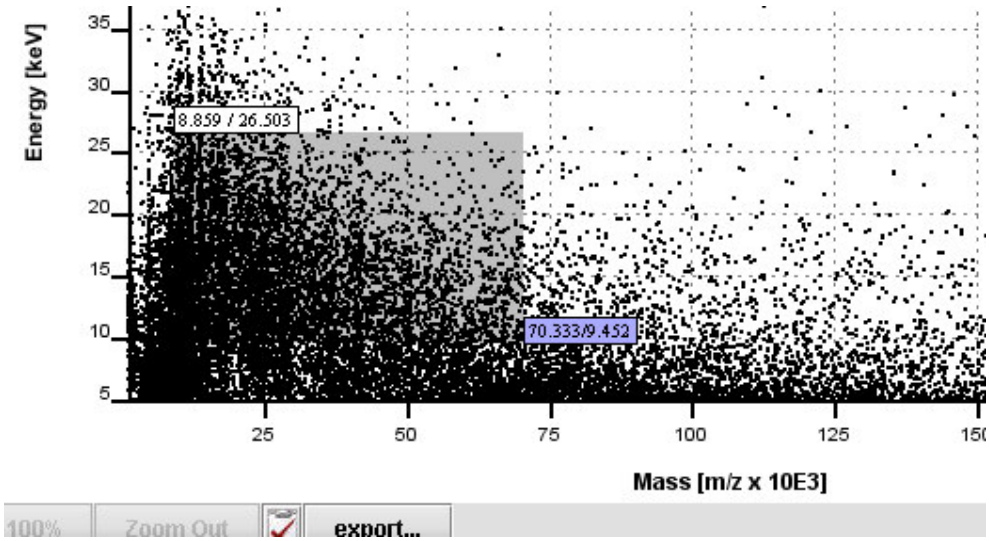
The full spectrum is shown by clicking the ’100%’ button.



By clicking 'Zoom out' the mass scale is zoomed out a single step and the signal axis is rescaled to show the complete spectrum for the mass range.



Zooming in the scatter plot is done by dragging a box while holding the left mouse button activated. A zoom to the selected area is done by releasing the button.

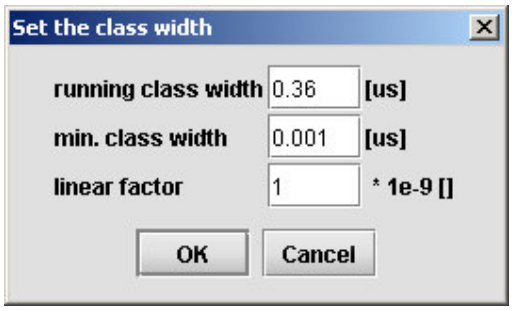


Zoom to full is done by clicking the '100%' button. Zooming out in steps is done by clicking the 'Zoom Out' button.

8.8. Setting the class width

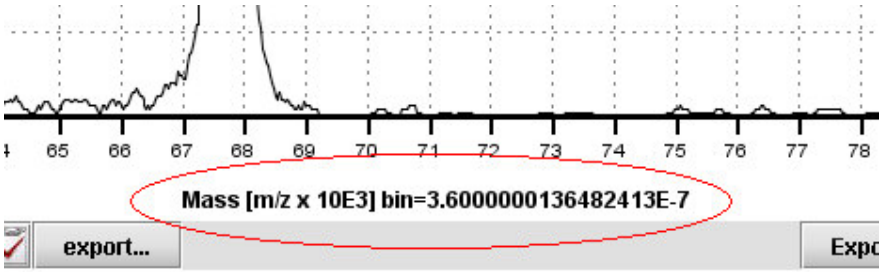
The class width can be set in the area labeled 'Histogram Control'.

When pressing the button 'set classwidth...' in the bordered area labeled 'Histogram Control' a popup dialog will appear with various parameters defining the binning of the histogram. The redefining of the class width will render the picked peak invalid.



The histogram has two different view styles:
One is a mass dependent histogram, which is chosen automatically when the screen resolution cannot resolve the whole histogram.
The other is a running histogram generated with the chosen class width and linear factor.

The lower two values ('min. class width' and 'linear factor') belong to that type of histogram calculation.
When zooming in, the display of the histogram will change to a running histogram at a certain stage. This change can be seen when the x-axis label additionally displays the bin width. The first parameter belongs to the running histogram calculation.



8.8.1. Mass dependent histogram

The mass dependent histogram is for convenience only and does not influence the peak picking. The first parameter called 'min. class width' is the minimum class width to begin to bin the histogram at the low mass end. For each next bin the value of the 'linear factor' is added, thus resulting in a growing bin size of the factor 'linear factor'.

8.8.2. Running histogram

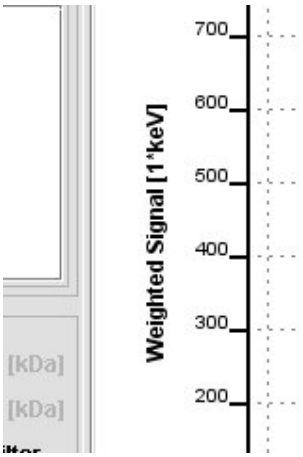
The running histogram consists of overlapping bins. The bin step is smaller than the bin size. The parameter 'running class width' will set the size of the bin. The step per bin is set automatically by the application. The peak picking is done with a running histogram with the bin size provided here. Changing this parameter may result in slightly different peak masses.

8.9. Weighted histogram spectrum

The weighted histogram can be chosen in the area labeled 'Histogram Control'.

In the bordered area called 'Histogram Control', the checkbox to the right, labeled ‚Weighted Histogram' controls the weight of the counts in the histogram.

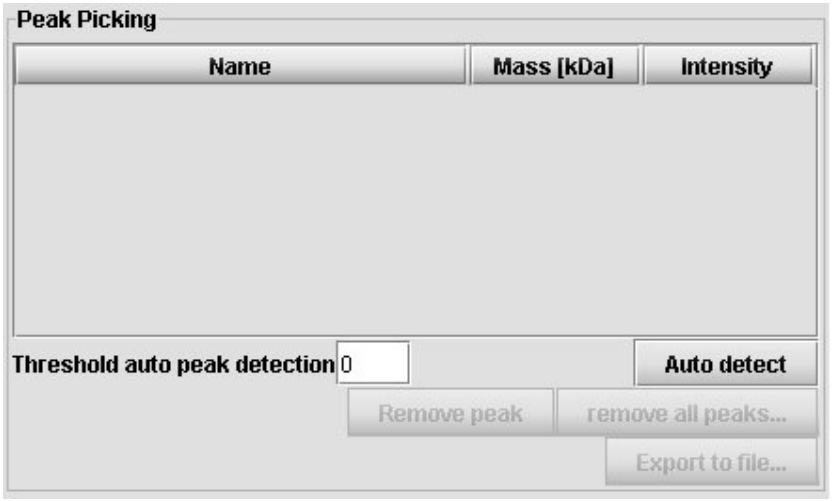
When the checkbox is not checked, each event counts as one. If the checkbox is checked each event is weighted by its energy, and events with higher energy (typically multiple ion impact events) are given higher account.



The label of the y-axis of the histogram changes when the checkbox is checked or unchecked.

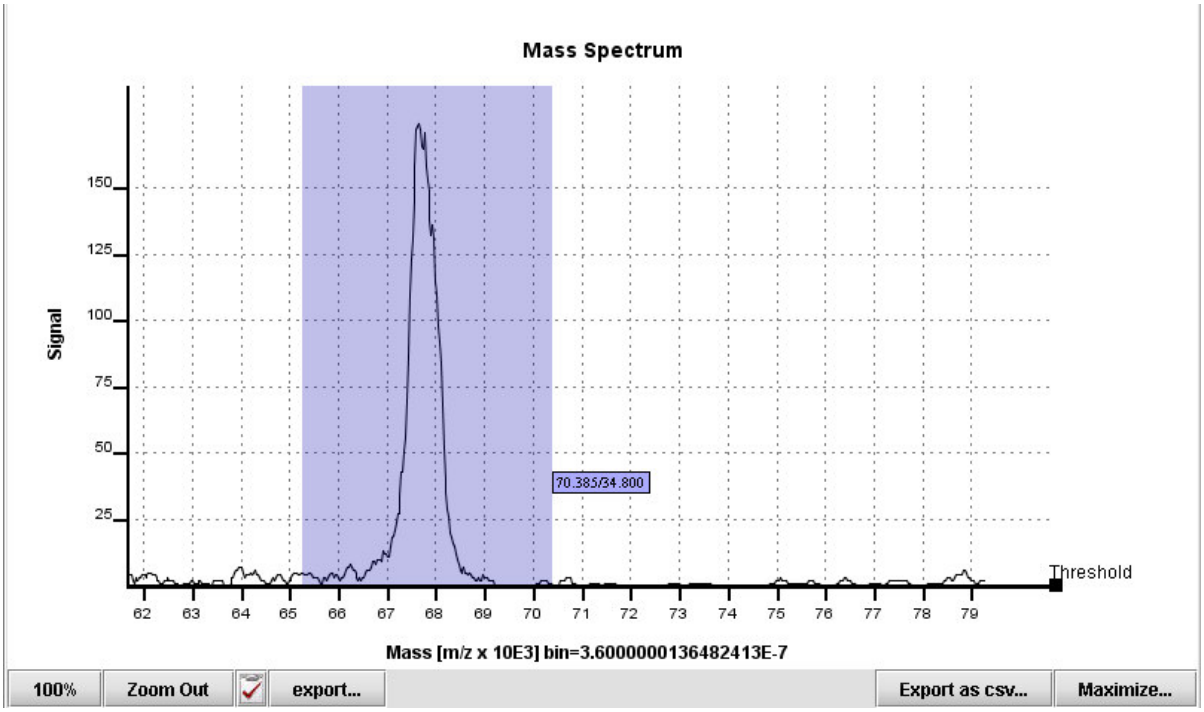
8.10. Picking peaks manually and automatically

After picking a peak, the peak data will be displayed in a table in the bordered area called 'Peak Picking'. The name of the peak can be set in the column 'Name'. The default name is given as a string representation of the mass. The column 'Mass [kDa]' displays the mass of the peak in kiloDalton. The column 'Intensity' displays the number of events forming that peak.

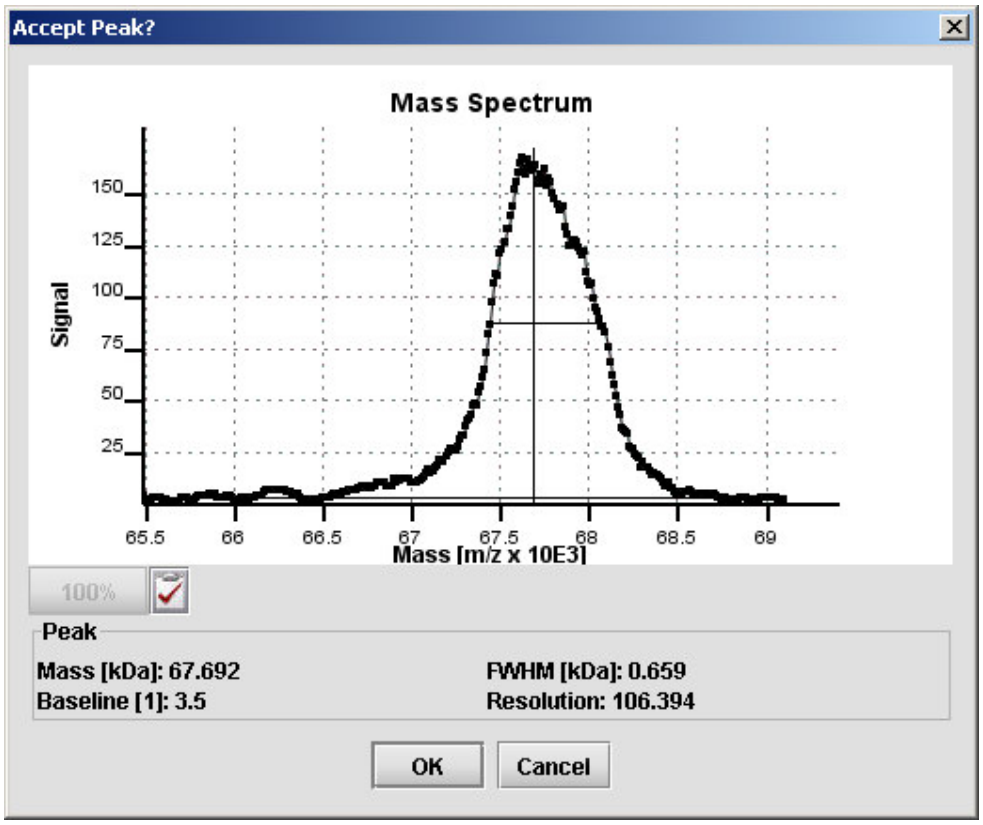


8.10.1. Picking peaks manually

Holding down the right mouse button in the histogram chart and dragging it over a peak will select that particular peak.



When the right mouse button is released a popup dialog will appear to confirm the picked peak.



The peak characteristics are displayed in the bordered area labeled 'Peak': the mass in kiloDalton, the baseline, the full width at half maximum (FWHM) in kiloDalton, and the resolution.
Clicking 'OK' will place that peak into the peak table and the picked peak receives a label.

8.10.2. Picking peaks automatically

The threshold value has to first be set either by providing a value in the number box or by dragging the threshold line in the chart. All peaks above that threshold will be processed. Pressing the 'Auto detect' button will pick the peaks and store them in the peak list table.

8.10.3. Maintainance of the peak list

When a peak row in the table is selected the 'Remove Peak' button will remove the selected peak. Pressing the 'Remove all peaks...' button will remove all peaks. A warning popup dialog will be shown first to ask for certainty.

8.10.4. Exporting the peak table ('Export to file...')

When pressing the 'Export to file...' button a file chooser dialog will appear. The peak table will be saved in the '.csv' format at the selected directory.

8.11. The peak picking algorithm

The peak picking mechanism consists firstly of processing the histogram data to determine the region of the peak. Secondly the region will be gauged to obtain the exact peak mass, the FWHM (full width half maximum) and further parameters. When the histogram is weighted, different formulas are required. for this case a separate chapter is done.

8.11.1. Peak Determination

The region to focus on where a peak shall be processed is set by the user (see getting started peak picking). The algorithms will be applied to that region.

1. The histogram of the selected region is recalculated internally as a running histogram with the highest resolution (params...).
2. That histogram will be searched for the highest point p_{top}, which gives a first impression of the peak mass.
3. A least square fit with power 2 is determines the exact mass of p_{top}.
4. The baseline is calculated
5. Determination of the left and right slope.
6. The FWHM is calculated.

Assuming a chosen area with these variables:

Variable	Range	Unit	Comment
n	-	1	The number of selected points
p(mass, counts)	$p_0..p_n$	p(Da, 1)	The data points

The minimum of n must be three.

Used notation for the technical terms:

p_k means the kth point (in the range of 0 to n).

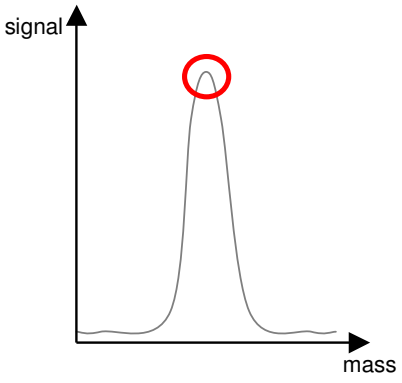
p_k^{mass} means the mass of the kth point (in the range of 0 to n)

p_k^{counts} means the counts of the kth point (in the range of 0 to n).

p_k^{tof} means the time of flight of the kth point (in the range of 0 to n).

Finding the highest point:

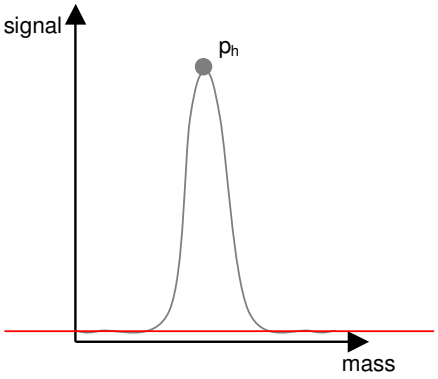
While browsing from point 0 to point n the point p_h with the highest count will be searched.



8.11.2. The baseline

The baseline is calculated according to

$$baseline = \frac{p_n^{counts} + p_0^{counts}}{2}.$$

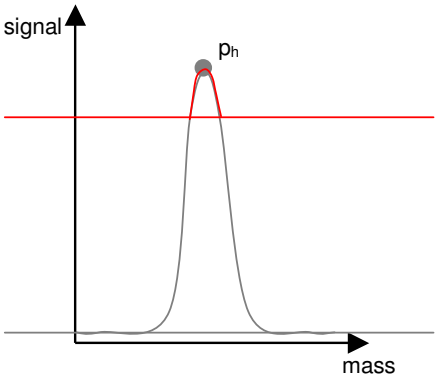


Caution:
The baseline is thus dependent on the user selection since p_0 and p_n are the borders of the mentioned selection. Assuming a 'reasonable' selected range this assumption will work.

With the highest point and the baseline a peak is now outlined. It will be referenced as the 'first order peak'.

Peak Top

The points near p_h and with a count value which is in the upper 20% of the first order peak height are selected. The peak height *height* = p_h^{counts} - *baseline*.



$$q(mass, counts) = p_h^{counts} > p_k^{counts} > p_h^{counts} - height * 0.2$$

with k = h...n and k= h...0.

q must be continuous. At a minimum the two adjacent points to p_h are selected.

A least square fit with power 2 (parable) is performed with the selected q's.
The point of inflection (?) becomes the actual peak top p_{top}.

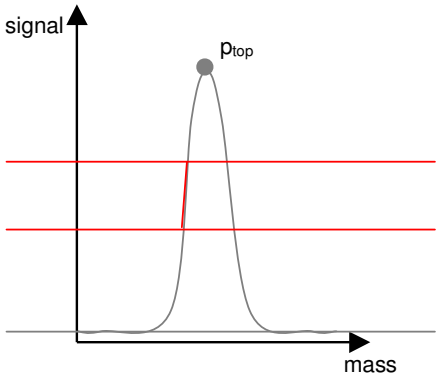
To determine the FWHM, two line fits are done on each side of the first order peak.

The left slope

All points lying between 40% and 60% of the peak height are selected:

$q(mass, counts) = p_h^{counts} - height * 0.4 > p_k^{counts} > p_h^{counts} - height * 0.6$ with k = h...0

A least square fit with power 1 (line) is performed with the selected q's to gain the sloping line.
At the y value of $baseline + (p_h^{counts} - baseline)$ the mass is determined with the line equation resulting the point p_{ls} as the beginning of the FWHM.

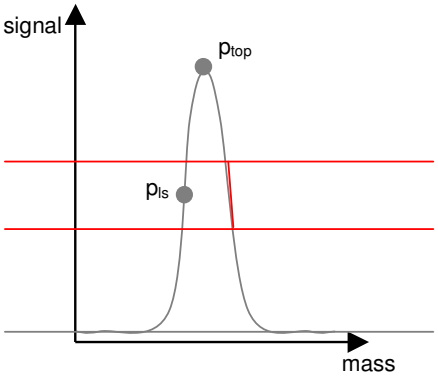


The right slope

All points lying between 40% and 60% of the peak height are selected:

$q(mass, counts) = p_h^{counts} - height * 0.4 > p_k^{counts} > p_h^{counts} - height * 0.6$ with k = h...n

A least square fit with power 1 (line) is performed with the selected q's to gain the sloping line.
At the y value of $baseline + (p_h^{counts} - baseline)$ the mass is determined with the line equation resulting the point p_{rs} as the beginning of the FWHM.



The FWHM is then: $FWHM^{tof} = p_{rs}^{tof} - p_{ls}^{tof}$.

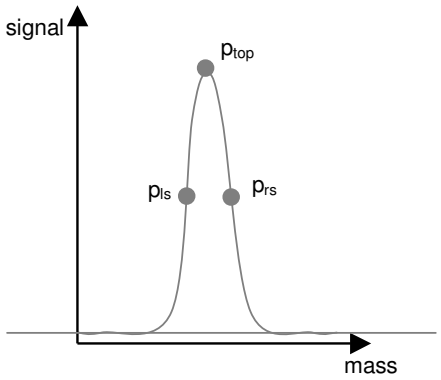
Caution:

The peak picking is processed with a running histogram. By that the FWHM is slightly broader because of the blurring effect of the running histogram.

The resolution

The resolution is calculated according to

$$R = \frac{p_{top}^{tof}}{FWHM^{tof}}$$



8.11.3. Weighted Peak Determination

When using a weighted histogram, the peak picking procedure is the same as with a default histogram.
The results though need not be the same. In every formula where the *count* value is used, the results will differ.

8.11.4. Multipeak picking

In this version of software multiple peaks are recognised but not processed. This feature will be implemented in a newer version. If there is a case of multi peak the program shows an error. This chapter describes the mechanism how a multiple peak is identified.

The Peak Processing method described in a previous chapter only gives good and reliable results for a single peak. Therefore before processing the data, it is checked for multiple peaks.

8.11.5. Abstract

1. Finding maxima
2. Finding minima
3. Compare delta

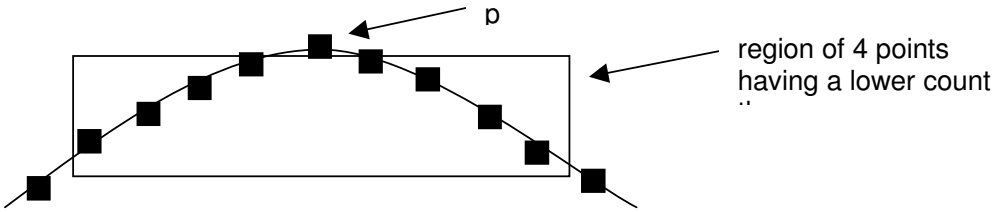
8.11.6. Method

The base data consists of all the events chosen by the user by right mouse dragging a region in the spectrum. *(See chapter 8.10 Picking peaks manually and automatically, page 108).*

Variable	Range	Unit	Comment
n	-	1	The number of selected points
p(mass, counts)	$p_0..p_n$	p(Da, 1)	The data points

1. Finding maxima

A maximum is defined as a point with a lower environment. n (default n=4) points next to the examined point p have to have lower counts to qualify p as a maximum.



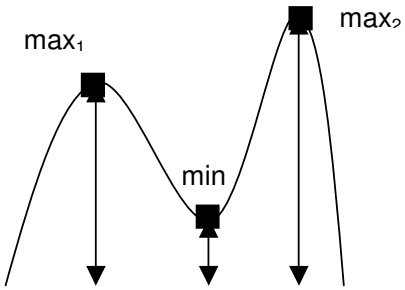
Browsing through the data from lower TOF to higher TOF. Only counts above the 40%-limit of the global maximum are looked at. The area below 40% is not used when processing a peak and thus not interesting. The possible peaks are stored temporary in a list.

2. Finding minima

If there are more than one maximum in the above mentioned list, minima are searched in the following manner: Between two maxima the lowest point is seeked out.

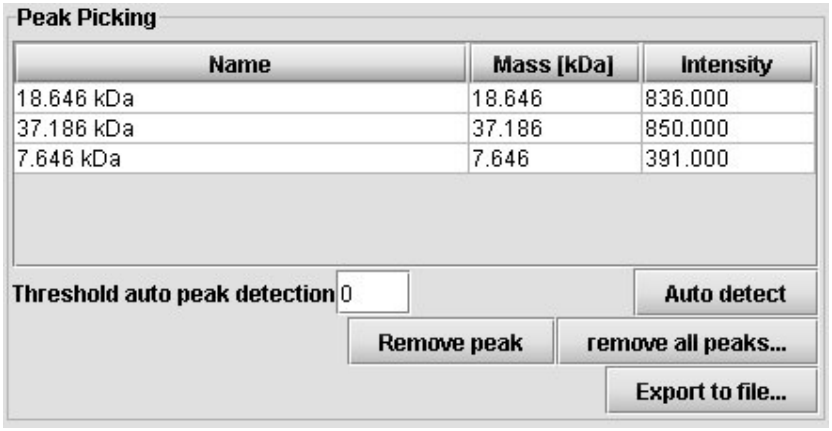
3. Comparing the delta

If the relative delta of the minimum and the smaller maximum is bigger than 20% the right maximum is counted as a peak.



$$\text{delta} = (\text{max}_1 - \text{min})/\text{max}_2$$

8.12. Peak list and peak data



The peak list consists of three columns:

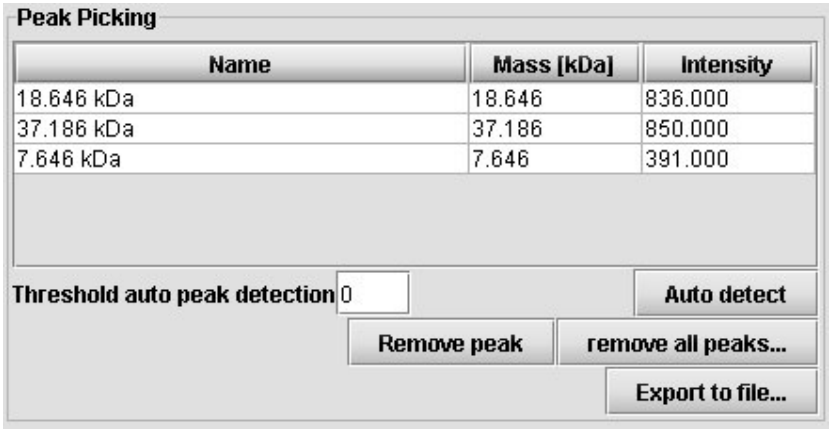
1. **The Name**: The name field is editable and contains the mass with the unit as a default. By double clicking the name field a new name can be entered.
2. **The Mass**: The mass field contains the mass in units of kDa.
3. **The Intensity**: Intensity is a value describing the height of the peak. The number is calculated by counting all the events between the picked mass plus/minus half of the FWHM.

The 'Threshold auto peak detection' value and the 'Auto detect' button are explained in the *chapter 8.10 Picking peaks manually and automatically, page 108.*

By pressing the 'Remove peak' button the selected peak will be deleted.
By pressing the 'Remove all peaks...' button a dialog appears asking to remove all peaks. If confirmed, all peaks are deleted.
By pressing the 'Export to file...' button the peak list can be saved as a '.csv' file.

8.13. Peak file export and format

8.13.1. Peak file export



The peak list can be exported by pressing the button labeled ‘Export to file...’ in the ‘Peak Picking’ area. The next section describes in detail the format of the exported file.

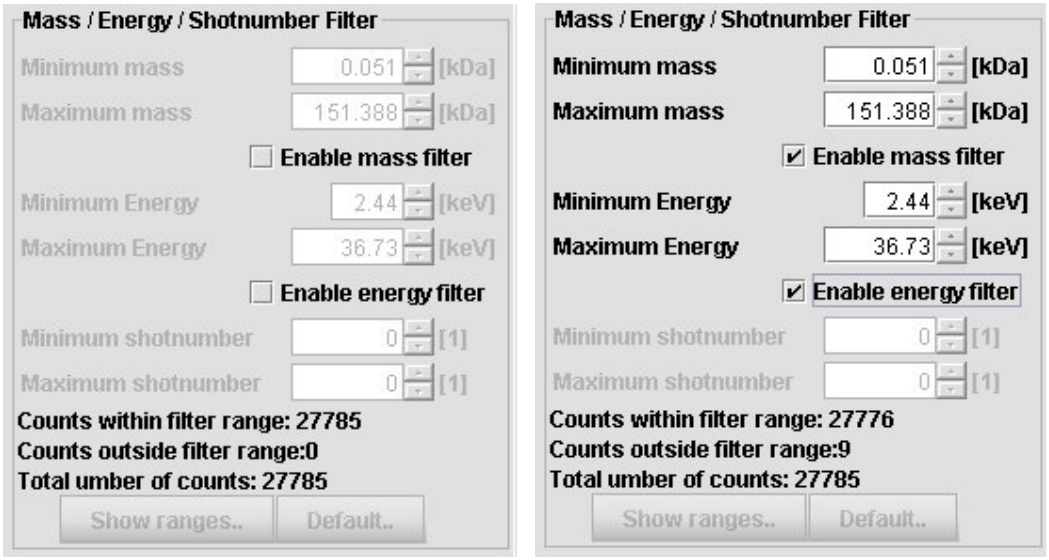
8.13.2. Peak file format

The file is saved in the .csv format (**c**haracter **s**eparated **v**alues), meaning a table style format. The format is known to various programs such as Microsoft Excel.
Since the file is saved in ASCII it is also possible to view the file with a simple text editor (such as Notepad on Windows).
The first line consists of the column names of the table: ‘Name’, ‘Mass [kDa]’ and ‘Intensity’, which are divided by a semicolon (‘;’). The following lines contain the data of the table, which are again separated with a semicolon. An example is shown below (it does not correspond with the table shown in the section ‘Peak file export’):

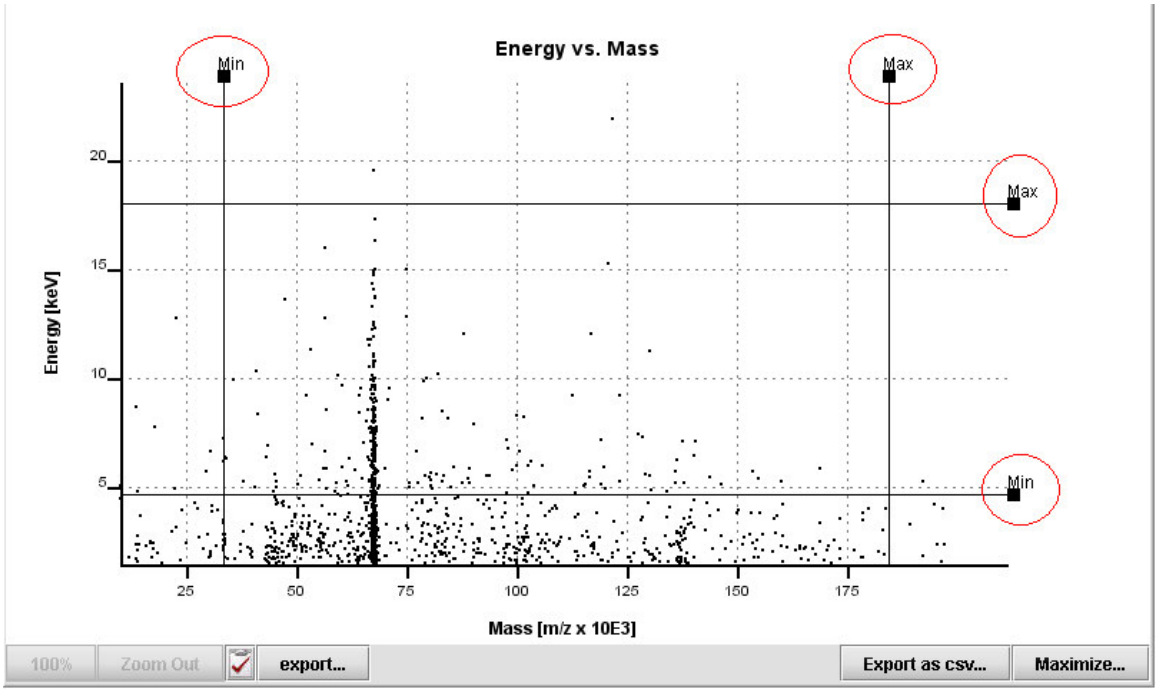
```
Name;Mass [kDa];Intensity
13.941 kDa;13.941;483.0;
5.065 kDa;5.065;683.0;
8.661 kDa;8.661;298.0;
```

8.14. Using filters

It is possible to apply a mass filter, an energy filter or both. In the bordered area labeled 'Mass / Energy / Shotnumber Filter', the respective filter(s) can be activated by checking the box 'Enable mass filter' and/or 'Enable energy filter'. When doing so, the two spinboxes above the checkbox become enabled and filter lines appear on the scatter plot. The value next to the label 'Counts within filter range:' shows the number of events which are inside the filter range. The value next to the label 'Counts outside filter range:' shows the number of events which are outside of the filtered range. The value next to the label 'Total number of counts' shows the total number of events.



The filter position can be changed by either typing a value into the spinbox, using the spinners of the spinbox (step is 1), or by dragging the filter lines in the chart at the marked spots shown in the screen shot. The histogram will recalculate automatically.



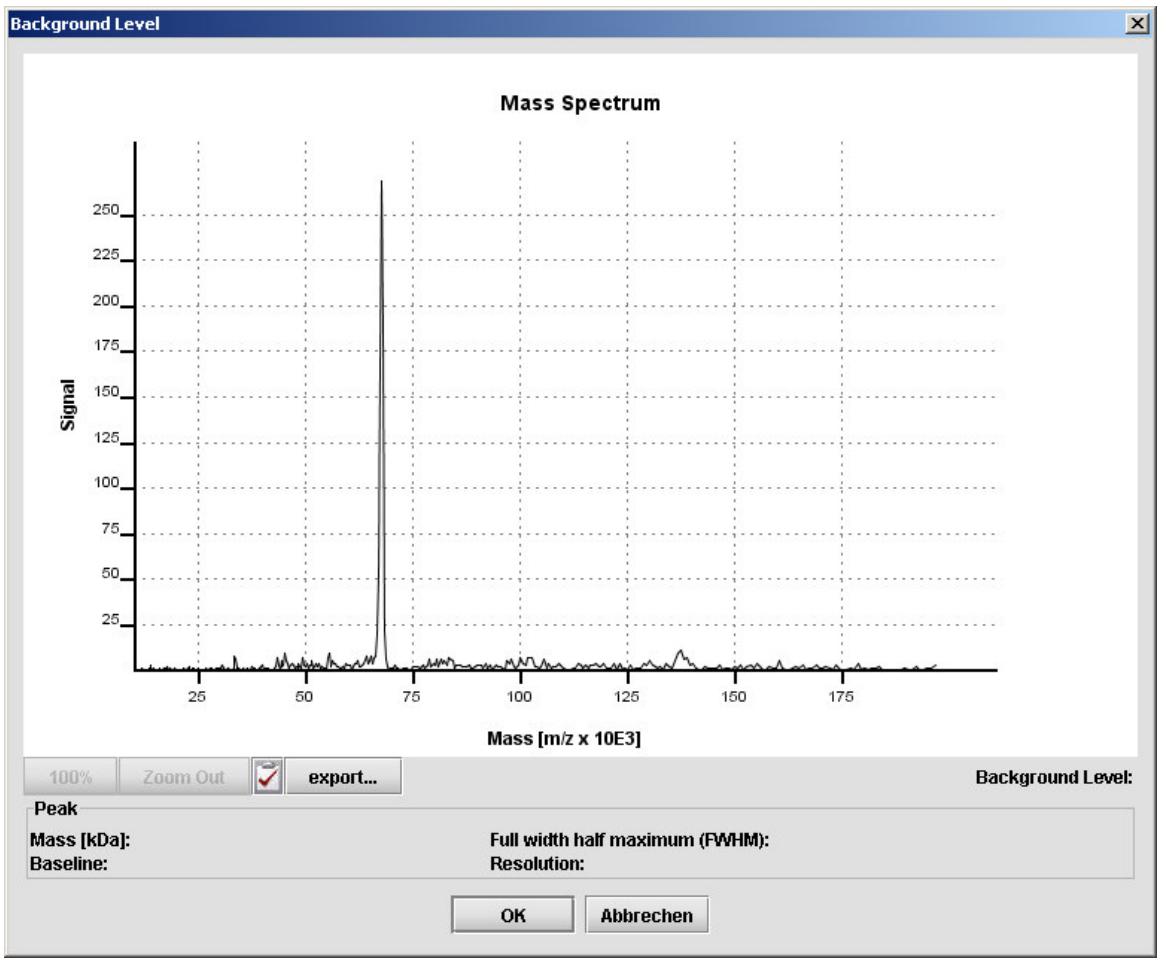
8.15. Background level calculation

The background level calculation can be setup in the areas 'Background Level'.

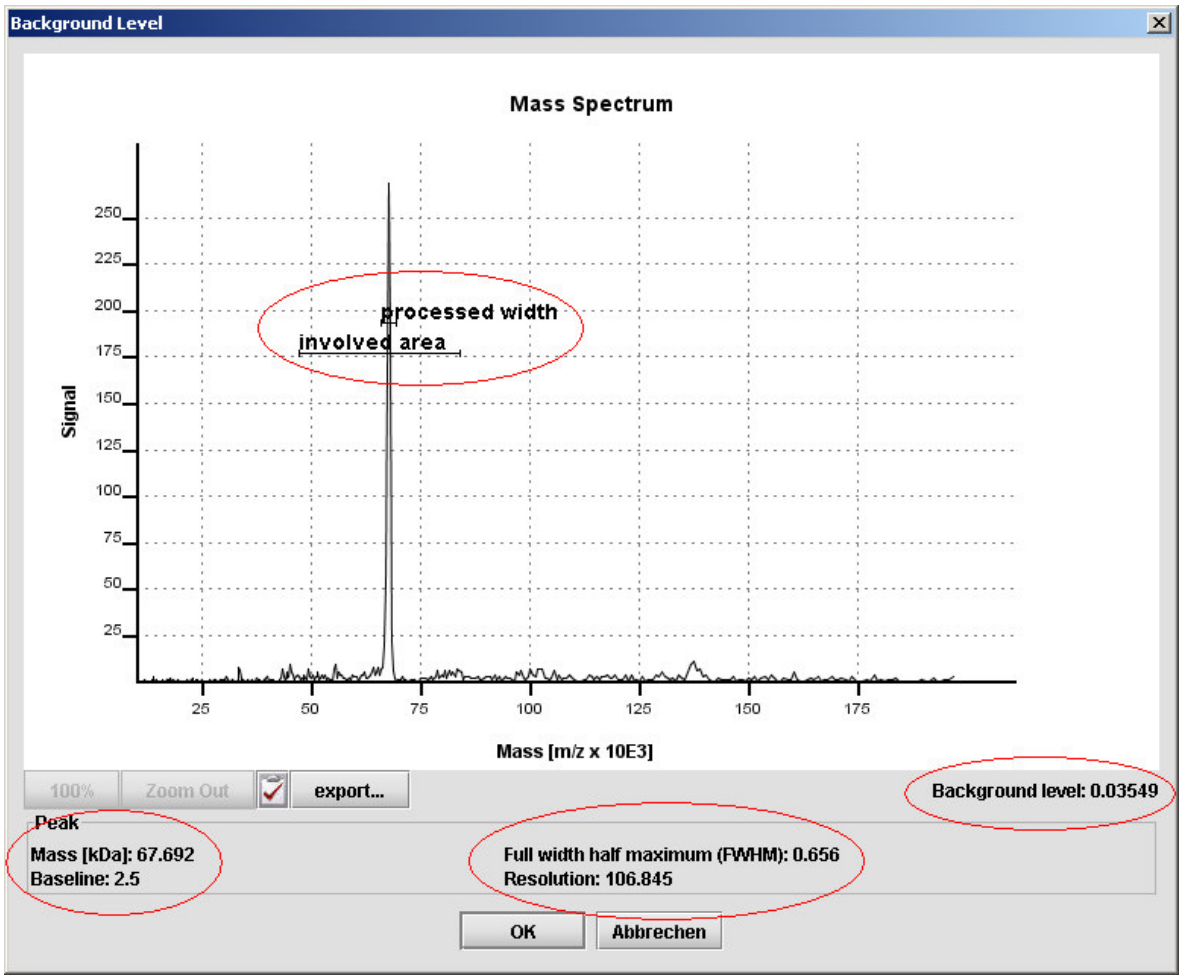
The background level value describes the quality of the peak. The background level is always positive and can be zero. The smaller the background level, the better the quality of the peak.

The 'set Area...' button will become enabled when there is a spectrum loaded. The 'Clear' button becomes enabled when an area is set for the calculation of the background level. When selected, the 'Clear' button will clear this area.

Pressing the 'set Area...' button brings up the following dialog:

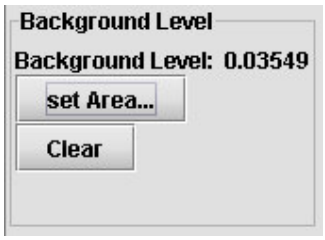


The chart has the usual function properties. The area that will be included in calculating the background level is set by dragging the mouse, with the right button pressed, over a peak, then releasing the button. Additionally, the involved peak is selected and the background level is calculated and shown.



The involved area and the width of the picked peak is drawn on the chart. The background level is shown in the left corner below the chart. In the bordered area labeled 'Peak', the peak values are displayed. The meaning of these values is described in the *chapter 8.11. The peak picking algorithm, page 110.*

Pressing 'OK' will set this area and the value will be displayed on the data analyzer panel.

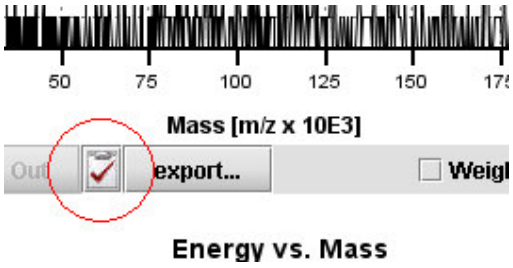


The background level will be updated automatically when changing the spectrum or manipulating its data.

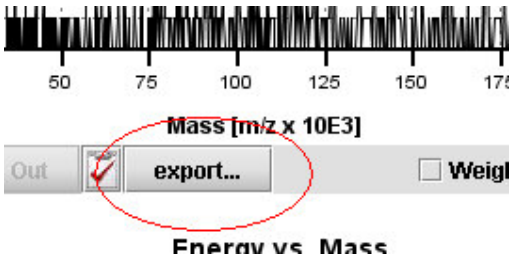
8.16. Exporting data

In the data analyzer panel there are, for both the mass spectrum and for the scatter plot, three possibilities for transferring the graphics to another tool.

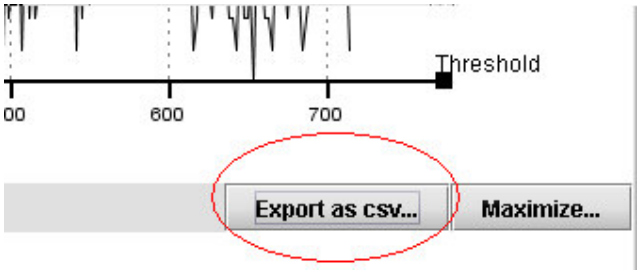
The first possibility is transferring the graphic to the Windows clipboard. Please refer to the section regarding acquiring spectra for details.



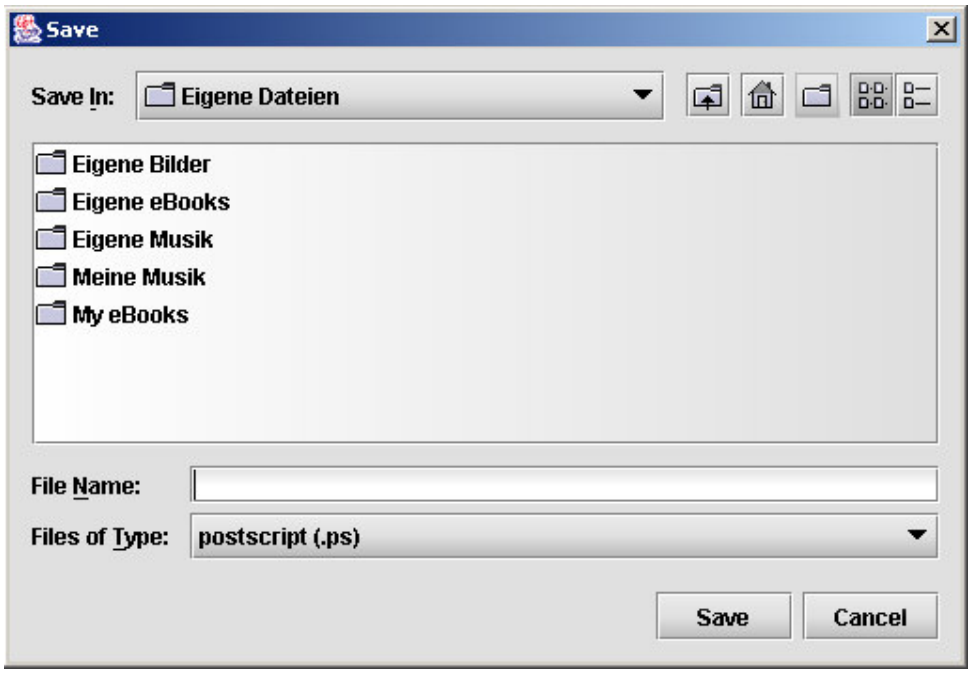
The second possibility is exporting the graphic to a file. Please refer to the section regarding acquiring spectra for details.



The third possibility is exporting the graphics to a '.csv' file by clicking the button 'Export as csv..'. The format of this file is a semicolon separated text file containing a list of each entry in the graph (Mass, intensity, energy, etc.). The exact data format is explained in the user's manual, and the exported file can be imported in Microsoft Excel for further data analysis.



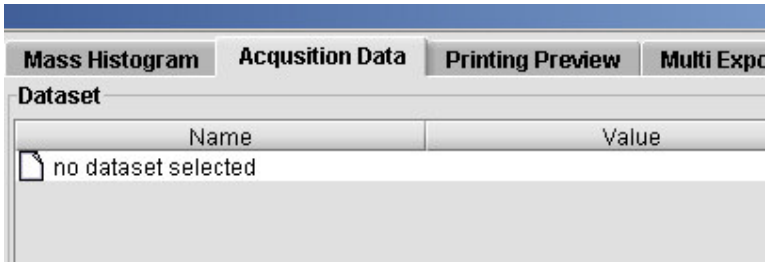
The file path and filename have to be selected in the export dialog. After the selections are made, click 'Save' to store the graphics in the file, or click 'Cancel' to cancel the operation.



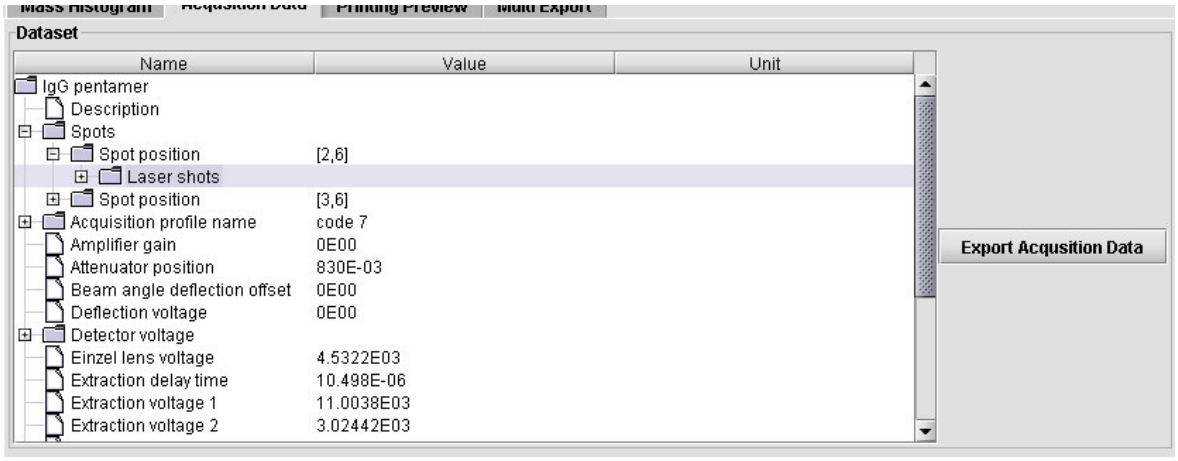
The filename is entered in the text box 'File Name' and the path is selected in the 'Save in' pull down box. The data type is preselected.

8.17. Managing Acquisition Data and methods

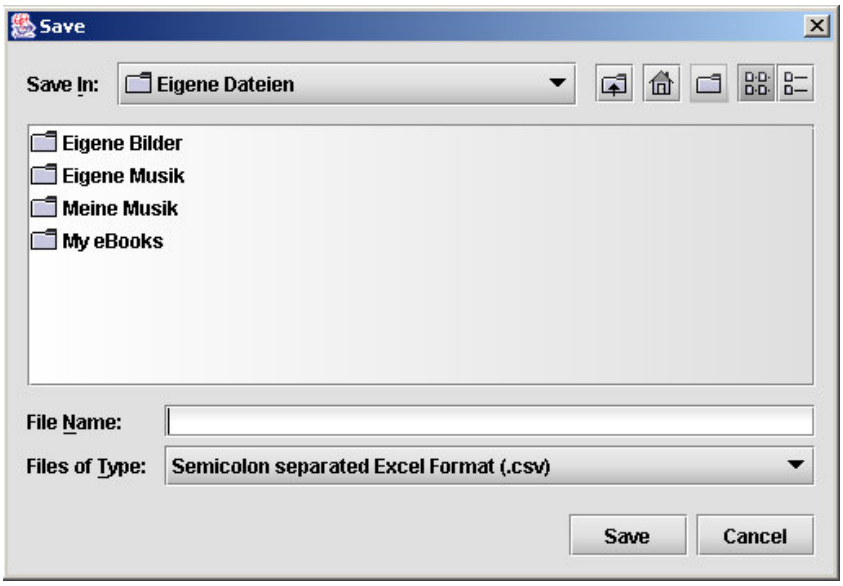
When analyzing a spectrum, it may be necessary to know under what conditions the acquisition was made. In the 'Acquisition Data' panel this information can be found after loading the spectrum. When no spectrum is loaded the lists in this panel are empty.



The Acquisition Data is presented in a tree structure in the upper part of this panel, where each level of information can be examined. The information is organized in columns where the first column is the parameter name, the next is the value of this parameter, and the third is the unit. By clicking the '+' icon the next level is expanded, and by clicking '-' it is closed.

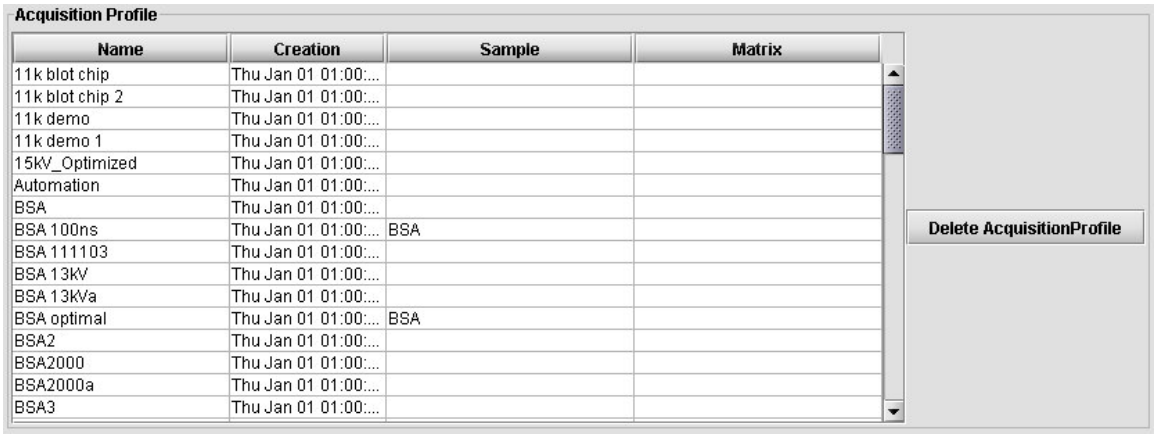


The Acquisition Data can be exported in a semicolon separated file by clicking 'Export Acquisition Data', and can be imported in Microsoft Excel.



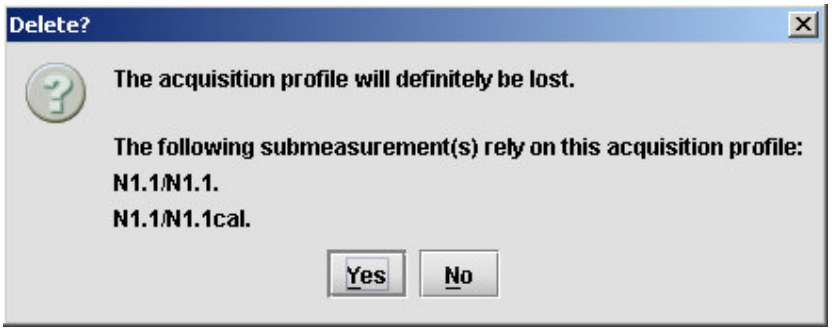
These parameters can also be printed together with the spectrum and scatter plot. Please see *chapter 8.18. Printing, page 124* for more information.

After having created several acquisition methods, it may be necessary to remove some of them. The deletion of an acquisition method is done in the middle part of the Acquisition Data panel.



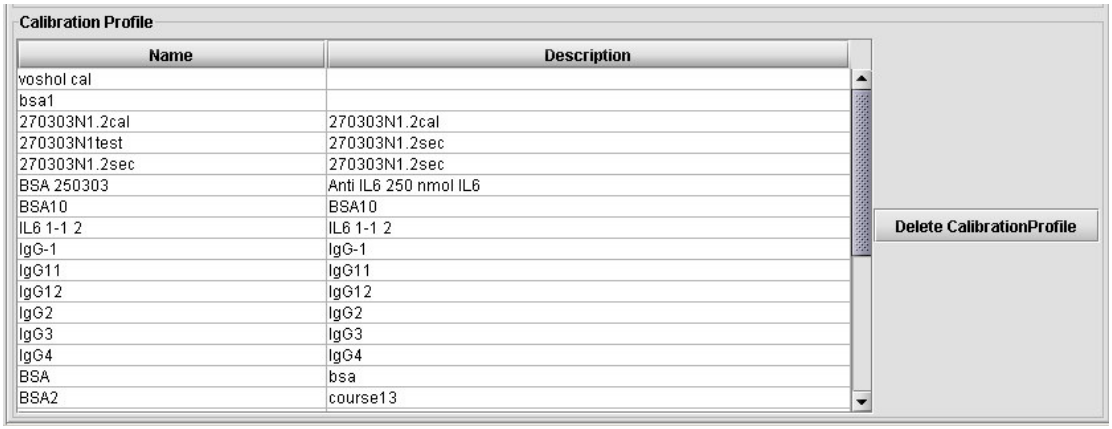
By selecting one of the methods and clicking 'Delete Acquisition Profile' a confirmation message is shown that lists the connected submeasurements.

By clicking 'Yes' in the confirm dialog the selected acquisition method is deleted. By clicking 'No' the action is canceled.



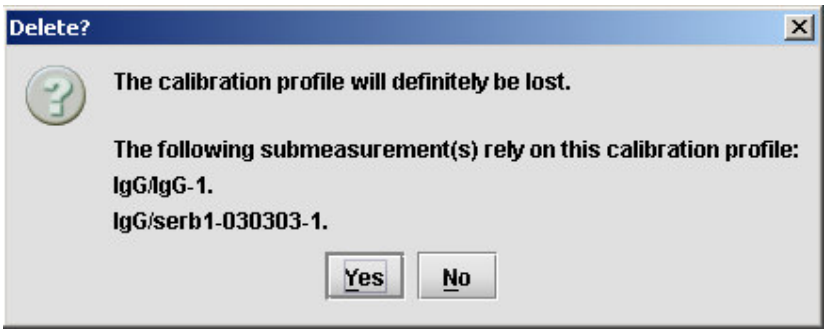
The acquisition data for the submeasurements is not lost by deleting the acquisition method. However, it is not possible to determine afterwards on what basis (method) the submeasurement was made, but the actual parameters used during the acquisition will still be available.

After having created several calibration files it may be necessary to remove some of them. The deletion of a calibration file is done in the lower part of the Acquisition Data panel.



By selecting a calibration file and clicking ‘Delete Calibration Profile’ a confirmation message is shown that lists the connected submeasurements.

By clicking ‘Yes’ in the confirmation dialog the selected calibration file is deleted, and by clicking ‘No’ the action is canceled.



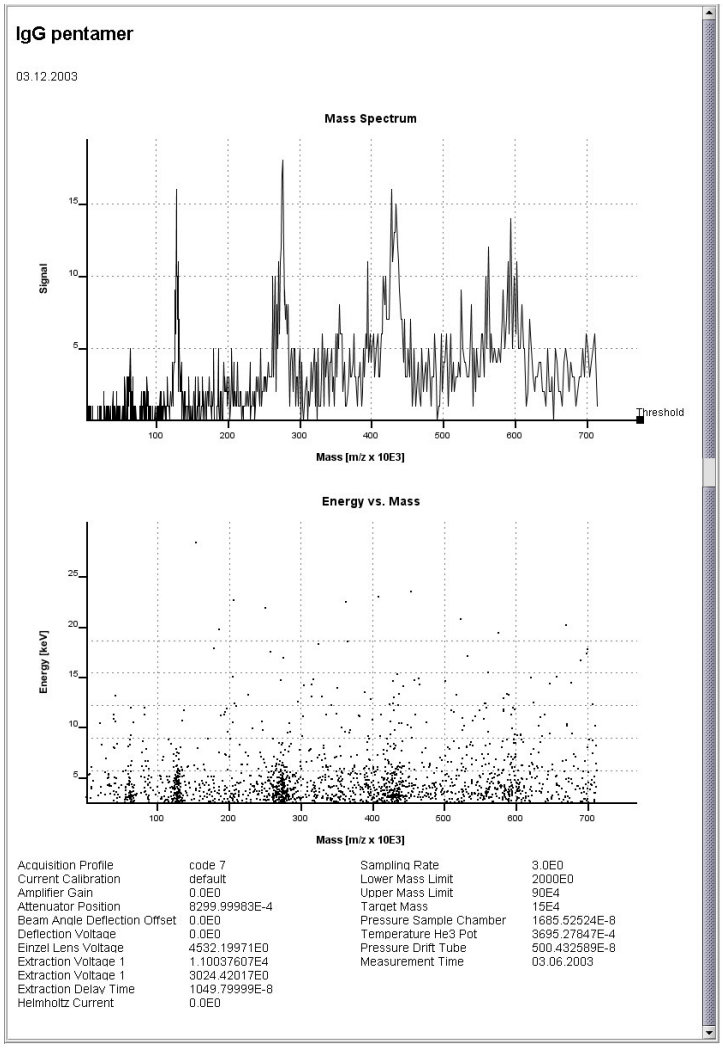
When a submeasurement is analyzed that was using the deleted calibration, the default calibration will be used in its place.

8.18. Printing

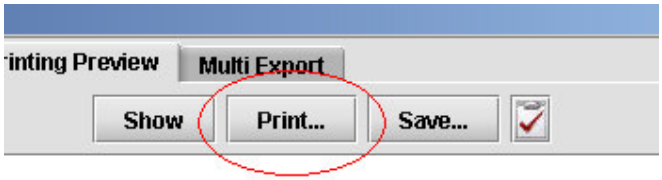
In the Data Analyzer, a dedicated ‘Printing Preview’ panel is used for generating a hard copy of the analyzed spectrums. This panel is selected by clicking the ‘Print Preview' tab above the mass spectrum.

The print preview is automatically (or by clicking the 'Show' button) updated with the name of the acquisition, the comment (empty if no comment is stored with this acquisition), the print date, mass spectrum, and the scatter plot from the Mass Histogram panel as they are seen in this panel.

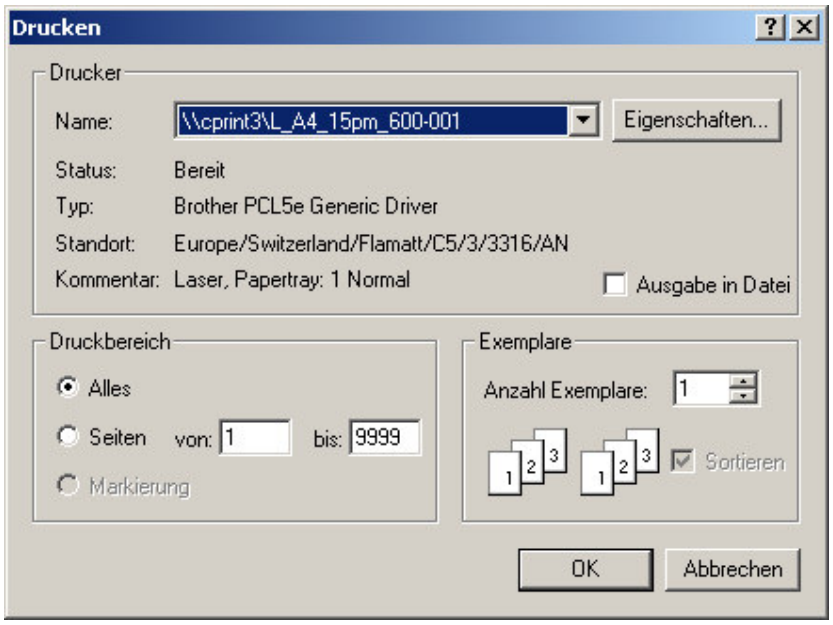
Additionally, the acquisition data is listed below the scatter plot, giving all information about the parameters used during the acquisition.



For outputting the previewed graphics directly to a printing device (which must be installed on the client's PC Windows as a Windows printer), click the 'Print..' button.

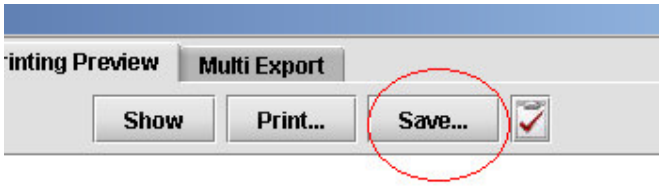


When clicking 'Print' a popup dialog appears where the printer can be selected (normal dialog as in office applications). Network printers can also be used for printing from the Data Analyzer.

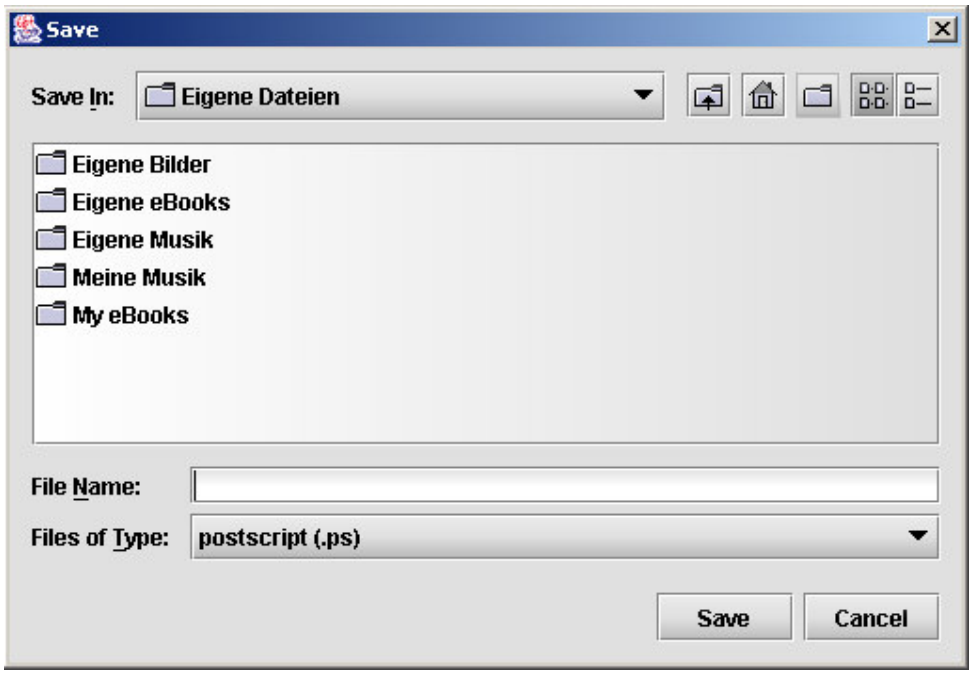


Click ‘OK’ to start printing or click ‘Cancel’ to cancel printing.

For outputting the previewed graphics to a ‘.png’ file click the ‘Save’ button.

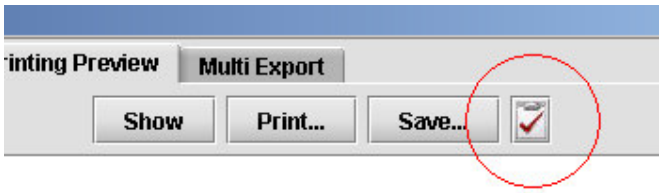


When clicking ‘Save’ a save popup dialog appears.



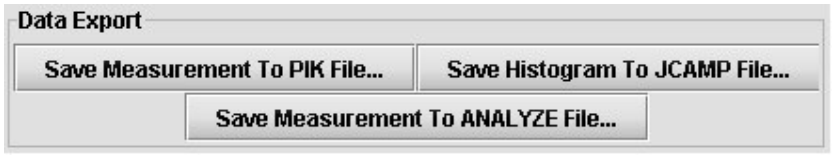
The filename is entered in the text box ‘File Name’ and the path is selected in the ‘Save in’ pull down box. The data type is preselected.

Finally, the previewed graphics can be transferred to the clip board for pasting into other applications such as Microsoft Word. The graphics are transferred by clicking the clip board icon in the printing preview panel. No popup will be shown.



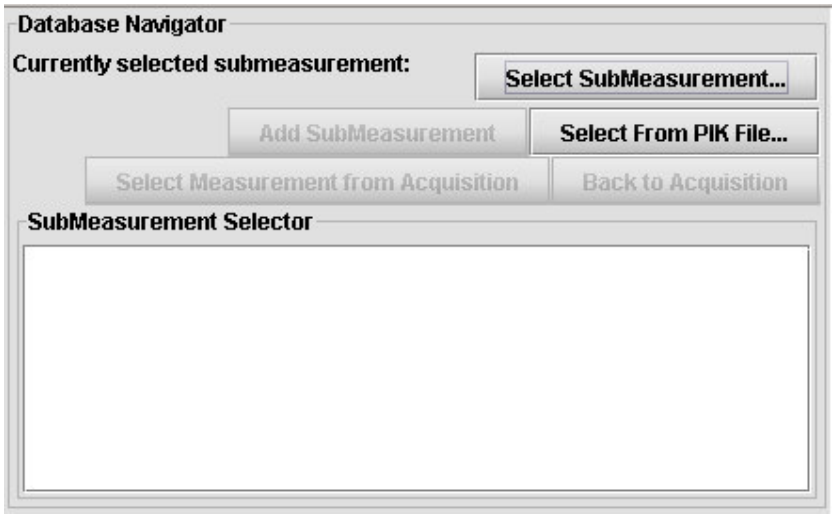
8.19. Exporting, importing and format of .PIK files

8.19.1. Exporting



Pressing the ‘Save Measurement To PIK File...’ button in the ‘Data Export’ panel saves the measurement in the PIK format on the hard disk.

8.19.2. Importing

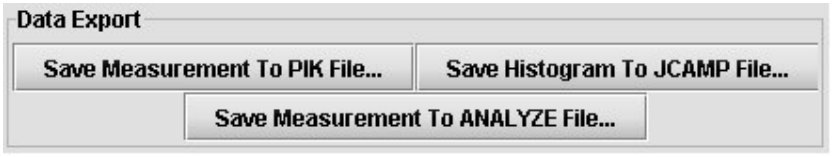


Pressing the ‘Select From PIK File...’ button in the ‘Database Navigator’ panel loads a PIK file into the Data Analyzer. It is not possible to execute all functions bound to a database.

8.19.3. Format

The PIK format is mainly a ASCII file containing all the data from the measurement, which includes the acquisition method, the calibration, the measurement parameters and all events.

8.20. Exporting and format of .JCAMP files



8.20.1. Exporting

Pressing the ‘Save Histogram To JCAMP file..’ button in the ‘Data Export’ panel saves the spectrum in the format of a JCAMP file on the hard disk.

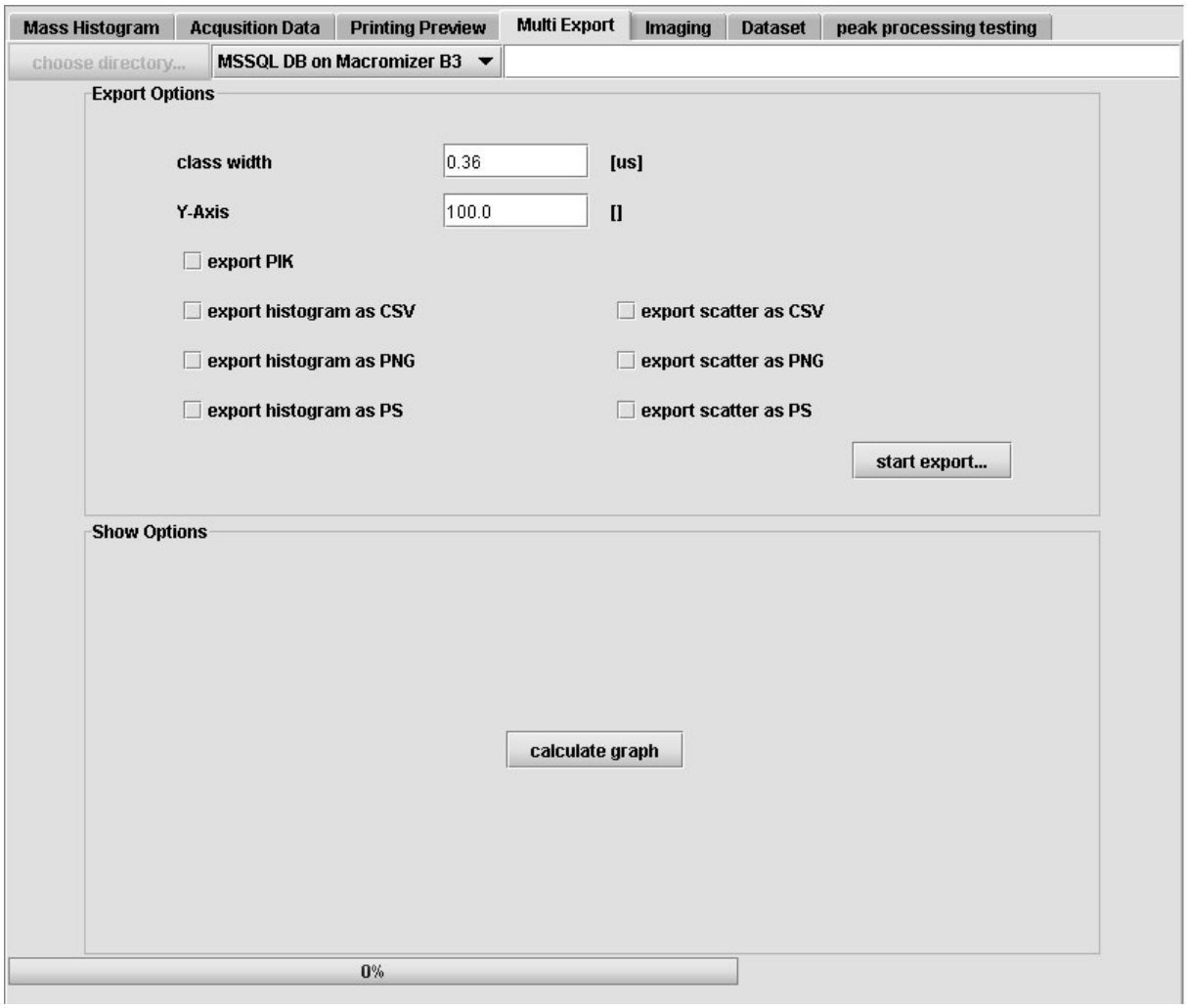
8.20.2. Format

The format of JCAMP is defined by the International Union of Pure and Applied Chemistry (IUPAC). For more information please visit: www.jcamp.org

8.21. Multi export panel

This part of the software is still under development and should not be used if not explicitly encouraged by the macromizer™ team.

A small commentary about the functionality of this part of the software is appended below.



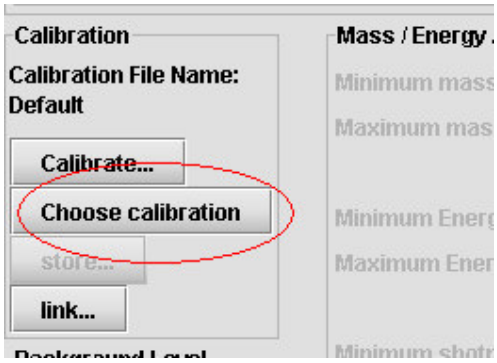
The 'Multi Export' tab is used to export multiple submeasurements and, in rare cases, to show them in a graph. The submeasurements are selected by means of a path, which has to be entered manually. Later on different export schemes can be chosen. These functions will become a part of multimeasurement processing.

9. Using and creating calibration methods

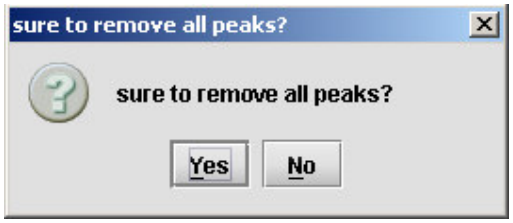
9.1. Selecting a calibration method

After loading a new acquisition into the Data Analyzer, the spectrum is calibrated with the calibration file chosen by the acquisition process. If no calibration file was chosen, the default calibration is automatically applied.

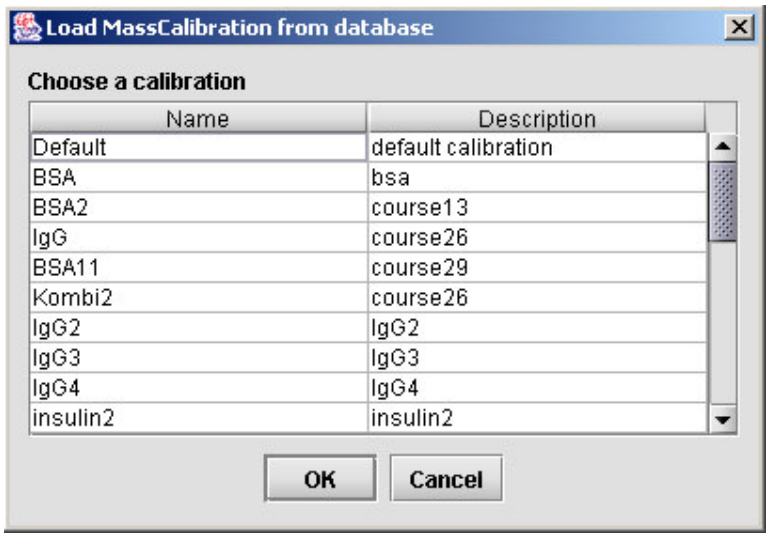
In the ‘Calibration’ area of the Data Analyzer panel it is possible to choose another calibration file for the spectrum. For the description on how to create a new calibration method please refer to the *chapter 9.2. Creating a calibration method, page 132.*



By clicking the ‘Choose Calibration’ button in the ‘Calibration’ area, a popup message appears to confirm if the picked peaks should be removed. If ‘Yes’ is clicked the selection list appears. If ‘No’ is clicked the selection of a new calibration file is canceled.

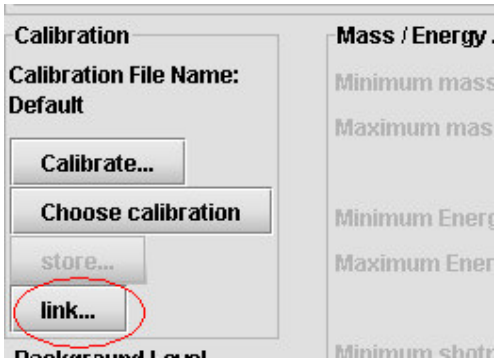


The ‘Choose a Calibration’ list show all previously stored calibration files (the list is sorted by clicking the top bar). By choosing a file and clicking ‘OK’ this calibration file is selected and the spectrum recalibrated. By clicking ‘Cancel’ the selection of a new calibration file is canceled.

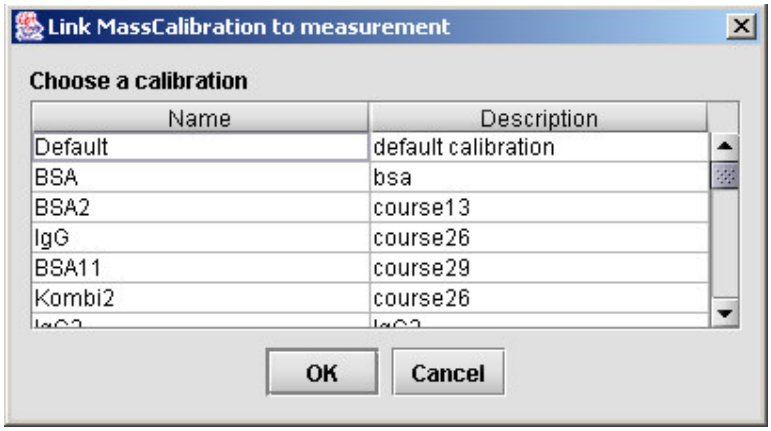


After choosing a new calibration file the name of this file is shown in the ‘Calibration’ area.

Please note that the calibration file is not permanently linked to the spectrum. To permanently link a calibration file to a spectrum click the ‘link..’ button in the 'Calibration' area.



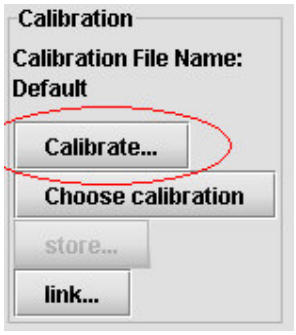
By clicking 'link..' the calibration file selection dialog appears.



The ‘Choose a Calibration’ list show all previously stored calibration files. By choosing a file and clicking ‘OK’ this calibration file is permanently linked to the spectrum and the spectrum is recalibrated. By clicking ‘Cancel’ the link is canceled.

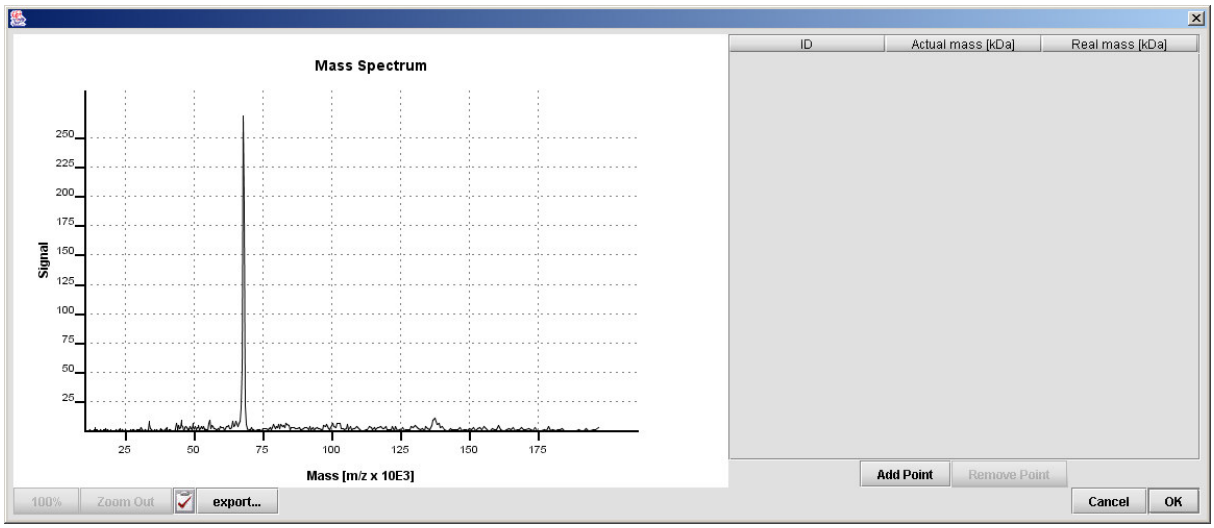
9.2. Creating a calibration method

Pressing the ‘Calibrate...’ button in the bordered area called ‘Calibration’ will bring up a calibration dialog.



A dialog is shown first, warning that all peaks will be removed.

This dialog will also appear even when there are no picked peaks. By pressing ‘Yes’ the actual calibration dialog is shown.



On the left side is the same histogram with the same functionality as the histogram on the Data Analyzer panel. On the right side a table is shown where the picked calibration peaks and the corrected mass are displayed. The table has three columns; the first is the ID of the calibration point, the second (Actual mass [kDa]) is the mass in kDa of the picked peak and the third (Real mass [kDa]) is an editable column containing the user defined masses.

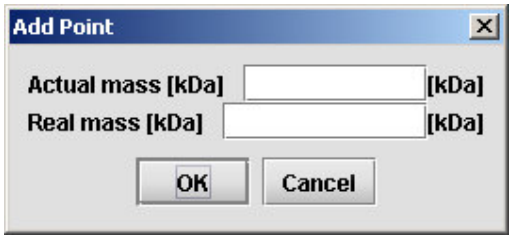
It is possible to assign up to eight calibration points. The points can be set either by picking them in the histogram or by adding them manually. Once the calibration points are set, pressing the 'OK' button assigns this calibration on the actual spectrum. Pressing the 'Cancel' buttton will abort the calibration.

ADDING CALIBRATION POINTS BY MEANS OF THE HISTOGRAM

Pressing the right mouse button and dragging over a peak will show the 'Accept Peak?' dialog. Choosing 'OK' will place the picked peak into the table. By providing the real mass in the third column (the field is editable) a calibration point is defined.

9.2.1. Adding calibration points manually

Pressing the 'Add Point' button will show an input dialog.



The first input field (Actual mass [kDa]) is the mass displayed on the histogram. The second field (Real mass [kDa]) is the supposedly correct mass provided by the user. Pressing 'OK' will insert the defined point into the table.

9.2.2. Removing a calibration point

When removing a calibration point a table row must be selected first. Pressing the ‘Remove Point’ button will remove the selected row.

9.3. The calibration algorithm

The calibration is used to convert the time of flight of a target into a mass. The algorithm is a polynom conversion of the kth order, where 8 >= k >= 1. The generic polynom conversion is

$$M^{analyte} = \sum_{k=0} a \cdot \left(t^{analyte}\right)^k$$

$t_{analyte}$ consists of the measured time of flight and a machine dependent time shift called t_{offset}.

1-point calibration:

$$M = a_2\left(t^{DSP} - t_{offset}\right)^2$$

2-point calibration:

$$M = a_2\left(t^{DSP} - t_{offset}\right)^2 + a_1\left(t^{DSP} - t_{offset}\right)$$

n-point calibration:

$$M = \sum_{k=0}^{n-1} a_{n-k}\left(t^{DSP} - t_{offset}\right)^{n-k}$$

9.3.1. The default calibration

The default calibration is a 3-point calibration. The three masses are the lower, the upper and the target mass provided by the user. The time of flight is calculated acc. to

$$t^{Ion} = t_s + t_d + t_D + t_{delay} = \frac{v_s - v_0}{a_s} + \frac{v_d - v_s}{a_d} + \frac{D}{v_d} + t_{delay}$$

with

$$t_s = \frac{v_s - v_{mean}}{a_s}; a_s = \frac{eU_s}{Ms}; v_s = \sqrt{\frac{2eU_s}{M}\frac{s - v_{mean}t_{delay}}{s} + v_{mean}^2}$$

$$t_d = \frac{v_d - v_s}{a_d}; a_d = \frac{eU_d}{Md}; v_d = \sqrt{\frac{2eU_d}{M} + \frac{2eU_s}{M}\frac{s - v_{mean}t_{delay}}{s} + v_{mean}^2}$$

$$t_D = \frac{D}{v_d}$$

t_{delay} is the delay time set by the user.
D is the distance between the sample plate and the detector
v_{mean} initial mean velocity of the molecule
e the charge of an electron.
U_s is the extraction voltage pulse.
U_d is the acceleration pulse.
M is the mass
s is the distance between the sample plate and the plate with U_s.
d is the acceleration distance.

Constant values table

Parameter name	Value	Unit	Comments
D	1.566	[m]	
v_{mean}	750	[m/s]	
e	1.602189*10^-19	[As]	
s	0.007	[m]	
d	0.009	[m]	

With the resulting times the conversion coefficients c_1, c_2 and c_3 are calculated:

$$M_{low} = c_3\left(t_{low}^{Ion}\right)^3 + c_2\left(t_{low}^{Ion}\right)^2 + c_1\left(t_{low}^{Ion}\right)$$
$$M_{high} = c_3\left(t_{high}^{Ion}\right)^3 + c_2\left(t_{high}^{Ion}\right)^2 + c_1\left(t_{high}^{Ion}\right)$$
$$M_{\tau} = c_3\left(t_{\tau}^{Ion}\right)^3 + c_2\left(t_{\tau}^{Ion}\right)^2 + c_1\left(t_{\tau}^{Ion}\right)$$

The conversion coefficient are then used for the default time to mass conversion:

$$M_{event} = c_3\left(t_{event}^{DSP} - t_{offset}\right)^3 + c_2\left(t_{event}^{DSP} - t_{offset}\right)^2 + c_1\left(t_{event}^{DSP} - t_{offset}\right)$$

where t_{event}^{DSP} is the measured time and t_{offset} is the time offset of the machine.

9.3.2. The custom calibration

By calibrating in the data analyzer panel, pairs of time of flight and mass are generated. The time of flight of a particular peak is given due to the measurement; the mass is set by the user.
By means of the formulas presented in the chapter “Calibration” the conversion coefficients are gained.

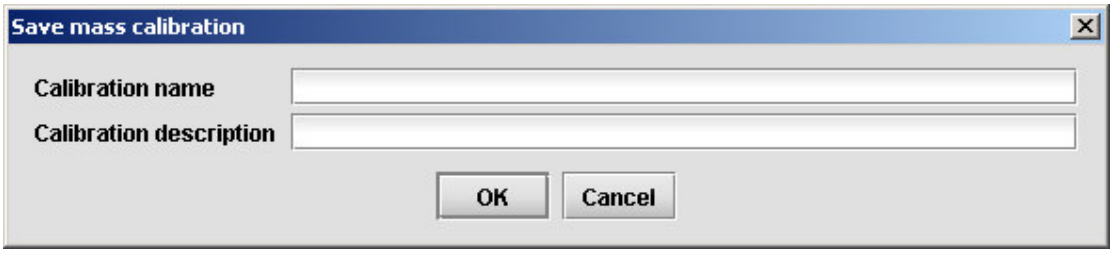
The gained peak pairs from the calibration dialog are called m_{actual} and m_{real}. m_{actual} will be converted back to the time of flight. The two adjacent mass values of m_{actual} in the histogram are lookup up in the histogram table to convert them back to time of flight. m_{actual} will be the interpolated time of flight between the two adjacent histogram points, using a linear relation in the mass units.

At that point, the calibration data is composed of the real mass m_{real} and the actual time of flight tof_{actual}. With these two values the equation in the chapter calibration can be solved for the coefficients a. These coefficients in turn will be used to calibrate further data.

9.4. Saving a calibration method

When a custom calibration is performed (Please see the chapter ‘Creating a calibration method’) it is possible to save it in the repository.

Pressing the ‘store...’ button in the bordered area labeled ‘Calibration’ will show an input dialog.



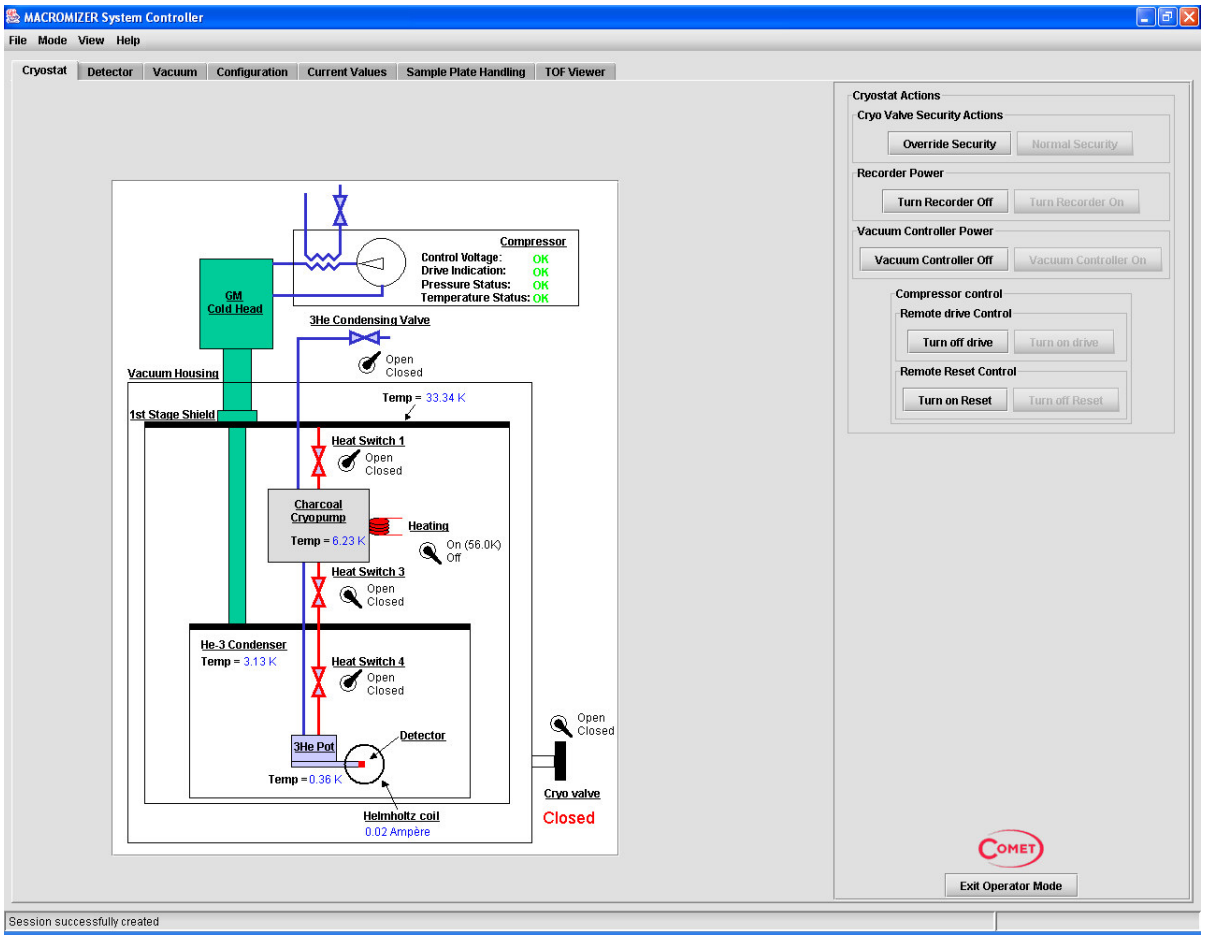
After providing the name and a voluntary description, pressing the ‘OK’ button will store the calibration in the repository. Pressing ‘Cancel’ will abort.

10. System settings

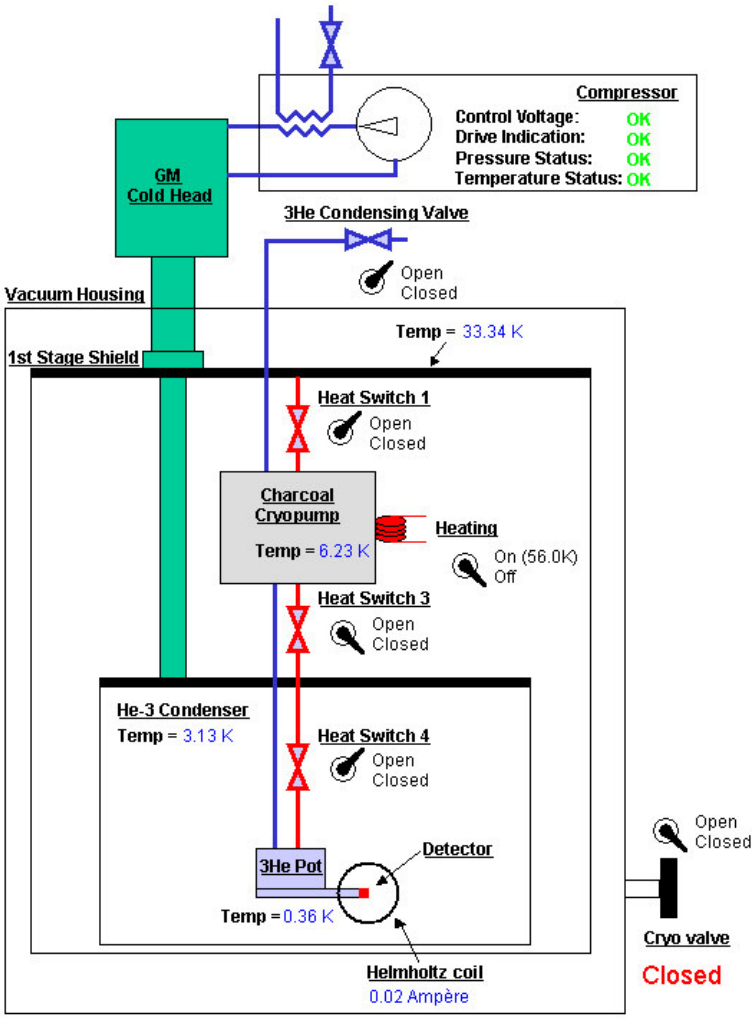
The system settings can be activated by clicking ‘View->System Settings->Cryostat’. It is possible that a popup will appear that asks for a user name. If this is the case, ask your person responsible for macromizer™ information for the data.

10.1. Croystat controls

In the ‘Cryostat’ tab you can control and view all the actions and states of the cryostat.
On the left side on the window is graphical representation of the cryostat with all its heats witches and temperature levels.
On the right side are commands or which the cryostat controller is responsible for setting or clearing.



10.1.1. Graphical representation of cryostat

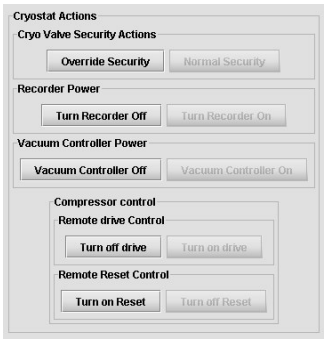


At the top of the graphic is a schematic of the compressor. You can see whether or not the control voltage is okay, if the drive is running (shown by Drive Indication), if the pressure of HE4 is okay (shown by Pressure Status), and if the temperature of cooling water is okay.

On the 'First stage shield' the temperature T1 is measured (33.34K in example above). On the 'Charcoal Cryopump' the temperature T2 is measured (6.23K). There you can also switch the 'Heating' with a power off about 5 W. The second stage shield, or 'He-3 Condenser', temperature is called T3 (3.13K). The '3He Pot' is the coldest point with the red dot, which is the symbol for the detector. Its temperature is called T4 (0.36K). The Helmholtz coil is responsible for the magnetic field, which is necessary for the detector work. The current can be set on the detector tab and its actual value is displayed here (0.02 Ampère).

On the right side of the schematic there is a switch of the cryostat controller for the cryovalve. Below is the actual state of the valve (closed). For further explanation consult chapter 'Function of cryovalve'.

10.1.2. Controls



On the right side of the cryostat tab are different button to control action of the cryostat controller.

Below the title 'Cryo Valve Security Actions' you could find the buttons to override security or switch back to normal security. If state is overridden, it's possible to do things in the cryostat tabs which are normally not allowed. Normally not allowed:

- Open cryovalve if He-3 temperature is above 5 Kelvin
- (Re-) Start compressor if temperature is above 50 Kelvin

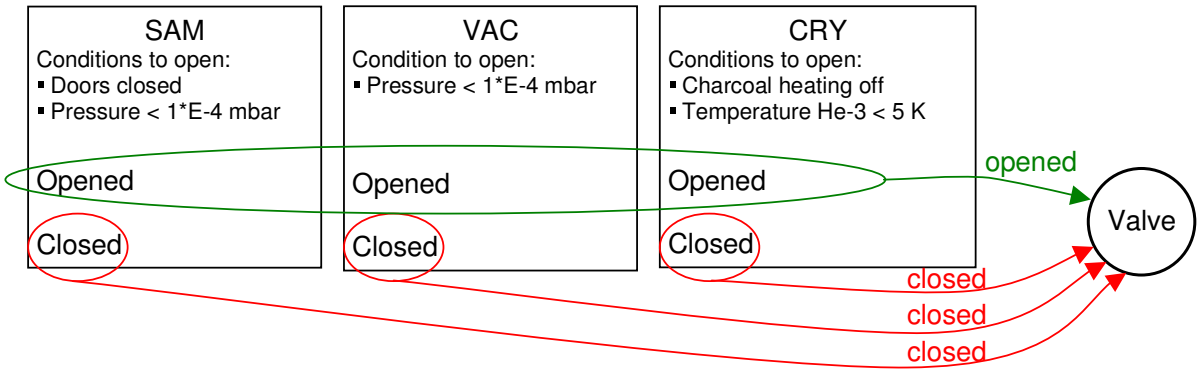
With 'Recorder Power' and 'Vacuum Controller Power' it's possible to power-off and on the selected module. This is used to make a proper restart of one of the modules.

The controls for the compressor are used to turn off and on the drive. Normally this should work with 'Turn off drive' and 'Turn on drive'. But it's possible that the compressor couldn't start. If this happens, turn off the drive and press 'Turn on Reset', wait 10 seconds and press 'Turn off Reset'. After this steps try again to 'Turn on drive'.

The drive is automatically observed by the cryostat controller. If the He3 temperature is above 50 Kelvin the controller automatically switches off the compressor. To start it you should either use the cooldown task or override security as described above.

10.1.3. Function of cryovalve

For security reasons the cryovalve is controlled by a serial bus. Each of the three modules SAM (Sample Stage Controller), VAC (Vacuum Controller) and CRY (Cryostat controller) have to enable the opening of the cryovalve before it could effectively be open. If one of them has its own state closed, the cryovalve is automatically closed. So it could happen for example that VAC and CRY say that the cryovalve is opened, but physically it's closed. The cause is that SAM has its own state on closed.



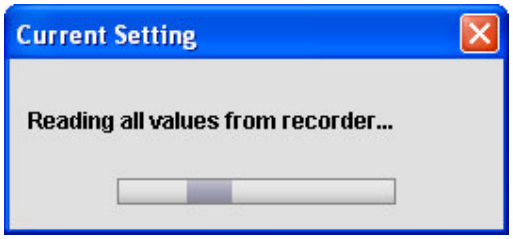
10.2. Detector controls

In the ‘Detector’ tab it is possible to set and configure the behavior of the transient recorder and detector. The main topics in this area the U/I characteristics and detector working point.

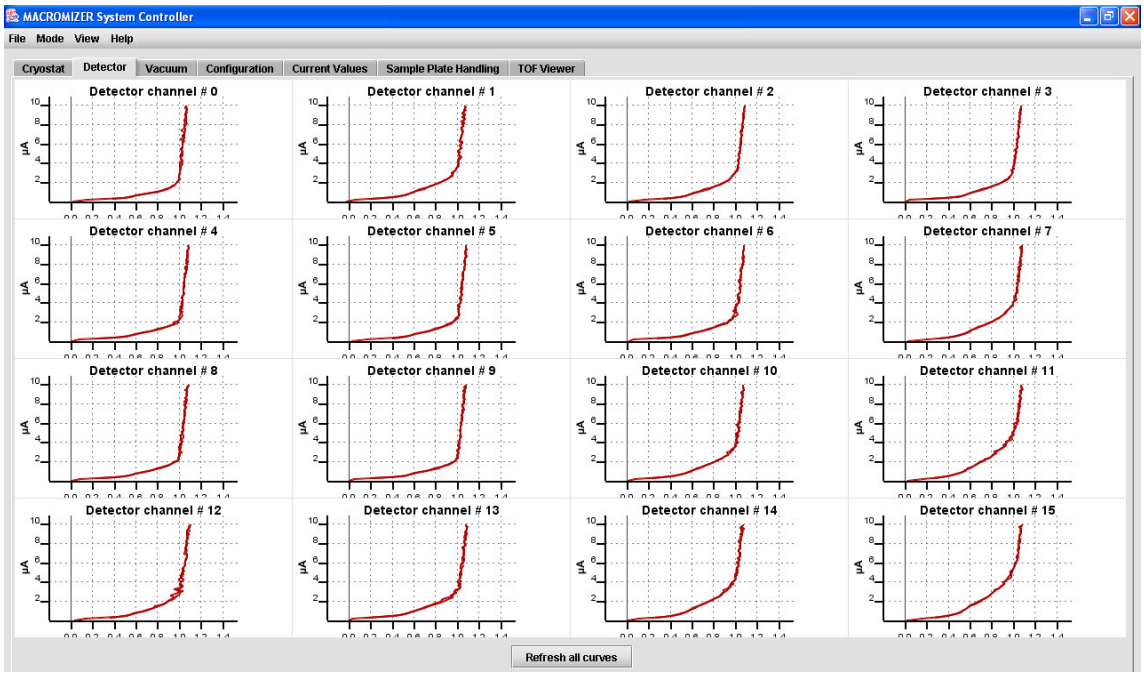
10.2.1. U/I Characteristics

To start a new measurement of the curve press following button:

While the U/I characteristics are being measured the following window appears:



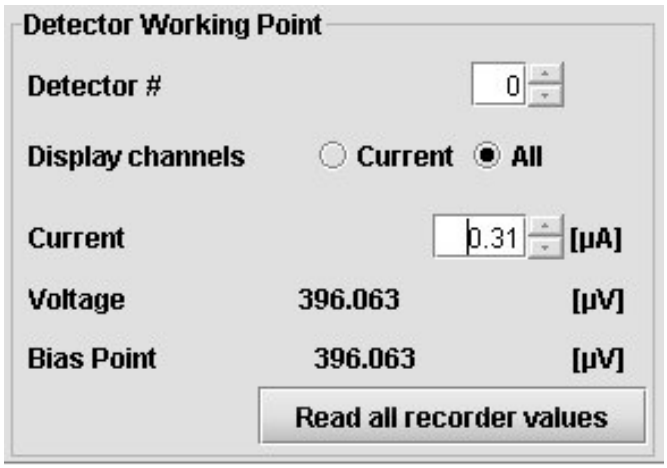
It takes approximately 25 seconds to measure all the curves. Under normal circumstances they look comparable to the curves shown in the following picture.



If the characteristics are linear (current changes from 0 to 10µA and voltage 0 to 200 µV) for all channels, no Helmholtz current is usually applied. See the chapter titled ‘Helmholtz Current’ to learn how to apply a working value.

10.2.2. Detector Working Point

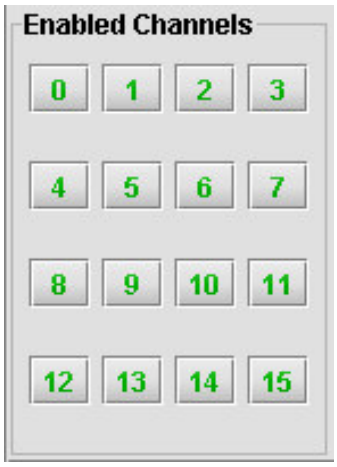
The working point of the detectors is set from the software through adjustment of the current. The ideal working point of the detector is at a voltage of 350 to 400mV. To find the optimal point adjust the current until the read back voltage is in the previously mentioned range (350 to 400mV).



The bias point is read back voltage directly measured after setting a new current. For example, by pressing the 'Read all recorder values' button, the current, voltage and bias point are read back from the transient recorder.

10.2.3. Enabled Channels

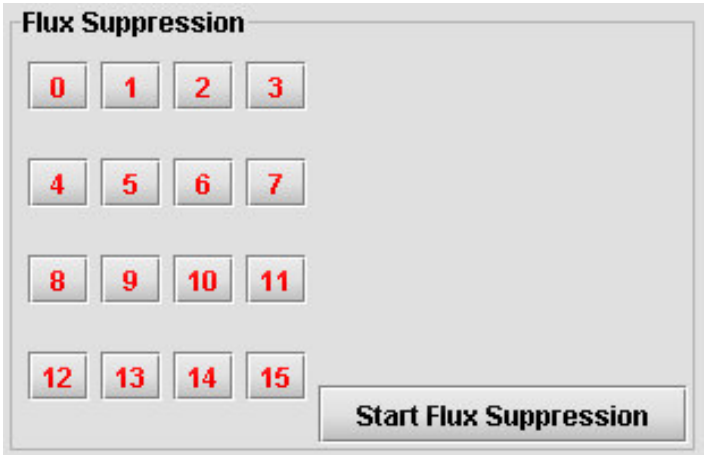
The enabled channels affect the measurement of ions. Only those channels which are enabled (will appear green) return a signal; disabled channels will not return anything. This feature is used to mask channels that return a lot of noise or other unexpected results.



10.2.4. Flux Suppression

It is possible for the detector to get trapped in the flux state. To solve this problem select one or more channels by enabling them (the button will turn green), then press the 'Start Flux Suppression' button. The Helmholtz current is automatically set to zero and a high current is set to the detector for approximately 30 seconds. After this time the Helmholtz current is automatically set

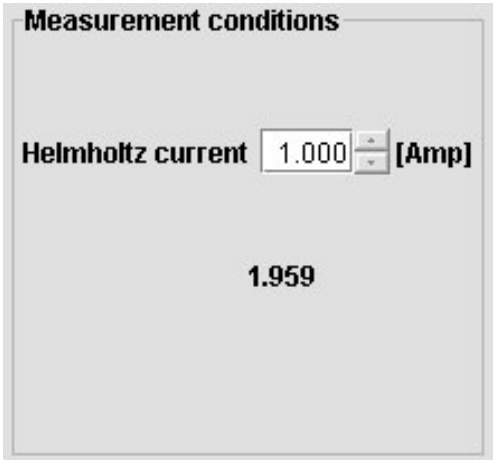
to the old values. Be aware that after pressing the ‘Start Flux Suppression’ button you are unable to measure for the next 50 seconds.



10.2.5. Helmholtz Current

Surrounding the detector is a coil for generating a magnetic field. This magnetic field is needed for the operation of the detector and affects the U/I characteristics, and thereby the working points.

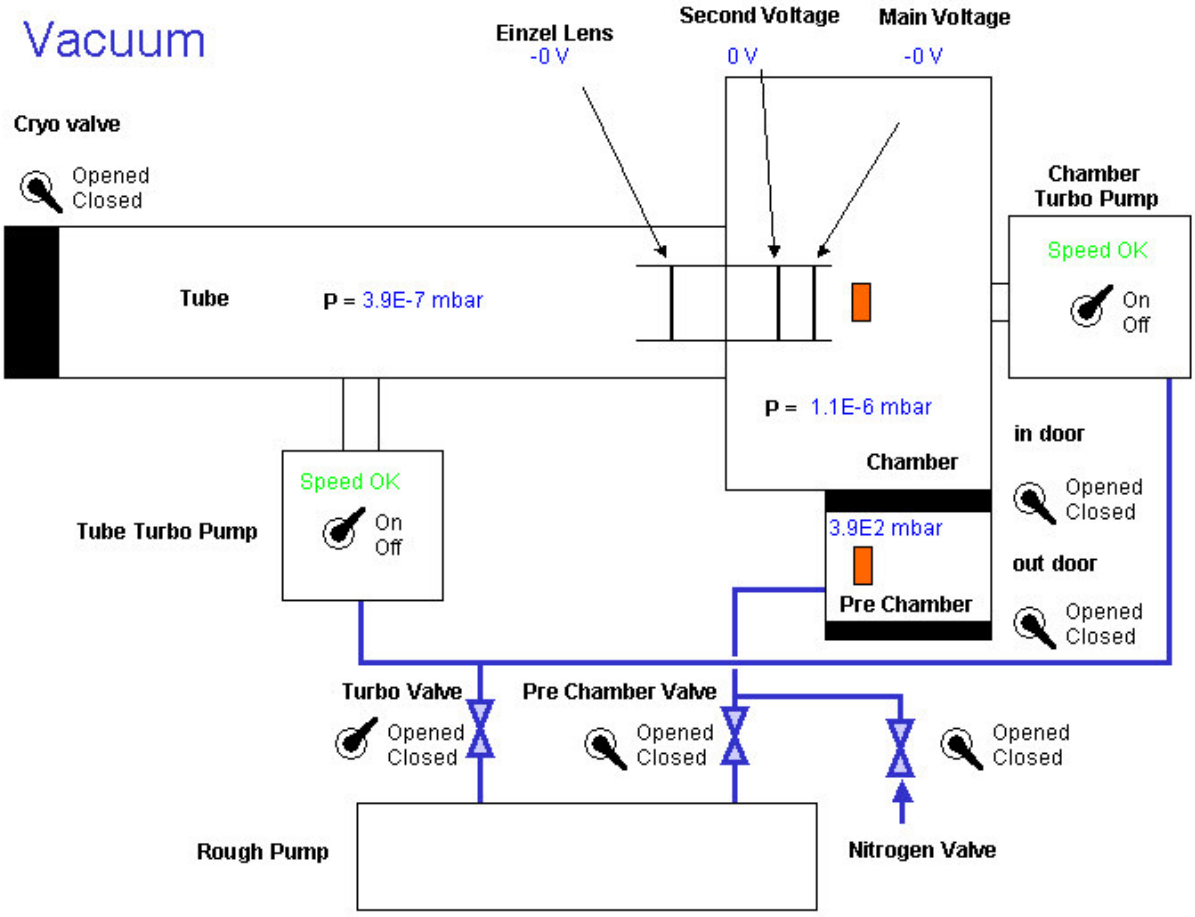
The current through the coil is called the Helmholtz current. To adjust the current input a new value and press enter. Using the read back value (1.959 in example below means HHCurrent=1.959 A) you can check if the correct value is set to the coil. (Note: It could be that there is a problem with the measuring of this value. Therefore its possible that the read back is always 0 or double the actual measured value.)



10.3. Vacuum controls

10.3.1. Graphical representation of vacuum system

The 'Vacuum' tab shows macromizer™'s entire vacuum system, including flight tube, sample stage and pre-chamber. The pressure is measured at two positions: one in the flight tube, near the cryovalve, and the other in the sample chamber. It is normal for the pressure in the sample chamber to be higher (less than a factor of 10) than the pressure in the flight tube.



Attached to the rough pump are two valves: the turbo valve and the pre-chamber valve. Only one of these valves can be open at any one time. The pre-chamber valve is only used during a load cycle in order to evacuate the pre-chamber. The remainder of the time the turbo valve is opened, allowing the rough pump to assist the two turbo pumps (flight tube turbo and chamber turbo). The nitrogen valve is there to flood the pre-chamber during an unload cycle.

If you try to switch the turbo pumps on while the pressure in the flight tube/sample chamber is above 10 mbar the controller will not start them.

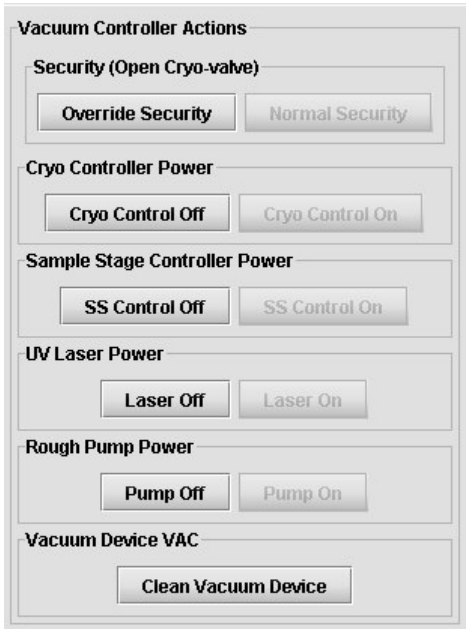
Not all controls in this window are controlled by the vacuum controller. The inner and outer doors and the chamber turbo pump are controlled by the sample stage controller. If you want to open the doors of the vacuum system during non-permitted stages, you can do that only with overridden security in the sample stage controller.

10.3.2. Controller Actions

The ‘Security’ button allows you to open the cryovalve in the vacuum system during stages when the procedure is normally not allowed.

With the four buttons that follow the security button you can make a power cut on the cryo-controller, sample stage-controller, UV laser or rough pump. This function is normally used to make a hard reset on the modules.

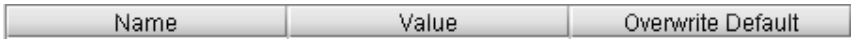
The ‘Clean Vacuum Device’ button allows you to clean the vacuum device in the flight tube, which is necessary if the pressure in the flight tube has unreasonable values (compared to sample stage pressure). To perform the cleaning the pressure must be below 10mbar, otherwise the device will not be cleaned.



10.4. Configuration

All macromizer™ configurations are stored in two config-files. One is stored on the server and the other on the clients hard drive. The settings for running the machine and system constants are stored on the server, while the parameters that are required for the GUI (graphical user interface) are stored on the client side.

It is important to select the accurate configuration file before changes are made. Click the tab labeled ‘Client’ on the right side to make your selection, then check if the red label matches your selection (‘Client’ in the example above).



There are three columns listed: ‘Name’, ‘Value’ and ‘Overwrite Default’

The name is the title for the configuration parameter and the value is the actual set value. If no check mark appears in the overwrite default checkbox then the default value of the software is taken. If you change a parameter the check mark automatically appears. If you are not sure what the default parameter of the software was before your changes, click on the check mark and the default value is automatically set.

10.4.1. Client configurations

One parameter on the client side which may require a change by the user is the preset value for the helmholtz-current. This is the value which is preset to the instrument upon connecting to the server and is labeled 'param.rec_hhc_s'.

param.rec_hhc_s	1	☑

10.4.2. Server configurations

On the server side are two parameters of interest to the user. The first is the 'machinesaver.idle', which represents the time in minutes after which the macromizer™ goes into sleep mode. Sleep mode is the state where the connection to the client is closed, the high voltage system is shutdown and the cryovalve is closed.

machinesaver.idle	30	☑

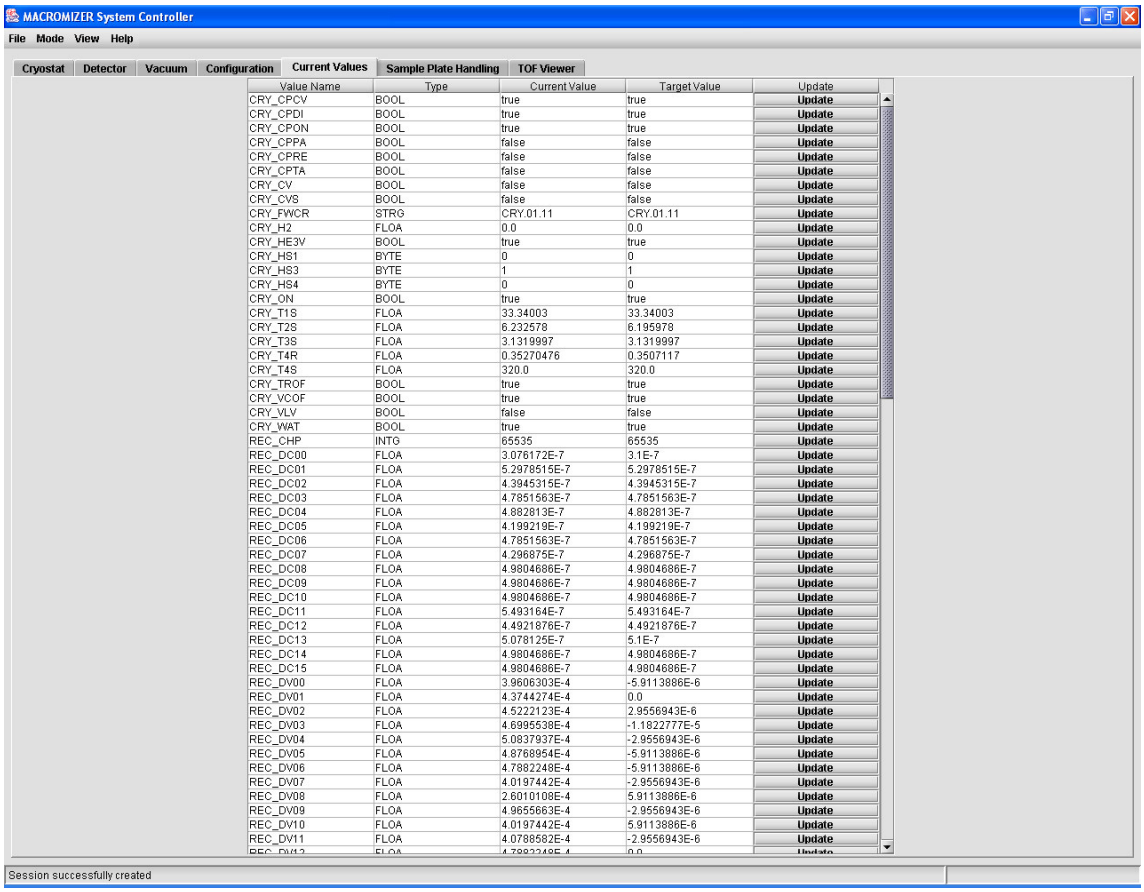
The second parameter of interest is the 'rec.peakalgo.version', which is there to switch between two peak-algorithms in the transient recorder.

If the number is '1' the older peak-algorithm is used. To use the derivation peak-algorithm enter a '3'. Be aware that entering any number other than 1 or 3 could cause the instrument to detect no peaks or incorrect peaks.

rec.peakalgo.version	1	☑

10.5. Current values

The current values tab could be used to ask the control modules (SAM,CRY,VAC,REC) for the actual measure values by pressing the ‘Update’-button on each row.

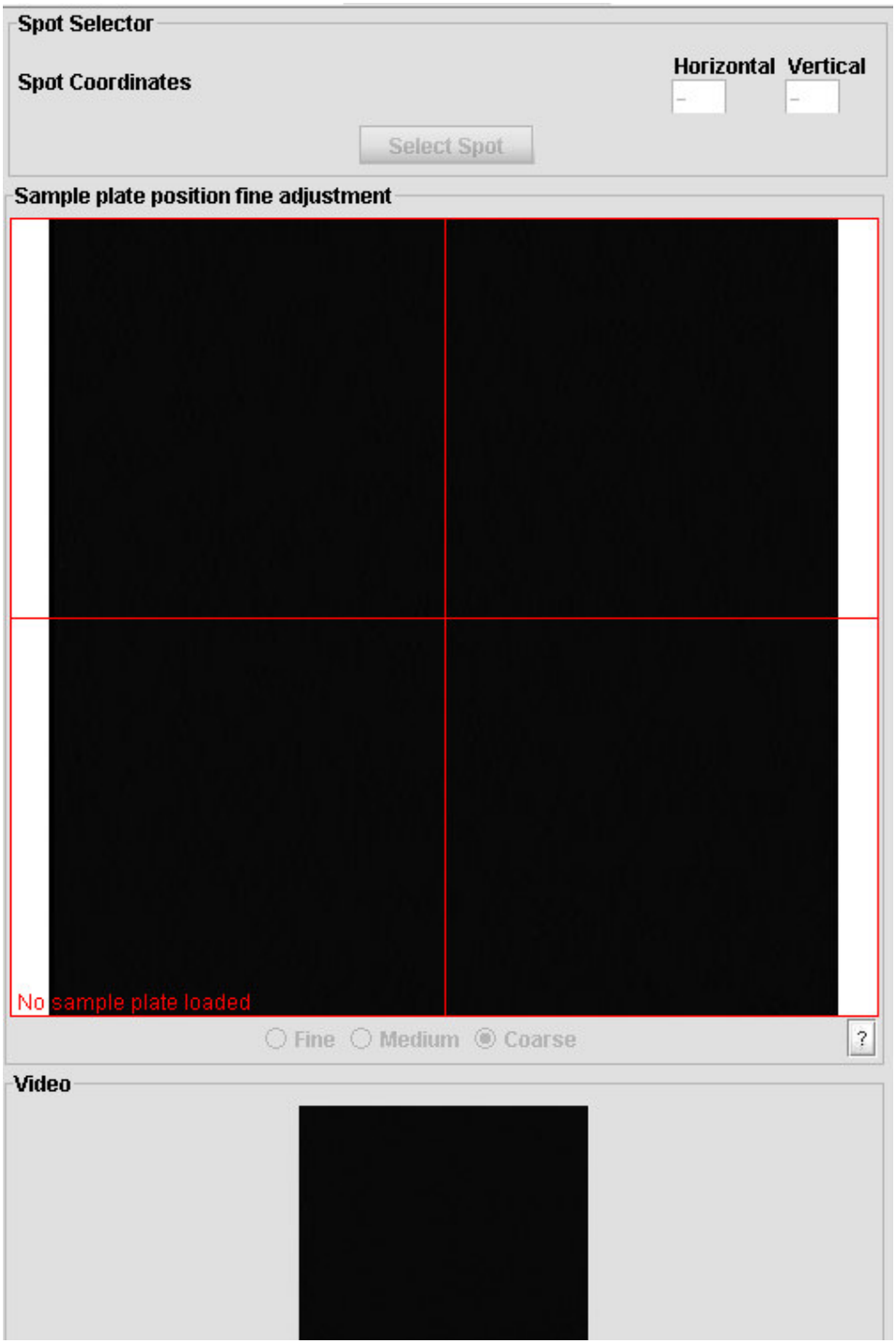


Value Name	Type	Current Value	Target Value	Update
CRY_CPCV	BOOL	true	true	Update
CRY_CPDI	BOOL	true	true	Update
CRY_CPON	BOOL	true	true	Update
CRY_CPPA	BOOL	false	false	Update
CRY_CPRE	BOOL	false	false	Update
CRY_CPTA	BOOL	false	false	Update
CRY_CV	BOOL	false	false	Update
CRY_CVS	BOOL	false	false	Update
CRY_FWCR	STRG	CRY.01.11	CRY.01.11	Update
CRY_H2	FLOA	0.0	0.0	Update
CRY_HE3V	BOOL	true	true	Update
CRY_HS1	BYTE	0	0	Update
CRY_HS3	BYTE	1	1	Update
CRY_HS4	BYTE	0	0	Update
CRY_ON	BOOL	true	true	Update
CRY_T1S	FLOA	33.34003	33.34003	Update
CRY_T2S	FLOA	6.232578	6.195978	Update
CRY_T3S	FLOA	3.1319997	3.1319997	Update
CRY_T4R	FLOA	0.35270476	0.3507117	Update
CRY_T4S	FLOA	320.0	320.0	Update
CRY_TROF	BOOL	true	true	Update
CRY_VCOF	BOOL	true	true	Update
CRY_VLV	BOOL	false	false	Update
CRY_WAT	BOOL	true	true	Update
REC_CHP	INTG	65535	65535	Update
REC_DC00	FLOA	3.076172E-7	3.1E-7	Update
REC_DC01	FLOA	5.2978515E-7	5.2978515E-7	Update
REC_DC02	FLOA	4.3945315E-7	4.3945315E-7	Update
REC_DC03	FLOA	4.7851563E-7	4.7851563E-7	Update
REC_DC04	FLOA	4.882813E-7	4.882813E-7	Update
REC_DC05	FLOA	4.199219E-7	4.199219E-7	Update
REC_DC06	FLOA	4.7851563E-7	4.7851563E-7	Update
REC_DC07	FLOA	4.296875E-7	4.296875E-7	Update
REC_DC08	FLOA	4.9804686E-7	4.9804686E-7	Update
REC_DC09	FLOA	4.9804686E-7	4.9804686E-7	Update
REC_DC10	FLOA	4.9804686E-7	4.9804686E-7	Update
REC_DC11	FLOA	5.493164E-7	5.493164E-7	Update
REC_DC12	FLOA	4.4921876E-7	4.4921876E-7	Update
REC_DC13	FLOA	5.078125E-7	5.1E-7	Update
REC_DC14	FLOA	4.9804686E-7	4.9804686E-7	Update
REC_DC15	FLOA	4.9804686E-7	4.9804686E-7	Update
REC_DV00	FLOA	3.9606303E-4	-5.9113886E-6	Update
REC_DV01	FLOA	4.3744274E-4	0.0	Update
REC_DV02	FLOA	4.5222123E-4	2.9556943E-6	Update
REC_DV03	FLOA	4.6995538E-4	-1.1822777E-5	Update
REC_DV04	FLOA	5.0837937E-4	-2.9556943E-6	Update
REC_DV05	FLOA	4.8768954E-4	-5.9113886E-6	Update
REC_DV06	FLOA	4.7882248E-4	-5.9113886E-6	Update
REC_DV07	FLOA	4.0197442E-4	-2.9556943E-6	Update
REC_DV08	FLOA	2.6010108E-4	5.9113886E-6	Update
REC_DV09	FLOA	4.9655663E-4	-2.9556943E-6	Update
REC_DV10	FLOA	4.0197442E-4	5.9113886E-6	Update
REC_DV11	FLOA	4.0788582E-4	-2.9556943E-6	Update

10.6. Sample plate handling

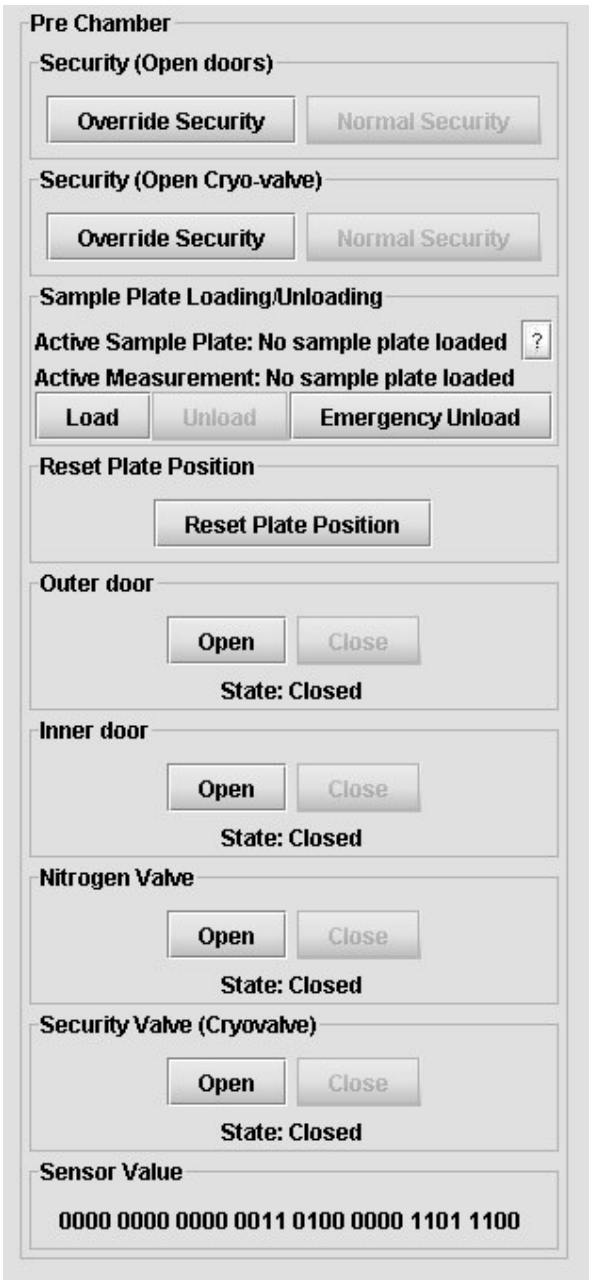
10.6.1. Spot Selector and Camera

The ‘Sample plate position fine adjustment’ is a zoomed window of the camera that allows you to see more detail than in the acquisition window. It is possible to select the actual position of the window by setting the horizontal and vertical representation values of the plate.



10.6.2. Pre Chamber

The control panel shown below can be used to control the sample stage controller.



10.6.2.1. Override Security

The ‘Security (Open doors)’ buttons allow you to open doors during stages when normally not allowed in the vacuum system. For example, in the overridden state it is possible to open both doors at the same time or to move doors at higher pressures.
The ‘Security (Open Cryo-valve)’ buttons allow you to open the cryovalve during stages when normally not allowed in the vacuum systems. If the cryovalve security is not overridden both doors must be closed before the cryovalve can be opened.

10.6.2.2. Sample Plate Loading/Unloading

These controls are the same as in the Acquisition; you are able to load or unload a plate. If there is an error during one of these actions it is often only possible to perform an emergency unload.
On a failure during an unload it is possible that there is a plate loaded but that the “Unload” button is disabled. To correct this, try to close and restart the application. If this does not help you must perform an emergency unload.

10.6.2.3. Reset Plate Position

This button triggers the sample stage to search the zero position of the sample plate.

10.6.2.4. Outer/Inner Door

The buttons in this section are used to open and close the doors. The current state of the door is mentioned below the buttons and can be either Closed, Open, Moving or Error.

To correct an error try to open and close the door several times or restart the sample stage controller. Remember to switch the ‘override security’ of the doors.

10.6.2.5. Nitrogen Valve

The nitrogen valve is used to vent the pre-chamber during a normal unload. With these buttons it is also possible to vent the flight tube by opening the inner door while the nitrogen valve is open.

Do not forget to close the **cryovalve** and to shutdown the **turbo pumps** before venting the instrument!!

10.6.2.6. Security Valve

These buttons are used to toggle the state of the cryovalve in the sample stage controller. An exact description of the cryovalve can be found in *chapter 10.1.3. Function of cryovalve, Page 139*.

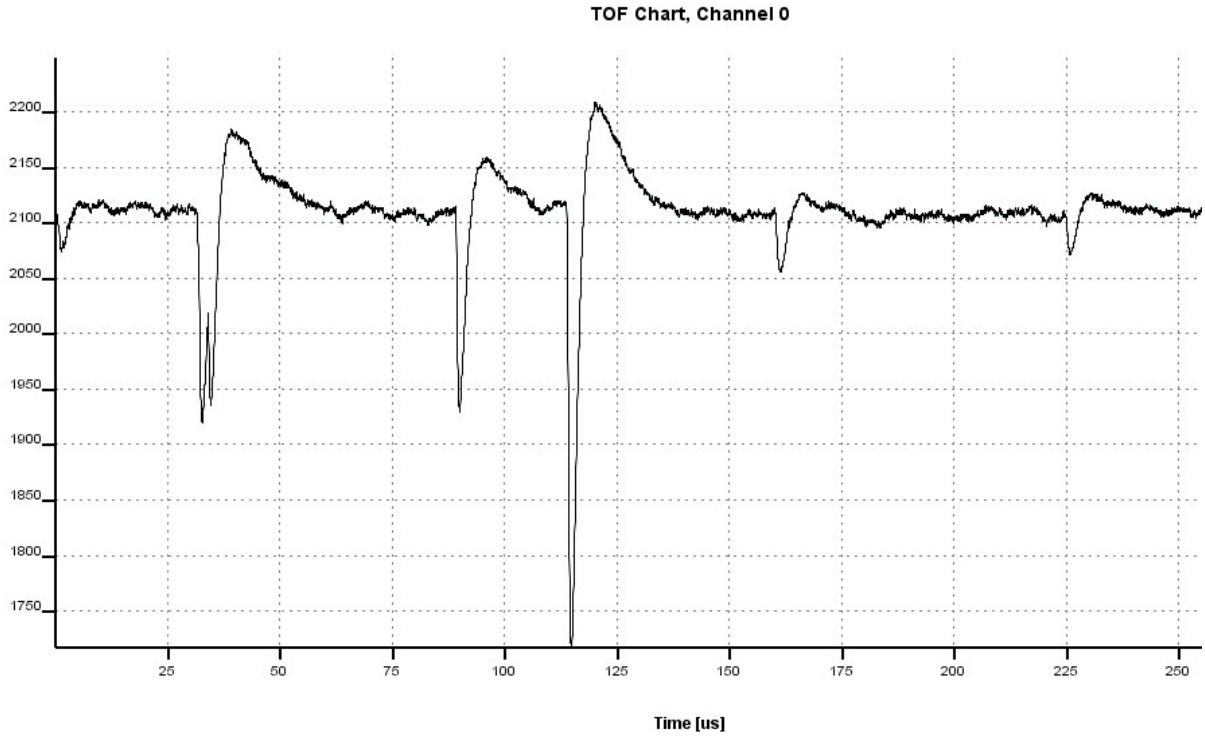
10.6.2.7. Sensor Value

These are the read back values of the all the sensors of the sample stage controller. This information could be used to detect an error.

10.7. TOF viewer

10.7.1. Functions

The peaks that appear in the scatterplot are calculated from the signal that comes from the detector. This signal is normal invisible but with the TOF viewer this signal can be sent to the client and monitored.



The transient recorder calculates peaks for the scatterplot from spectra similar to the one shown above. Keep in mind that this is not a mass spectrum. However, this and similar spectra can be used to derive mass spectra.

10.7.2. How to acquire a TOF spectra

To acquire a TOF spectra prepare a normal acquisition and find the working conditions for your sample. To see the time scale from trigger point to the upper limit, set the minimal mass to 0.001 kDa. Select the 'TOF Viewer' tab and select the checkbox 'TOF Data'. Go back to the acquisition tab and select either single shot or a burst shot sequence with a low shot rate (approximately 1 Hz). Start the measurement by pressing the 'Start New' button.

10.7.3. How to analyze a TOF spectra

Before you can analyze a TOF spectra you must do an acquisition as described in the previous chapter (‘How to acquire a TOF spectra’). Following the acquisition press the ‘Show Chart’ button for each channel. Only one channel can be shown at a time. It’s also important to note that you can only see the data of the last shot.

By pressing the ‘Save’ button the measurement can be stored in a PLS-file. This is a binary Comet-specific format. In this file all the channels and the acquired spectra are saved, resulting in very large size files. Occasionally, problems are caused by saving large files directly onto a network drive. To prevent these problems it is better to save these files on a local hard disk.