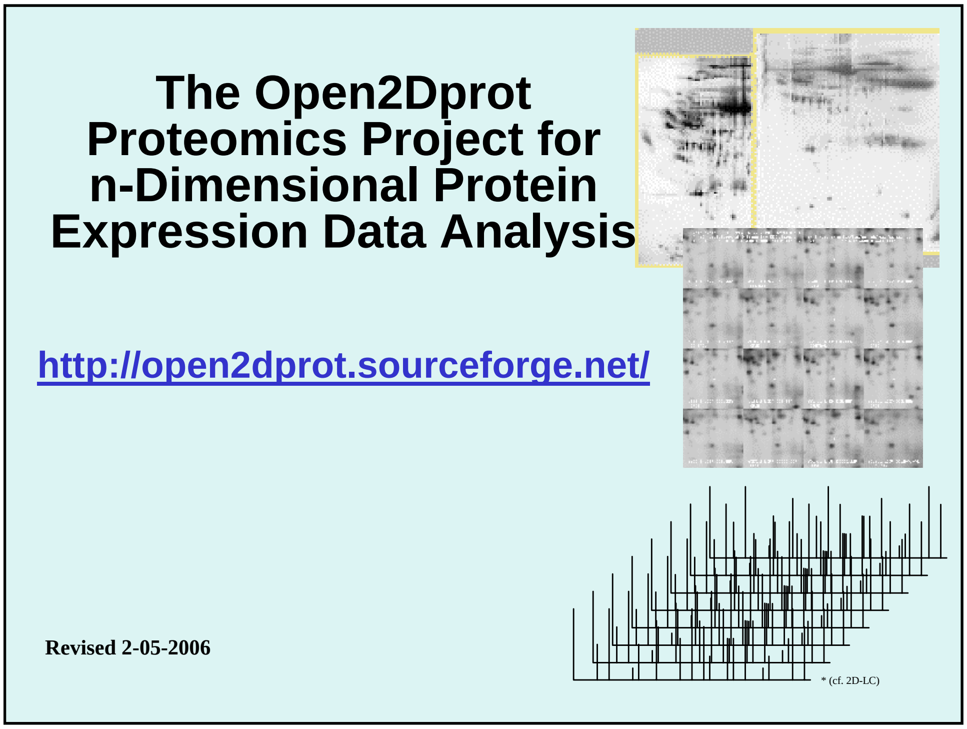
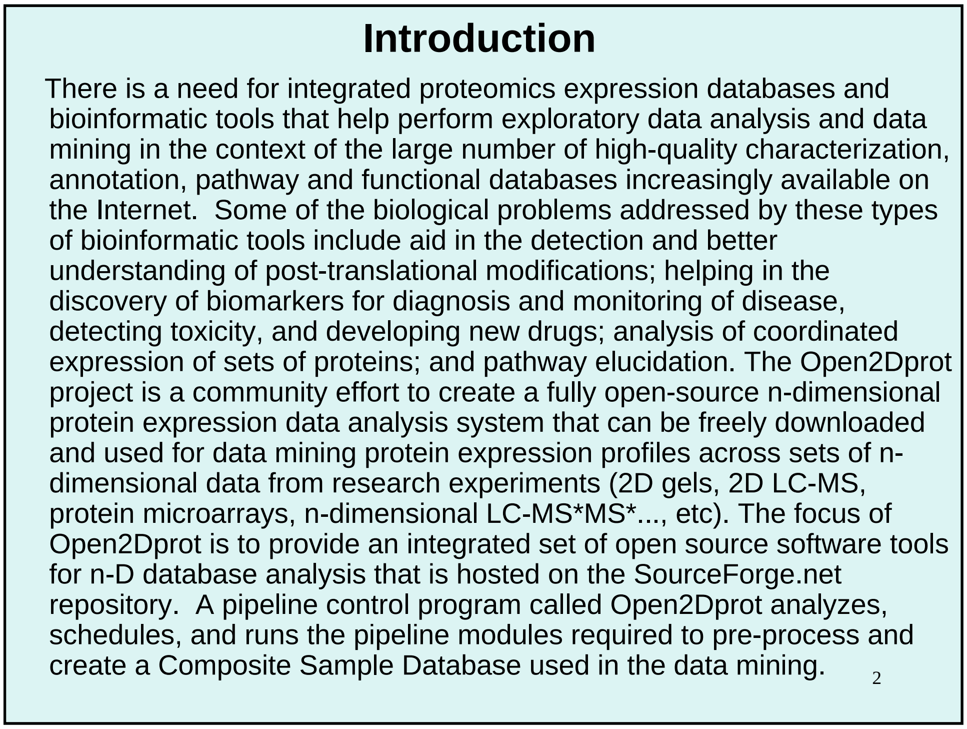


The Open2Dprot Proteomics Project for n-Dimensional Protein Expression Data Analysis

http://open2dprot.sourceforge.net/

Revised 2-05-2006

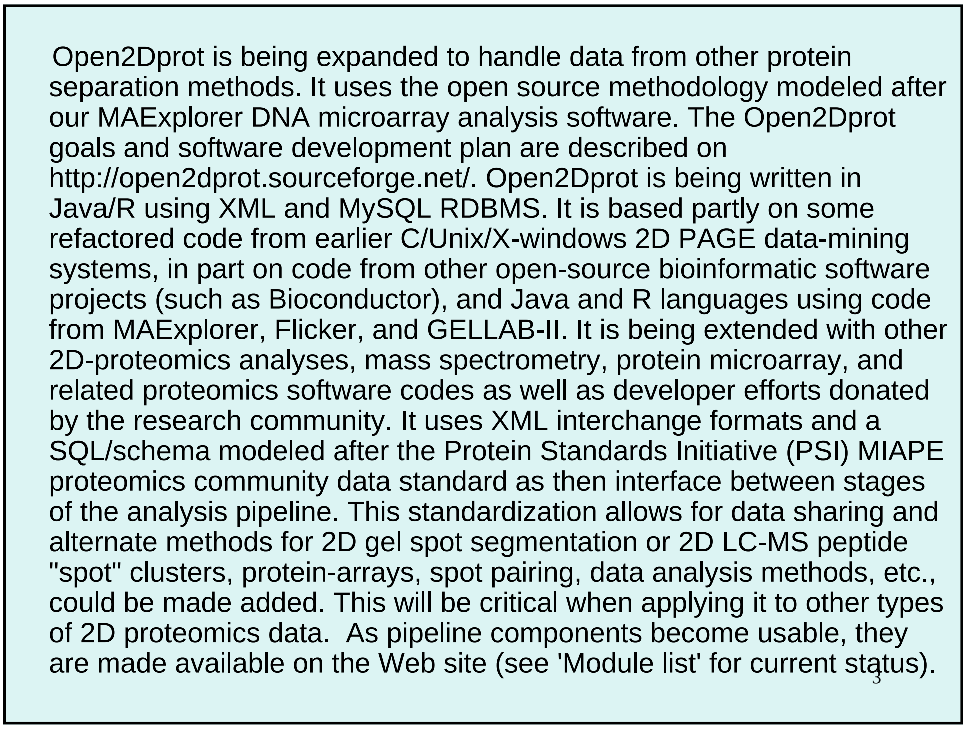
* (cf. 2D-LC)



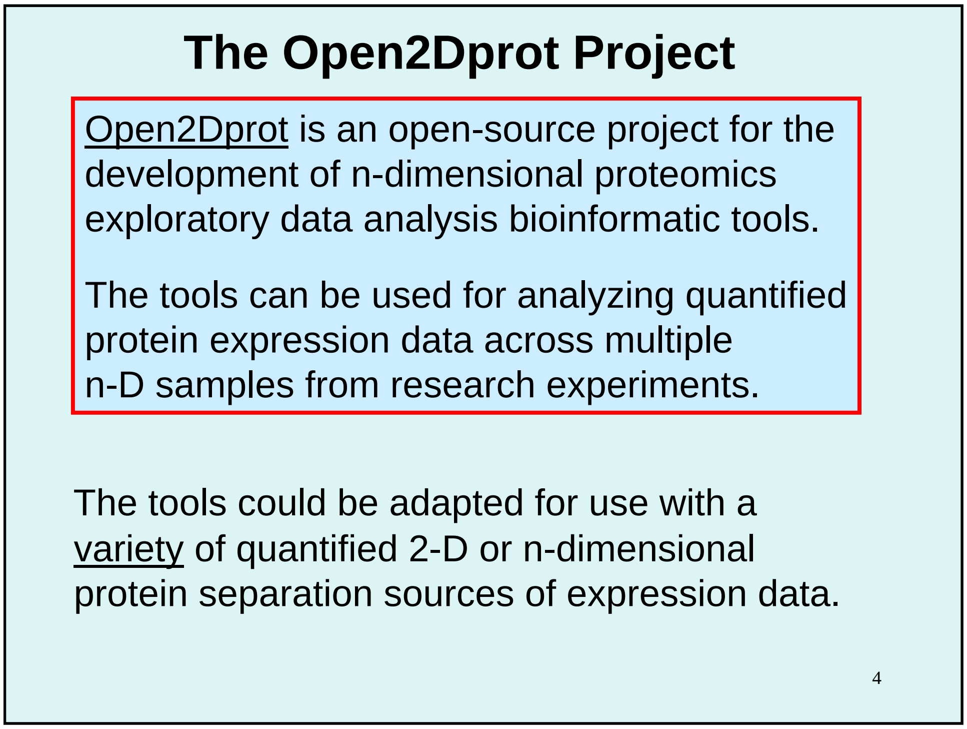
Introduction

There is a need for integrated proteomics expression databases and bioinformatic tools that help perform exploratory data analysis and data mining in the context of the large number of high-quality characterization, annotation, pathway and functional databases increasingly available on the Internet. Some of the biological problems addressed by these types of bioinformatic tools include aid in the detection and better understanding of post-translational modifications; helping in the discovery of biomarkers for diagnosis and monitoring of disease, detecting toxicity, and developing new drugs; analysis of coordinated expression of sets of proteins; and pathway elucidation. The Open2Dprot project is a community effort to create a fully open-source n-dimensional protein expression data analysis system that can be freely downloaded and used for data mining protein expression profiles across sets of n-dimensional data from research experiments (2D gels, 2D LC-MS, protein microarrays, n-dimensional LC-MS*MS*..., etc). The focus of Open2Dprot is to provide an integrated set of open source software tools for n-D database analysis that is hosted on the SourceForge.net repository. A pipeline control program called Open2Dprot analyzes, schedules, and runs the pipeline modules required to pre-process and create a Composite Sample Database used in the data mining.

2



Open2Dprot is being expanded to handle data from other protein separation methods. It uses the open source methodology modeled after our MAExplorer DNA microarray analysis software. The Open2Dprot goals and software development plan are described on http://open2dprot.sourceforge.net/. Open2Dprot is being written in Java/R using XML and MySQL RDBMS. It is based partly on some refactored code from earlier C/Unix/X-windows 2D PAGE data-mining systems, in part on code from other open-source bioinformatic software projects (such as Bioconductor), and Java and R languages using code from MAExplorer, Flicker, and GELLAB-II. It is being extended with other 2D-proteomics analyses, mass spectrometry, protein microarray, and related proteomics software codes as well as developer efforts donated by the research community. It uses XML interchange formats and a SQL/schema modeled after the Protein Standards Initiative (PSI) MIAPE proteomics community data standard as then interface between stages of the analysis pipeline. This standardization allows for data sharing and alternate methods for 2D gel spot segmentation or 2D LC-MS peptide "spot" clusters, protein-arrays, spot pairing, data analysis methods, etc., could be made added. This will be critical when applying it to other types of 2D proteomics data. As pipeline components become usable, they are made available on the Web site (see 'Module list' for current status).



The Open2Dprot Project

Open2Dprot is an open-source project for the development of n-dimensional proteomics exploratory data analysis bioinformatic tools.

The tools can be used for analyzing quantified protein expression data across multiple n-D samples from research experiments.

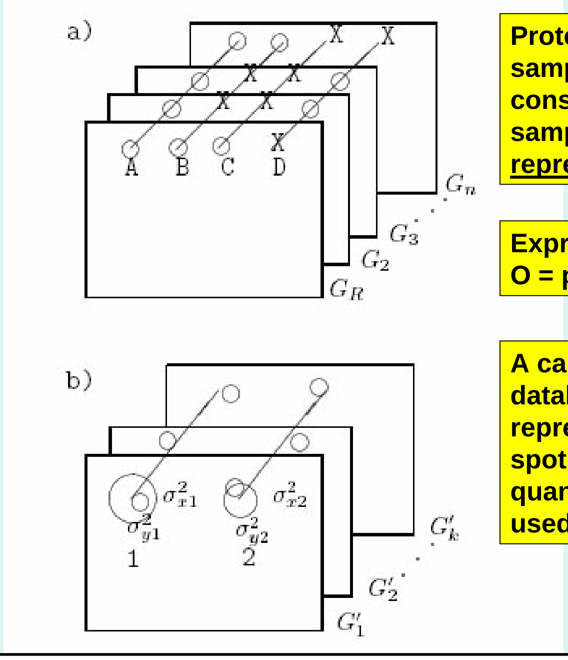
The tools could be adapted for use with a variety of quantified 2-D or n-dimensional protein separation sources of expression data.

Proteomic Separation Methods

- **2D-PAGE** (P. O'Farrell, 1975) pIe vs Mm (mass), 2D-gels
  2D LC-MS retention-times vs m/z (mass)
  2D IPG-MS pIe vs m/z (mass)
  2D LC-LC pIe vs RP-HPLC
  n-D (e.g., LC-MS*MS*MS …)
  Protein arrays (analytes vs antibodies**)**
- All share a common paradigm: proteins separated by orthogonal features
- Some of these methods are semi-quantitative
- Data represented as protein expression profiles lends itself to exploratory data analysis
- Open2Dprot could be used as part of a broader set of integrated tools

5

Composite Samples Database (CSD) Paradigm



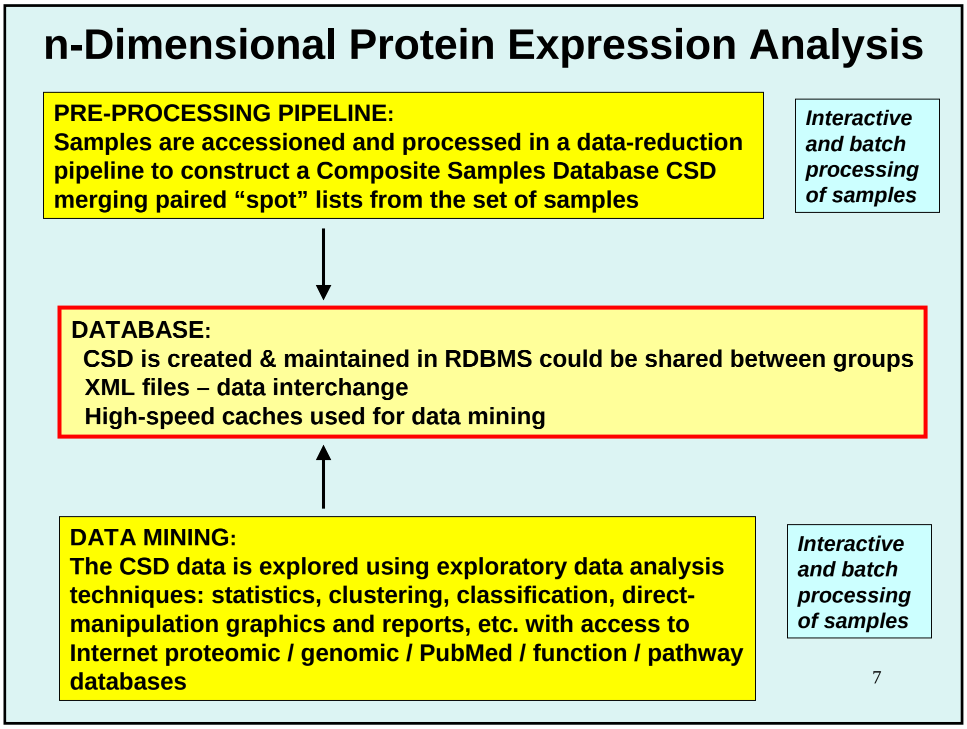
Proteomic composite samples database (CSD) consisting of a set of n samples G_1, G_2, …,G_n with representative sample $G_r = G_1$

Expression profiles A,B,C, ... O = present, X = missing

A canonical sample database is a statistical representation of the CSD spot geometry and quantification that could be used for data mining

Lemkin & Lester
***Clinical Chemistry*, 1982**

6



n-Dimensional Protein Expression Analysis

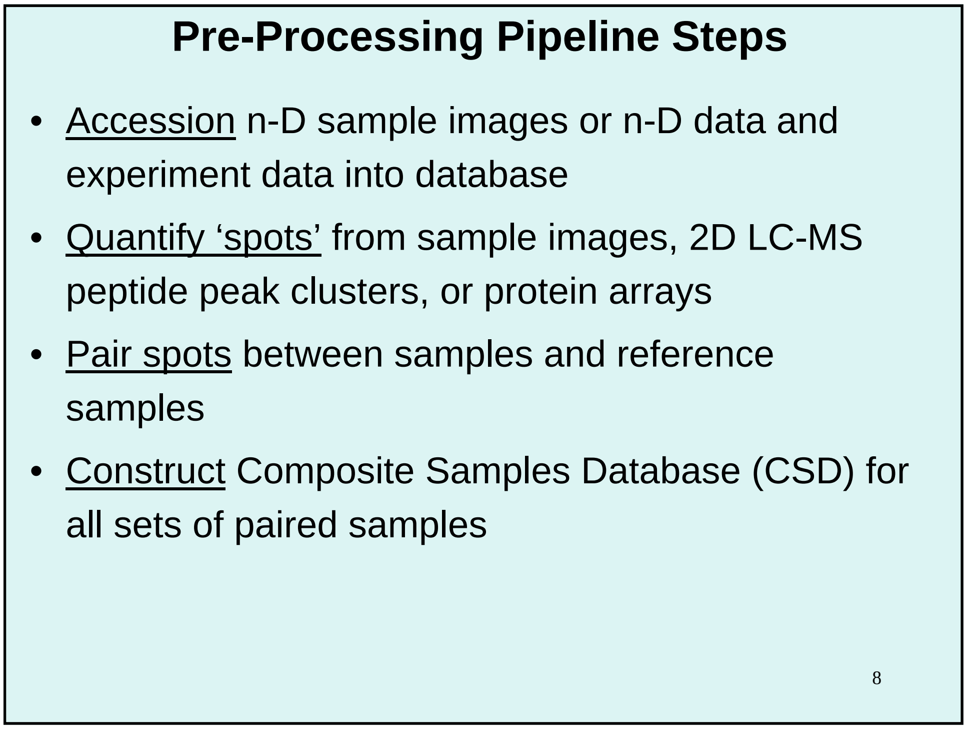
PRE-PROCESSING PIPELINE:
Samples are accessioned and processed in a data-reduction pipeline to construct a Composite Samples Database CSD merging paired "spot" lists from the set of samples

Interactive and batch processing of samples

DATABASE:
CSD is created & maintained in RDBMS could be shared between groups
XML files – data interchange
High-speed caches used for data mining

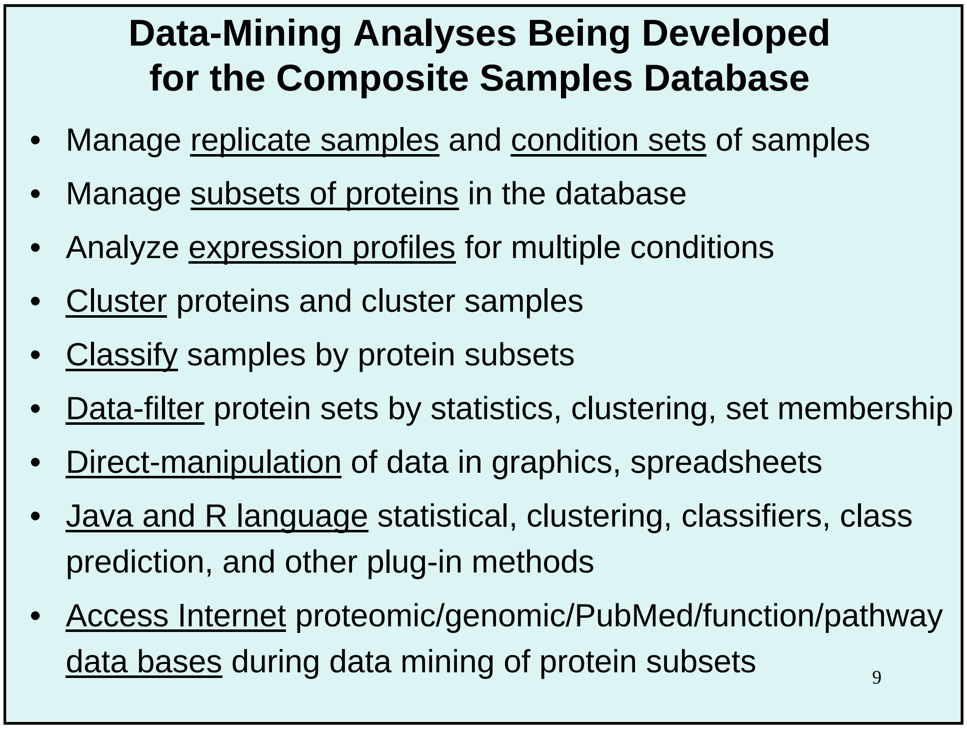
DATA MINING:
The CSD data is explored using exploratory data analysis techniques: statistics, clustering, classification, direct-manipulation graphics and reports, etc. with access to Internet proteomic / genomic / PubMed / function / pathway databases

Interactive and batch processing of samples



Pre-Processing Pipeline Steps

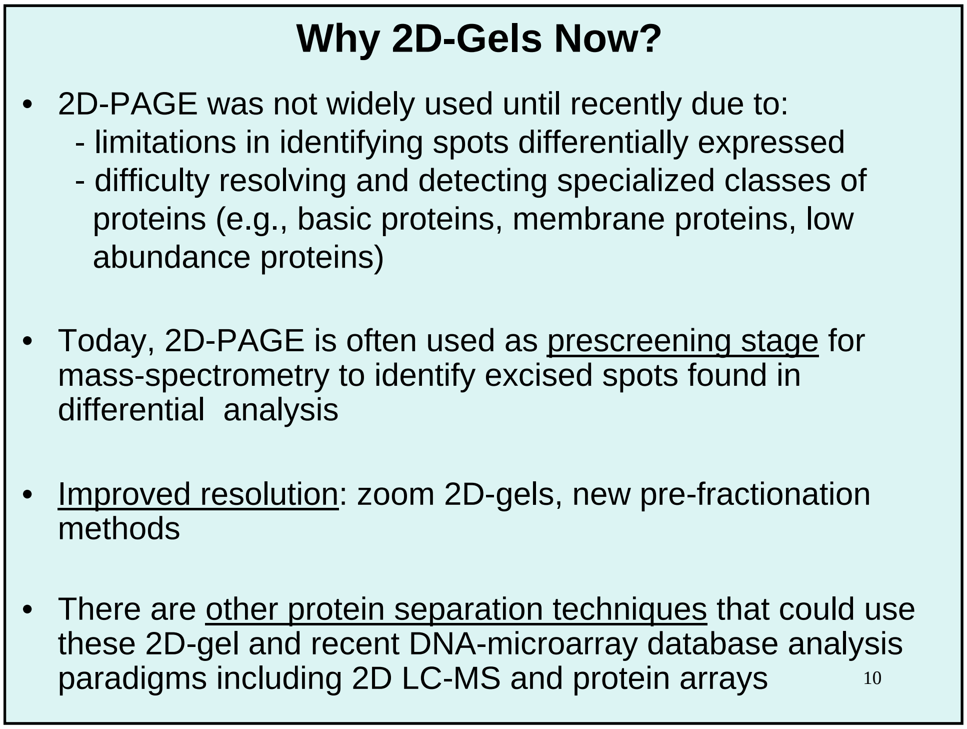
- Accession n-D sample images or n-D data and experiment data into database
- Quantify 'spots' from sample images, 2D LC-MS peptide peak clusters, or protein arrays
- Pair spots between samples and reference samples
- Construct Composite Samples Database (CSD) for all sets of paired samples



Data-Mining Analyses Being Developed for the Composite Samples Database

- Manage replicate samples and condition sets of samples
- Manage subsets of proteins in the database
- Analyze expression profiles for multiple conditions
- Cluster proteins and cluster samples
- Classify samples by protein subsets
- Data-filter protein sets by statistics, clustering, set membership
- Direct-manipulation of data in graphics, spreadsheets
- Java and R language statistical, clustering, classifiers, class prediction, and other plug-in methods
- Access Internet proteomic/genomic/PubMed/function/pathway data bases during data mining of protein subsets

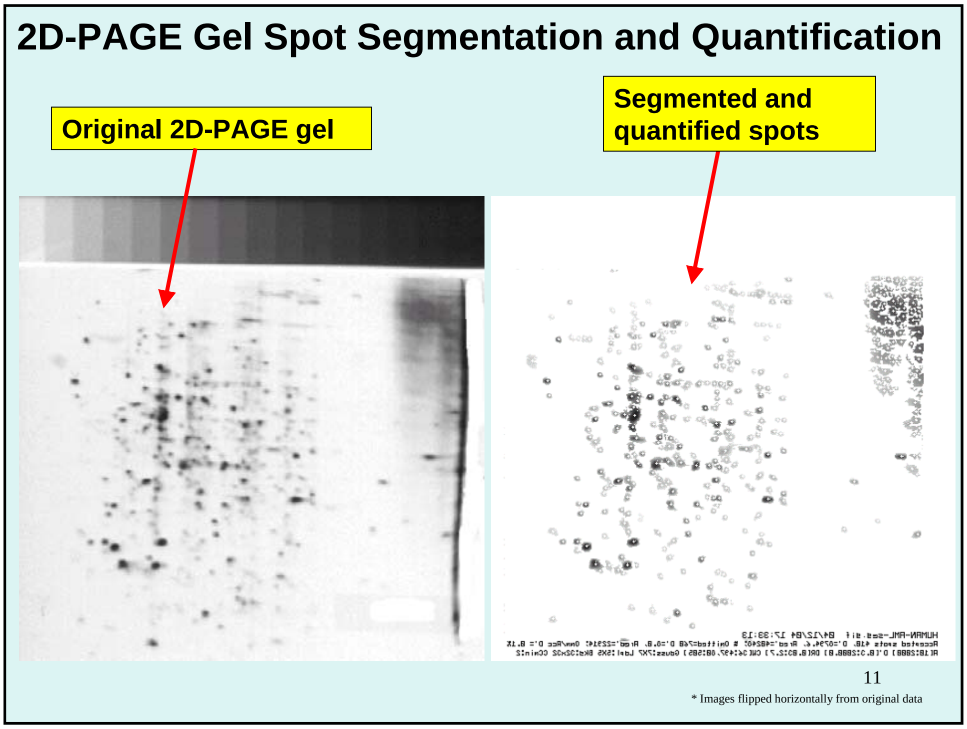
9



Why 2D-Gels Now?

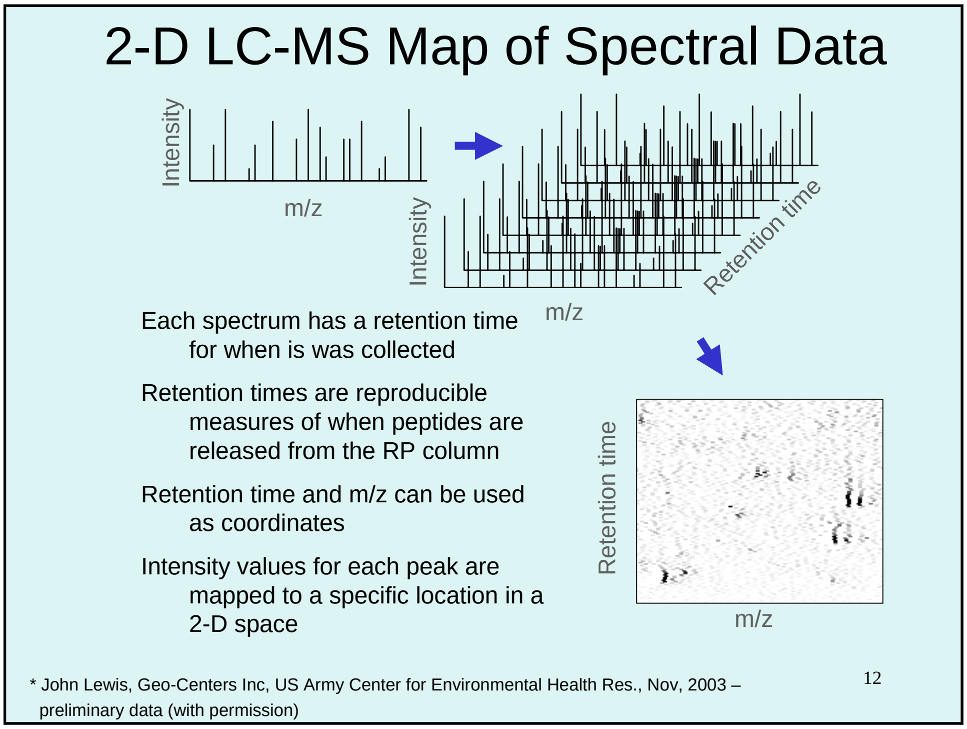
- 2D-PAGE was not widely used until recently due to:
  - limitations in identifying spots differentially expressed
  - difficulty resolving and detecting specialized classes of proteins (e.g., basic proteins, membrane proteins, low abundance proteins)
- Today, 2D-PAGE is often used as prescreening stage for mass-spectrometry to identify excised spots found in differential analysis
- Improved resolution: zoom 2D-gels, new pre-fractionation methods
- There are other protein separation techniques that could use these 2D-gel and recent DNA-microarray database analysis paradigms including 2D LC-MS and protein arrays

10



2D-PAGE Gel Spot Segmentation and Quantification

* Images flipped horizontally from original data



2-D LC-MS Map of Spectral Data

Each spectrum has a retention time for when is was collected

Retention times are reproducible measures of when peptides are released from the RP column

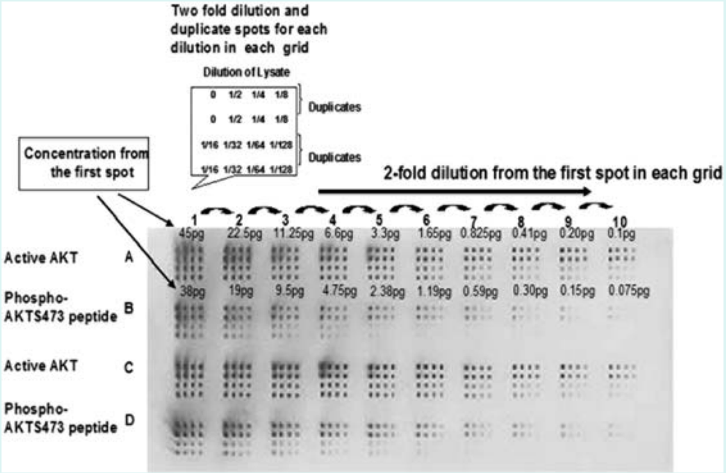
Retention time and m/z can be used as coordinates

Intensity values for each peak are mapped to a specific location in a 2-D space

* John Lewis, Geo-Centers Inc, US Army Center for Environmental Health Res., Nov, 2003 – preliminary data (with permission)

Protein Array

Example of sensitivity and reproducibility analysis of the reverse phase protein microarrays. From Sheehan, K. M. (2005) Mol. Cell. Proteomics 4: 346-355. With RP arrays, analytes are immobilized in solid-phase on the array.


Two fold dilution and duplicate spots for each dilution in each grid
Dilution of Lysate
0 1/2 1/4 1/8
0 1/2 1/4 1/8
1/16 1/32 1/64 1/128
1/16 1/32 1/64 1/128
Duplicates
Duplicates
Concentration from the first spot
2-fold dilution from the first spot in each grid
1 2 3 4 5 6 7 8 9 10
45pg 22.5pg 11.25pg 6.6pg 3.3pg 1.65pg 0.825pg 0.41pg 0.20pg 0.1pg
38pg 19pg 9.5pg 4.75pg 2.38pg 1.19pg 0.59pg 0.30pg 0.15pg 0.075pg
Active AKT A
Phospho-AKTS473 peptide B
Active AKT C
Phospho-AKTS473 peptide D

13

Why Open Source?

“The basic idea behind open source is very simple: When programmers can read, redistribute, and modify the source code for a piece of software, the software evolves. People improve it, people adapt it, people fix bugs. And this can happen at a speed that, if one is used to the slow pace of conventional software development, seems astonishing.”

“We in the open source community have learned that this rapid evolutionary process produces better software than the traditional closed model, in which only a very few programmers can see the source and everybody else must blindly use an opaque block of bits.”

From the Open Source Initiative (OSI)

http://www.opensource.org/

14

Why an Open-Source nD-Data Proteomics Effort?

- "***An open-source project can be advantageous to the community at large, since there is a far greater likelihood of progress in algorithm design in an academic style collaboration than a closed-source business model***."

- Researchers can more rapidly adapt new methods to existing software without waiting for release of commercial products

- Use contributed expertise and code of proteomics experts and bioinformaticians to help build and test open software

- Algorithms more transparent, so researchers can verify results more easily

- More opportunity to share data in standard non-proprietary formats

Why Open Source Proteomics? (continued)

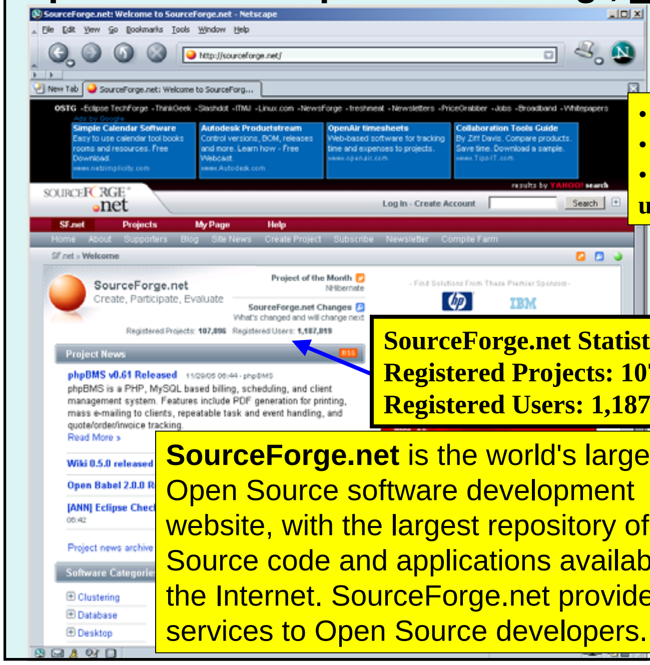
- No expensive software licenses required - reduces deployment costs within large organizations and small labs

- Using proper open-source licenses can encourage adoption and collaboration between industry, academic, and government interests (e.g., Linux, FireFox, Apache, Eclipse etc.)

- Many free open-source repositories available

- Repositories offer tools to support collaboration, software development, documentation, forums, and distribution

Open Source Repositories - E.g., SourceForge.Net


SourceForge.net: Welcome to SourceForge.net - Netscape
File Edit View Go Bookmarks Tools Window Help
http://sourceforge.net/
New Tab
SourceForge.net: Welcome to SourceForg...
OSTG -Eclipse TechForge -ThinkGeek -Slashdot -ITMJ -Linux.com -NewsForge -freshmeat -Newsletters -PriceGrabber -Jobs -Broadband -Whitepapers
Ads by Google
results by YAHOO! search
SOURCEFORGE.net
Log In - Create Account
Search
SF.net
Projects
My Page
Help
Home
About
Supporters
Blog
Site News
Create Project
Subscribe
Newsletter
Compile Farm
SF.net » Welcome
SourceForge.net
Create, Participate, Evaluate
Project of the Month
NHibernate
SourceForge.net Changes
What's changed and will change next
- Find Solutions From These Premier Sponsors -
hp
IBM
Project News
RSS
Software Categories
Clustering
Database
Desktop

Simple Calendar Software Easy to use calendar tool books rooms and resources. Free Download. www.netsimplicity.com

Autodesk Productstream Control versions, BOM, releases and more. Learn how - Free Webcast. www.Autodesk.com

OpenAir timesheets Web-based software for tracking time and expenses to projects. www.openair.com

Collaboration Tools Guide By Ziff Davis. Compare products. Save time. Download a sample. www.Tips-IT.com

Registered Projects: 107,096 Registered Users: 1,187,819

phpBMS v0.61 Released 11/29/05 06:44 - phpBMS
phpBMS is a PHP, MySQL based billing, scheduling, and client management system. Features include PDF generation for printing, mass e-mailing to clients, repeatable task and event handling, and quote/order/invoice tracking.
Read More »

Wiki 0.5.0 released

Open Babel 2.0.0 R

[ANN] Eclipse Chec
06:42

Project news archive

- Free code
- Repositories
- Developer, collaborator, user environments

SourceForge.net Statistics
Registered Projects: 107,096
Registered Users: 1,187,819

SourceForge.net is the world's largest Open Source software development website, with the largest repository of Open Source code and applications available on the Internet. SourceForge.net provides free services to Open Source developers.

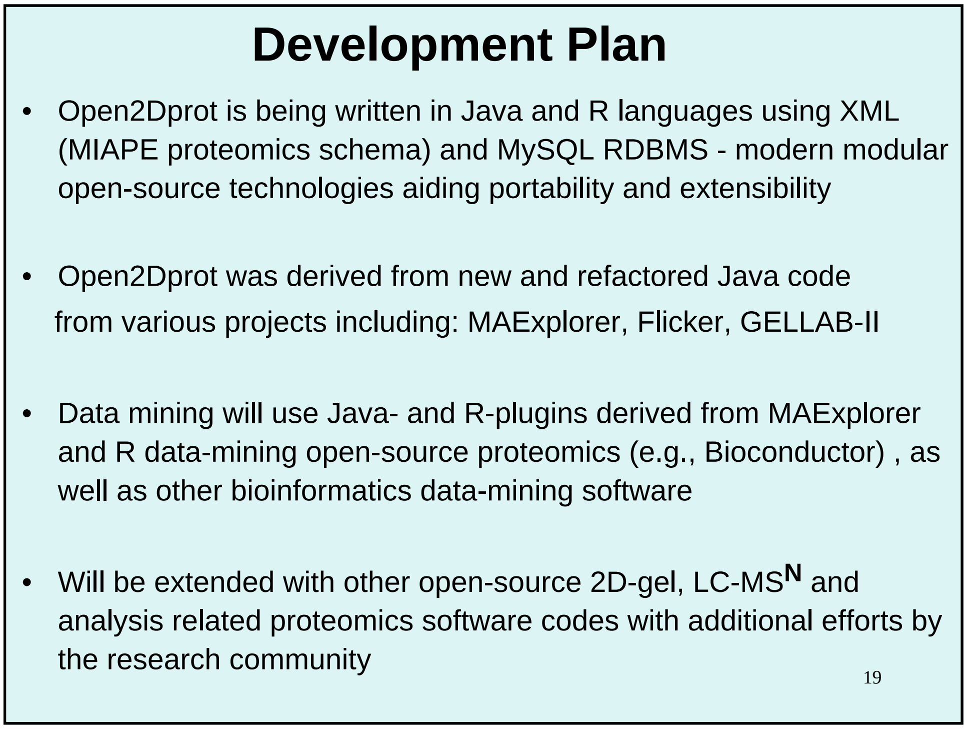
12-01-2005

17

Open2Dprot - Project Goals

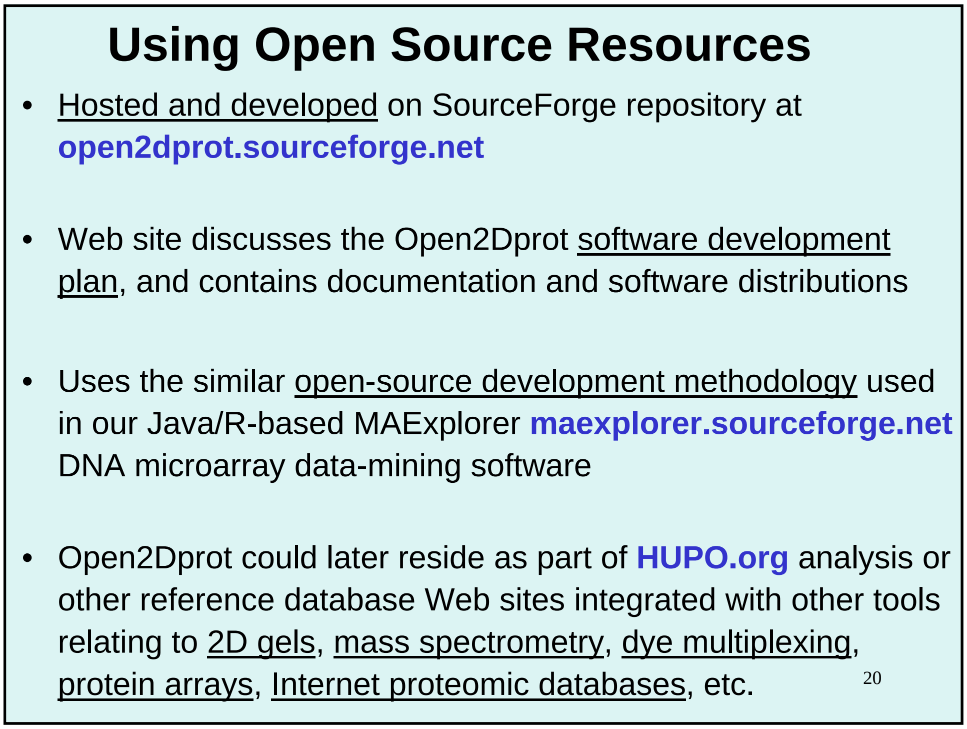
- An international community effort to create an open-source n-D quantitative data analysis system
- A stand-alone downloadable system that can connect to DBs
- Use for data mining protein expression of sets of samples from researcher's experiments to investigate and find significant protein expression differences from multiple experimental conditions
- Will provide integrated set of software tools, analysis methods and data structures for quantitative and system biology protein expression
- Will handle protein expression data from 2D-gel, 2D LC-MS, protein arrays, and other protein separation methods

18



Development Plan

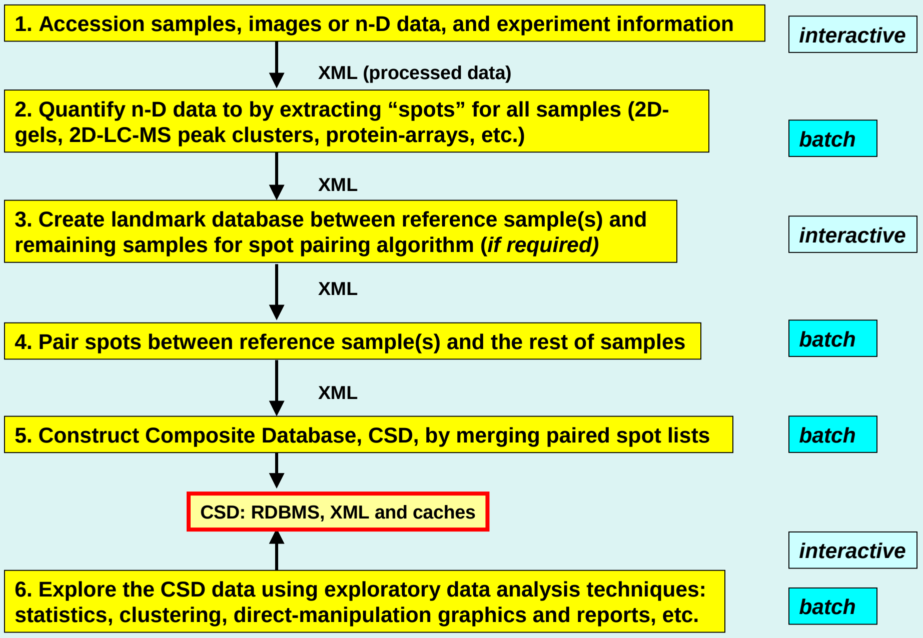
- Open2Dprot is being written in Java and R languages using XML (MIAPE proteomics schema) and MySQL RDBMS - modern modular open-source technologies aiding portability and extensibility
- Open2Dprot was derived from new and refactored Java code from various projects including: MAExplorer, Flicker, GELLAB-II
- Data mining will use Java- and R-plugins derived from MAExplorer and R data-mining open-source proteomics (e.g., Bioconductor) , as well as other bioinformatics data-mining software
- Will be extended with other open-source 2D-gel, LC-MS$^{\mathbf{N}}$ and analysis related proteomics software codes with additional efforts by the research community



Using Open Source Resources

- Hosted and developed on SourceForge repository at **open2dprot.sourceforge.net**
- Web site discusses the Open2Dprot software development plan, and contains documentation and software distributions
- Uses the similar open-source development methodology used in our Java/R-based MAExplorer **maexplorer.sourceforge.net** DNA microarray data-mining software
- Open2Dprot could later reside as part of **HUPO.org** analysis or other reference database Web sites integrated with other tools relating to 2D gels, mass spectrometry, dye multiplexing, protein arrays, Internet proteomic databases, etc.

n-D Protein Expression Analysis Pipeline



1. Accession samples, images or n-D data, and experiment information — *interactive*

XML (processed data)

2. Quantify n-D data to by extracting “spots” for all samples (2D-gels, 2D-LC-MS peak clusters, protein-arrays, etc.) — *batch*

XML

3. Create landmark database between reference sample(s) and remaining samples for spot pairing algorithm (*if required)* — *interactive*

XML

4. Pair spots between reference sample(s) and the rest of samples — *batch*

XML

5. Construct Composite Database, CSD, by merging paired spot lists — *batch*

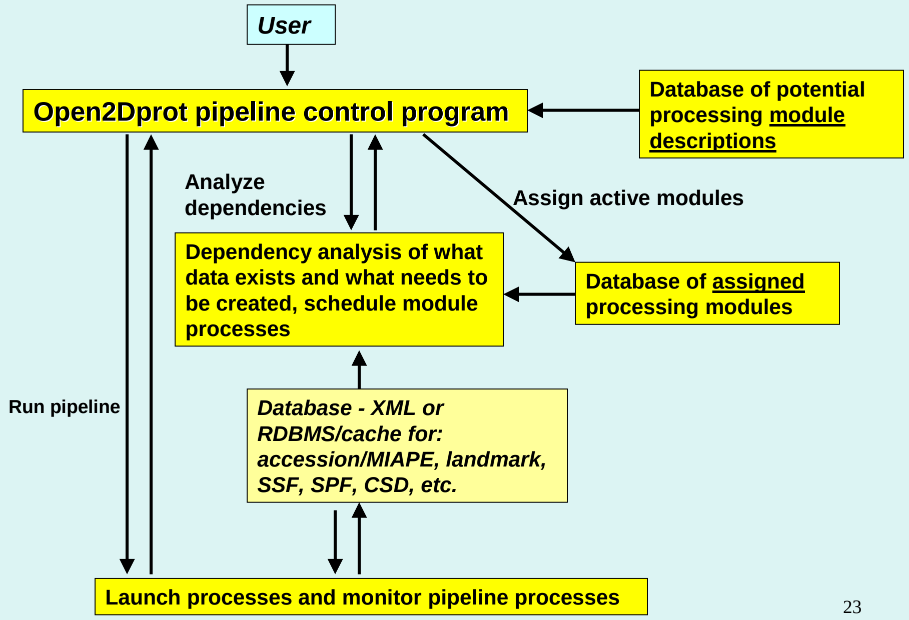
CSD: RDBMS, XML and caches

6. Explore the CSD data using exploratory data analysis techniques: statistics, clustering, direct-manipulation graphics and reports, etc. — *interactive* *batch*

Pipeline Control Program – Open2Dprot

- The pre-processing is controlled by the pipeline control program “Open2Dprot”
- Modules are assigned to Processing stages
- It determines what data exists, then from that dependency determines what data needs to be created from existing data, and creates the “target” data
- It then schedules and runs the required dynamically assigned modules in the pipeline to create the target data. Multiple processors could be used
- This is repeated until the desired data is created

Open2Dprot Pipeline Control Program



User

Open2Dprot pipeline control program

Database of potential processing <u>module descriptions</u>

Analyze dependencies

Assign active modules

Dependency analysis of what data exists and what needs to be created, schedule module processes

Database of <u>assigned</u> processing modules

Run pipeline

Database - XML or RDBMS/cache for: accession/MIAPE, landmark, SSF, SPF, CSD, etc.

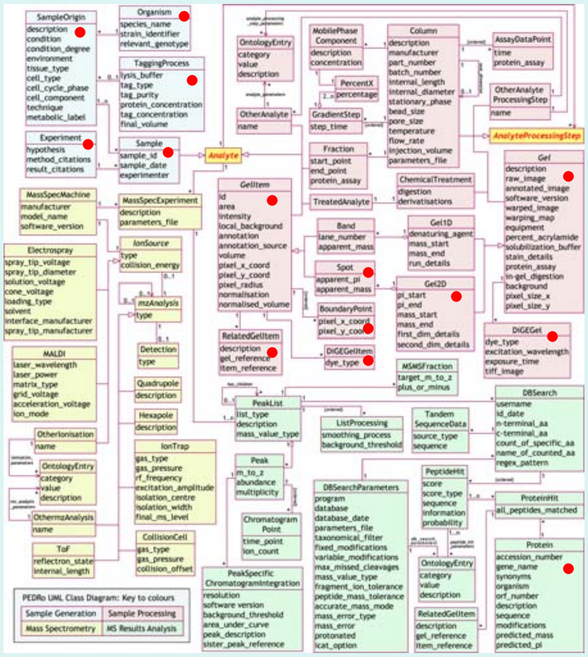
Launch processes and monitor pipeline processes

Data-Mining the Composite Sample Database

- The previous slide shows some of the types of tools that will be developed for Open2Dprot CSD data mining analysis as we have done previously for MAExplorer DNA microarray software using Java- and R-plugins

- In Open2Dprot, many of the R-plugins will use methods developed for or derived from Bioconductor (see bioconductor.org, DNA microarray analysis system written in the R language, r-project.org)

Early MIAPE (PEDRo) UML Schema n-D Data



- Classes that could be used with 2D-gels

Additional fields / classes are needed for Open2Dprot

in Taylor et.al., *Nature Biotechnology*, March 2003.

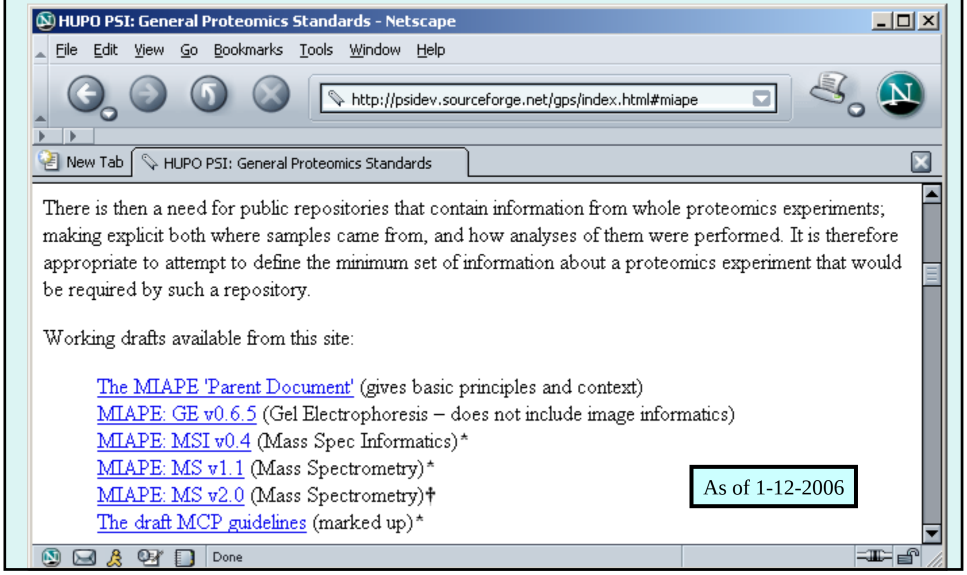
PEDRo has been renamed MIAPE "Minimal Information About a Proteomics Experiment" (Oct. 2003, HUPO-II) by EMBL-EBI

See psidev.sf.net

25

MIAPE –
Minimal Information About a Proteomics Experiment

psidev.sourceforge.net/gps/#miape


HUPO PSI: General Proteomics Standards - Netscape
File
Edit
View
Go
Bookmarks
Tools
Window
Help
http://psidev.sourceforge.net/gps/index.html#miape
New Tab
HUPO PSI: General Proteomics Standards
Done

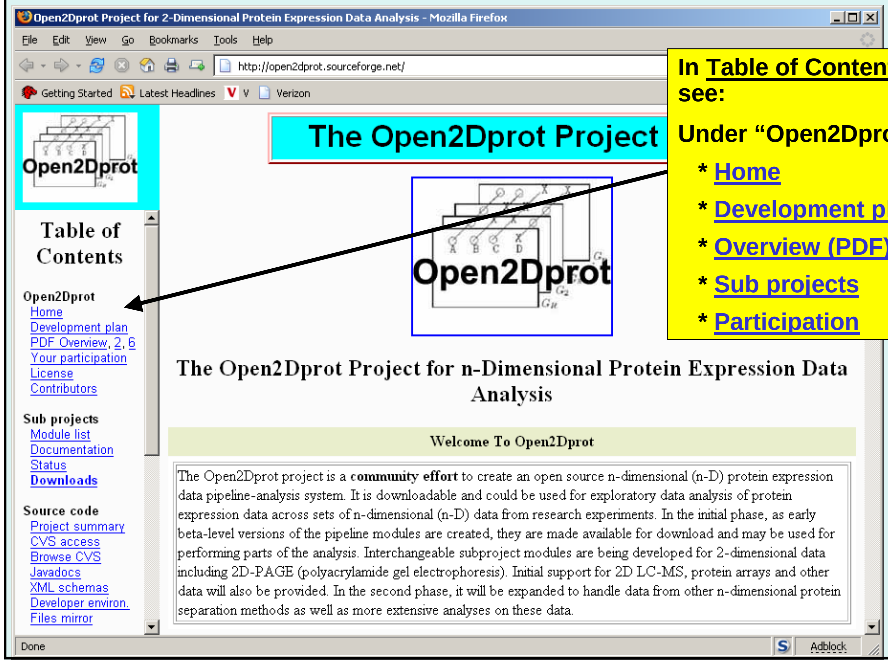
There is then a need for public repositories that contain information from whole proteomics experiments; making explicit both where samples came from, and how analyses of them were performed. It is therefore appropriate to attempt to define the minimum set of information about a proteomics experiment that would be required by such a repository.

Working drafts available from this site:

The MIAPE 'Parent Document' (gives basic principles and context)
MIAPE: GE v0.6.5 (Gel Electrophoresis – does not include image informatics)
MIAPE: MSI v0.4 (Mass Spec Informatics)*
MIAPE: MS v1.1 (Mass Spectrometry)*
MIAPE: MS v2.0 (Mass Spectrometry)†
The draft MCP guidelines (marked up)*

As of 1-12-2006

Home: http://open2dprot.sourceforge.net/



The Open2Dprot Project for n-Dimensional Protein Expression Data Analysis

Welcome To Open2Dprot

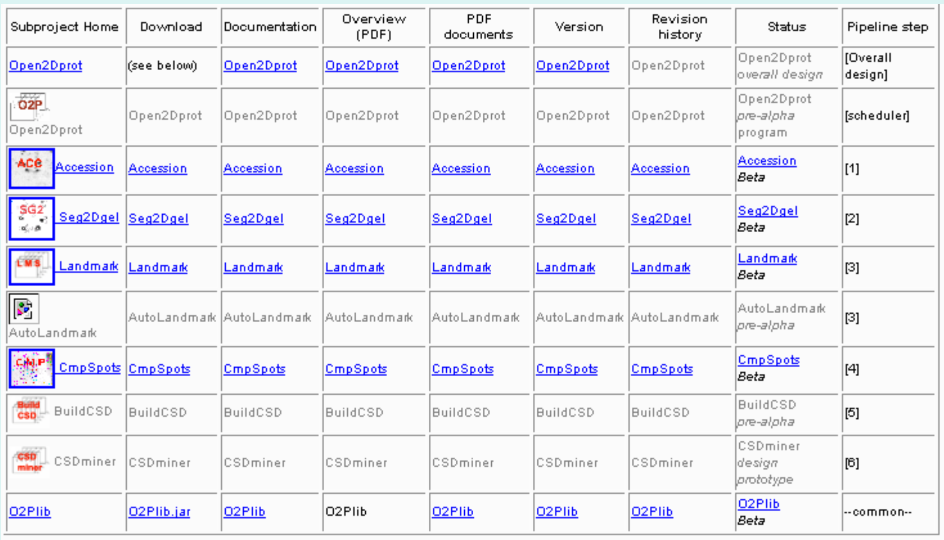
The Open2Dprot project is a **community effort** to create an open source n-dimensional (n-D) protein expression data pipeline-analysis system. It is downloadable and could be used for exploratory data analysis of protein expression data across sets of n-dimensional (n-D) data from research experiments. In the initial phase, as early beta-level versions of the pipeline modules are created, they are made available for download and may be used for performing parts of the analysis. Interchangeable subproject modules are being developed for 2-dimensional data including 2D-PAGE (polyacrylamide gel electrophoresis). Initial support for 2D LC-MS, protein arrays and other data will also be provided. In the second phase, it will be expanded to handle data from other n-dimensional protein separation methods as well as more extensive analyses on these data.

In Table of Contents, see:

Under "Open2Dprot"

- *** Home**
- *** Development plan**
- *** Overview (PDF)**
- *** Sub projects**
- *** Participation**

Open2Dprot Pipeline Subprojects - Status



Subproject Home	Download	Documentation	Overview (PDF)	PDF documents	Version	Revision history	Status	Pipeline step
Open2Dprot	(see below)	Open2Dprot	Open2Dprot	Open2Dprot	Open2Dprot	Open2Dprot	Open2Dprot *overall design*	[Overall design]
Open2Dprot	Open2Dprot	Open2Dprot	Open2Dprot	Open2Dprot	Open2Dprot	Open2Dprot	Open2Dprot *pre-alpha* program	[scheduler]
Accession	Accession	Accession	Accession	Accession	Accession	Accession	Accession *Beta*	[1]
Seg2Dgel	Seg2Dgel	Seg2Dgel	Seg2Dgel	Seg2Dgel	Seg2Dgel	Seg2Dgel	Seg2Dgel *Beta*	[2]
Landmark	Landmark	Landmark	Landmark	Landmark	Landmark	Landmark	Landmark *Beta*	[3]
AutoLandmark	AutoLandmark	AutoLandmark	AutoLandmark	AutoLandmark	AutoLandmark	AutoLandmark	AutoLandmark *pre-alpha*	[3]
CmpSpots	CmpSpots	CmpSpots	CmpSpots	CmpSpots	CmpSpots	CmpSpots	CmpSpots *Beta*	[4]
BuildCSD	BuildCSD	BuildCSD	BuildCSD	BuildCSD	BuildCSD	BuildCSD	BuildCSD *pre-alpha*	[5]
CSDminer	CSDminer	CSDminer	CSDminer	CSDminer	CSDminer	CSDminer	CSDminer *design prototype*	[6]
O2Plib	O2Plib.jar	O2Plib	O2Plib	O2Plib	O2Plib	O2Plib	O2Plib *Beta*	--common--

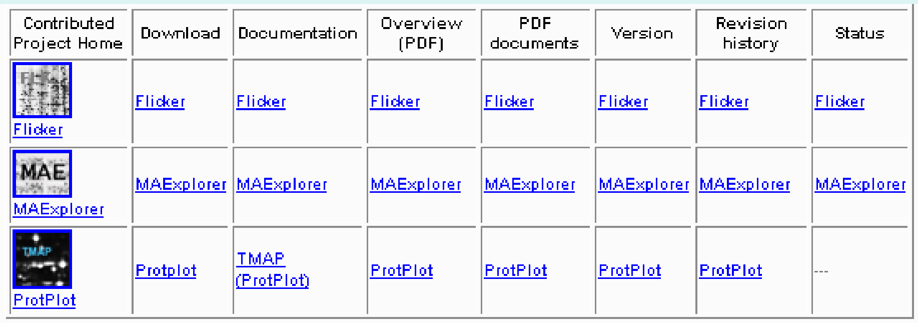
Additional alternative modules are being developed for all pipeline stages

01-12-2006

28

Contributed Associated or Related Projects

We added some additional non-pipeline open source projects that may use similar data or common software modules. They may be useful for performing other types of analysis on data used by Open2Dprot or provide other types of analyses.



Contributed Project Home	Download	Documentation	Overview (PDF)	PDF documents	Version	Revision history	Status
Flicker	Flicker	Flicker	Flicker	Flicker	Flicker	Flicker	Flicker
MAExplorer	MAExplorer	MAExplorer	MAExplorer	MAExplorer	MAExplorer	MAExplorer	MAExplorer
ProtPlot	Protplot	TMAP (ProtPlot)	ProtPlot	ProtPlot	ProtPlot	ProtPlot	---

01-12-2006

Summary of Open2Dprot

- Open2Dprot is a fully open-source n-D proteomics data-mining project for a variety of proteomic expression data sources and is being developed at **http://open2dprot.sourceforge.net/**
- It has a flexible pipeline-modules design using XML data interchange and /RDBMS-caches and portable Java and R using existing code where possible
- As parts of the project pipeline become usable, they are being released as stand-alone programs