# Sample Size Considerations
## for
# Raman Spectroscopic Cell Identification

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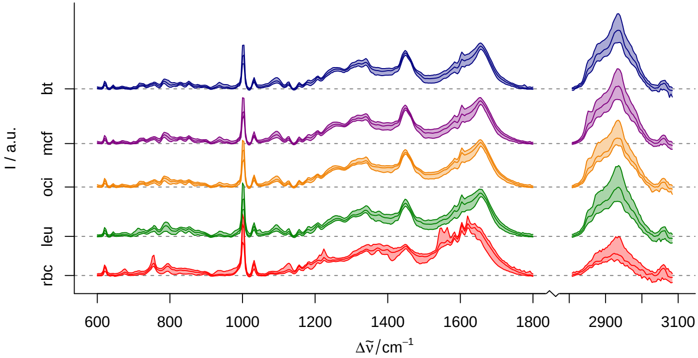
# Raman Spectra of Single Cells




| | | | |
|---|---|---|---|
| rbc | normal red blood cells | 5 donors | 372 spectra |
| leu | normal leukocytes | 5 donors | 569 spectra |
| oci | acute myelotic leukemia cell line OCI-AML | 5 batches | 518 spectra |
| mcf | breast cancer cell line MCF-7 | 5 batches | 558 spectra |
| bt | breast cancer cell line BT-20 | 5 batches | 532 spectra |
| total | | | 2549 spectra |

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- Samples are ever too few...
- But: how many samples (per class) do we **really** need?
- ...to train a good classifier:
  $\Rightarrow$ **learning curve** model performance $p = f(n_{train})$
- ...to **precisely** measure the classifier's performance:
  $\Rightarrow$ **confidence interval for test results**: $\sigma^2(\hat{p}) = \frac{p(1-p)}{n_{test}}$

### Take home message I

Training a good classifier is not enough: performance must be **demonstrated**, too.

# Data Analysis Set Up

- PLS-LDA, 25 latent variables (for $n_{train}$ / class < 10: $\frac{1}{2}$ $n_{train}$)
- 50$\times$ iterated 5-fold cross validation
- 100 growing "small" data sets
- "large" test with 320 – 520 spectra / class
  $\rightsquigarrow$ 95 % confidence interval: $p = 0.5 \pm 0.055$
  $\approx 1 : 9$ split for "small" : "large" sets

## Measuring a Learning Curve

- Learning curve:

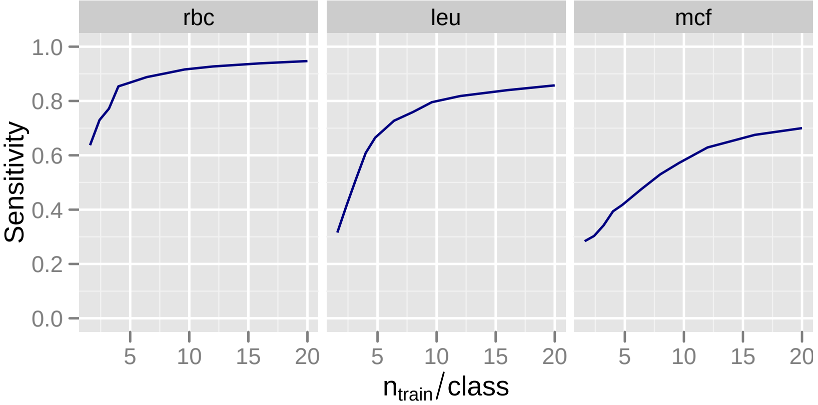
- true performance $p$ of model trained with $n_{train}$ samples:

$$\text{error}^2 = \underbrace{\text{Bayes-error}^2 + \text{bias}^2(n_{train})}_{\text{learning curve}} + \underbrace{\text{var}(n_{train})}_{\text{model instability}}$$

- observed performance $\hat{p}$ = true performace $p$

$$+ \text{systematic test error}(n, \text{method})$$
$$+ \text{random test error}(n_{test})$$

# Learning Curve




- Confidence band: $5^{th}$ – $95^{th}$ percentile of observations
- 100 repetitions
- tested with large test set

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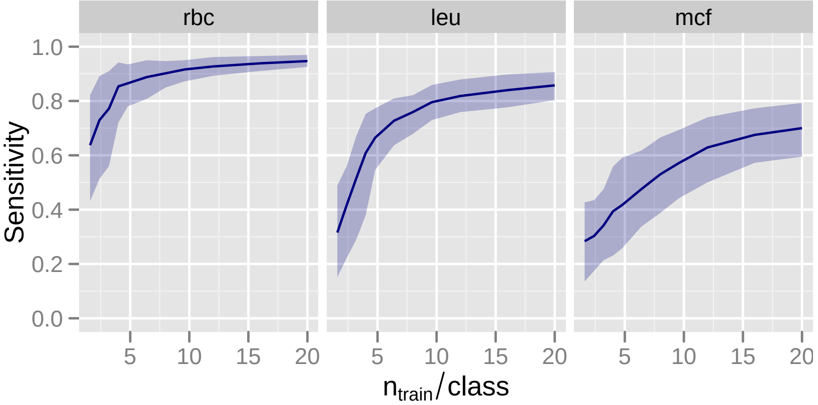


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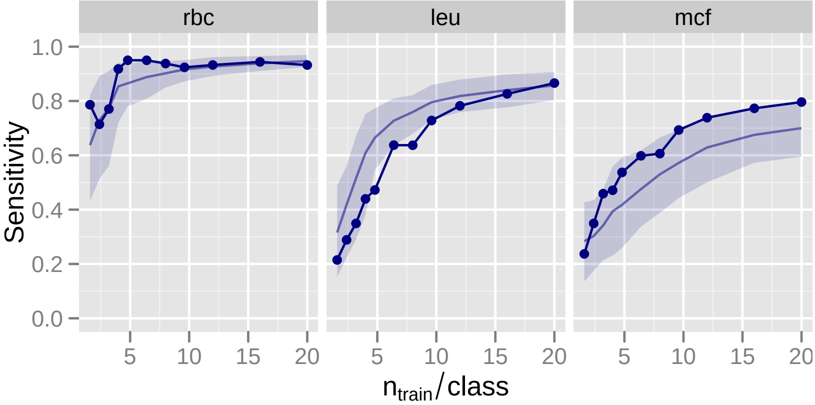


- Confidence band: 5th – 95th percentile of observations
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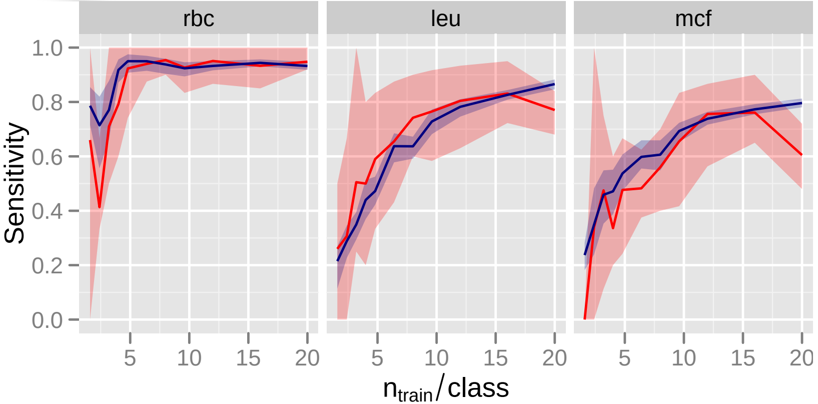
## Take home message II

The learning curve of **particular** small data set can differ substantially from average performance for data set of given size.

# Learning Curve: small set

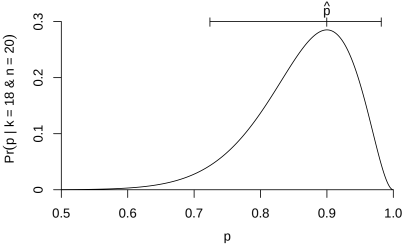



- Confidence band: $5^{th} - 95^{th}$ percentile of observations
- single, growing data set: iteration no. 17
- blue: tested with large test set
- red: 50× 5-fold cross validation

## Take home message III

For estimating the learning curve of **particular** small data set, performance estimation uncertainty is **huge**.

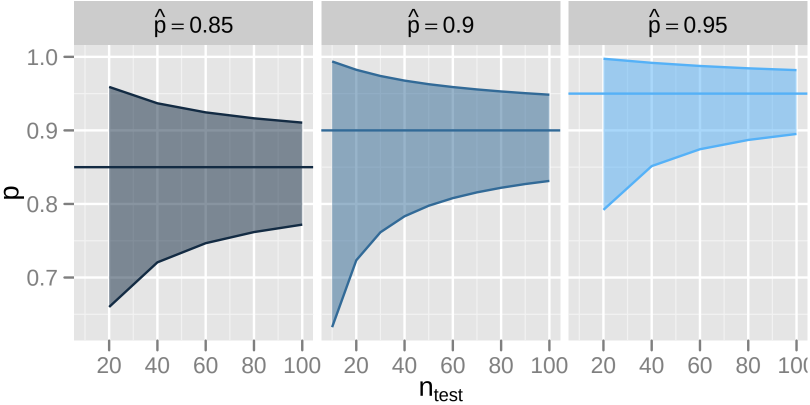
# Confidence Intervals for Proportion



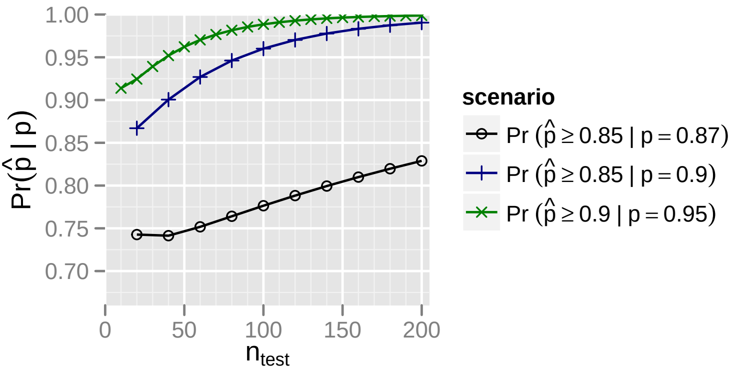

- Classifier performance: proportions
- Statistical description: Bernoulli trial
- Uncertainty on proportion: $\mathrm{var}(\hat{p}) = \frac{p(1-p)}{n_{test}}$

$\leadsto$ Estimate necessary $n_{test}$

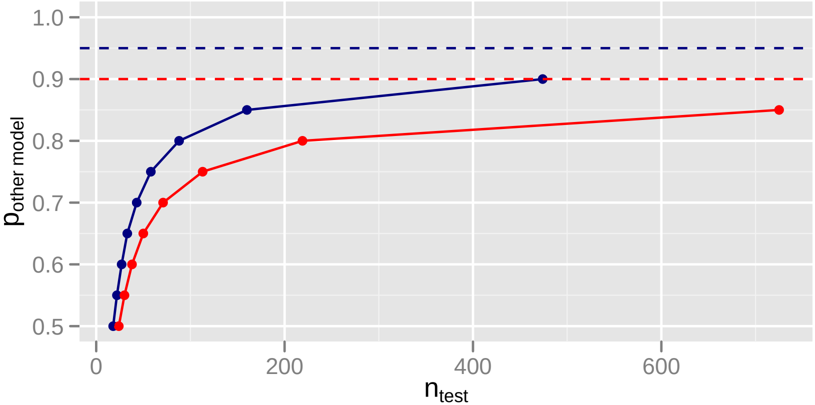
# Confidence Interval Width



# Observing at least $\hat{p}$



# Proving Advantage over Other Model




$\alpha = 5\,\%, \beta = 20\,\%$

from: Fleiss "Statistical Methods for Rates and Proportions"

- More powerful tests available for **paired** test

# Summary

- Learning curve: check variance as well as expected performance
- Performance of data set of size n vs. particular data set
- Learning curve is difficult to measure from small sample set:
  Uncertainty dominated by **testing**.
- Calculating necessary test sample size
  - Confidence interval width $p \leq \Delta p$
  - Observe $\hat{p} \geq x$
  - Show advantage over model with $\hat{p}_A$
- Necessary $n_{test}$ often $\gg$ necessary $n_{train}$

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