

# Practical Applications for AI in High-Content Analysis: Dose-Response and Quantitative Assays

**Featuring Dr. Ilya Goldberg**

*Presented by*

**ViQi**

Debris  
point

# Your Webinar Host | ViQi

## Cloud-based large-scale image analysis software and expertise.

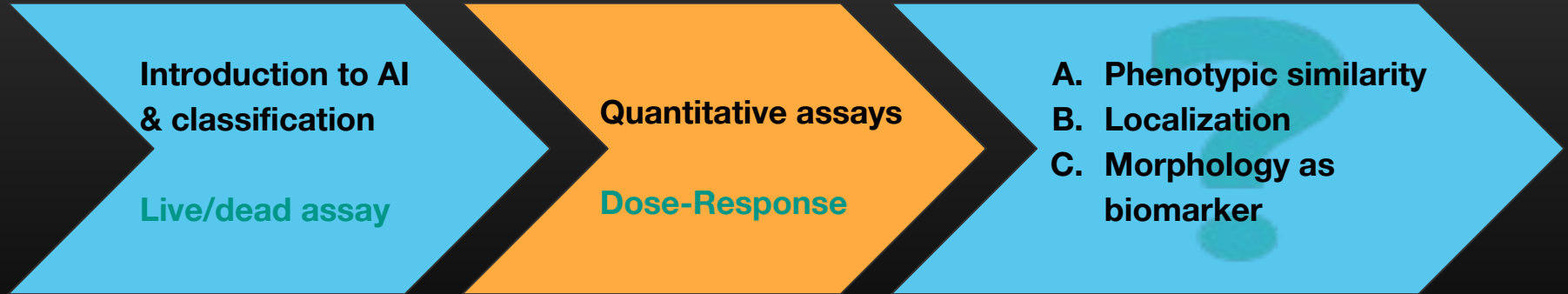
Our partners use ViQi to . . .

- Visualize, analyze, annotate, and store over 250 types of image and data formats in one central repository (including individual files over a terabyte in size)
- 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 Ilya brings to the table)



# A series of webinars for experimental biologists

How to use AIs for imaging problems in an experimental setting



# The Speaker | Dr. Ilya Goldberg

**Chief Science Officer, ViQi**

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

- Co-founded a startup that used AI to improve diagnosis of lung nodules in CT scans.
- Led research group at the NIH National Institute on Aging: Basic biology of aging, AI for biomedical images,
- At MIT, co-founded the OME project: Infrastructure for large image repositories and analysis.
- PhD in Cell Biology, Johns Hopkins School of Medicine
- 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

- Train and evaluate AIs for scoring a continuous morphological process.
- Score a multi-compound screen based on a dose-response standard curve.
- Score any type of phenotypic response without knowing what to look for a priori.

# Continuous variables from marginal probabilities: Risk estimation in case-control



Benign

| Class                          | Marginal Probability |
|--------------------------------|----------------------|
| Benign                         | 0.85                 |
| Malignant                      | 0.15                 |
| Malignant probability - "risk" | <b>0.15</b>          |



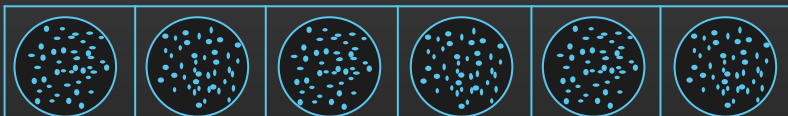
Malignant

| Class                          | Marginal Probability |
|--------------------------------|----------------------|
| Benign                         | 0.1                  |
| Malignant                      | 0.9                  |
| Malignant probability - "risk" | <b>0.9</b>           |

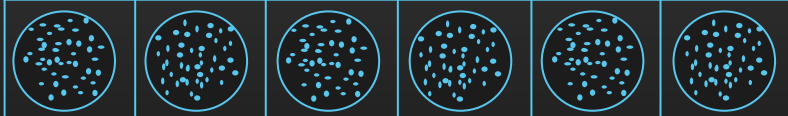
# Typical dose-response setup on an imaging plate

Concentrations: 0.0 0.1 1.0 10 100 1000

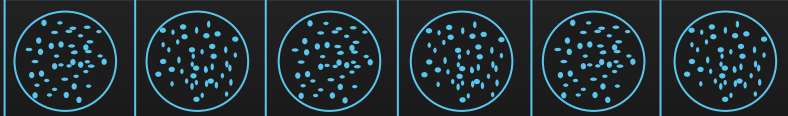
Positive Control



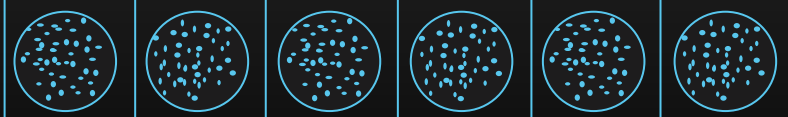
Negative Control



Drug #1 rep 1



Drug #1 rep 2



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# Compounds that affect neurons

The screenshot displays the ViQI software interface. The main window shows a fluorescence microscopy image of neurons, with a control panel on the left and a metadata table on the right.

**Control Panel (Left):**

- Layers:** Overlay, Annotations, Image.
- Enhancements for Image:** Histogram (0% to 100%), Channels (FTC, DAPI), Enhancement (Brightness, Contrast, Threshold), Layer (Transparency).

**Metadata Table (Right):**

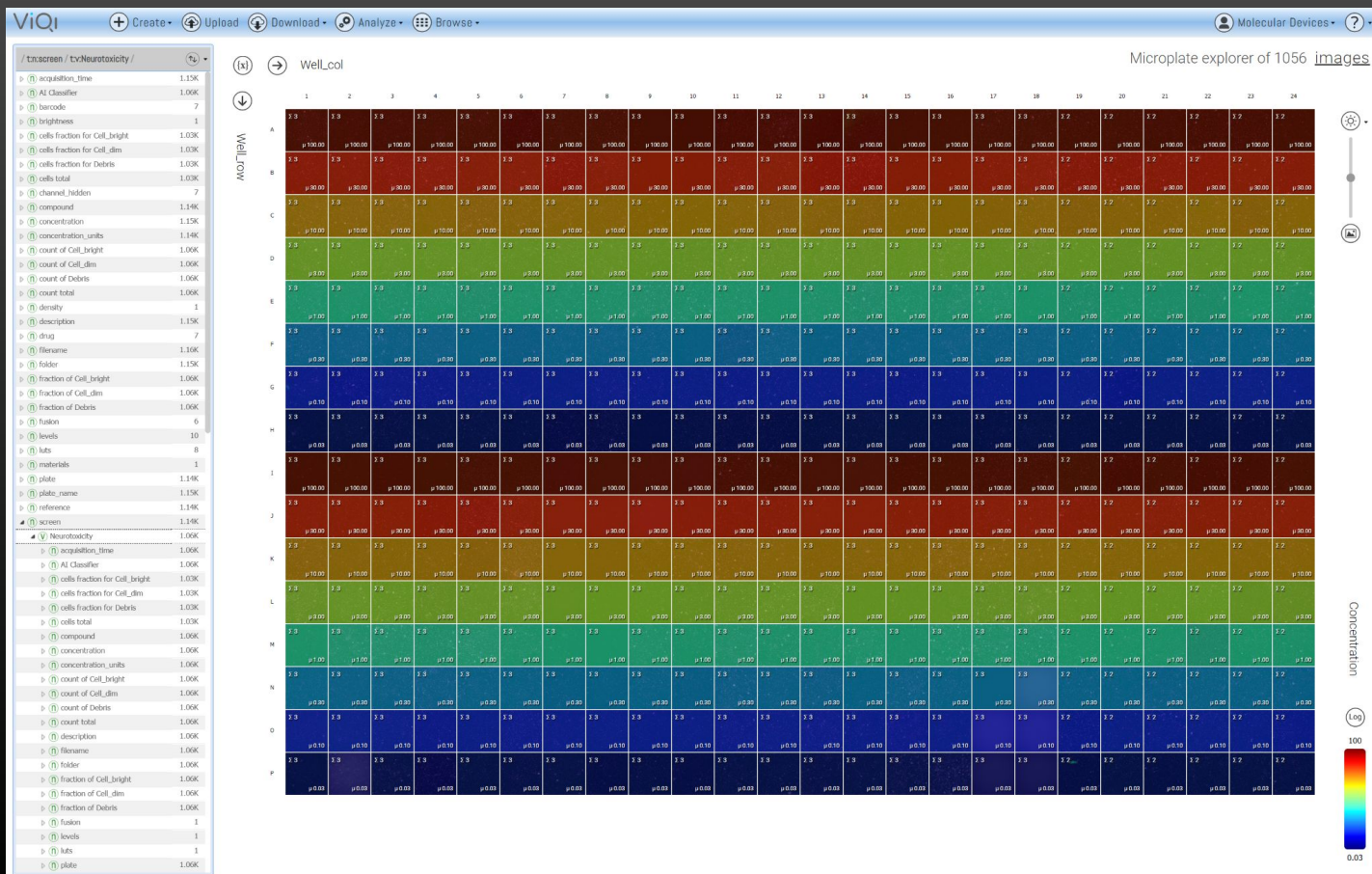
| Name                             | Value                                      |
|----------------------------------|--|
| T filename                       | neuropl1_con_F13                           |
| T upload_datetime                | 2020-05-02 21:39:53.955616                 |
| T screen_name                    | neuropl1 con                               |
| T description                    | plate1 Acquired from Andor-S83 Camera...   |
| T plate_name                     | neuropl1 con                               |
| T acquisition_time               | 20151102 12:31:12.673                      |
| T folder                         | neuropl1 con                               |
| T UniquePlateIdentifier          | 4c5ed798-f5dd-4d1d-62e4-812819546778       |
| T screen                         | Neurotoxicity                              |
| T plate                          | neuropl1 con                               |
| T well_label                     | F13  |
| T well_row                       | F  |
| T well_col                       | 13   |
| T well_ij                        | 6,13                                       |
| T compound                       | Dibenzaztracem                             |
| T concentration                  | 0.3  |
| T concentration_units            | uM   |
| T reference                      | https://www.ncbi.nlm.nih.gov/pmc/articl... |
| T cells_total                    | 1533                                       |
| T cells_fraction_for_Cell_bright | 0.20                                       |
| T cells_fraction_for_Cell_dim    | 0.45                                       |
| T cells_fraction_for_Debris      | 0.35                                       |

**Bottom Status Bar:** 28.8174 μm ( 1225.58, 867.25)px ( 840.91, 595.04)μm Values:( 1020, 396) ( 2, 10)%

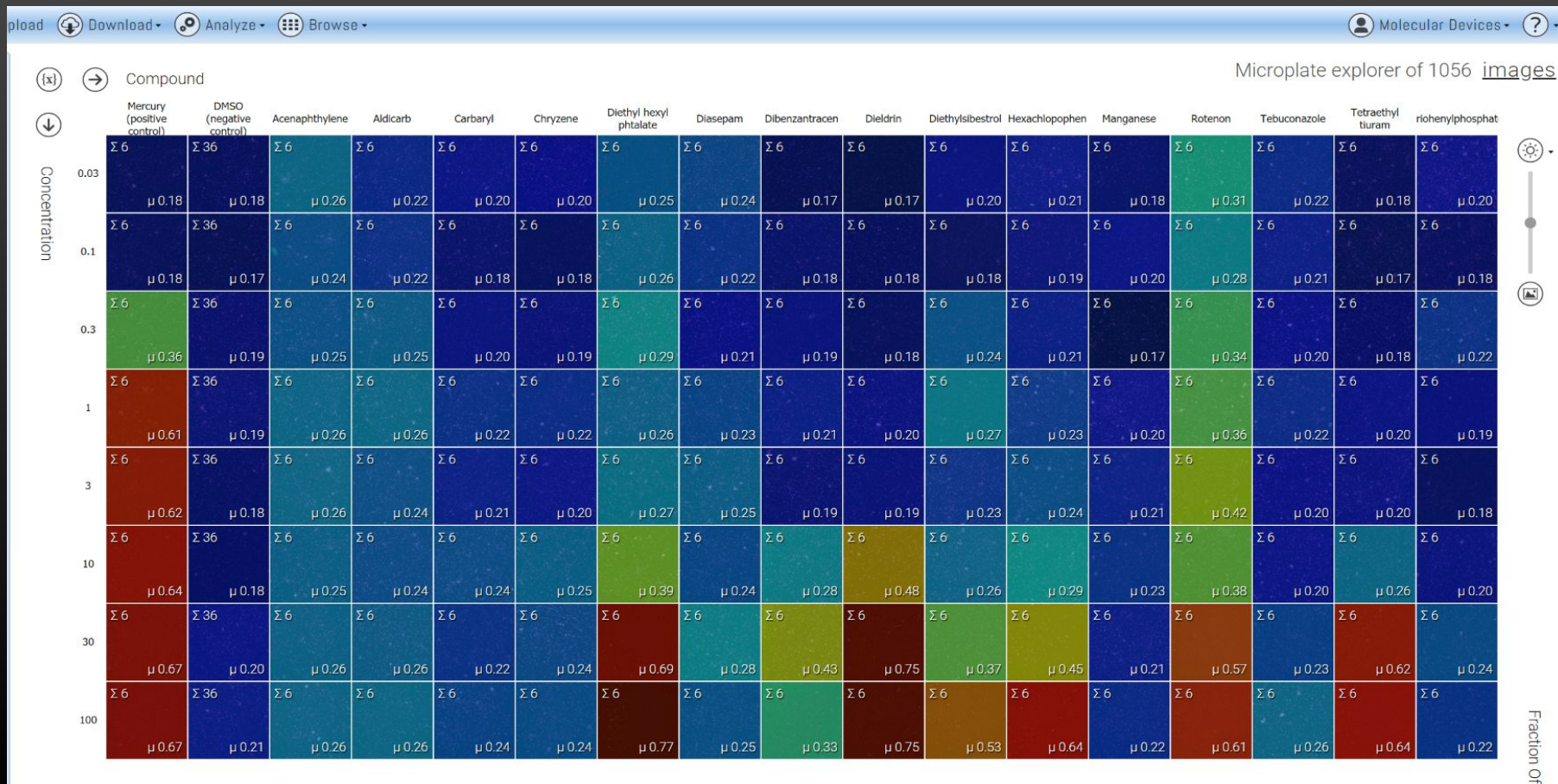
Data from:  
Molecular Devices



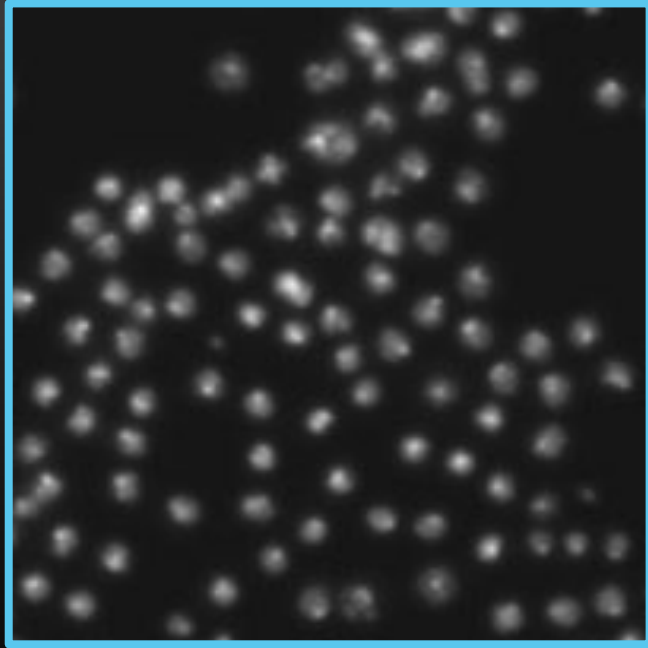
# Plate view: Rows, columns, concentrations



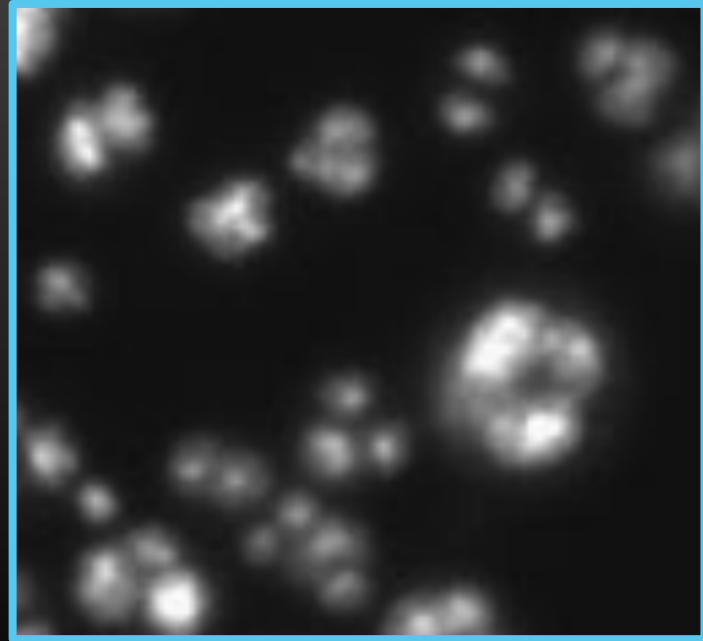
# Plate view: Compounds, concentrations and assay results



# Binucleate cells: An important target phenotype



**Normal**



**Binucleate**

# Continuous variables from marginal probabilities: Equivalent dose in dose-response

| Class Value          | Marginal Probability   | Formula  |
|----------------------|------------------------|--|
| Drug Concentration   | Single sample AI Score | Weighted Equivalent Drug Concentration:<br>Drug Concentration X Marginal Probability |
| 0.0                  | 0.001                  | $0.0 \times 0.001 = 0.0$   |
| 0.1                  | 0.001                  | $0.1 \times 0.001 = 0.0001$  |
| 1.0                  | 0.15                   | $1.0 \times 0.15 = 0.15$   |
| 10                   | 0.85                   | $10 \times 0.85 = 8.5$   |
| 100                  | 0.00001                | $100 \times 0.00001 = 0.001$   |
| 1000                 | 0.00001                | $1000 \times 0.00001 = 0.01$   |
| Equivalent Drug Dose |                        | <b>8.66</b>  |

# AI-Trainer user interface

ViQI + Create ↻ Upload ↓ Download 🔍 Analyze 📁 Browse 👤 Molecule

## AI Trainer

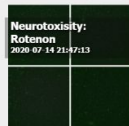
Version: 3 Authors: ViQI

*Train ML classification models on various data types.*

### 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: Image w/ objects (well)

Objects origin: ai\_classifier

Image (Well) level annotation: concentration

Discard classes: Debris

#### Annotations

Update training with latest annotations:

Update images with computed measures:

### 3. Run algorithm:

Run This may take some time, progress will be shown here.

## 2. Parameters:

Annotation level: Image w/ objects (well)

Objects origin: ai\_classifier

Image (Well) level annotation: concentration

Discard classes: Debris

#### Annotations

Update training with latest annotations:

Update images with computed measures:

## 3. Run algorithm:

Run

This may take some time, progress will be shown here.

# Confusion matrices: Positive controls

## Mercury

| Label | 0.03 | 0.1 | 0.3 | 1   | 3   | 10  | 30  | 100 | Accuracy |
|-------|------|-----|-----|-----|-----|-----|-----|-----|----------|
| 0.03  | 358  | 273 | 171 | 56  | 30  | 13  | 43  | 56  | 0.358    |
| 0.1   | 334  | 268 | 183 | 61  | 30  | 14  | 56  | 54  | 0.268    |
| 0.3   | 105  | 137 | 397 | 112 | 66  | 58  | 57  | 68  | 0.397    |
| 1     | 10   | 23  | 56  | 302 | 248 | 100 | 155 | 106 | 0.302    |
| 3     | 5    | 11  | 33  | 209 | 354 | 155 | 121 | 112 | 0.354    |
| 10    | 7    | 11  | 16  | 153 | 228 | 270 | 177 | 138 | 0.27     |
| 30    | 7    | 14  | 19  | 158 | 69  | 126 | 328 | 279 | 0.328    |
| 100   | 10   | 16  | 12  | 112 | 82  | 110 | 385 | 273 | 0.273    |

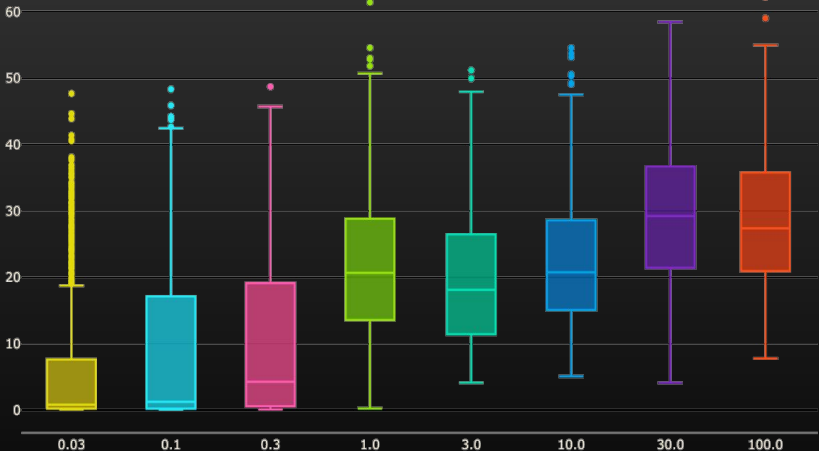
## Rotenone

| Label | 0.03 | 0.1 | 0.3 | 1   | 3   | 10  | 30  | 100 | Accuracy |
|-------|------|-----|-----|-----|-----|-----|-----|-----|----------|
| 0.03  | 161  | 200 | 187 | 121 | 148 | 128 | 25  | 30  | 0.161    |
| 0.1   | 224  | 124 | 207 | 124 | 141 | 116 | 34  | 30  | 0.124    |
| 0.3   | 130  | 138 | 228 | 193 | 171 | 107 | 13  | 20  | 0.228    |
| 1     | 111  | 88  | 258 | 187 | 166 | 115 | 38  | 37  | 0.187    |
| 3     | 127  | 65  | 214 | 186 | 170 | 148 | 47  | 43  | 0.17     |
| 10    | 131  | 56  | 111 | 121 | 148 | 299 | 93  | 41  | 0.299    |
| 30    | 6    | 0   | 1   | 22  | 38  | 31  | 443 | 459 | 0.443    |
| 100   | 2    | 1   | 1   | 7   | 25  | 19  | 429 | 516 | 0.516    |

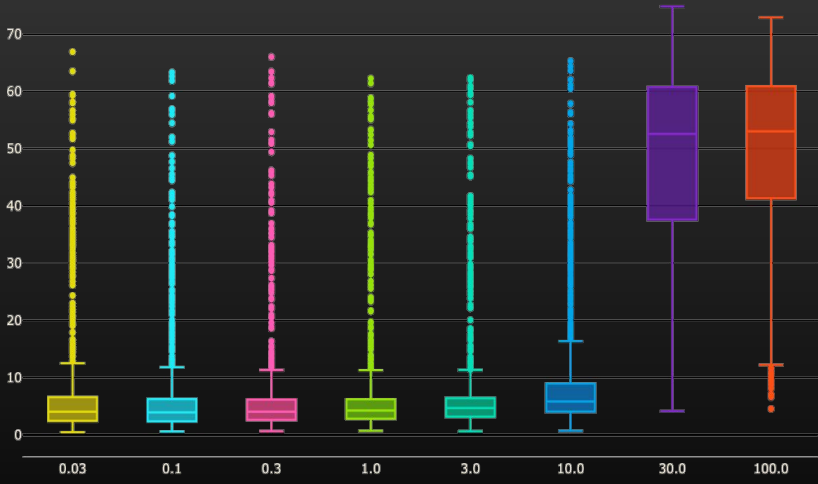


# Box plots for cell scores on a continuous scale

## Mercury

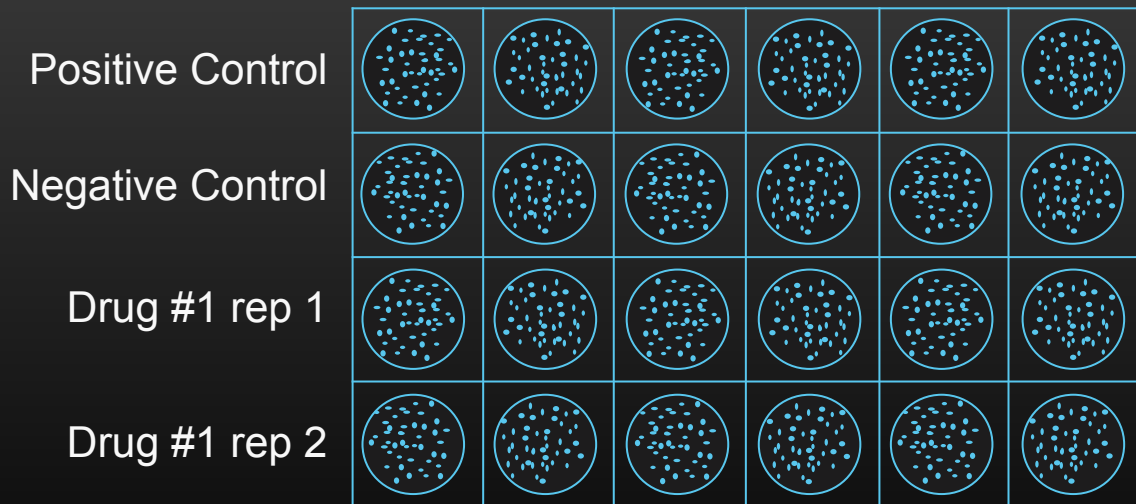


## Rotenone



# Scoring any phenotype

Concentrations: 0.0 0.1 1.0 10 100 1000



AI #1

AI #2

AI #3

AI #4

Each AI is trained independently and only used to score the drug it was trained with

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# Cell-based cross-validation

Concentrations:

0.0

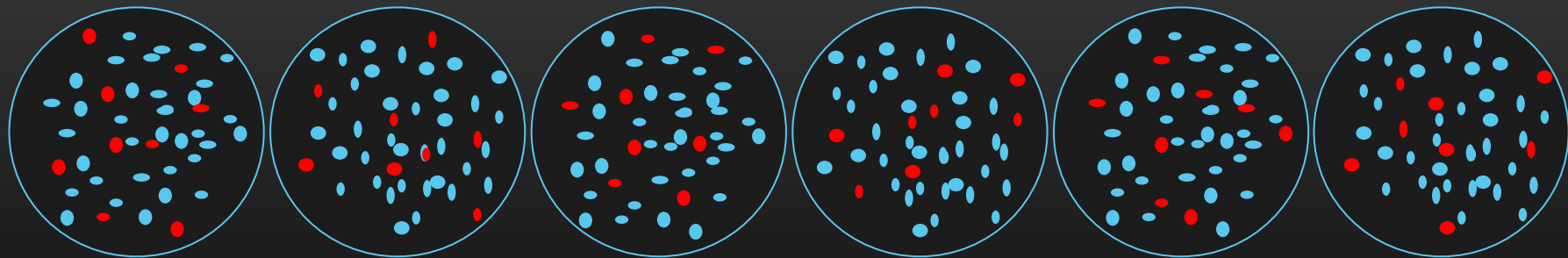
0.1

1.0

10

100

1000



**Training**

**Testing**

# Replicate-based cross-validation

Concentrations:

0.0

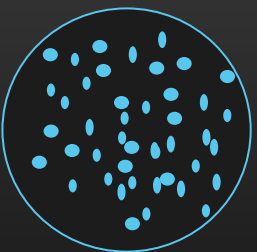
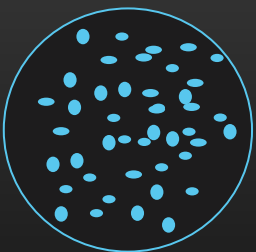
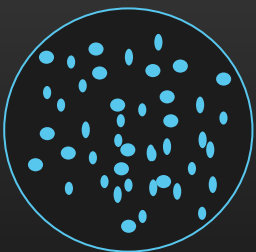
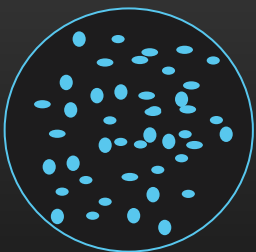
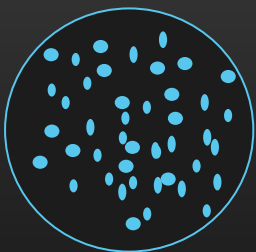
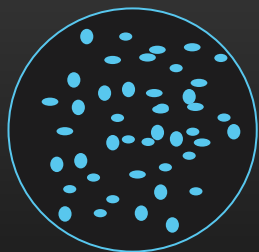
0.1

1.0

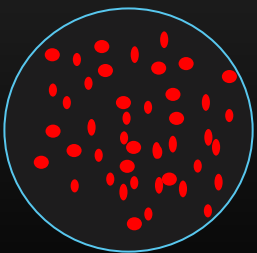
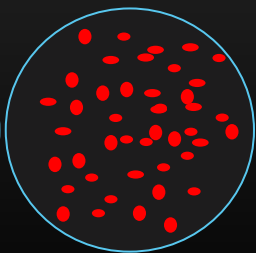
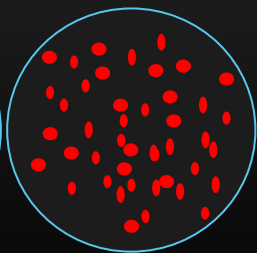
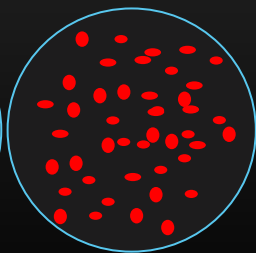
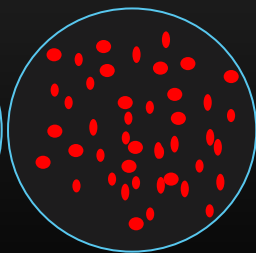
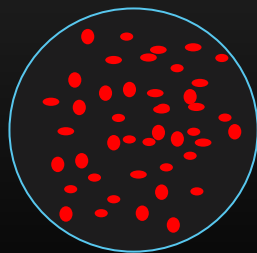
10

100

1000



Replicate #1  
Training



Replicate #2  
Testing

# Confusion Matrices: Negative control and non-responder

## DMSO

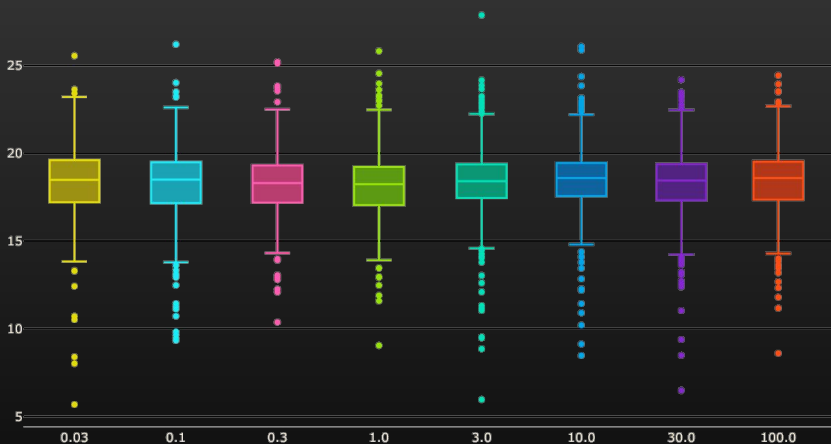
| Label | 0.03 | 0.1 | 0.3 | 1  | 3   | 10  | 30  | 100 | Accuracy |
|-------|------|-----|-----|----|-----|-----|-----|-----|----------|
| 0.03  | 90   | 139 | 178 | 69 | 124 | 97  | 72  | 231 | 0.09     |
| 0.1   | 115  | 167 | 122 | 50 | 80  | 148 | 100 | 218 | 0.167    |
| 0.3   | 123  | 52  | 169 | 42 | 80  | 163 | 135 | 236 | 0.169    |
| 1     | 102  | 135 | 129 | 44 | 94  | 167 | 125 | 204 | 0.044    |
| 3     | 137  | 114 | 115 | 36 | 104 | 162 | 112 | 220 | 0.104    |
| 10    | 107  | 91  | 185 | 81 | 132 | 86  | 73  | 245 | 0.086    |
| 30    | 90   | 77  | 179 | 50 | 109 | 102 | 108 | 285 | 0.108    |
| 100   | 74   | 89  | 143 | 23 | 98  | 111 | 114 | 348 | 0.348    |

## Tebuconazole

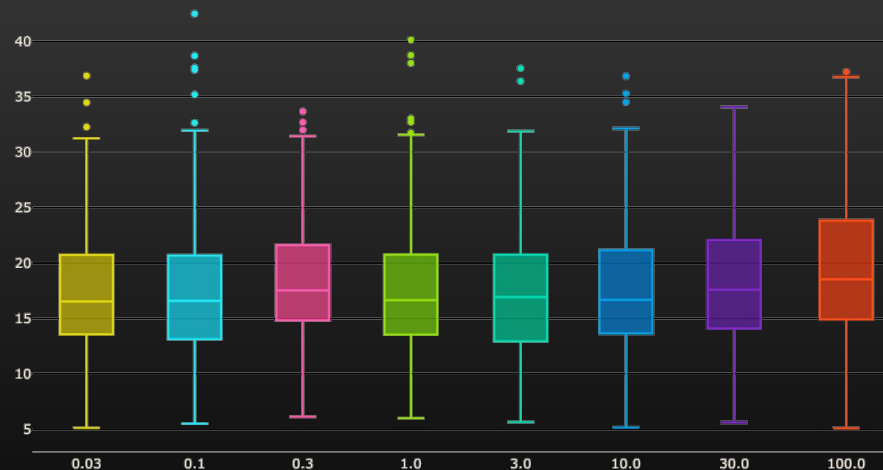
| Label | 0.03 | 0.1 | 0.3 | 1   | 3   | 10  | 30  | 100 | Accuracy |
|-------|------|-----|-----|-----|-----|-----|-----|-----|----------|
| 0.03  | 127  | 106 | 99  | 142 | 106 | 152 | 107 | 147 | 0.129    |
| 0.1   | 101  | 110 | 102 | 119 | 119 | 189 | 110 | 136 | 0.112    |
| 0.3   | 99   | 116 | 109 | 149 | 106 | 140 | 103 | 138 | 0.114    |
| 1     | 107  | 112 | 104 | 148 | 141 | 131 | 123 | 120 | 0.15     |
| 3     | 124  | 110 | 130 | 143 | 100 | 136 | 126 | 117 | 0.101    |
| 10    | 131  | 117 | 102 | 135 | 123 | 118 | 134 | 126 | 0.12     |
| 30    | 106  | 118 | 85  | 143 | 120 | 145 | 120 | 149 | 0.122    |
| 100   | 123  | 110 | 112 | 126 | 121 | 132 | 119 | 143 | 0.145    |

# Box plots: Negative control and non-responder

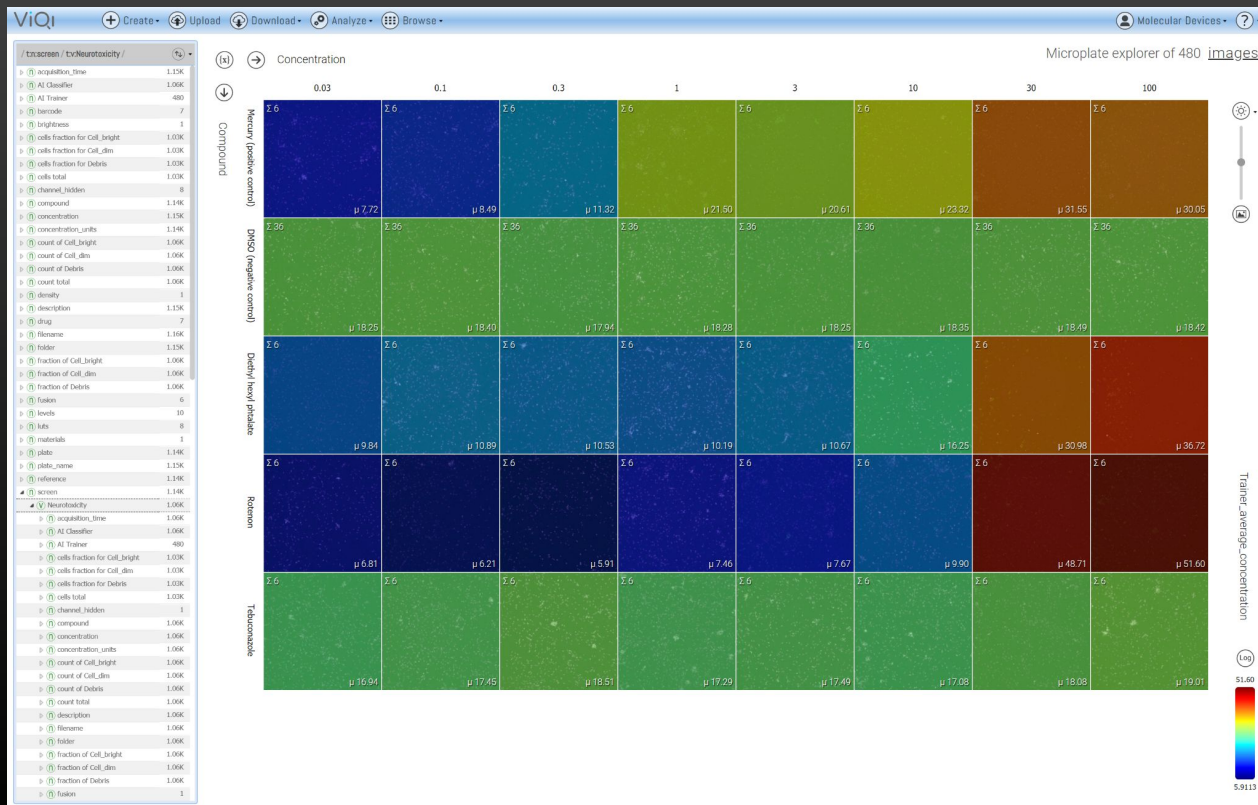
## DMSO



## Tebuconazole



# Assay summary for per-compound AIs



# Parting thoughts

- **You don't need preconceived notions about the phenotypes you expect in order to use AIs for quantitative assays. You don't even need to know if there will be an effect or not. The AI will tell you.**
- **With automated AI training, using AIs is conceptually more similar to typical experimental considerations compared to conventional image processing.**
  - Use positive controls or standard curves to train AIs. Always compare to negative controls.
  - Account for sample bias and sample variability when training.
  - Don't allow controls to contaminate your unknowns when validating and testing your AI.

ViQi |

Would you like to learn more?

Contact us at [info@viqui.org](mailto:info@viqui.org) or  
visit [viqui.org](http://viqui.org)

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