

# DIGE versus Coomassie Staining and Silver Staining

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# Overview:

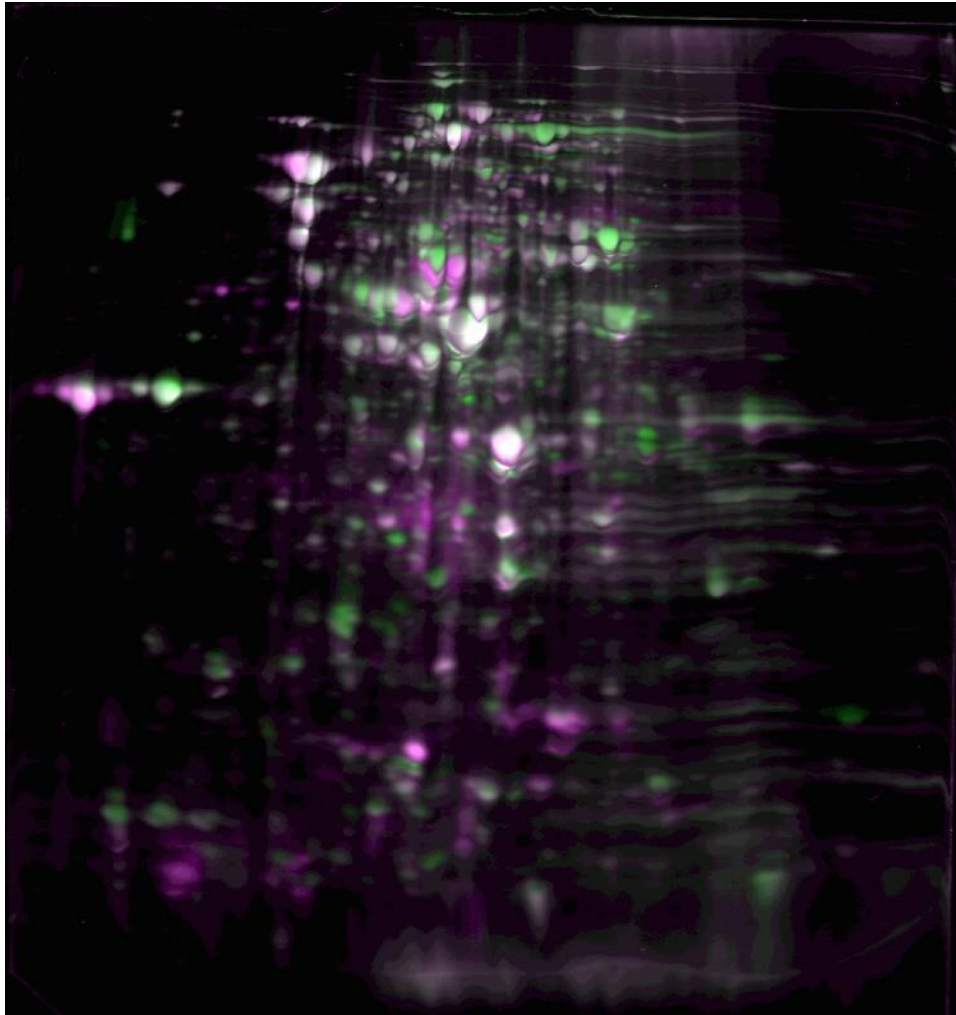
The following slides present a comparison of 2D (Two-Dimensional) Difference Gel Electrophoresis (DIGE) utilizing Cyanine Dyes (Cy Dyes: Cy3 & Cy5) versus 2D Coomassie blue-stained gels and 2D silver-stained gels.

For the 2D DIGE, an E.coli WT sample labeled with Cy5 and an E.coli Mutant sample labeled with Cy3 were run on the same gel. Images from each dye were obtained by scanning at 2 different wavelengths using a Typhoon FLA 9000 scanner.

Additionally, the E.coli WT samples and Mutant samples were run on separate gels, and the gels were Coomassie blue stained or silver stained. Patterns on the stained gels were digitized using a laser densitometer calibrated to be linear over 0-3 OD.

2D gel images were aligned using SameSpots software from TotalLab to show WT/Mutant overlays.

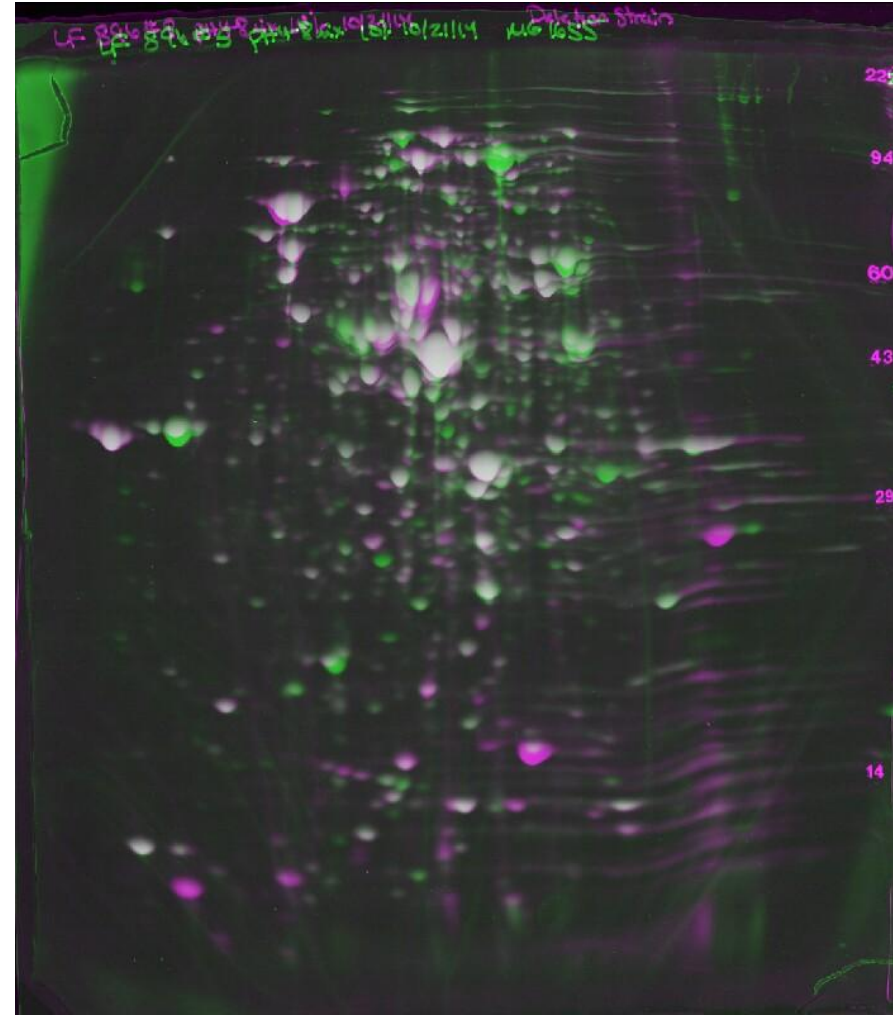
## DIGE Overlay



WT (Cy5-labeled, 200  $\mu\text{g}$ ) in green overlaying Mutant (Cy3-labeled, 200  $\mu\text{g}$ ) in magenta (400  $\mu\text{g}$  total). Spots of similar intensity appear white.

The overlay of Cy Dyes looks similar to the overlay of Coomassie gels. Proteins with large differences between samples are the same between the two approaches. Quantification of smaller fold differences, however, is straightforward with the laser densitometer, less so with DIGE. Cy dye wavelength overlap and different energies add variability to DIGE quantification.

## Coomassie Overlay

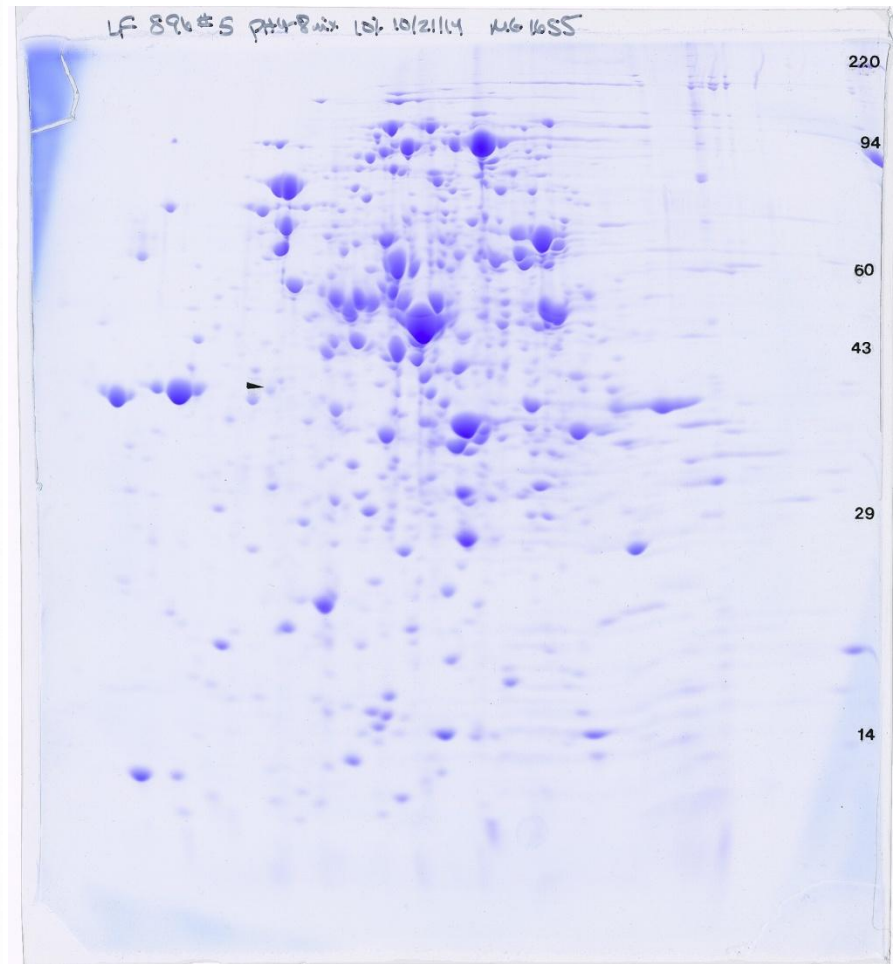


WT (400  $\mu\text{g}$ ) in green overlaying Mutant (400  $\mu\text{g}$ ) in magenta. Spots of similar intensity appear white.

## Cy5 Dye versus Coomassie



WT (Cy5-labeled, 200  $\mu$ g) on DIGE Gel



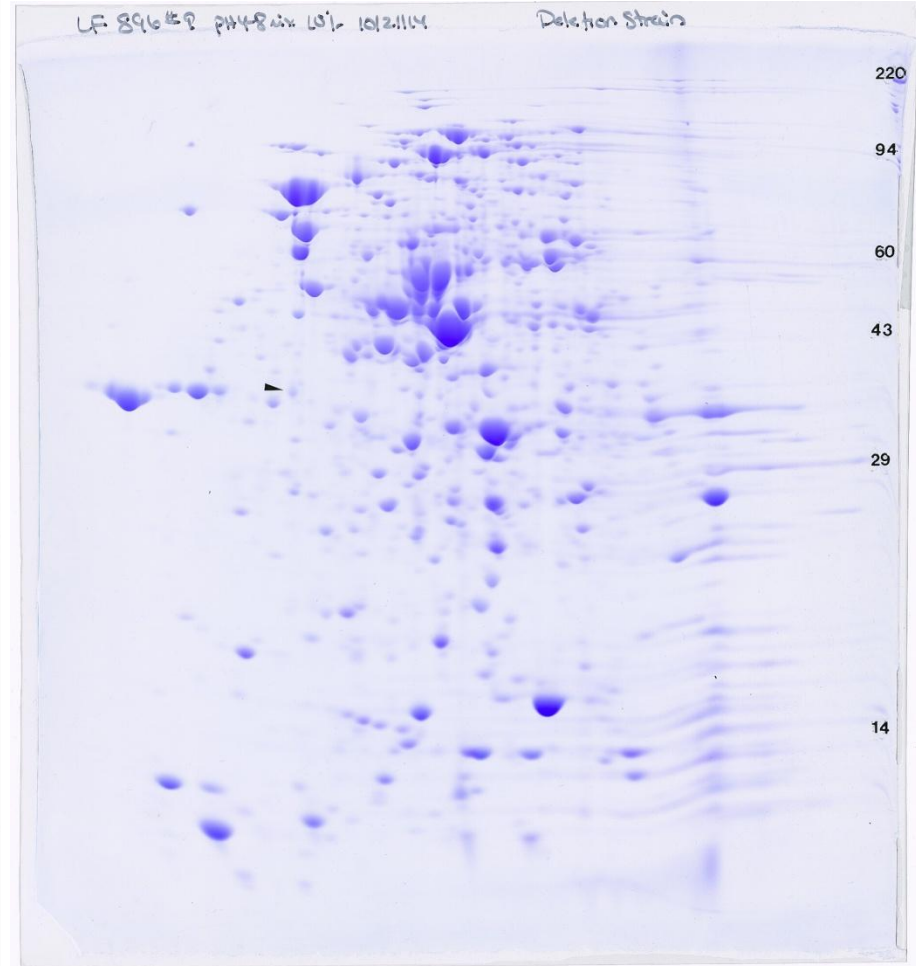
WT (400  $\mu$ g) on Coomassie Blue-Stained Gel

The protein spots on the Coomassie gel are sharper than the DIGE spots. Some protein spots on the Cy5 image may be false due to overlap of the excitation and emission spectra of Cy3 with Cy5 and due to the natural fluorescence of some proteins. Because Cy3 and Cy5 are combined before loading, this Cy5 sample is at half the load of the corresponding Coomassie gel. Given the load differences, the Cy5 sensitivity looks comparable to Coomassie blue.

## Cy3 Dye versus Coomassie



Mutant (Cy3-labeled, 200  $\mu$ g) on DIGE Gel



Mutant (400  $\mu$ g) on Coomassie Blue-Stained Gel

Again, the Cy3 protein spots are less sharp than the Coomassie spots. Some protein spots seen on the Cy3 image may be false due to overlap of the excitation and emission spectra of Cy5 with Cy3 and due to the natural fluorescence of some proteins. Given that the load is half, the Cy3 sensitivity looks comparable to Coomassie blue.



## Cy5 Dye versus Silver



WT (Cy5-labeled, 200 µg) on DIGE Gel



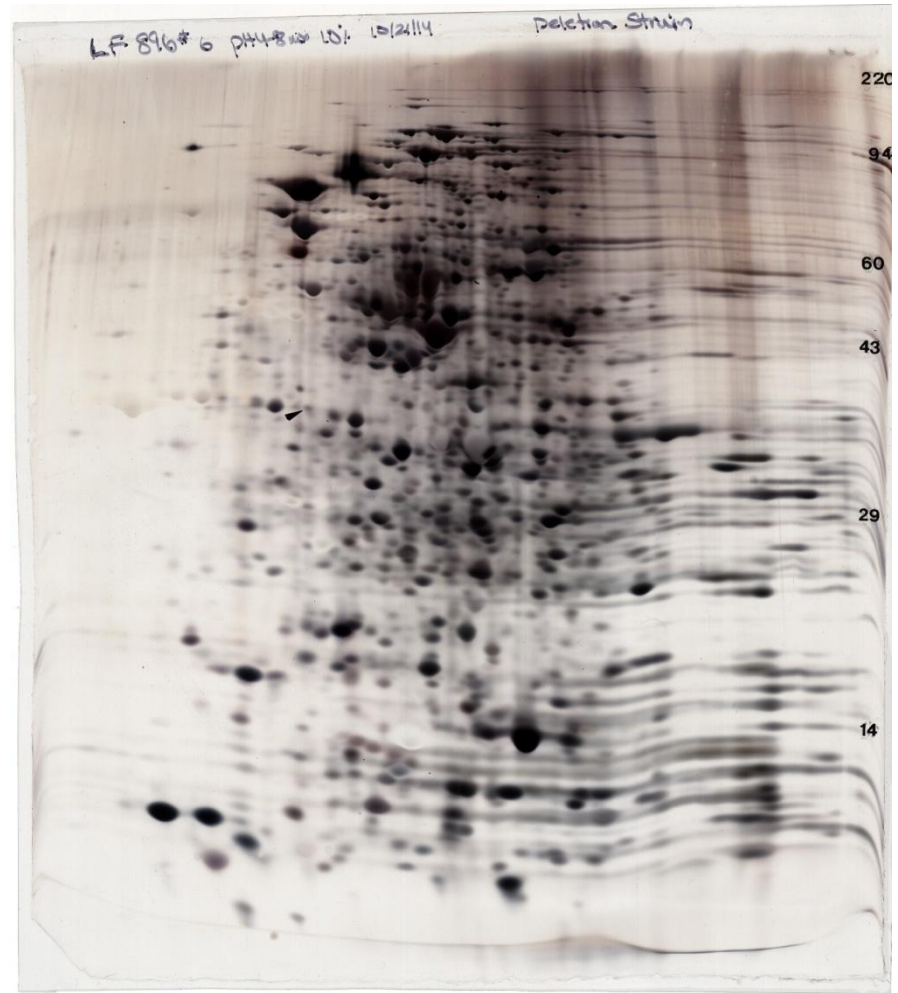
WT (200 µg) on Silver-Stained Gel

The protein spots on the silver-stained gel are much sharper and more distinct than those on the DIGE gel. Some protein spots seen on the Cy5 image may be false due to overlap of the excitation and emission spectra of Cy3 with Cy5 and due to the natural fluorescence of some proteins. Silver staining is clearly more sensitive than Cy5-labeling.

## Cy3 Dye versus Silver



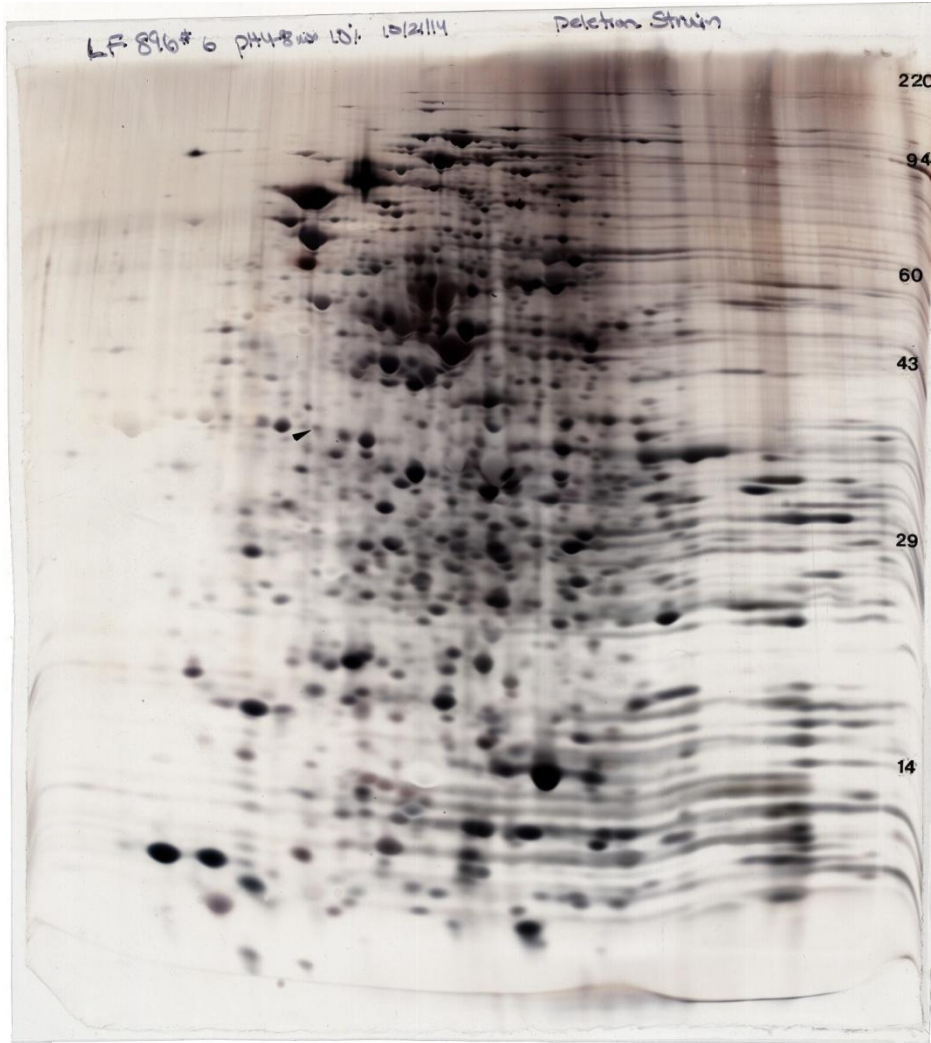
Mutant (Cy3-labeled, 200  $\mu$ g) on DIGE Gel



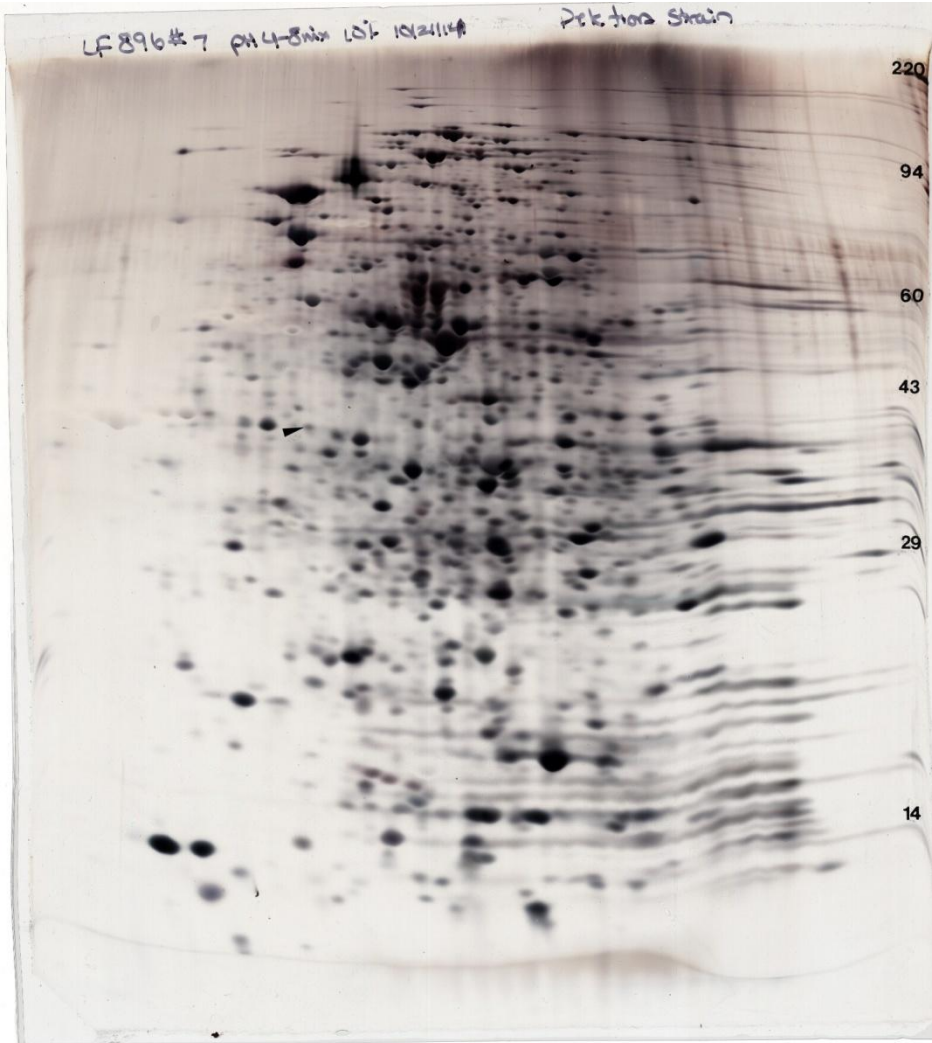
Mutant (200  $\mu$ g) on Silver-Stained Gel

Again, the protein spots on the silver-stained gel are much sharper and more distinct than those on the DIGE gel. Some protein spots seen on the Cy3 image may be false due to overlap of the excitation and emission spectra of Cy3 with Cy5 and due to the natural fluorescence of some proteins. Silver staining is clearly more sensitive than Cy3-labeling.

## 200 $\mu$ g Load versus 100 $\mu$ g Load on Silver-Stained Gels



200  $\mu$ g of Mutant on Silver-Stained Gel



100  $\mu$ g of Mutant on Silver-Stained Gel

The 200  $\mu$ g load is an overload for the best silver-stained gel. The protein pattern of the 100  $\mu$ g loaded gel shows more fine detail than the 200  $\mu$ g loaded gel.



# Conclusions:

Although DIGE allows two samples to be loaded on a single gel, the overlap of the excitation and emission spectra of Cy Dyes and the natural fluorescence of some proteins may result in false positive spots. This makes determination of the presence or absence of spots difficult.

Coomassie Blue-staining has a detection limit of  $\sim 20$  ng and is a reliable staining method that results in few artifacts. The detection sensitivity is similar to Cy Dyes. Coomassie staining with laser densitometry is the best method for quantifying fold differences.

Silver-staining is much more sensitive (detection limit  $\sim 2$  ng) than Coomassie or Cy Dyes and detects the most proteins.