Pushing the limits of fluorescent 2-D gel scanning by HDR imaging

Poster 350

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Introduction

In biological samples (e.g. in plasma) protein concentrations may differ by 10 orders of magnitude (Anderson et al., 2002). Available scanning devices for the detection of fluorescence stained proteins on 2D gels only distinguish 100 000 signal levels. Because there is no appropriate file format for storing 100 000 grey levels at hand data is transformed to 16 bit by using a calibration curve. If we assume spot sizes of 50 pixels maximum diameter a 2D gel scan is able to maximally cover 6 orders of magnitude protein concen-trations.

Because sensitivity of many scanning devices may be adjusted by changing their PMT voltages we decided to use a HDR imaging like approach to improve the dynamic range of 2D gel scans. For this purpose gels have been scanned in several iterations with increasing PMT sensitivity. Due to the low scanner speed gels undergo shrin-kage during the scan process, which hindered for immediate HDR-processing.

By gel shrinkage compensation (image warping by Delta2D), consi-dering the scanner specific calibration function, and combination of several image exposures in one HDR image we were able to improve the 65 536 grey level space about fourfold. The shown HDR image covered a dynamic range of more than 230 000 signal levels. We have implemented our method as a Web 2.0 application which allows the user to employ any low-end internet connected PC to combine multiple positionally corrected image exposures obtaining the highest achievable quality.

Anderson et al. The human plasma proteome: history, character, and diagnostic prospects. Molecular & cellular proteomics : MCP (2002) vol. 1 (11) pp. 845-67

Methods

In order to get an overall view the different information per pixel must be combined. Several methods of weighting the pixel values are already established, however only for photographs with different exposure times and not for fluorescence gels with various excitations.

To compute a charging formula , the following must be considered:
A spot, which can be distinguished within the exposure bracketing series for the first time, will also be represented in all images with a higher sensitivity. But in these it might be larger, possibly fusing with others and also the chance of artefacts in an image rises proportionel to the PMT value. On the other hand the chance to detect new spots also increases with the sensitivity, so there has to be a compromise between getting most of the spots and still being able to distingusih them.

In order to minimize the disadvantages of high sensitivity scanning, saturated pixels - those who reached the maximum black level in an image and might so lead to quantitative distortions - will be ignored and replaced with extrapolated values from the images scanned at lower PMT voltage.

This leads to the following algorithm:

1. Convert all 16 bit raw data images to text files, e.g. by ImageJ.
2. Recalibrate the so recieved integer values in every file with the inverse function of the one used by the scanner.
3. Calculate the multiplicativity factor for each image. That means comparing the average value of all pixels except the saturated ones in an image with the average value of the image with the highest PMT by forming the quotient.
4. Extrapolate the values from each image to the level of the one with the highest PMT by using the determined factor.
5. Figure out the mean of all extrapolated values for every single pixel, of course leaving out the values of the saturated ones.
6. Store these values in a new text file.
7. Convert the file back to a tiff image (ImageJ).

Results

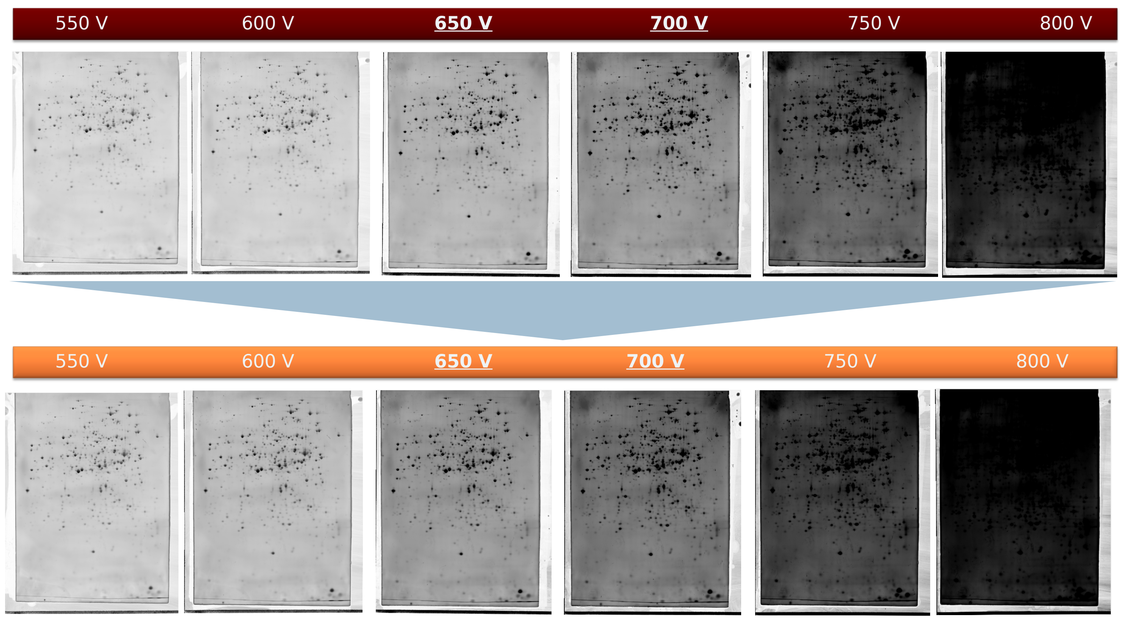
Ignoring the saturated pixels in every image of the exposure bracketing and replacing them by the average value of the extrapolated pixel values of the images with lower sensitivity has the following consequences:

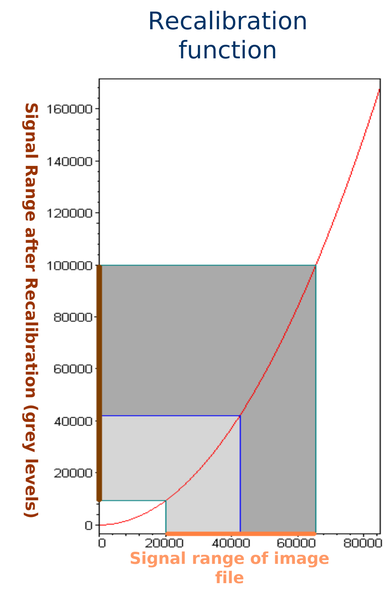
More visible spots: Spots that arise in images with a low PMT voltage will naturally be represented in the overall view. The other ones which appear only in images scanned with higher sensitivity can also be recognized. This results from the fact that every real spot appears at least once unsaturated. So the belonging pixels will not be ignored in the calculation and therefore the resulting values of the spot will be above the background noise in the HDR image.

Spots can be distinguished much better: In high intensity images spots can merge. This could lead to qualitative and quantitative misinterpretations. Because average values are used and saturated pixels ignored, the values of the pixels in the merging area will be significantly below the pixel values of the real spots.

Artefacts become alleviated: Especially dark regions that occur in only a few images of the exposure bracketing or noise can disappear completely, because the average value of the remaining images will replace them.

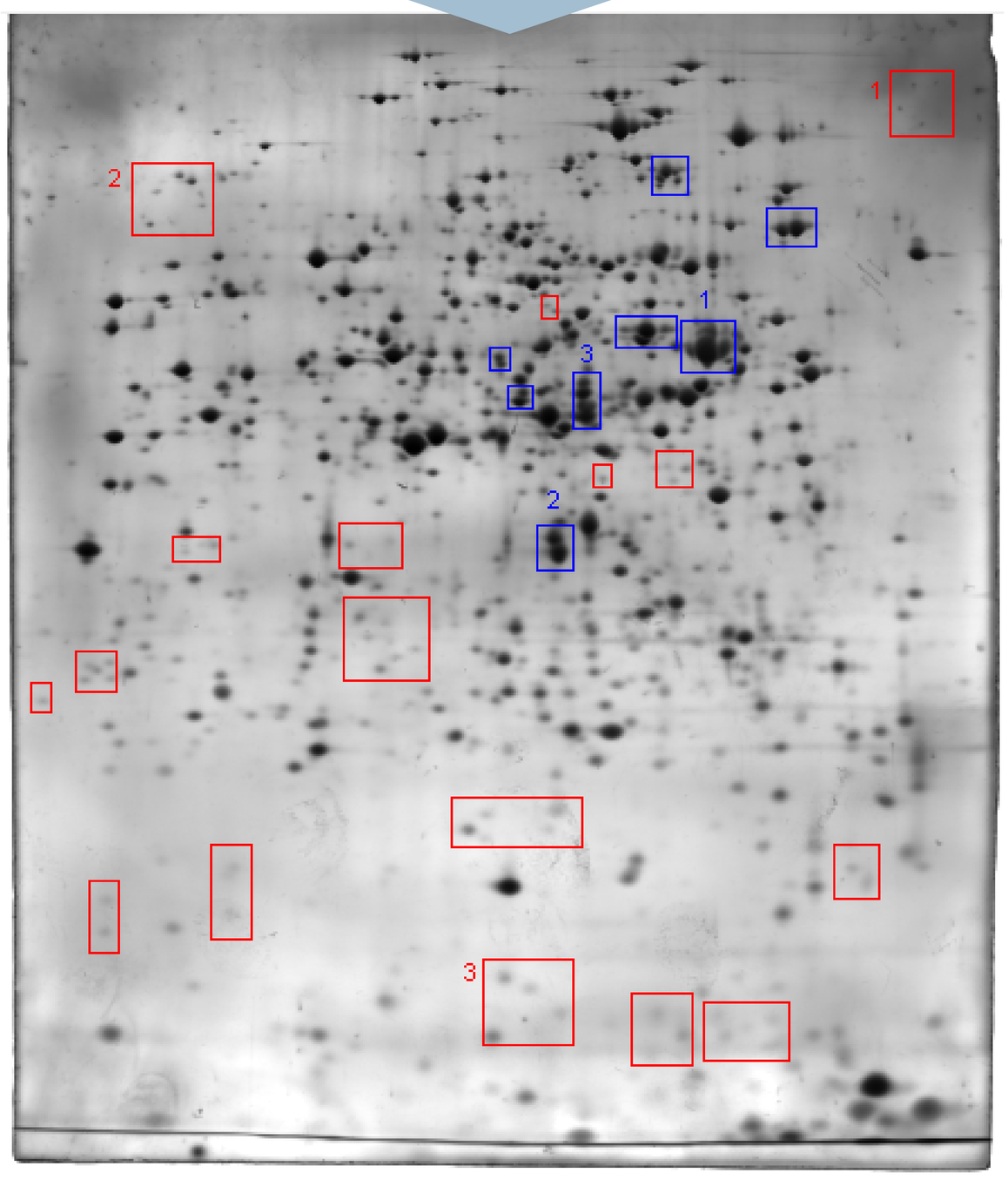
Larger contrast range: Combining all information from the exposure bracketing to one image by evaluating a function for every single pixel we get an eightfold increase of the contrast range. And even more important also the percentage of covered values inside this range actually arise from about 20 – 60 % in the raw data and the recalibrated images up to 90% in the HDR image . If there would be an appropriate software available the resulting 32 bit tiff image can be used for biological interpretations.





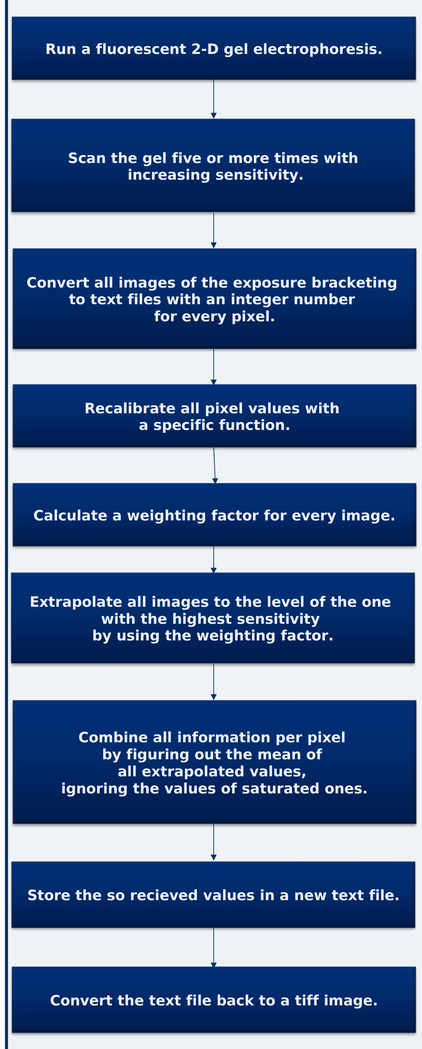
The upper gel image row shows the original exposure bracketing recieved from the scanner; the buttom row shows the images after recalibration
The contrast range increases in every image of the exposure bracketing. Thus the spots differ slightly more from the background than before.

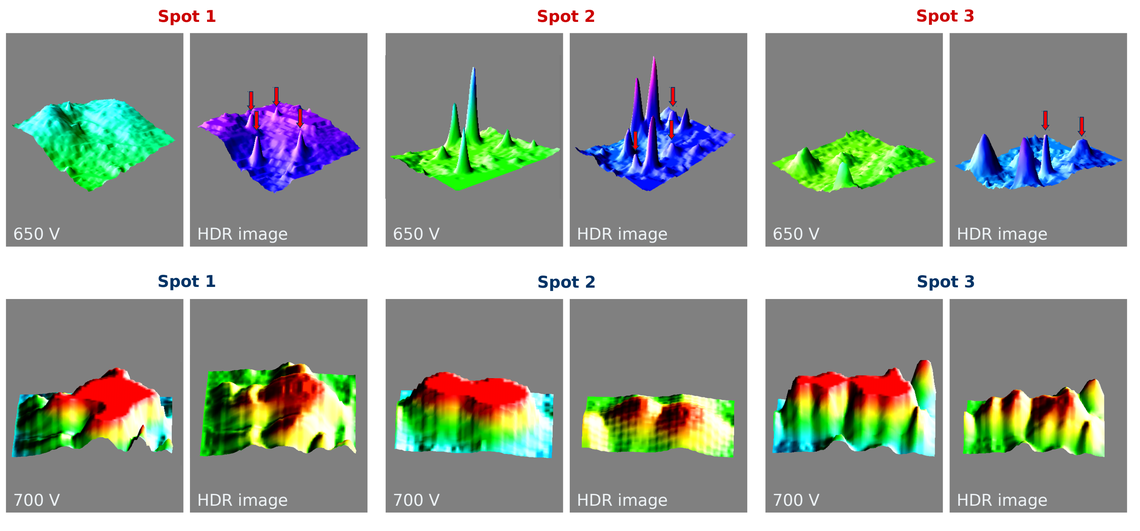
Resulting Image



HDR image – **Note**: An appropriate visualiziation of 270 000 grey levels with current printer technology is not possible for the blue and red boxes please see below.

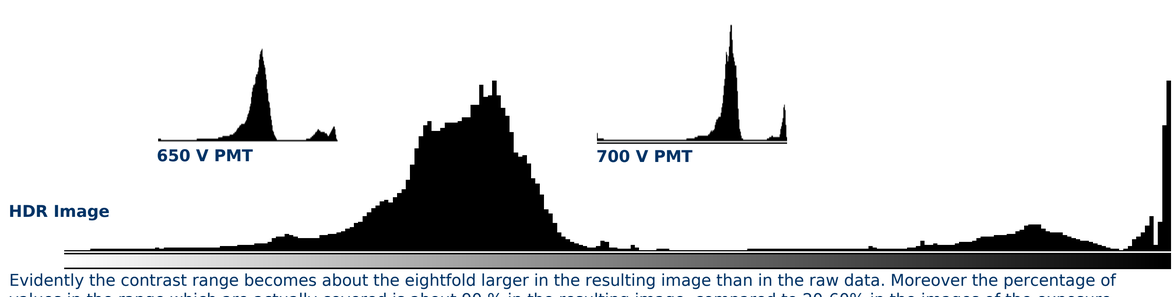
Algorithm





Spots in red boxes can be detected in the HDR image but not in the 650 PMT voltage image

In blue boxes spots merge in the 700 PMT voltage but can be distinguished in the HDR image



Evidently the contrast range becomes about the eightfold larger in the resulting image than in the raw data. Moreover the percentage of values in the range which are actually covered is about 90 % in the resulting image, compared to 20-60% in the images of the exposure bracketing.