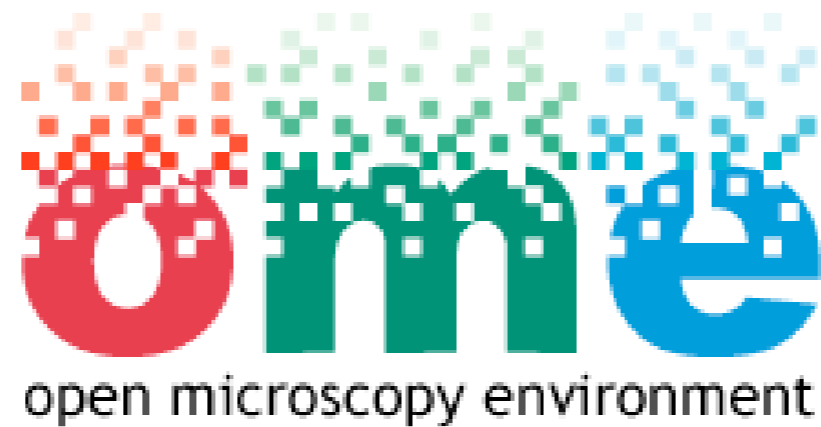


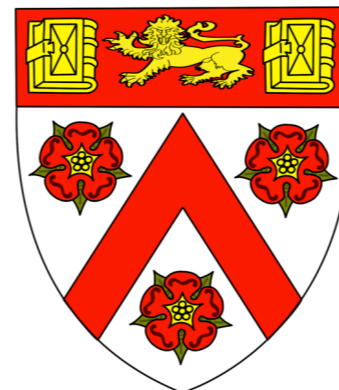
Pattern analysis of mouse liver: Morphological effects of gender, aging and diet

(doing Science with OME)



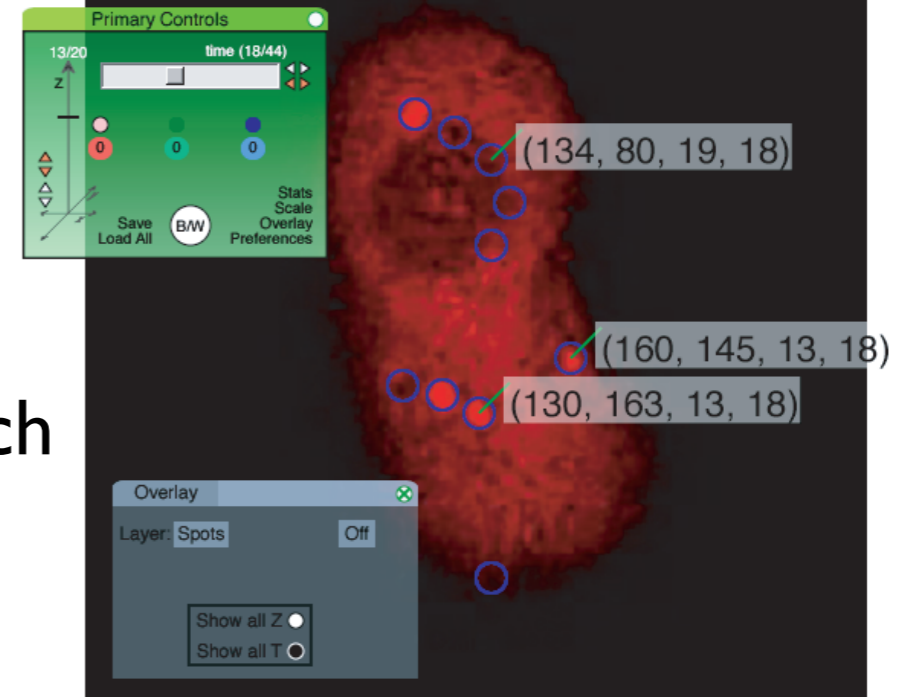
Tom Macura

Image Informatics and Computational Biology Unit



OME for Image Management

OME is a centralized image management solution enabling scientists to store, view, annotate, and search their and their collaborators' microscopy images.



Search for Image

Dataset: Worms

Welcome Ilya Goldberg
Most recent project: Demos (Popup)
Most recent dataset: Test Images (Popup)

Home -> Analysis -> Image

Search

Look for: Image

That match criteria: Created

Description

Group: All

Name: %day4%

Owner: All

Display Results as: Summaries

Results:	Results:	Results:
day4.n2._1_.30.a Harry Hochheiser OME 2004-07-24 21:10:49	day4.n2._1_.30.head Harry Hochheiser OME 2004-07-24 21:11:01	day4.n2._1_.31.a Harry Hochheiser OME 2004-07-24 21:11:02
day4.n2._1_.31.head Harry Hochheiser OME 2004-07-24 21:11:04	day4.n2._1_.32.a Harry Hochheiser OME 2004-07-24 21:11:05	day4.n2._1_.32.head Harry Hochheiser OME 2004-07-24 21:11:07
day4.n2._1_.33.a Harry Hochheiser OME 2004-07-24 21:11:09	day4.n2._1_.33.b Harry Hochheiser OME 2004-07-24 21:11:10	day4.n2._1_.33.c Harry Hochheiser OME 2004-07-24 21:11:12
day4.n2._1_.33.head Harry Hochheiser OME 2004-07-24 21:11:13	day4.n2._1_.34.a Harry Hochheiser OME 2004-07-24 21:11:14	day4.n2._1_.34.b Harry Hochheiser OME 2004-07-24 21:11:16
day4.n2._1_.34.c Harry Hochheiser OME 2004-07-24 21:11:17	day4.n2._1_.34.head Harry Hochheiser OME 2004-07-24 21:11:19	day4.n2._1_.35.a Harry Hochheiser OME 2004-07-24 21:11:20

Dataset: All Worms

Welcome Tom Macura
Most recent project: Nameless Project (Popup)
Most recent dataset: All Worms (Popup)

Home -> Dataset Detail

Dataset: All Worms

Id: 17, Owner: Tom Macura, Group: OME, Locked: False

Name: All Worms

Description [Save]
Dataset of Day1, Day2, Day4, Day6 and Day8 worms.

Your Current Annotation [Save | Mark Invalid | View all 0 Annotations]

Create a custom annotation of [Select a Semantic Type]

To cluster thumbnails by Category, select a CategoryGroup. Age

Can't find what you want in that list? You may want to Search or Create a new one.

Images are arranged by Age. To add a Category click on Age and refresh this page when you are done.

Clicking a thumbnail will: Open the Image Viewer Show Image Metadata Declassify the Image Classify the Image as: Day 1

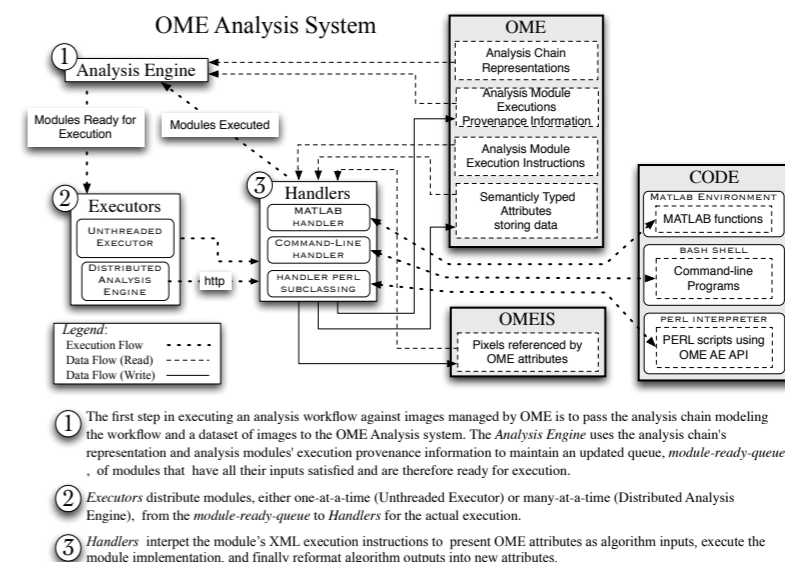
Unclassified (none)

Day 1

Day 2

OME for Quantitative Analysis

- **Modeling Workflows in OME:**
 - Semantic Types are OME's extensible ontology for data-modeling.
 - Analysis Modules are an language-independent, modular, re-usable computational algorithm representation.
 - Groups of analysis modules can be combined, by linking their inputs/ outputs, into OME Analysis Chain workflows.
- **Executing Workflows in the OME Analysis System**
 - The Analysis System provides managed algorithm execution (discussed later)



Complicated Schematic of the OME Analysis System

OME + WND-CHARM

OME = Informatics Platform for Visual Assays

WND-CHARM = Pattern Analysis to Score Visual Assays

OME + WND-CHARM = End to End Solution for High Throughput Imaging

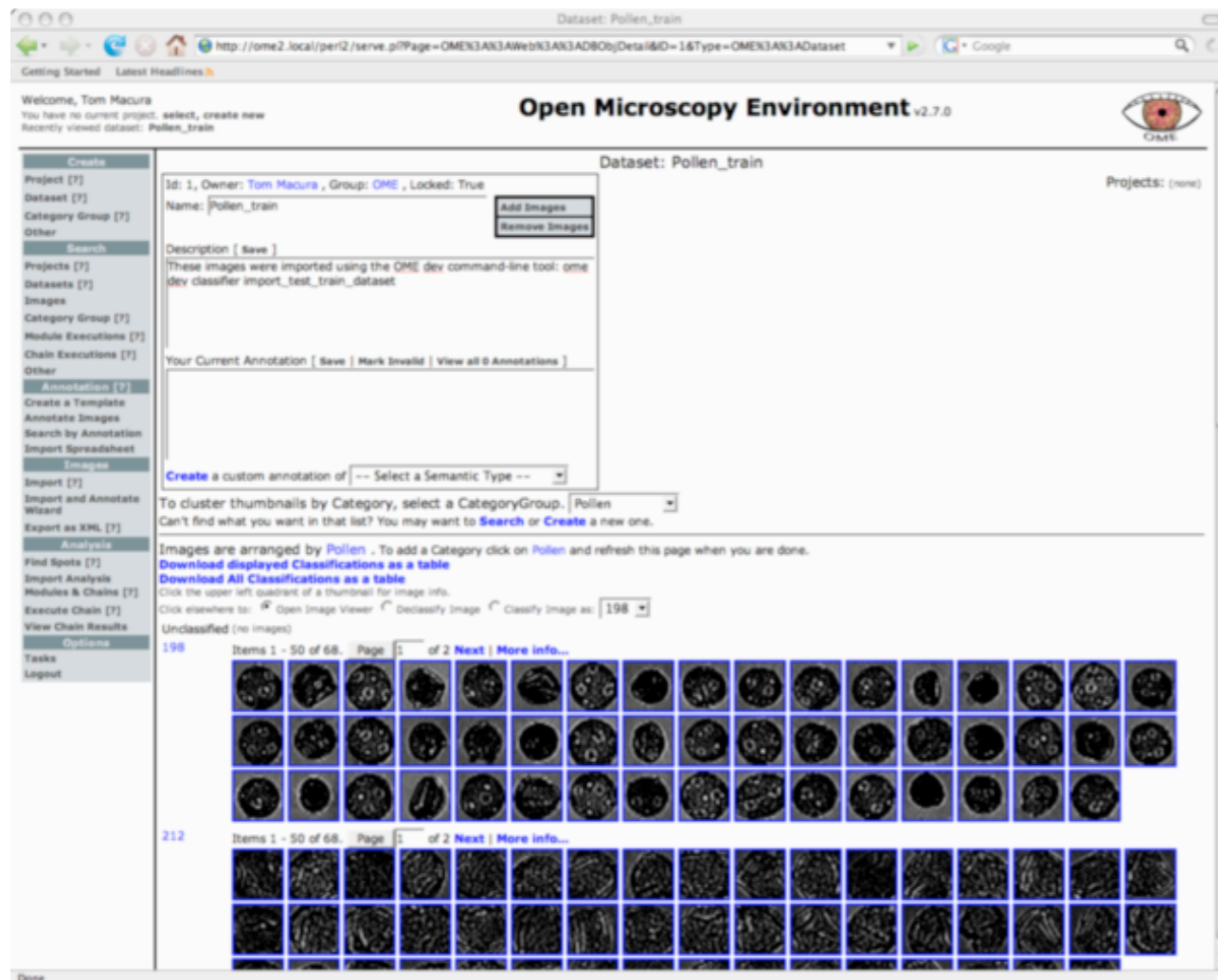
Integration of OME with WND-CHARM was finished in March.

Public Announcement to OME developers' lists was made on April 2nd.

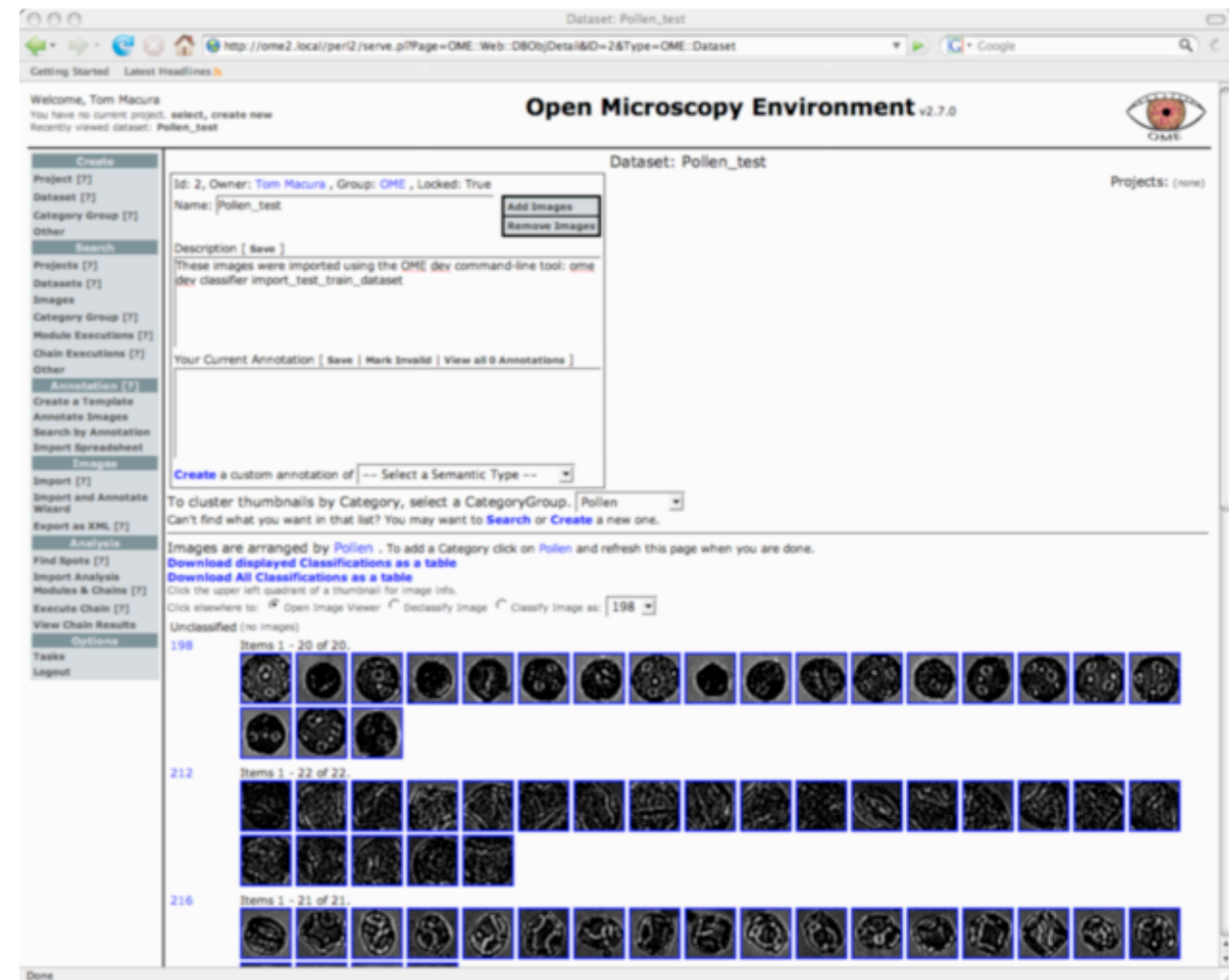
The "Full" OME+WND-CHARM release will use Lior's C/C++ implementation, eliminating the dependence on MATLAB.

OME+WND-CHARM (screenshots)

Training Pollen images with known categories



Pollen images predictions made with WND-CHARM



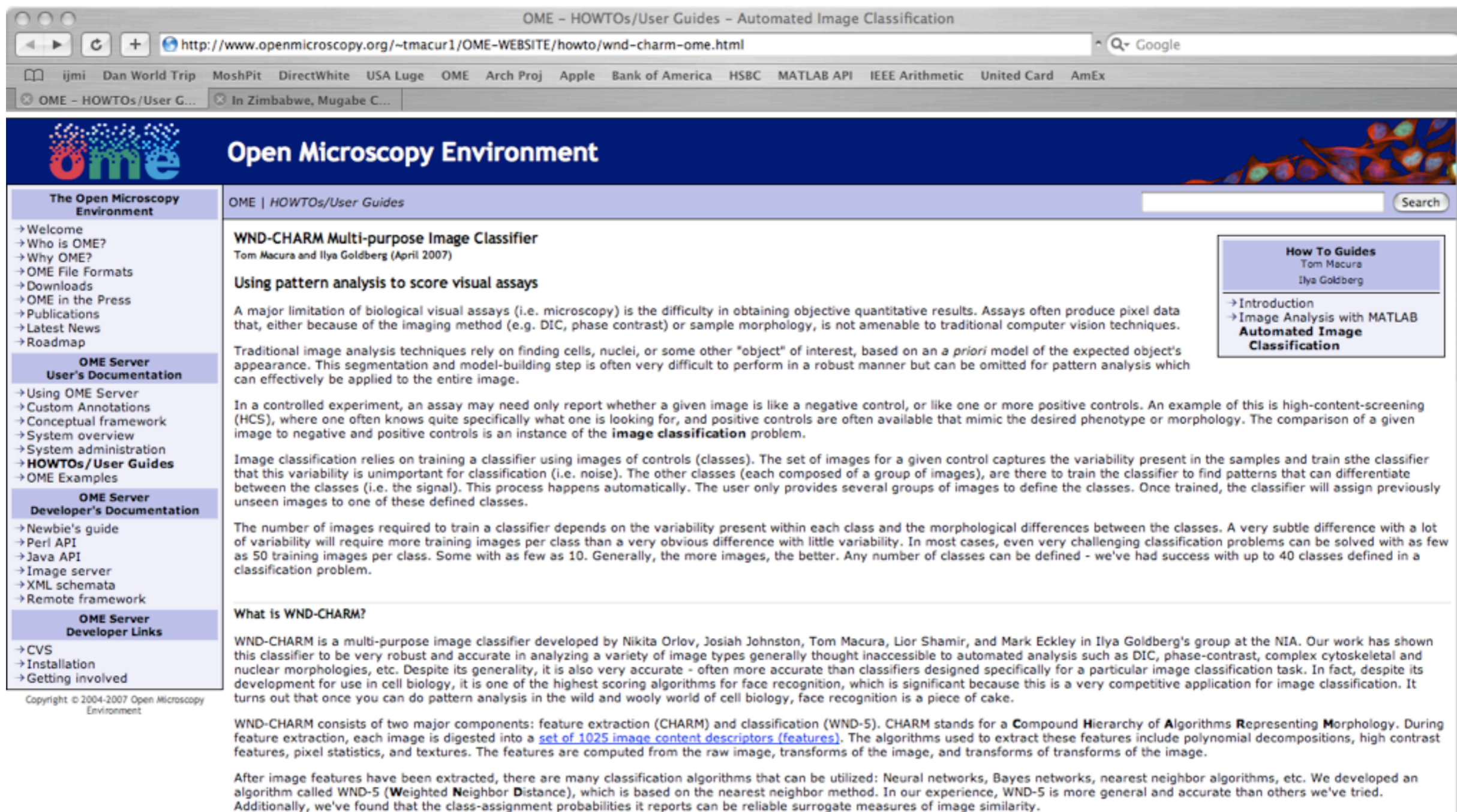
→
Several Command-Line Scripts Later

OME+WND-CHARM (How-To)

Step-by-step instructions for using OME+WND-CHARM are available on-line:

<http://www.openmicroscopy.org/~tmacur1/OME-WEBSITE/howto/wnd-charm-ome.html>

By following the How-To's instructions, Lior has reproduced my results.



The screenshot shows a web browser window displaying the Open Microscopy Environment (OME) website. The browser's address bar shows the URL: <http://www.openmicroscopy.org/~tmacur1/OME-WEBSITE/howto/wnd-charm-ome.html>. The website header features the OME logo and the text "Open Microscopy Environment". The main content area is titled "WND-CHARM Multi-purpose Image Classifier" and includes a search bar. The page is organized into several sections:

- The Open Microscopy Environment**: A sidebar menu with links to Welcome, Who is OME?, Why OME?, OME File Formats, Downloads, OME in the Press, Publications, Latest News, and Roadmap.
- OME Server User's Documentation**: A sidebar menu with links to Using OME Server, Custom Annotations, Conceptual framework, System overview, System administration, HOWTOs/User Guides, and OME Examples.
- OME Server Developer's Documentation**: A sidebar menu with links to Newbie's guide, Perl API, Java API, Image server, XML schemata, and Remote framework.
- OME Server Developer Links**: A sidebar menu with links to CVS, Installation, and Getting involved.

The main content area includes the following sections:

- OME | HOWTOs/User Guides**: A breadcrumb trail.
- WND-CHARM Multi-purpose Image Classifier**: The title of the page, with authors Tom Macura and Ilya Goldberg (April 2007).
- Using pattern analysis to score visual assays**: A sub-section title.
- Text**: A paragraph explaining the difficulty of obtaining objective quantitative results in biological visual assays and the role of pattern analysis.
- Text**: A paragraph discussing traditional image analysis techniques and their limitations.
- Text**: A paragraph describing high-content-screening (HCS) and the image classification problem.
- Text**: A paragraph explaining image classification and the training process.
- Text**: A paragraph discussing the number of images required for training a classifier.
- What is WND-CHARM?**: A section explaining the classifier's development and accuracy.
- Text**: A paragraph describing the components of WND-CHARM and the feature extraction process.
- Text**: A paragraph discussing classification algorithms and the WND-5 algorithm.

On the right side of the page, there is a "How To Guides" section with a search bar and a list of guides:

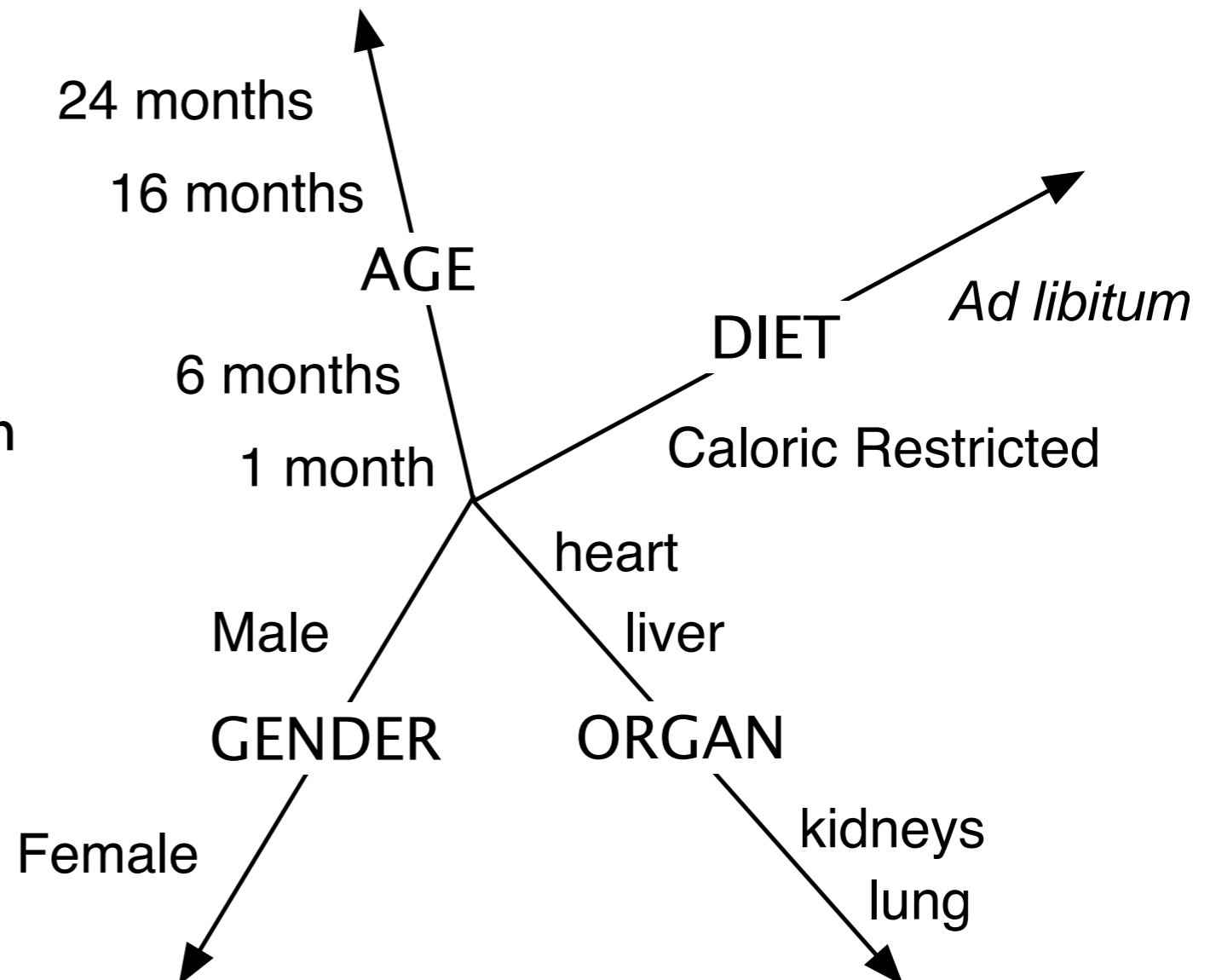
- How To Guides**: Tom Macura, Ilya Goldberg
- Introduction**
- Image Analysis with MATLAB Automated Image Classification**

At the bottom of the page, there is a copyright notice: "Copyright © 2004-2007 Open Microscopy Environment".

AGEMAP

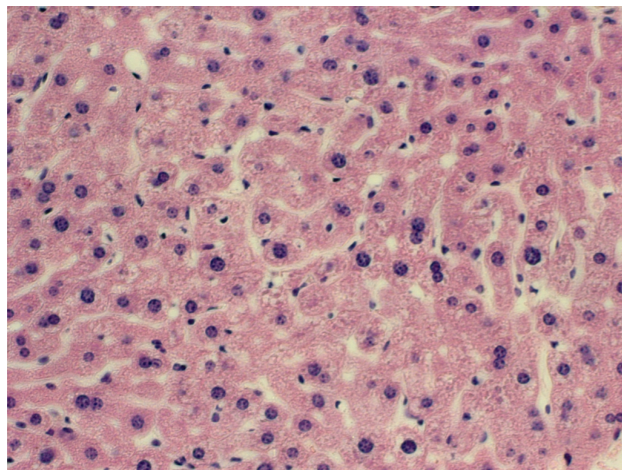
- **AGEMAP** (*Atlas of Gene Expression in Mouse Aging Project*)
 - initiated to investigate molecular bio-markers of aging and diet
 - 3 mice per data-point (gender, age, diet). 48 mice in total
 - individual organs were harvested, sectioned, stained and put on slides
 - we are using the tissue sections to search for structural biomarkers of aging and diet

- our initial investigations focus on livers because it is a uniform tissue that is expected to display age effects
- but our future plans are to analyze all organs.

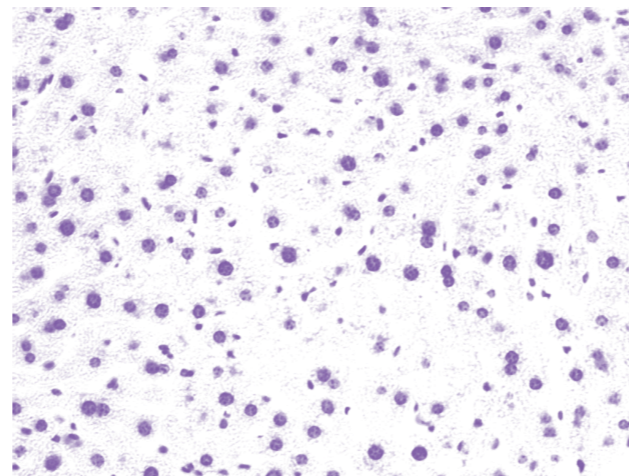


Collecting AGEMAP Images

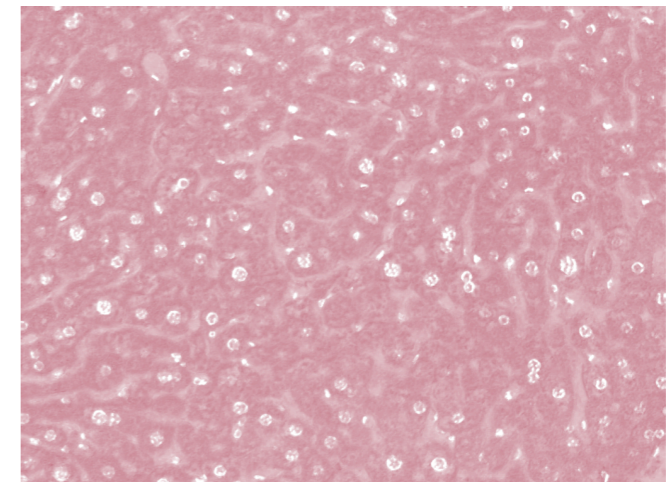
- 29 mouse livers, sectioned and stained with H&E
- ~50 RGB images of each liver acquired at 40x. Each image is 1388x1040 pixels with 12bits of quantization.
- “Colour Deconvolution” was used to convert camera’s Red, Green, Blue channels into “Hematoxylin” and “Eosin” channels.



Original RGB Image



Computed Hematoxylin Channel



Computed Eosin Channel

- Images were divided into 4x4 tiles and CHARM signatures were computed separately on tiles’ H and E components.

Quantitative Analysis of AGEMAP requires BIG!! Machinery

- Organizational Challenges:
 - 29 Mice => 1500 RGB Images => 3000 H&E Images => 50,000 tiles => 50 million “Features”
 - All 50 million features need to be mapped to meta-data (Mouse ID, Image ID, Age, Diet, Gender) that is essential to data analysis
- Computational Challenges:
 - 8GB of raw pixels => 125 GB of ‘computed pixels’ (e.g. Fourier/Wavelet/Chebysev transforms)
 - Each tile takes approximately 22 seconds to calculate signatures => 275 hours in total (using 6 processors!).
- OME’s “managed analysis”, and a network of computers, are essential to dealing with current challenges that will grow 5x as we proceed to analyze other organs. — *50 days of 24-7 computation and 600 GB of disk space.*

**After all the data processing, we have
preliminary results**

WND-CHARM detects structural differences between Male and Female livers

- Classifiers were trained on age/diet matched Male and Female mice (2560 tiles).
- Remaining tiles were used for testing.
- The training/testing splits were done per image (so all tiles for a particular image were either used for testing or training).

1 Months AL: **93%**

	Female	Male
Female	276	44
Male	9	311

16 Months AL: **95%**

	Female	Male
Female	1269	43
Male	31	289

6 Months AL: **95%**

	Female	Male
Female	466	14
Male	33	447

24 Months AL: **86%**

	Female	Male
Female	430	82
Male	52	412

WND-CHARM detects structural changes in mouse livers that are due to aging

- Classifiers were trained on ad-libitum mouse by age classes.
- Male and Female mice were analyzed separately (5 | 20 training tiles).

Female AL: **70%**

	1 Month	6 Months	16 Months	24 Months
1 Months	270	34	16	0
6 Months	69	855	131	65
16 Months	114	149	1022	27
24 Months	126	303	150	573

Male AL: **70%**

	1 Month	6 Months	16 Months	24 Months
1 Months	276	28	10	6
6 Months	67	931	80	42
16 Months	4	40	273	3
24 Months	37	95	149	823

- When we performed randomized Negative Controls we obtained expected results.

WND-CHARM detects structural differences between CR and AL livers

- We only have complete CR data points for 6 and 16 Month Female mice (1293 training tiles).

6 Months F: **88%**

	AL	CR
AL	404	76
CR	43	485

16 Months F: **95%**

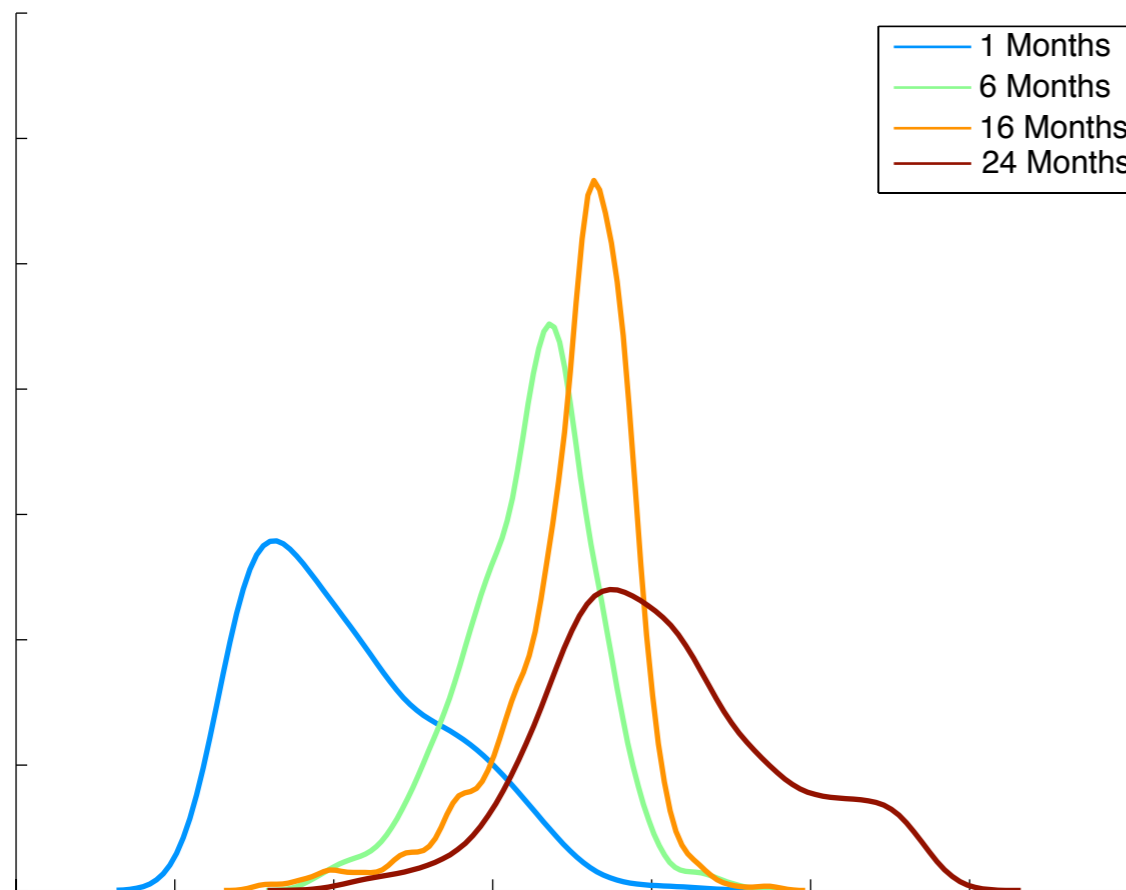
	AL	CR
AL	1219	80
CR	6	317

- When we performed randomized Negative Controls we obtained expected results.

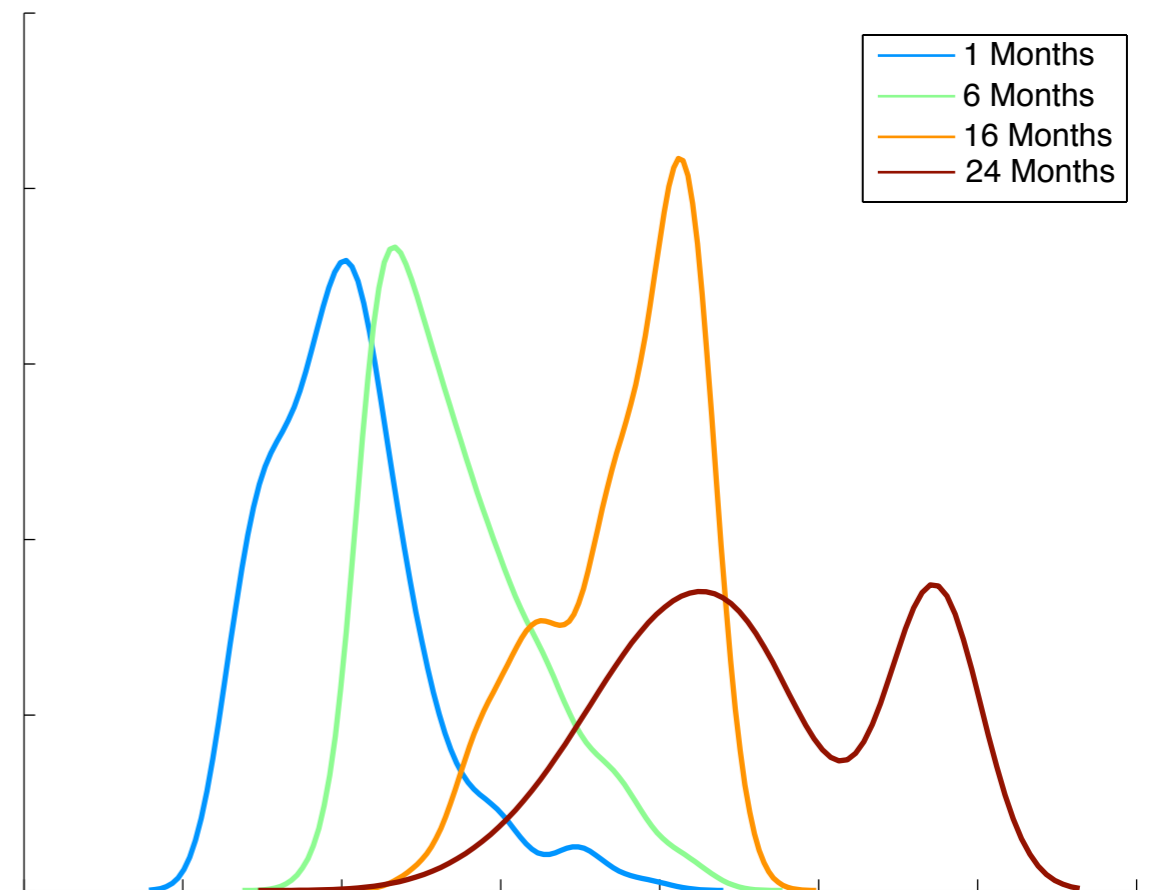
WND-CHARM Similarity Measure

- We've applied Josiah's Image Similarity tools to AGEMAP data
- There appear to be three states of aging

Female AL Probability Distributions



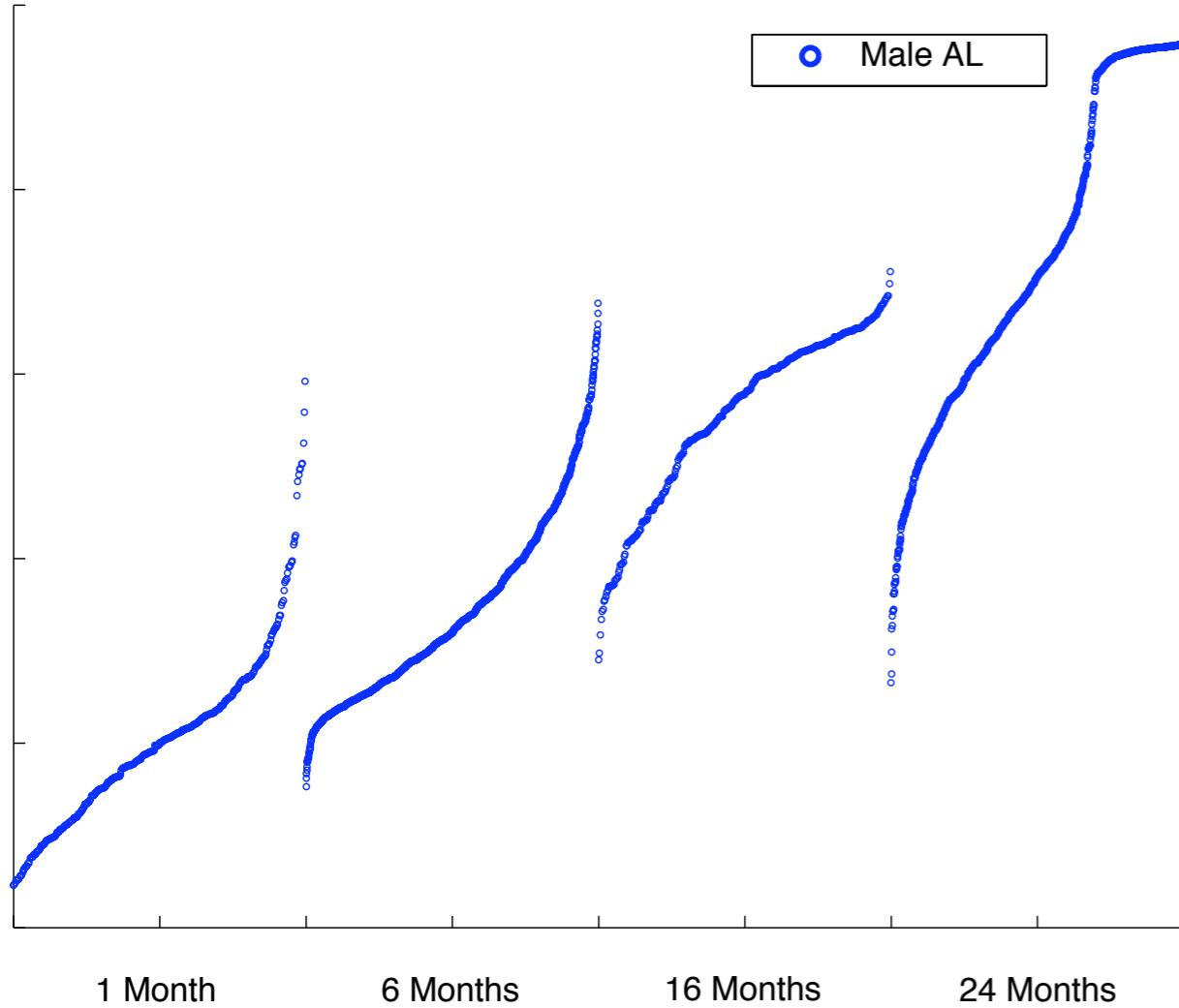
Male AL Probability Distributions



WND-CHARM Similarity Measure

- Female CR livers appear younger than Female AL livers.

Rank Order of Age Predictions for Males on AL Diets



Rank Order of Age Predictions for Females on AL and CR Diets

