

Technical Note # TN-12

Innovative smartbeam Laser Technology Enhances MALDI-TOF Based Proteomics Applications

The new smartbeam™ laser technology outperforms all previous laser technologies by combining the application flexibility of a nitrogen laser with the speed of a classical Nd:YAG laser. Comparison of smartbeam with traditional laser technologies has revealed fundamental differences in performance resulting in significant enhancements for a wide variety of applications including: Protein Profiling, Biomarker Discovery, Large Protein Applications, MALDI Imaging and classical 2D-Gel and LC based proteomic research.

Introduction

Smartbeam laser technology provides substantial performance enhancements for MALDI-MS based Life-Science research. It is the ideal laser-technology for cutting-edge applications such as MALDI Imaging, Large Protein Applications or Clinical Proteomics (CLINPROT™). Smartbeam combines the speed of a solid-state-laser with the wide range of applications associated with nitrogen lasers. The use of conventional Nd:YAG lasers is restricted to applications which use dried droplet techniques as the method of matrix preparation. Smartbeam provides excellent results from all matrices and all preparation methods, such as dried droplet and thin layer preparations and is ideal in combination with Prespotted AnchorChips™.

The advantages of smartbeam are particularly evident with thin layer applications where incredible improvements in sensitivity and resolution are observed.

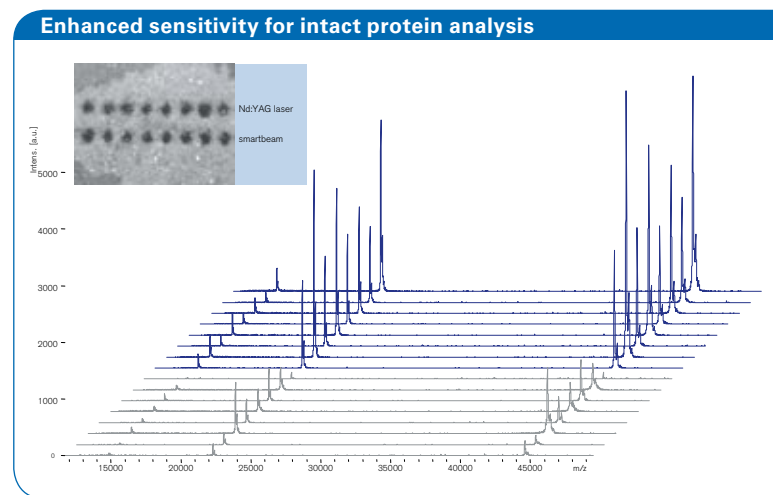


Fig. 1: Protein A spectra generated with Nd:YAG laser on the depleted locations on the spot from figure 1 (grey) and generated with the smartbeam technology on the corresponding spots (blue). Insert: seeded dried droplet of Protein A in Sinapinic acid. Complete desorption of sample and matrix on the spot. Upper row Nd:YAG laser, lower row: smartbeam technology.

Smartbeam expands sample preparation options

Common Nd:YAG lasers are restricted to limited sample preparation options in proteomics, i. e. only dried droplet HCCA (α-Cyano-4-hydroxycinnamic acid) preparations work well. With alternative matrices, such as sinapinic acid, the sample is exhausted after a few laser shots, whereas the same sample is more durable and results in significantly better spectra quality using a nitrogen laser for desorption/ionization. For HCCA dried-droplet preparations in a standard protocol, almost no difference in performance of smartbeam versus a Nd:YAG laser can be observed. However, on thin layer and sandwich preparations, dramatic improvements in results are obtained.

Intact protein analysis – MALDI Imaging and Clinical Proteomics at extended mass range

To validate the improvements of smartbeam technology over Nd:YAG lasers, Protein A in sinapinic acid matrix has been examined. Spectra were generated from 8 spots until each spot was completely depleted of sample. By visual inspection of the spots, it was obvious that the same amount of sample has been used in both series (Figure 1, insert). Since both, Nd:YAG laser and smartbeam laser desorption/ionization was performed on the same instrument, on the same spot and even on comparable positions on the spot, a most accurate comparison of the technologies was possible. The intensities of the spectra from the intact Protein A were about three times higher when using smartbeam, compared to a conventional Nd:YAG laser (Figure 1), thus smartbeam offers enhanced sensitivity for intact protein analysis.

Smartbeam permits thin layer preparations for gel- and LC-based proteomics

Thin layer preparations on MALDI targets such as the Prespotted AnchorChip (Fig. 2) permit the in-situ purification of either protein digests or LC-eluates by simply rinsing the sample spot after the analyte has bound to the matrix surface. This process removes the requirement for additional purification steps such as ZipTips, which are associated with increased sample loss and increased cost per experiment. On thin layer preparations, the Nd:YAG laser dramatically depleted the sample. 1 fmole BSA digest was prepared on a Prespotted AnchorChip™, pre-spotted with a thin layer of HCCA. On each position the entire sample was desorbed to gain as much signal intensity as possible (Fig. 3a. insert). The laser power was adjusted individually to collect as much signal as possible into the sum buffer. By visual inspection it is clear that in total a similar amount of sample was ionized from both rows. Figure 3 compares three spectra from each laser, which were recorded from these spots. Again smartbeam produced significantly more signal from the same amount



Fig. 2: The Prespotted AnchorChip. 384 anchor spots covered with HCCA matrix and 96 calibrants are prespotted.

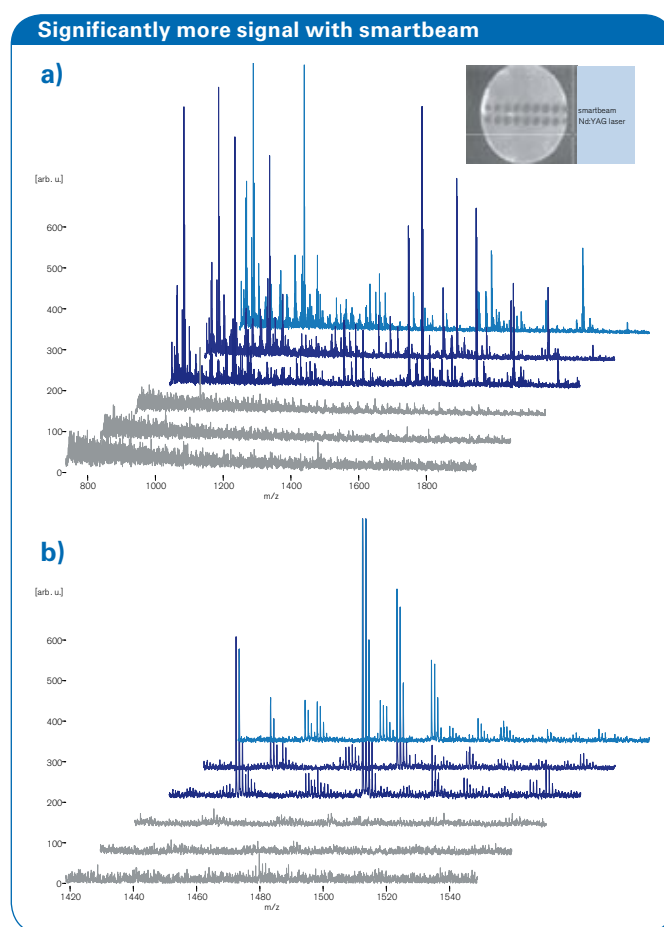


Fig. 3: a) Stack plot view of spectra acquired with or without smartbeam technology. Each spectrum represents the total signal which was recorded for each of the spots. grey: YAG laser; blue: smartbeam technology. Insert: Visual inspection of depleted amount of sample. Upper row: smartbeam, lower row: YAG laser. In both cases the same amount of material was used. b) zoomed representation of spectra from Figure 3a, representing m/z 1420-1540.

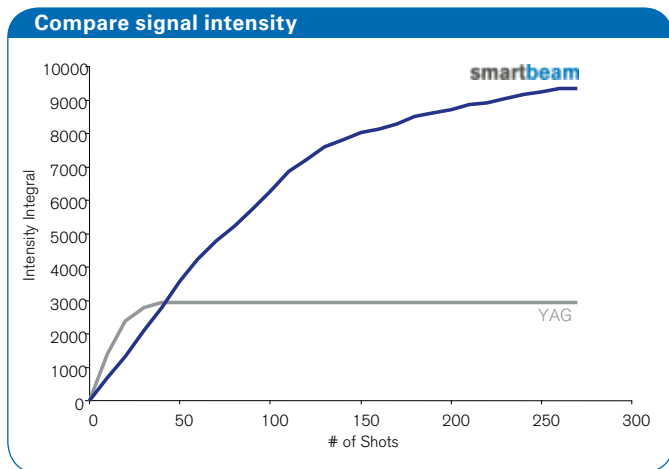


Fig. 4: BSA digest m/z 1479.8 on Prespotted AnchorChip: Integral signal increase at constant laser power, as function of number of shots.

of sample, and again the Nd:YAG laser depleted the sample very quickly. The overall signal intensity with smartbeam compared to Nd:YAG laser is shown in figure 4. A 100 fmole BSA digest sample on Prespotted AnchorChip was used to evaluate the maximum number of laser shots obtainable before exhausting the sample. Applying only a few shots, the Nd:YAG laser generates more intense spectra. However, a decrease in resolution was observed. For Nd:YAG lasers, 80% of the maximum integral intensity is reached after only 20 shots. Smartbeam takes off more gently, showing good resolution from the first shot onwards. After 40 shots, the accumulated signal intensity of smartbeam has reached that of the Nd:YAG laser, ultimately resulting in a 3-fold amplification after 250 shots compared to the Nd:

YAG laser. Smartbeam extracts information from the same sample much more efficiently providing higher sensitivity and improved data quality. For the first time ever smartbeam can take advantage of the all-solid-state laser for thin layer applications. Furthermore, smartbeam is easier to control in manual mode and automatic measurements are easier to set up and are more successful, as it is more tolerant to variations in laser power.

High performance MALDI Imaging

Smartbeam is particularly suited to MALDI Imaging applications due to the combination of the speed of a solid-state laser and the extraordinary spectra quality of a N2 laser. In order to demonstrate the good performance of smartbeam compared to nitrogen lasers, a rat brain tissue spotted with sinapinic acid was measured by MALDI Imaging on an ultraflex II mass spectrometer equipped with both, smartbeam and nitrogen laser. With a very comparable spot diameter of about 50 μm , 300 laser shots were accumulated without moving the laser relative to the tissue using 50 Hz with the N2 laser and 200 Hz with smartbeam. As shown in figure 5, both results are identical showing the typical reproducibility of such experiments. The intensity distribution and the signal to noise ratio are very comparable in both cases. Conveniently accessed with Bruker Daltonics flexImaging™ software, figure 6 shows a tissue section from rat testis with a colour-coded mass distribution. Smartbeam outperforms the nitrogen laser in terms of protein resolution. In figure 7, Protein A was measured on the same instrument and the same sample spot. With Smartbeam a twofold increase in resolution was observed for the 45 kDa protein.

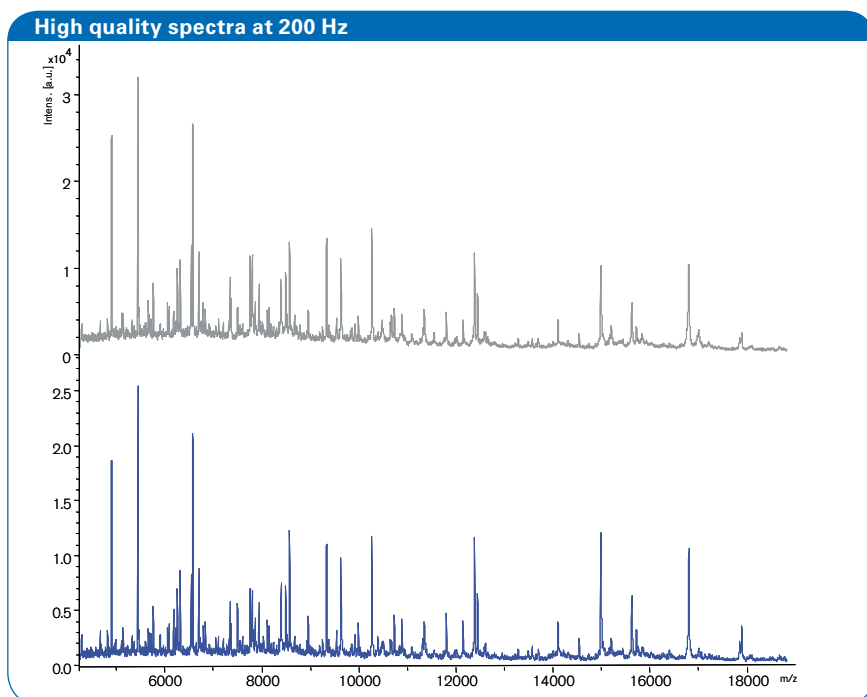


Fig. 5: MALDI Imaging spectra comparison of the nitrogen laser (upper, grey; 50 Hz) and the smartbeam laser (lower, blue; 200 Hz) from two adjacent spots on a tissue sample, using sinapinic acid.

Summary: Combining the advantages of nitrogen- and all-solid-state lasers

smartbeam on the ultraflex II exclusively combines the high reliability and high repetition rate of all-solid-state lasers with the efficient sample usage and the broader application range of nitrogen lasers. With its variable repetition rate from 1 to 200 Hz and the capability to handle various MALDI-matrices, smartbeam establishes the next-generation MALDI TOF(/TOF) performance for a variety of applications like gel-based or LC-based proteomics in combination with Bruker Daltonics Prespotted AnchorChip targets, MALDI Molecular Imager™, Biomarker detection with CLINPROT or Top-Down sequence analysis of intact proteins by ISD and T³-sequencing. smartbeam enhancements to conventional all-solid-state lasers:

- maintain uncompromised dried- droplet HCCA performance
- achieve highest sensitivity from thin layer preparations (for on-target sample purification, Proteomics, Biomarker- and Imaging MALDI applications)
- maximize linear instrument performance for large protein applications
- increase the variety of useable matrices (e. g. DHB, SA)
- accelerated automation, robust acquisition due to more tolerance for laser power settings
- gain perfect access to MALDI Imaging with highest repetition rates

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Keywords	Instrumentation & Software
MALDI laser	smartbeam
MALDI resolution	autoflex
Protein Analysis	ultraflex
Spectra Quality	flexImaging
	Anchor Chip

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MALDI tissue imaging at high resolution

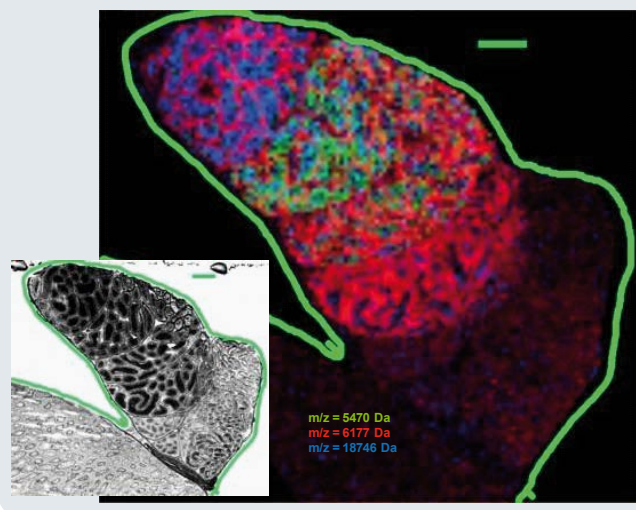


Fig. 6: MALDI Imaging of Rat Epididymis. The spatial distribution and intensities of selected mass signals are visualized. The distribution and intensities of the blue and the green signal are related to sperm maturation while the red signal shows the tubuli walls. Data acquisition was performed at 200 Hz repetition rate on an autoflex III in linear mode. Lateral resolution 80µm. Scalebar: 1mm. Sample by courtesy of Charles Pineau, Rennes, France.

High resolution of proteins

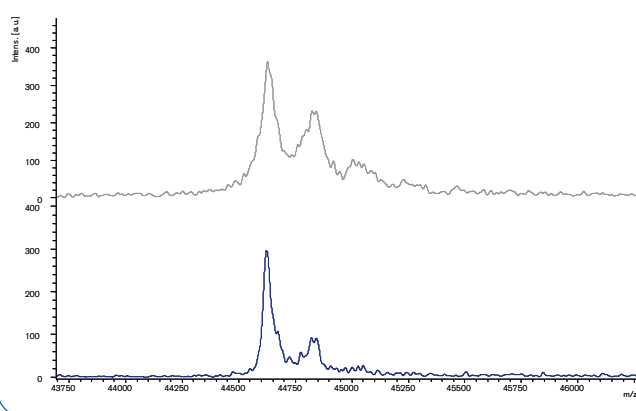


Fig. 7: Spectra from Protein A recorded with a nitrogen laser (upper spectrum) and with smartbeam (lower spectrum). Both spectra were recorded on the same sample and on the same instrument, with similar effort and similar, individually optimized conditions.

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