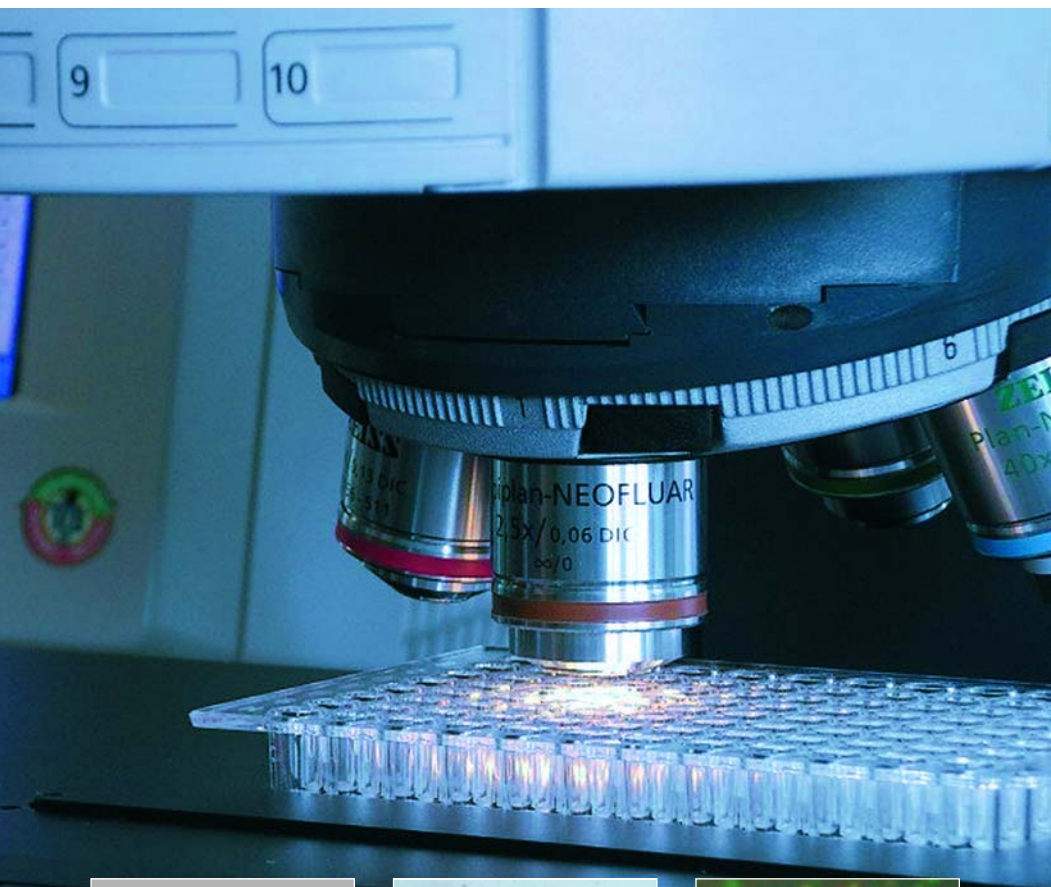


KS ELISPOT KS ELISPOT compact



Fast evaluation of standard and fluorescence Elispot specimen

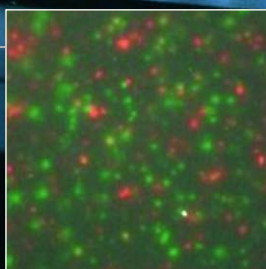
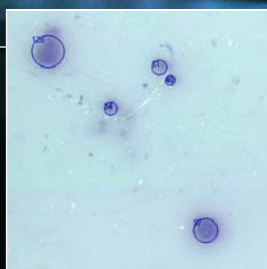
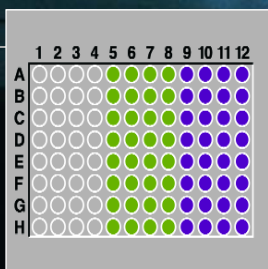
Efficient and reproducible method for Enzyme Linked Immuno SPOT assay evaluations

Complete package for immediate high speed measurements with high resolution

Re-evaluation of stored well images

Evaluation of multiple labels

Evaluation of fluorescence specimen



**Routine Systems for
Enzyme Linked Immuno SPOT
Assay Evaluations**



We make it visible.

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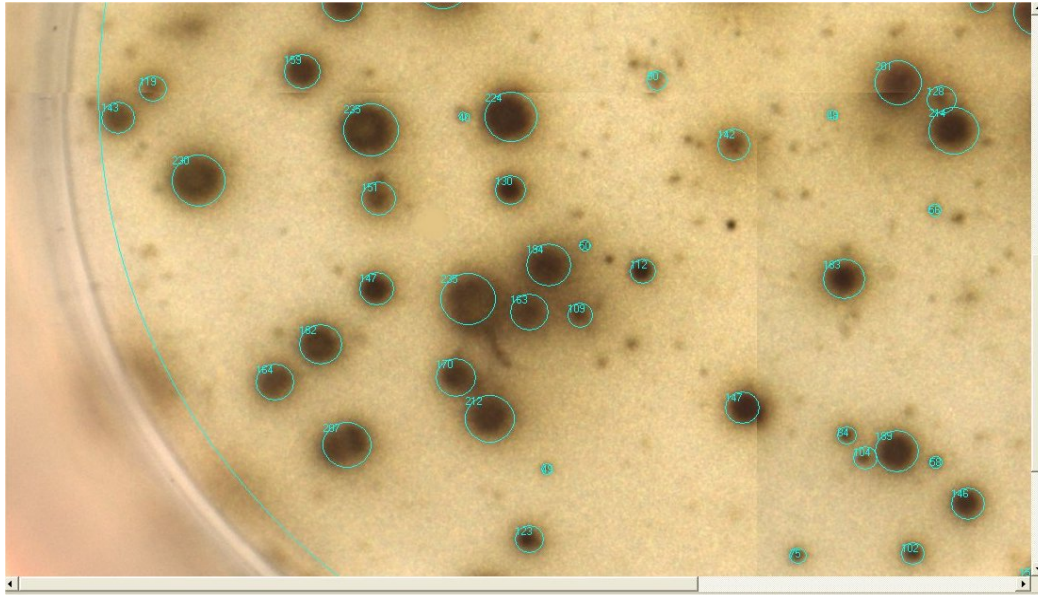
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Performance Features

- **Easy handling**
Especially developed for use in routine laboratories, easily adaptable to new preparation methods using the unique spot learning procedure.
- **Complete package**
Microscope, scanning stage, control unit, computer, and software: all from one source - assembled for maximum performance.
- **Minimum adjustments**
Define the start and end-position and then run the evaluation – it's that simple.
- **Overview or full resolution image**
Permanent display of overview image for control purposes with access to the full resolution image including the display of results.
- **Storage of measurement results**
Spot results (number, single areas, and single intensities) are stored as text files for external processing and graphic presentation.
- **Automatic re-evaluation of stored images**
All well images stored in a folder under the position name in the plate can be re-evaluated automatically. This is of advantage with the evaluation of fluorescence Elispot specimen with different labels to avoid bleaching.
- **Any choice between ultra high speed and ultra high resolution**
Each system offers the option to evaluate each well with either one image per well or in an image collage per well of 2x2, 3x3 or 3x4 images per well, depending on the preference in speed or resolution.
- **Data output**
All measurement results can be stored in the internal RTF format. As an alternative a Word protocol may be produced showing experiment description, measurement data, and parameter settings, as well as a plate image of all measured wells and a plate image with all detected spots in each measured well.
- **Storage of images**
Images of all measured wells may be saved for documentation purposes, if needed.
- **Stereo microscope or high end research microscope**
For a routine laboratory the KS ELISPOT compact based on a stereo microscope offers the best price performance. The KS ELISPOT using an upright research microscope is already prepared for all future developments.
- **Integrating digital color camera or standard 3 CCD color camera**
For ultra high speed the standard camera is the first choice, for speed and high resolution or evaluation of fluorescence Elispot specimen the digital camera is essential.

The KS ELISPOT Method

The enzyme linked immuno spot assay has been developed as a marker for individual antibody secreting B-type cells. Meanwhile, the method has been improved so that cells can be identified producing only about 100 molecules per second of a specific protein (e.g. cytokine). High protein concentrations in the region of the cells are detected with specific antibodies. The spots of the Elispot method are generated with specific staining methods producing only imprints of the original cells generating these spots. The advantage of this technique is the fact that the spots are permanent and can be evaluated visually and by means of image analysis.



The Elispot method consists of 5 steps:

- Adding a cytokine specific antibody to the nitrocellulose membranes of a microtiter plate.
- Exclusion of non-specific absorption of other proteins.
- Adding cytokine secreting cells in various concentrations.
- Addition of a second anti-cytokine antibody.
- Detection of the antibody-cytokine complex.

At present two staining techniques are used. The alkaline phosphatase (AP) marker produces blue spots while the horseradish peroxidase (HRPO) marker produces brownish ones. The method is also used for the detection of cytokine secretions of specific sub-groups of leukocytes or T-cells from peripheral blood and for monocytes and granulocytes.

Meanwhile also double labeling is of interest. Two cytokines labeled in red and in blue or cells with both cytokines appearing in violet should be detected. As an alternative fluorescence markers may be used resulting in red or green spots, respectively yellow spots in case of both expressions.

The Elispot assay faces two background problems. First, a variable background intensity of the membrane may be seen. To overcome this problem specific algorithms have been developed, taking into account the varying background conditions in the region when detecting the spots. It is no longer necessary to adjust different threshold values for various positions of a well.

With the aid of the unique spot learning procedure all system parameters, necessary for a correct spot recognition, are adjusted by simple cursor clicks to desired spots. A further advantage of this new method is the improvement of measurement reproducibility.

The second problem that occurs are small and very dark spots that were not generated by secreted cytokines. In the visual evaluation these spots are differentiated from "true" spots by their sharper edges. True spots always have a dark center with fading color intensity towards the edges. False spots are usually small with a homogeneous intensity. Functions for the differentiation of true spots have also been implemented in the detection algorithm of the KS ELISPOT software.

The shape can be tested to decide whether, or not, a spot exists. Cytokine spots are always round but due to "floating edges" with the background they have a "non-smooth" borderline.

All these conditions demand certain minimum and maximum magnifications for accurate use of the system. The recognition of differences in edge intensities needs a certain minimum spot diameter regarding the pixel resolution. Acquiring a complete well as one image requires a high resolution. Evaluating more single images with higher resolution raises problems with spots touching the frame edges of an image, as well as with spots at the edge of the well. Therefore the KS ELISPOT software first generates a complete image from numerous single images resulting in a high resolution, which is then evaluated with the correct pixel resolution without any edge problems using a circular measurement frame.

As shown in a current investigation a comparison of different methods evaluating 1, 4, or 12 images per well, one single image per well is not sufficient to clearly recognize true spots. For IFN-g spots with approximately 30 µm smallest spot diameter and 5 pixel necessary diameter for a true spot recognition a minimum pixel size of 6 µm is necessary. For a 6 mm well diameter 1000 pixels are therefore a must. This will result in at least 4 images from a standard 3 CCD color camera with 760x580, or 1 image from a digital camera with 1300x1000 pixel resolution.

For further details see:

Gazagne A, Malkusch W, Vingert B, Fridman WH, Tartour E (2004): Fluorospot assay: methodological analysis. In: Kalyuzhny A: Handbook of ELISPOT – methods and protocols, Humana Press, Totowa, USA.

Herr W, Schneider J, Lohse AW, Meyer zum Büschenfelde K-H, Wölfel T (1996): Detection and quantification of blood-derived CD8+ T lymphocytes secreting tumor necrosis factor α spots in response to HLA-A2.1-binding melanoma and viral peptide antigens. Journal of Immunological. Methods 191, 131-142.

Janetzki S, Cox JH, Oden N, Ferrari G (2004): Standardization and validation issues of the ELISPOT assay In: Kalyuzhny A: Handbook of ELISPOT – methods and protocols, Humana Press, Totowa, USA.

Janetzki S, Palla D, Rosenhauer V, Lochs H, Lewis JJ, Srivastava PK (2000): Immunization of cancer patients with autologous cancer-derived heat shock protein gp96 preparations: a pilot study. International Journal of Cancer 88, 232-238

Klinman DM, Nutman TB (1994): Elispot Assay to Detect Cytokine-Secreting Murine and Human Cells. In: Current Protocols in Immunology, Suppl. 10, Unit 6.19, 1-8

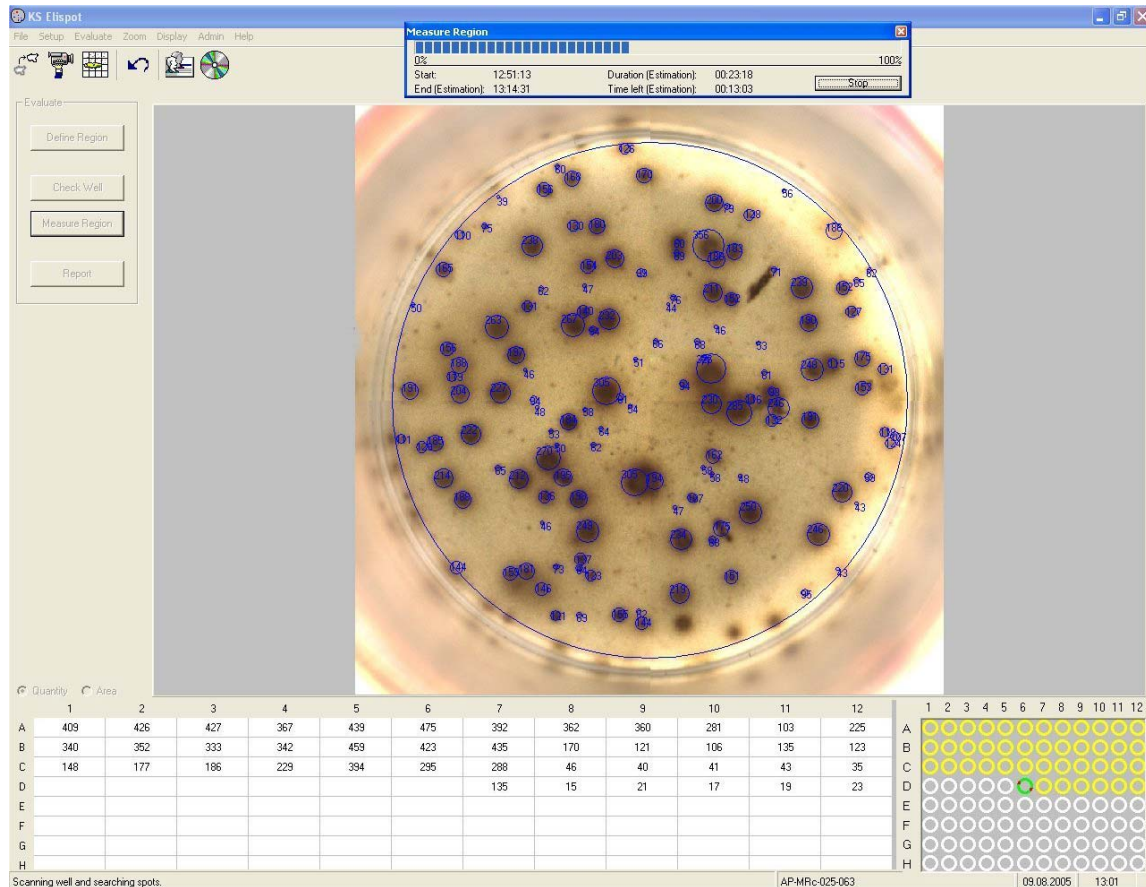
Lewis JJ, Janetzki S, Schaed S, Panageas KS, Wand S, Williams L, Meyers M, Butterworth L, Livingston PO, Chapman PB, Houghton AN (2000): Evaluation of CD8+ T-cell frequencies by the Elispot assay in healthy individuals and in patients with metastatic melanoma immunized with tyrosinase peptide. International Journal of Cancer 87, 391-398

Malkusch W (2004): High resolution as a key feature to perform accurate ELISPOT measurements using Zeiss KS ELISPOT readers. In: Kalyuzhny A: Handbook of ELISPOT – methods and protocols, Humana Press, Totowa, USA.

*Schaed S, Klimek V, Panageas K, Musseli C, Butterworth L, Hwu WJ, Livingstone P, Williams L, Lewis JJ, Houghton A, Chapman P (2002): T-cell responses against Tyrosinase 368-376 (370D) peptide in HLA *AO201* melanoma patients: randomized trial comparing incomplete Freud's adjuvant, granulocyte macrophage colony-stimulating factor, and Q-21 as immunological adjuvants. Clinical Cancer Research 8, 967-972.*

The KS ELISPOT Software

The user-friendly system interface guarantees minimum training time and very easy adjustments of the few control parameters necessary. In this way results can be obtained quickly and accurately, saving time and laboratory costs.



KS ELISPOT is a system with a complete solution for precise and automatic evaluation of Elispot assays. It has been developed especially for use in routine laboratories. All components, from the camera, the scanning stage, down to the microscope, are assembled for optimum performance.

Specimen Mounting

The specimen should be mounted very flush with the plate of the stage.

Suitable specimens are original 96 well microtiter plates, filtration plates with nitrocellulose membranes, or nitrocellulose membranes mounted on sticky foils. The latter should lay flat on the scanning stage (best applied with spray adhesive and a roller squeeze).

Special stage plates with markers for the driving ranges are available for use with peeled off nitrocellulose membranes. For use with complete nitrocellulose plates suitable specimen holders are available.

Tools for Routine Evaluation



Define Region: Definition of the plate region for the evaluation, resulting in the number of wells to be measured. At the same time the well positions and the stage co-ordinates are synchronized.

Check Well: Test evaluation of one selected well to check the system settings and to perform the learn procedure.

Measure Region: Evaluation of all selected wells of a microtiter plate.

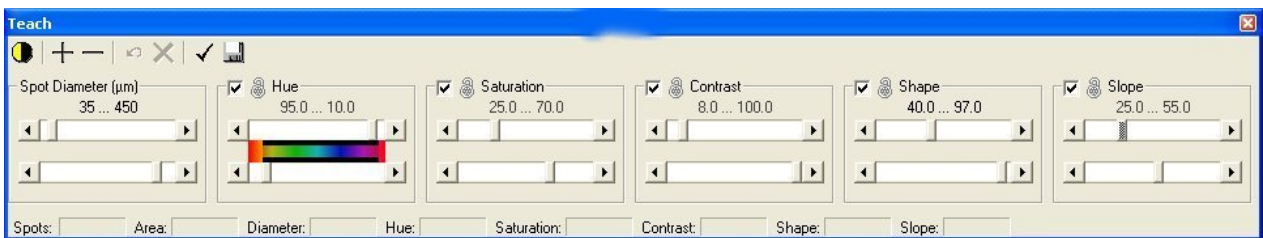
Report: Display of a results page with the option to print and save the data.

Selection of Specimen Type

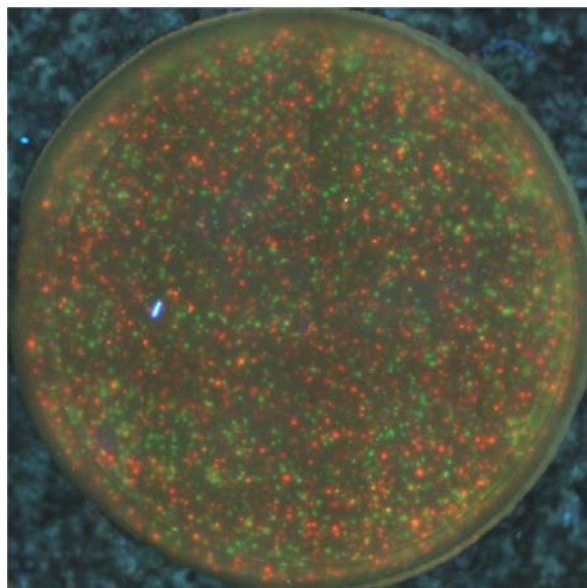
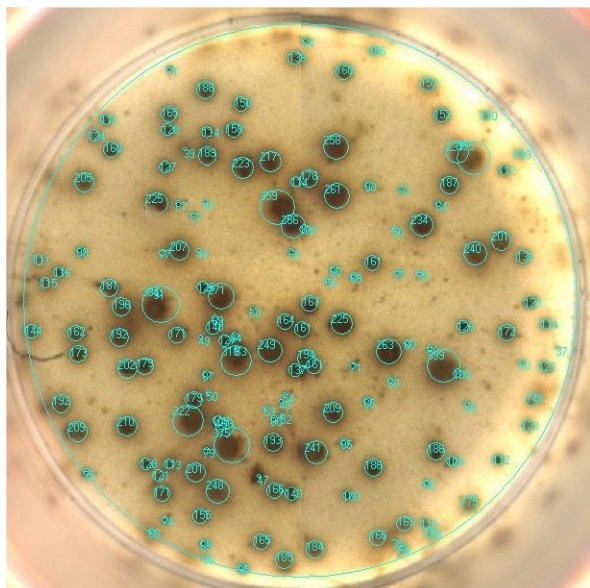
Special user defined configuration files will determine, whether a standard specimen has to be evaluated, or if it will be a fluorescence specimen. The switch between on-line evaluation and re-evaluation of stored images is a CD symbol in the icon toolbar. All systems without available hardware like microscope or stage control will automatically start up in re-evaluation mode.

Learning Method for Spot Recognition

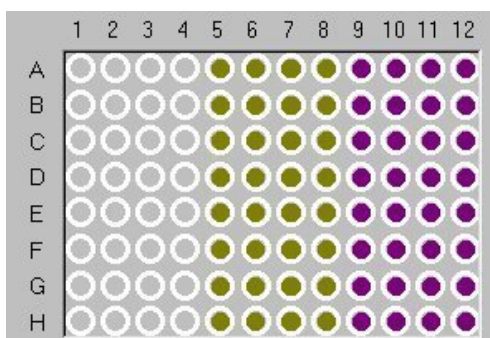
KS Elispot uses a specifically developed learning method to recognize the spots. This learning algorithm extends all its parameters for spots that were selected from clicks on not yet recognized spots using the cursor and the left mouse key. In this way all spot parameters are adapted automatically. Spot definitions created in this way can be stored for future use.



All recognized spots are graphically displayed together with their diameters. The spot recognition algorithms have been further improved to also detect very accurately fluorescent spots.



Routine Use



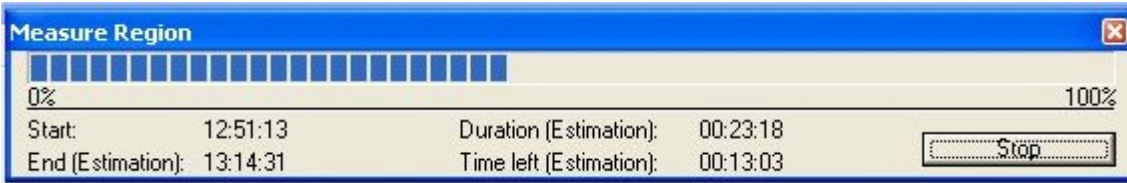
The region for evaluation is defined in the plate table on the screen using the mouse. To start a measurement the definition of only the start and end well is necessary.

Positions not required can be eliminated from the measurement by mouse clicks.

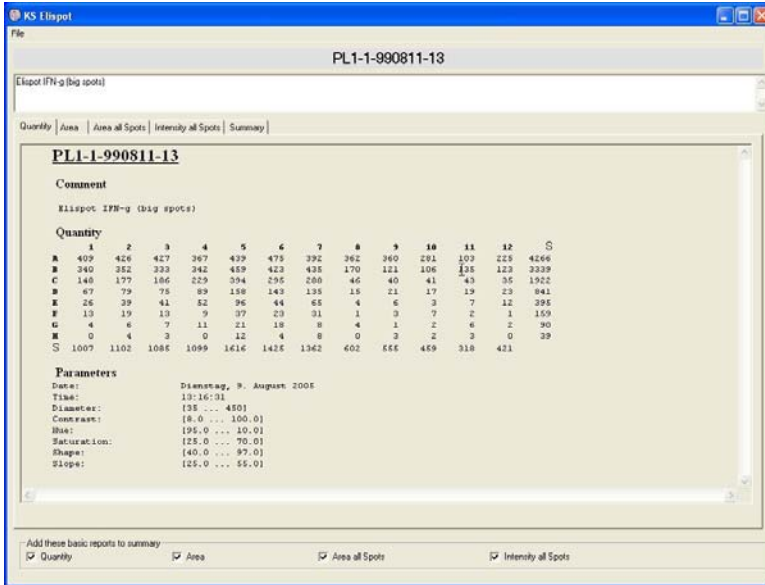
Per plate various regions can be defined using different configurations for evaluation.

	Quantity											
	1	2	3	4	5	6	7	8	9	10	11	12
A	409	426	427	367	439	475	392	362	360	281	103	225
B	340	352	333	342	459	423	435	170	121	106	135	123
C	148	177	186	229	394	295	288	46	40	41	43	35
D	67	79	75	89	158	143	135	15	21	17	19	23
E	26	39	41	52	96	44	65	4	6	3	7	12
F	13	19	13	9	37	23	31	1	3	7	2	1
G	4	6	7	11	21	18	8	4	1	2	6	2
H	0	4	3	0	12	4	8	0	3	2	3	0

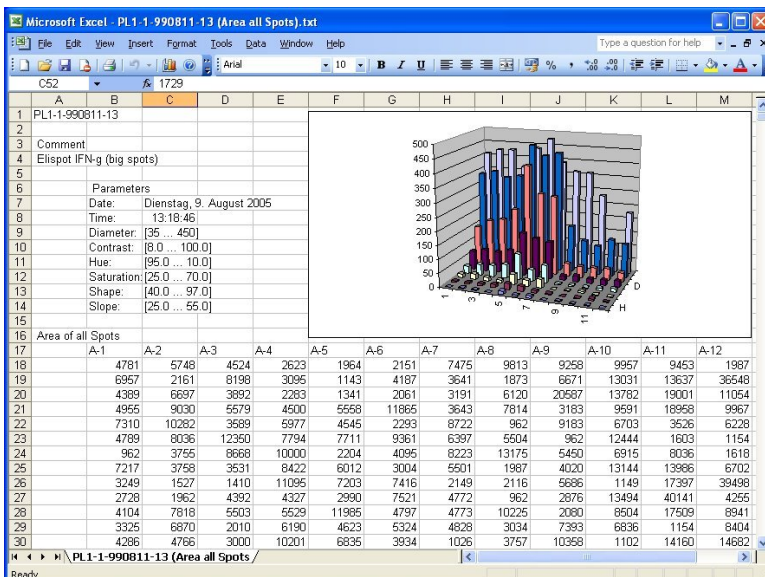
The results of an analysis (spots per well) are immediately presented on the screen.



During the evaluation period the likely time for the complete evaluation of the entire plate is calculated and permanently updated, together with the estimated time for the completion of the evaluation - as a value and as a progress bar.



Number, size and intensity of each single spot can be printed via the results page, or stored in data files for further statistical evaluations or graphic displays. Images of single wells can be stored for documentation purposes.



Apart from title, date and spot size limits all single spot values are stored in well number order. For each well the number of spots, the areas and the intensities are available. In addition to the measurement data all adjustment parameters will be stored.

As a true 32-bit application KS ELISPOT operates under Windows 2000 or Windows XP.

ZEISS Carl Zeiss - internal / confidential - PL1-1902011-13-eng-44.doc

Carl Zeiss EliSPOT Report

Description: EliSpot IFN- γ
PL1-4-990301-13

Employer: IFM

Date: 09.04p.2005

Comments: Big spots

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- 2 Summary area per well 2
- 3 Overview image spots per well 3
- 4 Overview image results per well 4
- 5 Parameters of Classifier 5

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Printed: 09.09.2005 13:54:00

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1 Summary Spots per well

W	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
N	430	430	431	397	430	430	390	390	390	390	390	390	390	390	390	390	390	390	390	390
U	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340
S	240	240	240	240	240	240	240	240	240	240	240	240	240	240	240	240	240	240	240	240
G	4	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Z	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340	340

2 Summary area per well

W	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
N	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024	1767024
U	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024
S	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024	555024
G	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024
Z	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024	1111024

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3 Overview image spots per well

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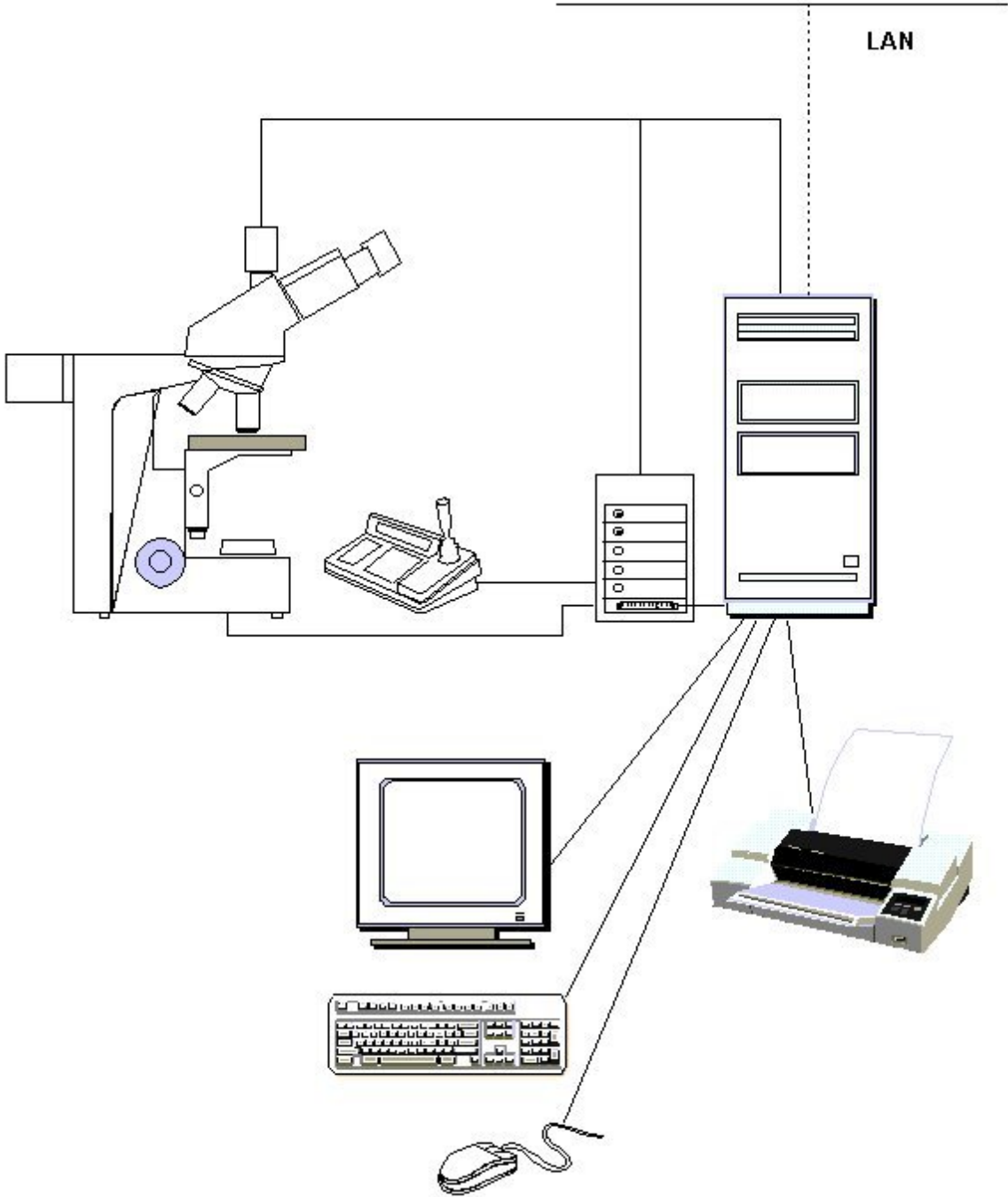
ZEISS Carl Zeiss - internal / confidential - Document

4 Overview image results per well

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As an alternative to the internal data format a result protocol may be automatically generated under Word. This protocol exists of a title page with the general experiment description, the results of spot counting and area measurements, as well as the parameter settings for the spot definition. Furthermore a color overview image of all evaluated wells in a plate is created and additionally an overview image showing all recognized spots in each measured well.

The KS ELISPOT System



Microscope, Scanning Stage, Camera, Joystick

The immuno chemically stained cytokine spots are either scanned with a high resolution digital integrating color camera (AxioCam MRc) or as an alternative with a 3 chip CCD true color camera (Hitachi) on a microscope with incident light (Axio Imager equipped with motorized Z-drive, TV adapter, and EC Epiplan Neofluar 2.5x and 5x objectives) with motorized stage. The images are digitized with 24-bit color resolution and evaluated with the KS ELISPOT software (KS ELISPOT).

In the KS ELISPOT compact the scanning stage is controlled by a Ludl MAC5000 control unit. Changes in the regions for evaluation are made using the joystick.

For fluorescence specimen the Axio Imager microscope is equipped with fluorescence illumination and adequate filter sets.

System Computer

The KS ELISPOT computer is equipped with everything necessary for a trouble-free operation. The INTEL Pentium processor offers more than adequate computing power for the application. The equipment, with sufficient MB RAM, is designed for the evaluation of even larger images. In this way the system is already equipped for future developments. Installed in robust tower housing, there is enough space for system upgrades. This is also guaranteed by the 3.5 inch floppy drive, a CD-rewritable recorder, a SCSI adapter, serial, parallel, and USB ports. Mouse, keyboard, and operating system (Windows 2000 or Windows XP) complete the system.

The hard disk provides capacity for large amounts of data and images. The graphics card, with 24-bit color resolution, allows the parallel display of images and control elements on the high-resolution monitor. The image input is achieved by the AxioCam interface or the Matrox Meteor 2 frame grabber.

System Components

There are 4 different configurations available, 2 for the KS ELISPOT and 2 for the KS ELISPOT compact.

Hardware

"Axio Imager.M1" Microscope for KS ELISPOT

430004-0000-000	"Axio Imager.M1" microscope stand with Z-drive mot., 6-position reflector turret mot.
430051-0000-000	Z-drive operation on the right
424501-0000-000	7-position objective nosepiece, HD M27 cod.
423603-0000-000	Reflected-light illuminator for FL and HD
423632-0000-000	Diffusion disk for reflected-light illumination
428300-0000-000	Double filter wheel, discrete, man.
487931-0000-000	Neutral-density filter set D/A, d=25
447825-0000-000	Conversion filter 3200-5500 K, d=25x2
424928-0000-000	Reflector module H P&C for reflected light
000000-1046-277	Reflector module DIC P&C
425503-0000-000	Binocular phototube 15°/25 Mat (100:0/0:100), upright image
434304-0000-000	Dust protection set L
423000-0000-000	100 HAL lamp housing with collector
380079-9540-000	Halogen lamp 12V 100W
422322-9900-000	Objective EC "Epiplan-Neofluar" 2.5x/0.06 M27 (a=15.1 mm)
000000-1156-511	Objective EC "Epiplan-Neofluar" 5x/0.13 DIC M27 (a=15.8 mm)
455043-0000-000	Eyepiece W-PL 10x/23 Br. foc.
444801-0000-000	Eyepiece eyecup
426113-0000-000	Camera adapter 60N-C 2/3" 0.63x

490014-0002-000 "Axio Imager.M1" Microscope for KS ELISPOT

"Stemi 2000 C" Microscope for KS ELISPOT compact

455053-0000-000	"Stemi" 2000-C microscope body
455094-0000-000	Stemi mount with drive for column 32
455101-0000-000	Stand base 32 (330x380) without column
475120-0000-000	Column 32/450
000000-1148-287	Clamp piece 0° + 5,5° / 32
000000-1063-181	Cold-light source KL 1500 LCD (230V) (D)
417068-0000-000	Slit-ring illuminator, d=58 mm (D)
455184-0000-000	Holding ring d=58 mm for ring illuminator
417053-0000-000	Halogen lamp 15V 150W (D)
455043-0000-000	Eyepiece W-PL 10x/23 Br. foc.
444801-0000-000	Eyepiece eyecup
456105-9901-000	Camera adapter 60 C 1" 1.0x
459306-0000-000	Dust cover G

495005-0001-000 "Stemi" 2000-C for KS ELISPOT compact

Color Cameras

426808-0000-000	High resolution microscopy-camera Zeiss AxioCam MRc Rev. 2 / 3.3 V (D)
000000-1078-403	Matrox Meteor2/MC4, RGB, b/w, Trig, built in Zeiss Computer (D)
000000-0468-392	Meteor2/MC2 cable for Hitachi HV-D20P (D)
000000-0468-336	Video-Camera HITACHI HV-D20P 1/2" 3-CCD C-Mount (D)

Scanning Stage and Control

For Axio Imager	
432022-0000-000	Scanning stage 130x85 PIEZO (D)
432901-9901-000	XY-Stage controller PIEZO (D)
432903-9901-000	XY-Joystick for stage controller PIEZO (D)
For Stemi 2000 C	
000000-0431-478	Ludl MAC5000 XY stage controller stepper incl. Joystick (D)
410121-0026-000	Maerzhaeuser scanning stage set for "Axioplan" 2 imaging (D)
452327-0000-000	Stage carrier with condenser carrier
Accessories	
432315-0000-000	Mounting frame for slide 76x26 (D)
432312-0000-000	Glass insert plate (D)
432313-0000-000	Metal insert plate (D)
432314-0000-000	Mounting frame for Elispot plates HA/IP (D)
432316-0000-000	Mounting frame for Elispot plates HTS (D)
474025-0000-000	Stage micrometer 25 + 50/10 mm "Stemi"

Computer, Camera and Software

KS ELISPOT Rel. 4.8 AxioCam MRc	
410203-0000-000	Image Analysis XEON Workstation 2,8 GHz multilingual (D)
410303-0100-000	Memory 1024 MB DDR-RAM 800 MHz ECC (D)
000000-1221-885	Language Package Windows XP English (D)
000000-0461-284	LCD TFT Monitor B17 – 1, 17" (43 cm) (D)
426808-0000-000	High resolution microscopy-camera AxioCam MRc Rev. 2 / 3.3 V (D)
456108-0000-000	Adapter Video 60 C 1/3" 0.4x
000000-1060-449	Manual Squeezer 25 x 150 mm
000000-1060-450	Adhesive Spray 400 ml
000000-1202-307	Elispot Puncher Kit (D)
410134-0100-000	KS ELISPOT Software Rel. 4.8
490002-0002-000	KS ELISPOT Rel. 4.8 / AxioCam MRc
KS ELISPOT Rel. 4.8 / Hitachi HV-D20P	
410203-0000-000	Image Analysis XEON Workstation 2,8 GHz multilingual (D)
410303-0100-000	Memory 1024 MB DDR-RAM 800 MHz ECC (D)
000000-1221-885	Language Package Windows XP English (D)
000000-0461-284	LCD TFT Monitor B17 – 1, 17" (43 cm) (D)
000000-1078-403	Matrox Meteor2/MC4, RGB, b/w, Trig, built in Zeiss Computer (D)
000000-0468-392	Meteor2/MC2 cable for Hitachi HV-D20P (D)
000000-0468-336	Video-Camera HITACHI HV-D20P 1/2" 3-CCD C-Mount (D)
000000-1060-449	Manual Squeezer 25 x 150 mm
000000-1060-450	Adhesive Spray 400 ml
000000-1202-307	Elispot Puncher Kit (D)
410134-0100-000	KS ELISPOT Software Rel. 4.8
490002-0001-000	KS ELISPOT Rel. 4.8 / Hitachi

Options

000000-0468-312 **Instead of 17" TFT Monitor on request:**
LCD TFT Monitor P19 – 1 S, 19" (48 cm)(D)

410104-0033-000 **Printer:**
Inkjet printer HP DeskJet 970Cxi, 220V
410164-1343-000 Parallel (Centronics) printer cable (1.8 m)

Fluorescence Equipment (Option)

423010-0000-000 **HBO 100 illuminator**
Illuminator HBO 100 with lamp mount and collector
380301-9350-000 Super-pressure mercury lamp
HBO 103 W/2
432604-0000-000 Power supply unit for HBO 100, 90...250V, 50...60 Hz, 265 VA
For each filter set:
424920-0000-000 Reflector module FL P&C
Filter sets:
488009-0000-000 Filter set 09 shift free
EX BP 450-490, BS FT 510, EM LP 515
or
488024-0000-000 Filter set 24 FITC + Texas Red shift free
EX DBP 485/20+578/14, BS FT 500+600, EM BP (515-540)+LP
610
or
488025-0000-000 Filter set 25 DAPI + FITC + Texas Red shift free
EX TBP 400+495+570, BS FT 410+505+585, EM TBP
460+530+610

000000-1103-997 Storing case for 6 pieces of reflector modules P&C

Software

410134-0100-000 KS ELISPOT Software Rel. 4.8
for Windows 2000, and Windows XP,
incl. documentation and security device
410134-1100-000 KS ELISPOT Software Rel. 4.8
site license
000000-1282-463 Elispot Manual English (O)

Carl Zeiss
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