Chapter 7
Matrix Application with ImagePrep

Martin Schuerenberg and Soeren-Oliver Deininger

Abstract In this chapter, matrix application to tissue samples with the ImagePrep device is explained. The operating principle of vibrational vaporization of the matrix solvent and gravitational deposition of the droplets is presented. An important feature of the device is the control of the deposition process with an optical sensor; the principle of this detection is described. The chapter includes an introduction to the simple user interface with the adjustment of wetness, incubation time, and matrix thickness as intuitive parameters. A brief introduction to method development possibilities for detailed control over the matrix deposition process is given.

7.1 Introduction

ImagePrep is a stand-alone preparation device used to apply matrix to tissue samples for MALDI imaging (Fig. 7.1).

A fine mist of matrix droplets is generated through the vibration of a pinholed metal sheet. The fine matrix droplets are allowed to sink into the tissue by gravitational force alone and to incubate under controlled conditions. The ImagePrep is operated via an integrated touch-panel screen. The preparation is in real time; it is controlled by an internal optical sensor to achieve the highest possible reproducibility. Because of its unique design, the ImagePrep produces small droplets with a 25 μm diameter; additionally, they are produced simultaneously, to ensure efficient incubation. This capability leads concurrently to a high lateral resolution and good mass spectra (Fig. 7.2), which are mutually exclusive aims when other sample preparation devices are used. The instrument allows two levels of operation: a simple user interface and a method development graphic user interface (GUI) for expert users.

M. Schuerenberg and S.-O. Deininger (✉)
Bruker Daltonik GmbH, Fahrenheitstrasse 428359, Bremen, Germany
e-mail: Soeren-Oliver.Deininger@bdal.de

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Fig. 7.1 The ImagePrep preparation device

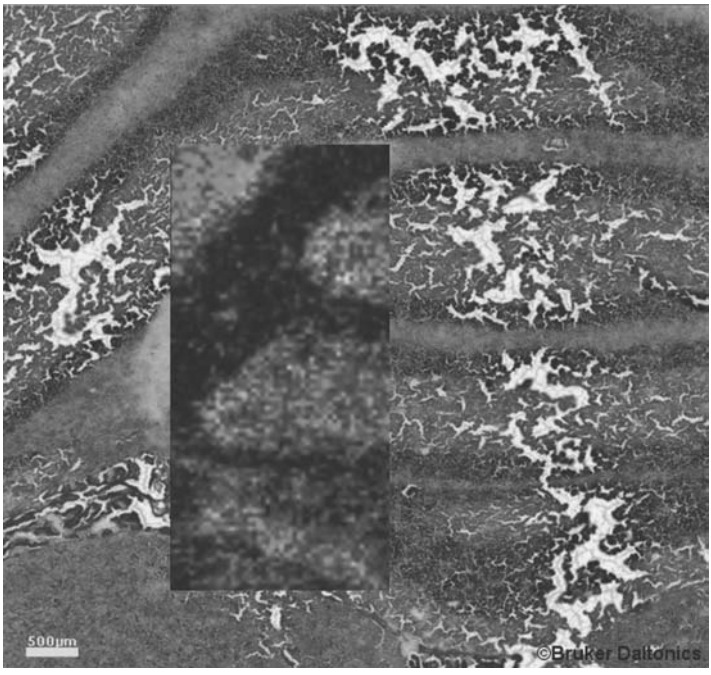

Fig. 7.2 Correlation between histological staining and matrix-assisted laser desorption/ionization (MALDI) image (*insert*). The MALDI image shows the distribution of two different phospholipids at a 25-µm spatial resolution

7.2 The Simple GUI

This mode is considered the standard mode of operation. It allows a true push-button operation and does not require a detailed understanding of the underlying technology. The user must choose a predefined method, according to the matrix he or she has chosen for the imaging experiment. Upon choosing a method, an information screen with the correct matrix concentration and solvent composition is shown on the screen. After filling the reservoir with matrix, preparation can be started immediately. The matrix application can be customized by changing three intuitive parameters: incubation time, matrix layer thickness, and wetness during preparation. Because these parameters may need to be optimized for different tissue types, customized settings can be saved for later use.

7.3 The Operation

To build up a sufficient matrix layer thickness without the lateral delocalization of analytes, it is necessary to apply the matrix in multiple cycles. Each cycle consists of three phases. In the first phase, the spray is generated. During the second phase, the matrix is incubated on the tissue section. In the third phase, a soft nitrogen flow is used to dry the sample and therefore ensure a consistent incubation time between cycles. The matrix preparation is ended after the desired thickness of matrix, as defined by the method, is reached. After this, the sample can be directly measured. The instrument can be conveniently cleaned by running a cleaning program and wiping the Teflon-coated spray chamber.

7.4 The Optical Sensor

The heart of the ImagePrep is an optical sensor that enables the real-time monitoring of a scattered light signal. The sensor and the light source are located under the sample, which needs to be mounted onto a glass slide equipped with an electrically conductive ITO coating. To understand the principle of the optical sensor (Fig. 7.3), it is best to image a sample that is already covered with matrix crystals. These crystals will scatter the light to a certain extent. When the spray is started, the surface of the sample becomes wet; because the liquid droplets smooth out the craggy crystals (i.e., refractive index matching), the matrix layer becomes more transparent. Therefore, less light is scattered and the scattered light signal decreases. The decrease of the scattered light signal is a direct measurement of the wetness of the sample. As the sample dries, the scattered light signal increases again and the drying rate can be directly monitored by the scattered light signal. If the drying is

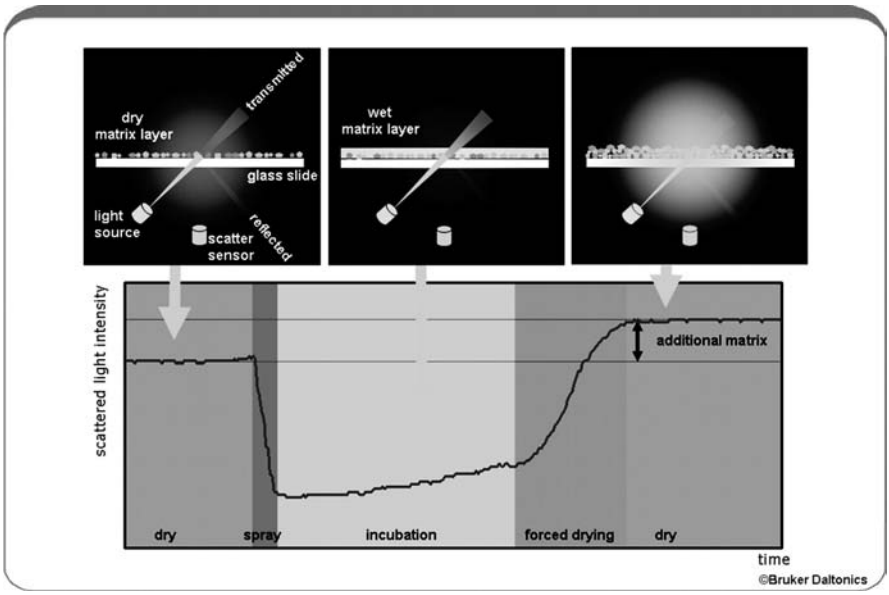

Fig. 7.3 Function of the optical sensor

completed, additional matrix crystals are formed, so the scattered light signal will be higher than in the beginning. When the sample is dry, the scattered light signal will therefore serve as a direct measure for the thickness of the matrix layer.

During a typical preparation, the ImagePrep will not dry the sample completely after each matrix application but instead applies the next matrix layer while the sample is still wet, which ensures a maximum extraction efficiency and good spectra quality in the imaging experiment. After a certain number of spray cycles, a complete drying is performed, to estimate the absolute matrix layer thickness. The next spray cycle is then applied until a defined wetness is achieved.

7.5 The Method Development GUI

For the routine use of ImagePrep with standard matrices such as sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB), or cyano-4-hydroxy cinnamic acid (CHCA), no understanding of the internal workflow of the instrument operation is necessary. Users can choose special matrices or create their own methods, via the method development GUI. Because the operation of ImagePrep is controlled by the scattered light sensor, it is necessary to have access to the scattered light output of the sensor during method development. An analog/digital converter and suitable software are provided with ImagePrep, allowing access to the scattered light curves. Through the method development GUI, every relevant sample preparation parameter

is accessible, and the entire preparation process can be divided into different phases. For each phase, the experimenter can decide whether a fixed number of spray cycles shall be used or if the matrix layer thickness will be controlled via the sensor. For each spray cycle within a phase, the experimenter can define how long the spray shall be on and whether this spray-on time will be controlled by the sensor; he or she can next decide how long the sample will be incubated before the nitrogen flow starts, as well as at which level of dryness the next spray cycle will be applied. There are more parameters that can be controlled. A detailed manual for method development is provided with ImagePrep.