



## Infrared Electrospray-assisted Laser Desorption Ionization Mass Spectrometry: Backside Illumination Mode

### Introduction

The ablation potential of IR versus UV lasers can be many orders of magnitude greater due to lower absorption coefficients for many materials. This translates into deeper penetration depths and higher fluence requirements. Faster sample material depletion is a drawback of this process; however, there are some useful advantages of greater ablation. Analytes embedded in thick media such as 2D gels, tissues and cells can be analyzed. Another advantage is the possibility of backside illumination where samples are deposited on transparent substrates and desorbed and ionized from the back of the sample at normal incidence as opposed to front irradiation (45° incidence). Novel ion source geometries as well as better mass spectrometry imaging resolution through better focusing are possible benefits of this technique. In this application note, IR ELDI is combined with backside illumination for the analysis of the protein, human insulin.



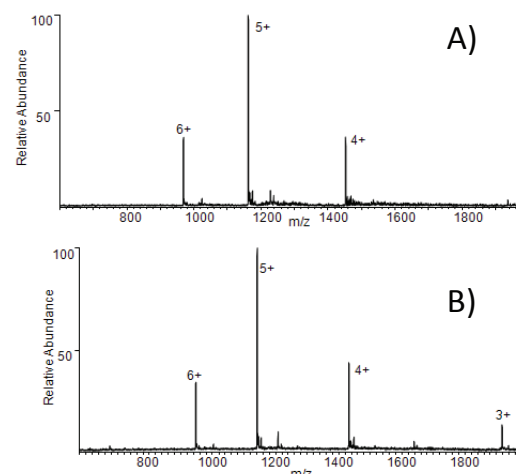
**Figure 1.** Backside illumination configuration with sample target between the laser focusing lens and the electrospray.

### Experimental Conditions

The protein human insulin (MW = 5733 Da) was dissolved in water at a final concentration of 100  $\mu$ M. In order to produce a thick film, 10  $\mu$ L of the protein solution was deposited on a 117.6 lpi Ni mesh target or CaF<sub>2</sub> substrate and dried with hot air. The resulting film was irradiated with 2.94  $\mu$ m mid-IR laser light at 20 Hz using an IR Opolette (Figure 1) focused to 150  $\mu$ m and analyzed on a Thermo Finnigan LTQ ion trap mass spectrometer. The laser fluence was approximately 3 J/cm<sup>2</sup>.

### Results

IR ELDI in backside illumination mode is demonstrated for human insulin deposited on a glass slide and grid (Figure 2). The laser is focused onto the surface where the protein thin film is located; therefore only sample material is irradiated. Since electrospray is used to ionize the material desorbed by the laser light from the backside, multiple charges up to 6+ are detected.



**Figure 2.** IR ELDI MS in backside illumination mode of a dried thin film of human insulin from 10  $\mu$ L of 100  $\mu$ M 30/70% water/methanol solution deposited on A) glass slide and B) 117.6 LPI grid.

### Conclusions

IR ELDI of human insulin in backside illumination mode was successfully demonstrated using a tunable mid-IR OPO. Future work will focus on minimizing the laser spot size using a microscope objective and optimizing desorption plume and electrospray interaction.

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