

How to Use AI for Classifying Cell Types & Phenotypes in the Cloud: The Live/Dead Assay

Featuring Dr. Ilya Goldberg

Presented by



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- Automate and scale unique research workflows through machine learning and AI (we can help build them or integrate our partners' existing analyses)
- Problem-solve complex image and data challenges (we have a team of experts who have been developing this infrastructure for over 15 years starting at UCSB in addition to 20+ years in bio-imaging informatics and AI that Dr. Goldberg brings to the table)

The Speaker | Dr. Ilya Goldberg

Dr. Goldberg has a long career that lies at the intersection of biology, imaging, and AI.

- Co-founded a company that developed the first medical device to receive regulatory clearance for using an AI to predict malignancy in lung nodules in CT exams.
- The research group he led at the NIH National Institute on Aging developed machine learning software for image processing in biology and medicine.
- At MIT he co-founded the OME project, which is still used for imaging infrastructure in large image repositories.
- He has over 60 peer-reviewed scientific articles from time at Johns Hopkins, Harvard, MIT, and NIH in molecular and cell biology, pattern recognition, image informatics and the basic biology of aging.



Agenda |

What You'll Learn

- Different kinds of AIs
- How to train an AI to perform a simple classification task
- How to evaluate AI performance
- What to look out for

Let's start with an **audience poll**

A series of webinars for experimental biologists

How to use AIs for imaging problems in an experimental setting

Not focusing on the details of how AIs work

- **Introduction to AI & classification**
 - Simple classification problems e.g. Live/dead assay
 - Multiclass problems
- **Quantitative problems**
 - Ordered class problems, regression, dose-response
- **Phenotypic similarity**
 - Clustering, dendrograms
- **Localization**
 - AI-based segmentation, heat-maps, etc.

Two types of machine learning

Supervised: Training on known answers, controls

- Typical experimental setups are a natural fit
- Positive and negative controls
- Standard curves

Unsupervised: No known classes/groups

- Open-ended, Exploratory, Clustering, PCA
- Completely different AI technologies

Semi-supervised: Really just supervised, but bootstrapping from known classes to new classes/clusters

Two AI systems: Deep-learning and Feature-based

Deep Learning

Neural networks, "Perceptrons", the first AIs

- 1961** Bernard Widrow. Earliest learning rule for networks with multiple elements.
- 1971** Paul Werbos. Backpropagation, multilayer networks. Published in his doctoral dissertation, remained almost unknown until 1986!
- 2010** Dan Ciresan. Backpropagation on GPUs.
- 2011** "Superhuman" handwritten digit recognition
- 2012** Alex Krizhevsky ImageNet Large Scale Visual Recognition Challenge

Feature-based AIs

"Handcrafted features"

Feature extraction: Numerical representations of image content

Dimensionality reduction

Classification

- 2001** Boland and Murphy. Bank of feature algorithms + neural network. Superhuman subcellular localization.
- 2002** Murphy lab: PSLID
- 2008** Goldberg lab: WND-CHARM
- 2008** Carpenter lab: CellProfiler-Analyst

Two AI systems: Technology overview

Deep Learning

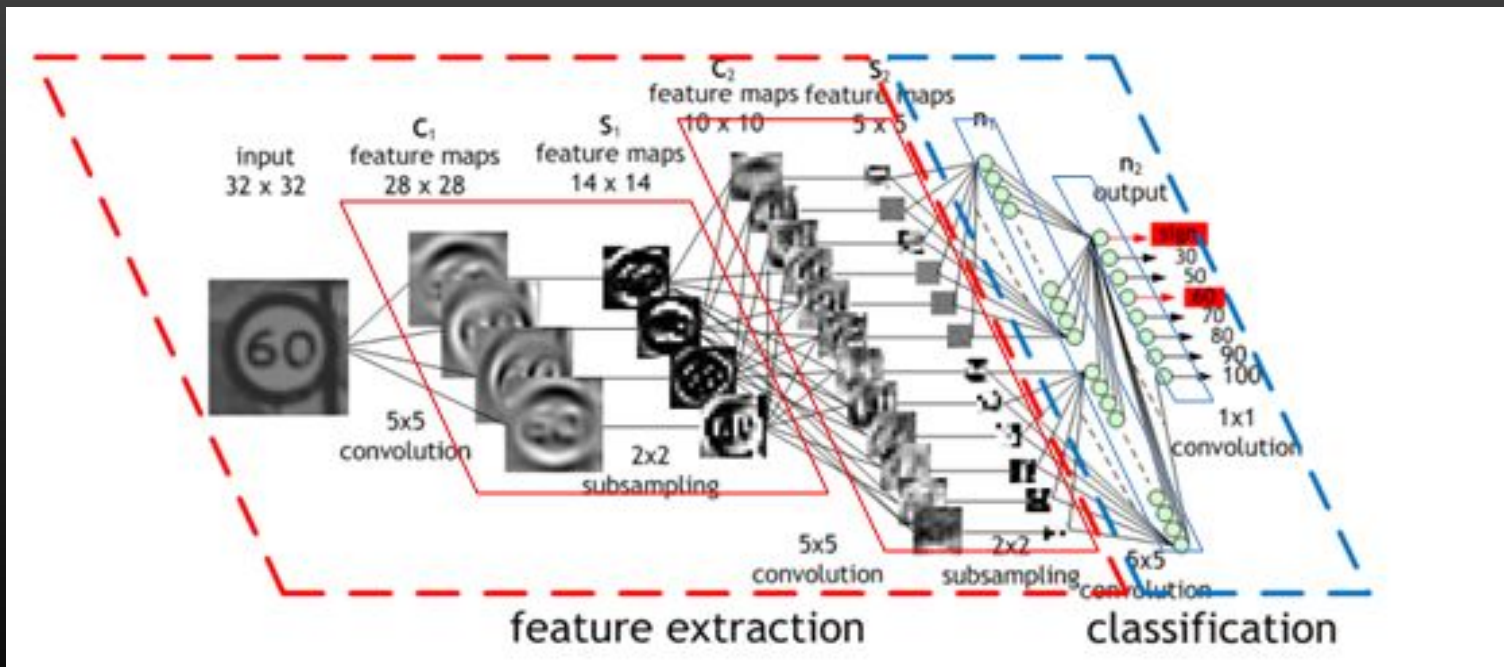


Image credit: Maurice Peemen
parse.ele.tue.nl/mpeemen

Two AI systems: Technology overview

Feature-based AIs

- **Feature Extraction & Normalization**
 - Statistics, texture descriptors, shape descriptors, etc.
- **Scoring, Weighting, & Reducing**
 - Pearson correlation, Fisher discriminant, mRMR, etc.
- **Classifying**
 - Random Forest, Support Vector Machines, Distance methods, etc.

Two AI systems: Pros and Cons

Deep Learning

Pro: No feature algorithms, no feature reduction algorithms, no classifier algorithms.

Pro: Feature reduction occurs in-network.

Con: Classification prone to over-training (too many degrees of freedom). This requires a lot of data to overcome.

Con: Hard to train. Need GPU clusters.

Con (for now): Still requires manual model assembly and training. No "push-button" AI. Yet.

Pro: Large community of developers.

Feature-based AIs

Pro: Lots of 2D features. Decades of digital signal processing for 1D and 2D data.

Con: 3D+ requires "tricks" to extract features.

Con: Fast feature reduction uses filters - each feature evaluated one-at-a-time. Features are not evaluated in-classifier.

Pro: Modern classifiers are robust to overtraining (too many features). Can use less data.

Pro: Conventional CPUs, fewer resources.

Pro: Several models can be tried and parameters optimized automatically.

Con: May be considered "retro".

Hybrid and Ensemble AIs

Why not take the best parts of each?

- 2D Features + Deep Learning dimensionality reduction + Classifiers
- Multi-D CNNs + dimensionality reduction + Classifiers
- Multiple AIs "voting"

Next: Training AIs.

How to train an AI - binary classifiers

- **Live/Dead**
- **Positive/negative controls**
- **Treated/untreated**
- **Case/control: benign/malignant**

Tag areas of an image (e.g. cells) with labels

Tag whole images with class (e.g. treatment)

Manual labeling

Layers

- Overlay 1
- Annotations 1
- Image 1

Enhancements for Image

Histogram

0% 100%

Channels

1	<input type="checkbox"/>	906
1	<input type="checkbox"/>	454
1	<input type="checkbox"/>	631
1	<input type="checkbox"/>	844
1	<input type="checkbox"/>	4849

Enhancement

Brightness:

Contrast:

Threshold:

Layer

Transparency:

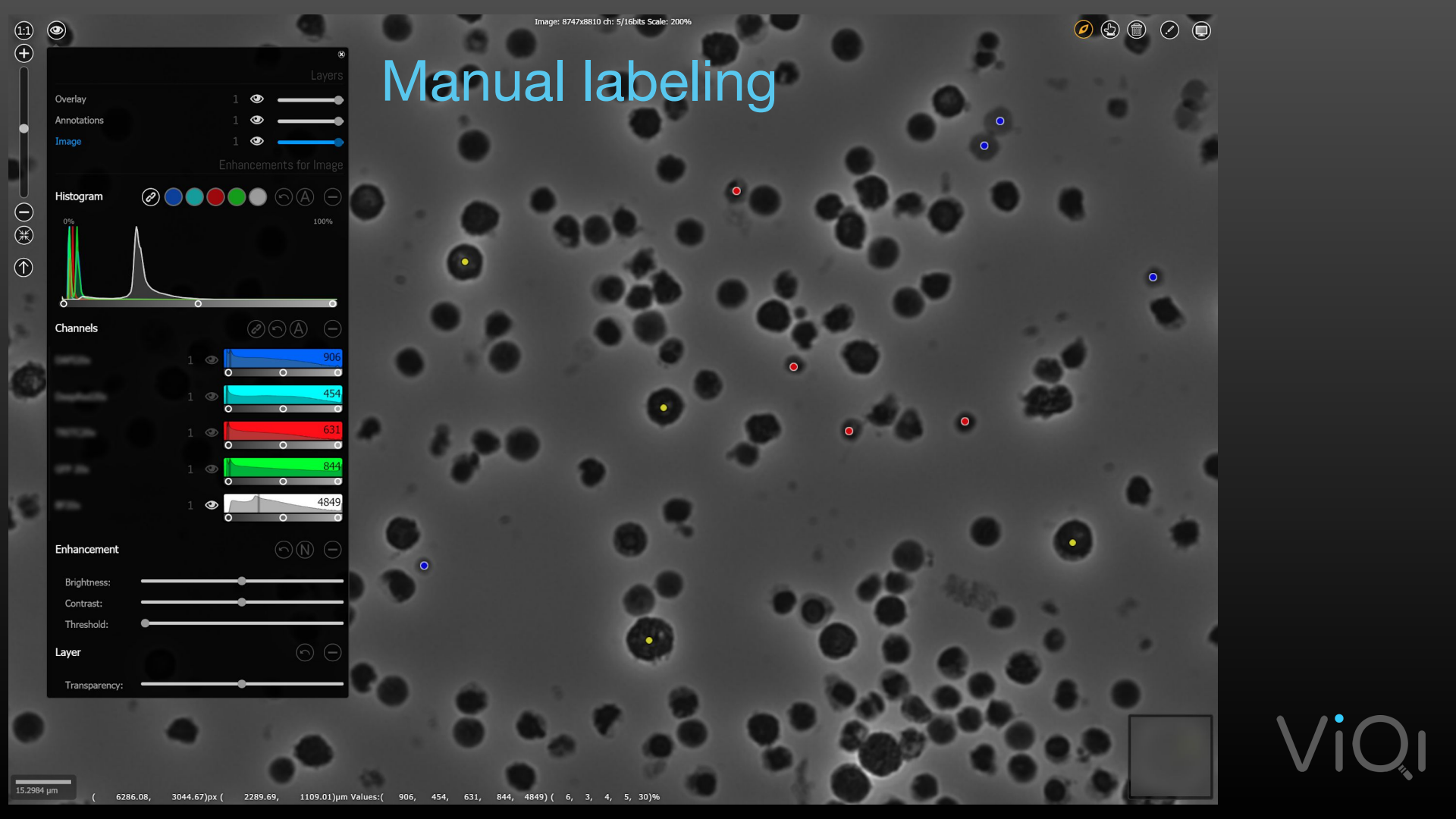


Image: 8747x8810 ch: 5/16bits Scale: 100%

Automated segmentation: Color per cell

Layers

- Overlay 1
- Annotations 1
- Cell masks 1
- Image 1

Enhancements for Image

Histogram

Channels

Channel	Count
1	1187
1	656
1	708
1	898
1	6677

Enhancement

Brightness: [Slider]

Contrast: [Slider]

Threshold: [Slider]

Layer

Transparency: [Slider]

Feature table

table: 14.nd2_00-DJgUx62xajMj5fzboVMVgK.45646437_classified.hs

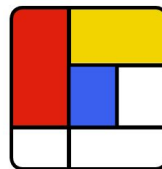
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object	mask	x (ms)	y (ms)	area	perim	A2P	diam	reguli	eccen	solidit	radial	euler	exten	major	minor	orient	class	confid	ch1.m	ch1.s	ch1.k	ch1.e	ch1.h	ch1.l	ch1.c	ch1.i	ch2.m	ch2.s	ch2.k	ch2.e	ch2.h	ch2.c	ch2.i	ch3.m	ch3.s	ch3.k	ch3.e	ch3.h	ch3.c	ch3.i	ch4.m	ch4.s	ch4.k	ch4.e	ch4.h	ch4.c	ch4.i	
0	854	26.63	97	35.31	2.7	11.11	0.9	0.2	0.6	1.5	1	0.8	13.85	8.9	-1.55	367	0.6	408	-1.74	1.0	4.0	0.4	0.1	0.7	883	326	-1.75	1.0	2.8	0.6	0.2	0.7	914	372	-1.75	1.0	4.0	0.4	0.1	0.7	895	445	-1.75	1.0	4.1	0.4	0.1	
1	209	41.42	145	43.80	3.3	13.59	0.9	0.7	0.9	1.2	1	0.7	13.90	13.36	0.70	367	0.8	312	-1.09	-0.81	3.7	0.5	0.2	0.6	111	292	-1.09	-0.81	3.1	0.5	0.2	0.6	113	299	-1.09	-0.81	3.3	0.5	0.2	0.6	110	348	-1.09	-0.81	3.9	0.4	0.2	
2	246	87.76	5607	46.80	9.8	18.49	0.4	0.6	0.7	1.2	9	0.6	97.77	91.56	1.30	367	0.6	286	-0.69	-1.52	3.8	0.6	0.3	0.9	336	261	-0.69	-1.52	2.9	0.6	0.3	0.9	345	272	-0.69	-1.52	3.5	0.6	0.3	0.9	340	309	-0.69	-1.52	3.8	0.5	0.2	
3	786	60.88	128	41.31	3.1	12.77	0.7	0.3	0.7	1.4	1	0.7	14.50	11.42	-0.03	367	0.7	440	-1.23	-0.49	0.4	0.4	0.2	0.7	862	300	-1.23	-0.49	3.1	0.5	0.2	0.7	893	319	-1.23	-0.49	3.7	0.5	0.2	0.7	871	383	-1.22	-0.49	4.4	0.3	0.1	
4	4545	68.49	415	73.70	5.6	22.99	0.5	0.5	0.8	1.1	1	0.8	24.56	21.53	0.04	4524	0.7	341	0.4	-1.32	5.6	0.1	0.1	0.9	117	386	-1.69	0.8	4.6	0.4	0.1	0.7	466	716	-0.85	0.30	5.7	0.2	0.1	0.8	169	748	0.00	-0.82	5.6	0.2	0.1	
5	654	71.43	106	43.01	2.4	11.62	0.8	0.3	0.7	1.2	1	0.6	13.85	10.43	-0.96	367	0.6	276	-0.54	-1.71	3.4	0.5	0.2	0.7	127	257	-0.54	-1.71	3.2	0.5	0.3	0.7	127	258	-0.54	-1.71	3.1	0.6	0.3	0.7	127	311	-0.54	-1.71	3.6	0.5	0.2	
6	456	74.72	220	55.36	3.9	16.74	0.9	0.3	0.7	1.1	1	0.6	19.88	14.39	-1.05	367	0.6	503	-0.06	-1.49	4.7	0.3	0.2	0.9	220	307	-0.61	-1.62	4.0	0.5	0.2	0.7	794	508	-0.60	-1.62	4.4	0.4	0.2	0.8	729	188	0.00	-1.29	4.7	0.3	0.2	
7	287	84.94	123	40.04	3.0	12.51	0.7	0.4	0.8	1.1	1	0.6	13.80	11.46	-1.06	367	0.7	314	-0.75	-1.44	3.7	0.5	0.2	0.6	111	268	-0.75	-1.44	3.2	0.6	0.2	0.6	113	288	-0.75	-1.44	3.4	0.5	0.2	0.6	112	326	-0.75	-1.44	3.7	0.5	0.2	
8	450	94.63	798	70.18	4.2	19.48	0.8	0.4	0.7	1.1	1	0.5	21.93	18.21	1.40	367	0.7	396	0.2	-1.52	4.5	0.3	0.3	0.9	160	304	-0.39	-1.84	4.0	0.5	0.3	0.8	539	532	-0.37	-1.84	4.4	0.5	0.3	0.8	533	272	-0.14	-1.66	4.5	0.4	0.3	
9	468	94.16	257	56.53	4.5	18.09	0.9	0.6	0.9	1.1	1	0.7	18.66	17.63	1.12	4524	0.8	571	-1.16	-1.23	5.1	0.2	0.1	0.9	141	399	-1.15	-0.66	4.7	0.4	0.2	0.7	479	968	-0.79	-0.94	5.0	0.2	0.1	0.8	248	211	-0.26	-1.02	5.0	0.2	0.1	
10	4371	92.73	184	47.80	3.8	15.31	0.9	0.3	0.7	1.1	1	0.8	17.50	13.40	1.5	4524	0.7	697	-0.79	0.46	5.0	0.1	0.1	0.13	0.8	237	420	-1.71	1.0	4.2	0.4	0.1	0.7	374	869	-1.62	0.8	5.0	0.2	0.1	0.7	430	178	-1.22	0.3	4.9	0.2	0.1
11	666	143	5448	69	8.0	83.29	0.3	0.6	0.7	1.2	-11	0.5	97.04	92.55	-0.09	367	0.6	263	-0.40	-1.84	3.5	0.6	0.3	0.9	375	245	-0.40	-1.84	3.4	0.6	0.3	0.9	379	245	-0.40	-1.84	3.2	0.6	0.3	0.9	378	255	-0.40	-1.84	3.6	0.6	0.3	
12	804	95.13	139	42.38	3.2	13.30	0.9	0.2	0.7	1.4	1	0.7	15.80	11.27	-1.25	367	0.6	349	-1.30	-0.32	0.4	0.4	0.2	0.7	851	304	-1.30	-0.32	4.4	0.5	0.2	0.7	850	326	-1.30	-0.32	3.9	0.4	0.2	0.7	853	387	-1.30	-0.32	3.9	0.4	0.2	
13	405	105	805	85.84	5.8	25.31	0.9	0.2	0.6	1.1	1	0.7	30.45	21.68	1.4	4524	0.6	327	0.6	-1.01	5.2	0.3	0.2	0.9	977	325	-0.90	-0.19	4.5	0.5	0.2	0.8	504	845	-0.06	-1.14	5.3	0.3	0.2	0.9	104	816	-0.13	-1.48	5.1	0.3	0.2	
14	446	106	685	96.91	7.0	29.53	0.7	0.5	0.8	1.0	1	0.7	31.25	27.97	-1.54	622	0.7	110	-1.23	-0.46	5.1	0.4	0.2	0.8	320	778	-0.59	-0.97	5.8	0.2	0.1	0.9	134	192	-0.72	-0.89	5.7	0.3	0.1	0.9	131	123	-1.17	-0.49	5.2	0.4	0.2	
15	478	100	105	36.14	2.9	11.56	0.9	0.4	0.8	1.1	1	0.7	12.80	10.54	0.55	367	0.7	103	-1.46	-1.1	4.4	0.3	0.1	0.6	104	406	-1.46	-1.1	3.9	0.4	0.1	0.6	105	714	-1.38	0.00	4.5	0.2	0.1	0.6	778	277	-1.01	-0.39	4.5	0.1	0.1	
16	401	113	298	61.70	4.8	19.48	0.8	0.5	0.8	1.1	1	0.7	20.77	18.21	0.5	4524	0.7	548	-0.67	-1.04	5.3	0.2	0.1	0.9	214	365	-1.35	-0.05	4.4	0.4	0.1	0.7	309	114	-0.89	-0.70	5.3	0.2	0.1	0.8	241	247	-0.84	-0.64	5.3	0.2	0.1	
17	448	114	292	71.36	4.0	19.28	0.8	0.4	0.7	1.0	1	0.6	21.56	18.21	0.55	4524	0.7	461	-0.16	-1.38	5.0	0.2	0.2	0.9	215	417	-1.25	4.6	0.5	0.2	0.7	345	605	-0.82	-1.29	4.8	0.4	0.2	0.8	589	262	-0.46	-1.37	5.0	0.3	0.2		
18	419	121	455	82.98	5.4	24.07	0.7	0.6	0.9	1.0	1	0.7	25.26	23.27	-0.29	622	0.8	233	0.1	-1.34	5.3	0.3	0.2	0.9	117	650	-0.47	-1.38	5.4	0.3	0.2	0.9	133	248	0.1	-1.48	5.4	0.3	0.2	0.9	100	101	-1.03	0.91	5.0	0.4	0.2	
19	440	121	227	60.04	4.6	18.78	0.9	0.6	0.9	1.0	1	0.8	19.49	18.38	-1.36	622	0.8	252	-0.36	-1.18	5.3	0.2	0.1	0.8	233	645	-1.03	-3.0	5.3	0.2	0.1	0.8	271	308	-0.23	-1.51	5.3	0.2	0.1	0.8	252	140	-1.03	0.2	5.0	0.4	0.1	
20	463	127	389	71.94	5.4	22.26	0.6	0.6	0.9	1.0	1	0.7	23.04	21.65	0.9	4524	0.8	302	0.0	-1.23	5.5	0.2	0.1	0.9	139	686	-0.84	-0.77	5.5	0.2	0.1	0.8	242	362	0.00	-1.41	5.5	0.2	0.1	0.9	124	140	-0.74	-0.02	4.6	0.5	0.2	
21	398	126	279	59.70	4.6	18.85	0.9	0.6	0.9	1.0	1	0.7	19.53	18.21	-0.62	4524	0.8	524	-0.20	-1.52	5.2	0.2	0.1	0.9	199	250	-1.30	-3.1	4.1	0.4	0.1	0.7	631	619	-1.26	-0.35	5.1	0.3	0.1	0.7	513	469	-0.91	-0.65	5.2	0.2	0.1	
22	409	131	387	73.25	5.2	22.78	0.9	0.5	0.8	1.0	1	0.6	23.97	20.77	0.44	4524	0.7	395	0.3	-1.37	5.1	0.3	0.2	0.9	126	355	-0.69	-1.46	4.7	0.5	0.2	0.8	428	708	-0.54	-1.46	5.0	0.3	0.2	0.8	324	779	-0.11	-1.51	5.0	0.3	0.2	
23	403	128	339	67.36	4.6	20.80	0.8	0.3	0.6	1.0	1	0.7	25.25	18.06	1.3	4524	0.6	478	0.4	-1.30	4.9	0.3	0.2	0.9	135	359	-0.86	-1.11	4.6	0.5	0.2	0.8	349	635	-0.89	-1.13	5.0	0.4	0.2	0.8	498	319	-0.22	-1.25	5.1	0.3	0.2	
24	444	130	263	66.77	3.9	18.30	0.8	0.2	0.6	1.0	1	0.6	22.25	15.81	-0.42	622	0.6	334	-0.15	-1.74	4.6	0.3	0.2	0.8	346	340	-1.11	-4.6	0.4	0.3	0.8	263	365	0.00	-1.70	4.6	0.3	0.2	0.8	284	105	-0.49	-1.72	4.4	0.5	0.3		
25	437	130	263	60.53	4.3	18.30	0.9	0.5	0.8	1.0	1	0.6	19.49	17.53	-0.41	4524	0.7	397	-0.37	-1.47	4.8	0.3	0.2	0.8	251	436	-1.78	-1.31	4.7	0.4	0.2	0.8	497	620	-0.82	-1.31	4.4	0.5	0.2	0.8	562	336	-0.65	-1.38	4.8	0.3	0.2	
26	455	132	224	58.28	3.8	16.80	0.9	0.3	0.7	1.0	1	0.7	19.48	15.14	-0.26	622	0.6	312	-0.45	-1.32	5.1	0.2	0.1	0.8	401	388	-1.07	-0.85	4.6	0.4	0.2	0.8	739	540	-0.69	-1.15	5.1	0.2	0.1	0.8	299	540	-0.59	-1.15	5.1	0.2	0.1	
27	406	137	488	82.43	5.9	24.93	0.6	0.6	0.9	1.0	1	0.7	25.85	24.31	-0.98	622	0.8	343	-0.23	-1.29	5.7	0.2	0.1	0.9	109	478	-0.88	-0.89	5.7	0.2	0.1	0.9	177	371	-0.02	-1.48	5.7	0.2	0.1	0.9	121	113	-1.04	0.18	5.2	0.4	0.1	
28	421	139	92	89.25	6.6	27.95	0.4	0.4	0.8	1.0	1	0.7	29.71	25.46	-0.27	1161	0.7	323	-0.36	-1.24	5.7	0.2	0.1	0.9	115	783	-0.93	-0.87	5.6	0.3	0.1	0.9	210	137	-0.78	-1.02	5.7	0.2	0.1	0.9	202	115	-1.08	-0.62	5.4	0.4	0.2	

AI Trainer

Version: 2 Authors: ViQI

Train ML classification models on various data types.

Training the AI



1. Select data for processing:

Input data:

Select an Image

or

Select a set of Images

or even

Upload local Images



2. Parameters:

Annotation level: Objects (cells)

Objects origin: cell_segmentation

Specific annotations:

Ground truth

Update training with latest annotations:

3. Run algorithm:

STOP

Progress: 80.0% of Testing on *Two Head test*, (80/100)

Classified cells: Colored by class

Layers

- Overlay 1
- Annotations 1
- Cell masks 1
- Image 1

Enhancements for Image

Histogram

Channels

Channel	Count
Blue	3188
Cyan	567
Red	1689
Green	3339
White	2871

Enhancement

Brightness: [Slider]

Contrast: [Slider]

Threshold: [Slider]

Layer

Transparency: [Slider]

1:1

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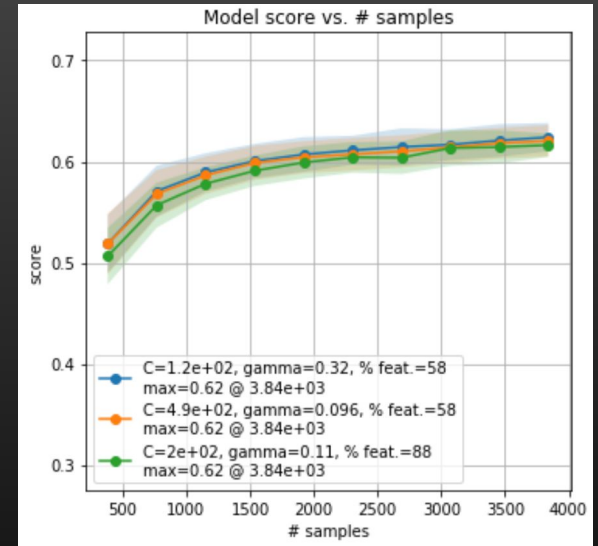
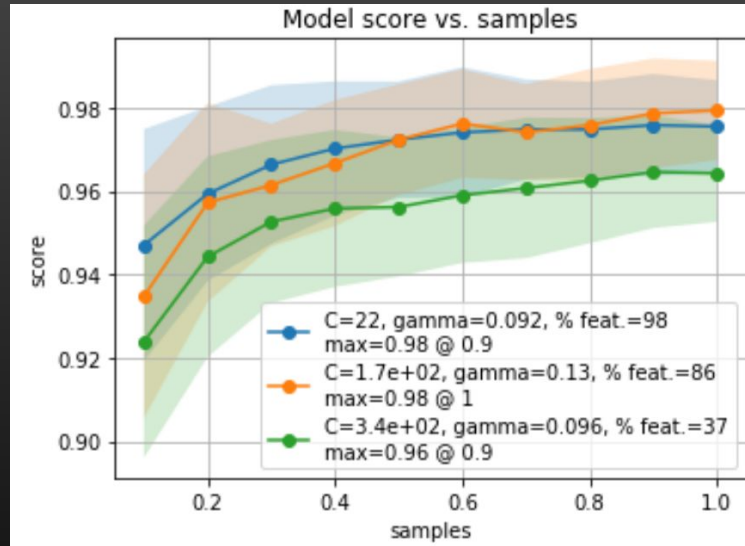
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AI Performance: Things to look for.

Training curve



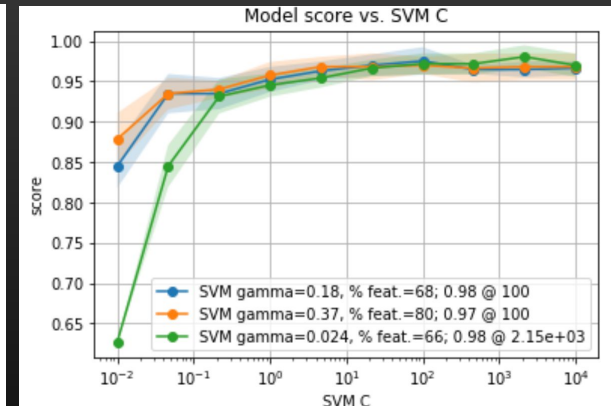
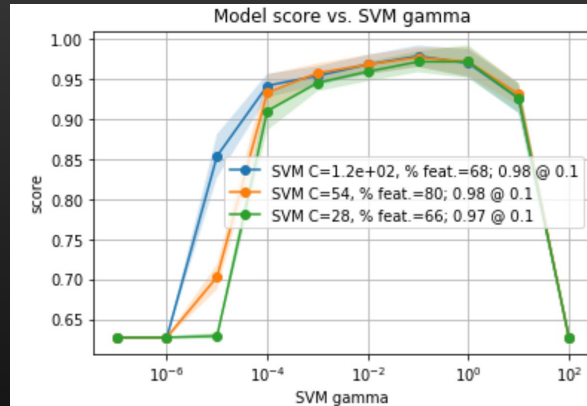
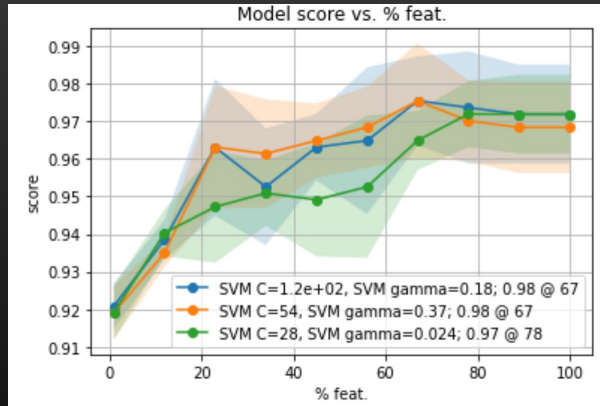
Was there enough data? Is it saturated?

Error bars: Is each subsample substantially different from the others?

If so, not enough data, or too much variation.

AI Performance: Things to look for.

Parameter Sensitivity (feature-based classifiers)



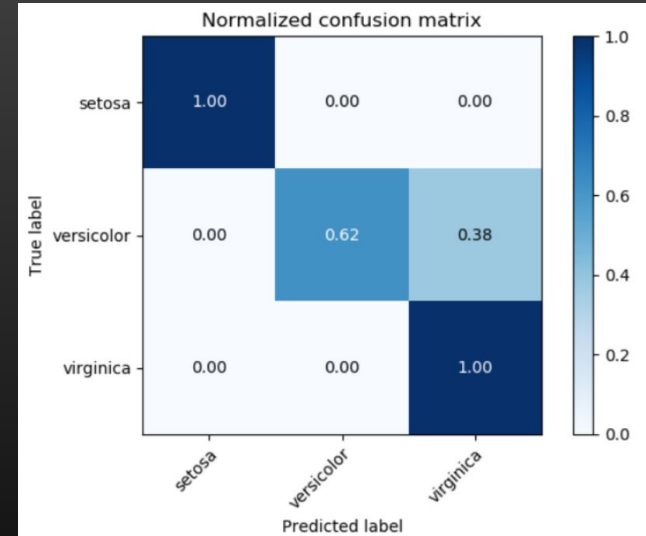
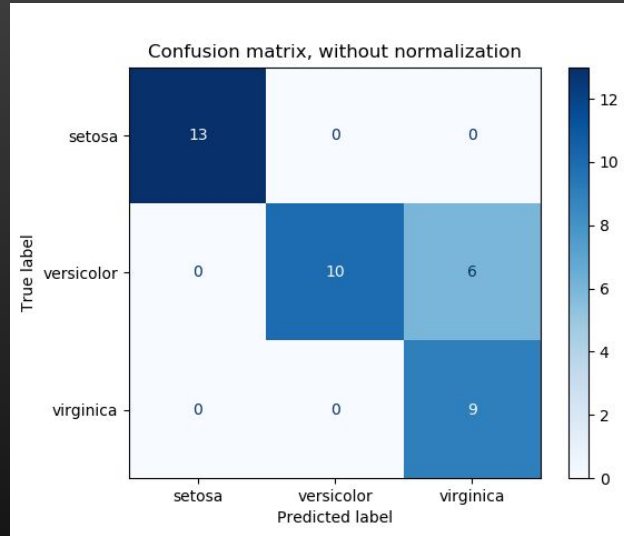
Are any parameters in sharp peaks?

May not generalize to a different sample.

Parameters in plateaus result in more robust models.

AI Performance: Things to look for.

Confusion matrix



Source: Scikit-learn

- Should confused classes be combined into a single class?
- Should a confused class be separated into subclasses?
- Should a classifier be trained just on the confused classes?

AI Performance: Things to look for.

Overtraining

How well does the predictor perform on new data?

- Cross-validation.
- Reserve some data and never let the AI see it during training: 10% at a bare minimum.
- Especially important for Deep Learning, because it is more prone to overtraining.
- Modern feature-based classifiers are very resistant to overtraining, or excess features.

Testing vs. validation in different fields.

- ML/AI/DL/DS:
 - Validation set: Internal subset used by the AI training algorithm.
 - Test set: an externally withheld set used to test the final trained AI.
- Regulatory, common use:
 - Validation is a higher standard than testing.
 - Validation set: Used to validate the final product.
 - Test sets: Used for "bench tests" - internal tests of the AI as it's being developed.

AI Performance: Things to look for.

Bias: Disproportionate class sizes

If a dataset is 90% Class A, and 10% Class B,
a predictor can be 90% accurate by always guessing Class A.

Some AIs claim to compensate for this, but it requires making assumptions that may not hold in your case.

Best defense is to force the classes to be equal sizes.

AI Performance: Things to look for.

Biases in data - hidden, known & unknown.

- Can result in biased models
- Random sampling may not be enough to correct for bias.

Measure the bias:

- Ask the AI if it can distinguish classes based on your bias variable
- If it can't then you have nothing to worry about.

Correct for known sources of bias:

- Confuse the AI with regards to bias.
 - Distribute the bias evenly in each class
- "Train out" known bias variables

Parting thought:

An AI will only perform as well as the accuracy of the class labels and the degree to which the training set represents reality.

Let's have another **audience poll**

Q&A |

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