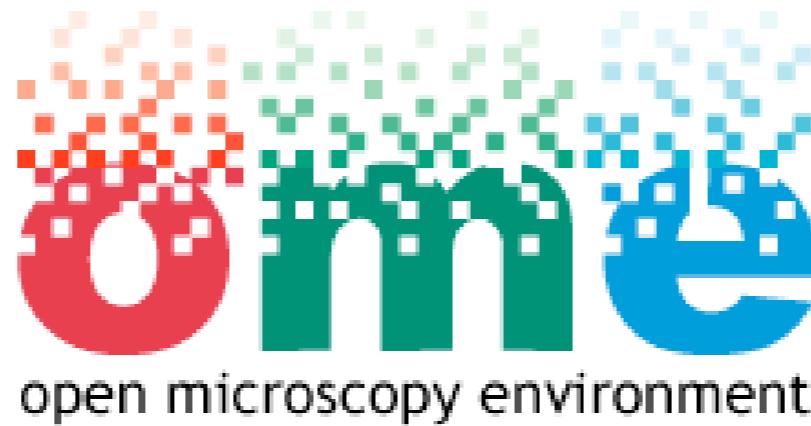
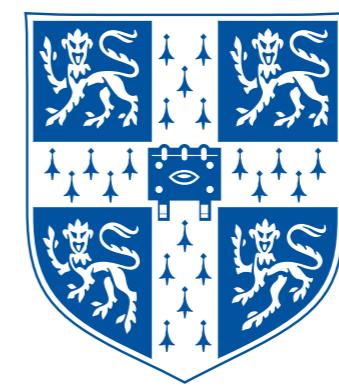
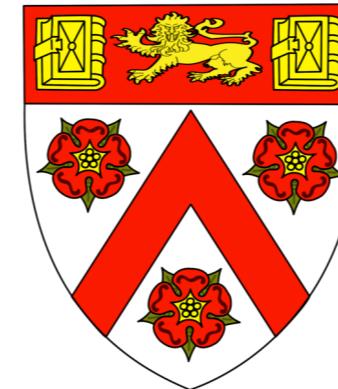


Pattern analysis of mouse liver: Morphological effects of gender, aging and diet

(*doing Science with OME*)

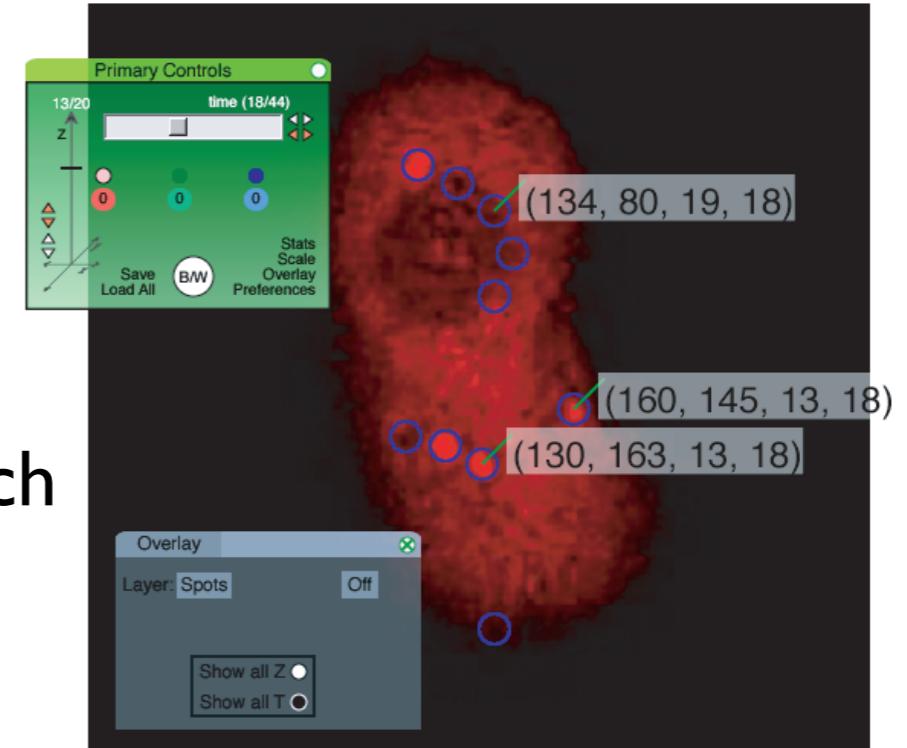


Tom Macura
Image Informatics and Computational Biology Unit



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

http://localhost/perl2/serve.pl?Page=OME::Web::Search&Type=OME::Image

Dataset: Worms

Open Microscopy Environment v2.003

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

Create

Project
Dataset
Other

Search

Projects
Datasets
Images
Module Executions
Chain Executions
Other

Images

Import
Export Image(s)

Analysis

Find Spots
Import Modules
Execute Chain
View Chain Results

Options

Tasks
Logout

Search

Look for: Image

That match criteria:

Created

Description

Group

Name

%day4%

Owner

All

Display Results as:

Summaries
Names

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)

Open Microscopy Environment v2.4.0

Create

Project
Dataset
Other

Search

Projects
Datasets
Images
Module Executions
Chain Executions
Other

Images

Import
Export Image(s)

Analysis

Find Spots
Import Modules
Execute Chain
View Chain Results

Options

Tasks
Logout

Dataset: All Worms

Projects: More info...
Nameless Project

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

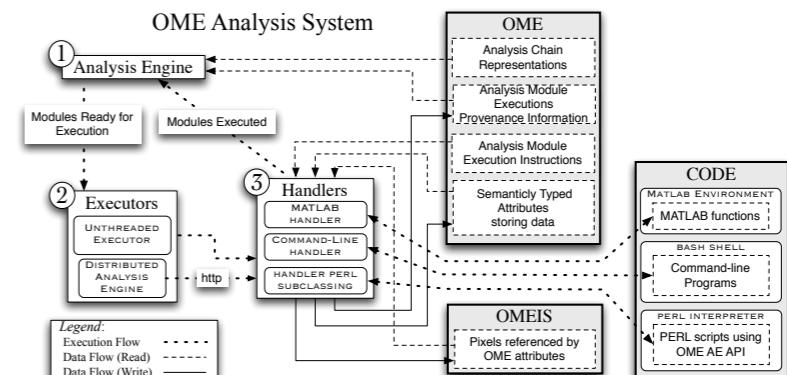
Unclassified (none)

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 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)



- ① The first step in executing an analysis workflow against images managed by OME is to pass the analysis chain modeling the workflow and a dataset of images to the OME Analysis system. The *Analysis Engine* uses the analysis chain's representation and analysis modules' execution provenance information to maintain an updated queue, *module-ready-queue*, of modules that have all their inputs satisfied and are therefore ready for execution.
- ② *Executors* distribute modules, either one-at-a-time (Unthreaded Executor) or many-at-a-time (Distributed Analysis Engine), from the *module-ready-queue* to *Handlers* for the actual execution.
- ③ *Handlers* interpret the module's XML execution instructions to present OME attributes as algorithm inputs, execute the module implementation, and finally reformat algorithm outputs into new attributes.

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

Dataset: Pollen_train
Welcome, Tom Macura
You have no current project; select, create new
Recently viewed dataset: Pollen_train

Open Microscopy Environment v2.7.0

Dataset: Pollen_train
Projects: (none)

Description [Save]
These images were imported using the OME dev command-line tool: ome dev classifier import_test_train_dataset

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

Images are arranged by Pollen . To add a Category click on Pollen and refresh this page when you are done.
Download displayed Classifications as a table
Download All Classifications as a table
Click the upper left quadrant of a thumbnail for image info.
Click elsewhere to: Open Image Viewer Declassify Image Classify Image as: 198
Unclassified (no images)
198 Items 1 - 50 of 68. Page 1 of 2 Next | More info...
212 Items 1 - 50 of 68. Page 1 of 2 Next | More info...

Done

Pollen images predictions made with WND-CHARM

Dataset: Pollen_test
Welcome, Tom Macura
You have no current project; select, create new
Recently viewed dataset: Pollen_test

Open Microscopy Environment v2.7.0

Dataset: Pollen_test
Projects: (none)

Description [Save]
These images were imported using the OME dev command-line tool: ome dev classifier import_test_train_dataset

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

Images are arranged by Pollen . To add a Category click on Pollen and refresh this page when you are done.
Download displayed Classifications as a table
Download All Classifications as a table
Click the upper left quadrant of a thumbnail for image info.
Click elsewhere to: Open Image Viewer Declassify Image Classify Image as: 198
Unclassified (no images)
198 Items 1 - 20 of 20.
212 Items 1 - 22 of 22.
216 Items 1 - 21 of 21.

Done

→
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.

OME – HOWTOs/User Guides – Automated Image Classification

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

OME | HOWTOs/User Guides

Open Microscopy Environment

The Open Microscopy Environment

OME Server User's Documentation

- Welcome
- Who is OME?
- Why OME?
- OME File Formats
- Downloads
- OME in the Press
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OME Server Developer's Documentation

- Using OME Server
- Custom Annotations
- Conceptual framework
- System overview
- System administration
- HOWTOs/User Guides
- OME Examples

OME Server Developer Links

- Newbie's guide
- Perl API
- Java API
- Image server
- XML schemata
- Remote framework

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WND-CHARM Multi-purpose Image Classifier

Tom Macura and Ilya Goldberg (April 2007)

Using pattern analysis to score visual assays

A major limitation of biological visual assays (i.e. microscopy) is the difficulty in obtaining objective quantitative results. Assays often produce pixel data that, either because of the imaging method (e.g. DIC, phase contrast) or sample morphology, is not amenable to traditional computer vision techniques.

Traditional image analysis techniques rely on finding cells, nuclei, or some other "object" of interest, based on an *a priori* model of the expected object's appearance. This segmentation and model-building step is often very difficult to perform in a robust manner but can be omitted for pattern analysis which can effectively be applied to the entire image.

In a controlled experiment, an assay may need only report whether a given image is like a negative control, or like one or more positive controls. An example of this is high-content-screening (HCS), where one often knows quite specifically what one is looking for, and positive controls are often available that mimic the desired phenotype or morphology. The comparison of a given image to negative and positive controls is an instance of the **image classification** problem.

Image classification relies on training a classifier using images of controls (classes). The set of images for a given control captures the variability present in the samples and train the classifier that this variability is unimportant for classification (i.e. noise). The other classes (each composed of a group of images), are there to train the classifier to find patterns that can differentiate between the classes (i.e. the signal). This process happens automatically. The user only provides several groups of images to define the classes. Once trained, the classifier will assign previously unseen images to one of these defined classes.

What is WND-CHARM?

WND-CHARM is a multi-purpose image classifier developed by Nikita Orlov, Josiah Johnston, Tom Macura, Lior Shamir, and Mark Eckley in Ilya Goldberg's group at the NIA. Our work has shown this classifier to be very robust and accurate in analyzing a variety of image types generally thought inaccessible to automated analysis such as DIC, phase-contrast, complex cytoskeletal and nuclear morphologies, etc. Despite its generality, it is also very accurate - often more accurate than classifiers designed specifically for a particular image classification task. In fact, despite its development for use in cell biology, it is one of the highest scoring algorithms for face recognition, which is significant because this is a very competitive application for image classification. It turns out that once you can do pattern analysis in the wild and wooly world of cell biology, face recognition is a piece of cake.

WND-CHARM consists of two major components: feature extraction (CHARM) and classification (WND-5). CHARM stands for a Compound Hierarchy of Algorithms Representing Morphology. During feature extraction, each image is digested into a [set of 1025 image content descriptors \(features\)](#). The algorithms used to extract these features include polynomial decompositions, high contrast features, pixel statistics, and textures. The features are computed from the raw image, transforms of the image, and transforms of transforms of the image.

After image features have been extracted, there are many classification algorithms that can be utilized: Neural networks, Bayes networks, nearest neighbor algorithms, etc. We developed an algorithm called WND-5 (Weighted Neighbor Distance), which is based on the nearest neighbor method. In our experience, WND-5 is more general and accurate than others we've tried. Additionally, we've found that the class-assignment probabilities it reports can be reliable surrogate measures of image similarity.

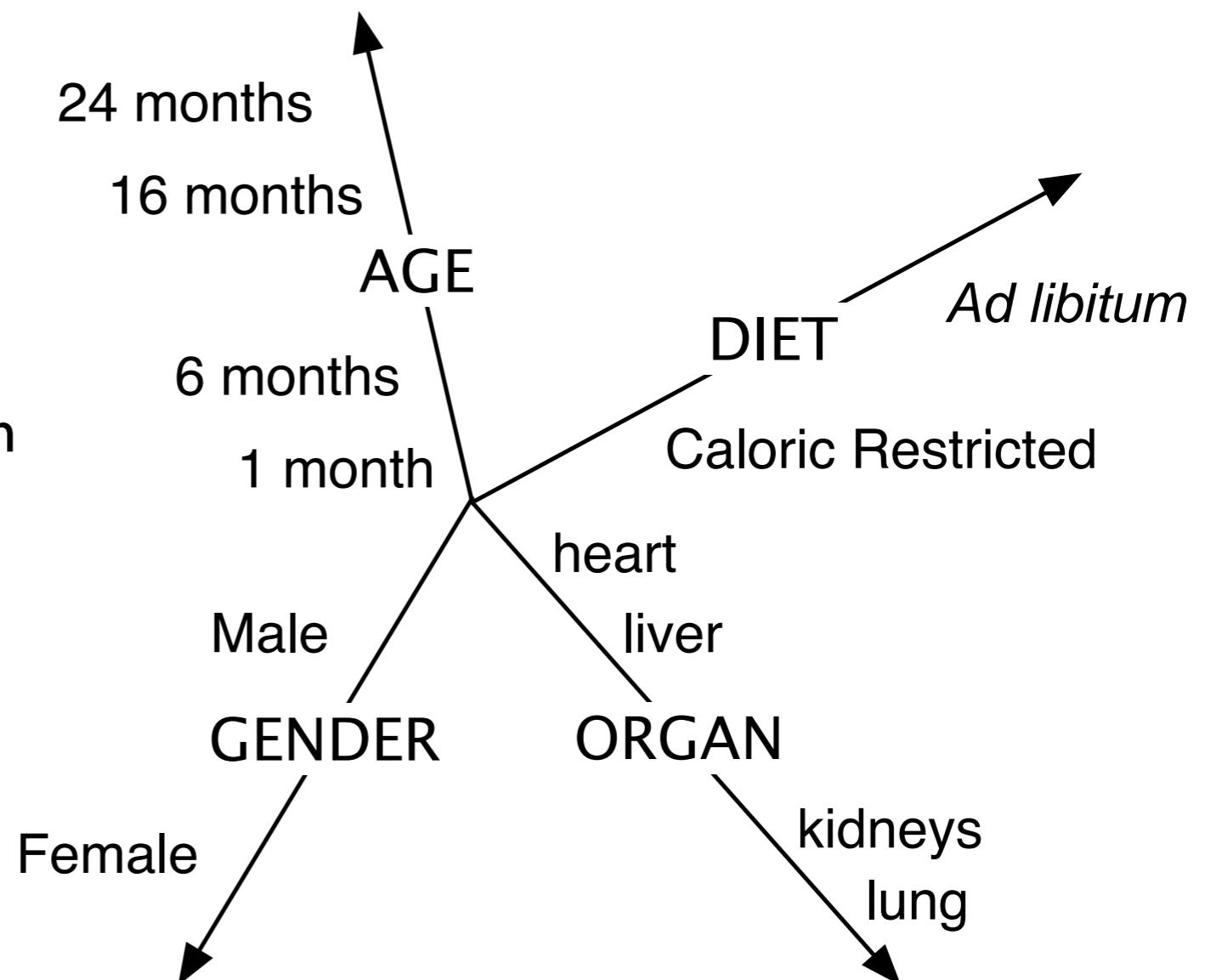
How To Guides

Tom Macura
Ilya Goldberg

Introduction
Image Analysis with MATLAB
Automated Image Classification

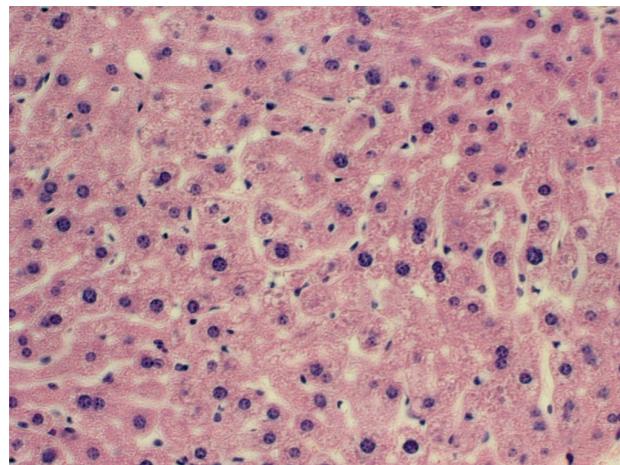
AGEMAP

- 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.

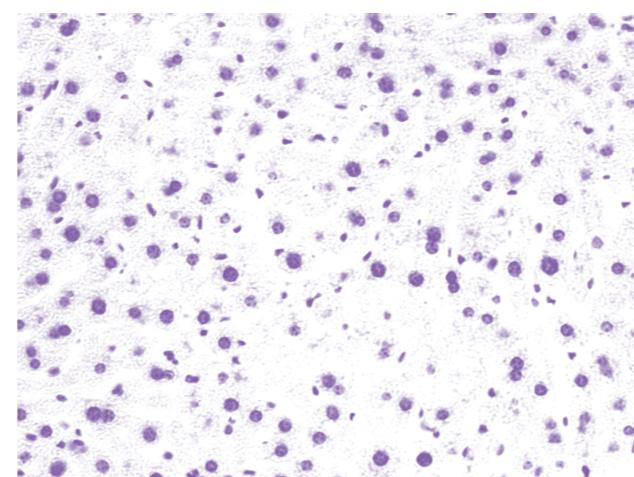


Collecting AGEMAP Images

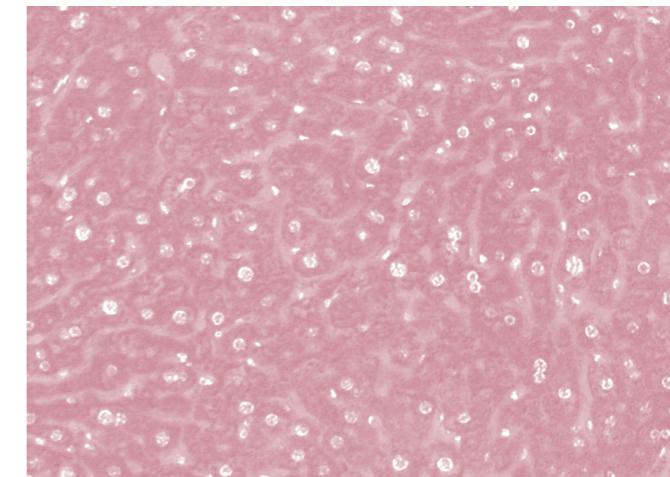
- 29 mouse livers, sectioned and stained with H&E
- ~50 RGB images of each liver acquired at 40x. Each image is 1388x1040 pixels with 12bits of quantization.
- “Colour Deconvolution” was used to convert camera’s Red, Green, Blue channels into “Hematoxylin” and “Eosin” channels.



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 (5120 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

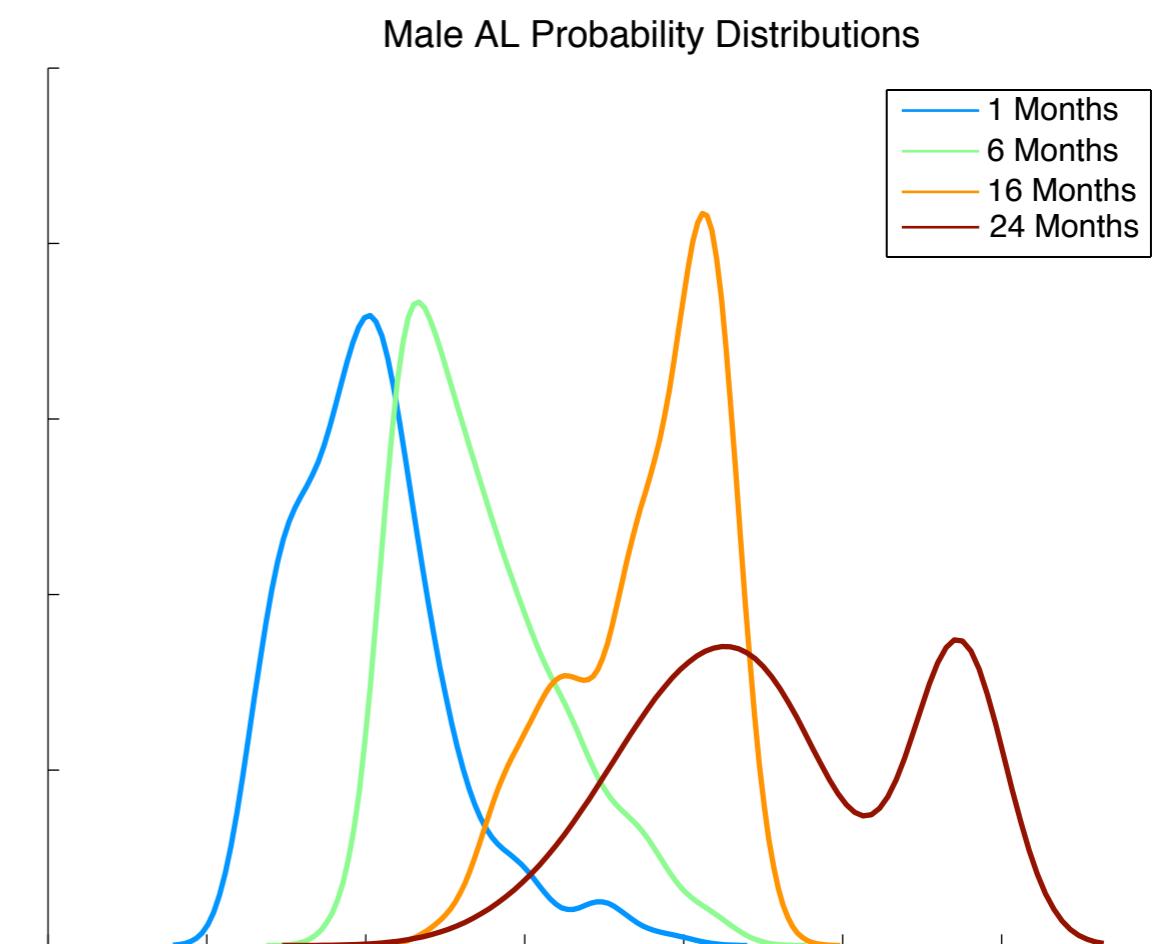
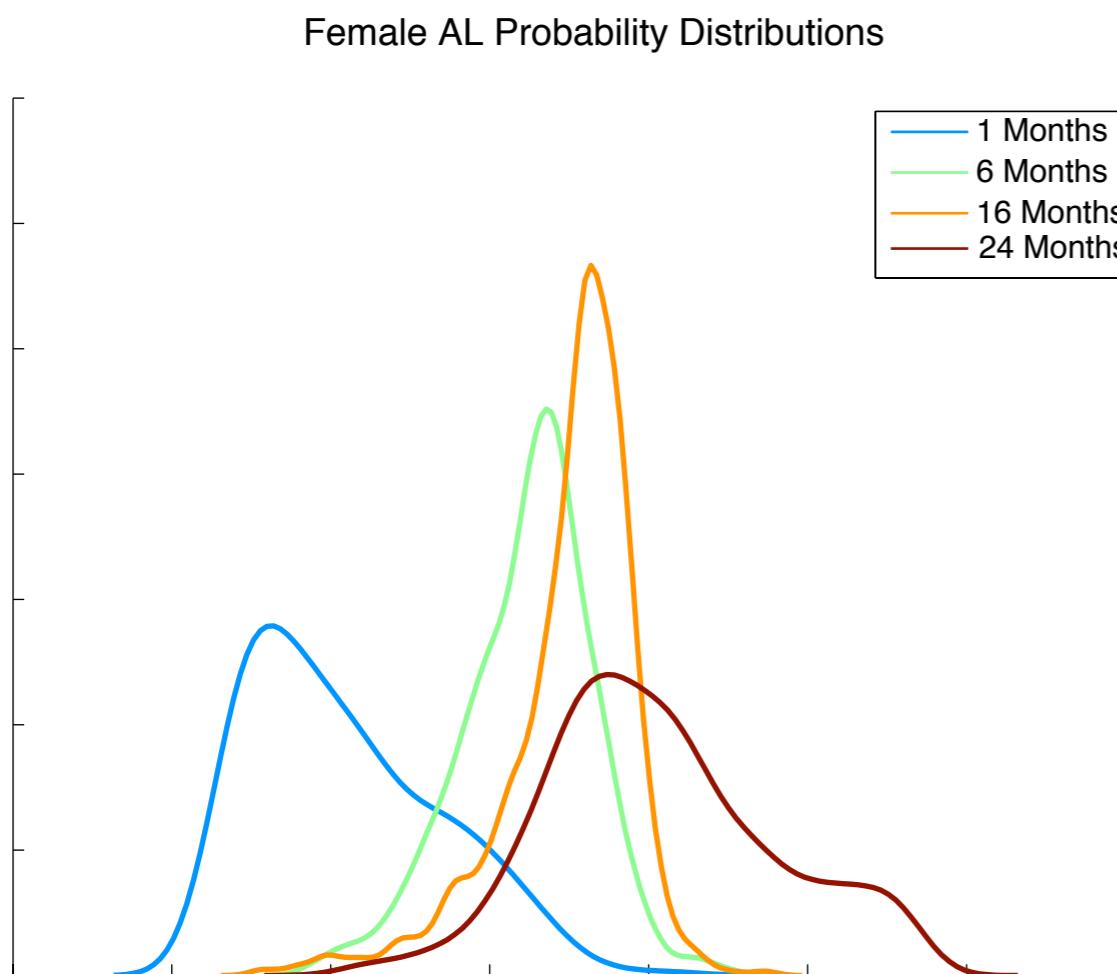
16 Months F: **95%**

	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



WND-CHARM Similarity Measure

- Female CR livers appear younger than Female AL livers.

