



## 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** is extremely successful in collecting fractions using either approach. If desired, a single fraction can be saved in both formats at the same time. 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. 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 design.

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

#### Outstanding Features of the SunCollect MALDI-Spotter

The **SunCollect MALDI-Spotter** provides the following features:

- 1) High precision
- 2) Simultaneous collection of a sample on a microtiter plate and a MALDI target
- 3) Extremely rapid; the probe moves from one spot to another in less than 1 second
- 4) It is a compact unit that requires a small amount of space
- 5) The spots can be dried very rapidly (option)
- 6) The unit is completely biocompatible (quartz capillary probe)
- 7) Very small, precise spots are possible (260  $\mu\text{m}$  OD probe as option for 10 nl spots)
- 8) Perfect mixing of the eluate with the matrix
- 9) MALDI imaging and continuous collecting of eluates in lines instead of discrete spots
- 10) Controllable dosing pump (optional)
- 11) 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
- 12) It is very reasonably priced, compared to other present systems.

The **superb precision** of the system ensures that there is no contamination between neighboring spots. In addition, it allows the user to shorten the time between measurements since the time required to sample a spot by the laser of the MALDI-MS is minimized.



**Figure 1 Spotting Precision for difficult Targets with 100 spots, 192 spots and even with 5625 Spots on only 5 x 5 cm (2x2 inch) area**

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 the target is very small.

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

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

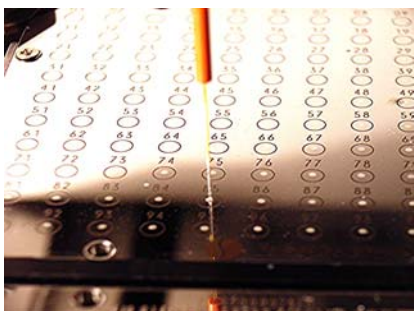
**Collection of sample on two targets / MTP** allows for the simultaneous deposition of the sample on both MALDI plates and microtiter plates when high flow rates are employed. This is shown in Figure 2.



**Figure 2 Simultaneous Collection on MALDI Plate and Microtiter Plate**

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 situation, commonly most of the sample from ion exchange separation (1st dimension) is saved in a microtiter plate and used for a second 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 rapid 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 1 were collected with a fraction time of 1 sec. The probe needle is made from 360  $\mu\text{m}$  OD quartz to ensure very small and precise spots. This leads to superb positioning of the spot as the spots are exactly in the middle of the assigned area and have the same size. Also increased precision is observed as an additional benefit when the matrix-dosing pump is used.



**Figure 3 Biocompatible Quartz Capillary Probe**

Since the eluent is only in contact with PEEK and Quartz (see Figure 3), 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.



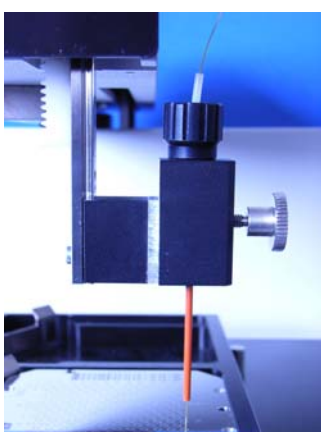
The speed of drying of the spots has a significant impact on the size of the crystals that are formed in the spots. To obtain small, homogeneous crystals, we offer an optional device (see Figure 4) to control the flow of air for drying.

*Precise air stream for fast drying of spots*

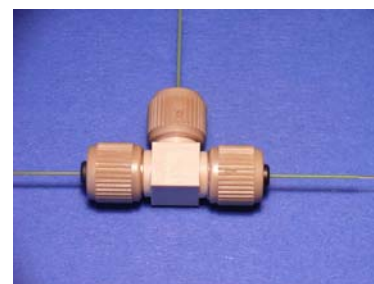
A further benefit of the rapid evaporation of the solvent is that the compounds are often not stable in the solution, but in solid form.

**Figure 4 The SunCollect with a very small foot print equipped with an air blower on the right**

The optional matrix pump allows for extremely precise and reproducible delivery of reagent in the picoliter range. Relatively large flow rates between millilitres and picolitres can be precisely delivered by the pump since the flow rate resolution of the pump is extremely high. This pump is used to replenish the syringe as shown in Figure 4. The length of the capillary between the detector cell and T junction is an important dimension in terms of delay time and peak broadening. Due to the very close distance of SunCollect, the delay time is very short and outer column peak broadening negligible.



A micro T with a dead volume of few nano litres connects the quartz capillary from the HPLC and the metering pump with the probe needle and provides for maximum mixing with minimum peak broadening. To avoid undesirable absorption effects, the polyimide layer of the tube is removed. A specially designed needle holder is used to prevent damages to the needle (Figure 5).



**Fig. 5 The patented probe (sample needle) in its special holder (left) and PEEK T-connector / mixer with only few nano litres of dead volume (right)**

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## Continuous fractionation



**Figure 6** Continuous collecting of eluates in lines instead of discrete spots

The collection of the eluate in drops or spots can be very often disadvantageous because of possible remixing of compounds which were separated on the column just before. In comparison to online HPLC-ESI/API-MS technique MALDI is an offline approach with a discontinuous interfacing HPLC to MS.

In order to overcome this disadvantage SunChrom offers special developed targets for ABI and Bruker MALDI MS, where the sample needle remains on the target surface and moving continuously on the surface, drawing meander like lines. An air blower dries these lines rapidly without remixing of compounds due to diffusion.

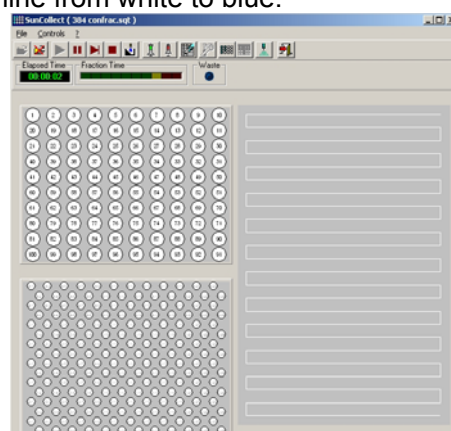
The special surface without any disturbing coating ensures creating continuous lines without interruption at any eluent composition from 100% water up to 100% acetonitrile. If desired, more than one separation can be placed on a single target. After drying and inserting the target in the MALDI, the laser 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.

Within the sequence table all collection parameters can be selected, Fig. 7a. Depending on the target geometry more than one single analysis can be placed on the same target. If the continuous collection mode is chosen, the fraction time setting describes the velocity of needle movement. In Fig. 7a shown example the needle moves every 0.5 second 0.125 mm toward the X axis (minimum resolution).

After loading a sequence table containing the “continuous collection mode”, the target display will change according the programmed movement, Fig. 7b. During collection the user can follow the position of the needle by turning the colour of the line from white to blue.

Wait	Delay	Run Time	Fraction Time	Start Pos.	Rack No.	Toggle	Cont. Frac.	Flow Rate
(min)	(min)	(min)	(sec)					(µl/min)
man. start	0	30	0.5	A-1 (to E-7)	Rack 2	no toggle	X	5
man. start	0	45	0.5	E-10 (to O-20)	Rack 2	no toggle	X	5
man. start	0	15	1	O-22 (to K-23)	Rack 2	no toggle	X	5

**Fig. 7a** Sequence table for continuous fractionation



**Fig. 7b** Target display for continuous fractionation mode



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## MALDI Imaging

The first step of proteomics is discovering the constitution of proteins. The next step is the knowledge about their biological function. Toward to this goal there are many possible strategies. One of very promising way is to find out what kind of proteins can be found in a specific area. 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 during ASMS 2006, an exciting paper titled "**MALDI tissue direct analysis and imaging on Formalin Fixed Paraffin-Embedded Tissues**" dealing with applications to Parkinson disease.

They fixed a very thin slice of rat brain on a glass target and spotted first with a trypsin solution all over the slice. Using a 260  $\mu\text{m}$  quartz needle the digestion fluid could apply in very high density of spots. The needle was kept on the surface and injected approx. 100 nl of enzyme. The advantage is that the fluid could penetrate in the substrate without drying.

After the incubation process the MALDI matrix (HCCA at 10mg/mL) was applied to the same spots made before and than analysed with MALDI-LIFT TOF/TOF.

Fig. 8 shows the amazing results of the distribution of some selected proteins in different regions of rat brain. They could clearly show, which proteins are dominantly present in different anatomic areas.

These results were possible because of deeply penetration of trypsin sealing the spot area from ambient during application without drying. SunCollect offers this unique feature opening new horizons to the proteomics community.

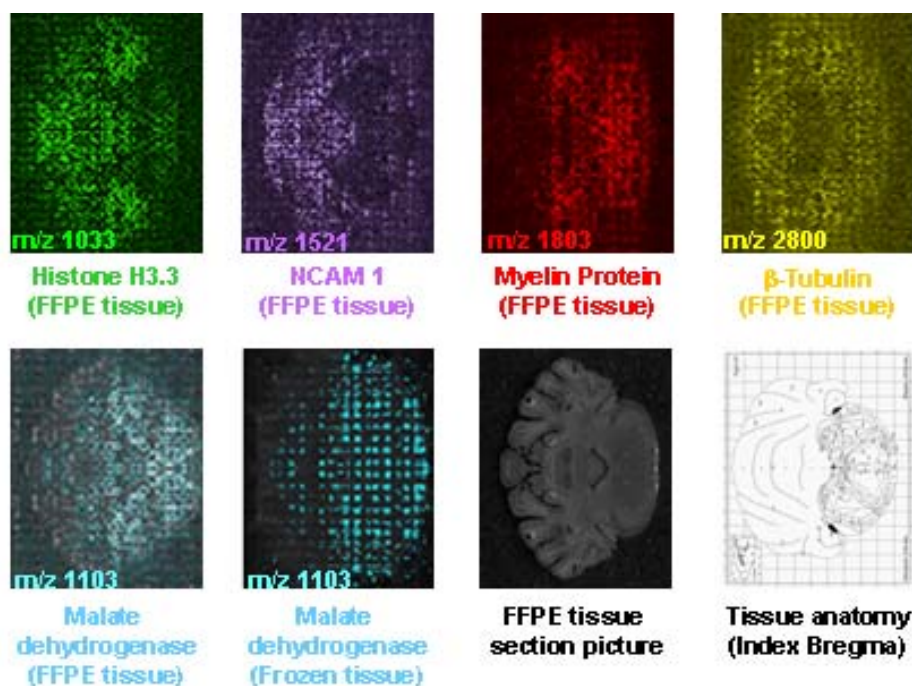


Fig. 8 Distribution of some selected proteins in different anatomic regions of rat brain

(Courtesy of Prof. Salzet and Dr Fournier, Université des Sciences et Technologies de Lille, France)

## The SunCollect Control Software

The special characteristics of the control software include:

- 1) Up to four MALDI targets can be used
- 2) Targets may have different geometries
- 3) Microtiter plates can be employed
- 4) For other targets like films, the spot geometry can be defined
- 5) Standard targets and newly developed targets can be employed
- 6) Different modes of sampling for each target is provided
- 7) Simultaneous fractionation with two targets (MALDI target and microtiter plate) is possible
- 8) Separation of a target into segments for optimal usage of targets is provided.
- 9) Manual initiation of fractionation via the mouse, automatically via an external signal or a Windows DDE connection
- 10) Extremely simple training program for new targets
- 11) MALDI imaging and continuous fractionation in lines instead of discrete spots

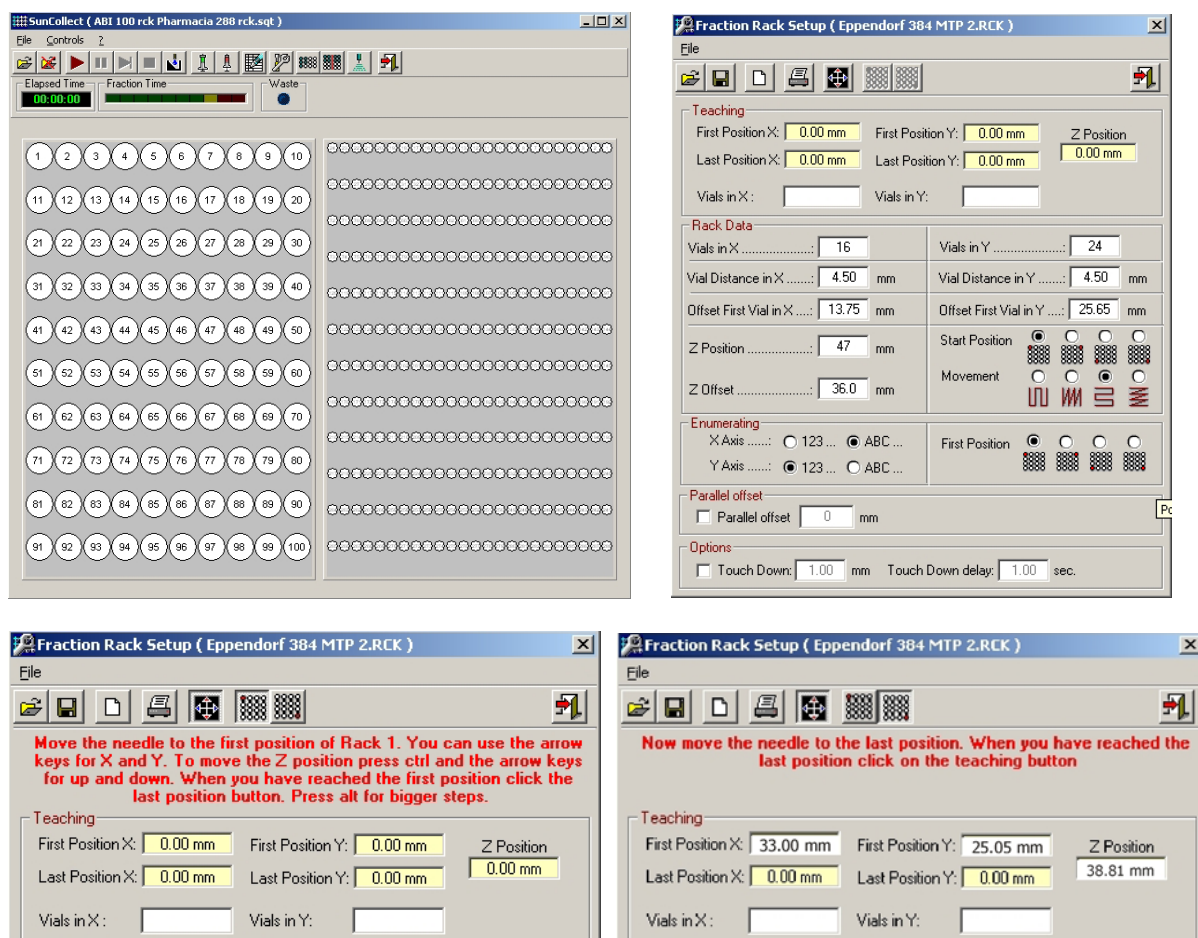


Figure 9 SunCollect Main Menu (top, left), Rack Setup (top, right) and Training Program (above)

The present targets placed within the instrument are showed on the monitor to simplify the use of the software and hardware. When the fractionation is started, the colour of the corresponding spots is changed. 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 9, top, left).

The geometry of the collection plate in the Z-axis can be stored as shown in Figure 9 (middle). In addition, the user can see a report of the sequence of the spots with respect to the rows, numbering, position of the starting points, etc. The definition of the droplets is also presented.

The upper half of the *Rack-Setup* window is part of the *Learn* program. Specific test combinations are used to drive the probe needle to the two diagonal corner positions (X and Y axes). After the 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 (Figure 9, top, right).

Wait	Delay (min)	Run Time (min)	Fraction Time (sec)	Start Pos.	Rack No.	Toggle	Cont. Frac.	Flow Rate (µl/min)
man. start	0	10	3	A-1 (to last)	Rack 1	no toggle		5
ext. start	0	50	20	A-1 (to G-6)	Rack 3	no toggle		1

**Figure 10 Sequence Table**

The individual fractions are programmed via a **timetable** named **Sequence Table**; Fig. 10. In this table, the start position of the microtiter plate or MALDI target, the receiver position, the delay time, the desired collection time for each fraction and the overall collection time are entered as shown in Figure 9. This allows for the possibility to optimize the spots on a microtiter plate or MALDI receiver. Once the overall fractionation time and the time for each fraction is indicated, the position of the last spots are determined and displayed. This allows the operator to enter the next sequence without lengthy calculations or without having to leave empty spots for security. When the system starts, the program automatically checks the system for consistency.

After a sequence table has been generated, the fraction collection can be initiated by a mouse click of the Start button, by a contact closure or via the Windows DDE connection. All these three alternatives can be selected by an entry in the sequence table. When the system is started, the dosing pump is also automatically started.

**Note:**

*Many of the features described in this document are the subjects of patent applications, even if that fact is not indicated. The features described herein are the property of SunChrom. The user cannot use this program in part or complete with other instruments. The pictures and text of this document cannot be copied, duplicated or transmitted electronically without the written permission from SunChrom.*

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## Technical Specifications

<b>Instrumental Characteristics</b>	
<b>Number of Targets</b>	max. 4 ABI Targets (100 or 192 spots)
<b>Targets to which the system can be interfaced (ready or adaptable)</b>	Bruker; ABI; MTP (96 or 384 Positions) and any others
<b>Needle Material</b>	Quartz; 360 µm OD. Please request information for other materials or diameters.
<b>Time to skip to the next spot</b>	< 1 s (measured by change with ABI100 target between two neighboring spots and 2 mm Z-axis movement)
<b>Resolution of the Axis Movement</b>	X-Axis: 0.0038 mm (0.00015")
	Y-Axis: 0.03 mm (0.0012")
	Z-Axis: 0.0049 mm (0.000193")
<b>Software Characteristics</b>	
<b>Number of Programmable Targets</b>	4 simultaneous collection and spotting
<b>Number of Fractionation Sequences</b>	No limit (dependent on capacity of hard disk)
<b>Computer Memory Requirement</b>	Approx. 2 MB (256 MB RAM recommended)
<b>Windows Versions supported</b>	Windows 2000; XP
<b>General</b>	
<b>Dimensions</b>	36W x 49D x 40H cm (14.4x19x16")
<b>Weight</b>	16 kg
<b>Power Requirements</b>	100 – 240 VAC 50/60 Hz (external power supply)
<b>Dosing Pump (option)</b>	Chemyx 24 x 17 x 14 cm (9.5x6.7x5.5 in) 2 kg Harvard 22 x 12 x 16 cm; (8.8x4.8x6.4") 1,5 kg

We reserve the right to make changes in the specifications, design, comments in the above information or price at any time without notice.