

*Probot*<sup>TM</sup>

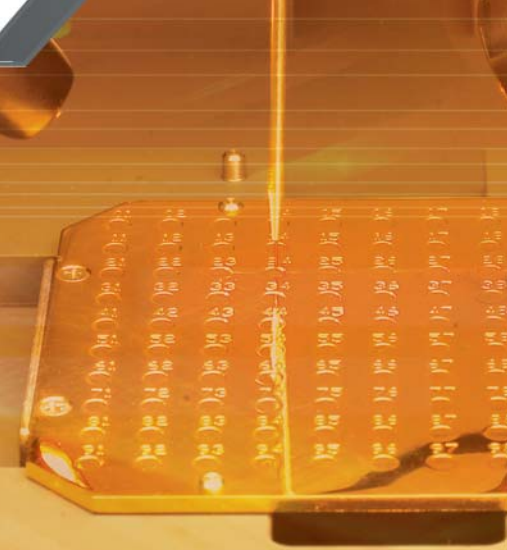
PROBOT MICROFRACTION COLLECTOR



**LC PACKINGS**  
A DIONEX Company

LC/MALDI is rapidly gaining ground in the analysis of highly complex proteomic samples. With the development of the MALDI/TOF/TOF mass spectrometers, real MS/MS can now be performed with MALDI ionization.

On-line Nano LC/MALDI target preparation brings together the high-resolution separation power of nano LC with the high resolving power of MALDI/MS. This power combined with Probot's high sample capacity is ideal for high-throughput proteomics.



## COMBINE HIGH-RESOLUTION NANO LC SEPARATIONS

Probot specifically is designed for on-line collection of Microliter and Nanoliter fractions eluting from capillary and nano LC systems with highest reproducibility. Collection is possible on a wide variety of substrates, such as MALDI targets, PVDF membranes, 69, 384, 1536 well plates, and microchip structures. Additionally, the instrument can pipette or dispense samples and reagents. With its versatility, Probot is capable to handle almost any MALDI target from different vendors.

### On-line Nano LC/MALDI Target Preparation

Lowest nanoliter volumes are collected onto MALDI targets with zero chromatographic dispersion, maintaining the high resolution of the Nano LC separation system. For high throughput, up to six high-density targets can be placed on the table, allowing for the collection of up to 3456 spot in a fully automated run.

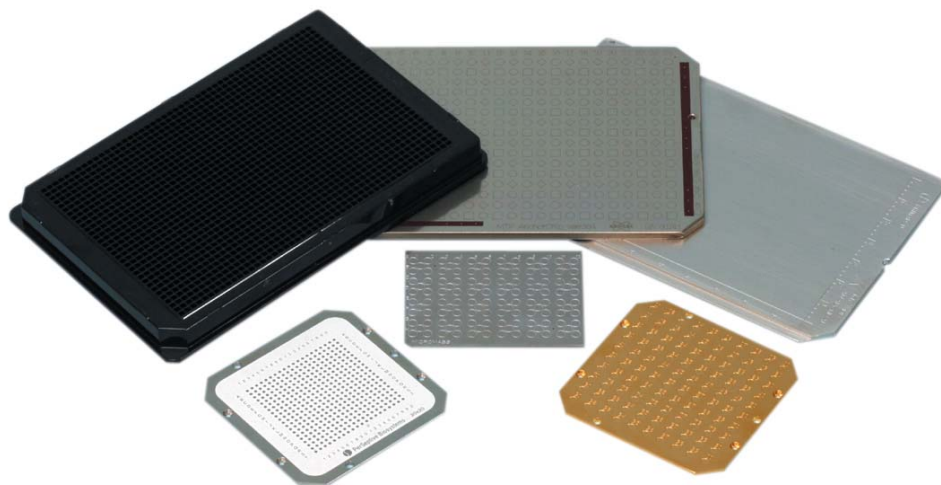
### Integrated Matrix Addition

Probot can be equipped with a fully integrated matrix pump. This microdosage pump allows for matrix or make-up flow addition during Nano LC spotting onto MALDI targets. The dosage pump can be used in two modes: continuous flow from nL/min to  $\mu\text{L}/\text{min}$  or dosage mode with fixed amounts starting at 0.2  $\mu\text{L}$ .

### Software control

Probot comes with its own user-friendly software control that allows to program the MALDI spotting through easy-to-use wizards. All required steps to perform fractionation are covered with these wizards. In no more than five easy steps, the complete fractionation set-up can be made from scratch.

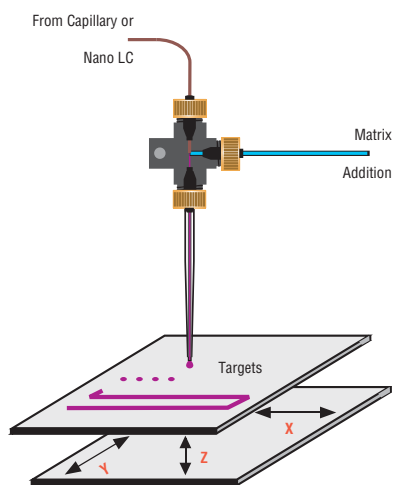
The software allows the user to take full advantage of Probot's flexibility.



The Probot is capable of collecting fractions on a wide variety of MALDI targets and microtiter plates.

**High-Resolution**  
Nano LC Separations

## WITH HIGH MALDI/MS RESOLVING POWER



*In nano LC, the matrix is mixed with the LC flow in a micro-T piece before spotting.*

### Performance Features:

- Robotic system for fraction collection of nanoliter and microliter spots
- User-selectable spotting frequency for zero chromatographic dispersion
- Integrated matrix addition
- Continuous MALDI target preparation monitoring
- Reagent addition, and pipetting
- Dual collection mode on targets, membranes, and other substrates

### Dual-Collection Mode

In dual-collection mode, a fraction can be split over two different sample carriers, such as MALDI targets and well plates or PVDF membranes. The split ratio is programmable.

### Collection, Sweet Spot, and Sample Storage

For small volume collection (less than one microliter), Probot can perform the matrix addition prior to the needle to transport the nanoliter fractions onto the MALDI target and generate a perfect sweet spot. Using nano LC with typical 200 nL/min flow rates, collection times as small as five seconds are possible, resulting in collection (spotting) volumes down to 7 nL. Under these conditions, no chromatographic dispersion is observed (zero dead volume), achieving the highest MALDI/MS sensitivity.

For large volume collection (greater than one microliter), the matrix can be added coaxially through the needle. Another advantage from interfacing capillary or nano LC with MALDI/MS is the storage capability of targets for up to several months, allowing optimizing work flows and sample reanalysis.

### Spotting Frequency

The spotting frequency is user-selectable to match chromatographic resolution. For complex samples with numerous peaks and fast separations (e.g., using monolithic nano and capillary columns), fast spotting frequencies are recommended.



*For multidimensional LC applications, the Probot can be used to collect fractions into microtiter plates.*



*Fraction collection on a Bruker AnchorChip MALDI target. The Probot is equipped with an optional video camera for viewing the spotting remotely.*

Probot automates LC/MALDI interfacing. Fractionation, spotting and target preparation can now be performed in fully automated fashion, offering highest precision and reproducibility. Continuously spot LC eluent onto the MALDI target in volumes as low as 5 nL. With these low spot volumes, the ionization process is easily executed, greatly improving the MS sensitivity. Using Probot in conjunction with the UltiMate Plus and UltiMate 3000 Nano LC Systems, prior to MALDI/TOF/TOF instrumentation enables high precision and high sequence coverage in proteomics analysis.



Probot Microfraction Collector

## DISPERSION FREE MA

### Optimal Use of X, Y, Z, Moving Table for MALDI Targets and Well Plates

Probot moves its target table—not the needle, which remains in a fixed position—allowing precise spotting of nanoliter volumes. The use of a fixed needle allows for the collection and spotting of smallest nanoliter volumes, which is impossible with a moving needle due to capillary forces. In addition, the use of a fixed needle also allows for minimal dead-volumes, minimizing extra column-broadening.

Probot's deck table can be programmed to move freely in a cartesian space with dimensions of  $x = 195$  mm,  $y = 130$  mm, and  $z = 95$  mm, reaching any point with highest precision. All axes are controlled by reference switches with a reproducibility of  $\pm$  motor step ( $2.5 \mu\text{m}$ ), resulting in a table positioning precision of better than  $20 \mu\text{m}$ . Due to this excellent precision, high-density MALDI targets with several hundred spots or microchip substrates can be used routinely for fraction collection. In fact, Probot can handle any type of MALDI targets that fall within the above listed X, Y, Z dimensions.



**High-Resolution**  
Nano LC Separations



## ALDI TARGET SPOTTING

### Intuitive Software Control for

### Microfraction Collection— $\mu$ -Carrier

Probot is controlled by  $\mu$ -Carrier software, performing an intuitive programming of the microfraction process. The clear and easy-to-use interface displays all necessary information in one window.

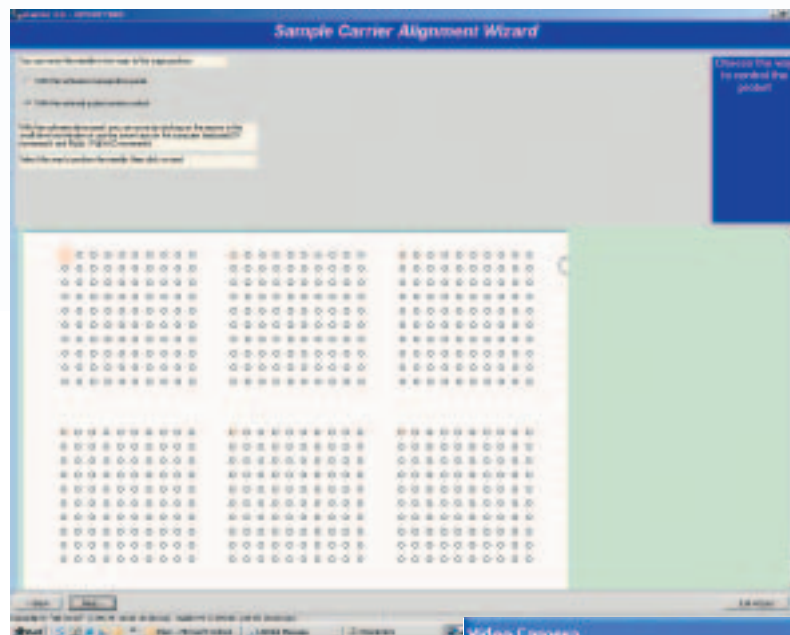
### Basic Fractionation Task

The Basic Fractionation Task enables users to fast and easily start the Probot. Task options have been set at an absolute minimum required to run the application:

- **How:** The conditions for matrix addition are specified here
- **When:** Specify the spotting frequency and the end time of chromatogram
- **Where:** Specify the target group, the position from where the needle should start and where it should go at run end [standby position] and the height of the needle during table movement.

### Wizard Function for More Advanced Applications

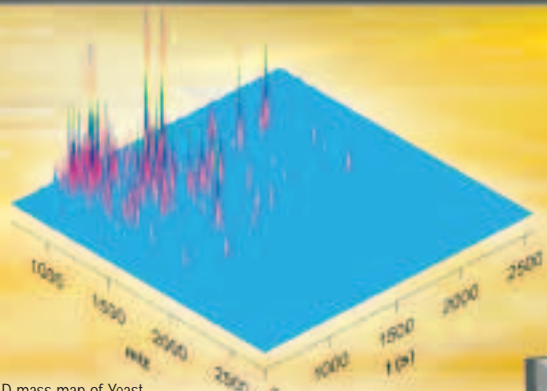
For more advanced spotting applications, self-explaining wizards will guide the user through the software, allowing full advantage of Probot's flexibility.



Software wizards allow for easy alignment and set-up of fraction collection programs.



The image of the optional video camera can be viewed in a separate window on the PC controlling the Probot.



3-D mass map of Yeast digest fraction #8 after 2-D separation on SAX and RP (5 s fractions).  
 Courtesy: Dr. Dale Patterson, Applied Biosystems, Inc., Framingham, MA USA.

Probot Microfraction Collector and UltiMate™ 3000 Nano and Capillary LC system.



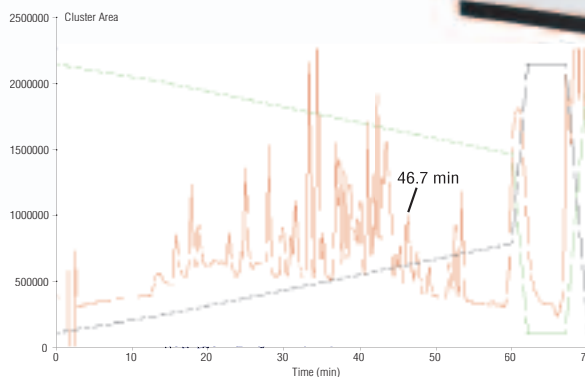
# PROBOT APPLICATIONS

## Experimental

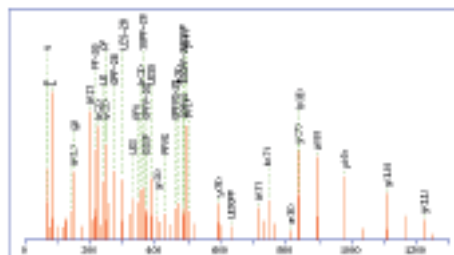
Nano LC system	UltiMate, FAMOS™, Switchos™, Probot
Sample	5 $\mu$ L of 200 fmol/ $\mu$ L transferrin digest
Flow rates	Loading 15 $\mu$ L/min Nano LC 800 nL/min
$\mu$ -Guard column	300 $\mu$ m $\times$ 1 mm, PepMap C18, 5 $\mu$ m
Nano LC column	100 $\mu$ m $\times$ 15 cm, PepMap C18, 3 $\mu$ m

## Typical Applications:

- On-line LC/MALDI target preparation
- Preparation of MALDI targets by sample pipetting from source, such as well plates, onto MALDI targets
- On-line collection of intact proteins after IEX or RP separations (top-down proteomics)
- Collection of peptides after 1-D or multidimensional nano LC (bottom-up proteomics)
- Dual collection mode, such as collection of intact proteins onto MALDI targets and collection of parts with same fraction into well plates.
- Interfacing capillary electrophoresis with MALDI/MS by direct collection of CE fractions onto MALDI targets
- Collections onto microchip substrates, such as microarrays, lab-on-chip, and SELDI ProteinChip

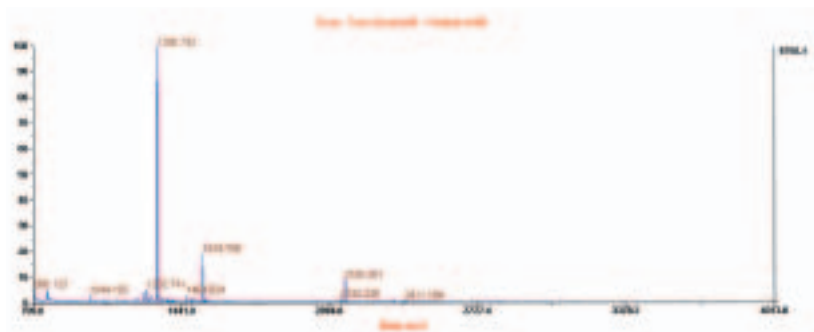


UV (214 nm) and MALDI (BPI) trace of 1 pmol transferrin digest illustrating zero chromatographic dispersion (frequency: 28 s/spot). Peptide eluting at 46.7 min collected on well #100.



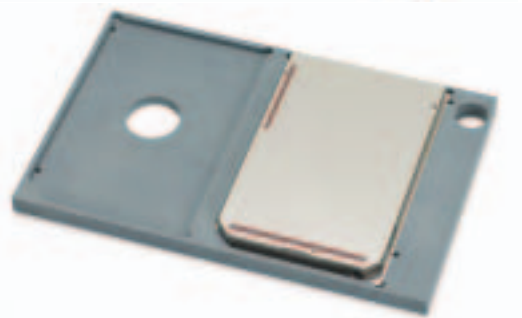
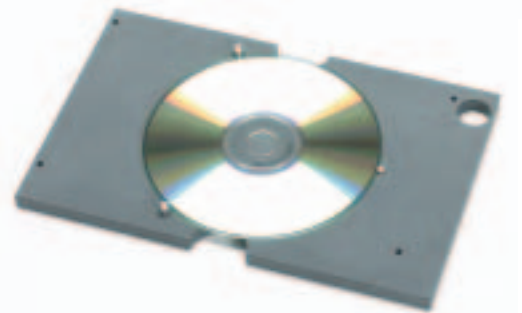
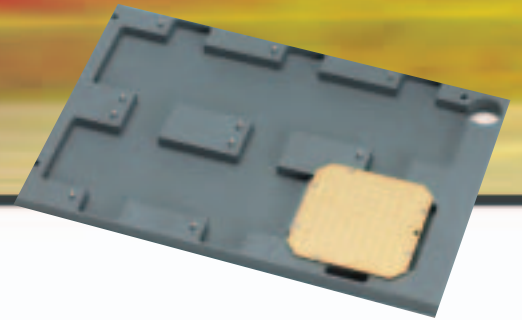
MS/MS Fragmentation of **ELKSTKPPVQIC**  
 Found in **TRIF\_8.FIN** (2344) SECYFQKIKRERQSH FKKVQKCR (K)KIKQVGLH  
 Match to Query:26 (20871),1-C:161(2344),Spot\_34 (46.7),Peak\_List\_14 (2344),MSMALDI\_34a\_34 (1336),Classical  
 Protein:559-E:1(2)and 1336 - Ultimate 1 pmol 1-20ppm\_transferrin\_34\_467111111.ms  
 Click mouse on the plot, see the zoomed-in plot of the above data point.  
 Da:  Da

MS/MS spectrum from peak m/z 1336. Very strong and complete y-ion series with immonium ions and internal fragments for sequence verification are obtained, allowing for unambiguous identification by database search.



MALDI/MS spectrum of spot #100 eluting at 46.7 min (m/z 1336).  
 From any spot, MS data can be recorded.

Adapters are available to accommodate most MALDI targets. A few examples are (top to bottom) Applied Biosystems, Micromass, PMMA (CD) disk, Bruker, and Ciphergen.



## COMPONENTS AND SPECIFICATIONS

Table Dimensions	210 × 135 mm or 8.27 × 5.31 in. (w × d)
Table Movement	X = 195 mm speed 0.250 to 20 mm/s Y = 130 mm speed 0.125 to 10 mm/s Z = 95 mm speed 0.125 to 10 mm/s
Precision of Table Positioning	Better than 20 μm
Spotting Frequency	User-selectable down to 5 s with minimal volumes of 5 nL
Lifting Force	>2 kp
Reference Position	All axes are controlled by reference switches Reproducibility is ±1 motor step (2.5 μm)
Safety	All functions stop when: <ul style="list-style-type: none"> <li>• The front door is opened</li> <li>• An axis runs over its end switch</li> <li>• The table exerts too much force on the needle</li> </ul> In CZE mode, the CZE voltage is interrupted when: <ul style="list-style-type: none"> <li>• The front cover is opened</li> <li>• Probot is unplugged or switched off</li> </ul>
Connections	Remote, X, Y, Z RS-232 communication Remote start input and TTL input Outputs: four relay outputs (optional) HV switch off (safety feature for CZE)
Dimensions (h × w × d)	30 cm × 48 cm × 50 cm (12 in. × 19 in. × 20 in.)
Weight	23 kg (51 lbs)
Software	Fully controlled by μ-Carrier
Collection Modes	Time-based collection and peak collection

### Ordering Information

Item Description	Part Number
Probot including software, microdosage unit, and manual control, without table	5713.0200
<b>Probot Tables</b>	
Universal table for AB4700 Proteomics Analyzer for six targets (allows for the use of Opti-TOF MALDI Plate System with reusable holder)	5713.6402
Table for AB4800 MALDI TOF/TOF Analyzer for two targets	6713.0022
Table for Bruker AutoFlex/UltraFlex for AnchorChip target plate with frame for two targets	5713.6307
Table Shimadzu/KRATOS Axima QIT for two targets	5713.6319
Table Micromass for six rectangular targets	5713.6328
Table for Ciphergen SELDI target for ten	5713.6340
Table for Compact Disk for one CD	5713.6395
Table for MassTech MALDI target for eight	5713.6401
Universal table with adjustment stripes	5713.6338
Table for two microtiter plates	5713.6348

#### Corporate Headquarters

Dionex Corporation  
1228 Titan Way  
P.O. Box 3603  
Sunnyvale, CA 94088-3603  
Tel: (408) 737-0700  
Fax: (408) 730-9403

#### LC Packings—a Dionex Company

Abberdaan 114  
1046 AA Amsterdam  
The Netherlands  
Tel: +31 20 683 97 68  
Fax: +31 20 685 34 52

#### U.S. Regional Offices

Sunnyvale, CA (408) 737-8522  
Westmont, IL (630) 789-3660  
Atlanta, GA (770) 432-8100  
Houston, TX (281) 847-5652  
Marlton, NJ (856) 596-0600

#### International Subsidiaries

Australia (61) 2 9420 5233  
Austria (01) 616 51 25  
Belgium (03) 353 42 94  
Canada (905) 844-9650  
China (852) 2428-3282  
Denmark 36 36 90 90  
France 01 39 30 01 10  
Germany (06126) 991-0  
Italy (06) 66 51 50 52  
Japan (06) 6885-1213  
Korea (2) 2653-2580  
Netherlands (0161) 43 43 03  
Switzerland (062) 205 99 66  
United Kingdom 01276 691722

[www.dionex.com](http://www.dionex.com)



Dionex products are designed, developed, and manufactured under an ISO 9001 Quality System.



AUTOMATION | HPLC | IC | EXTRACTION | PROCESS

AB4700, AB4800, and Opti-TOF are registered trademarks of Applied Biosystems. OmniFlex, AutoFlex, UltraFlex, and AnchorChip are registered trademarks of Bruker Daltonics. Axima QIT is a registered trademark of Shimadzu. SELDI is a registered trademark of Ciphergen Biosystems, Inc. FAMOS, Probot, Switchos, and UltiMate are trademarks of Dionex Corporation.

© 2005 Dionex Corporation LPN 1506-01 10M 5/05 Printed in U.S.A.