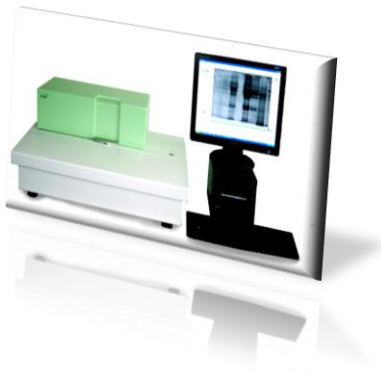


**What if you could visualize
your protein gels without staining?
Working with unmodified proteins!
Reduce time and costs for staining to zero!**



BioAnalyzer Gel –

***Proteomic Imaging System for
Unstained and Stained Gels***

Gel electrophoresis plays a key role in proteomics. Visualization of proteins is usually accomplished by the application of dyes (Coomassie Blue, silver staining). However, needed staining and washing steps are both time and cost consuming. Additionally, different dyes have limitations in linearity, sensitivity and affordability. The latest addition to our product line features the BioAnalyzer Gel, a novel proteomic imaging system for stained as well as for unstained protein gels. The BioAnalyzer Gel offers new perspectives, as no dyes are required to make the protein spots visible. The BioAnalyzer Gel utilizes native fluorescence of single amino acids (mainly tryptophan) to visualize their localization within the gel. The outstanding advantage is of course time reduction, as soon as the gel run is finished it can be imaged. Neither

lengthy diffusion based staining process nor covalent modifications are necessary. As all proteins contribute to the resulting fluorescence signal the method is highly quantitative. After imaging the gel can be directly processed by subsequent methods. Because of the unstained proteins no purifying process is required. Nevertheless color sensitive imaging of stains is also possible with a mouse click. DIGE applications as well as the detection of fluorochrome from 320 nm to 700 nm can be conducted with this system. Imaging different dyes just implies choosing another filter. There is no restriction to a narrow wavelength range. The system utilizes UV extended white light illumination, advanced filter techniques and parallelized photo multipliers. A flexible scan field adaptation allows imaging of any gel format up to 27 cm x 27 cm. The high image acquisition speed of 1 cm²/s facilitates rapid scanning with a spatial resolution of 40 µm over the entire sample area. Depicting unstained protein amounts down to 2 ng affirms a superb sensitivity. The manifold character of the BioAnalyzer Gel allows comparably scanning of unstained 1D and 2D gels, fluorochrome labeled protein gels, native gels, microtiter plates and pure protein solution.

Imaging unstained 1D protein gels

Defining lowest detectable concentration of midsized protein

For ascertaining the lowest detectable protein concentration a 10% bis-Tris gel was loaded with a dilution series of Mark12™ Unstained Standard (Invitrogen). The volume of each dilution step was 10 µl. The electrophoretic separation was performed with MOPS running buffer at constant voltage (200 V). Figure 1 displays on the top detail of the gel and the densitometric quantification graph of the β-galactosidase bands. The upper band series on the gel represents β-galactosidase while the bands below show phosphorylase b. Regarding to β-galactosidase 2 ng is the lowest detectable concentration on a 10% bis-Tris gel.

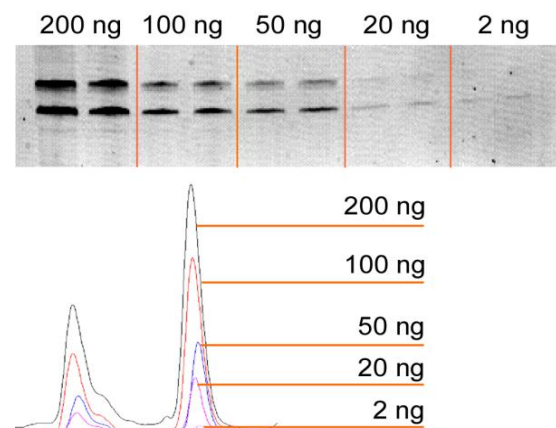


Fig. 1: The bis-Tris gel cutout with dilution series of β-galactosidase and phosphorylase b. The quantification graph of β-galactosidase evinces 2 ng as lowest detectable concentration on this gel.

Influence of Fixation

Normally protein gels are processed after electrophoretic separation. The most common step following is the fixation of the gel to avoid runaway diffusion. Here we tested whether this processing effects the scanning with the BioAnalyzer Gel. 10 µl of Mark12™ Unstained Standard (Invitrogen) pair wise diluted was separated on a 10% bis-Tris gel. After electrophoresis the gel was bisected. The first half was directly scanned with the BioAnalyzer Gel (Figure 2, A) while the second half was incubated at room temperature for 7.5 minutes with 20 % tri-chloro acetic acid (TCA). Before scanning the fixed gel (Figure 2, B) was washed three times for 5 minutes with distilled water. The best result for imaging protein gels

with the BioAnalyzer Gel does not assume any fixation (Figure 2). On the contrary fixation causes impairment of detected signals.

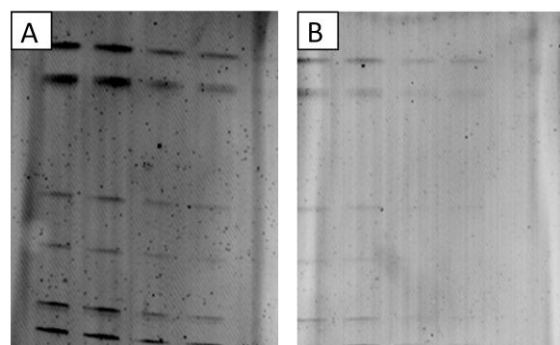


Fig. 2: The 10% bis-Tris gel cutout with pair wise diluted standard. The left half of the gel was scanned without fixation before scanning (A) while the right half was fixed with TCA (B).

Imaging small protein with one tryptophan

The BioAnalyzer Gel detects the native fluorescence of single amino acids (mainly tryptophan). A smaller protein with at least one tryptophan emits only a slight fluorescence signal. That raises the question: What is the lowest detectable concentration of a small protein with only one tryptophan? To answer this question we analyzed such an 11.2 kDa protein (Tab. 1). A dilution series reaching from 8 ng to 50 ng was

loaded on a 12.5% Tris-HCl gel. Electrophoresis and subsequent scanning of this gel with the BioAnalyzer Gel revealed 10 ng as the lowest educible concentration (Fig. 3). Further densitometric quantification with evaluation software (AlphaEaseFC, Alpha Innotech) evinced a conclusive linearity (Graph 1, Tab. 2).

Ala	11	Gln	5	Leu	6	Ser	4
Arg	3	Glu	7	Lys	9	Thr	9
Asn	6	Gly	3	Met	2	Trp	1
Asp	4	His	4	Phe	3	Tyr	3
Cys	2	Ile	10	Pro	3	Val	4

Tab. 1: Composition of analyzed 11.2 kDa protein.

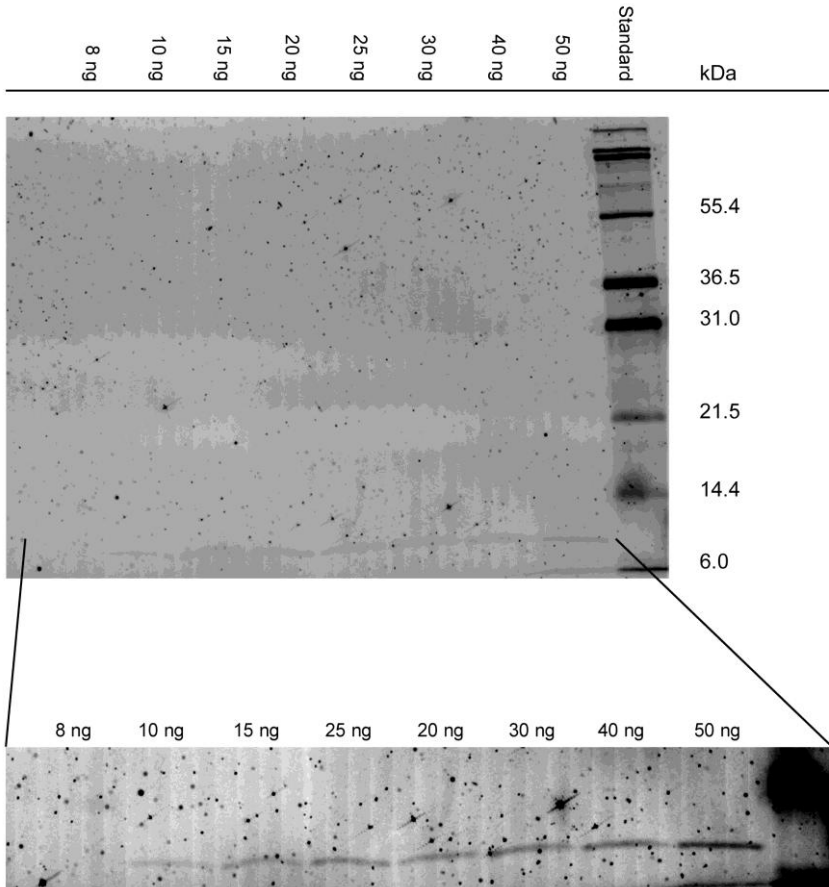
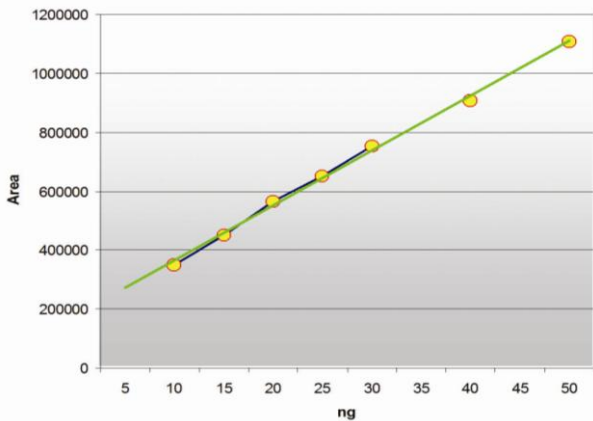


Fig. 3: 12.5% Tris-HCL gel loaded with 11.2 kDa dilution series and scanned with BioAnalyzer Gel.



Graph 1: Densitometric quantification of dilution series separated on 12.5% Tris-HCl gel reveals strong linearity.

PEAK	DIST	WIDTH	HEIGHT	AREA
1	94	19	16642	350071
2	84	21	20377	449720
3	82	26	21924	563979
4	67	31	21150	652025
5	18	20	20322	428720
6	47	32	24269	752973
7	32	39	26744	906636
8	32	36	36105	1107002

Tab. 2: Quantification of protein bands was conducted with evaluation software AlphaEase FC (Alpha Innotech).

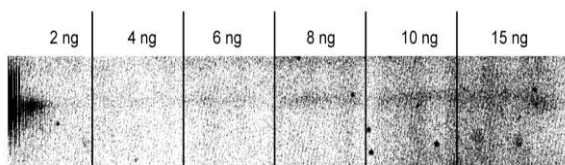
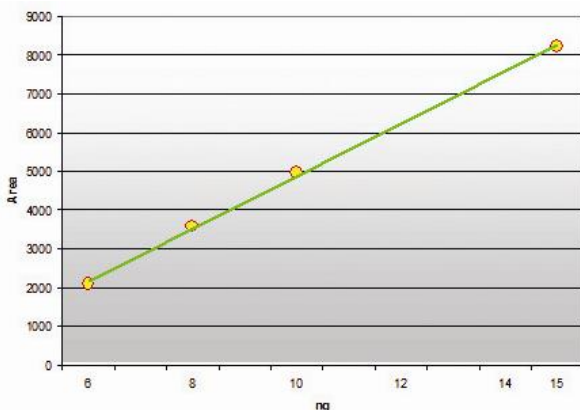


Fig. 4: 18% Tris-HCL gel loaded with 11.2 kDa dilution series and scanned with BioAnalyzer Gel.



Graph 2: Graphic quantification report of dilution series separated on an 18% Tris-HCl gel.

Detecting a lower concentration of this specimen is possible by employing an 18% Tris-HCl gel. The same protein was separated by electrophoresis and scanned with the BioAnalyzer Gel (Fig. 4). The dilution steps were: 2, 4, 6, 8, 10 and 15 ng. Beside the gel and the dilution, both electrophoresis were performed under the same circumstances (Tris/Glycin/SDS running buffer, 170 V). Once more the densitometric evaluation showed a strong linearity (Graph 2, Tab. 3). Regarding to the employed gel the lowest concentration of a small protein with only one tryptophan is about 8 ng.

PEAK	DIST	WIDTH	HEIGHT	AREA
1	61	41	60	2089
2	85	45	47	3575
3	60	43	132	4966
4	63	57	161	8244

Tab. 3: Evaluated data display dimension of protein bands. Area values were constituted in graph 2.

Imaging protein without tryptophan

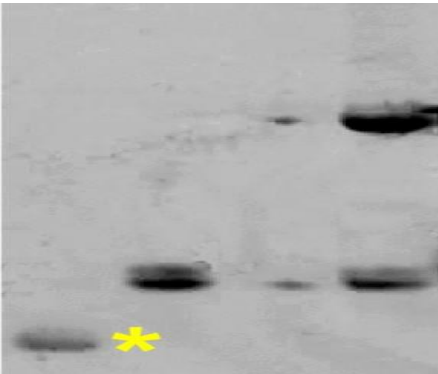


Fig. 5: SAH3 domain protein fraction from gel filtration separated on a precast 4–12% bis-Tris gel. Gel showing protein detected without any tryptophan (asterisk). (Song, EMBL Outstation Hamburg)

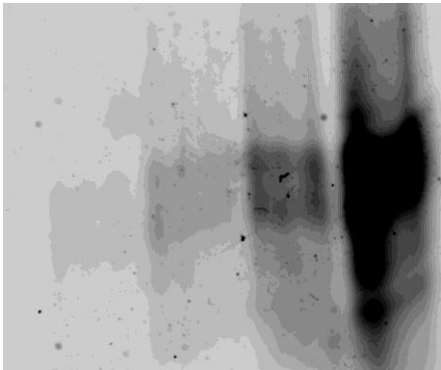


Fig. 6: Dilution series of a tryptophan-less protein scanned with BioAnalyzer Gel. (Knispel, Max-Planck-Institut, Martiensried)

Comparing Coomassie staining versus unstained gel scan

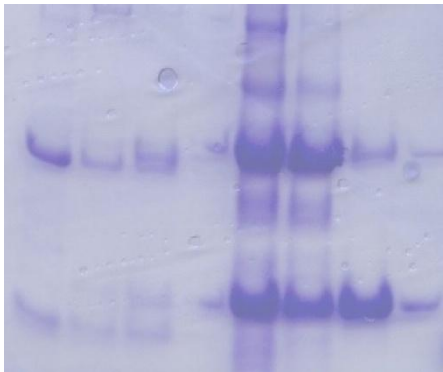
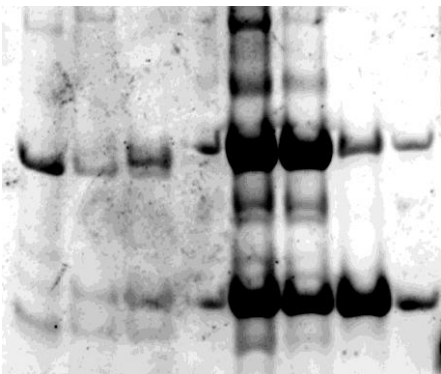


Fig. 7: Comparison of a 1D gel first imaged with BioAnalyzer Gel and thereafter stained with Coomassie. (Song, EMBL Outstation Hamburg)

Imaging stained 1D gels

The BioAnalyzer Gel offers the possibility to detect proteins labeled with different fluorochromes. The broad wavelength range enables the user to image the most common dyes by choosing the adequate filter set. The following comparison between a Sypro Ruby stained gel scanned with the BioAnalyzer Gel and a silver stained gel gives a short view on the capacities of the system within the field of

fluorescence imaging. Two sets of different proteins were electrophoretically separated on 10% bis-Tris gels. ADH was employed as internal standard. The concentration area from 5 to 625 fmol was covered by the analyzed dilution series. One gel was stained with Sypro Ruby while the second one was silver stained.

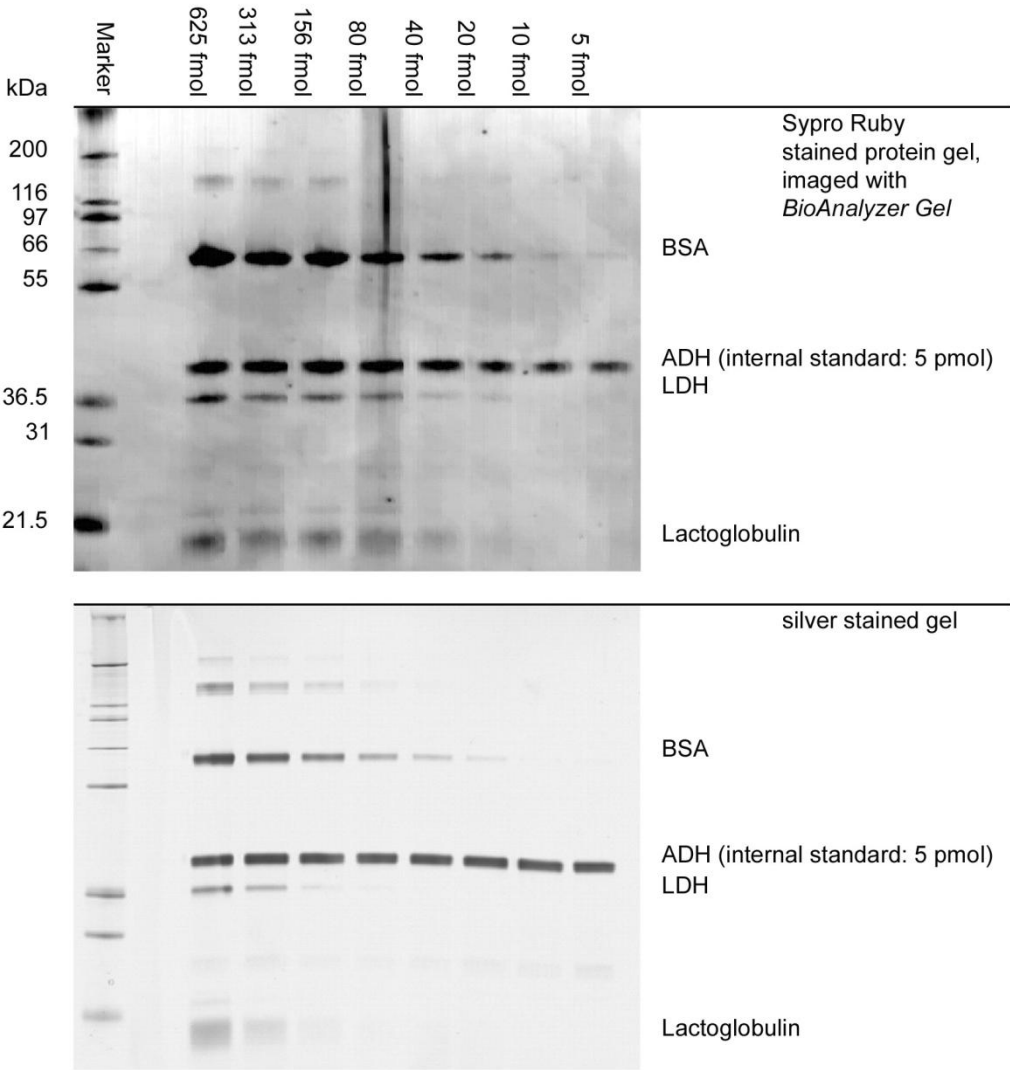
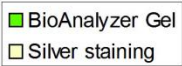


Fig. 8: Comparison of Sypro Ruby stained gel scanned with BioAnalyzer Gel versus silver stained gel. Quantification of bovine serum albumin (BSA), lactate dehydrogenase (LDH) and lactoglobulin was normalized to 50 pmol internal standard alcohol dehydrogenase. (Möbius & Sickmann, Rudolf-Virchow-Zentrum, Würzburg)

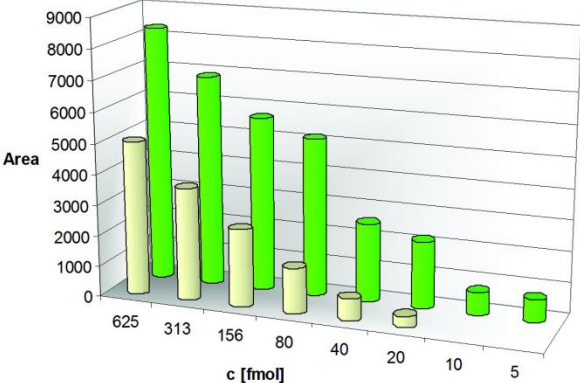
The Sypro Ruby stained gel scanned with the BioAnalyzer Gel revealed a higher sensitivity. The lowest concentration visualized by silver staining was 20 fmol BSA. A fourth of this concentration was at least

detected when scanning with the BioAnalyzer Gel. The scanned data of every Sypro Ruby stained protein revealed a higher sensitivity than the silver staining.

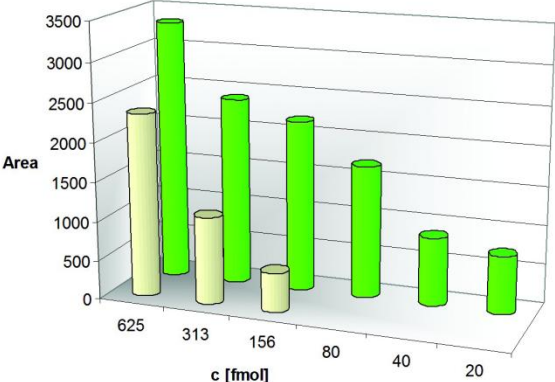
Evaluation Software: AlphaEaseFC, Version 4.0.0, Alpha Innotech



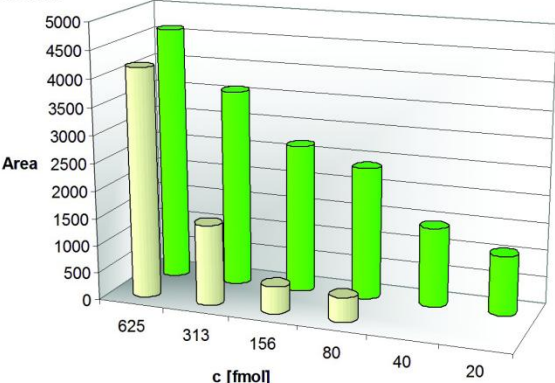
BSA



LDH



Lactoglobulin



Graph 3: Densitometric quantification of protein bands. The green-yellow columns display data of silver stained protein gel while the green columns reflect data obtained by scanning the Sypro Ruby stained gel with BioAnalyzer Gel.

	DIST	WIDTH	HEIGHT	AREA	AREA norm
BSA	53	90	128	7899	8314,48
	150	89	97	6852	6852
	254	82	82	5409	5663,21
	353	82	74	5000	5132,5
	460	78	35	2121	2570,23
	553	64	37	1695	2174,17
	655	67	10	530	753,98
	754	73	4	504	730,8
LDH	46	80	57	3152	3317,79
	145	85	37	2403	2403
	245	77	35	2091	2189,28
	351	81	41	1656	1699,89
	453	73	13	720	872,5
	549	69	11	578	741,41
Lactog.	42	70	89	4396	4627,23
	141	84	53	3588	3588
	245	74	50	2568	2688,7
	347	87	39	2341	2403
	455	92	25	1189	1440,82
	547	71	12	840	1077,47
ADH norm (internal standard)					% absl.
	49	84	76	4582	94,73
	147	87	65	4837	100,00
	248	77	78	4609	95,29
	351	80	79	4709	97,34
	455	75	67	3812	78,81
	555	64	61	3469	71,71
	659	69	66	2793	57,73
758	76	48	2660	54,98	

Tab. 4: Data evaluated with Alpha Ease FC reflecting size of the detected protein bands of the Sypro Ruby stained 1D gel scanned with the BioAnalyzer Gel. Area data were represented in graph 3 as green columns.

	DIST	WIDTH	HEIGHT	AREA	AREA norm
BSA	61	78	68	4565	5007,7
	159	80	47	3667	3667
	255	81	32	2307	2533,1
	353	75	17	1251	1470,67
	451	69	10	620	698,48
	545	75	5	346	364,86
LDH	59	74	30	2135	2342,1
	153	75	15	1115	1115
	255	80	7	455	499,6
Lg	54	86	56	3812	4181,75
	154	85	19	1463	1463
	254	82	6	449	493
	356	77	6	380	446,73
					% absl.
ADH norm (internal standard)	59	77	61	4608	90,3
	156	84	65	5103	100
	252	83	63	4602	90,2
	352	78	60	4207	82,43
	449	81	62	4457	87,33
	545	84	61	4825	94,55
	642	87	55	4571	89,57
	738	80	58	4196	82,23

Tab. 5: The silver stained gel was imaged and thereafter evaluated with quantification software like the Sypro Ruby stained gel. The area data were normalized to the internal standard ADH and then depicted as yellow columns in graph 3.

Imaging stained and unstained 2D gels

The 2D gel electrophoresis is a significant method within the field of proteomic research. The whole proteome of certain cells or tissue specimens can be characterized with this technique. Differences in expression profile of wild type and mutant can be easily identified. In many cases the different techniques for protein identification like MALDI are useful downstream applications. For this analysis gels are normally stained with Coomassie or other non-destructive staining systems. The staining procedure is often expensive and time-consuming. Working with the BioAnalyzer Gel saves you from all this. There is no need for staining procedures because your gel can be directly imaged. Nevertheless, if there is need for picking certain spots of interest, this can be done manually by the spot picking dialogue. Fig. 9 shows an unstained gel and a Flamingo stained gel below. The unstained gel (Fig. 9, A) was loaded with 150 μg cell lysate, fixed with TCA and washed for 2.5 h with distilled water.

Sveral 2D applications (e.g. DIGE) imply fluorescence staining. Because of the flexible filter set the BioAnalyzer Gel is predestinated for multi-color acquisition. Fig. 9, B displays a 50 μg cell lysate specimen stained with Flamingo.

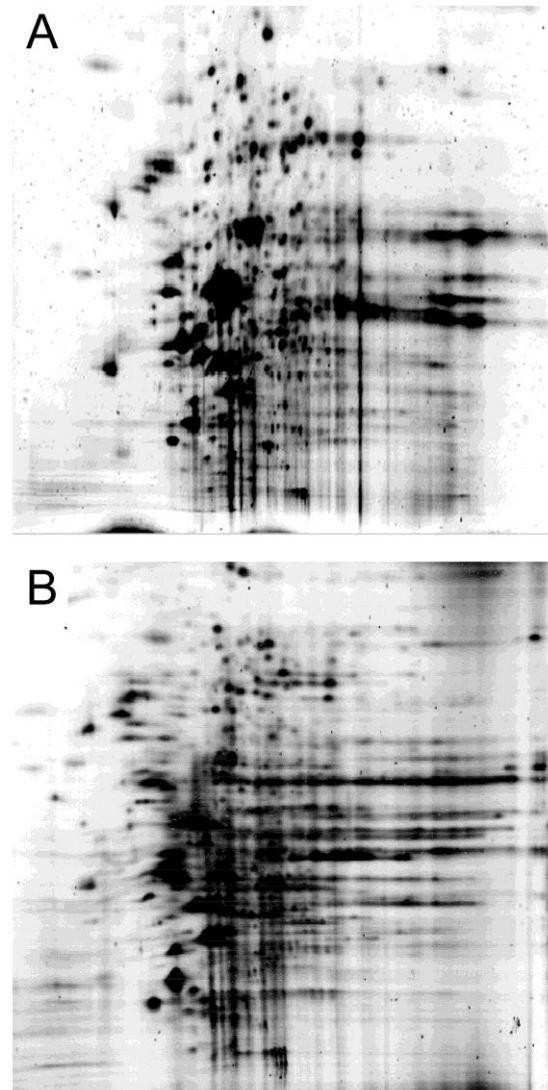
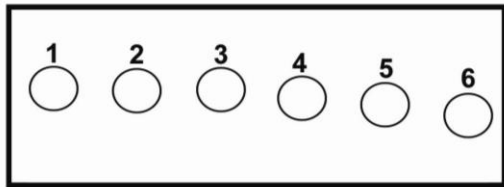


Fig. 9: Unstained (A) and stained (B) 2D gels scanned with the BioAnalyzer Gel. 150 μg of unstained protein mixture from cell lysate were separated and scanned (A). 50 μg of Flamingo stained protein separated on 2D gel (B).

Directly imaging of protein dilution series

Protein solution scanned with BioAnalyzer Gel



concentraion

- 1: 0 mg/ml (aqua dest.)
- 2: 6 mg/ml
- 3: 12 mg/ml
- 4: 18 mg/ml
- 5: 24 mg/ml
- 6: 30 mg/ml

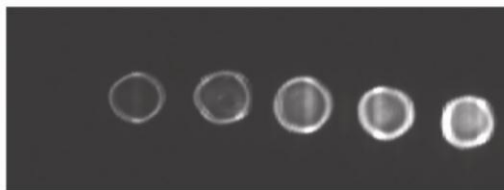
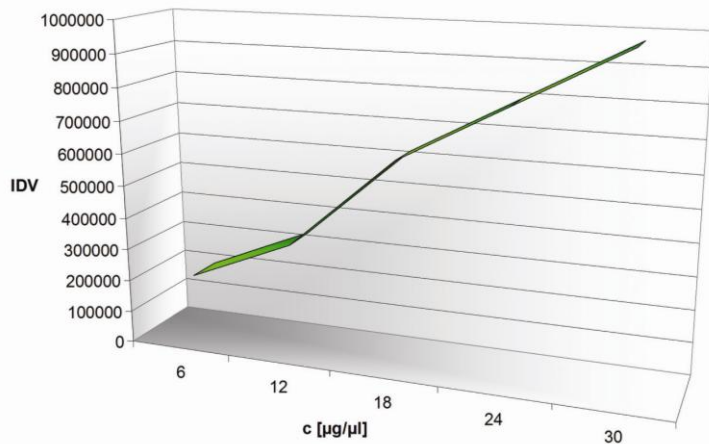


Fig. 10: Protein dilution series was spotted on the scanning plate of the BioAnalyzer Gel and imaged directly. (BMC, Uppsala)

Evaluation Software: AlphaEaseFC, Version 4.0.0, Alpha Innotech



Graph 4: Graphic delineation of evaluated data from Tab. 6 displaying densitometry quantification data of scanned spots.

#	IDV	%	AREA	AVG	BACK
1	977184	33,2	13572	72	62
2	800748	27,2	13572	59	63
3	624312	21,2	13572	46	62
4	339300	11,5	13572	25	61
5	203580	6,9	13572	15	61

Tab. 6: Spotted protein dilution series was evaluated with AlphaEase FC.

Imaging native gels

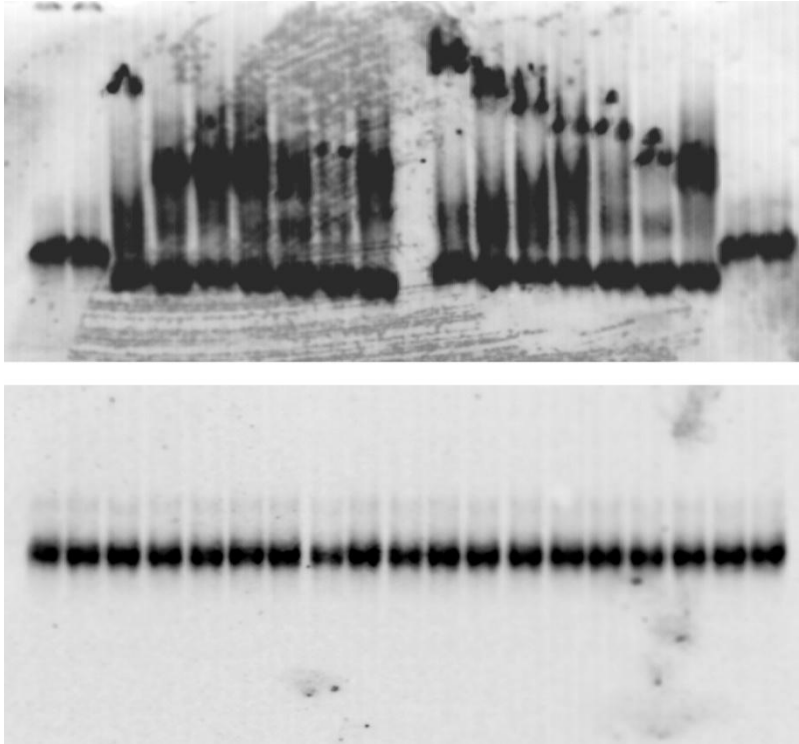


Fig. 11 & 12: Dried gels from a gel shift assay on a nitrocellulose membrane scanned with BioAnalyzer Gel. (BMC, Uppsala)

Scanning microtiter plates



Fig. 13: Scan of a microtiter plate with Hi5 insect cell line expressing GFP. (Song, EMBL Outstation Hamburg)

The main application of the BioAnalyzer Gel is scanning protein gels. For this reason the system was not optimized for scanning microtiter plates. In this case we recommend

using plates with low fluorescence and a maximum height of 7 mm. Scanning plastic disposals with UV light causes reflections which diminish the quality of image acquisition.

Illumination

Type	300W Xe UV White Light Source
Illumination Wave Length	265 – 680 nm 1 filter position for UV excitation 3 filter positions for visible excitation Free choice of wavelength by filter configuration

Detector

Type	Proprietary PMTray
Detection Wavelength	320 nm – 700 nm 1 filter position for UV fluorescence 3 filter positions for visible fluorescence Free choice of wavelength by filter configuration

Spatial resolution 50 µm over whole sample area

Sensitivity Unstained proteins < 5 ng/spot
Stained proteins < 0.1 ng/spot

Dynamic Range 16 bit

Sample Format Reading area up to 27 x 27 cm²

Image Acquisition Speed < 17.5 min for maximum area (1cm²/s)
≈ 1 minNu Page BisTris Gel (8.5 x 7.5 cm²)

Dimensions 77.5 cm (W) x 37 cm (H) x 76.5 cm (D)

Weight 65 kg

Computer Microsoft Windows based PC

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