Preprocessing, Management, and Analysis of Mass Spectrometry Proteomics Data*

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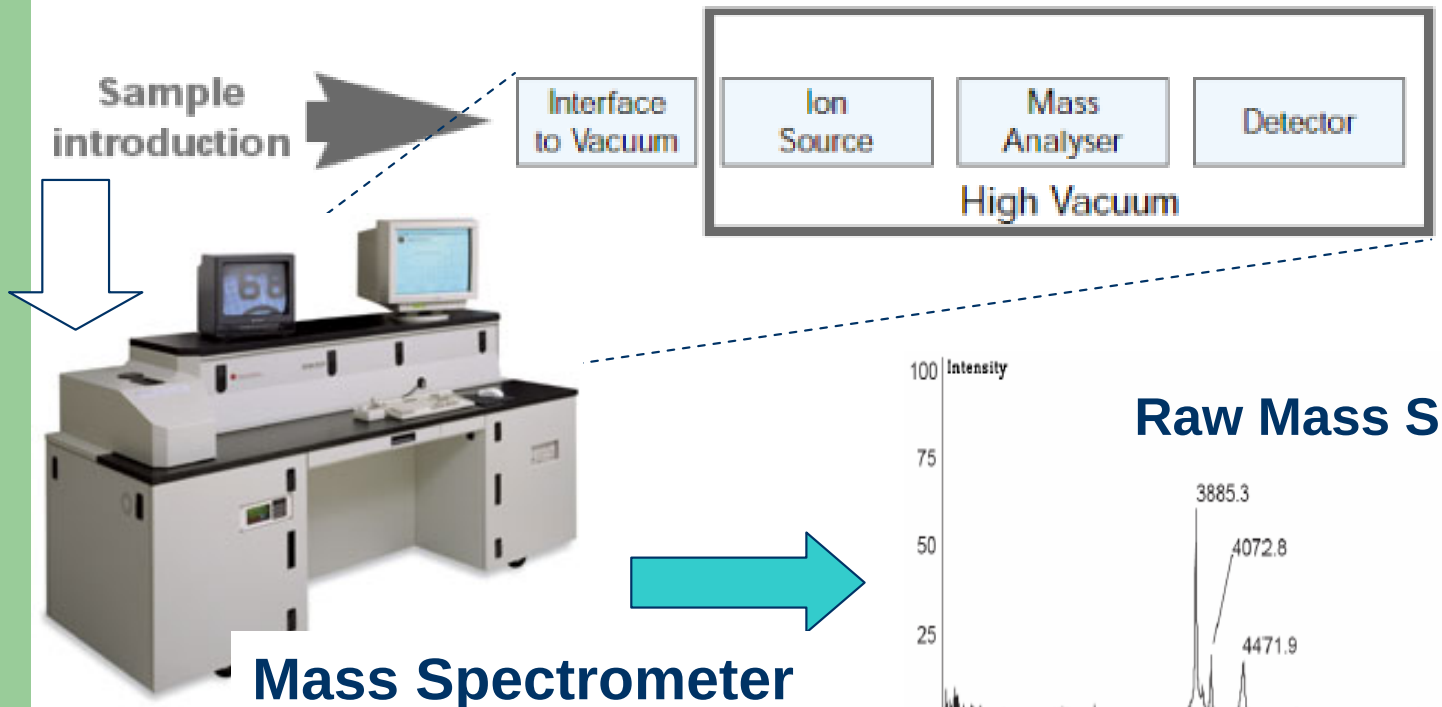
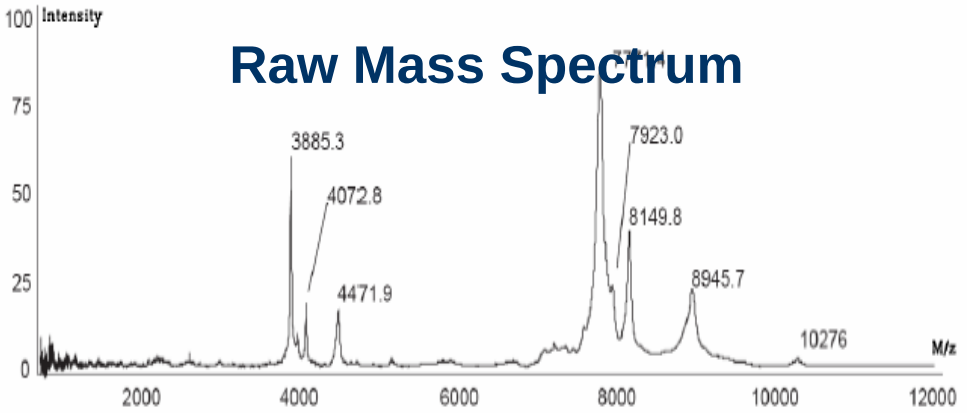
*Joint work with P. H. Guzzi, T. Mazza, P. Veltri

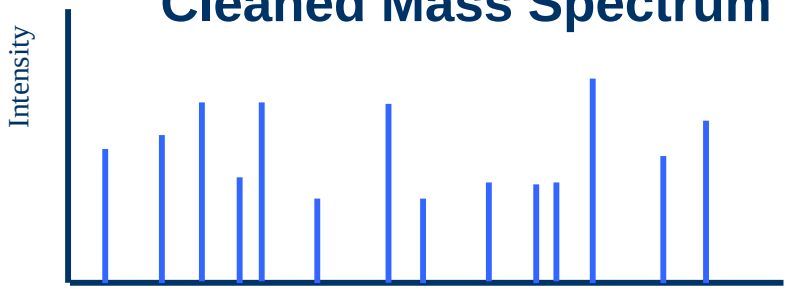
Outline

- Mass Spectrometry-based Proteomics
  - MS data
- Preprocessing of MS Data
  - Noise Reduction
  - Binning
  - Peaks Alignment
- MS-Analyzer, a platform for spectra analysis
  - Ontology+Workflow
- Conclusions and future work

Mass Spectrometry-based Proteomics







Data Preparation

Workflow Execution
(i.e. Supervised Classification)

Knowledge Models

Data Pre-Processing

Subtract Base Line
Normalization
Binning
Peak Identification
Peak Alignment

Preprocessed Cleaned Spectra Database

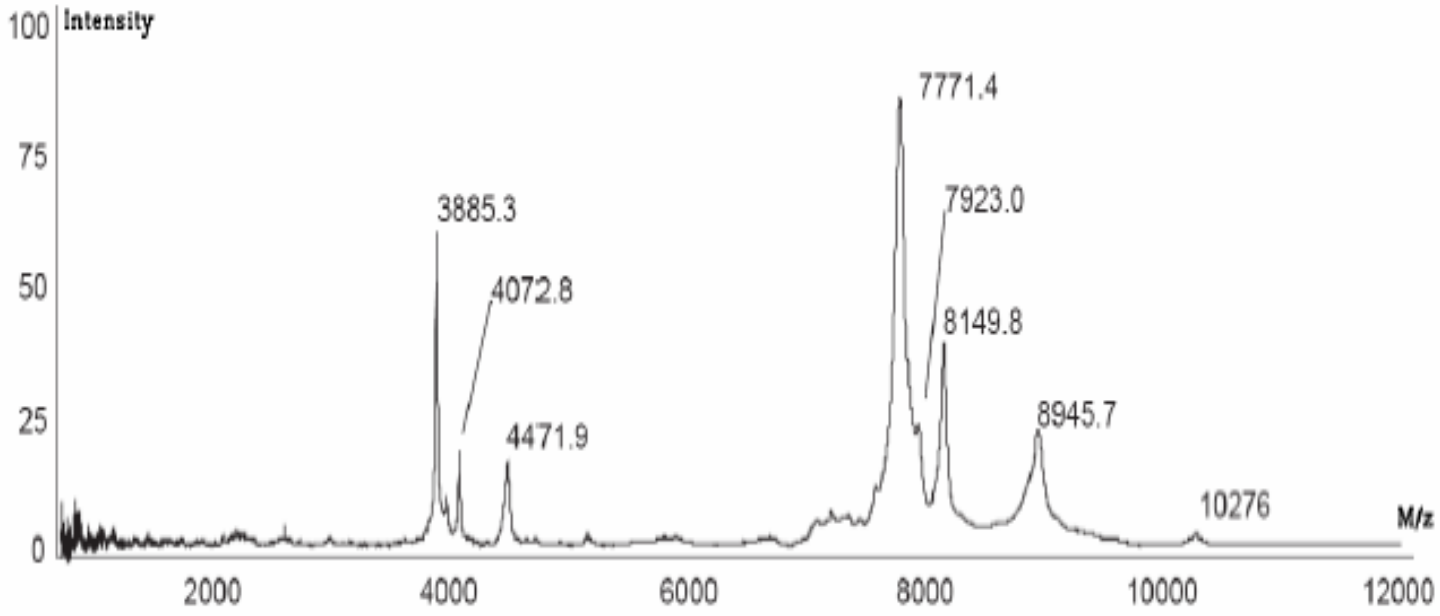
- **A spectrum is a large sequence of value pairs (*I, m/Z*):**
  - *I* (*intensity*) depends on the quantity of the detected biomolecules,
  - *m/Z* (*mass to charge ratio*) depends on the molecular mass of detected biomolecules

MALDI-TOF spectrum

...

700.003	9.000
700.015	3.000
700.026	2.000
700.038	3.000
700.050	1.000
700.062	2.000
700.073	2.000
700.085	4.000
700.097	3.000
700.109	2.000
700.121	5.000
700.132	8.000
700.144	7.000
700.156	12.000

...

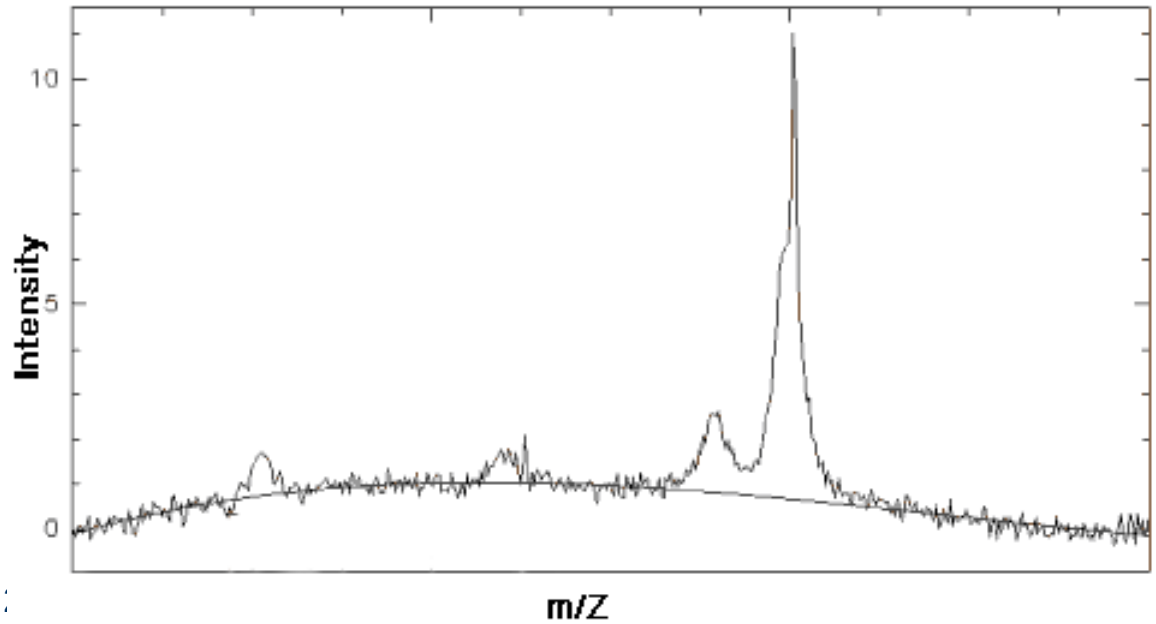


Preprocessing of MS Data

- Each spectrum data is the result of two measurements (*I, m/Z*) that are corrupted by noise
- Preprocessing aims to correct *intensity* and *m/Z* values in order to:
  - (i) reduce noise,
  - (ii) reduce amount of data, and
  - (iii) make spectra comparable (Data calibration or Peaks Alignment).

Noise Reduction

- Each mass spectrum exhibits a base intensity level (a baseline) which varies from fraction to fraction and consequently needs to be identified and subtracted.
  - ***Base line subtraction*** flattens the base profile of a spectrum
  - ***Smoothing*** reduces the noise level in the whole spectrum.

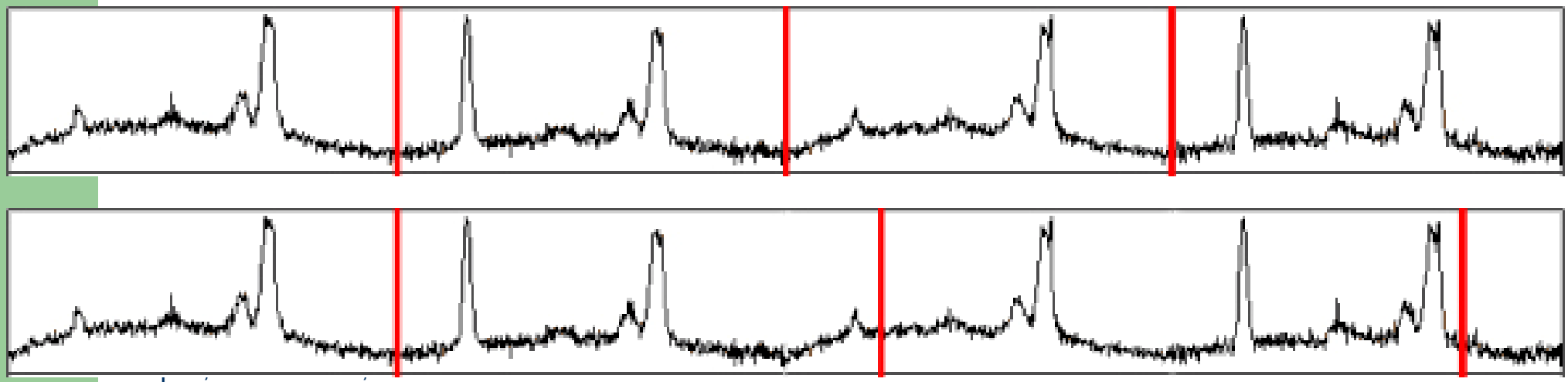


Normalization

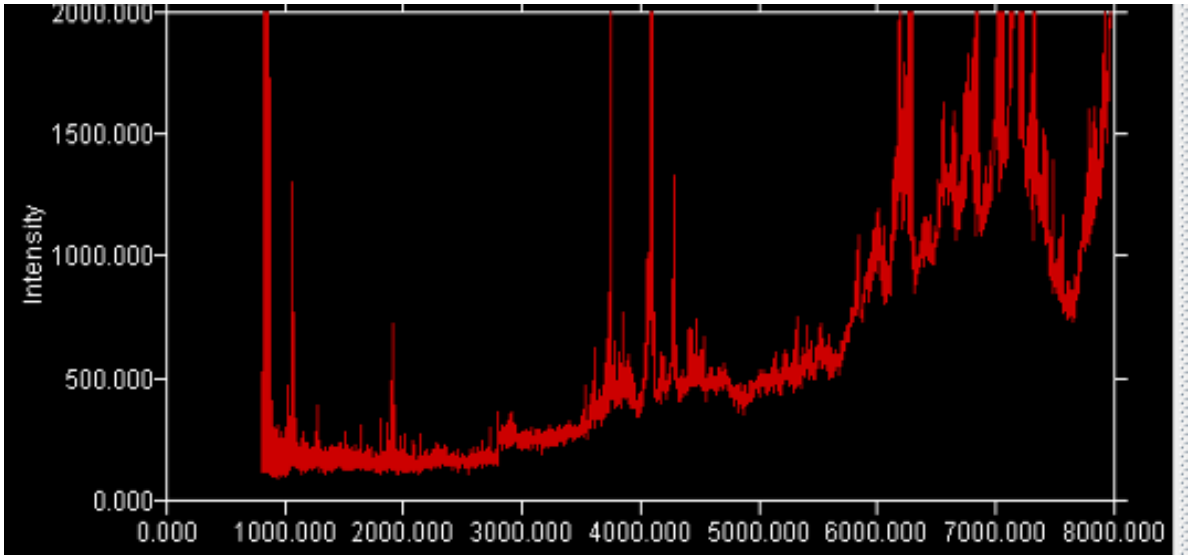
- Normalization aims to make intensity comparable across different spectra. It is usually applied to each spectrum.
- Different normalization scheme
  - Direct $I_{j\_norm} = \frac{I_j - I_{min}}{I_{max} - I_{min}}$
  - Inverse $I_{j\_norm} = 1 - \frac{I_j - I_{min}}{I_{max} - I_{min}}$
  - Canonical $I_{j\_norm} = \frac{I_j}{\sum I_j}$
  - Logarithmic
    - This method performs a logarithmic transformation on a set of *K* spectra. The intensity measure I_{hj} for sample *h*, (*h* = 1,…,*K*), at m/Z value *j*, is transformed because of its skewed distribution.

Data Reduction: Binning

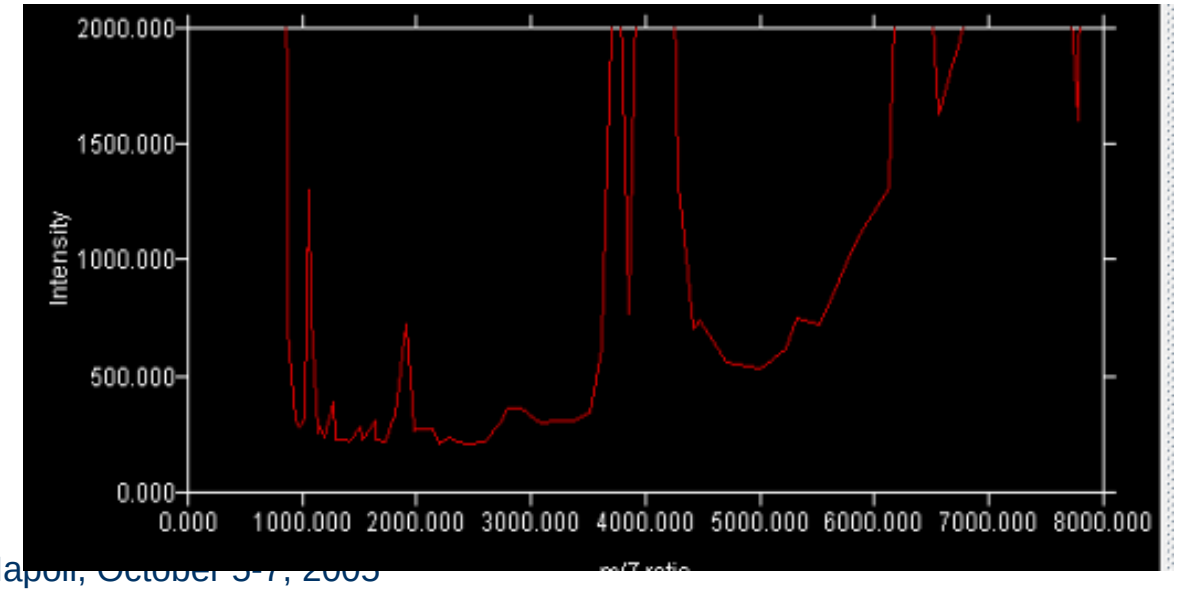
- Binning performs data dimensionality reduction by grouping measured data into *bins*.
- It calculates, for each interval of m/Z values (bin),
  - an aggregate intensity (e.g. the sum of the intensities in the bin)
  - and a representative m/Z value (e.g. the median or the one with maximum intensity)
- and elects this couple as representative of the bin.



Data Reduction: Binning



Raw Spectrum



Binned Spectrum:

- Window Size: 100
- Type: fixed

Peaks Alignment

- Without alignment, the same peak (e.g. the same peptide) may have different m/Z values across samples
- This method finds a common set of peak locations (i.e. m/Z values) in a set of spectra, so that all spectra have common m/Z values for the same biological entities.
  - Applied to an entire dataset.
- Window in which m/Z can be shifted is defined as *window of potential shift* (intrinsically related to instrument)
  - A precise definition of this value has to consider instrument data sheet or a set of measurement (a sort of calibration).
  - For MALDI-TOF MS instrument this values can be set as 0.5% of current m/Z values (variable window shift).

MS Data Mining Steps

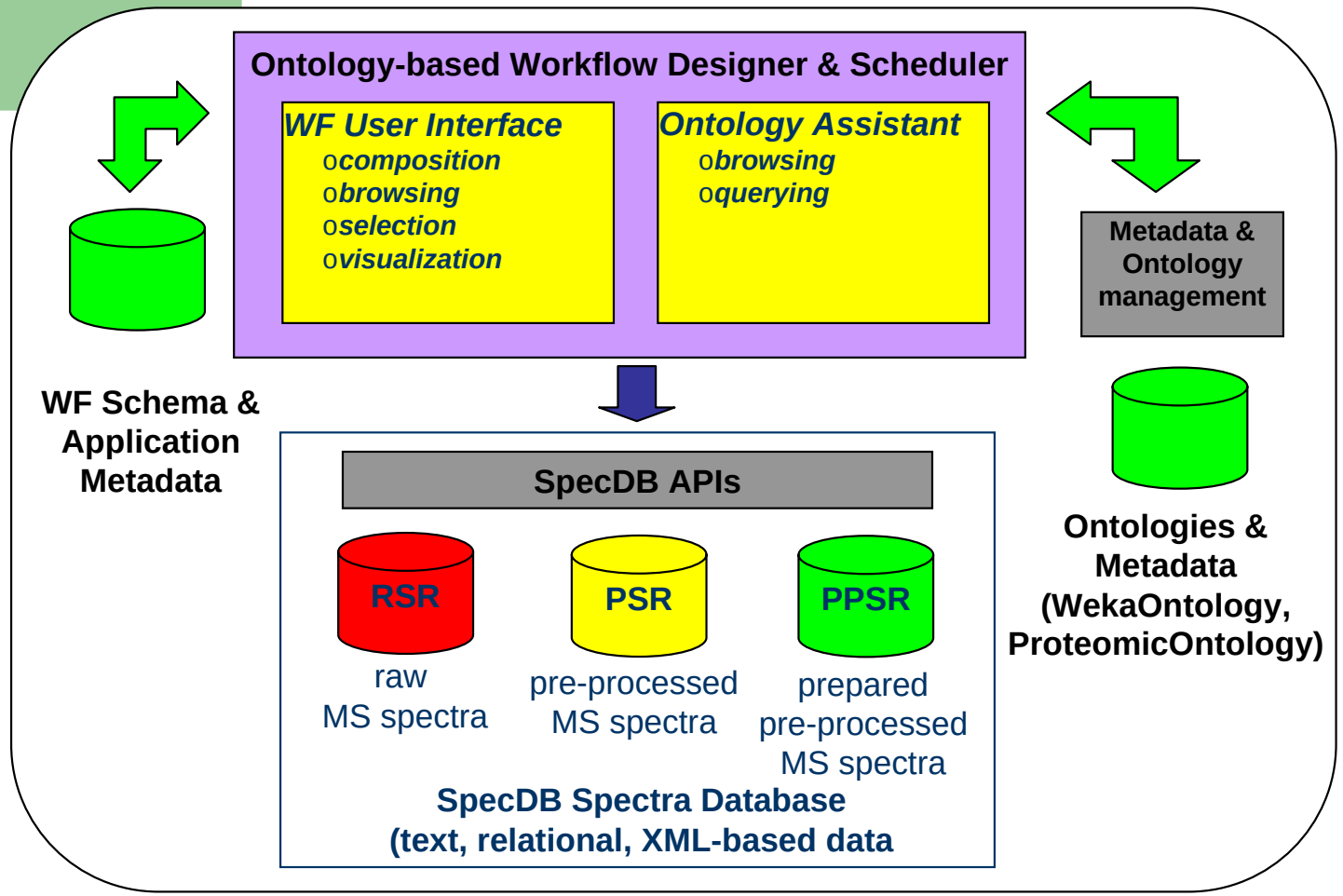
1. **Loading** of the raw spectra produced by mass spectrometer,
2. **Preprocessing** of the raw spectra data,
3. **Preparation** of the data mining input file (i.e. the Weka ARFF file),
4. Data Mining **analysis** (i.e. classification) of mass spectra,
5. Knowledge Models **visualization** (e.g. decision tree)
   - represented in a standard language as PMML

M. Cannataro, C. Comito, A. Congiusta, P. Veltri, PROTEUS: a Bioinformatics Problem Solving Environment on Grids, *Parallel Processing Letters*, World Scientific, 2004.

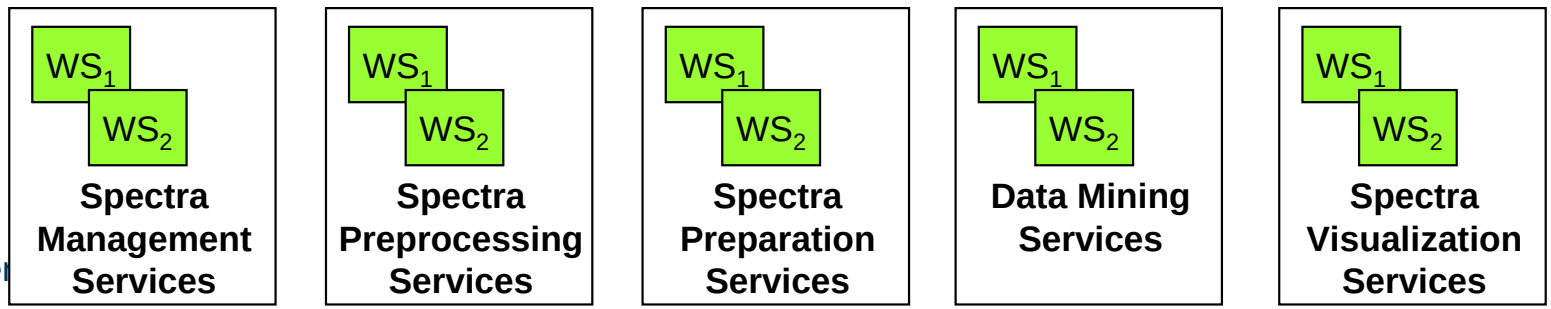
MS-Analyzer: a software platform supporting MS Analysis Workflows

- MS-Analyzer is a Grid-based Problem Solving Environment for the preprocessing, management and data mining analysis of MS data. It uses
  - domain ontologies to model
    - software tools (e.g. preprocessing and mining) and their relationships
    - data sources (e.g. MALDI-TOF, LC-MS/MS spectra datasets)
    - constraints (e.g. binning cannot be applied twice)
  - and workflow techniques to design complex in silico experiments.
- MS-Analyzer is able to:
  - interfacing mass spectrometers (i.e. spectra data sources)
  - acquiring, preprocessing and managing MS data on the Grid
  - offering composable filtering, preprocessing, and DM services
  - sharing experiments (data, workflows and knowledge)
- enabling a **Virtual Proteomics Laboratory**

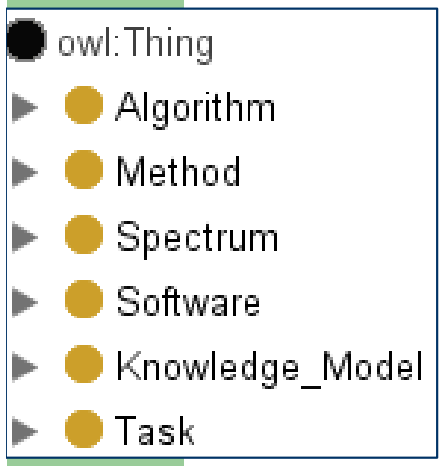
MS-Analyzer

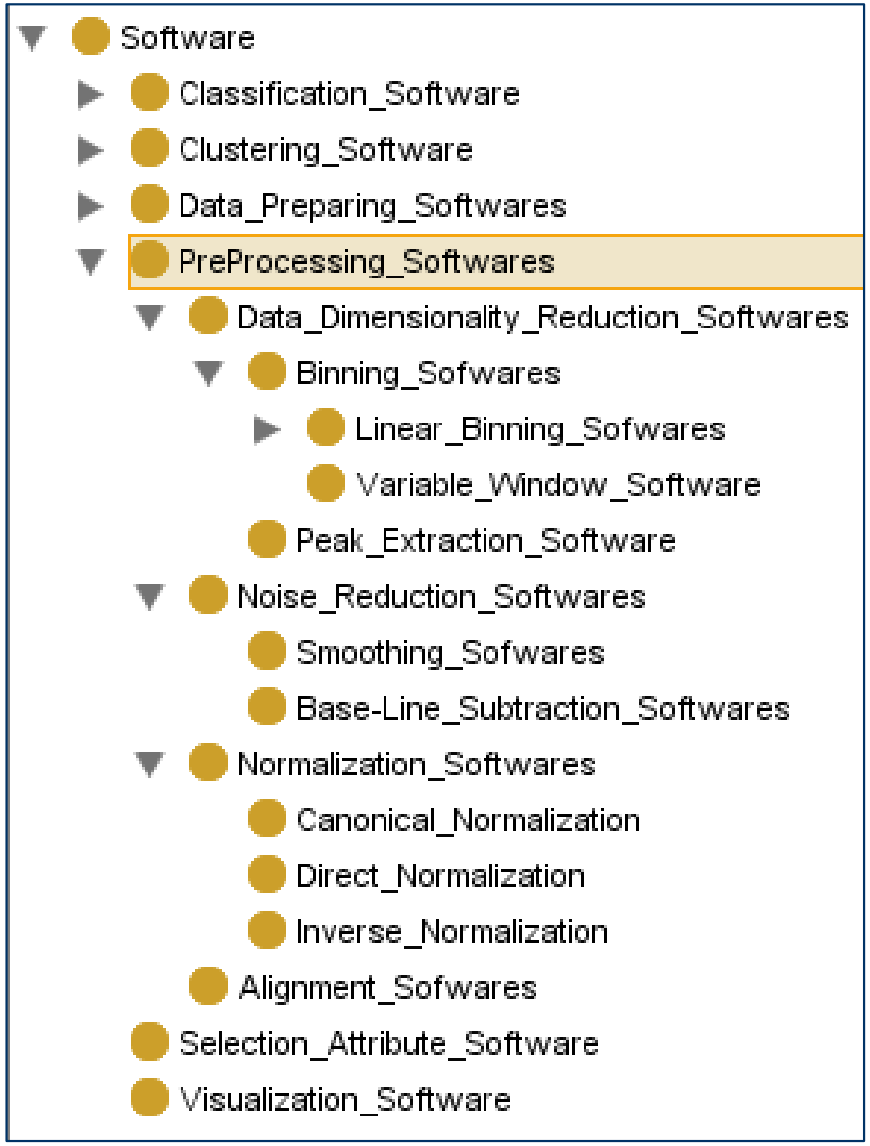

Ontology-based Workflow Designer & Scheduler
WF User Interface
ocomposition
obrowsing
oselection
ovisualization
Ontology Assistant
obrowsing
oquerying
Metadata & Ontology management
WF Schema & Application Metadata
SpecDB APIs
RSR
PSR
PPSR
raw MS spectra
pre-processed MS spectra
prepared pre-processed MS spectra
SpecDB Spectra Database (text, relational, XML-based data
Ontologies & Metadata (WekaOntology, ProteomicOntology)

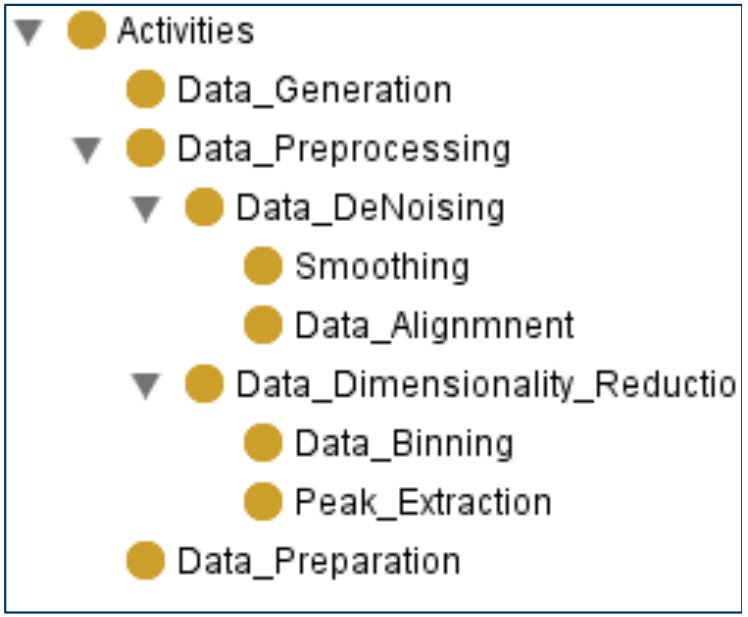

Grid Infrastructure (Globus)


WS1
WS2
Spectra Management Services
Spectra Preprocessing Services
Spectra Preparation Services
Data Mining Services
Spectra Visualization Services

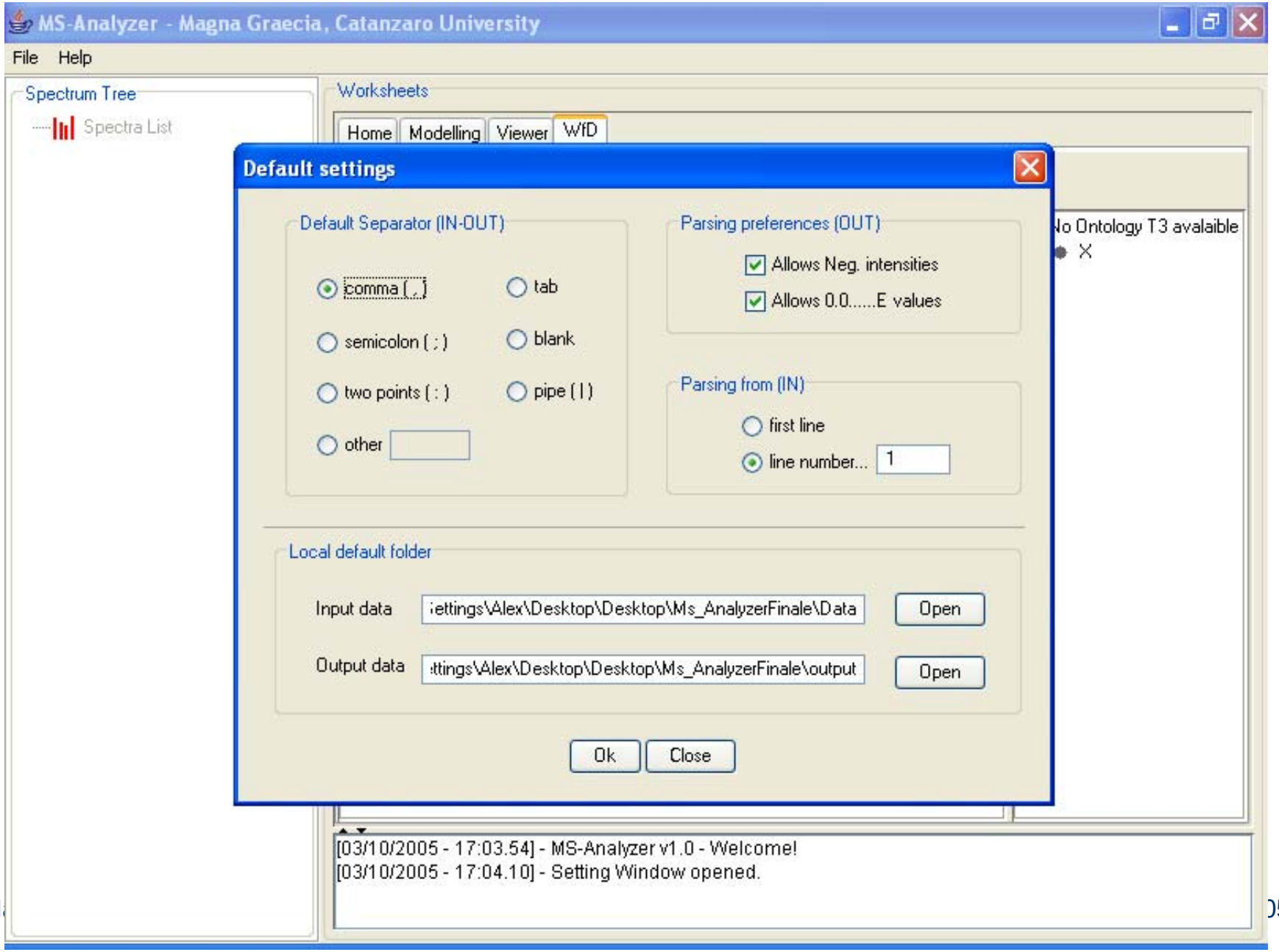
MS-Analyser Ontologies: WekaOntology & ProteomicOntology



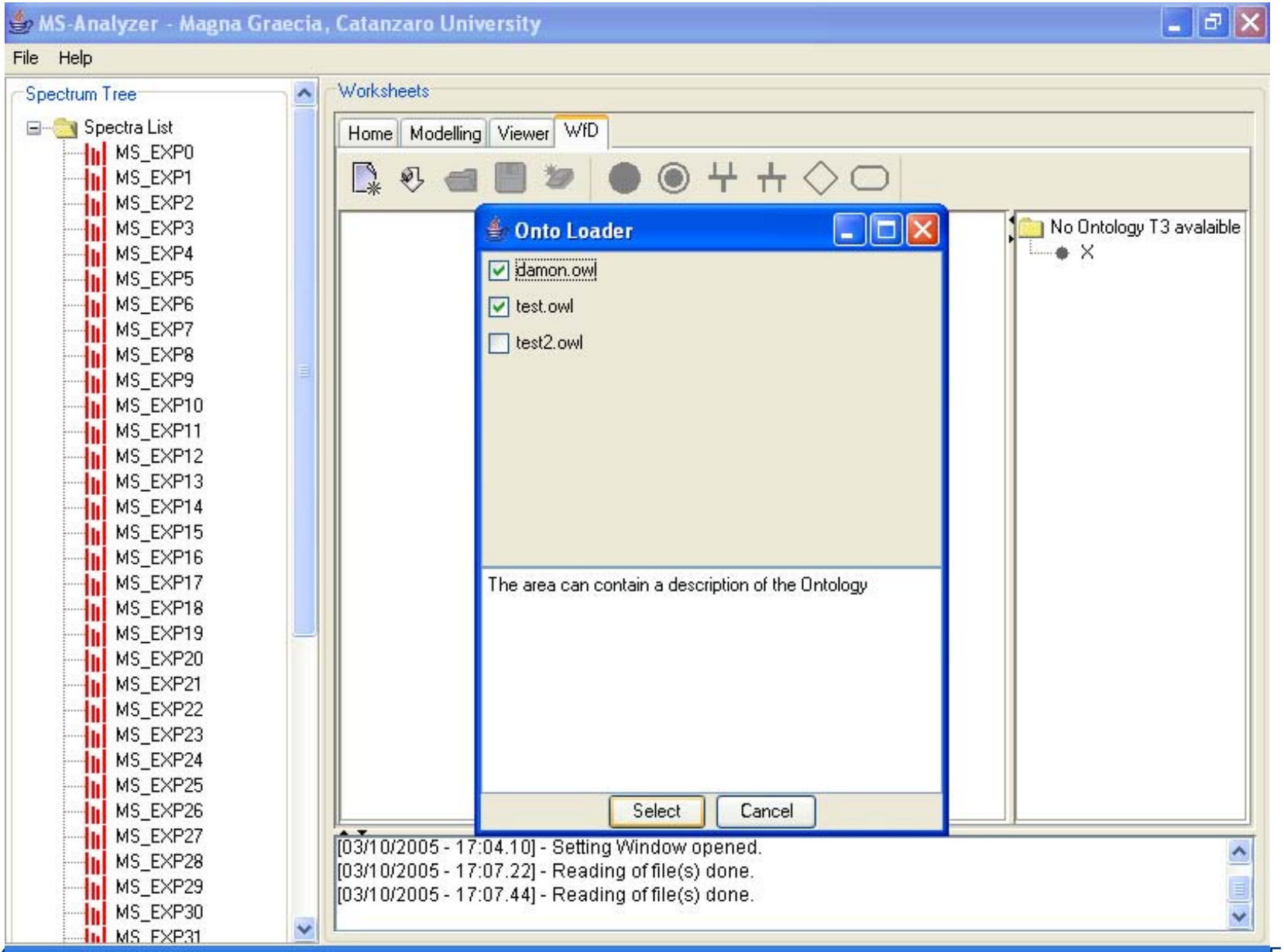




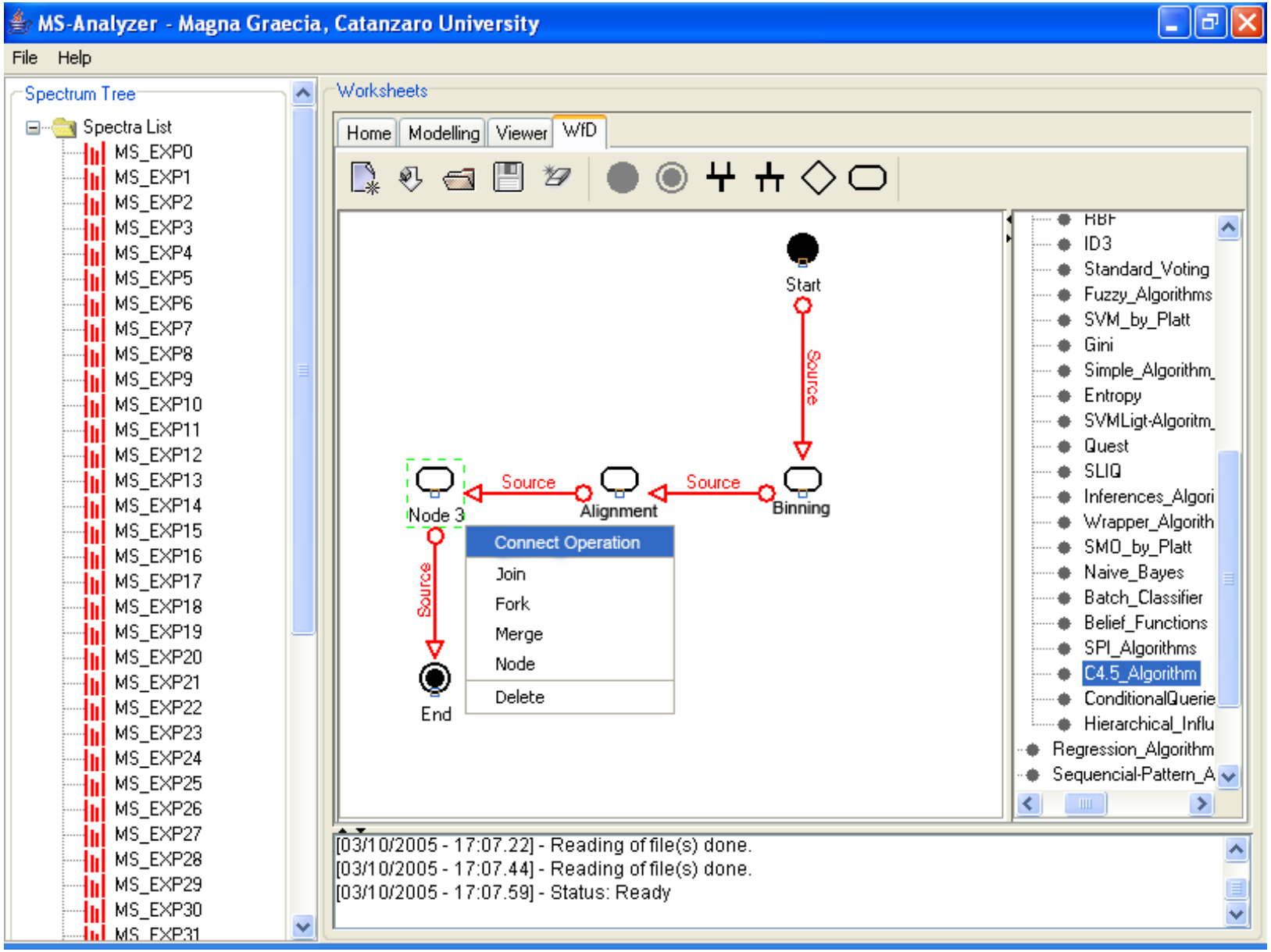
Loading Spectra in different Formats



Loading Domain Ontologies



Ontology-based Workflow Design

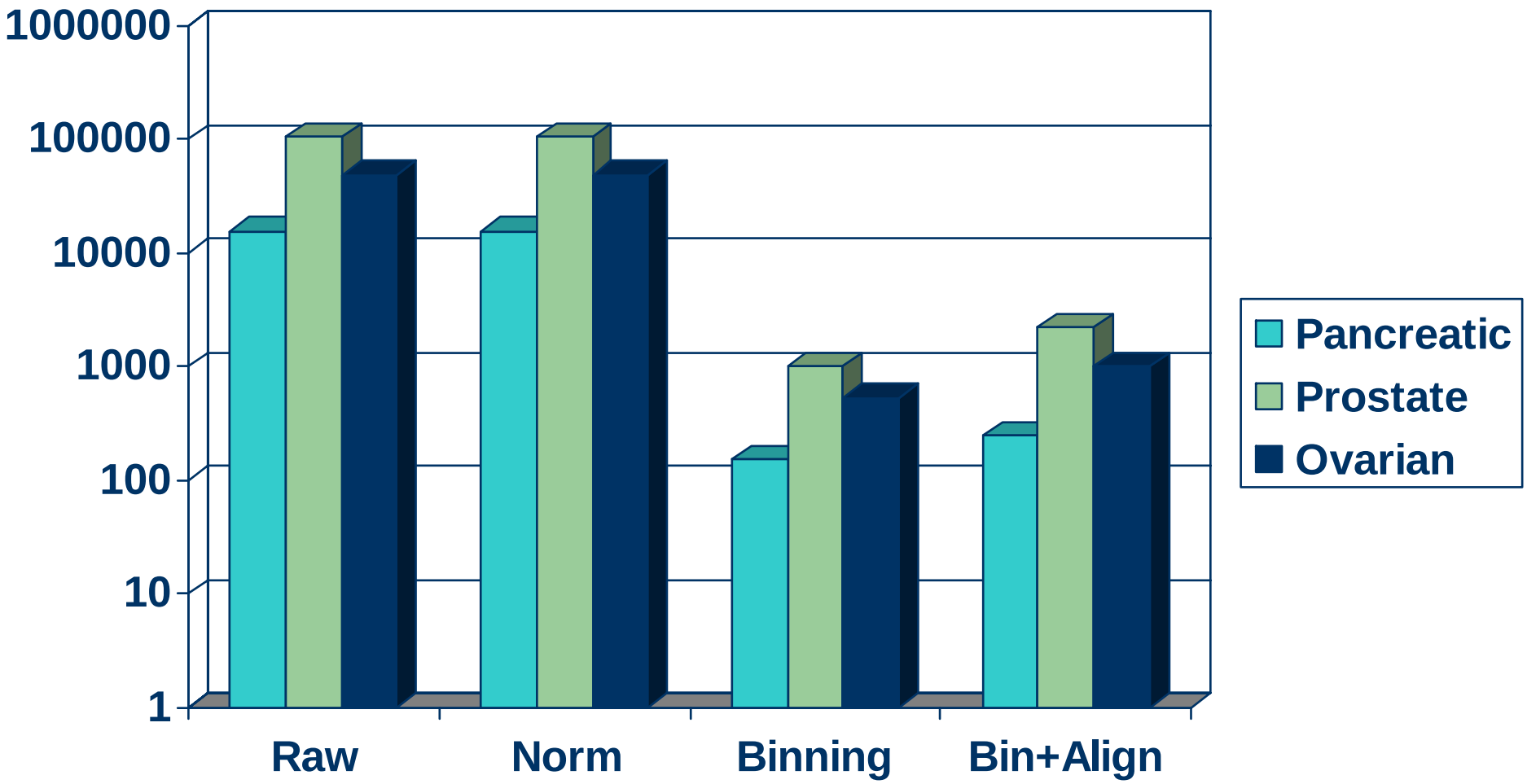


Performance evaluation of preprocessing techniques

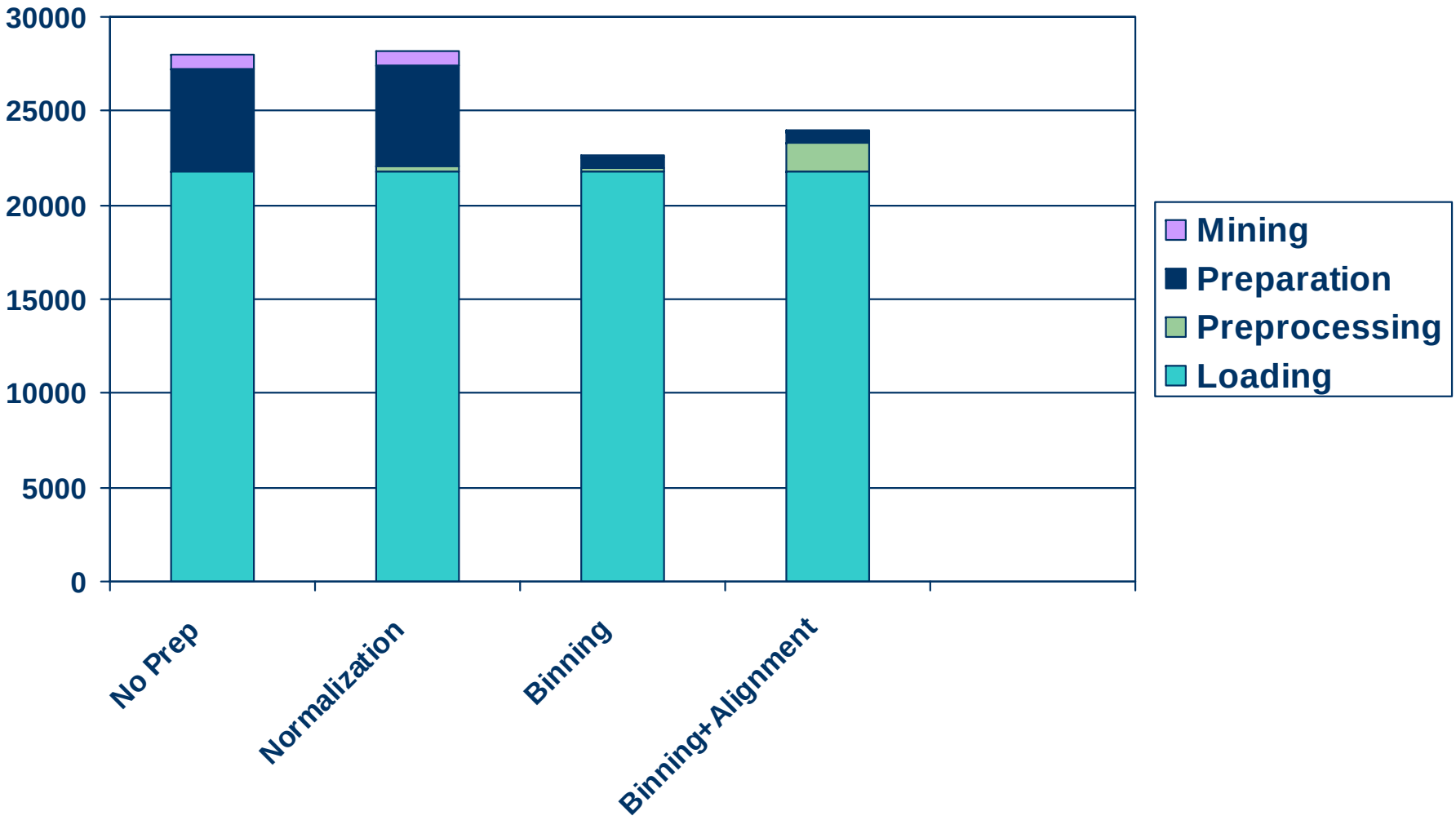
- We considered three different mass spectra datasets publicly available on Internet:
  - (i) *Pancreatic Cancer dataset*
    - 142 spectra each one with 6,772 (m/Z, intensity) measurements,
    - two classes: **healthy** and **diseased** patients;
  - (ii) *Prostate Cancer dataset*
    - 322 spectra each one with 15,154 measurements,
    - four classes: **no disease**, **benign cancer**, **pc410, pcg10** patients;
  - (iii) *Ovarian Cancer dataset*
    - 49 spectra each one with 59,386 measurements,
    - two classes, 25 **control** and 24 **disease**.

- We measured: execution times, memory occupancy, classification quality

Spectra size [Kbytes]



Execution time vs Preprocessing



Quality of classification

Table 7. Classification indexes with none pre-processing

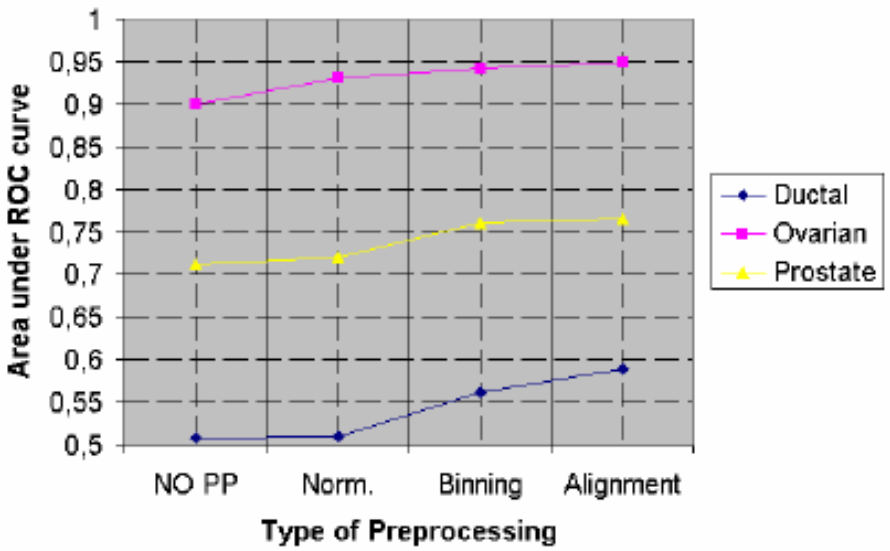
No Prep.	TP Rate	Precision	Recall
Pancreatic Cl1	0.513	0.5	0.513
Pancreatic Cl2	0.488	0.5	0.488
Prostate Cl1	0.774	0.786	0.774
Prostate Cl2	0.794	0.704	0.794
Prostate Cl3	0.269	0.269	0.269
Prostate Cl4	0.395	0.447	0.395
Ovarian Cl1	0.84	0.913	0.84
Ovarian Cl2	0.917	0.846	0.917

Table 9. Classification indexes with binning

Binning	TP Rate	Precision	Recall
Pancreatic Cl1	0.536	0.578	0.536
Pancreatic Cl2	0.614	0.573	0.614
Prostate Cl1	0.847	0.885	0.847
Prostate Cl2	0.825	0.813	0.825
Prostate Cl3	0.577	0.405	0.577
Prostate Cl4	0.558	0.615	0.558
Ovarian Cl1	0,849	0,913	0,845
Ovarian Cl2	0,934	0,944	0,917

Table 10. Classification indexes with binning and alignment

Bin.+Alig.	TP Rate	Precision	Recall
Pancreatic Cl1	0.613	0.563	0.613
Pancreatic Cl2	0.525	0.575	0.525
Prostate Cl1	0,937	0,922	0,937
Prostate Cl2	0,937	0,967	0,937
Prostate Cl3	0,577	0,556	0,577
Prostate Cl4	0,698	0,732	0,698
Ovarian Cl1	0.96	0.96	0.96
Ovarian Cl2	0,958	0,958	0,958



Conclusions and future work

- We surveyed some preprocessing techniques and presented a first prototype of MS-Analyzer
  - ontology-based workflow design simplifies workflow building and helps enforcing constraints
  - selective use of preprocessing techniques may improve execution times, memory occupancy and quality of data mining
- Future work will regard
  - the implementation of MS-Analyzer functions as Grid services and
  - the integration of a spectra relational database to allow “inside database” preprocessing

Questions?

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- MS-Analyzer (standalone version) can be downloaded at
  - www.icar.cnr.it/proteus