Comparison of SCHOTT NEXTERION® Slide E Slides and SCHOTT NEXTERION Slide HiSens E Using Sensovation's Fluorescent Array Imaging Reader (FLAIR™)

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1. Introduction

Sensovation's Fluorescent Array Imaging Reader (FLAIR™), was used to analyze microarrays printed on NEXTERION® Slide E and NEXTERION® Hisens E. Both slides types are coated with an epoxy silane. The HiSens slides contain an additional layer below the epoxy coating for selective signal enhancement in the green and red channel. The goal of this study was to determine whether the two substrates produced a significant difference in the fluorescent signals when using Sensovation's FLAIR™.

1.1 Slides and Microarrays

Four sets of slides were provided by SCHOTT and measured with FLAIR.
The slides contained several microarrays consisting of serial dilutions of proteins or oligonucleotides. Slide processing at SCHOTT: Oligonucleotides and antibodies were dissolved in NEXTERION® Spot PB and spotted with a Genetix QArray mini microarray printer using SMP3 pins of Arrayit®. The oligonucleotides were hybridized in a Tecan HS 4800™ Hybstation using NEXTERION® Hybsolution. Microarray layout as well as protein and oligonucleotide concentrations are summarized in Figure 2 below. The slides were scanned with a Tecan LS 400 Reloaded™ Laserscanner as well as with the FLAIR. The data obtained with the Tecan scanner were analysed with the Array-Pro® software of Media Cybernetics™.
In total, ten slides were analyzed: three NEXTERION® Slide E and three NEXTERION® Slide HiSens E with identical antibody arrays as well as two NEXTERION® Slide E and two NEXTERION® Slide HiSens E slides with identical oligonucleotide arrays.

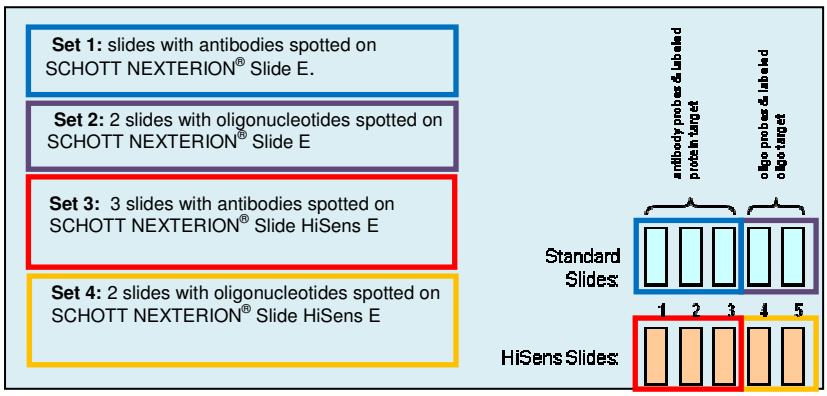


Figure 1: Schematic representation of slides measured with FLAIR.

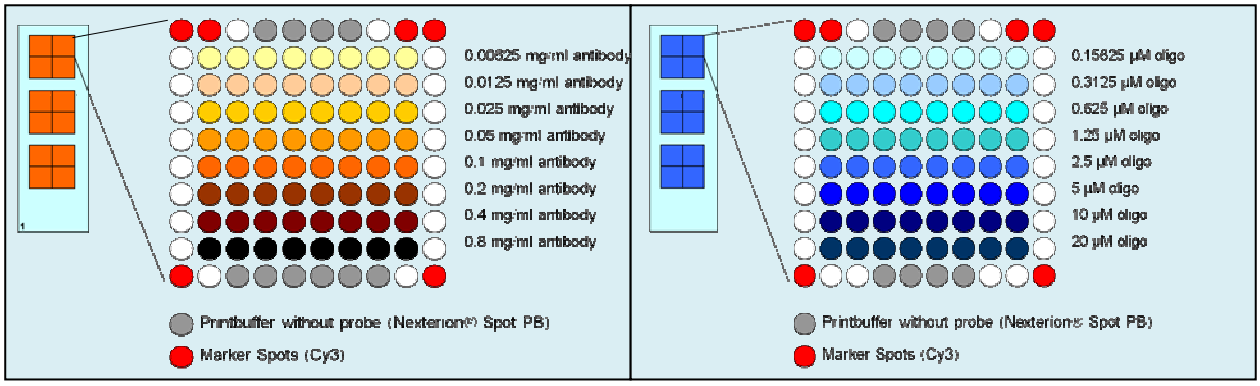


Figure 2: Layout of protein microarrays (left) and oligonucleotide microarrays (right). Each microarray contains an 8-fold serial dilution of antibodies and oligonucleotides, respectively at the concentration shown. All microarrays contain six positive marker spots for array detection and grid alignment by FLAIR.

Each slide contained 12 microarrays, organized in three groups of four replicate microarrays: the spotted oligos/antibodies were incubated with oligos/proteins labelled with a green Cy 3 compatible dye (ATTO 550, ATTO-TEC GmbH) in group one, labelled with a red Cy 5 compatible dye (ATTO 647N) in group 2 and labelled with both dyes in group 3 (Figure 4). Afterwards all microarrays were scanned and analyzed using FLAIR. Figure 3 shows the excitation and emission spectra of ATTO 550 and ATTO 647N. The microarrays were incubated with a 1 µg/mL solution of fluorescent labelled protein or 5 nM labelled target oligonucleotide (Figure 4).

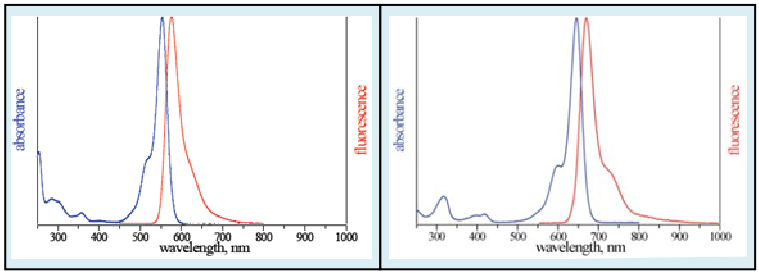


Figure 3: Absorbance / fluorescence spectra of ATTO 550 (left) and ATTO 647N (right). Absorbance maximum of ATTO 550 is 554 nm, absorbance maximum of ATTO 647N is 644 nm.

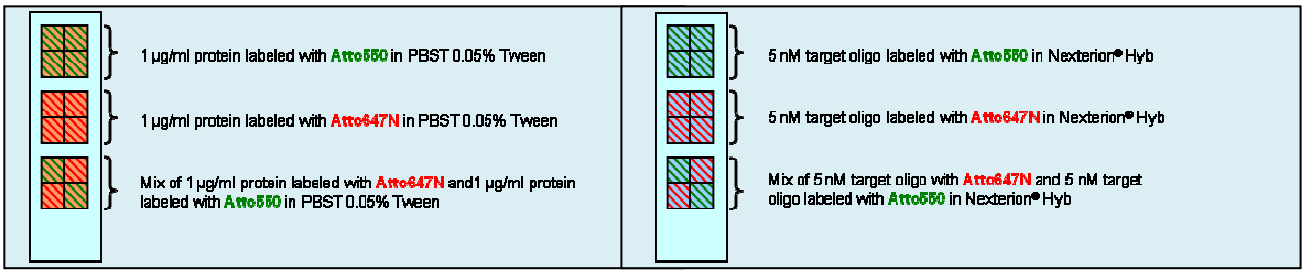


Figure 4: Fluorescence labelling of antibody microarrays (left) and oligonucleotide microarrays (right). Microarrays were incubated with protein/oligonucleotide solutions as per protocols. Each slide contained one group of four oligos/proteins labelled with ATTO550, one group of four labelled with ATTO 647N and one group of four labelled with both fluorophores.

1.2 Microarray Detection

Parallel to the measurements of SCHOTT the slides were measured and analyzed with FLAIR (Sensovation AG, Radolfzell, Germany). FLAIR is a compact, standalone imaging reader designed to measure fluorescent microarrays. It features an integrated processing unit, image acquisition- and array analysis software as well as a touch-screen monitor (Figure 5). FLAIR uses high sensitivity CCD imaging technology and high power LED illumination. FLAIR is ideally suited for read-out and analysis of multiplexed arrays, e.g. in diagnostic applications and research. The instrument is available in 2 versions with different excitation/emission filter sets: *FLAIR blue/red* and *FLAIR green/red*. For the experiments described here *FLAIR green/red* was used. *FLAIR green/red* has 549/647 excitation and the emission filters which match the spectral characteristics of the dyes used (Figure 3).

Originally designed to read arrays inside the wells of a 96-well-plate, FLAIR can also accommodate other sample formats. Sensovation provides a four slide holder as an accessory. This holder allows the user to read and analyze four slides with up to 16 arrays per slide. The size of the measurements fields is flexible up to 6mm x 6mm (Figure 6).

Figure 5: FLAIR standalone instrument with integrated PC, array analysis software, and touch-screen monitor.

1.3 Microarray Analysis

Microarray analysis was performed with Sensovation's Array Reader™ software. Array Reader software is an integral part of FLAIR; it offers full instrument control- and image acquisition capabilities, intelligent spot finding and array analysis as well as a spot evaluation software modules. Microarray analysis occurs in real time during measurement.

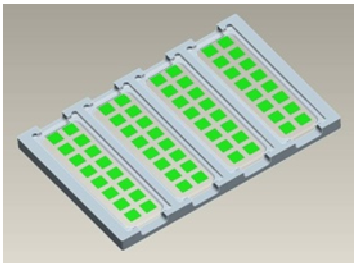


Figure 6: FLAIR 4-slide holder

Figure 7 shows a screen shot of the Array-Reader software during image acquisition, in this case a microarray on a NEXTERION® Slide HiSens E. The microarray has six reference spots which serve as anchor points for the software: two spots in the upper right corner, two spots in the upper left corner and one each in the lower right and left corners (compare figure 2).

Immediately after the array is acquired, the software identifies the reference spots, overlays the grid, detects the individual spots, and calculates spot and background intensity and a host of additional data. Calculation and analysis are extremely fast and happen while the array moves to the next measurement position. Scanning- and analysis time for a complete 96-well plate is generally well below 3 minutes per color.

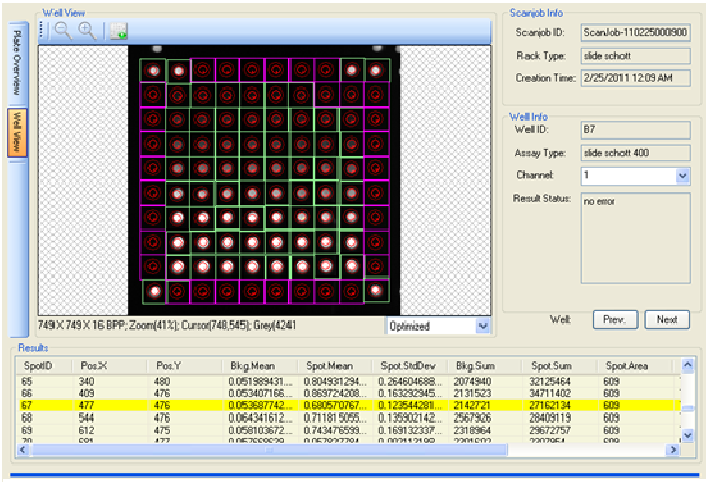


Figure 7: Screen-shot of Sensovation's Array Reader software during acquisition. Shown: microarray with grid on a NEXTERION® Slide HiSens E (position B7) and the resulting data.

An important feature of the integrated analysis software is the intelligent spot detection algorithm. After the software has performed the gridding it will search for the microarray spot within each grid sector and will adjust the analysis circle at the correct position over the spot (Figure 7, 8). The analysis diameter is then adjusted to the actual spot diameter. This is important if individual spots deviate from their designated position which happens with some frequency during spotting. FLAIR's intelligent spot finding software compensates for spotting errors and provides correct spot intensities regardless of deviation in spot positioning.

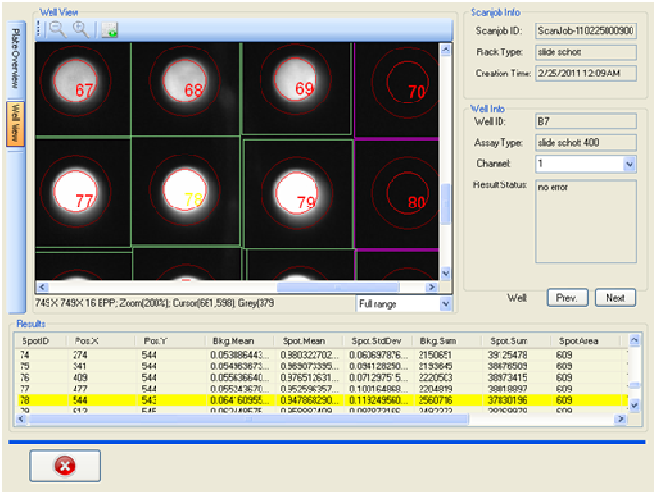


Figure 8: Screen-shot of the Array Reader software with a detailed view of the analyzed microarray. The intelligent spot detection algorithm has adjusted the analysis circle to the true spot position. Two circles are displayed: the inner circle represents the spot analysis circle used to calculate mean intensities. All pixel values outside of the outer circle represent the local background which is used for S/N calculations.

1.4 Purpose and Scope

NEXTERION® Slide E and NEXTERION® Slide HiSens E were spotted with identical microarrays. After incubation with the appropriate fluorescently labelled biomolecules, the slides were analyzed with a Tecan LS 400 Reloaded™ microarray scanner (TECAN) and with FLAIR (Sensovation).

Purpose of this study was to:

- compare the fluorescence intensities of microarrays spotted on NEXTERION® Slide E with the fluorescence intensities of the same microarrays spotted on NEXTERION® Slide HiSens E
- determine whether FLAIR provides the sensitivity required for the analysis of the oligonucleotide- and protein microarrays
- compare the results obtained from FLAR with those obtained with a conventional laser based microarray scanner.

2. Results

2.1 Microarray Spot Morphology

Microarrays spotted on standard glass slides and SCHOTT NEXTERION® Hisens slides were remarkably consistent and showed evenly distributed spots. Sensovation's spot detection algorithm only found very minor spot deviations from the designated positions (Figure 8). The spots were almost perfectly round and homogeneous and showed very few "donut-effects" or other deviations from the ideal shape (Figure 9).

Spot diameter was determined to be 200 µm (Figure 9) with very little deviation.

No significant difference regarding spot morphology could be observed between spots printed on standard slides and spots printed on Hisens slides. Additionally, no significant difference in spot morphology was observed between antibody and oligonucleotide spots.

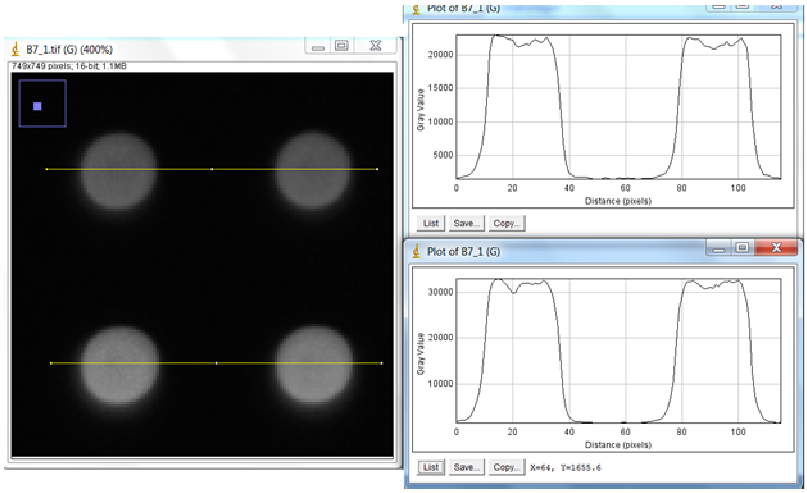


Figure 9: Four representative spots from an antibody microarray on a NEXTERION® Slide HiSens E slide were analyzed with FLAIR. The image and the line profiles show homogenous spot morphology, regular round shapes, and good positioning. Spot diameter is 30 pixel. Since FLAIR has a pixel resolution of 6.7 µm/pixel the true spot diameter can be calculated to be 200 µm.

2.2 Visual Comparison of Individual Microarrays

To compare the signal intensities on NEXTERION® Slide E and NEXTERION® Slide HiSens E random microarrays were selected and compared. Samples are displayed below for visual comparison. The original images are 16 bit grey scale TIFF images . Figures 10 – 13 show a side-by-side comparison of microarray images from NEXTERION® Slide E with the same microarrays printed on NEXTERION® Slide HiSens E. All images were acquired under identical conditions using FLAIR. The images are displayed at the identical contrast settings for accurate side-by-side comparison

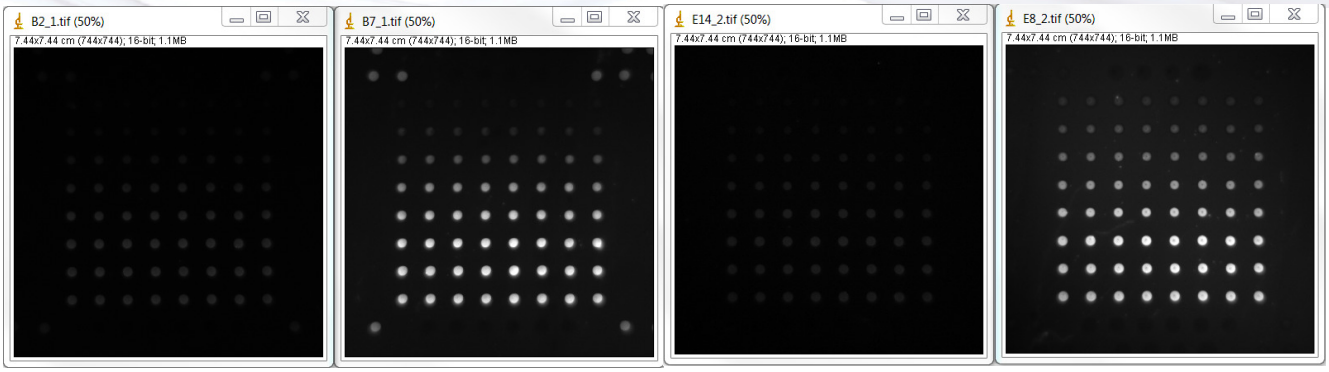


Figure 10: Visual comparison of an antibody array spotted on a standard slide (left) and NEXTERION® Slide HiSens E (right) . Contrast settings: 0 – 65,000 for both images. The arrays were measured under identical conditions with the FLAIR, in the green channel with 200 ms integration time.

Figure 11: Visual comparison of an antibody array spotted on a standard slide (left) and NEXTERION® Slide HiSens E (right) . Contrast settings: 0 – 65,000 for both images. The arrays were measured under identical conditions with the FLAIR, in the red channel with 30 ms integration time.

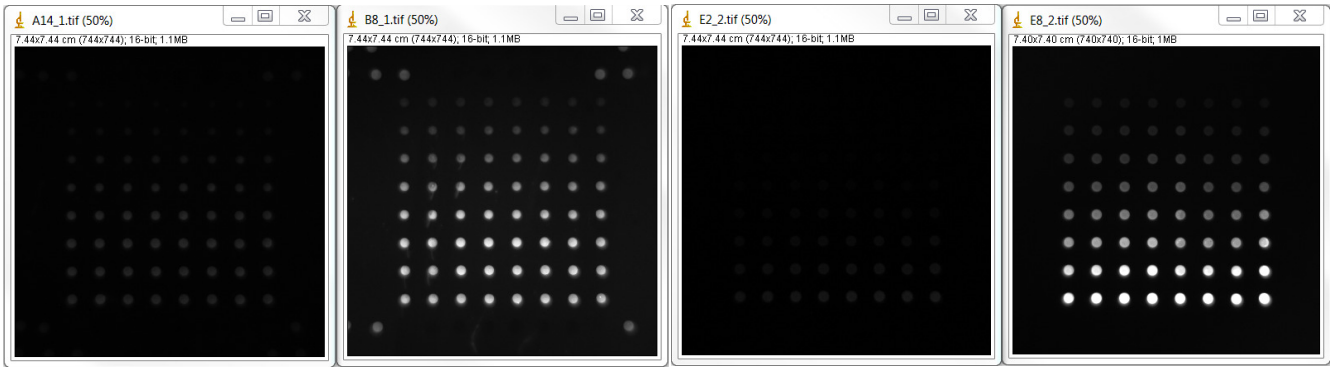


Figure 12: Visual comparison of an oligonucleotide array spotted on NEXTERION® Slide E (left) and NEXTERION® Slide HiSens E (right). Contrast settings: 0 – 65,000 for both images. The arrays were measured under identical conditions with the FLAIR, in the green channel with 200 ms integration time.

Figure 13: Visual comparison of an oligonucleotide array spotted on NEXTERION® Slide E (left) and NEXTERION® Slide HiSens E (right). Contrast settings: 0 – 65,000 for both images. The arrays were measured under identical conditions with the FLAIR, in the red channel with 30 ms integration time.

2.3 Intensity Comparison

Selected microarrays were acquired with FLAIR and automatically analyzed with the Array Reader software with automatic spot finding switched on.

The criteria of selecting representative microarrays were:

- Antibody microarray with green label only, NEXTERION® Slide E and NEXTERION® Slide HiSens E
- Antibody microarray with red label only, NEXTERION® Slide E and NEXTERION® Slide HiSens E
- Antibody microarray with dual label NEXTERION® Slide E and NEXTERION® Slide HiSens E (both colors analyzed).
- Oligonucleotide microarray with green label only, NEXTERION® Slide HiSens E and NEXTERION® Slide HiSens E
- Oligonucleotide microarray with red label only, NEXTERION® Slide E and NEXTERION® Slide HiSens E
- Oligonucleotide microarray with dual label NEXTERION® Slide E and NEXTERION® Slide HiSens E (both colors analyzed).

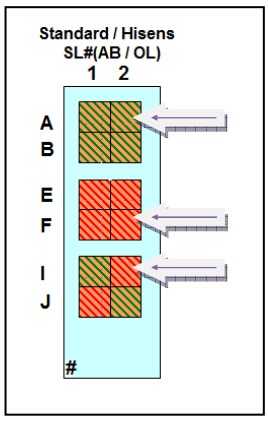


Figure 14: Micro-arrays selected for comparison

Figures 15 -18 and 19 – 22 show direct comparisons of spot net intensity values (spot intensity minus local background) obtained from NEXTERION® Slide E and NEXTERION® Slide HiSens E. Each bar in the diagram represents the mean value of eight spots of the same antibody or oligonucleotide concentration. Integration time of FLAIR was adjusted so that highest spot intensities were approximately 50,000. This made it possible to detect signals from the lowest concentration spots. The entire dynamic range was covered with FLAIR.

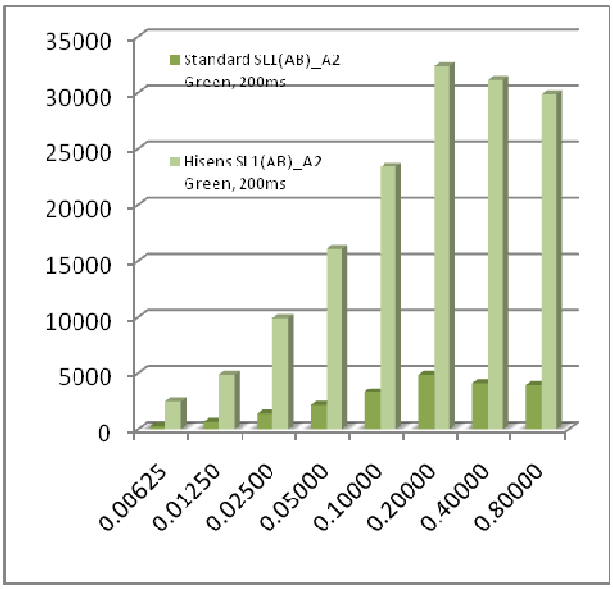


Figure 15: Comparison of NEXTERION® Slide E 1 with NEXTERION® Slide HiSens E 1, Array A2, ATTO 550 label only. Mean intensity calculated using 8 spots of the same concentration. Green excitation, 200 ms exposure. Antibody Array.

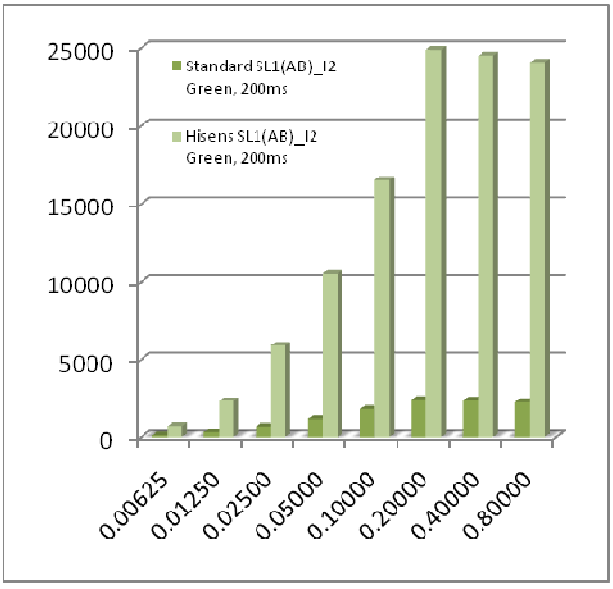


Figure 16: Comparison of NEXTERION® Slide E 1 with NEXTERION® Slide HiSens E 1, Array I2, ATTO 550 and ATTO 647N label. Mean intensity calculated using 8 spots of same concentration. Green excitation, 200 ms exposure. Antibody Array.

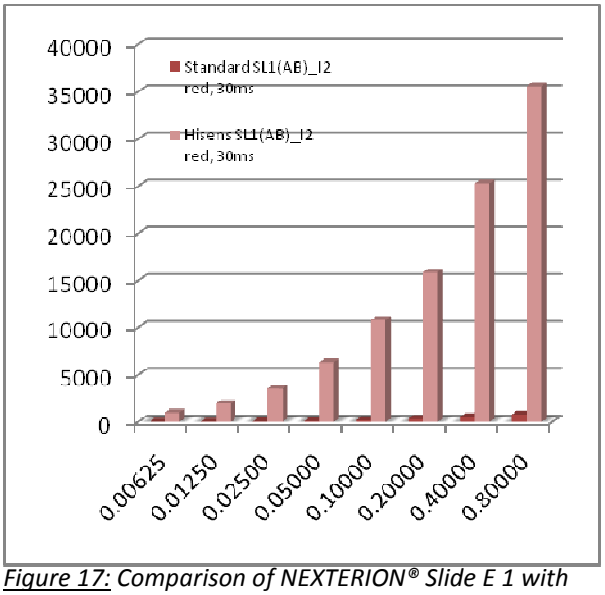


Figure 17: Comparison of NEXTERION® Slide E 1 with NEXTERION® Slide HiSens E 1, Array I2, ATTO 550 and ATTO 647N label. Mean intensity calculated using 8 spots of same concentration. Red excitation, 30 ms exposure. Antibody Array.

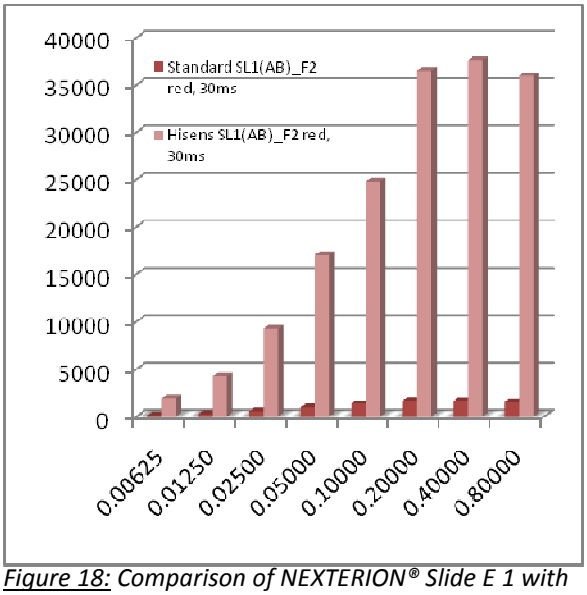


Figure 18: Comparison of NEXTERION® Slide E 1 with NEXTERION® Slide HiSens E 1, Array F2 - ATTO 647N label only. Mean intensity calculated using 8 spots of the same concentration. Red excitation, 30 ms exposure. Antibody Array.

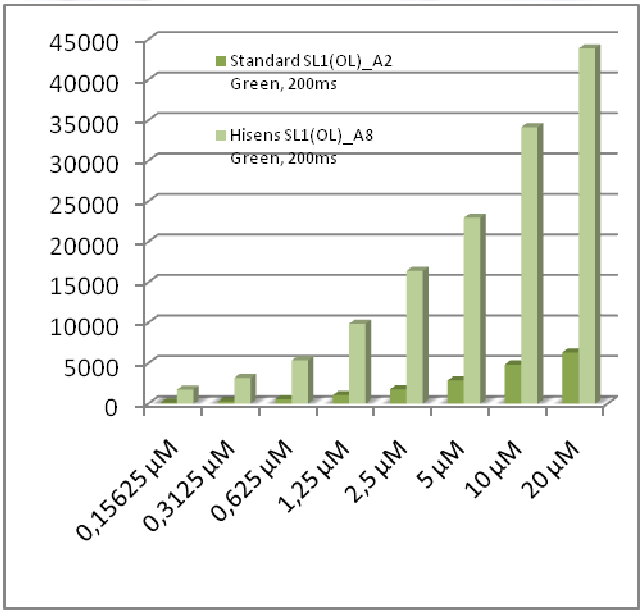


Figure 19: Comparison of NEXTERION® Slide E 4 with NEXTERION® Slide HiSens E 4, Array A2 – ATTO 550 label only. Mean intensity value calculated using 8 spots of the same concentration. Green excitation, 200 ms exposure.
Oligonucleotide Array

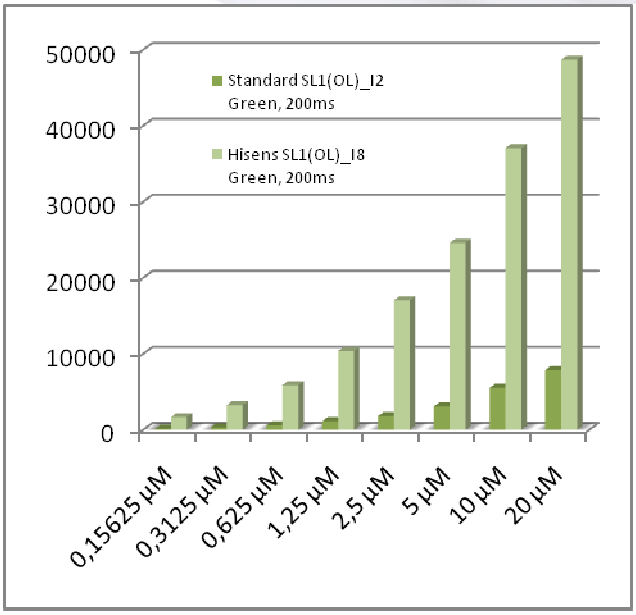


Figure 20: Comparison of NEXTERION® Slide E 4 with NEXTERION® Slide HiSens E 4, Array I2 - ATTO 550 and ATTO 647N label Mean intensity value calculated using 8 spots of the same concentration. Green excitation, 200 ms exposure.
Oligonucleotide Array.

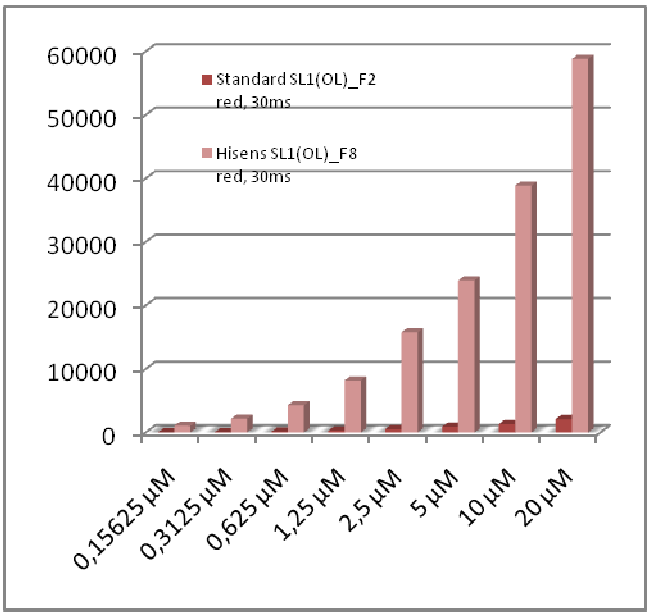


Figure 21: Comparison of NEXTERION® Slide E 4 with NEXTERION® Slide HiSens E 4, Array I2 - ATTO 647N label only. Mean intensity calculated using 8 spots of the same concentration. Red excitation, 30 ms exposure.
Oligonucleotide Array

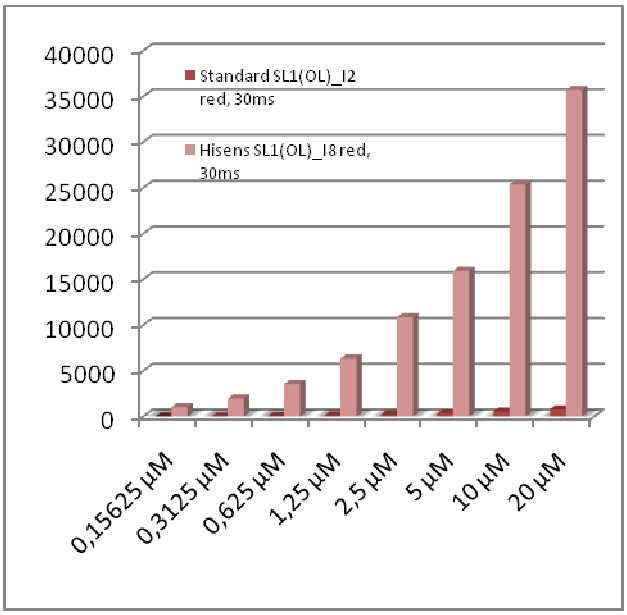
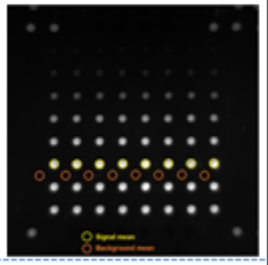


Figure 22: Comparison of NEXTERION® Slide E 4 with NEXTERION® Slide HiSens E 4, Array F2, ATTO 647N label only. Mean intensity calculated using 8 spots of the same oligonucleotide concentration. Red excitation, 30 ms exposure.
Oligonucleotide Array

2.4 Signal-to-Noise ratios

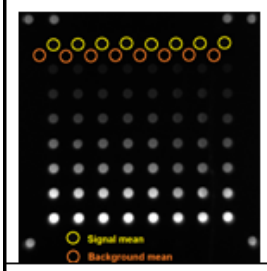
The signal-to-noise ratios (S/N) of selected microarrays were calculated. S/N is a good indicator for lower limit of detection. The Array Reader software automatically calculates mean spot intensities, background mean intensities, and background standard deviation. From these values S/N can be derived. The calculations were performed for low and high concentration spots, for both antibody and oligonucleotide microarrays for both colors. The results are shown in Table 1 – Table 4.

Table 1: Analysis of strong spots (Protein Concentration 2 mg/mL) in the antibody microarrays. Mean spot intensities and mean background intensities (green channel 200 ms) are calculated from 8 spots with the same concentration. Signal/Noise ratio is determined from these measurement results:

	Protein, Standard green 200ms (array B2)					Protein, Hisens Slide green 200ms (array B7)				
High Concentration	Spot Mean	Back-ground Mean	Back-ground Std. Dev.	Net Signal*	S/N**	Spot Mean	Backgro und Mean	Back-ground Std. Dev.	Net Signal*	S/N
0,2 mg/ mL	5656	539	69	5117	74,2	50613	4326	193	46287	239,8
	6072	557	93	5515	59,3	52716	4648	171	48068	281,1
	6285	574	87	5711	65,6	54649	4661	155	49988	322,5
	6156	582	72	5574	77,4	54127	4910	193	49217	255,0
	6450	602	73	5848	80,1	57802	4871	189	52931	280,1
	6096	577	72	5519	76,7	54129	5353	180	48776	271,0
	6001	571	70	5430	77,6	53630	5021	160	48609	303,8
	5876	583	91	5293	58,2	53584	5020	163	48564	297,9
Mean	**6074**	**573**	**78**	**5501**	**71,1**	**53906**	**4851**	**176**	**49055**	**281,4**

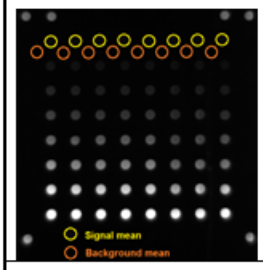
*Net signal = (Signal Mean spot intensity - mean background intensity)
**Signal / Noise = (Net signal / Background standard deviation)

Table 2: Analysis of weak spots (Protein Concentration 0.00625 mg/mL) in the antibody microarrays. Mean spot intensities and mean background intensities (red channel 30 ms) are calculated from 8 spots with the same concentration. Signal / Noise ratio is determined from these measurement results:

	Protein, Standard red 30ms (array E14)					Protein, Hisens Slide red 30ms (array E8)				
Low Concentration	Spot Mean	Back-ground Mean	Back-ground Std. Dev.	Net Signal*	S/N**	Spot Mean	Backgro und Mean	Back-ground Std. Dev.	Net Signal*	S/N
0,00625 mg/ mL	966	594	80	372	4,7	15998	9971	265	6027	22,7
	1017	650	94	367	3,9	16735	11111	263	5624	21,4
	1033	676	77	357	4,6	19520	11381	262	8139	31,1
	1051	663	92	388	4,2	17167	11533	296	5634	19,0
	1064	664	91	400	4,4	15876	11353	304	4523	14,9
	1036	672	78	364	4,7	15315	10376	259	4939	19,1
	1233	668	95	565	5,9	13866	9976	331	3890	11,8
	849	627	70	222	3,2	13245	9946	249	3299	13,2
Mean	**1031**	**652**	**85**	**379**	**4,4**	**15965**	**10706**	**279**	**5259**	**19,1**

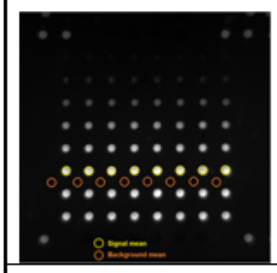
*Net signal = (Signal Mean spot intensity - mean background intensity)
**Signal / Noise = (Net signal / Background standard deviation)

Table 3: Analysis of weak spots (concentration 0.15625 µMol) of the oligonucleotide microarrays. Mean spot intensities and mean background intensities (green channel, 200 ms) are calculated from 8 spots with the same concentration. Signal / Noise ratio is determined from these measurements:

	Oligonucleotide, Standard green 200ms (array A14)					Oligonucleotide, Hisens Slide green 200ms (array A8)				
Low Concentration	Spot Mean	Back-ground Mean	Back-ground Std. Dev.	Net Signal*	S/N**	Spot Mean	Back-ground Mean	Back-ground Std. Dev.	Net Signal*	S/N
0,15625 µMol	251	103	55	148	2,7	3225	1498	86	1727	20,1
	252	100	51	152	3,0	3218	1398	99	1820	18,4
	253	104	54	149	2,8	3033	1306	100	1727	17,3
	244	95	53	149	2,8	2986	1264	97	1722	17,8
	267	96	56	171	3,1	3074	1256	103	1818	17,7
	273	101	55	172	3,1	3170	1275	85	1895	22,3
	274	99	55	175	3,2	3671	1317	86	2354	27,4
	276	102	46	174	3,8	3701	1397	89	2304	25,9
Mean	**261**	**100**	**53**	**161**	**3,0**	**3259**	**1339**	**93**	**1921**	**20,8**

*Net signal = (Signal Mean spot intensity - mean background intensity)
**Signal / Noise = (Net signal / Background standard deviation)

Table 4: Analysis of strong spots (Oligonucleotide Concentration 5 µMol) in the oligonucleotide microarrays. Mean spot intensities and mean background intensities (red channel, 30 ms) are calculated from 8 spots with the same concentration. Signal / Noise ratio is determined from these measurements:

	Oligonucleotide, Standard red 30ms (array A14)					Oligonucleotide, Hisens Slide red 30ms (array A8)				
High Concentration	Spot Mean	Back-ground Mean	Back-ground Std. Dev.	Net Signal*	S/N**	Spot Mean	Back-ground Mean	Back-ground Std. Dev.	Net Signal*	S/N
5 µMol	1876	230	71	1646	23,2	40126	7154	184	32972	179,2
	2103	275	67	1828	27,3	47546	7663	172	39883	231,9
	2274	305	57	1969	34,5	49113	7758	229	41355	180,6
	2409	317	67	2092	31,2	48876	7297	208	41579	199,9
	2415	333	93	2082	22,4	49963	6781	203	43182	212,7
	2386	320	78	2066	26,5	48103	6472	277	41631	150,3
	2210	308	74	1902	25,7	46931	5147	186	41784	224,6
	2070	283	77	1787	23,2	40972	5311	302	35661	118,1
Mean	**2217**	**296**	**73**	**1922**	**26,8**	**46453**	**6698**	**220**	**39756**	**187,2**

*Net signal = (Signal Mean spot intensity - mean background intensity)
**Signal / Noise = (Net signal / Background standard deviation)

2.5 Comparison FLAIR - Laser Scanner

In addition to FLAIR, a conventional laser microarray scanner, the Tecan LS 400 Reloaded™, was used to reanalyze the slides. To reduce the amount of data the four identical replicate microarrays were measured and averaged for further analysis. The following nomenclature was used: Well 1 refers to the average of four microarrays labeled with the green dye, Well 2 refers to the average of four microarrays labeled with the red dye, Well 3 refers to the average of four microarrays labeled with the both dyes. Figure 23 gives an overview over the well-designation of the combined microarrays.

The averaging of replicate microarrays resulted in the significant reduction of the amount of data. These data are shown in Figures 24 through 31. The results of the biomolecule concentrations in each well on the different slides are displayed as bar diagrams. For easy visual comparison the results obtained from FLAIR and the laser scanner are placed side-by-side.

The samples were measured and analyzed with FLAIR under slightly modified conditions in order to bring the net fluorescence signal to the same level as obtained from the laser scanner. To match the laser scanner intensities, an integration time of 250 ms in the green channel and an integration time of 25 ms in the red channel were used with FLAIR.

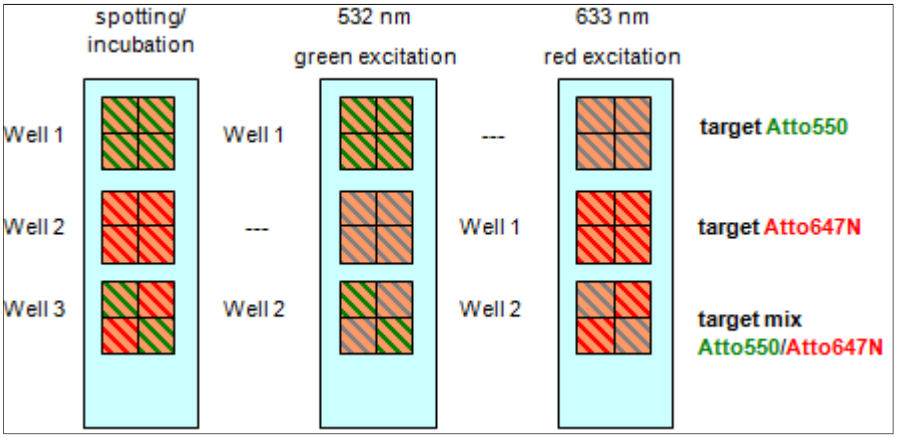


Figure 23: Schematic drawing of combination / averaging of microarrays and designation of "wells".

FLAIR Data	Laser Scanner Data

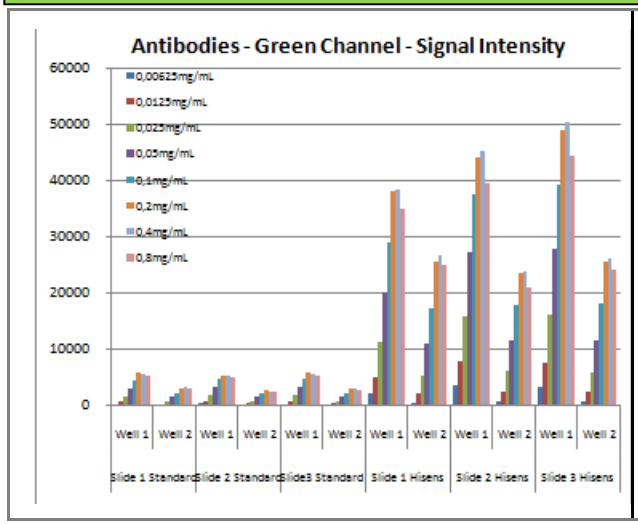


Figure 24: Signal intensity of antibody dilution series in combined microarrays comparing NEXTERION® Slide E with NEXTERION® Slide HiSens E slide; measured with Sensovation FLAIR, 549 nm excitation, 250 ms integration time, 6.7 µm resolution.

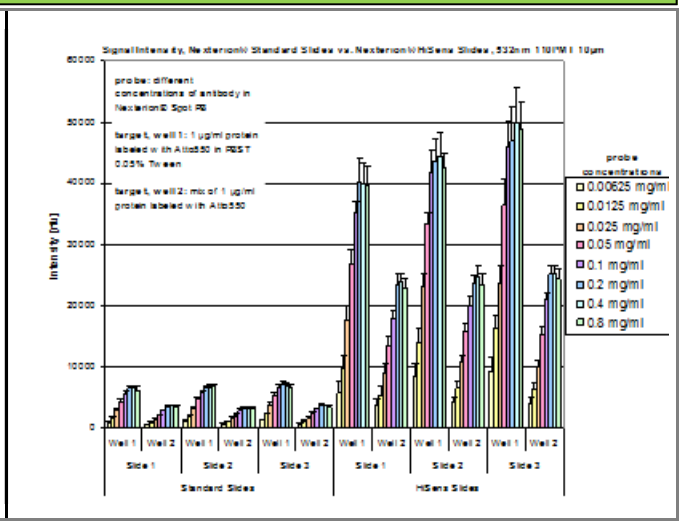


Figure 25: Signal intensity of antibody dilution series in combined microarrays comparing NEXTERION® Slide E with NEXTERION® Slide HiSens E; measured with Laser Scanner, 532 nm excitation, 110 gain, 10 µm resolution.

FLAIR Data	Laser Scanner Data

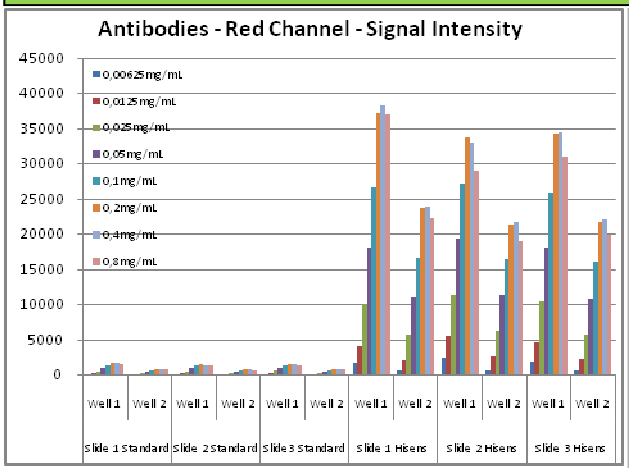


Figure 26: Signal intensity of antibody dilution series in combined microarrays comparing NEXTERION® Slide E with NEXTERION® Slide HiSens E; measured with Sensovation FLAIR, 647 nm excitation, 25 ms integration time, 6.7 µm resolution.

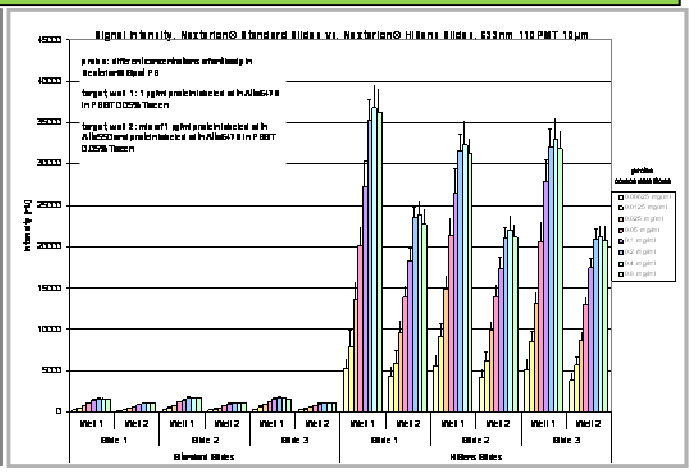


Figure 27: Signal intensity of antibody dilution series in combined microarrays comparing NEXTERION® Slide E with NEXTERION® Slide HiSens E; measured with Laser Scanner, 633 nm excitation, 110 gain, 10 µm resolution.

FLAIR Data	Laser Scanner Data

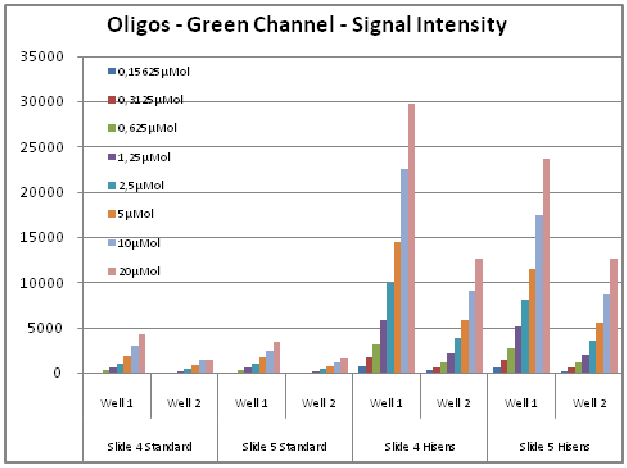


Figure 28: Signal intensity of oligonucleotide dilution series in combined microarrays comparing NEXTERION® Slide E with NEXTERION® Slide HiSens E; measured with Sensovation FLAIR, 549 nm excitation, 250 ms integration time, 6.7 µm resolution.

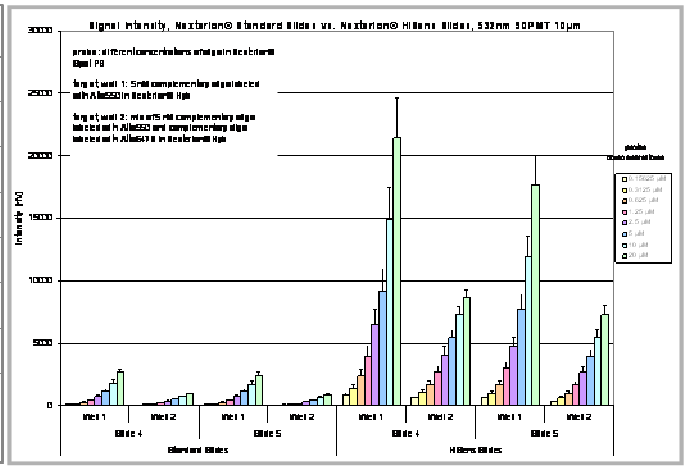


Figure 29: Signal intensity of an oligonucleotide dilution series in combined microarrays comparing NEXTERION® Slide E with NEXTERION® Slide HiSens E; measured with Laser Scanner, 532 nm excitation, 110 gain, 10 µm resolution.

FLAIR Data	Laser Scanner Data

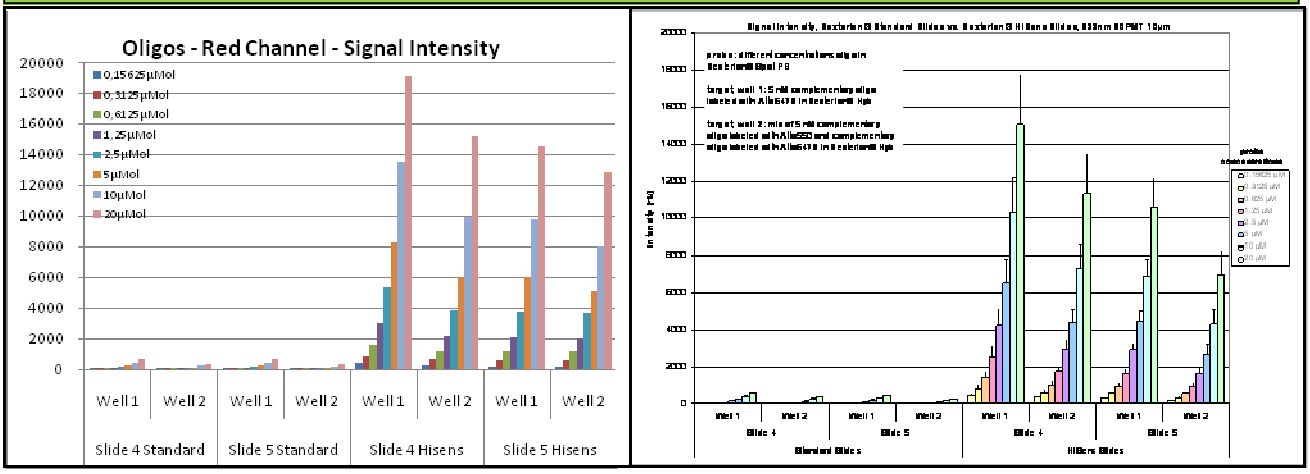


Figure 30: Signal intensity of oligonucleotide dilution series in combined microarrays comparing NEXTERION® Slide E with NEXTERION® Slide HiSens E; measured with FLAIR, 647 nm excitation, 25 ms integration time, 6.7 µm resolution.

Figure 31: Signal intensity of an oligonucleotide dilution series in combined microarrays comparing NEXTERION® Slide E with NEXTERION® Slide HiSens E; measured with Laser Scanner, 633 nm excitation, 110 gain, 10 µm resolution.

3 Results and Discussion

3.1 Instrument and Microarrays

We measured and analyzed a total of 10 slides (5 NEXTERION® Slide E, 5 NEXTERION® Slide HiSens E) with 120 Microarrays and 7,680 spots and created 10,240 data points (spot intensities) with FLAIR using the integrated Array Reader software. The process was quick and simple since the array configurations were defined and stored in FLAIR as a method upfront. Post run processing of the raw data was performed with Microsoft EXCEL, which is compatible with the Array Reader software.

Integration times of 200 ms for green channel and 30 ms for the red channel were used. These short integration times are ideal because dark current and bleaching are minimal. If required, integration times can be extended by a factor of 10 or 40, respectively, for higher sensitivity and lower detection limits. With the settings described, FLAIR was able to detect the entire concentration range of all microarrays measured.

Microarray layout was very precise and reproducible. All spots were located at their expected position. The 6.7 µm pixel-resolution of FLAIR allowed analysis of spot morphology in detail. The 200 µm spots were perfectly round with minimal ”donut-effects” (Figure 6). Spot morphology within the different microarrays was very reproducible. Fluorescence signals varied to certain extend between the microarrays. Especially the microarrays incubated with 2 differently labeled targets showed lower fluorescence intensity than the microarrays incubated with a single target label. These effects are likely due to competitive binding kinetics during incubation and are to be expected.

3.2 Slide Comparison

Spot morphology and microarray geometry was identical for NEXTERION® Slide E *and* NEXTERION® Slide HiSens E. No significant difference could be found. NEXTERION® Slide E *and* NEXTERION® Slide HiSens E were first compared visually under identical instrument settings. NEXTERION® Slide HiSens E show a bright, easily visible microarray pattern. In contrast, the microarrays on NEXTERION® Slide E were much weaker and only the brightest spots were visible (with identical contrast settings). This effect was observed with antibody arrays and oligonucleotide arrays, in both the red (excitation 647 nm) and green (excitation 549 nm) channels (see Figures 10 – 13).

A detailed analysis of data from selected microarrays confirmed this visual assessment: all microarrays printed on NEXTERION® Slide HiSens E gave a significantly higher net fluorescence signal (mean spot signal minus mean local background signal) compared to NEXTERION® Slide E. The factor was between 10 – 50, depending on the concentration of the biomolecule and the color (Figures 15 – 22). The maximal signal leveled off with some microarrays (Figures 15, 16, 18) which is likely due to concentration effects and not due to reader properties, because measured intensities were far from saturation and this effect was not observed in other samples from the same series.

Analysis of (net-) signal intensity, however, does not provide all the information, especially when background signal increases as well. A better indicator for sensitivity and limit-of-detection is signal to noise ratio (S/N). Assuming that the instrument performance is identical for all measurements, S/N can be used for slide comparison. S/N was calculated from randomly selected microarrays. Rows of spots with the same concentration were analyzed with a special emphasis on weak signals. S/N was calculated for each spot (Tables 1-4). We found that spot signal increased with NEXTERION® Slide HiSens E. We also observed a slight increase in background signal (Tables 1-4). The summary in Table 5, however, shows that signal-to-noise ratio of spots on NEXTERION® Slide HiSens E is higher by a factor 4 to 7. This improvement is impressive especially at low concentration levels. On NEXTERION® Slide E S/N ratios for these spots was just above the detection limit (S/N of 4.4 and 3.0). NEXTERION® Slide HiSens E increased S/N up to a value of approx. 20. This means that NEXTERION® Slide HiSens E allows detection of approximately 6 fold lower concentrations compared to a NEXTERION® Slide E.

	mean S/N of 8 spots with the same concentration - **standard slide**	mean S/N of 8 spots with the same concentration - **Hisens slide**	Ratio (Hisens / standard)
Protein 0.2 mg/mL (high signal), green channel	71,1	281,4	4,0
Protein 0.00625 mg/mL (low signal), green channel	4,4	19,1	4,3
Oligo 0.15625 µMol (low signal), red channel	3,0	20,8	6,8
Oligo 5 µMol (high signal), red channel	26,8	187,2	7,0

3.3 Reader Technology Comparison

Additionally, we compared FLAIR to a conventional laser scanner. Laser-based microarray scanners represent the state-of-the art imaging technology. They use confocal scanning with laser beam excitation and PMTs for detection. In contrast FLAIR uses a different technological approach. FLAIR is a CCD-detector based imager with high-power LED illumination. This design results in more compact and economical instruments.

The reduced data obtained from both instruments, FLAIR and the laser scanner were, presented as bar-diagrams and arranged side-by-side (Figures 24 – 31). This allows a visual comparison of the spot net-intensities. It can clearly be seen that FLAIR produces comparable results to a conventional laser scanner. A more detailed comparison will be published in a separate article.

4 Summary and Conclusion

Model microarrays printed for this study were very accurate and reproducible with homogenous and consistent spots. This is likely due to the SCHOTT NEXTERION® surface chemistry and the spotting technology used. The experiments clearly show that NEXTERION® Slide HiSens E produce a significantly higher fluorescence signal. Especially the detection limit is significantly improved when using NEXTERION® Slide HiSens, compared to NEXTERION® Slide E. These effects could be verified with protein and oligonucleotide microarrays – both in red channel as well as in green channel.

FLAIR, Sensovation's fluorescence array imaging reader, was successfully used to analyze the microarrays. Using the 4-slide holder the instrument provided sufficient sensitivity and linearity for measuring and analyzing all microarrays and slides from this study. A side by side comparison with data from a conventional microarray scanner (laser based) showed that the results of all arrays are very well comparable. An important component of a microarray analysis system is the microarray analysis software. With the Array Reader software package, FLAIR includes a powerful tool providing on-the-fly array analysis with intelligent spot detection. Only with this software it was possible to create reliable numerical data and finally reduce them to obtain a solid data base.