PUCL experimental flow platform

- Modified Coulter Elite flow sorter
- 32-channels of fluorescence
- 4 angles of forward scatter
- Side scatter
- Axial light loss

38 variables
Multicolor (polychromatic) vs. multispectral cytometry

- What is the difference between polychromatic and multispectral cytometry.
- Is it the number of colors?
Single intensity as a parameter (usually)

Spectrum as a parameter
Multianode PMT – sensitivity and uniformity

**Spatial Resolution and Cross-Talk Scan**

- **Dead Space**
- **Effective Space**
- **Signal**

**Cross-Talk Ratio (%)**

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**Cross-Talk Area B / Area A \times 100**

**Output Deviation (%)**

**Channel**

**Cathode Radiant Sensitivity (mA/W)**

**Quantum Efficiency (%)**

**Wavelength (nm)**
1. 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (CDCFA)
2. 5(6)-carboxy-4',5'-dimethylfluorescein (CDMFA)
3. 5-sulfofluorescein diacetate (SFDA)
4. Cell Tracker Green - 5-chloromethylfluorescein diacetate (CTG)
5. 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate, succinimidyl ester (DCF)
6. bis-(1,3-dibutylbarbituric acid)trimethine oxonol (DiBAC<sub>4</sub>(3))
7. 3,3'-dipentyloxacarbocyanine iodide (DiOC<sub>5</sub>(3))
8. 3,3'-dihexyloxacarbocyanine iodide (DiOC<sub>6</sub>(3))
9. Rhodamine 110
Scatter matrix

Matrix of 2-D scatterplot allow us to visualize all the possible combination of channels.

Result: we are overwhelmed by information, so the matrix is not very useful.
Principal components

The principal components with the highest variance, however, do not necessarily carry the greatest information to enable a discrimination between classes.
Linear discriminant analysis – can we use it for “gating”? 
Supervised vs. Unsupervised Learning

- Unsupervised learning (clustering)
  - The class labels of training data is unknown
  - Given a set of measurements, observations, etc. with the aim of establishing the existence of classes or clusters in the data

- Supervised learning (classification)
  - Supervision: The training data (observations, measurements, etc.) are accompanied by labels indicating the class of the observations
  - New data is classified based on the training set
Method: Automated classification using support vector machines

- The SVM algorithm creates a hyperplane that separates the data into *two* classes with the maximum-margin. The SVM idea was proposed by Vladimir Vapnik in 1963.

- For categorical variables a dummy variable is created with case values as either 0 or 1. Thus, a categorical dependent variable consisting of three levels, say (A, B, C), is represented by a set of three dummy variables: A: \{1 0 0\}, B: \{0 1 0\}, C: \{0 0 1\}.
A linear discriminant function divides the feature space by a hyperplane decision surface.

The discriminant function can be come quadratic or polynomial. The curse of dimensionality makes hard to capitalize on this flexibility in practice.
The final discriminant function is $g(x) = (x_1, x_2) = x_1 x_2$, and the decision hyperplane is defined by $g = 0$.

However, we preprocess the features mapping them to a space where they can be linearly separated.
Advantages of SVM
Confusion matrix

1. 5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate (CDCFA)
2. 5(6)-carboxy-4′,5′-dimethylfluorescein (CDMFA)
3. 5-sulfofluorescein diacetate (SFDA)
4. 5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate, succinimidyl ester (DCF)
5. Cell Tracker Green - 5-chloromethylfluorescein diacetate (CTG)
6. bis-(1,3-dibutylbarbituric acid)trimethine oxonol (DiBAC₄(3))
7. 3,3′-dipentyloxacarbocyanine iodide (DiOC₅(3))
8. 3,3′-dihexyloxacarbocyanine iodide (DiOC₆(3))
9. Rhodamine 110

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<th>SFDA</th>
<th>CTG</th>
<th>DCF</th>
<th>DiBAC43</th>
<th>DiOC₅(3)</th>
<th>DiOC₆(3)</th>
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<td>0.76%</td>
<td>2.72%</td>
<td>0.00%</td>
<td>6.04%</td>
<td>1.92%</td>
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<td>0.52%</td>
<td>0.04%</td>
<td>0.00%</td>
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<td>0.16%</td>
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Misclassified events

- Dichlorofluorescein diacetate
- Dimethylfluorescein diacetate
- Sulfofluorescein diacetate
- Dichlorofluorescein, succinimidyl ester
- Cell Tracker Green
- DiBAC4(3) - oxonol
- DiOC$_5$(3)
- DiOC$_6$(3)
- Rhodamine 110
SVM classification – summary

- Clean controls required (training samples)!
- One training and validation process in completed, classification can be performed “on fly”