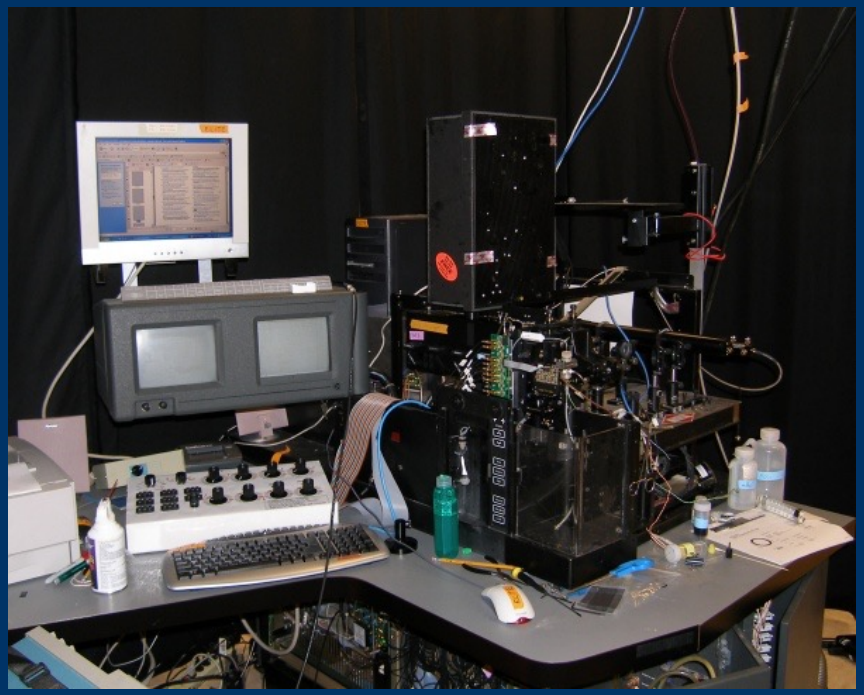
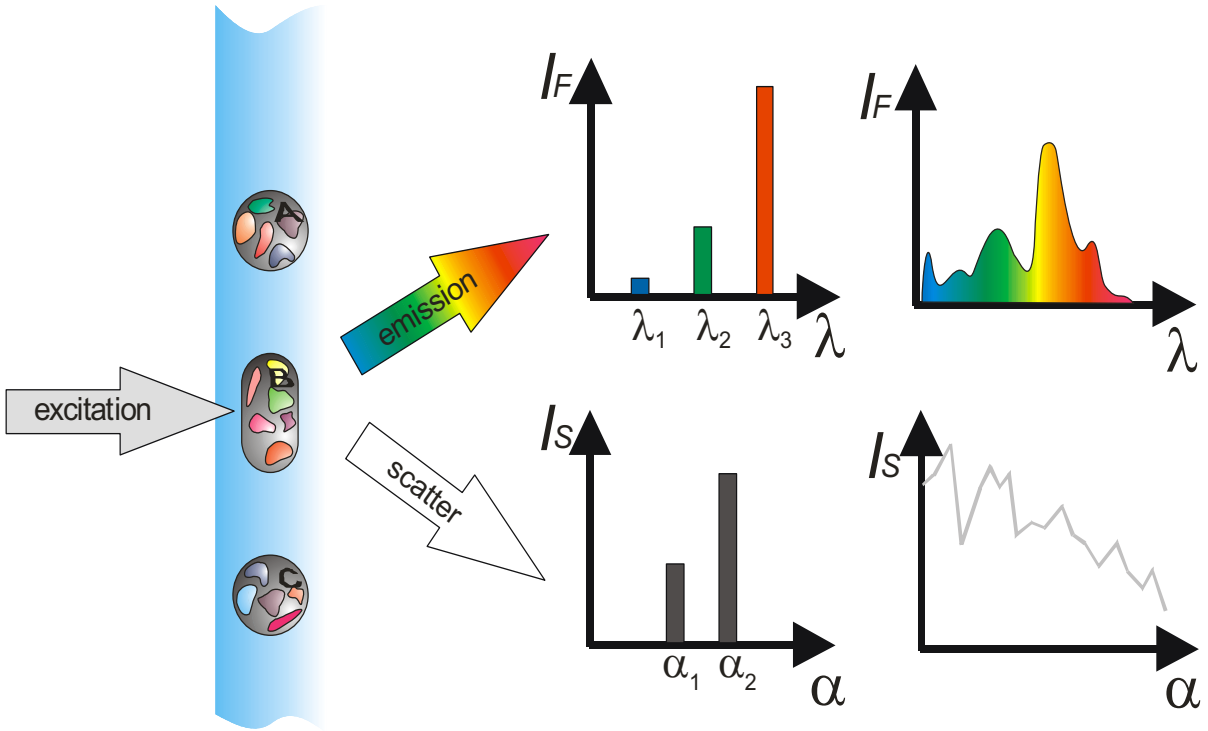
PUCL experimental flow platform



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

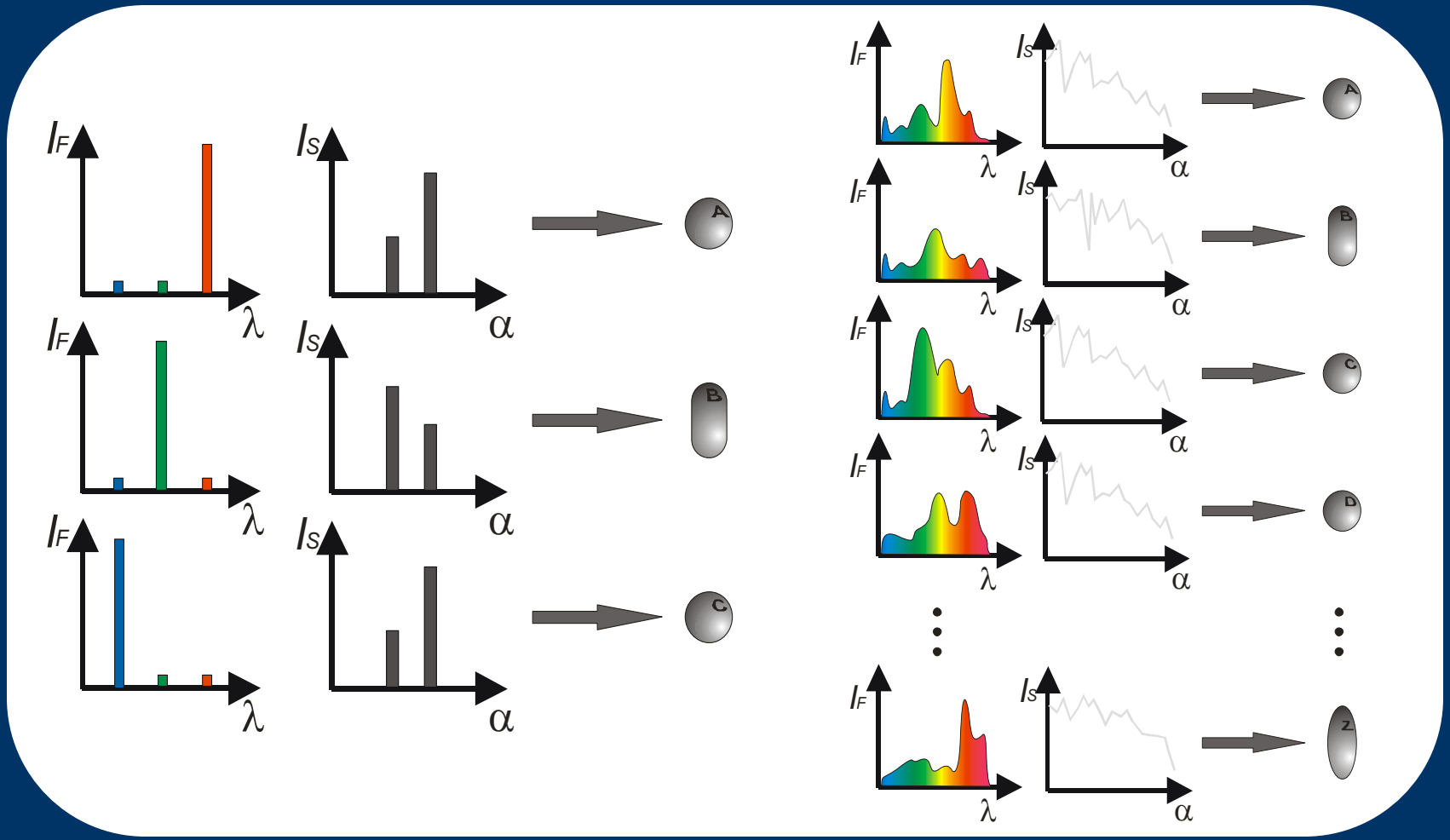
38 variables

Multicolor (polychromatic) vs. multispectral cytometry



- What is the difference between polychromatic and multispectral cytometry.
- Is it the number of colors?

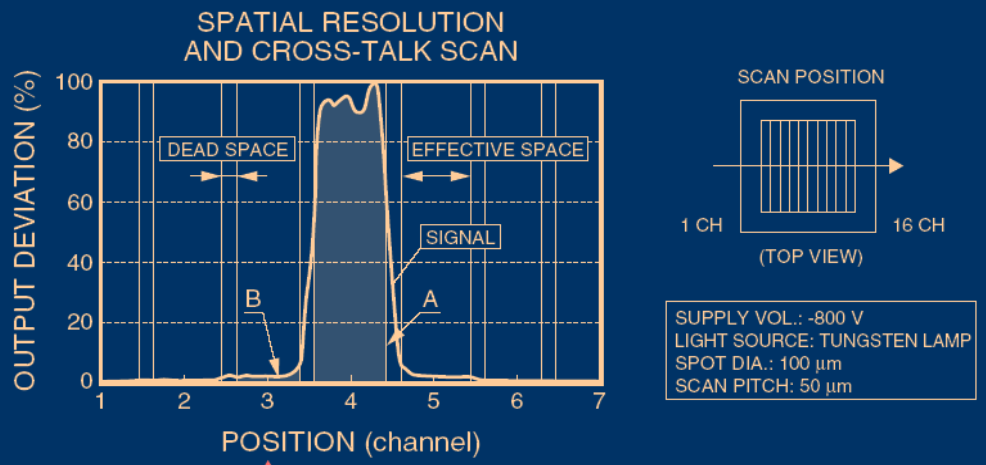
Multicolor (polychromatic) vs. multispectral cytometry (I)



Single intensity as a parameter (usually)

Spectrum as a parameter

Multianode PMT – sensitivity and uniformity

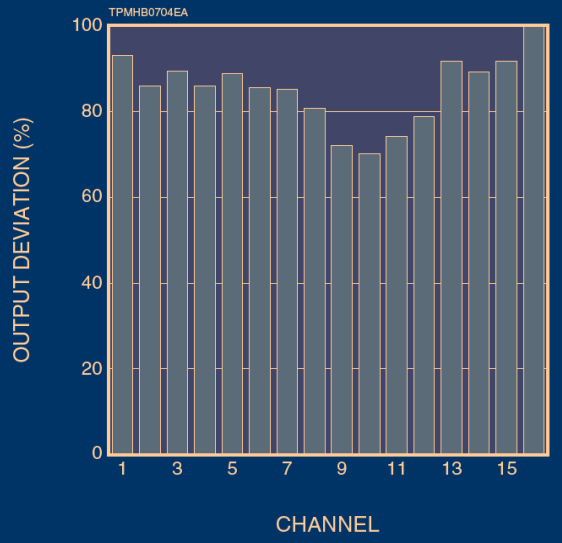


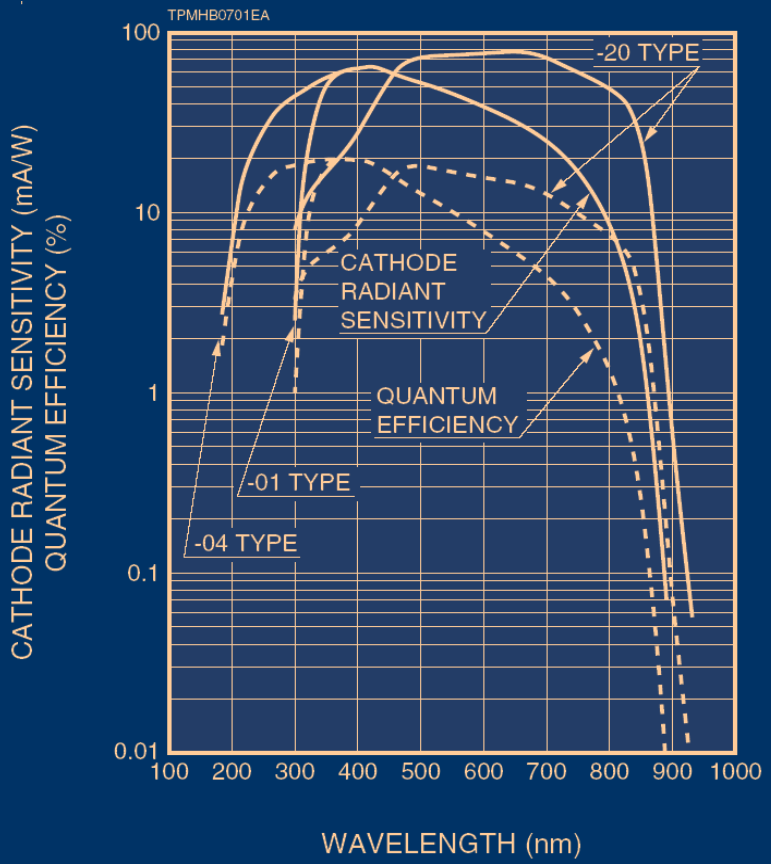
SUPPLY VOL.: -800 V
LIGHT SOURCE: TUNGSTEN LAMP
SPOT DIA.: 100 µm
SCAN PITCH: 50 µm

CROSS-TALK RATIO (%)

CH	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	100	2.9	0.6	0.2	0.1	—	—	—	—	—	—	—	—	—	—	—
2	2.9	100	3.1	0.5	0.2	0.1	—	—	—	—	—	—	—	—	—	—
3	0.8	2.8	100	2.8	0.6	0.2	0.1	—	—	—	—	—	—	—	—	—
4	0.3	0.8	2.7	100	3.2	0.6	0.2	0.1	—	—	—	—	—	—	—	—
5	0.1	0.3	0.8	2.9	100	3.1	0.6	0.2	0.1	—	—	—	—	—	—	—
6	—	0.1	0.3	0.8	2.7	100	3.0	0.6	0.2	0.1	—	—	—	—	—	—
7	—	—	0.1	0.3	0.8	2.7	100	3.0	0.6	0.2	0.1	—	—	—	—	—
8	—	—	—	0.1	0.3	0.8	2.9	100	2.9	0.6	0.2	0.1	—	—	—	—
9	—	—	—	—	0.1	0.3	0.8	2.9	100	2.9	0.6	0.2	0.1	—	—	—
10	—	—	—	—	—	0.1	0.3	0.8	3.1	100	2.7	0.6	0.2	0.1	—	—
11	—	—	—	—	—	—	0.1	0.4	0.8	3.3	100	3.8	0.6	0.2	0.1	—
12	—	—	—	—	—	—	—	0.1	0.4	0.9	3.2	100	2.8	0.6	0.2	0.1
13	—	—	—	—	—	—	—	—	0.1	0.4	0.8	3.1	100	2.8	0.6	0.3
14	—	—	—	—	—	—	—	—	—	0.1	0.4	0.8	3.1	100	2.7	0.6
15	—	—	—	—	—	—	—	—	—	—	0.1	0.4	0.9	3.2	100	2.9
16	—	—	—	—	—	—	—	—	—	—	—	0.1	0.4	0.9	3.1	100

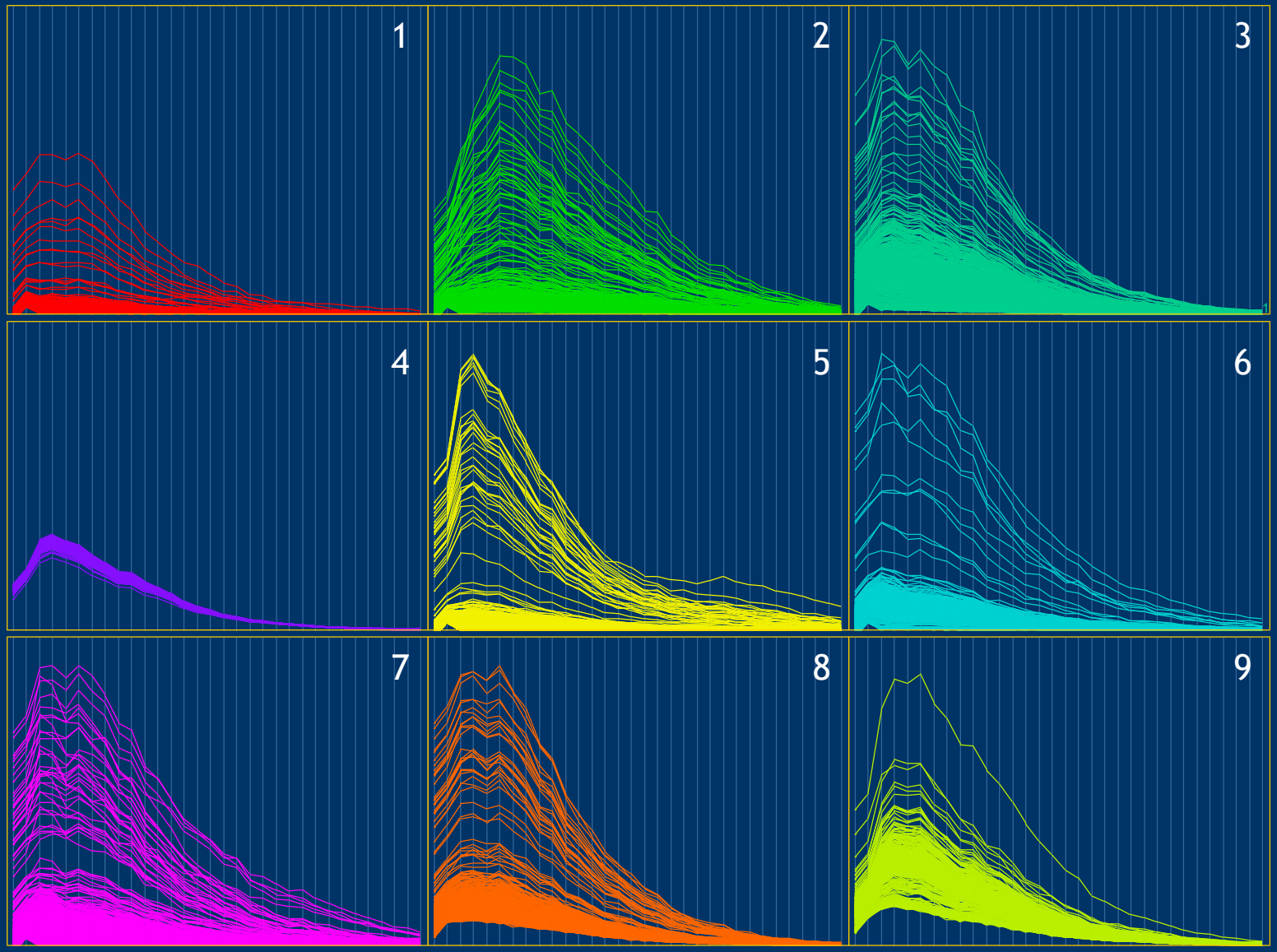
CROSS-TALK
AREA B / AREA A × 100



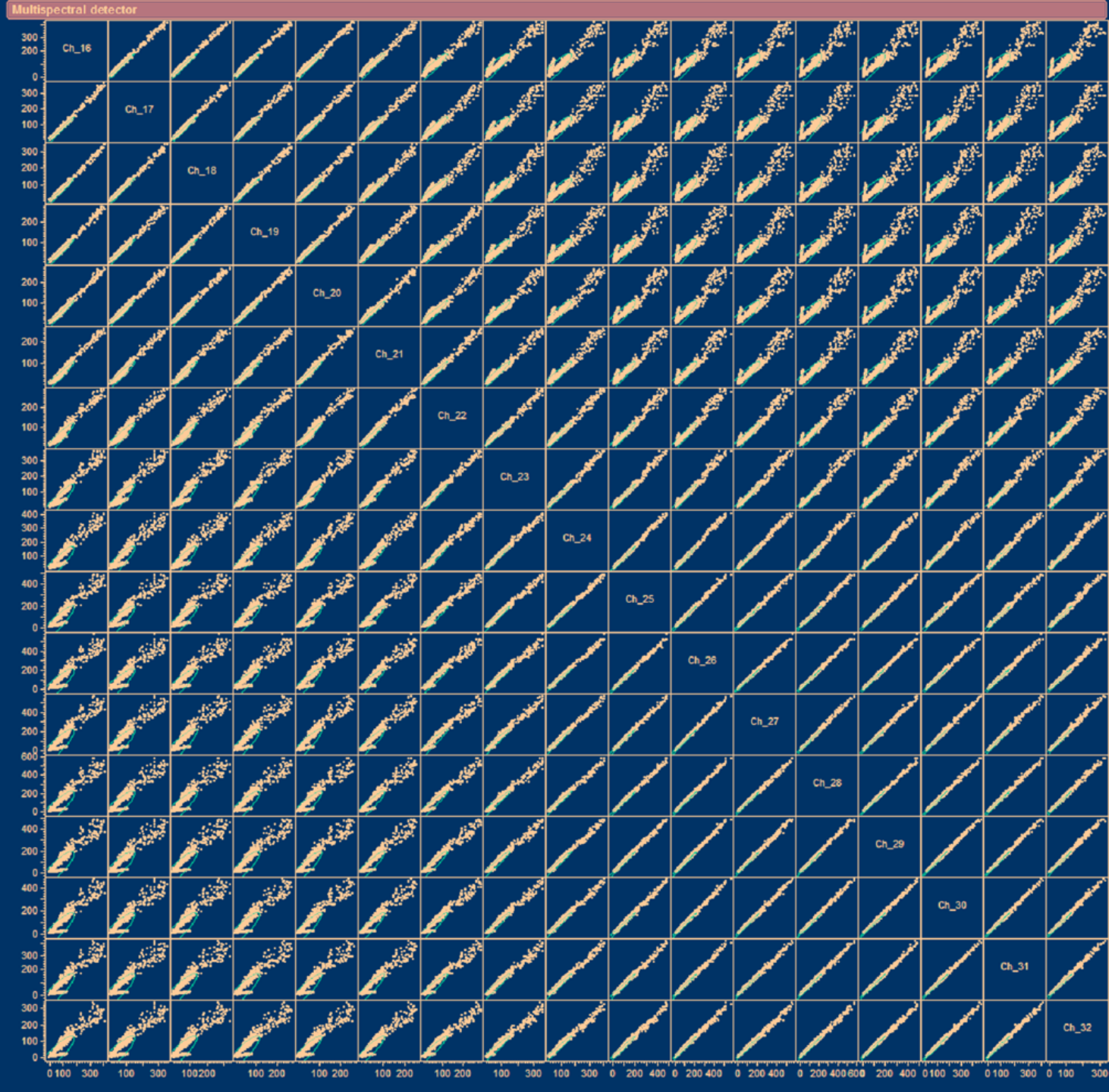


Spectral plots

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 ($DiBAC_4(3)$)
7. 3,3'-dipentyloxacarbocyanine iodide ($DiOC_5(3)$)
8. 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$)
9. Rhodamine 110



Scatter matrix

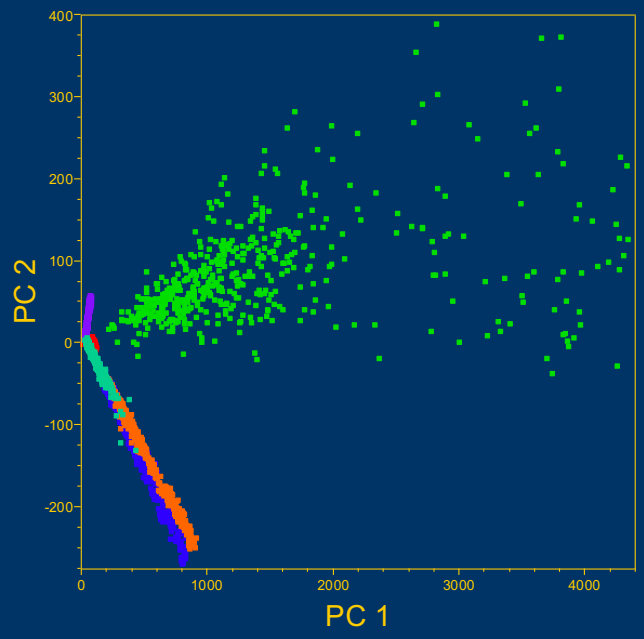


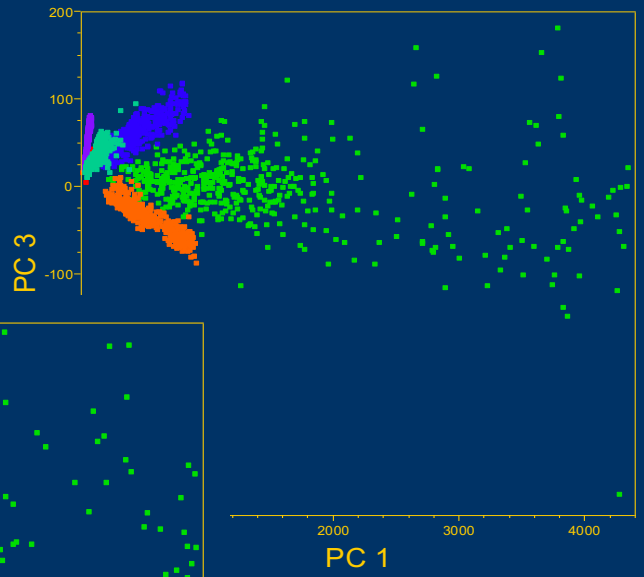
Matrix of 2-D scatterplot allow us to visualize all the possible combination of channels.

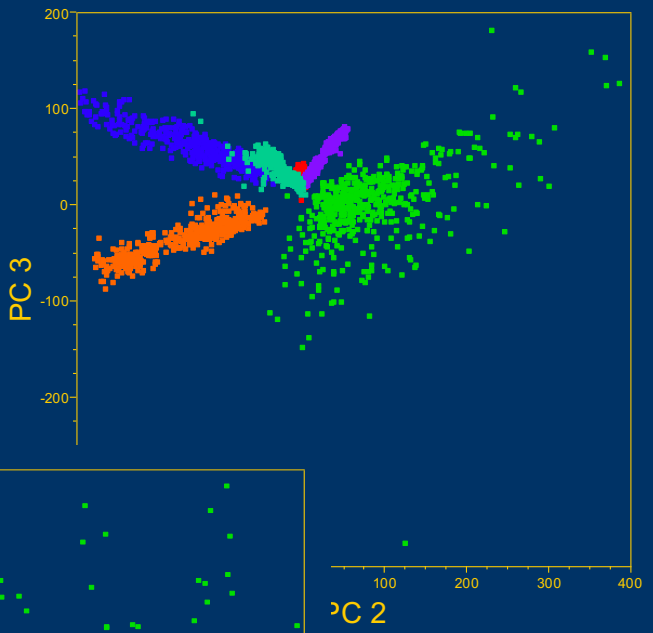
Result: we are overwhelmed by information, so the matrix is not very useful

Principal components

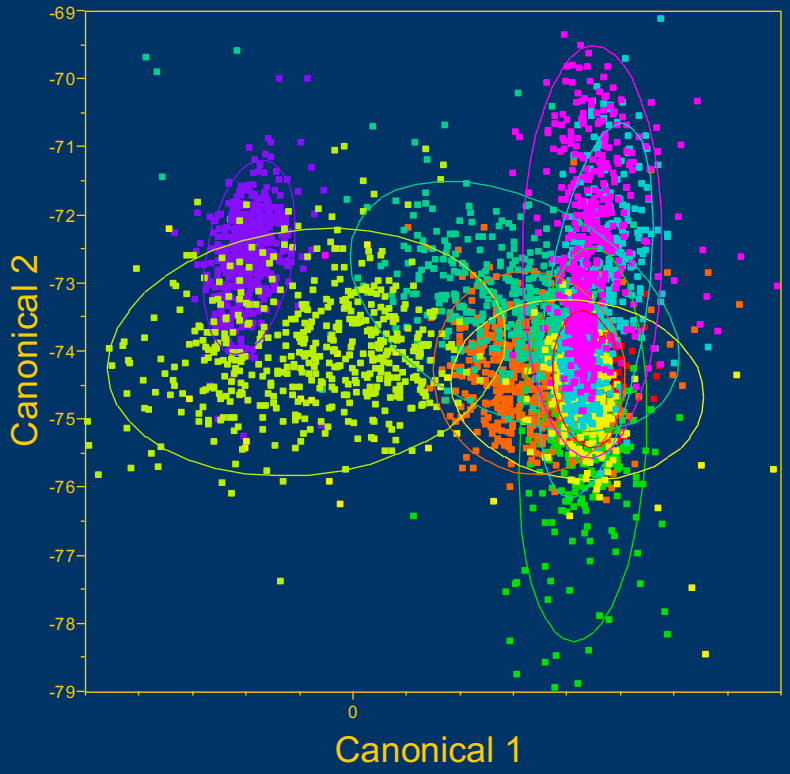
The principal components with the highest variance, however, do not necessarily carry the greatest information to enable a discrimination between classes.

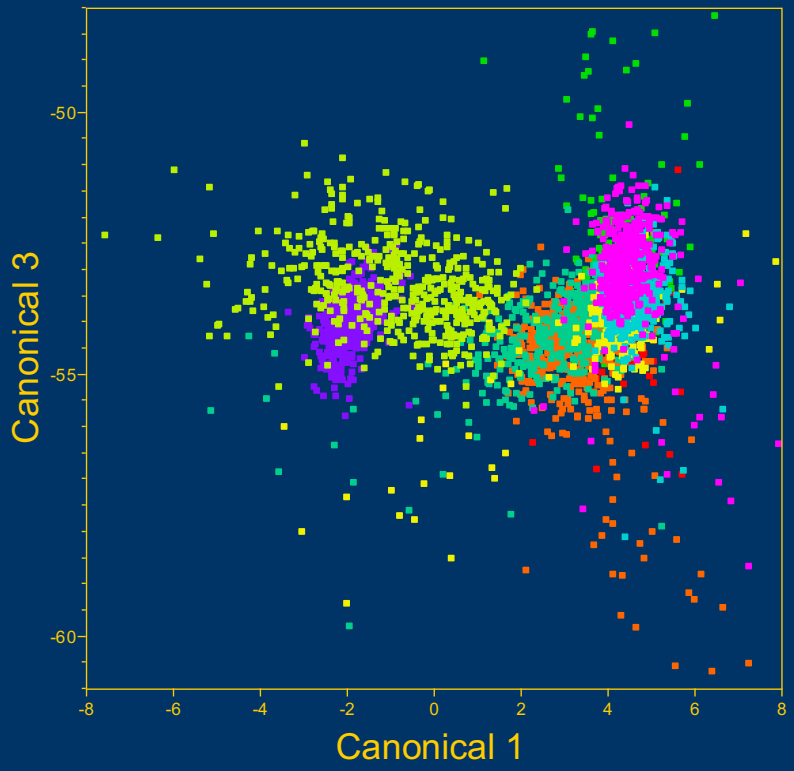






Linear discriminant analysis – can we use it for “gating”?





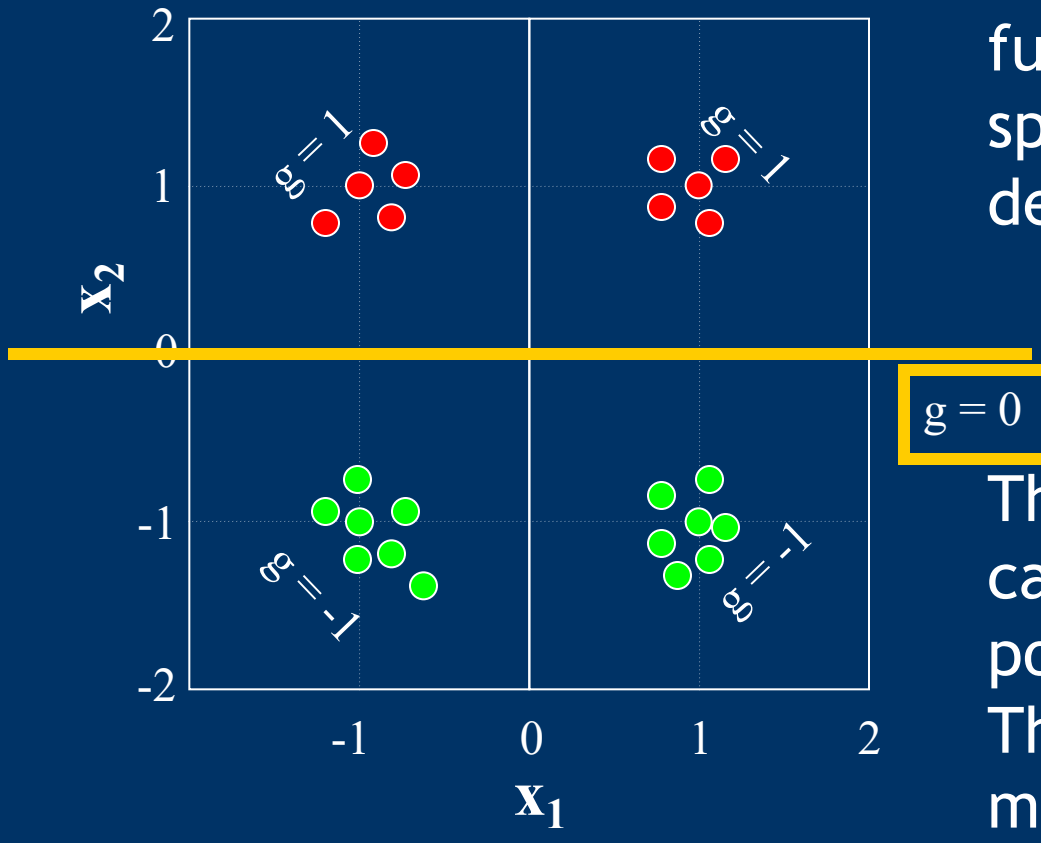
Supervised vs. Unsupervised Learning

- 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}

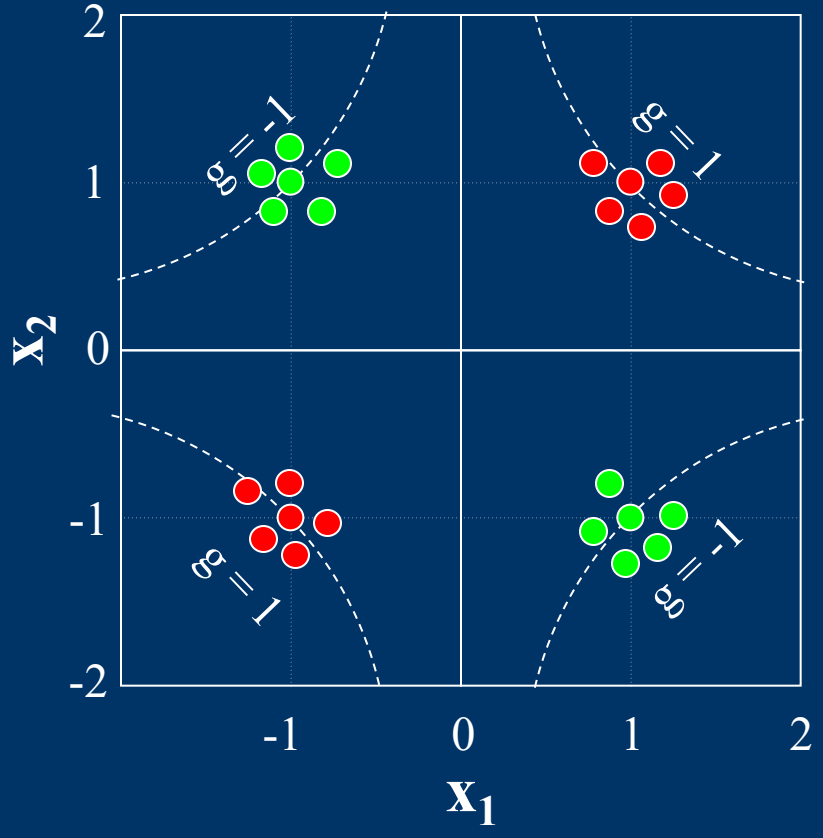
Classification problem



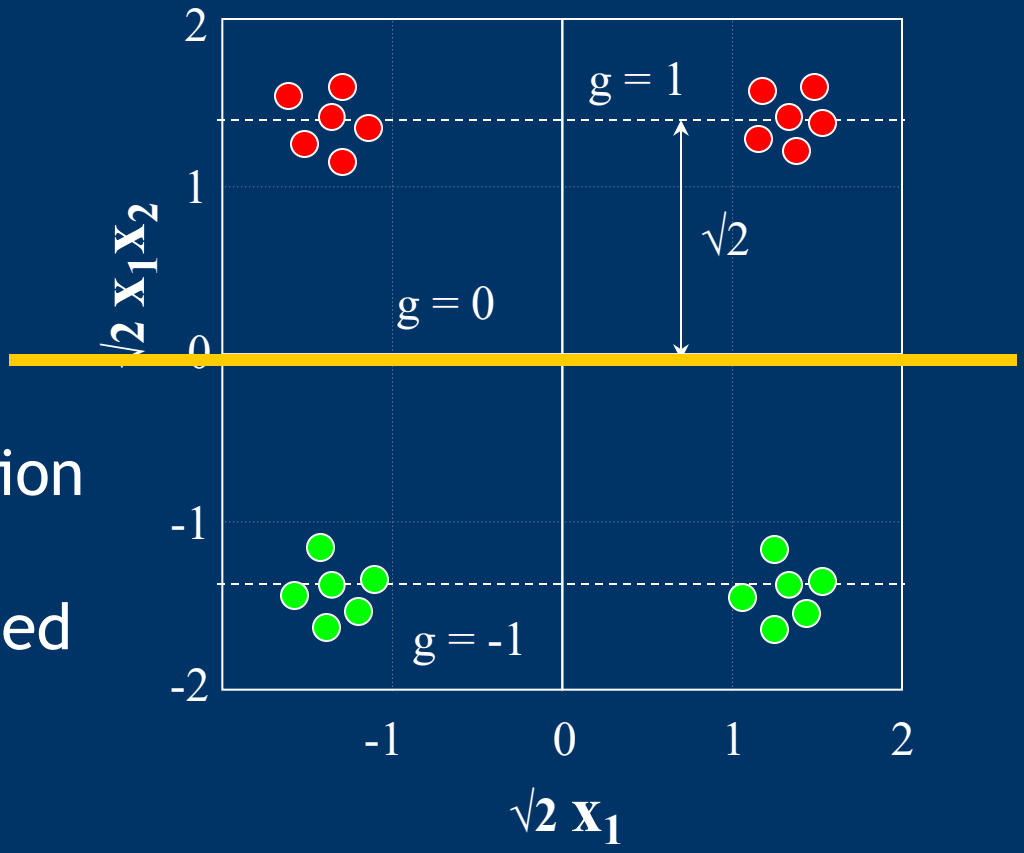
A linear discriminant function divides the feature space by a hyperplane decision surface.

The discriminant function can be come quadratic or polynomial.

The curse of dimensionality makes hard to capitalize on this flexibility in practice

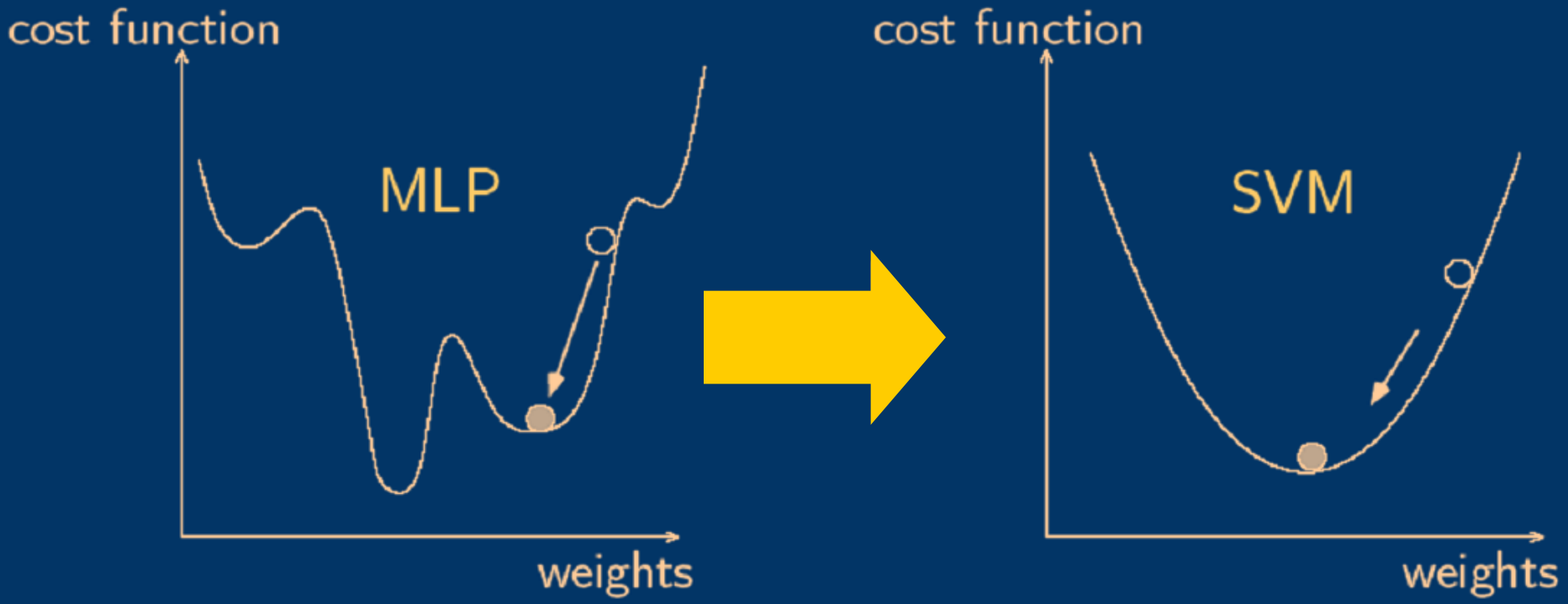


However, we preprocess the features mapping them to a space where they can be linearly separated



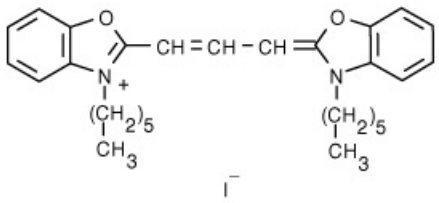
The final discriminant function is $g(\mathbf{x})=(x_1,x_2)=x_1x_2$, and the decision hyperplane is defined by $g=0$.

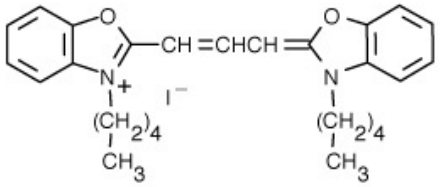
Advantages of SVM



Confusion matrix

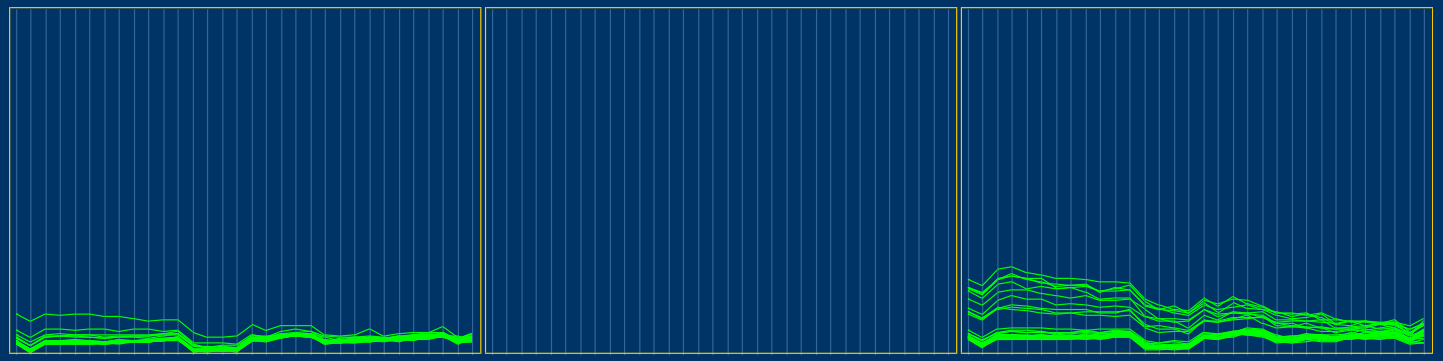
1. 5-(and-6)-carboxy-2´,7´-dichlorofluorescein diacetate (CDCFA)
2. 5(6)-carboxy-4',5'-dimethylfluorescein (CDMFA)
3. 5-sulfofluorescein diacetate (SFDA)
4. 5-(and-6)-carboxy-2´,7´-dichlorofluorescein diacetate, succinimidyl ester (DCF)
5. Cell Tracker Green – 5-chloromethylfluorescein diacetate (CTG)
6. bis-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4(3)$)
7. 3,3'-dipentyloxacarbocyanine iodide ($DiOC_5(3)$)
8. 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$)
9. Rhodamine 110



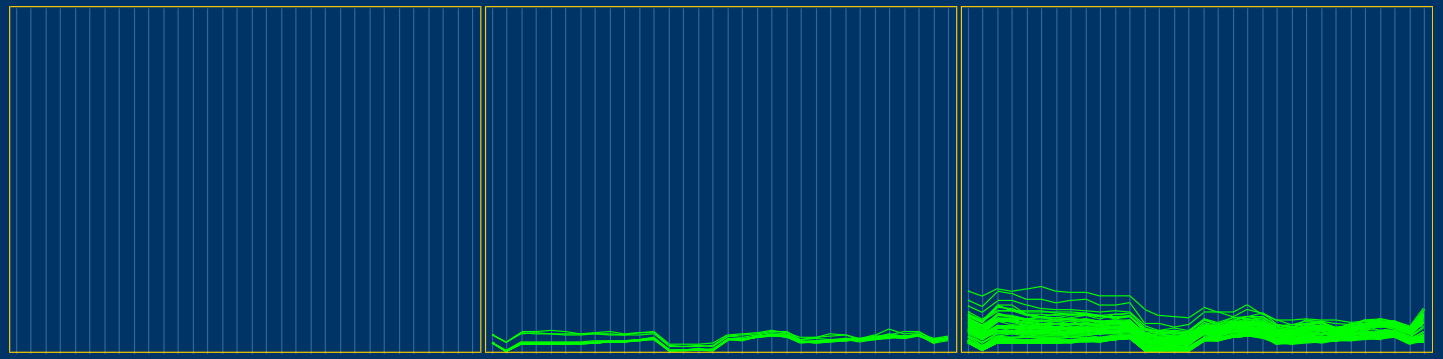


	CDCFA	CDMFA	SFDA	CTG	DCF	DiBAC43	$DiOC_5(3)$	$DiOC_6(3)$	RH110
CDCFA	**87.92%**	0.00%	0.76%	2.72%	0.00%	6.04%	1.92%	0.64%	0.00%
CDMFA	0.24%	**97.76%**	0.52%	0.04%	0.00%	0.00%	0.16%	0.88%	0.40%
SFDA	0.04%	0.00%	**94.36%**	4.88%	0.00%	0.00%	0.00%	0.72%	0.00%
CTG	5.44%	0.00%	5.04%	**86.44%**	0.00%	0.20%	0.80%	2.04%	0.04%
DCF	0.00%	0.00%	0.00%	0.00%	**100.00%**	0.00%	0.00%	0.00%	0.00%
DiBAC43	3.72%	0.20%	0.04%	0.40%	0.00%	**92.76%**	0.96%	1.92%	0.00%
$DiOC_5(3)$	4.12%	0.28%	0.56%	1.92%	0.00%	1.32%	**77.60%**	14.20%	0.00%
$DiOC_6(3)$	1.92%	0.12%	0.76%	1.72%	0.00%	1.24%	17.72%	**76.52%**	0.00%
RH110	0.00%	0.00%	0.08%	0.36%	0.00%	0.00%	0.00%	0.20%	**99.36%**

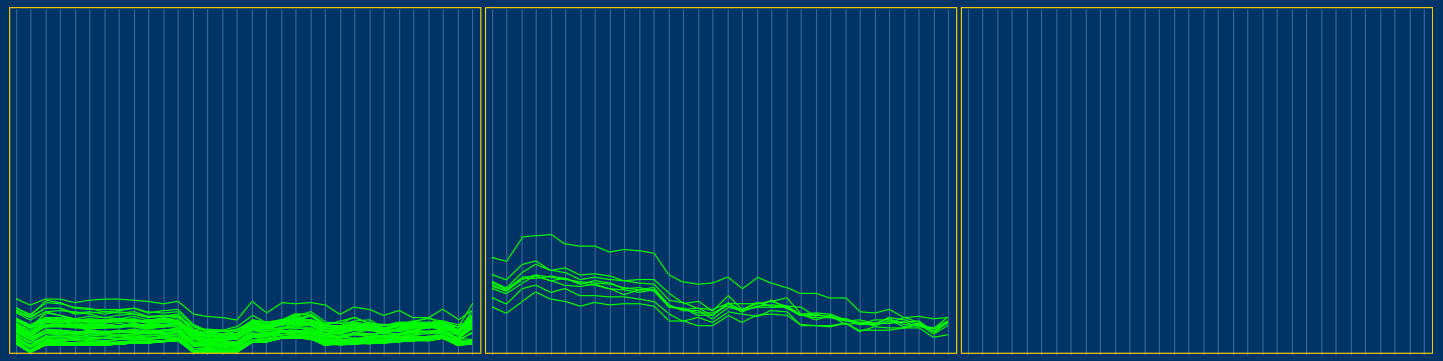
Misclassified events



Dichlorofluorescein diacetate | Dimethylfluorescein diacetate | Sulfofluorescein diacetate



Cell Tracker Green | Dichlorofluorescein, succinimidyl ester | 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”