

# The Introduction of the First MALDI Mass Spectrometer in a Biosafety Level 3 Facility

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## ABSTRACT

High containment laboratories can present challenges for the placement and maintenance of new technologies and specialized equipment due in part to facility design, restrictions for vendor entrance during normal operations, medical surveillance requirements and until a few years ago, decontamination materials that would not damage sensitive imaging equipment and computers. Researchers studying infectious disease and new, effective drug therapies are interested in the permeation of drugs into tissues and organs of infected animals and need imaging technology in order to understand the efficacy of these new treatments. However, many scientists have to fix their samples, to utilize equipment located outside of the containment laboratory. This presentation will describe the introduction of the first MALDI-MSI mass spectrometer into a BSL3 facility at the Rutgers Regional Biocontainment Laboratory. The use of this technology is the first known worldwide in a high containment (BSL3) setting, and will be groundbreaking in understanding drug penetration and metabolism in tuberculosis and select agent infected tissues. The use of the mass spectrometer in the BSL3 laboratory involves biological, chemical and laser safety and involves much coordination between the laboratory staff, laboratory managers, facility and safety departments.

## INTRODUCTION

❖ Matrix assisted laser desorption/ionization (MALDI) Mass Spectrometry Imaging (MSI) has been used over the past 10 years to map the distribution of drugs and metabolites in biological tissues.

❖ Tissue samples can be analyzed, multiple measurements can be taken from each location in the tissue, called a spot. A mass spectrum for each spot is acquired and a two-dimensional map is developed based on the original raster location on the sample.

❖ MALDI-MSI allows for unlabeled samples to be analyzed without prior information regarding the analytes, or their distribution.

❖ MALDI-MSI is useful in determining the spatial distribution of small molecules, peptides, drugs and metabolites in infected tissues, to determine the drug concentration in these tissues, rather than plasma alone.

❖ The introduction of the MALDI-MSI in a BSL3 facility allows for live, infected tissues to be analyzed, instead of fixed or irradiated tissue.

❖ Biosafety Level Three Facilities traditionally focus on biological materials and preventing exposure to infectious microorganisms. The introduction of a MALDI-MSI into this type of facility presents additional safety concerns; hazardous chemicals, laser safety, gas cylinders, exhaust for solvent vapors, etc.

## PLANNING FOR THE INSTALLATION OF THE MALDI-MSI

PHRI recruited a new faculty member to join their institute at NJMS. Dr. Dartois specializes in pharmacokinetics and will use MALDI-MSI to analyze the distribution of drugs in TB infected tissues.

In conjunction with Facilities Planning and Management, and Maintenance, it was determined that a room with cooling capabilities was needed due to the heat output(22,390 BTU/hr).

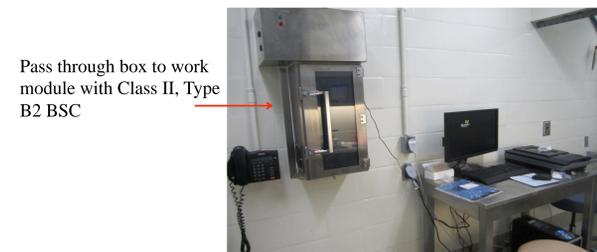
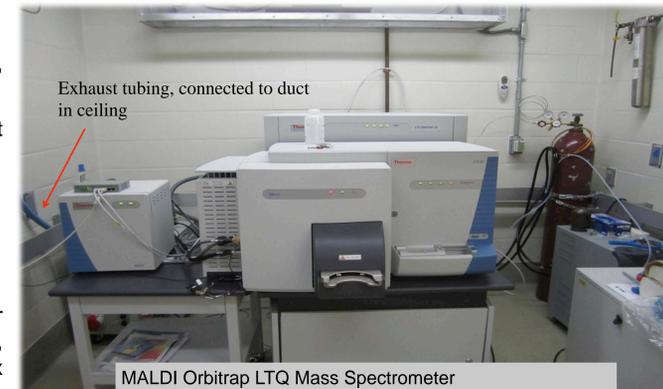
The Rutgers RBL was not built with the temperature and electrical requirements needed for a MALDI-MSI, thus facility modifications would be needed.

### Space requirements:

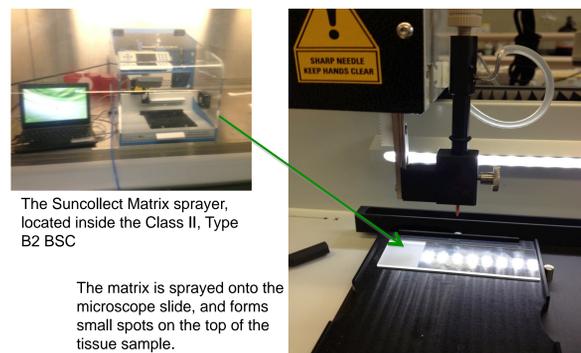
- ❖ Open floorplan to accommodate The LTQ Orbitrap, MALDI source, recirculating chiller, computer workstation, cryostat and matrix sprayer.
- ❖ Air conditioning to cool space due to high heat output, to maintain consistent temperature for optimal performance of the MALDI-MSI
- ❖ Ability to vent gases directly to exhaust
- ❖ Space suitable for work with organic solvents and matrices

### Final Installation:

- ❖ The MALDI-MSI was installed in the RBL "insectary." This area was designed, but never used for arthropod work, and was empty. A wall air conditioning unit was present, a larger, open room containing a Class II Type A2 biological safety cabinet with a pass through box to a work room with a Class II Type B2 biological safety cabinet.
- ❖ A few modifications were needed including:
  - ❖ Installation of several electrical outlets (220v)
  - ❖ Gas cylinder installation, Nitrogen generator installed, solvent vapor exhaust system
- ❖ Installation, initial runs performed during BSL3 shutdown
- ❖ Risk Assessment and SOP development for BSL3 work
- ❖ BSL2 Demos
- ❖ BSL3 Training for MALDI Technician



## SAMPLE PREP FOR MALDI-MSI AND THE ASSOCIATED RISKS



The Suncollect Matrix sprayer, located inside the Class II, Type B2 BSC

The matrix is sprayed onto the microscope slide, and forms small spots on the top of the tissue sample.

### SAMPLE PREPARATION:

1. Infect animals with appropriate microorganism
2. Treat infection with drug of interest
3. Harvest organs from infected animals at designated time points, snap freeze tissues
4. Use cryostat to cut thin sections of infected tissues
5. Affix sliced tissue to slide, allow to dry in dessicator
6. Spray UV absorbing matrix coating over sample
7. Place slide into MALDI-MSI, a laser is rastered across the surface of tissue, causing ionization of the matrix and the crystallized analyte.

### RISKS ASSOCIATED WITH SAMPLE PREPARATION

- ❖ Use of organic solvents: acetonitrile, methanol, and MALDI matrices in trifluoroacetic acid
  - ❖ Matrices include: 2,5-dihydroxybenzoic acid (THAP), 3,5-dimethoxy-4-hydroxycinnamic acid (SA) and  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA)
- ❖ Aerosolization of organic solvents
- ❖ Use of cryostat to cut infected tissues at BSL3

### MITIGATION OF RISKS

- ❖ All work with organic solvents in Class II, Type B2 BSC
  - ❖ Only very small volumes are used throughout the facility in various steps of the process, and for cleaning of the mass spectrophotometer
- ❖ Cryostat (Leica 1860) and -80C freezer in same room as the Class II, B2 cabinet to minimize movement throughout facility with samples.
- ❖ No storage of solvents in BSL3
- ❖ Practice run through of all procedures at BSL2 and also in BSL3 before shutdown concluded

## The Daily Life of MALDI Projects

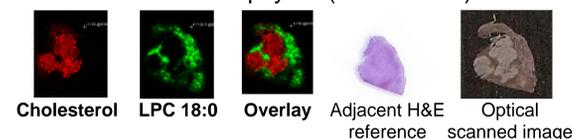
- ❖ The MALDI-MSI is located in a select agent registered facility, all technicians are SRA cleared
- ❖ Analysis of *M.tuberculosis* infected tissues is currently underway
- ❖ SOPs have been developed to harvest tissues for analysis with MALDI-MSI
- ❖ SA approval for MALDI-MSI for select agent infected tissues is pending.
- ❖ Temperature concerns remain. The existing air conditioner would cycle and not keep the temperature in the room consistent. Temperature monitoring difficult due to location of monitor in exhaust duct, not room itself. Portable air conditioner installed and cycling is less prevalent. Warm/ humid days can still present an issue.



- ❖ Solvents ideal for freezing samples (e.g. isopentane) are not ideal for use in a BSL3 facility. Chemical safety analysis is ongoing.

## Example of MALDI MSI Data

### Clinical Biopsy 5h (Chol & LPC)



The MALDI images above are from lung tissue biopsies taken from patients with severe pulmonary TB (lung resectioning surgery). These are two lipids which show contrasting distributions within caseum/necrotic tissue (cholesterol, red) and cellular/viable granuloma (lysophosphatidylcholine 18:0, green) The H&E reference is from an adjacent tissue section, whereas the optical image is the actual tissue scanned prior to matrix application and MALDI image acquisition.

### CONCLUSIONS

- ❖ The introduction of the MALDI-MSI in a BSL3 facility allows for live, infected tissues to be analyzed, instead of fixed or irradiated tissue.
- ❖ A temperature sensor/ alarm inside the room will assist in monitoring temperature. A new portable air conditioner helps to stabilize room temperature.

### ACKNOWLEDGEMENTS

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