C297-E016

Chemical Inkjet Printer (ChIP)

ChIP employs state-of-the-art chemical inkjet printing technology, enabling microprinting to be carried out on just a fraction of a protein spot by MALDI mass spectrometry.

- The ChIP is a unique tool for accessing proteins separated by gel electrophoresis, developed in partnership with Proteome Systems Ltd
- Pico-liter reagent delivery to specific protein microspots on PVDF membranes for faster and more effective enzyme digestion
- A new research tool for the study of separated isoforms
- Delivers highly localised 'on-membrane' reactions in protein microspots
- Allows multiple chemistries on a stable, archivable support
- ChIP opens new horizons for biomedical research and true micro-scale analysis supported by MALDI MS and fluorescence detection

SHIMADZU BIOTECH

bringing analysis to life

Chemical Inkjet Printer (ChIP)

The ChIP (Chemical Inkjet Printer) is a revolutionary new approach to Peptide Mass Fingerprinting and Protein Macroarray Analysis, developed in conjunction with leading proteomics company, Proteome Systems Ltd..

The strategy compliments established protocols in resolving proteins by 2-D gels. However, unlike classical approaches the ChIP reproducibly dispenses picoliter volumes of reagents to defined locations on a PVDF membrane opening new horizons for micro-scale protein research and repeated sub-analysis.

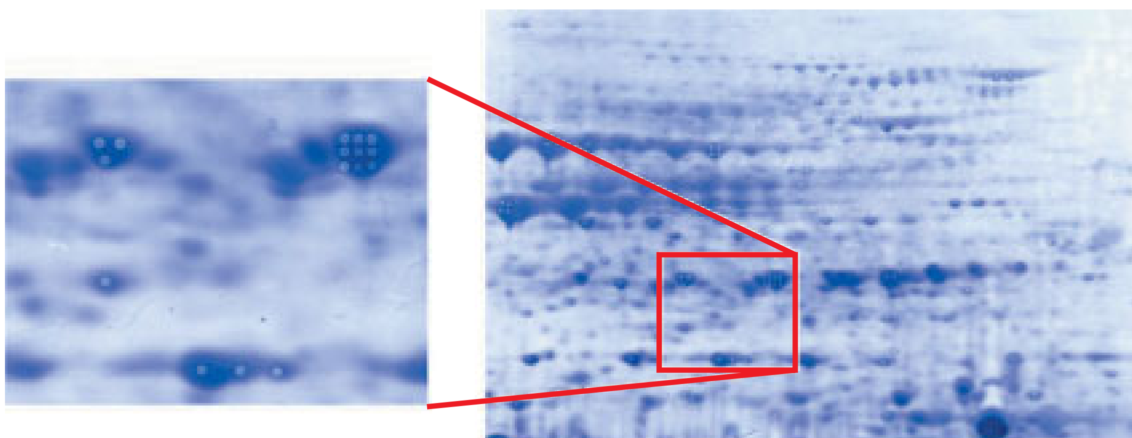
For the first time, micro-scale on-membrane digestion can be rapidly generated without the need for time consuming procedures of in-gel digestion, peptide extraction and C18 ZipTip® clean-up steps.

Micro-scale on membrane digestion has a number of significant advantages

- **Provides a new approach to critical sample analysis and long term storage.** In conventional 2D gel analysis the entire protein spot of interest is excised from the gel, digested and analysed by mass spectrometry, leaving no sample available for further analysis. Using ChIP technology picoliter volumes of reagent can be delivered to specific locations within the protein spot of interest opening up a new approach in identification by mass spectrometry. The membrane can then be archived for further analysis at a later stage. Alternatively the sample can be further characterized using multiple chemistries. The option to specifically select individual proteins for sub-analysis is a considerable advantage in increasing sequence coverage using multiple endoproteinases.
- **High sensitivity detection.** The sensitivity of on-membrane digestion using the chemical printer resulted in the successful peptide mass fingerprinting (PMF) identification of femtomole levels of BSA immobilised on an Immobilon-^{PSQ} PVDF membrane (Sloane,A.J. et al; Molecular and Cellular Proteomics 1; 490-499, 2002). This level of sensitivity is equal to, if not higher than other reported in-gel digestion procedures.
- **Results in fast, simple and effective analysis.** No need for in-gel digestion, peptide extraction, C18 ZipTip® clean-up steps or target spotting.
- **Automation.** Software control automates the process increasing sample throughput.

A typical workflow

- **Separate proteins using 2D PAGE.** Proteins are resolved using conventional gel electrophoresis.
- **Transfer proteins from 2D gel to a membrane.** This approach provides a simple protein transfer to membranes and removes gel based contaminants.
- **Micro-scale digestion of the protein spot.** The digestion zone is typically 200-300 μm in diameter. Once the reagents have been dispensed to the target locations it can be removed and re-hydrated in a separate humidified environment increasing the system flexibility and throughput. Once digestion is complete, the membrane is analysed directly by MALDI mass spectrometry using the precise protein co-ordinates used to deliver the reagents.



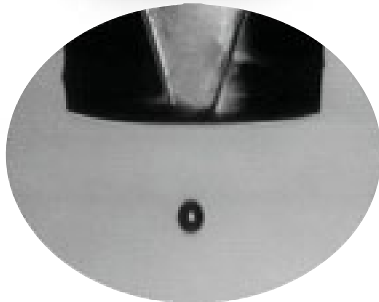
Each digestion zone is between 200-300 μm in diameter

Piezoelectric reagent dispensing for microscale protein research

- **4ch print head.** There are 4 piezoelectric printing devices. Each printing device has a detachable vacuum and pressure manifold that forms a seal on the reagent vessel. The one shot vessels have a 500μL reservoir together with in line filters. The microjets do not touch the PVDF membrane - it avoids possible sources of contamination by using a non contact form of dispensing reagents.

- **Image capture.** Target proteins can be visualized with the overhead scanner. Once the protein co-ordinates have been determined the membrane is positioned precisely under the piezoelectric head.

- **A side mounted camera** is used to optimize the printing conditions for each piezoelectric device without touching the PVDF membrane.



- **Reproducible and accurate reagent delivery.** The PVDF membrane is attached to the MALDI sample plate using electrically conductive tape. Reference points on the sample plate provide the 'lock' positions that support membrane archiving and future protein spot chemistry.

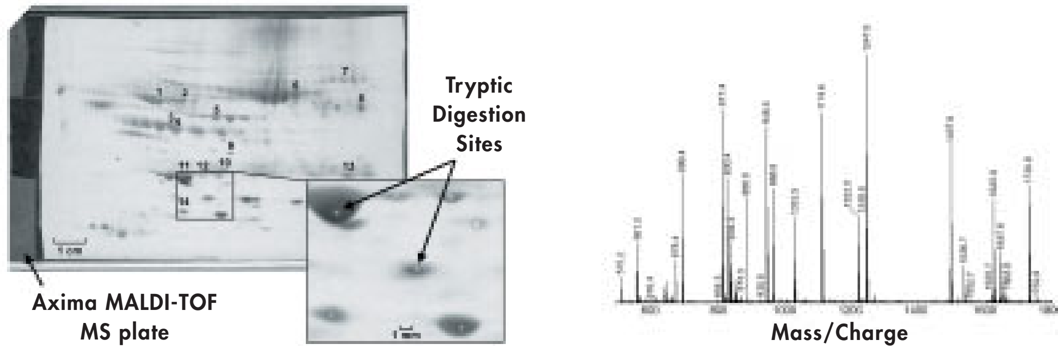
- **Piezoelectric reagent dispensing.** Each droplet delivered to the membrane is approximately 55μm in diameter with a volume of approx 87pL. The accuracy of dispensing is within ±120μm.

- **ChIP software** provides a flexible platform for optimizing membrane image analysis, peak detection, spot targeting, and digestion conditions. It also archives all information about the membrane for future characterization (method, protein co-ordinates used in micro-scale analysis).

Protein identification through peptide mass fingerprinting

MALDI mass spectrometry analysis is a key tool in protein identification. A mass spectrum of a protein digest results in a series of ions that are highly specific to individual proteins. This specificity in digestion patterns provides the basis for peptide mass fingerprinting (PMF) and data base searching for the identification of proteins for which sequences are already known.

- The electroblotted membrane was stained with Direct blue 71.
- Mass spectrum obtained for tryptic digestion of spot 9 identified as Apolipoprotein E.



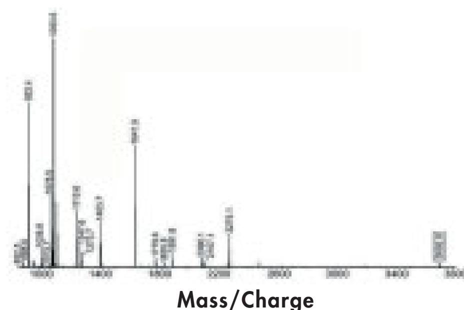
On membrane tryptic digestion and MALDI MS analysis was carried out on human plasma proteins.

- Reduced and alkylated human plasma proteins were separated by 2D PAGE and electroblotted onto PVDF membrane. A range of human plasma proteins of variable amounts, molecular weights and pI's were selected for identification. Tryptic digests were prepared as described and analysed by MALDI-MS to generate a peptide mass fingerprint (PMF) for each spot.
- All 14 spots were identified using chemical printing technology. The mass spectrum obtained for tryptic digestion of spot 9 was identified as apolipoprotein E.

Multiple chemistries

In protein research applying multiple enzymes to a protein spot can provide further characterization of the peptide.

- A spot on the membrane identified as α-1-antitrypsin was treated with PNGase F, followed by digestion with trypsin and analysis by MALDI MS. Tryptic peptide peaks representing 51.3% sequence coverage was obtained. By analysing the data for peptides with an increase in m/z of 1 Da, one peptide at m/z 3692.8Da was identified as a peptide containing an glycosylated Asn residue.



- Chemical printing can be used to carry out multiple endoproteinase digestions on a single spot. The use of a second protease not only independently confirms the proteins identity but the combined peptide mass fingerprints increases sequence coverage.

- The sequence coverage of Apolipoprotein A-IV was improved by combining trypsin and Glu-C digestion data.

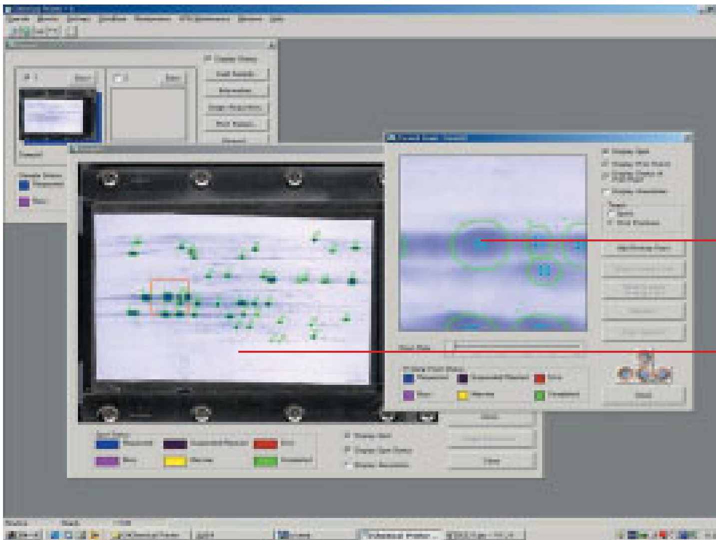
Apolipoprotein A-IV P06727
Mr 43374.5 pI 5.18

Trypsin	**29.8%**
Endo Glu-C	**21.5%**
Combined	**41.2%**

ChIP software support

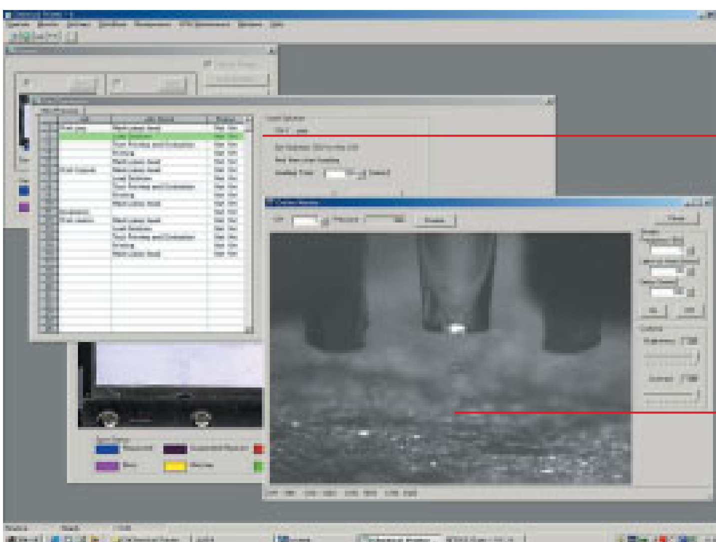
• **Method development.** A highly flexible method editor can be used to develop protocols for specific enzyme treatments. All reagent steps, positions and spot information are 'locked' into each data file to support sample archiving and later sub-analysis.

• **Printing.** A camera is used to both optimize sample delivery and check the efficiency of droplet production.



A specific print pattern (2x2) has been applied to target proteins.

Each spot location is defined as a protein co-ordinate and fixed to a MALDI template for archiving and later sub-analysis.



Each spot is assigned a specific sequence of 'instructions' such as enzyme used, volume dispensed and print pattern.

A camera is also used to optimize the reagent delivery from a micro-capillary.

Workflow schematic

- **Protein transfer to PVDF membranes.**

Proteins resolved by gel electrophoresis are electroblotted onto PVDF membranes.

- **Chemical Printing.**

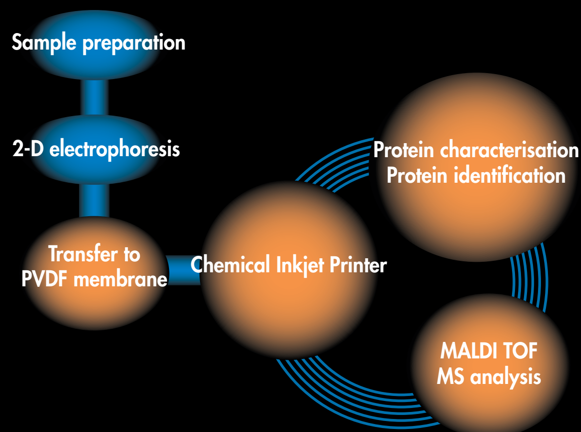
Micro-scale protein digestion is focused on specific protein co-ordinates.

- **Direct MALDI analysis.**

Once the digestion is complete the membrane is transferred to the MALDI for protein identification.

- **Sub-analysis and archiving.**

As a fraction of the protein spot is used in protein identification, further sub-analysis can take place using alternative chemistries. This cycle can be used to increase sequence coverage and characterization.



Key technology areas

Post-translational modifications:
- Particularly glycosylation, this technology coupled with LC-ESI MS, can be applied to the structural analyses of oligosaccharides released from a solid-phase membrane

Increase the sequence coverage of a protein:
- Analysing a single protein spot using multiple endoproteinases

Clinical proteomics:
- Conserves critical biological samples

Advantages of chemical printing

Gel is transferred to a PVDF membrane:
- Removes gel-based contaminants
- Robust storage method for samples, allows gel samples to be re-screened at a later date

Automated technique, can be used for:
- Screening
- Large scale protein identification and characterisation

Quantifiable dispensing of nanolitre volumes:
- Minimises quantities of reagents required
- Non-contact dispensing, samples do not 'bleed'

A range of enzyme treatments can be carried out on one protein spot:
- Maximising the amount of data obtained
- Allows identification and subsequent characterisation of a protein of interest

"The contents of this brochure are subject to change without notice"

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