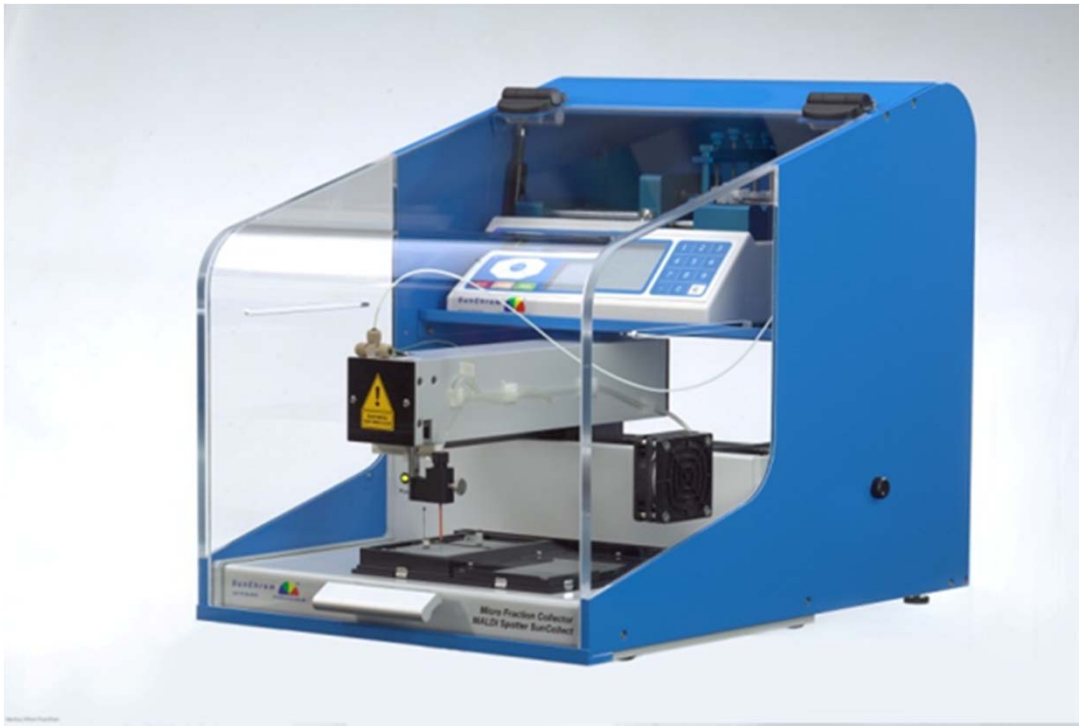
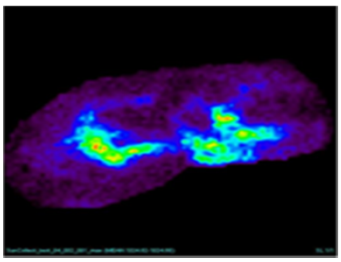
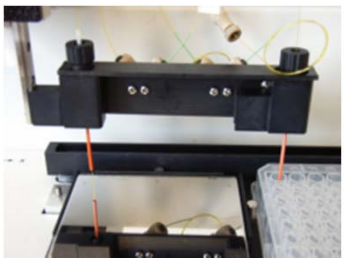
SunCollect



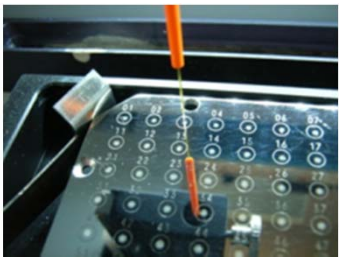
MALDI Imaging





Micro Fraction Collector

MALDI Spotting



Continuous Fractionation

SunChrom®

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MALDI Spotter / Micro Fraction Collector

SunCollect

MALDI Imaging and Continuous Deposition

Overview

When Micro HPLC or Nano HPLC is employed to separate samples, the components of the sample are isolated as fractions in the same way as when analytical scale HPLC is used. In many laboratories, the fractions are deposited into microtiter plate wells (MTP) or are spotted onto MALDI-MS targets for further investigation.

The SunCollect MALDI-Spotter offers outstanding performance for collecting fractions using either approach. If desired, a single fraction can be saved in both formats at the same time by a unique “dual needle-splitter” design.

When this approach is used, most of the fraction can be collected in the microtiter plate well and a small volume can be spotted on the MALDI target including the matrix solution. Even if the volume of the fraction is large, the entire fraction can be collected and no eluant is lost.

The software is designed to be extremely simple to use. It is very easy to enter new sample collection protocols or create new target formats.

The SunCollect MALDI-Spotter is biocompatible, because the sample and collected fractions are in contact only with PEEK and quartz.

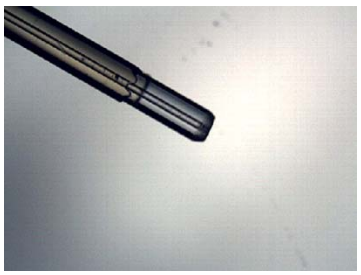

Figure 1 Patented fused silica needle (20x magnification)

Outstanding Features of the SunCollect MALDI-Spotter

- **Extremely flexible** with high precision: **SunCollect unifies 4 instruments** in one unit: Spotting; image preparation (spraying of enzyme or matrix solution), micro/nano fraction collection and continuous eluate and matrix deposition.
- **Simultaneous collection** of a sample on a microtiter plate and a MALDI target
- **Fast movement**; the needle switches from one spot to another in less than 1 second
- It is a **compact** unit that requires a small amount of space
- The spots can be **dried** very **fast** (option)
- The unit is completely **biocompatible** (quartz capillary probe)
- Very **small, precise spots** are possible (260 µm OD probe as option for <10 nl spots)
- **Perfect mixing** of the **eluate** with **matrix**
- **MALDI imaging** and continuous collecting of eluates in lines instead of discrete spots
- Controllable **dosing pump** for Matrix delivery
- Designed to be **easy to use**. The control software makes it simple to develop and edit new targets and collection protocols. In addition, the unit is easy to service
- It is very **reasonably priced**, compared to other present systems

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Optimized Shape

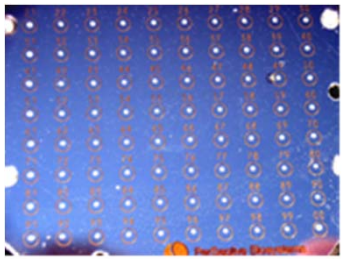
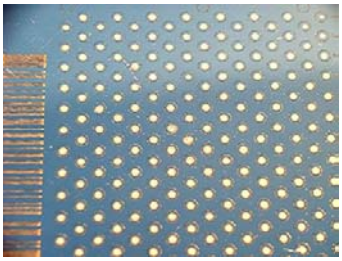
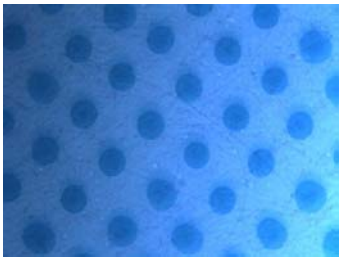

Figure 2a-c: Spotting Precision for difficult Targets with 192 spots and even with 5625 spots of 4 nl each on only 5 x 5 cm (2x2 inch) area (40x magnification)

The superb precision of the system ensures that there is no contamination between neighboring spots. In addition, it allows the user to shorten the measuring time, since the time required searching for „hot spots“ by the laser of the MALDI-MS is minimized.

Some targets employ a highly polished plate and therefore have a “hydrophobic” surface. In this situation, the distribution of small droplets can be difficult to control when the distance between the probe and target is very small.

Collection of sample on two targets or MTP allows the simultaneous deposition of the sample on both MALDI plates and micro titer plates, especially when high flow rates are employed. This is shown in Fig. 3.

A second application of the collection of fractions in two distinct devices leads to the use of 2-dimensional Micro or Nano HPLC. In this case, commonly most of the sample from ion exchange separation (1st dimension) is saved in a micro titer plate and used for second orthogonal separation (e.g. reversed phase).

Only a small amount of the sample is spotted on a MALDI target for a preliminary investigation.

An additional benefit of **extremely fast changeover** from spot to spot (less than 1 sec) allows for fractionation at high flow rates (e.g. with Micro-HPLC). As an example, the spots in Figure 2a were collected with a fraction time of 1 sec. The probe needle is made of 360 µm OD quartz to ensure very small and precise spots.

This leads to superb sample deposition with precise spot size and position; Fig. 2b. Spot volumes can be as low as 1 nl. 4 nl spots shown in Fig. 2c. Also the use of our high precision matrix dosing pump enhances the MS results in comparison to manual matrix pipetting.

New studies have clearly shown that the shape of the drop is optimized when:

- The needle does not touch the surface
- The droplets can be formed in a symmetrical shape by the tip of the probe
- Small droplets are deposited by the probe onto the plate
- Past adjustments to the system are kept in memory so that similar droplets can be treated in a common manner
- Sufficient time is provided for the deposition of the sample so that the liquid film can drain from the probe tip and minimized carryover.

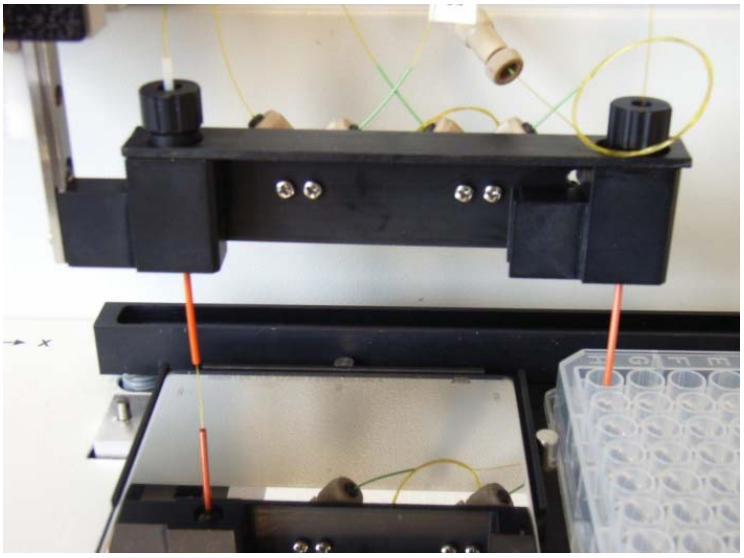

Figure 3: Simultaneous deposition of the sample on both MALDI plates and microtiter plate

Benefits

- Since the eluent is only in contact with PEEK and Quartz (see Figure 4), the SunCollect system is completely biocompatible. This is especially important when a sample containing an enzyme or a protein, which is sensitive to the presence of a metal, is being separated and fractionated.

- In addition to the benefit provided by the precise placement of the spots, it should be noted that the SunCollect is the smallest micro fraction collector on the market, Figure 6.

- The speed of drying of the spots has a significant impact on the size of the crystals that are formed in the spots. To obtain small, homogeneous crystals, an air blower is installed (see Figure 6).

- A further benefit of the rapid evaporation of the solvent is that the compounds are often not stable in the solution, but are more stable in solid form.

- The high precision matrix pump allows for extremely precise and reproducible delivery of reagent down to picoliter range. Syringes between 100 µl and 10 ml are accepted by the soft and hardware.

- This pump can be started before spotting to establish a constant flow rate from the first spot on.

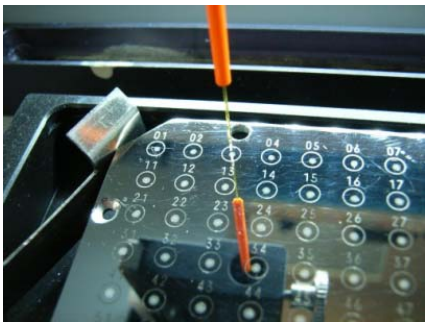

Figure 4 Biocompatible deposition needle

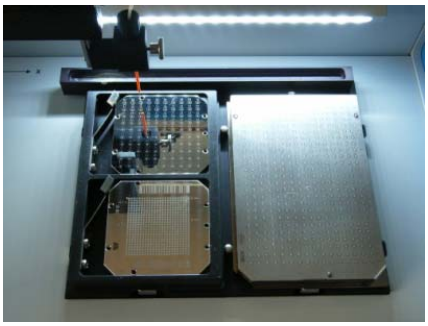

Figure 5 Illuminated target holder makes an expensive camera system needless

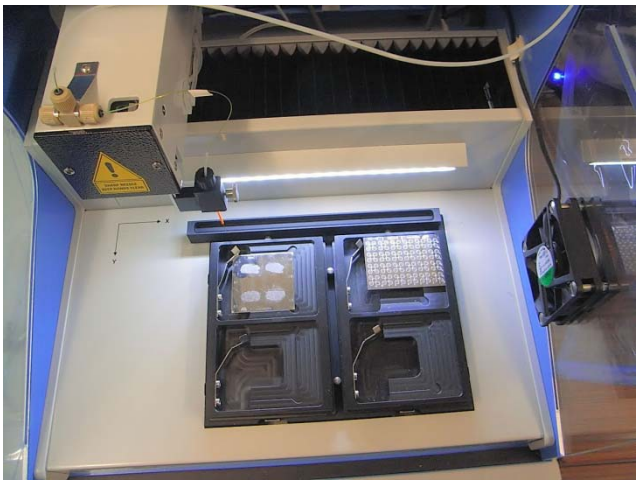

Figure 6 SunCollect has a very small foot print with an air blower on the right side

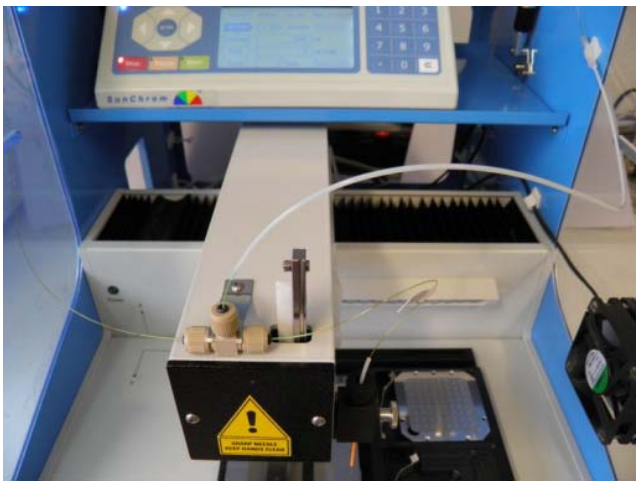

Figure 7 Micro T unifies and mixes eluent and matrix streams and directs to the spotting needle

Continuous Fractionation

The collection of the eluate in drops or spots can be very often disadvantageous because of possible remixing of compounds separated nicely on the column, but collected in a single spot. In comparison to online HPLC-ESI/API-MS technique MALDI is an offline approach with a discontinuous interface between HPLC and MS.

In order to overcome this issue SunChrom offers special developed targets for ABI and Bruker MALDI MS, where the sample needle remains on the target surface and moves continuously along the surface, depositing eluent with matrix in meandering line. An air blower dries these line rapidly without remixing of compounds due to diffusion, Fig. 7c.

This target has a special surface without any coating to ensure creating a continuous line without interruption at any eluent composition from 100% water up to 100% acetonitrile.

If desired, more than one separation can be placed on a single target. After drying and inserting the target in the MALDI, the laser beam of the mentioned ABI and Bruker MALDI can follow the lines in a continuous manner and the results are similar to online approach like HPLC-ESI-MS.

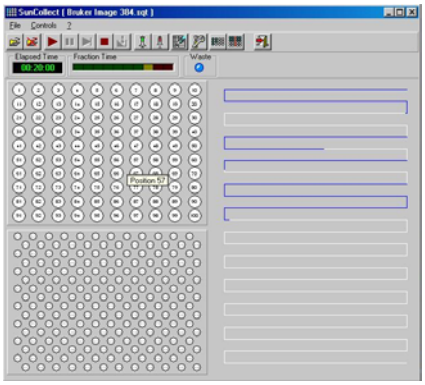

Figure 7a Changing of display during continuous eluate deposition

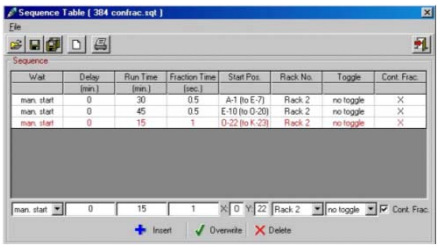

Figure 7b Sequence table for continuous eluate deposition

After loading a sequence table containing the “continuous collection mode”, the target display will change according the programmed movement, Fig. 7a.

During collection the colour of the line turns from white to blue.

All collection parameters can be selected within the sequence table, Fig. 7b. Depending on the target geometry multiple analysis can be deposited on a single target.

In the continuous collection mode, the fraction time setting describes the velocity of needle movement.

Fig. 7b shows an example where the needle moves every 0.5 second 0.125 mm toward the X axis (minimum resolution).

Figure 7c Continuous eluate deposition on special targets

MALDI Imaging

The first step of proteomics is discovering the constitution of proteins. The next step is to gain the knowledge about their biological function. Toward this goal there are many possible strategies. A very promising approach is to find out where specific proteins are located in a tissue sample. So correlations between proteins and their biological function can be made more easily.

R. Lemaire et.al. of "MALDI Imaging Team" of Université des Sciences et Technologies de Lille; France, presented an exciting paper at ASMS 2006 titled "***MALDI tissue direct analysis and imaging on Formalin Fixed Paraffin-Embedded Tissues***". This paper deals with the effect of Parkinson disease on the distribution of Proteins in tissue sample; Fig. 8. This was the beginning of MALDI tissue imaging.

This „single spot" procedure is very time consuming for large tissue areas. The limit of single spot distribution is 250 micron due to the needle OD of 260 micron.

Higher resolution can be achieved with our spray technology. More homogeneous coverage is also attained with this technique. Fig. 9 shows on tissue tryptic digestion of rat kidney proteins after CHCA coverage in a second step.

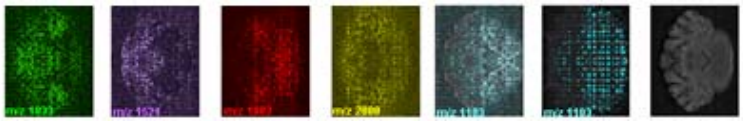

Figure 8 Distribution of some selected proteins in different anatomic regions of rat brain achieved by single spot application (Courtesy of Prof. Salzet and Dr. Fournier, Université Lille, France)

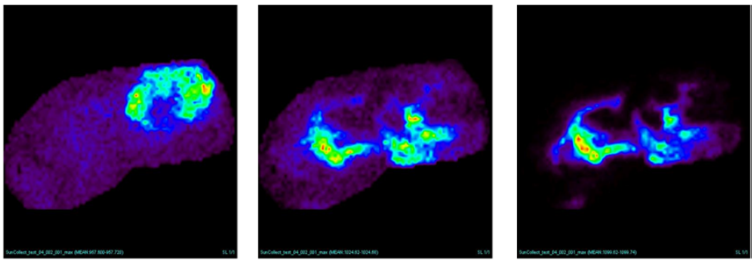

Figure 9 Distribution of some selected peptides in different anatomic regions of rat kidney achieved by spraying Trypsin and thereafter CHCA layer (Courtesy of Emmanuelle Claude; Waters; Manchester; UK)

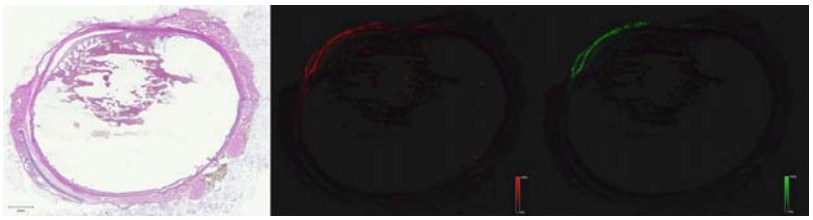

Figure 10 Distribution of two Benzyl-alkyl-ammonia homologues on rabbit eye after DHB spray application and its amazing ultra high resolution (Courtesy of ImaBiotech; Lille; France)

The spray technique is becoming more popular due to number of advantages:

- It is significant faster; instead of hours only few minutes are necessary for one layer
- The tissue surface coverage is more homogeneous
- The crystal size can be varied between amorphous and up to 50 micron by several parameters
- Enzyme and matrix solutions can be applied in the same manner

The SunCollect Control Software

The special characteristics of the control software include:

- Up to four MALDI targets can be used
- Targets may have different geometries
- Microtiter plates can be employed
- Spot geometry can be defined by user for other targets like films
- Standard targets and newly developed targets can be employed
- Different modes of sampling for each target is provided
- Simultaneous fractionation with two targets (MALDI target and microtiter plate) is possible
- Separation of a target into segments for optimal usage of targets is available
- External contact closure allows for automatic control by any HPLC system
- Extremely powerful, but simple training program for new target formats
- MALDI imaging and continuous fractionation in lines instead of discrete spots

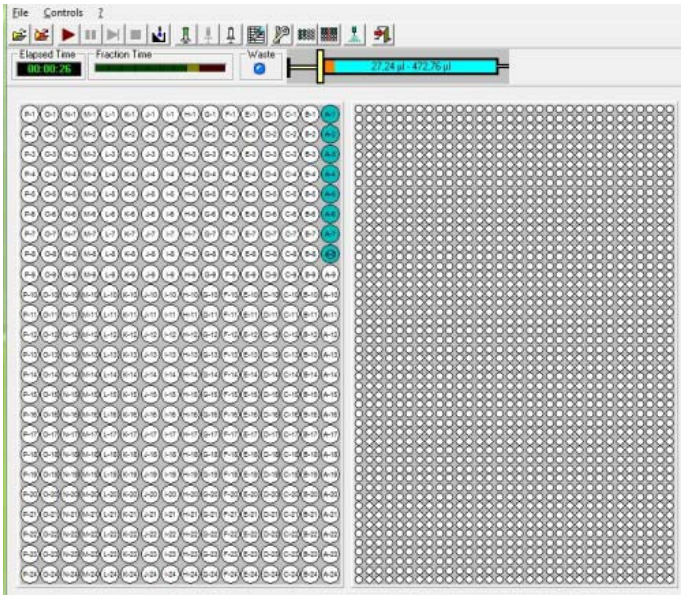

Figure 11a SunCollect main window with present targets and syringe

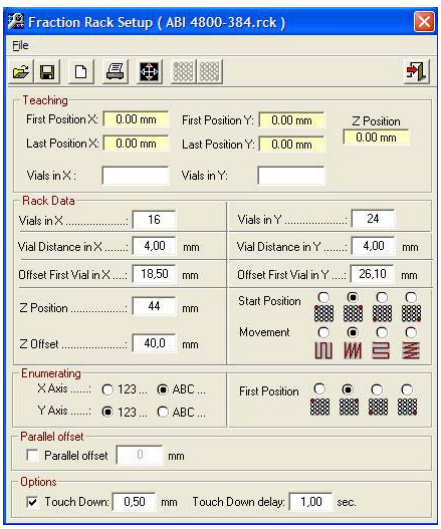

Figure 11b Rack Setup for target format and parameters

Targets placed on the instrument are showed on the monitor to simplify the use of the software and hardware. When the fractionation is started, the corresponding spots change from white to blue. Similarly, a manual change of the position of the probe needle can be performed via the mouse to move it to a specific position (Figure 11a).

unique “teaching” feature enables the user to create new target formats within few minutes instead of hours. A number of parameters are available to meet desired target features.

The main parameters like distances between spots in X and Y directions are calculated automatically by the software as well as the offsets for the first spot in X-Y directions from the home position.

There are also two movement types and directions available; two in zigzag and two meanders.

Beside the adjustment of the height of the probe in Z axis, a so called „touch-down“ features enables to lower the needle after the fractionation time in order to leave the solvent drop almost quantitavely on the target surface.

The user can program the length of the down stroke and waiting time required for the fluid to completely flow to the target surface.

Last-but-not-least all essential parameters can be protected from undesired operator changes, if the rack inputs are saved as fixed files.

Creating a new target format

The upper half of the *Rack-Setup* window is part of the teaching program. Specific test combinations are used to drive the probe needle to the two diagonal corner positions (X and Y axes).

After the two X and Y-axis positions are determined, the Z-axis position is established. In this way, the parameters for new microtiter plates and MALDI targets can be rapidly determined. The user needs only enter the total number of spots or wells in X and Y directions (Figure 11c).

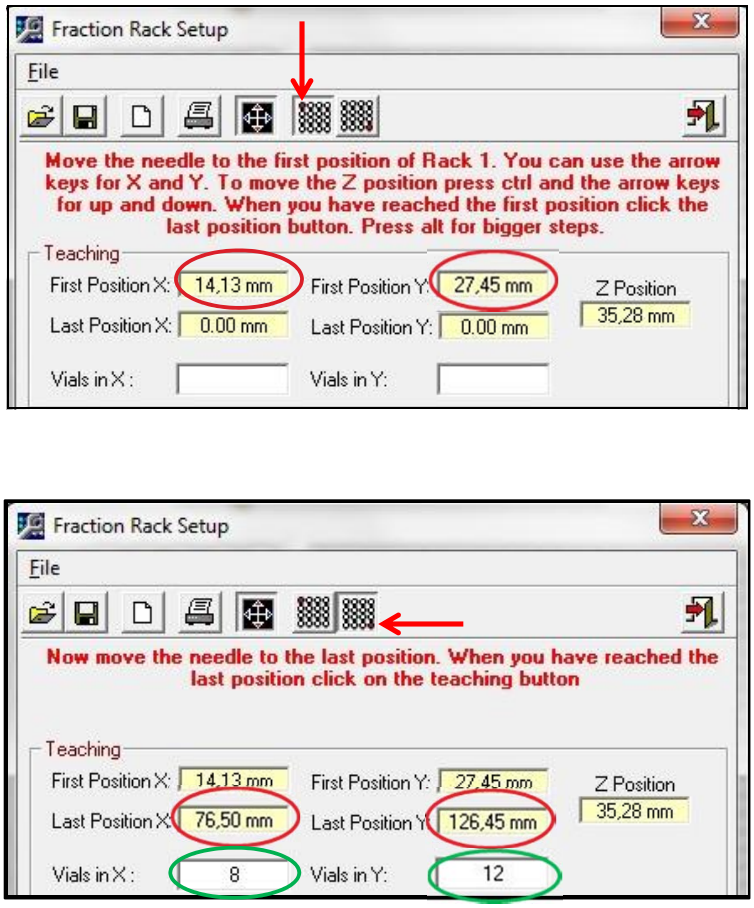

Figure 11c Teaching of new target format and parameters

Editing run parameters

The individual fractions are programmed via a **timetable** named ***Sequence Table***; Fig. 12. In this table, the start position of the microtiter plate or MALDI target, the delay time, the desired collection time for each fraction and the overall collection time (analysis time) are entered as shown in Figure 12.

Once the overall fractionation time and the time for each fraction is entered, the position of the last spot is calculated automatically , Fig. 12; see red arrow.

When the sequence table is loaded or system starts, the program automatically checks the system for consistency and total volume of matrix required for all runs.

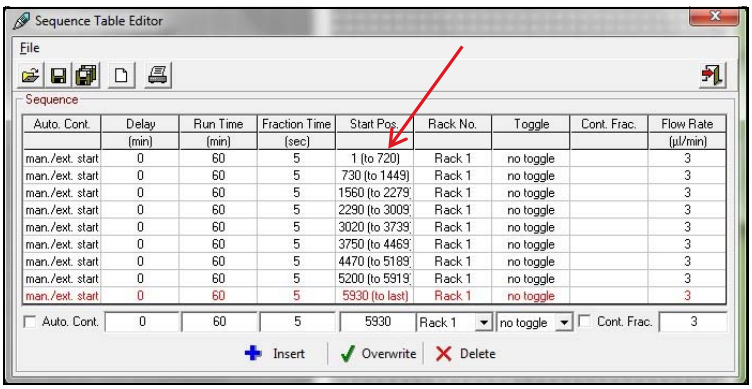

Figure 12 Sequence Table editor

Technical Specifications

Hardware

Number of Targets	max. 4 ABI Targets (100 or 192 spots)
Targets to which the system can be interfaced (ready or adaptable)	Bruker; ABI; MTP (96 or 384 Positions) and any others
Needle Material	Quartz; 360 µm OD. Please request information for other materials or diameters.
Time to skip to the next spot	< 1 s (measured by change with ABI100 target between two neighboring spots and 2 mm Z-axis movement
Resolution of the Axis Movement	X-Axis: 0.004 mm (0.00015“) Y-Axis: 0.016 mm (0.000625“) Z-Axis: 0.005 mm (0.000196“)

Software

Number of Programmable Targets	4 simultaneous collection and spotting
Number of Fractionation Sequences	No limit (dependent on capacity of hard disk)
Computer Memory Requirement	Approx. 2 MB (256 MB RAM recommended)
Windows Versions supported	Windows 2000; XP; Windows 7

General

Dimensions: SunCollect (closed lid) **SunCollect (open lid)**	W 36 cm (14.2“) x D 49 cm (19.3“) x H 40 cm (15.7“) W 36 cm (14.2“) x D 64 cm (25.2“) x H 85 cm (33.5“)
Weight	17 kg
Power Requirements	100 – 240 VAC 50/60 Hz (external power supply)
Dosing Pump	W 24 cm (13.4“) x D 18 cm (7.1“) x H 15 cm (5.9“); 3 kg 100 – 240 VAC 50/60 Hz

SunChrom GmbH . Industriestr. 27 . 61381 Friedrichsdorf . Germany
Contact: Catherine Obstétar . Tel.: +49 6172 953350 . E-Mail: cobstetar@sunchrom.de