



NATIONAL REFERENCE LABORATORY



UNIVERSITY OF UTAH
SCHOOL OF MEDICINE

Department of Pathology

MALDI-TOF MS:

Clinical Mycobacterial Laboratory

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R&D Special Operations

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University of Utah

Disclosures

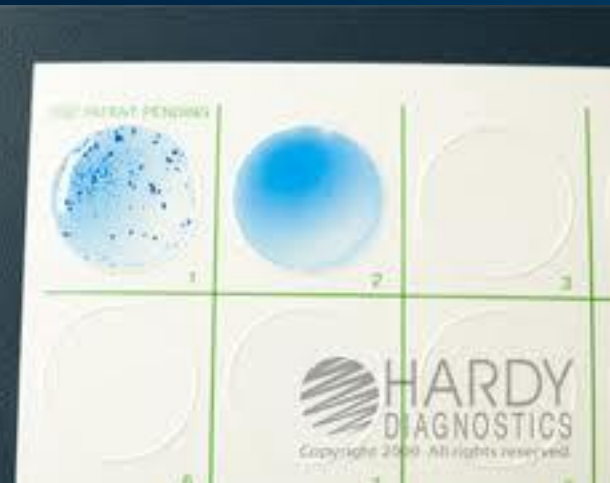
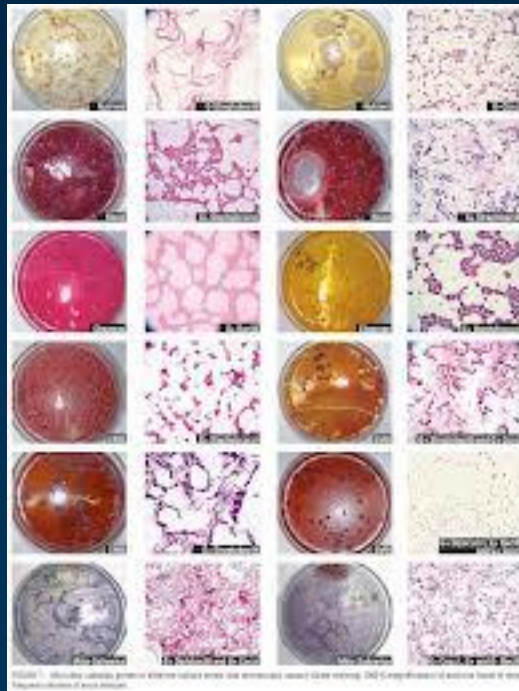
Clinical trial site for bioMerieux mycobacterial database

Under contract with Thermo Scientific Mass Spec group

Infectious Disease

What do we do?

We identify pathogens!!!



Automation



BD Phoenix™



Biomérieux Vitek 2™

- A chemistry test in the micro lab...

1. Whole bacteria
2. Extracted proteins
3. Extracted lipids

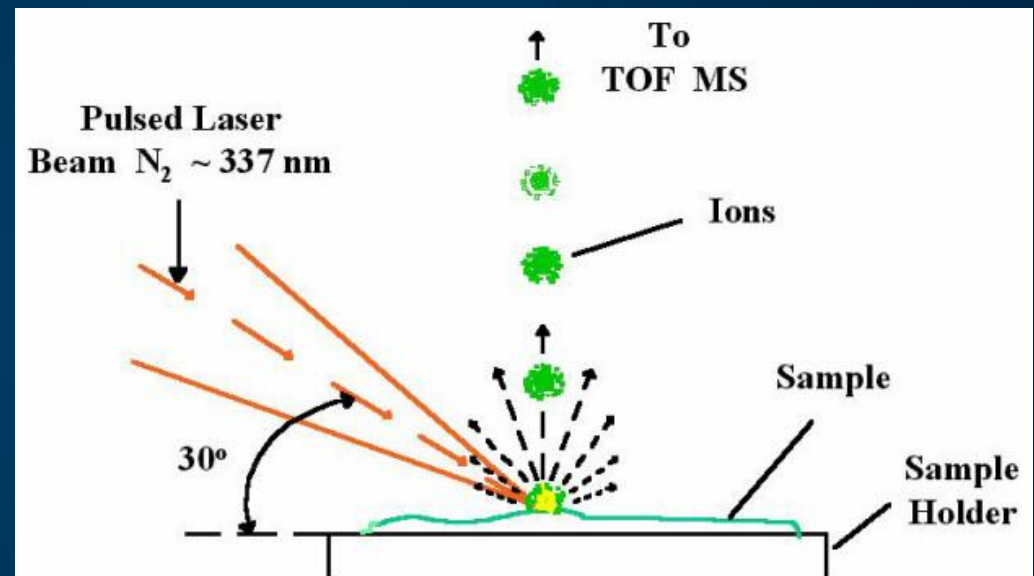
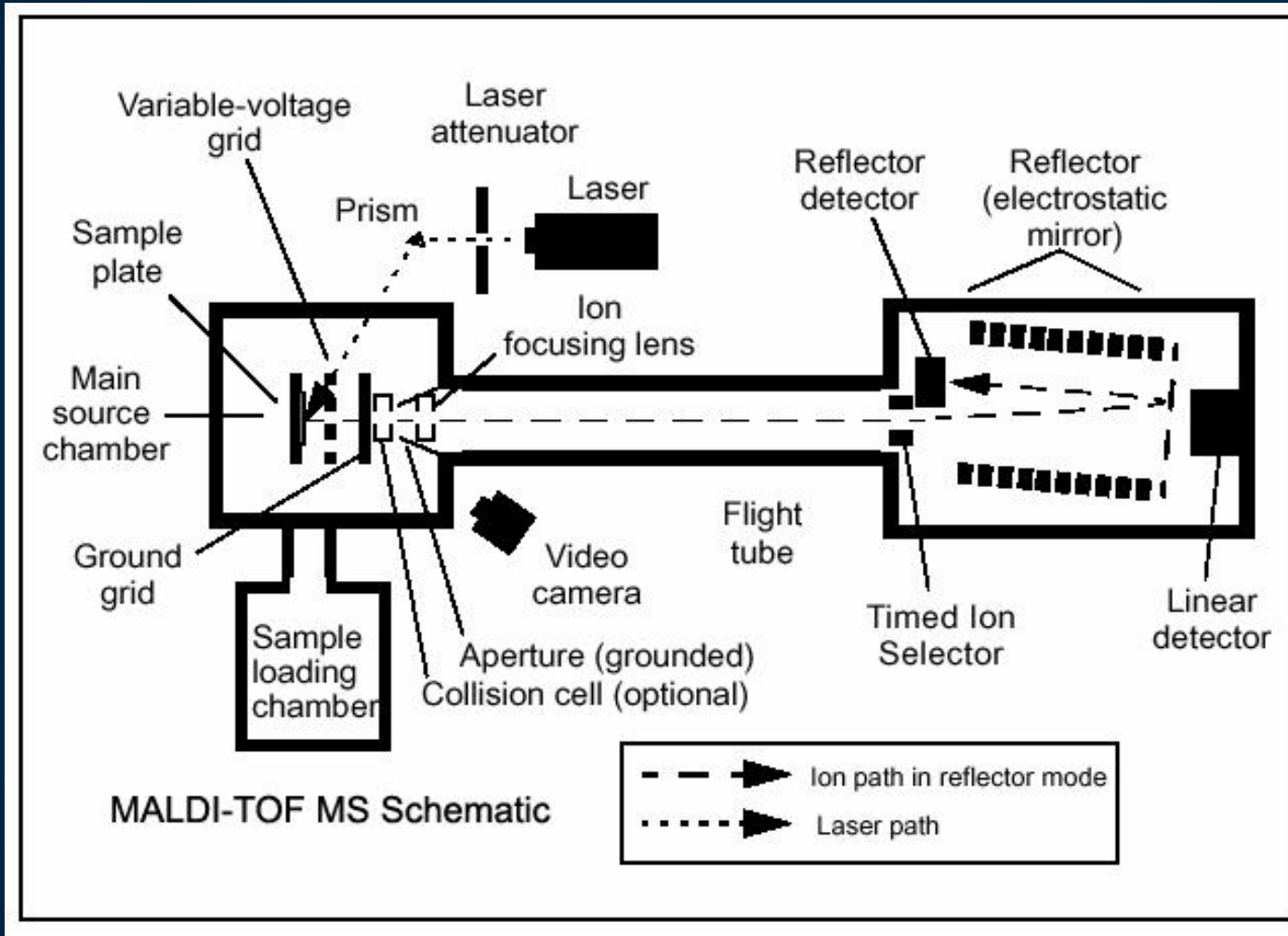
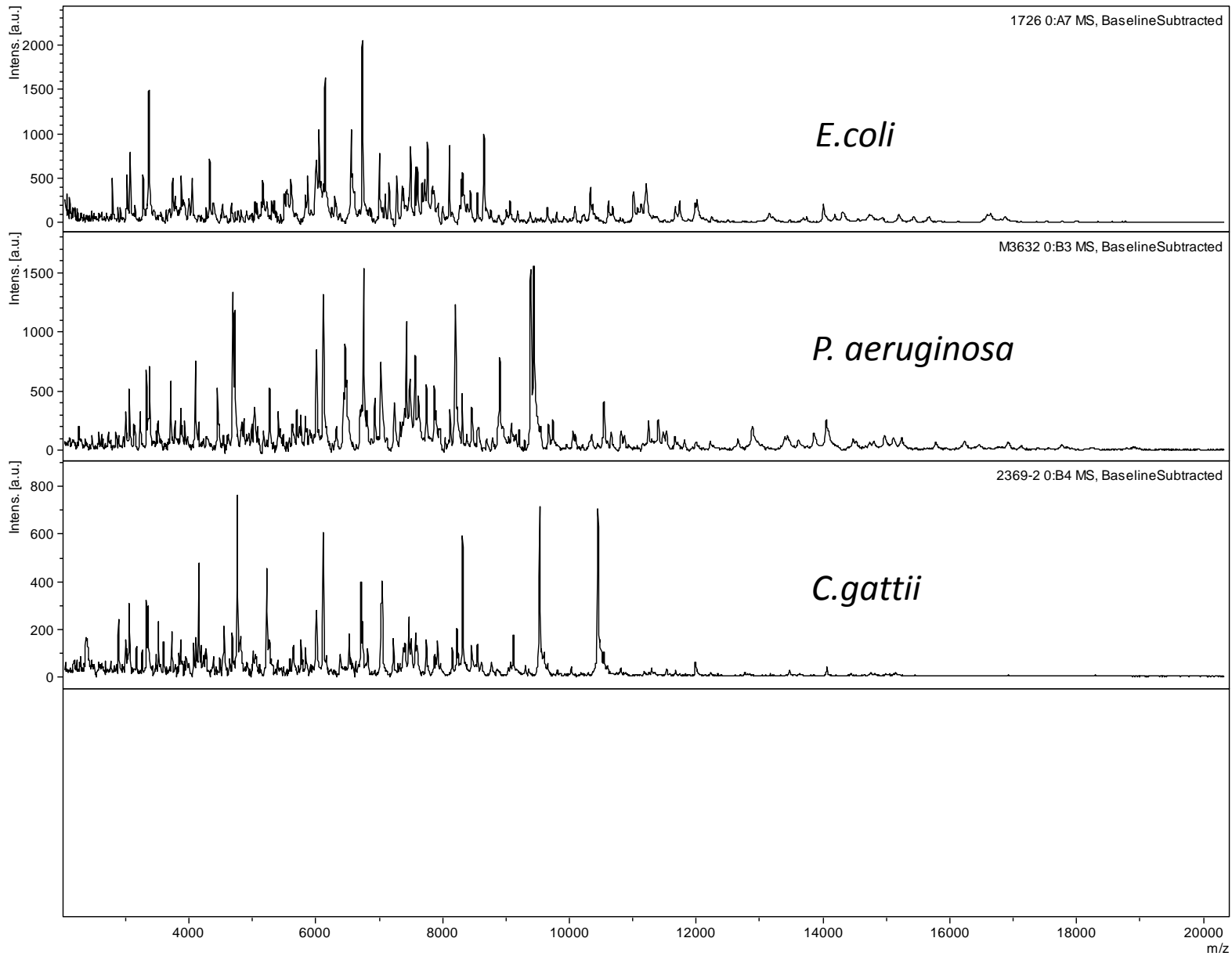


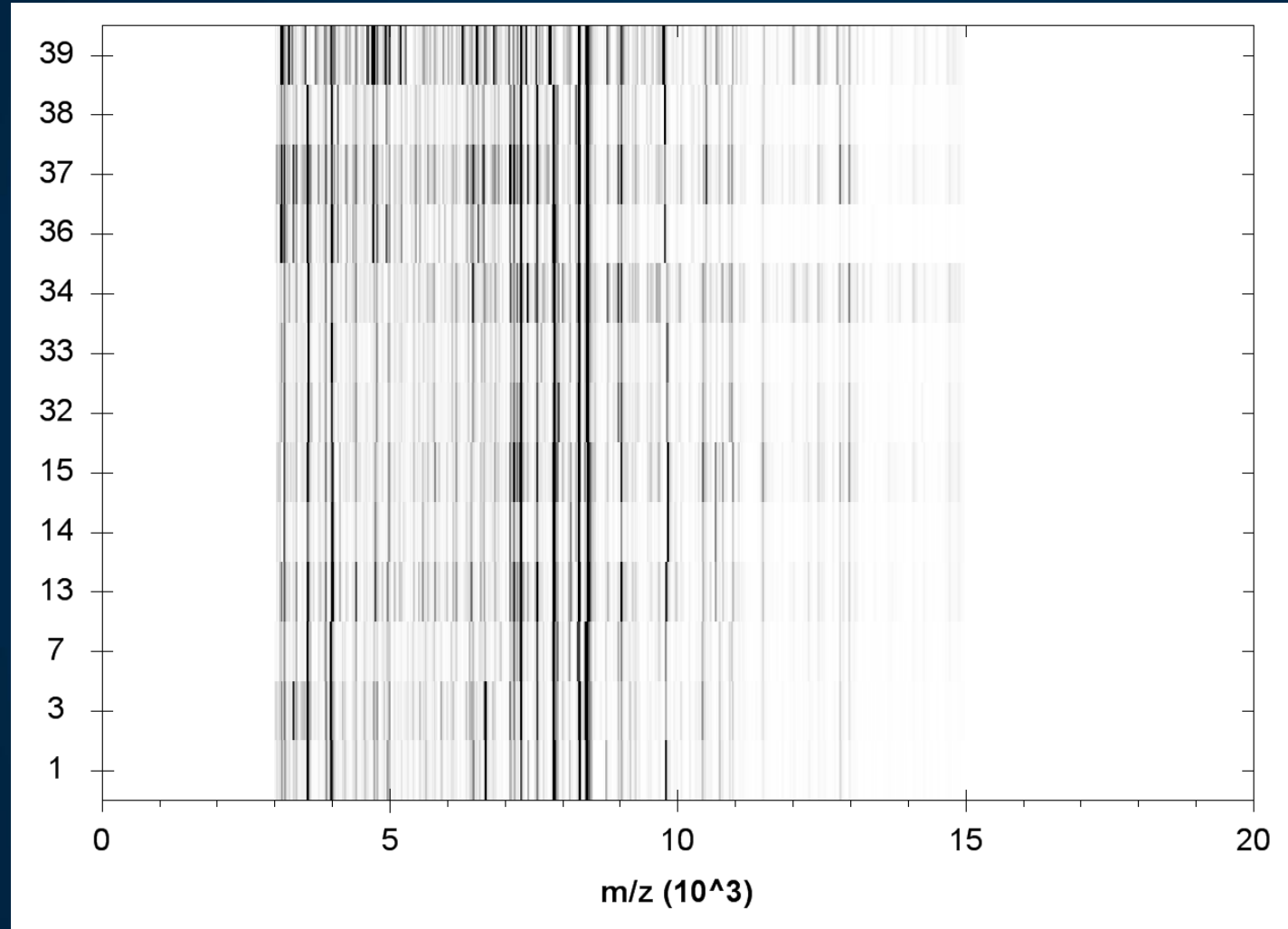
Image from Dr. Kenneth A. Mauritz, University of Southern Mississippi

Time of Flight





Protein Fingerprint



MALDI-TOF MS at ARUP

- Applications
 - Routine
 - Bacterial identification
 - Yeast identification
 - Database creation
 - More difficult
 - AFB lab
 - Fungi
 - Antimicrobial resistance



MALDI-TOF MS in MCORG

- 75-80% ID comes from MALDI
- Turn around time 1-2 days
- Cost savings
 - Switching paid for MALDI-TOF machine in 6 months
- Technicians can be trained in weeks (not as easy as it sounds)

Yeast Identification

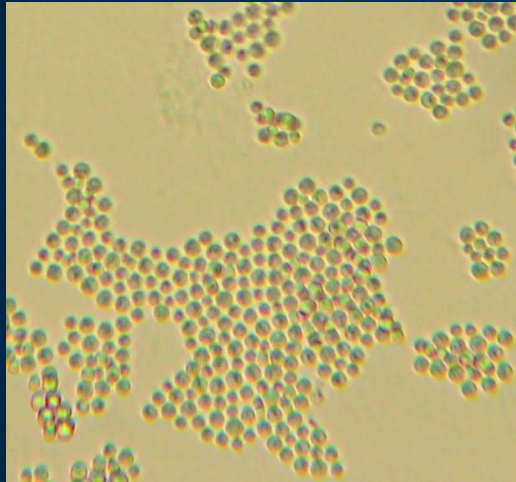
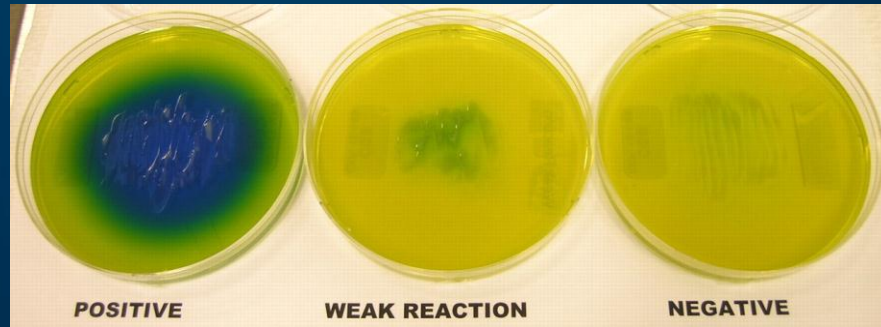


Image Courtesy of M. McGinnis
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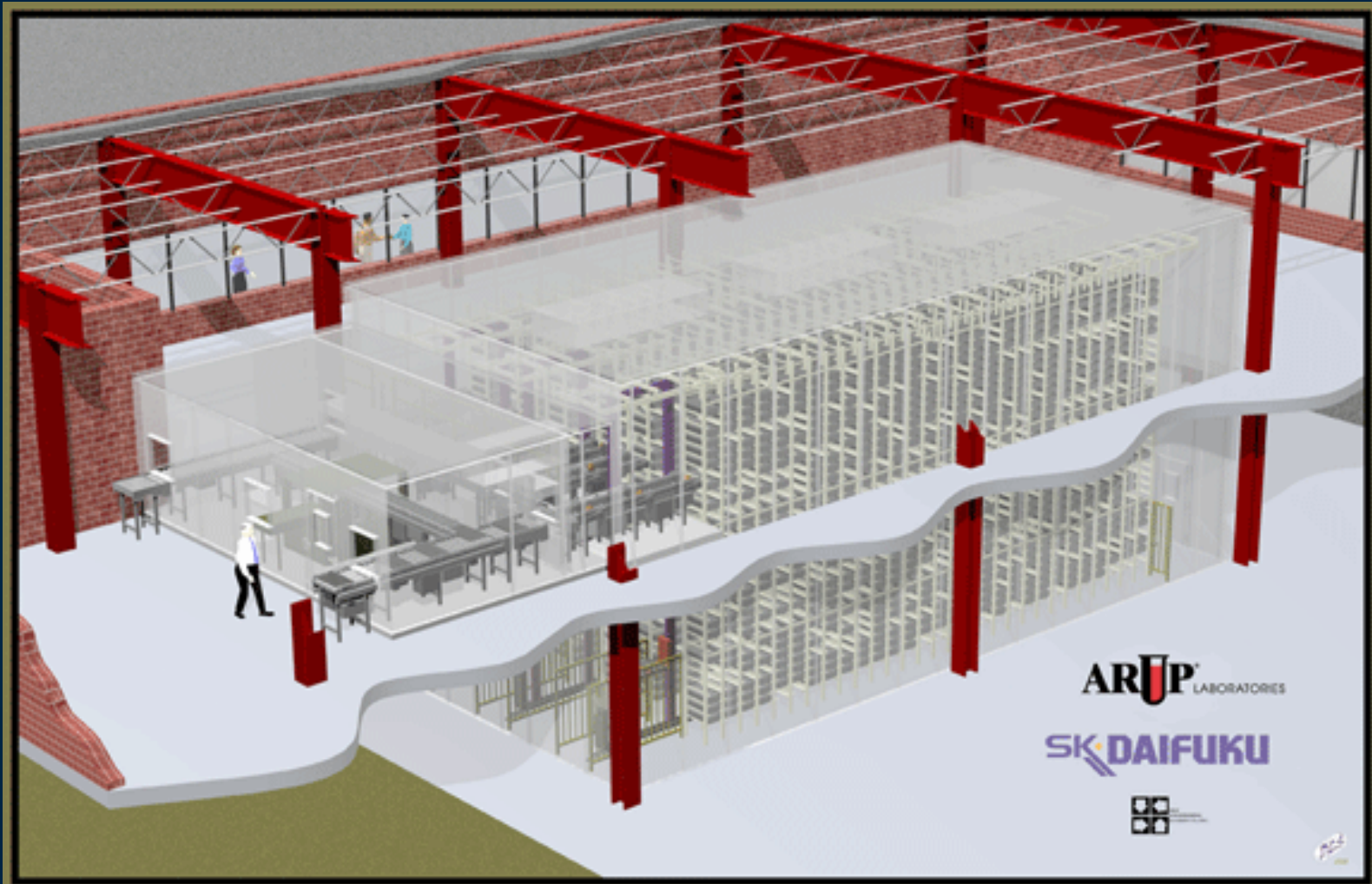
POSITIVE

WEAK REACTION

NEGATIVE

MALDI-TOF MS in Mycology

- All routine yeast identified by MALDI-TOF
- Internal validation > 350 isolates
 - 3 organisms failed to ID in the Bruker database®
 - Would actually ID contamination and freezer mistakes





MALDI-TOF MS in Mycology

- All routine yeast identified by MALDI-TOF
- Internal validation > 350 isolates
 - 3 organisms failed to ID in the Bruker database®
 - Would actually ID contamination and freezer mistakes
- Real world benefits
 - TAT Before: 3-4 days, After: 4-24 hours
 - Now we are waiting on susceptibility testing
 - Linear workflow

What is the true power of the system for us?

- Database creation!!!

Database

- Identify an organism that is not in the database
 - Confirm the true ID of that organism
 - Hard part
 - What is the gold standard?
 - Collect “perfect” spectra
 - 8 spots at 3 fires each for 24 separate spectra
 - Build MSP
 - Challenge the database



Acid Fast Bacterial Lab

Perform testing on all mycobacteria sent for identification

RGM ~40 isolates/day

SGM ~22-30 isolates/day

Identification

Smear, 16s sequencing, Gen-Probe[®], NAAT testing
culture, susceptibility

Now MALDI-TOF MS

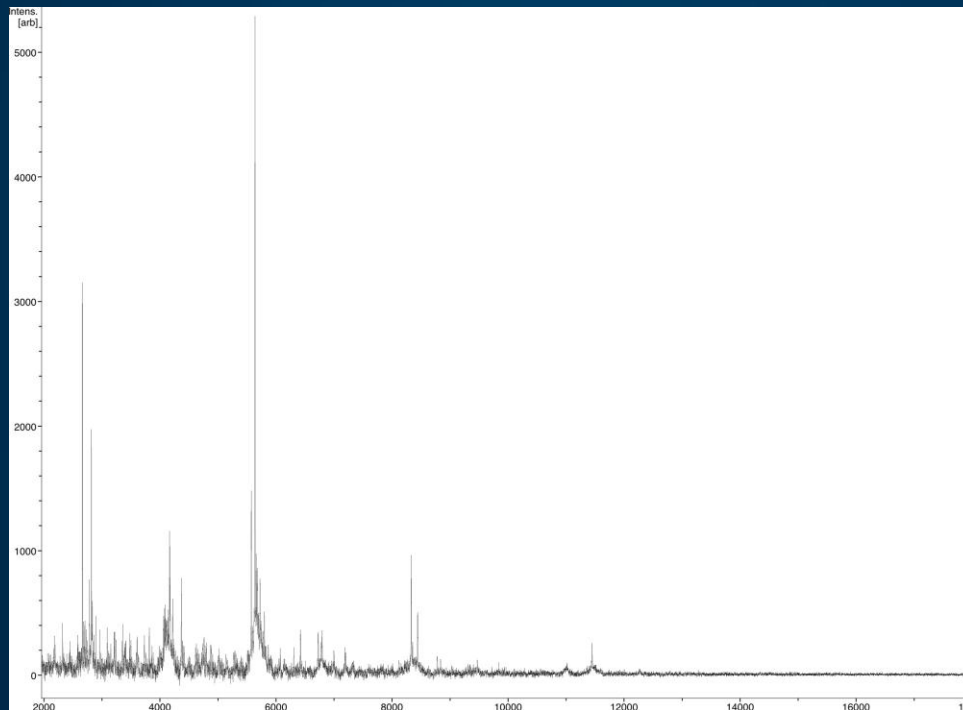
Bruker, bioMerieux

Rapidly Growing Mycobacteria

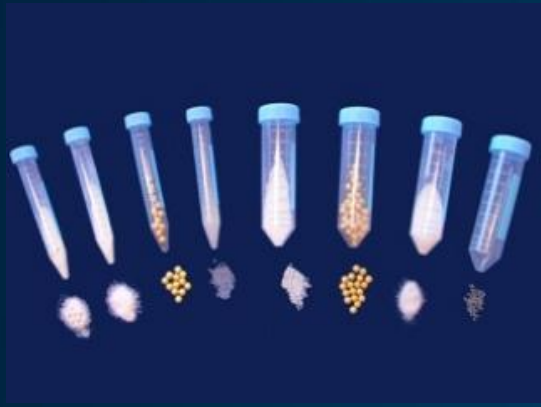
- They make up ~70% of all Mycobacteria in my lab
 - We have to sequence all of them
 - Some we still can not ID with 16s sequencing
 - *M. abscessus/M.chelonae/M.bolletii/M.massiliense*
- Mycolic acid makes everything worse
- Very similar genetically
- Emerging pathogens

It is all about the extraction...

- Extraction up to this point was simple
 - Ethanol wash, acetonitrile and formic acid

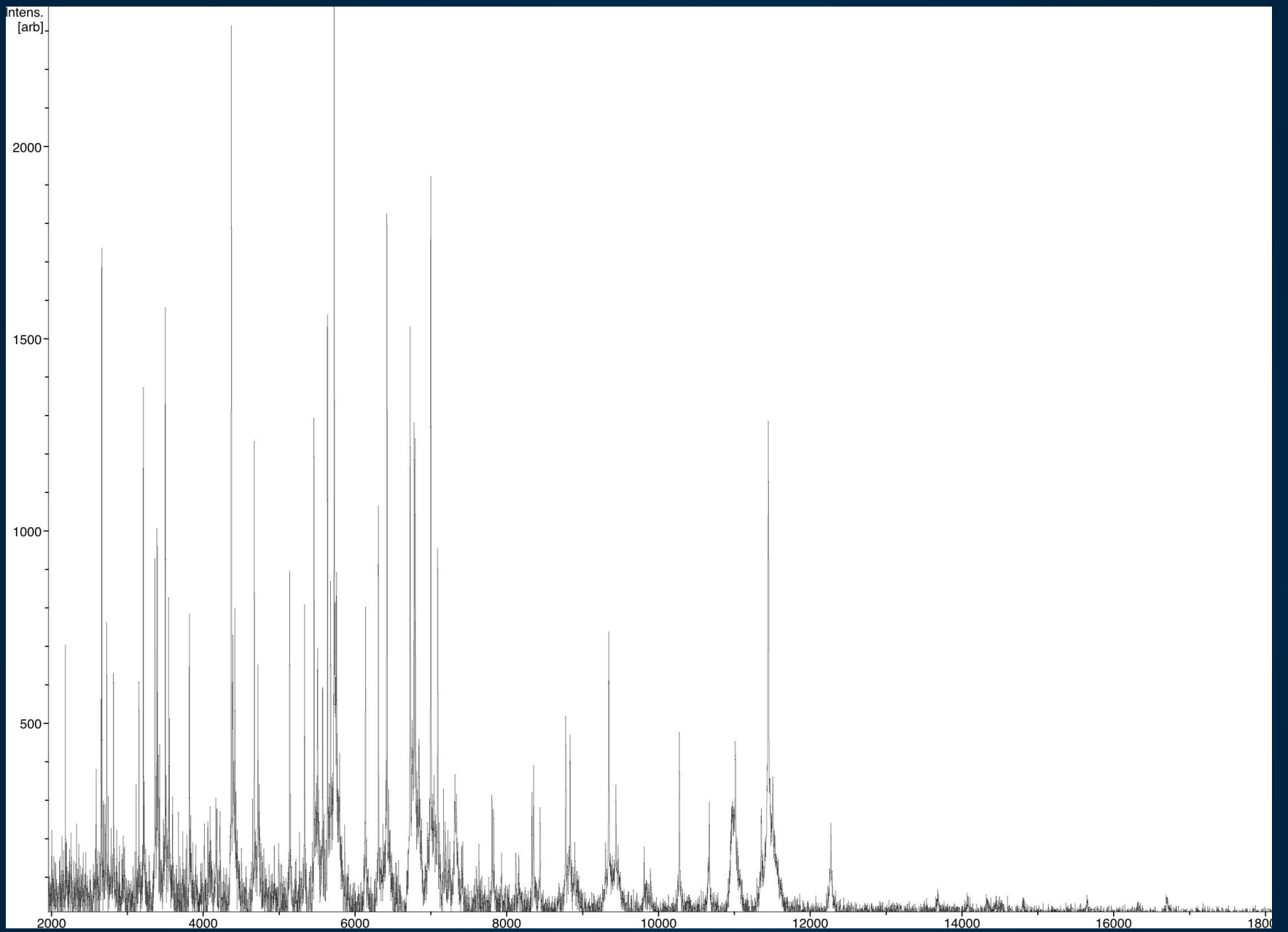


How to get more proteins?

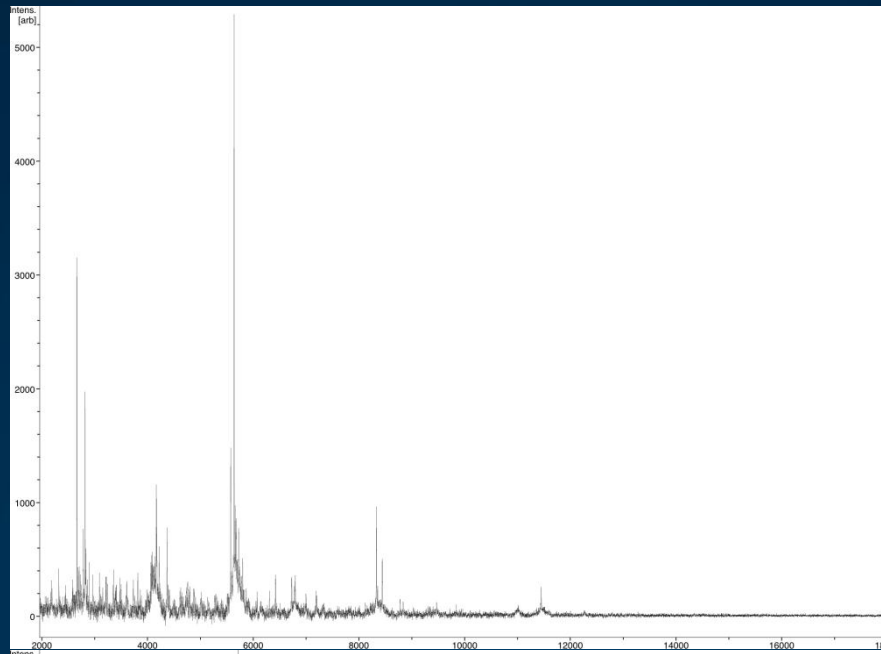


MO BIO®

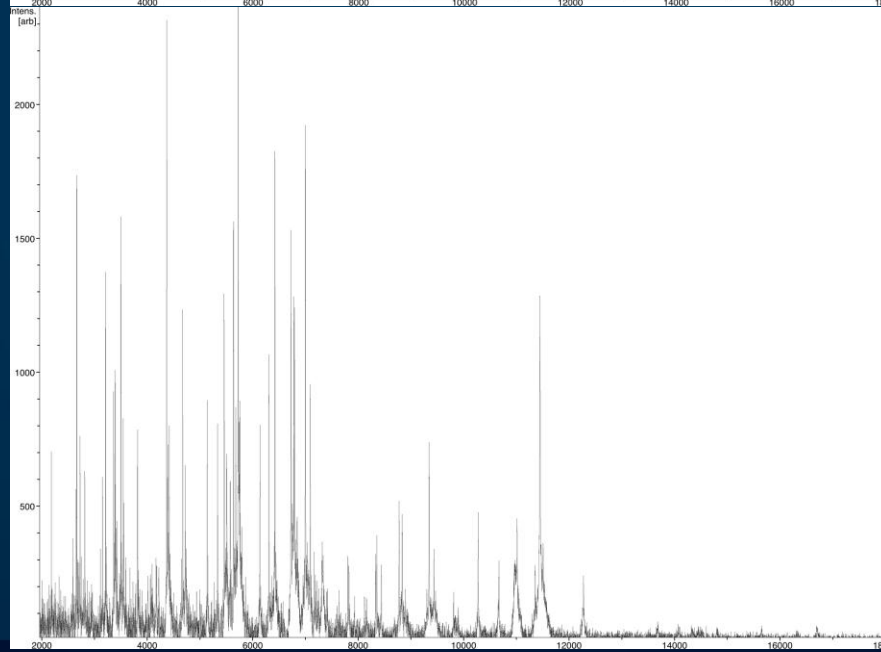
Extraction: Just like before with small changes

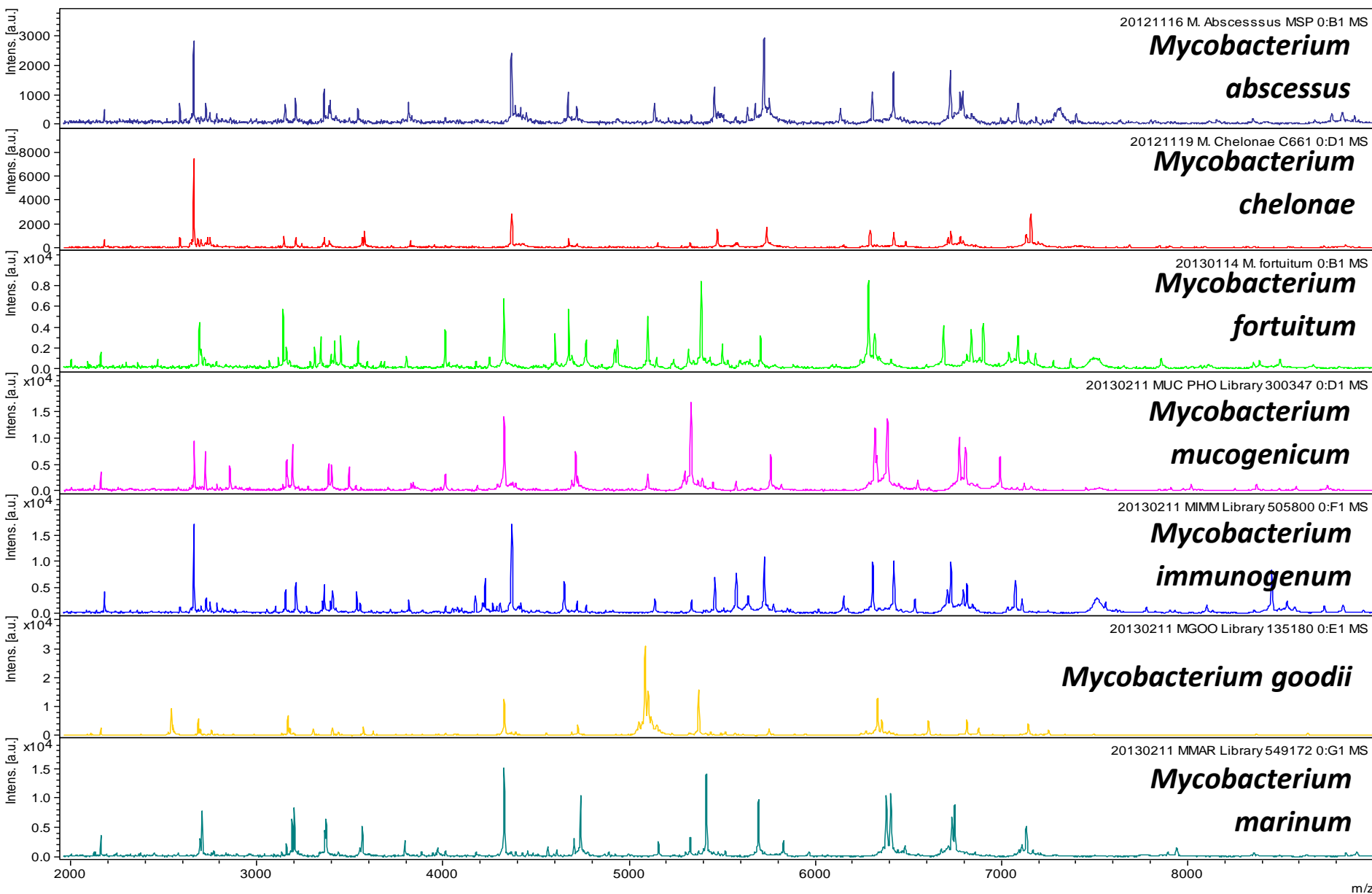


Before



After





Organism ID by Sequencing/ITS	MALDI Score Value					Total
	>2.0	1.9-2.0	1.8-1.9	1.7-1.8	≤1.7	
Mycobacterium abscessus*	87	4	0	0	1	92
Mycobacterium fortuitum	60	6	5	2	5	78
Mycobacterium mucogenicum/phocaicum	23	1	1	0	1	26
Mycobacterium chelonae*	23	0	0	1	0	24
Mycobacterium chelonae/abscessus complex	22	0	0	0	0	22
Mycobacterium goodii	5	0	0	0	0	5
Mycobacterium marinum	4	0	0	0	0	4
Mycobacterium immunogenum	3	0	0	0	0	3
Mycobacterium smegmatis	1	0	0	1	0	2
Total	228	11	6	4	7	256

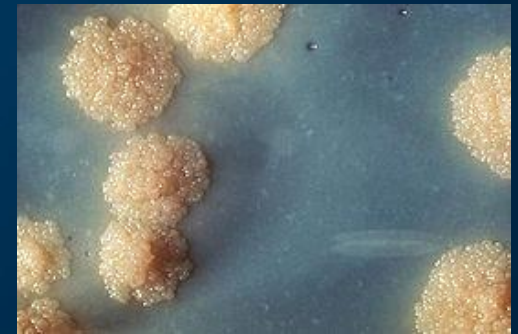
Move everything to MALDI-TOF MS!

- Downstream changes are always beneficial!
 - Good example: work flow
 - *M.abs/M.che* identification
 - 16s sequencing
 - RT-PCR to differentiate them
 - Results are not always correct!

Mycobacterium tuberculosis complex

ABCs of MTB Complex

Mycobacterium africanum, bovis, canetti, caprae, microti, tuberculosis, BCG

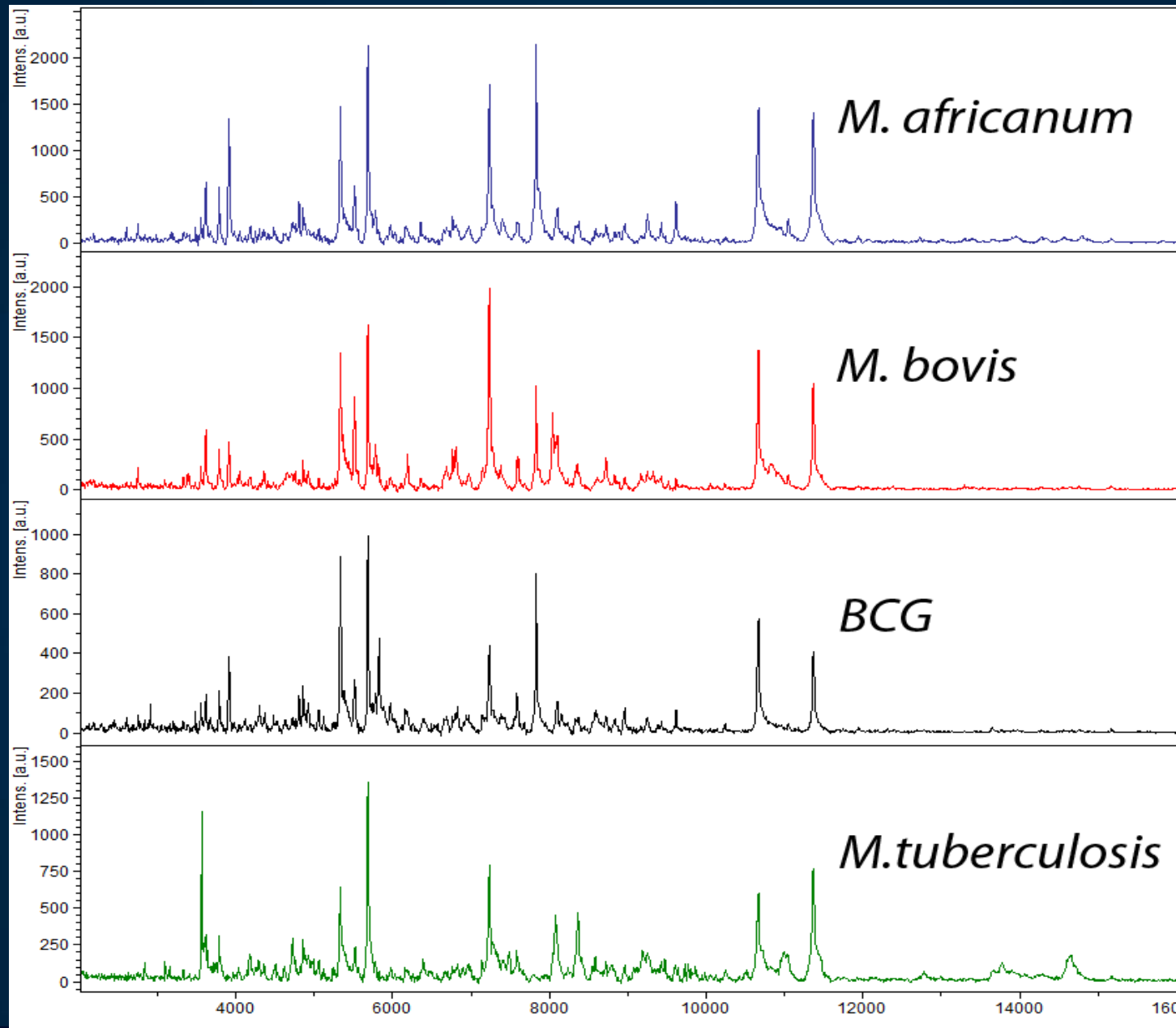


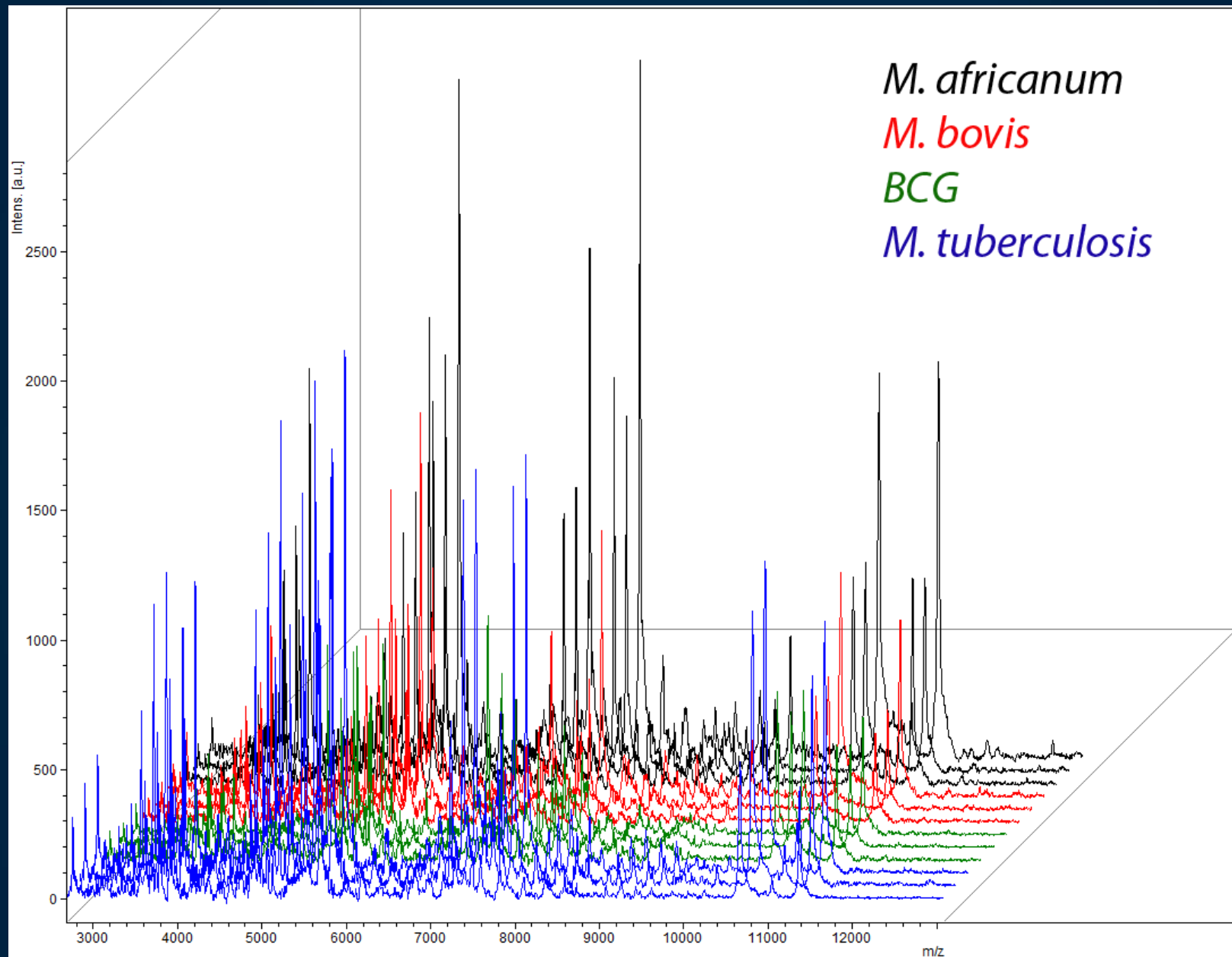
MALDI-TOF MS

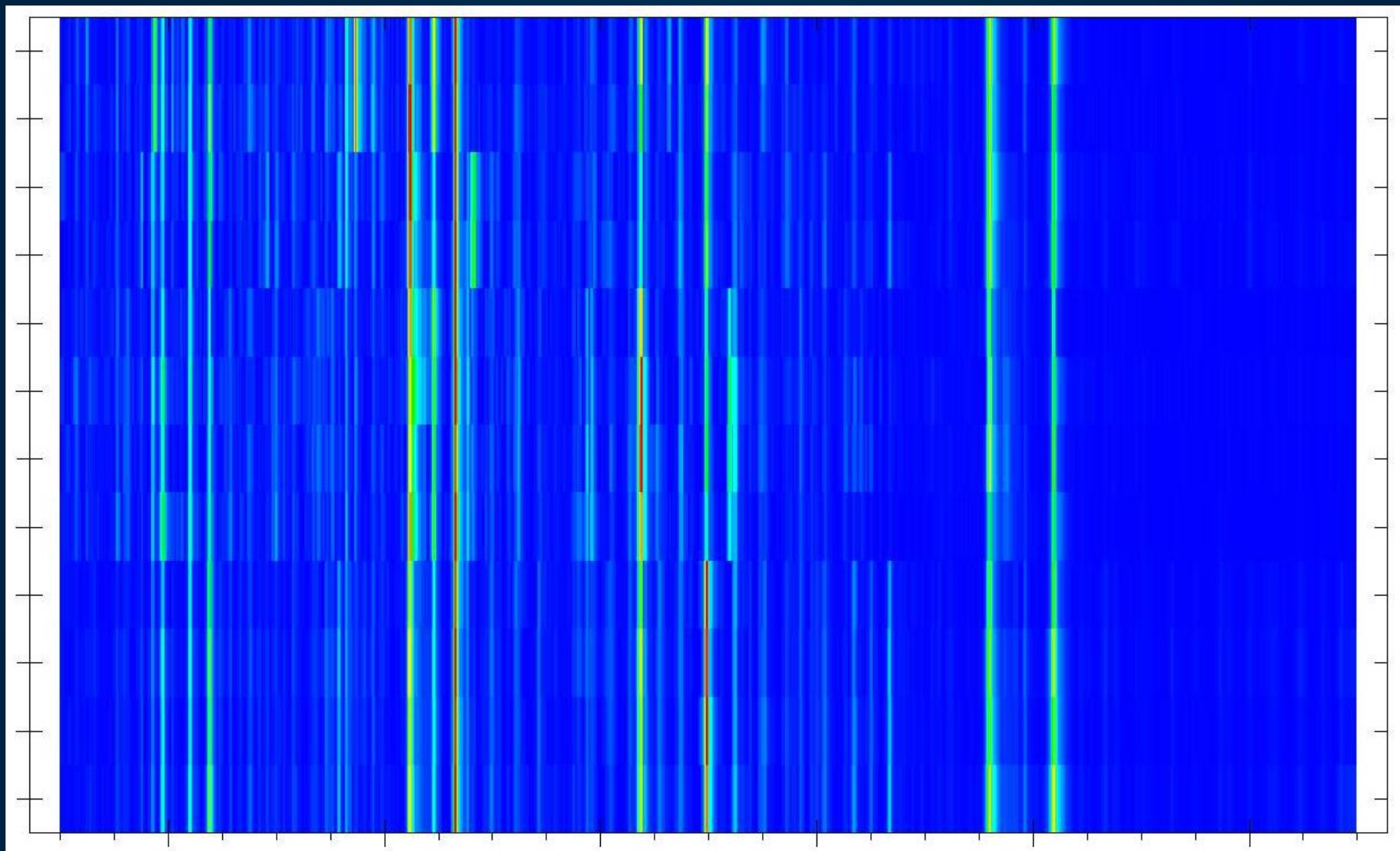
- Not going from patient sample yet...
 - Still need a fair amount of organism
- Really the increased turnaround comes from NTM
- MTBC identification could be useful if reliable
- Where are we at for LOD for MALDI

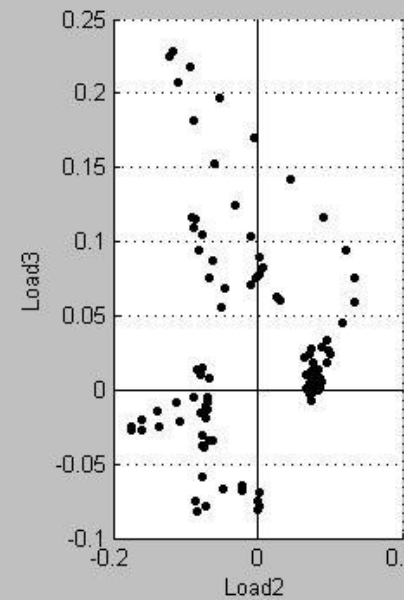
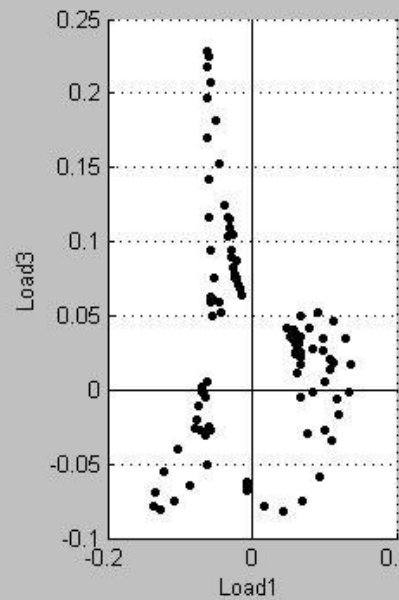
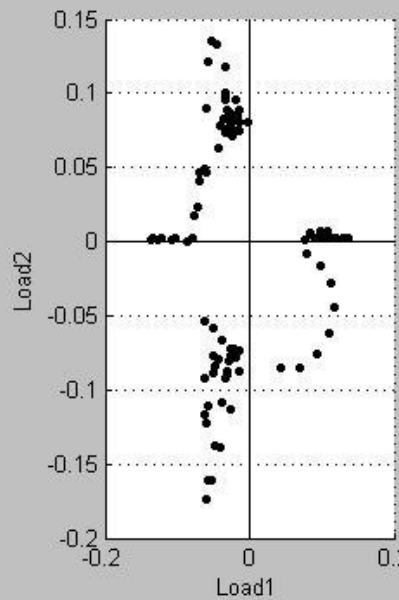
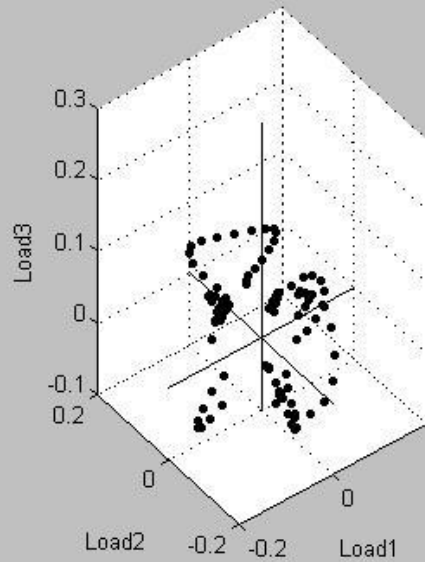
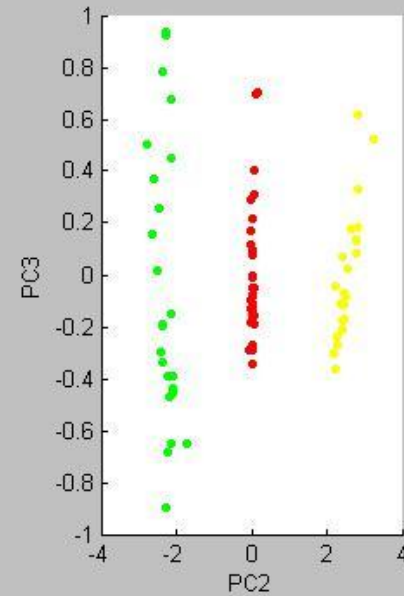
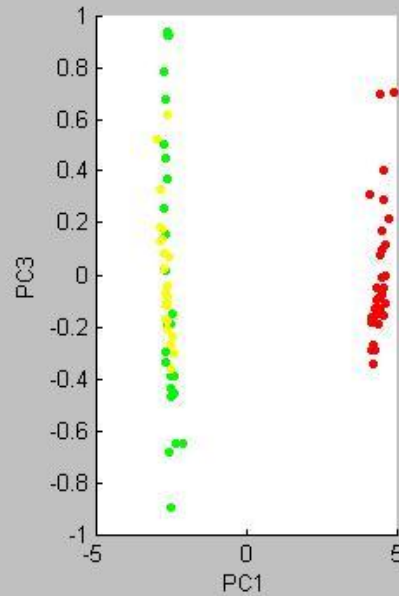
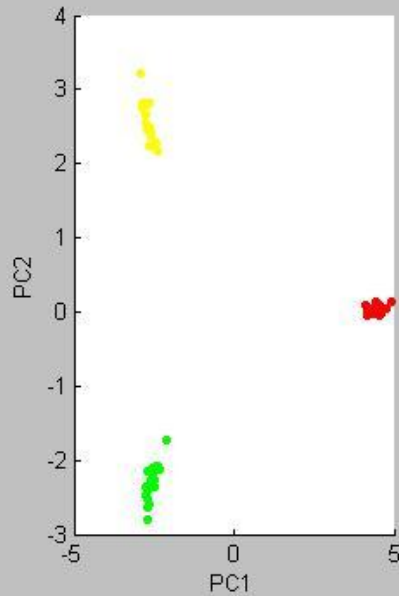
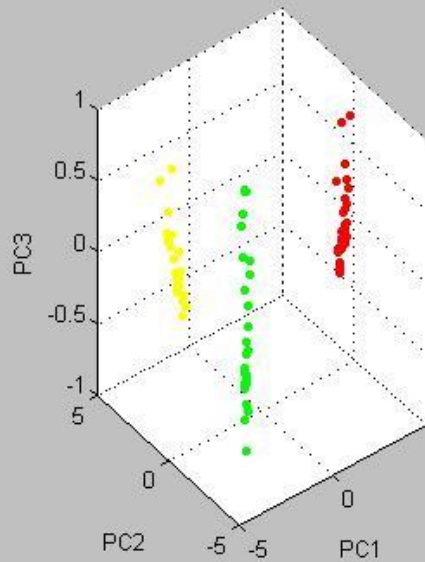
ARUP Workflow

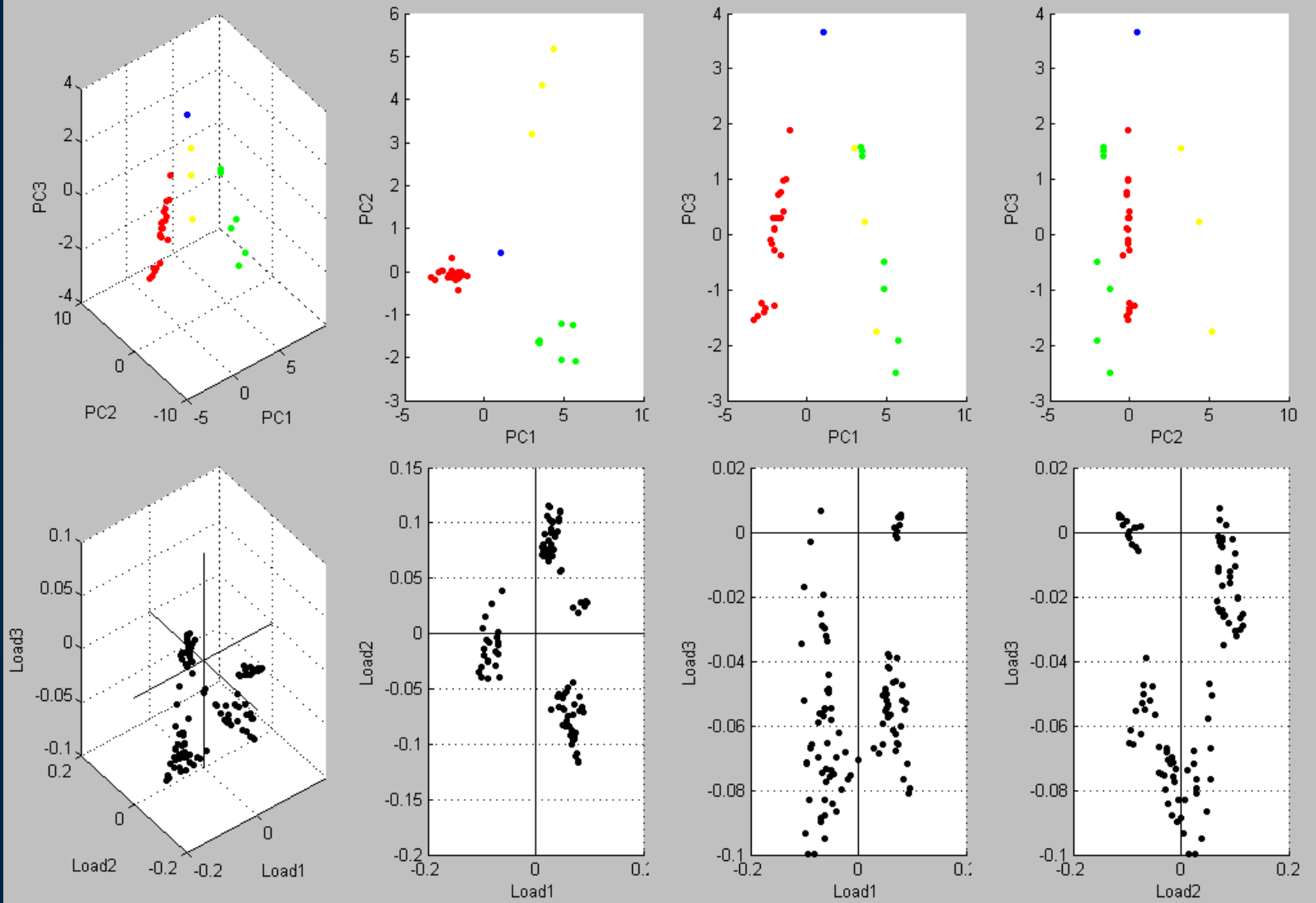
- Smear Positive
- MTD test GeneProbe[®]/Xpert[®] MTB/RIF
- Set for growth/susceptibility
- Growth required for susceptibility and complex differentiation
 - PCR/sequencing









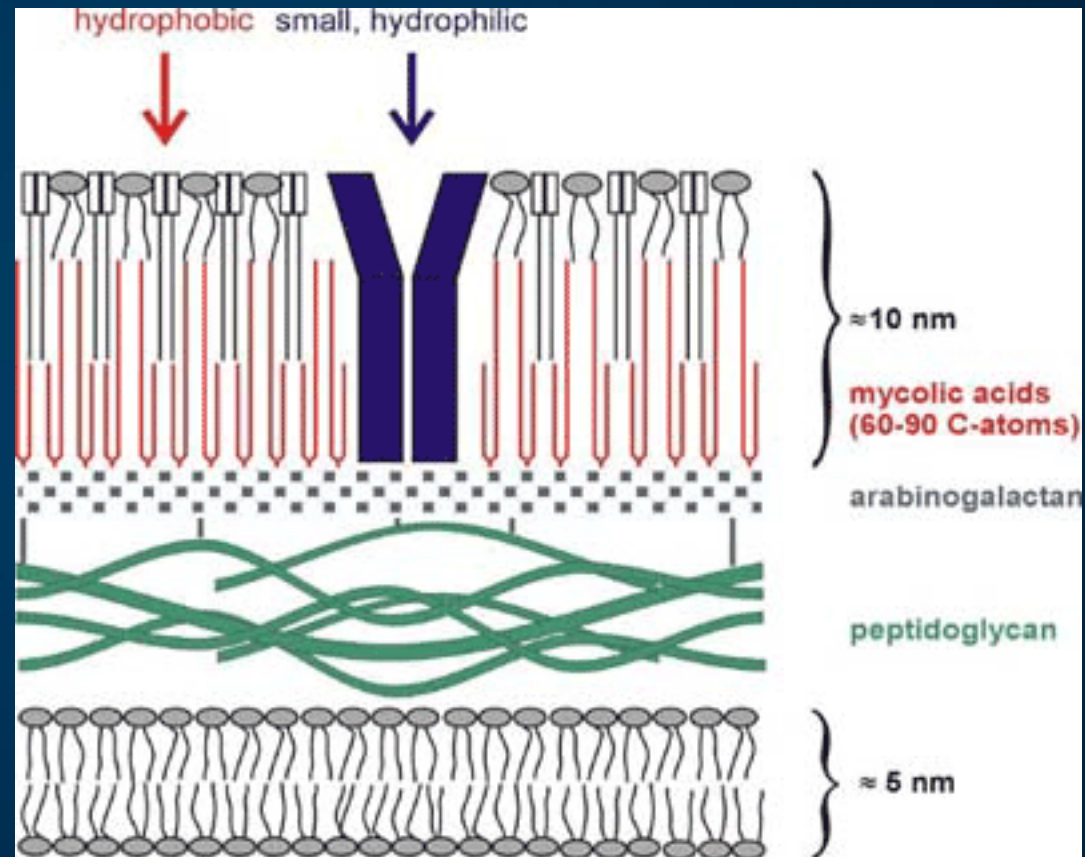


- In a blinded study using the in-house database, 100% (35/35) of Mycobacterial isolates were identified to the MTC (ID > 2.0), 86% (30/35) were identified to the correct species based solely on highest ID score. However, only 15% (5/35) could be differentiated to the species level when the percent difference was examined.

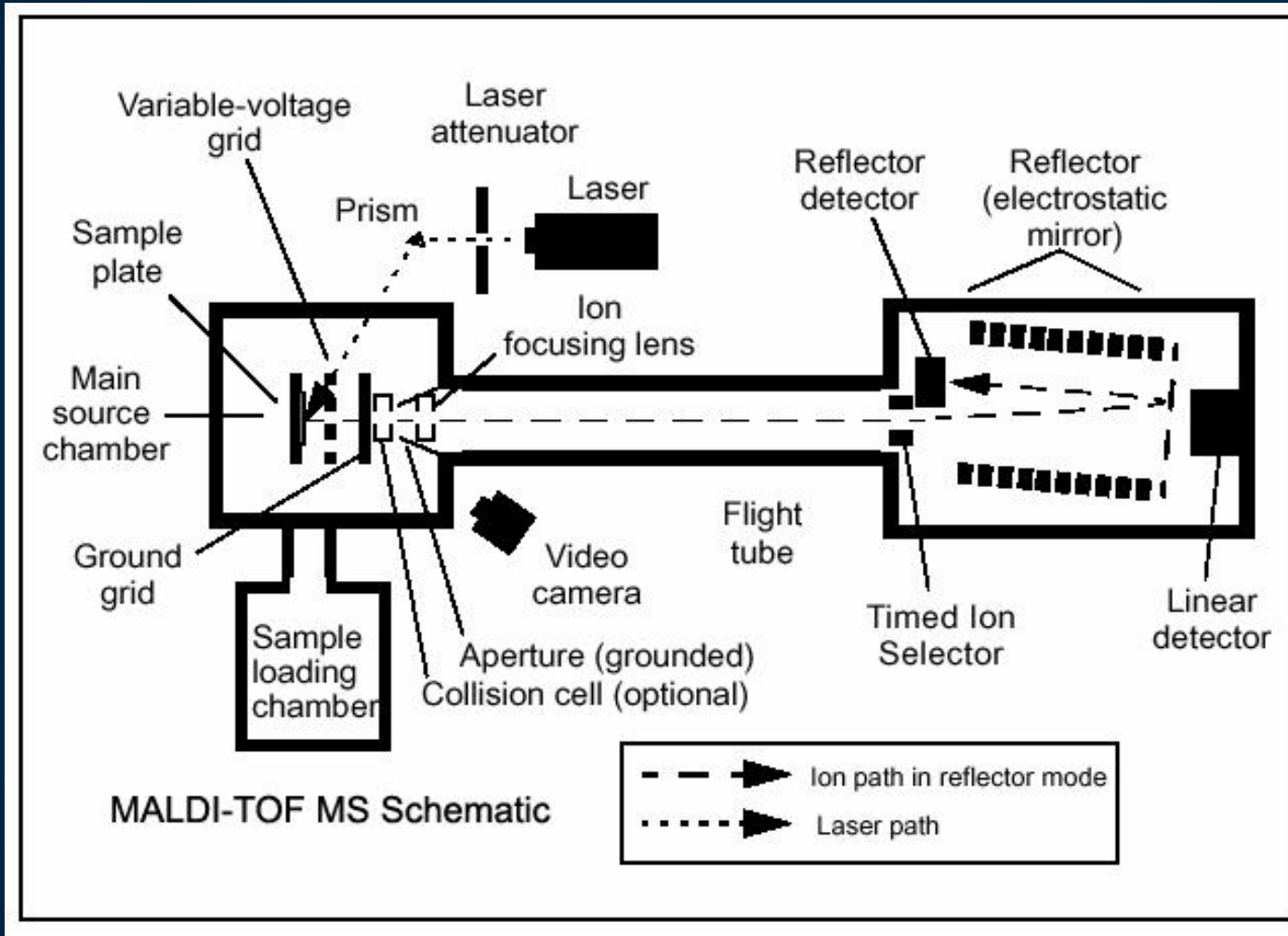
IT DID NOT WORK!!!

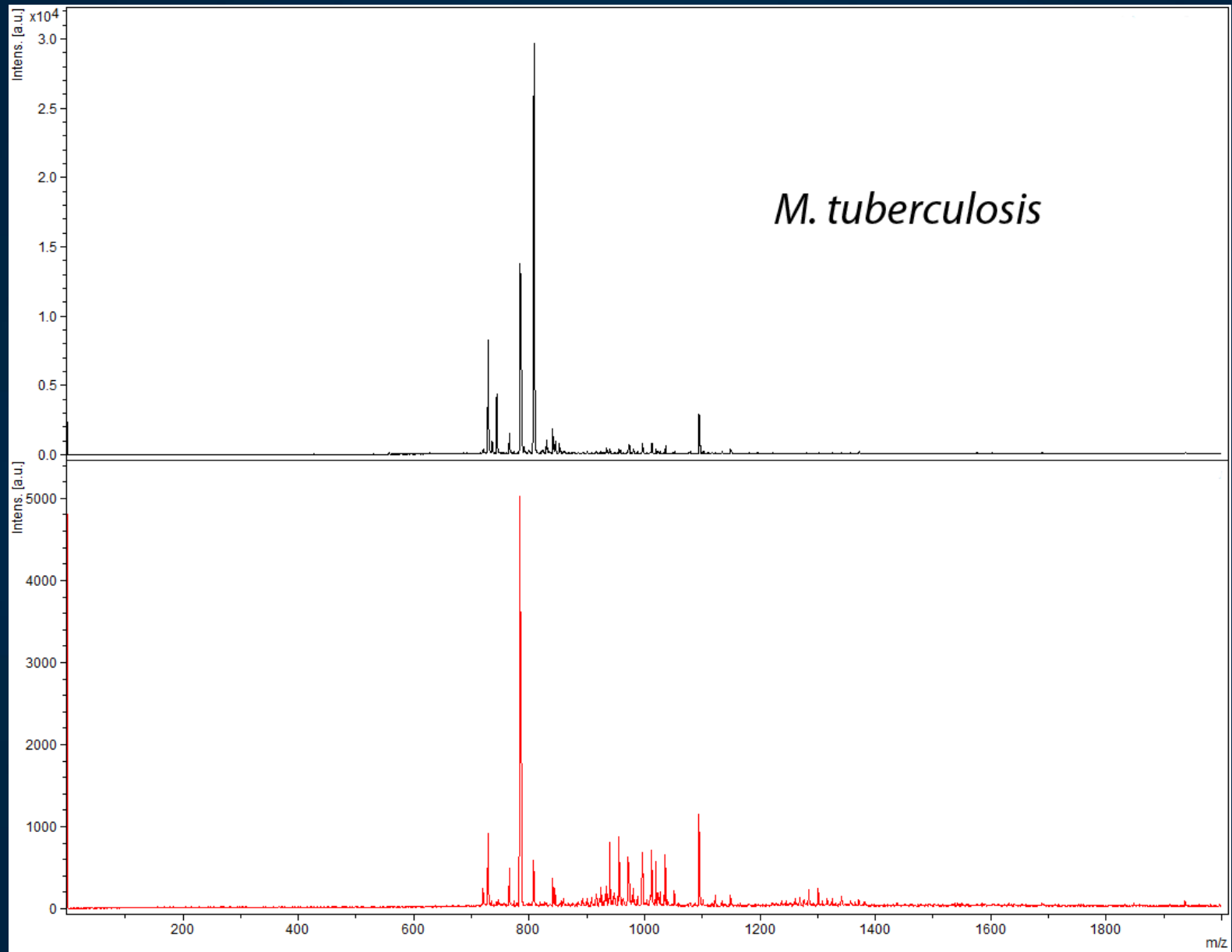
M. tuberculosis cell wall is amazing

Utilize the unique cell
To help us!



Time of Flight





Each organism has a unique fingerprint....

Coupling positive and negative ion spectra gives higher specificity

We can differentiate the entire complex

Could be applied to other difficult organisms (mold)

One problem...

- Negative ion mode is not going on the FDA machine!!!
- Complex test requiring mass spec personnel
- Gets expensive
- No database to use at all

The Future....

High resolution mass spectrometry

Europe is already testing new systems

So are we...

Added benefit of susceptibility testing

Typing

Virulence factors

Low limit of detection

Next Generation Sequencing

Just around the corner for all TB positive samples in our lab

Starting in house validation

Cost is less than all the other testing we have to bill

Summation

- MALDI-TOF MS has changed the way we work in Clinical Microbiology
 - It will never replace everything
 - Bring it online to as many tests as possible
 - Be careful with your database
 - This is the future of testing
 - You get to play with lasers!!!



Acknowledgements:

Dr. Hanson, Dr. Jensen, Dr. Slechta, Dr. Horan

