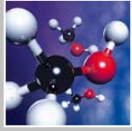
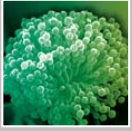
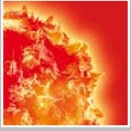


Zählung von Luftkeimen - eine Echtzeitmethode Reinraum Lounge

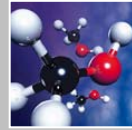
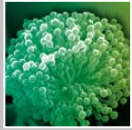
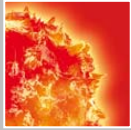
16 März 2010

Jörg Dressler -PMT GmbH



Inhalt

- Guidelines
- Klassische Methoden
- Fluoreszenz und Auto - Fluoreszenz
- Apparativer Aufbau
- Anwendungsbeispiele



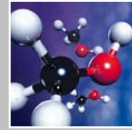
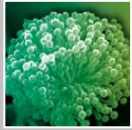
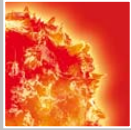
EU - Annex 1: VIABLE MONITORING

	Empfohlene limits für mikrobielle Kontamination (a)		
	Aktive Probenahme (cfu/m ³)	Sedimentationsplatten D: 90 mm // cfu /4 hr ^(b)	Kontaktplatten D: 55 mm cfu / Platte
Grades			
A	< 1	< 1	< 1
B	10	5	5
C	100	10	25
D	200	100	50

(a) Es handelt sich um Mittelwerte

(b) Einzelne Sedimentationsplatten können für weniger als 4 Stunden beprobt werden

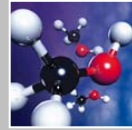
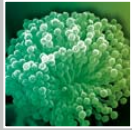
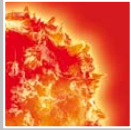
Es fehlen in dieser Präsentation die Werte für Handschuh Abklatsch



FDA guidance for industry

Clean Area Classification (0.5 um particles/ft ³)	ISO Designation	≥ 0.5 mm particles/m ³	Microbiological Active Air Action Levels (cfu/m ³)	Microbiological Settling Plates Action Levels (D: 90mm; cfu/4 hr)
100	5	3,520	1 ^e	1 ^e
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

“Air monitoring samples of critical areas should normally yield no microbiological contaminants. We recommend affording appropriate investigative attention to contamination occurrences in this environment. ”



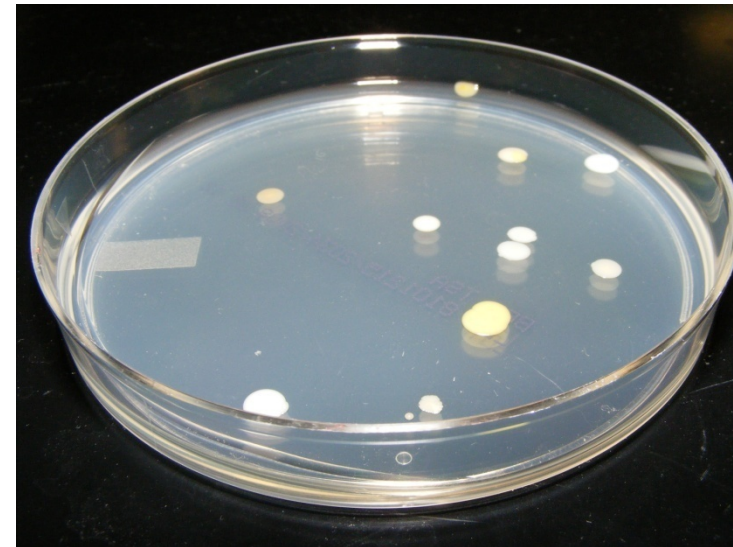
Klassische Methoden

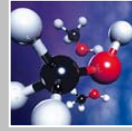
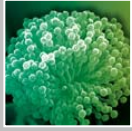
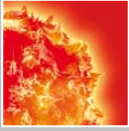
Sedimentationsplatte

Impaktionssysteme

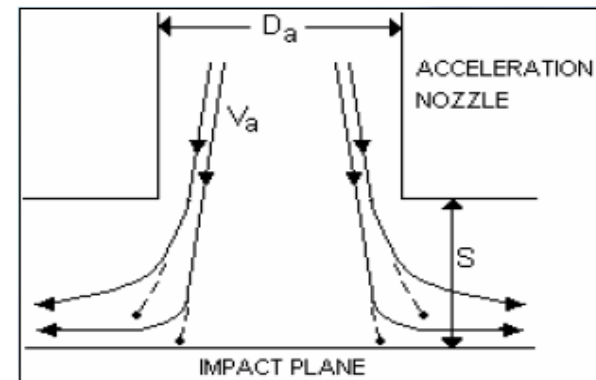
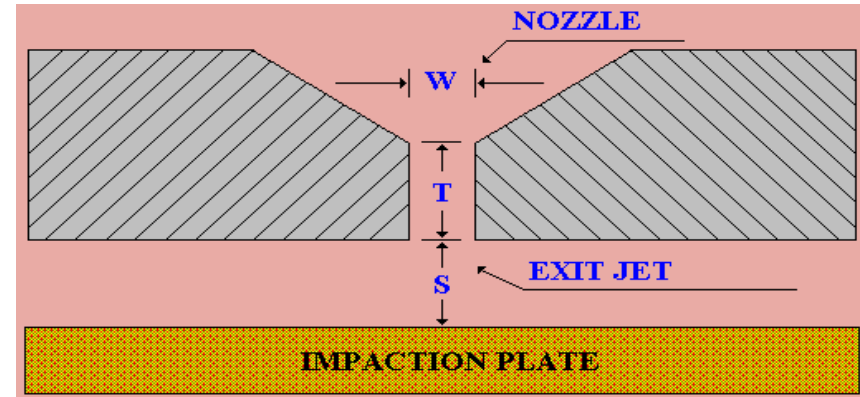
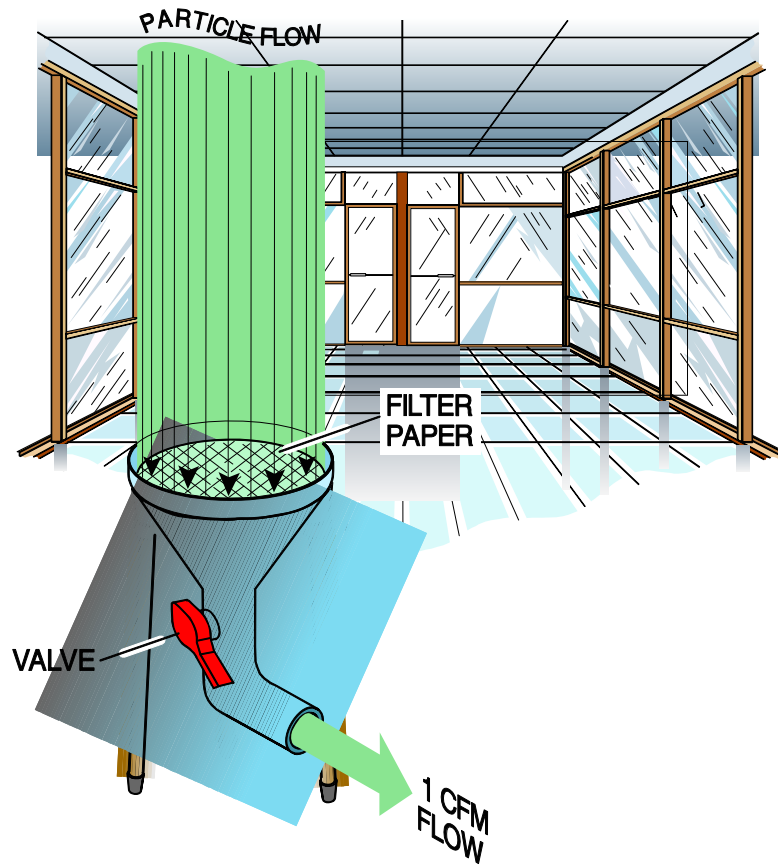
Filtrationssysteme

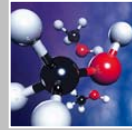
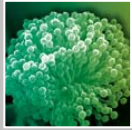
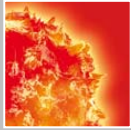
Liquid Impinger





Aktive Keimsammlung

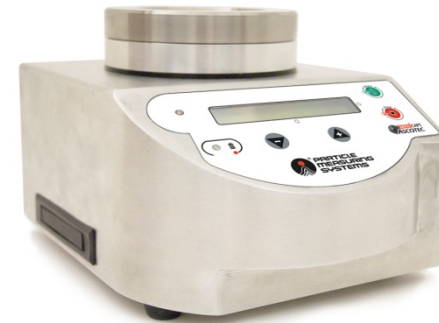




Impaktoren - Bauformen



© Biotest

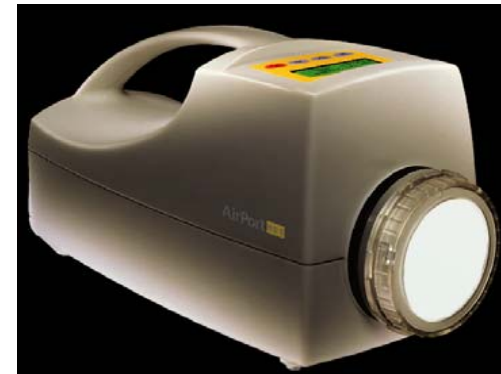


©PMS

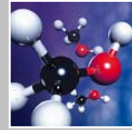
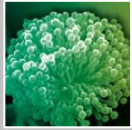
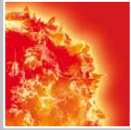
Filtrationssysteme



©VWR/Merck



© Sartorius



- Sterile Medien spezifisch für System

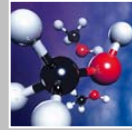
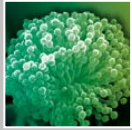
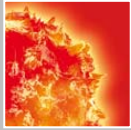
90 oder 100 mm Platten
Nährmedien Streifen

- Medien spezifisch für Organismen

- TSA Trypticase Soy Agar für universelles Monitoring

- SDA Sabouraud Dextrose Agar für Hefen und Pilze

- Bebrütung typisch 2 - 3 Tage
5 - 7 Tage



Übergang zur Echtzeitmethode – FDA sterile guidance

X. Laboratory controls

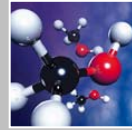
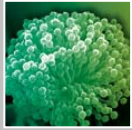
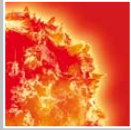
A. Environmental Monitoring

4. Monitoring Methods

- a. surface monitoring
- b. active air monitoring
- c. passive air monitoring

D. Alternate Microbiological Test

Other suitable microbiological test methods (e.g. Rapid test methods) can be considered for environmental monitoring, in process testing, and finished product release after it is demonstrated that the methods are equivalent or better than traditional methods (e.g. USP)



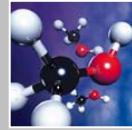
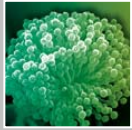
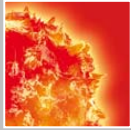
Floureszenz – bekannter Ansatz für Rapid Tests

Fluoreszenz

ist die kurzzeitige, spontane Emission von Licht beim Übergang eines elektronisch angeregten Systems in einen Zustand niedrigerer Energie, wobei das emittierte Licht im Regelfall energieärmer ist als das vorher absorbierte.

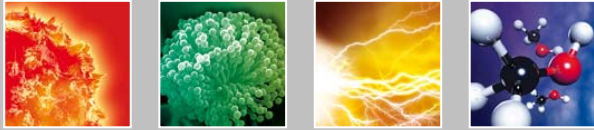


Euscorpius italicus

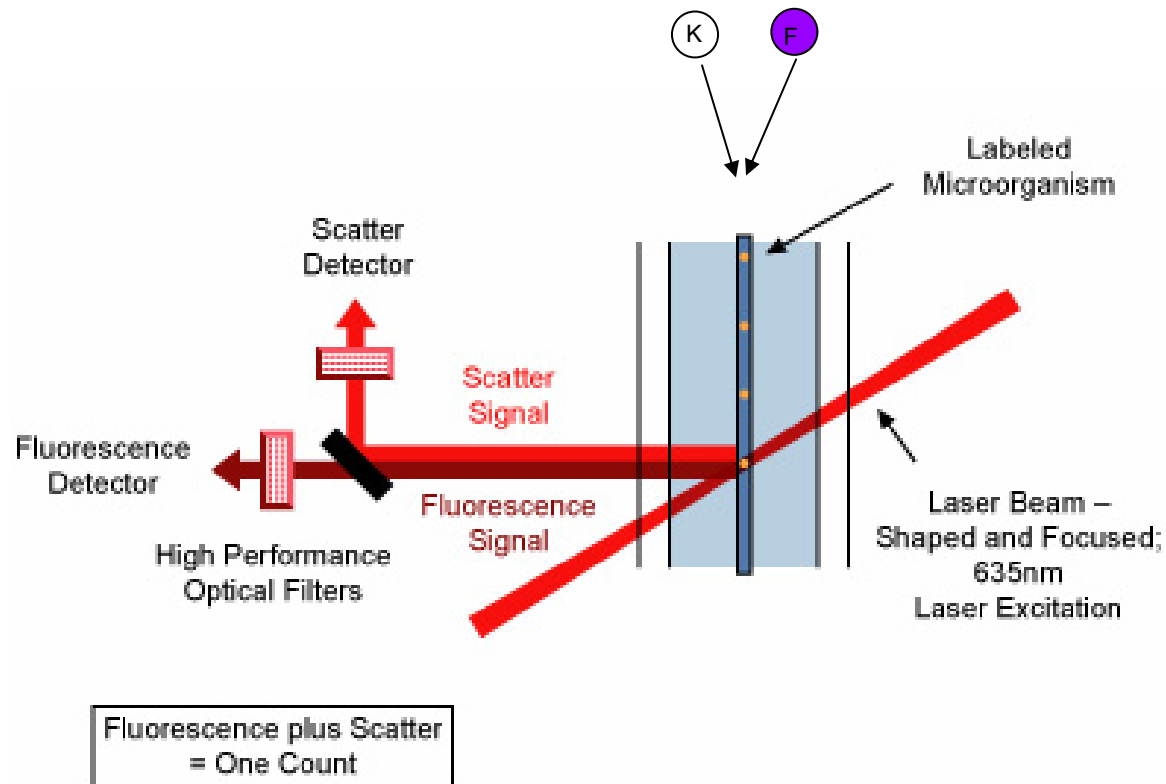


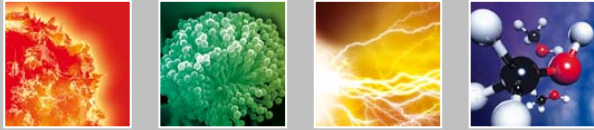
Beispiel: Durchflusszytometrie mit Farbstoffmarkierung

Fluorochrom	Absorptions- maxima [nm]	Emmissions- maxima [nm]	Abkürzung
APC-Cy7	743	767	PharRed
Fluoreszeinisot hiozyanat	495	519	FITC
PE-Cy5	480; 565; 649	670	CyChrome, Red670
PerCP	490	675	Peridin Chlorophyll
Phycoerythrin	480; 565	578	PE
Propidiumjodid	550	650	PI
Red613	480;565	613	PE-TexasRed



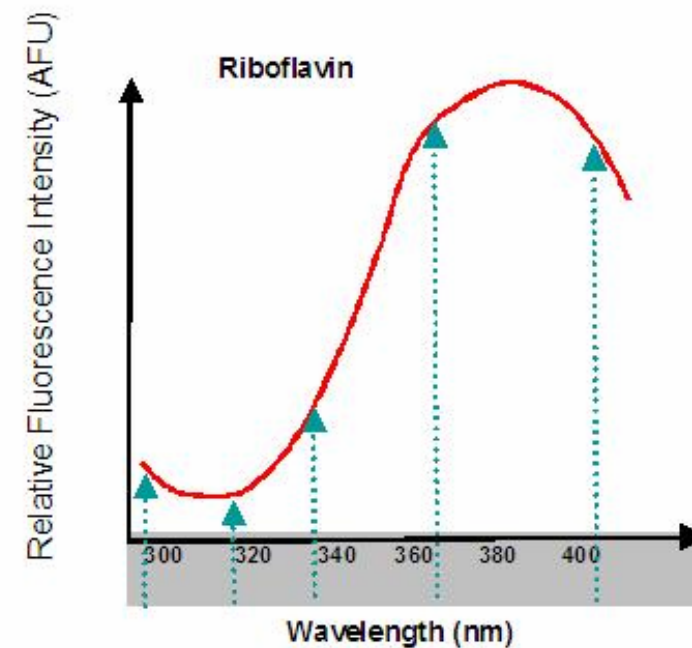
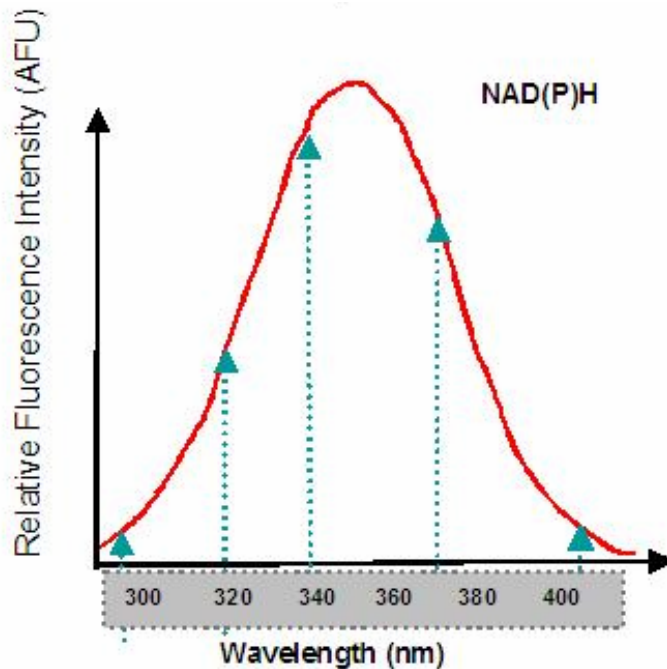
Durchfluss Zytometrie mit Farbstoffmarkierung

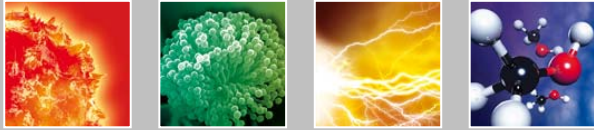




Auto Fluoreszenz – Wegfall externer Farbstoffe

NADH und Riboflavin sind zwei wichtige Fluoreszenzstoffe in vegetativen Bakterien

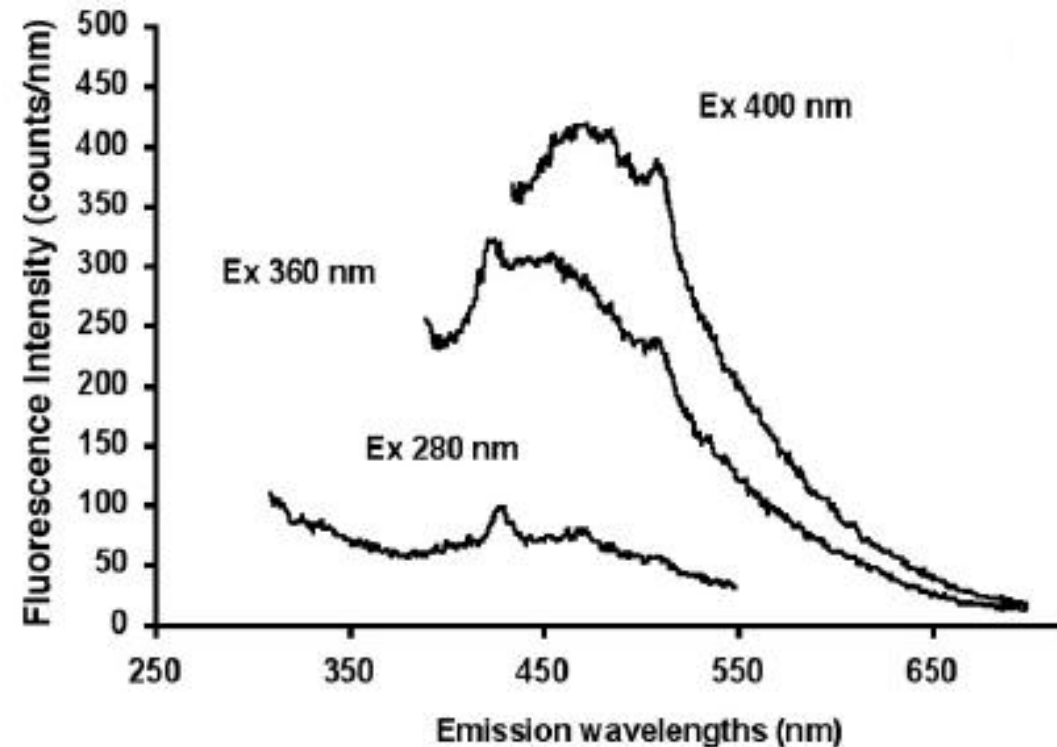


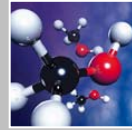
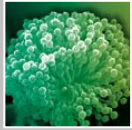
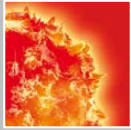


Auto Fluoreszenz – Wegfall externer Farbstoffe

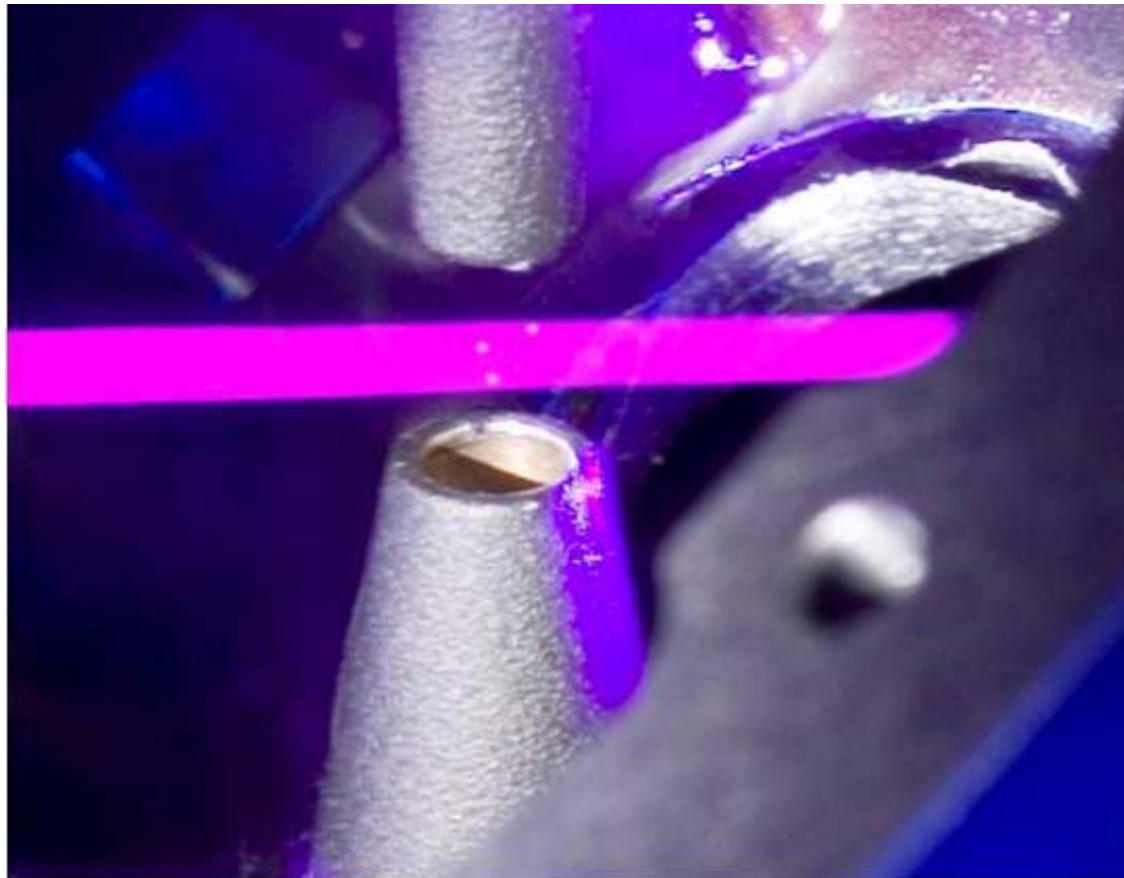
Bakterien im Sporenstadium lassen sich bei etwa 400 nm effizient zur Fluoreszenz anregen :

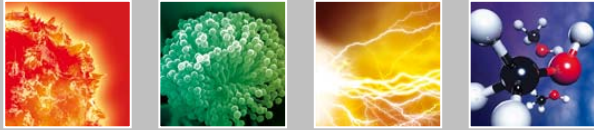
DPA
Dokosa - pentaensäure



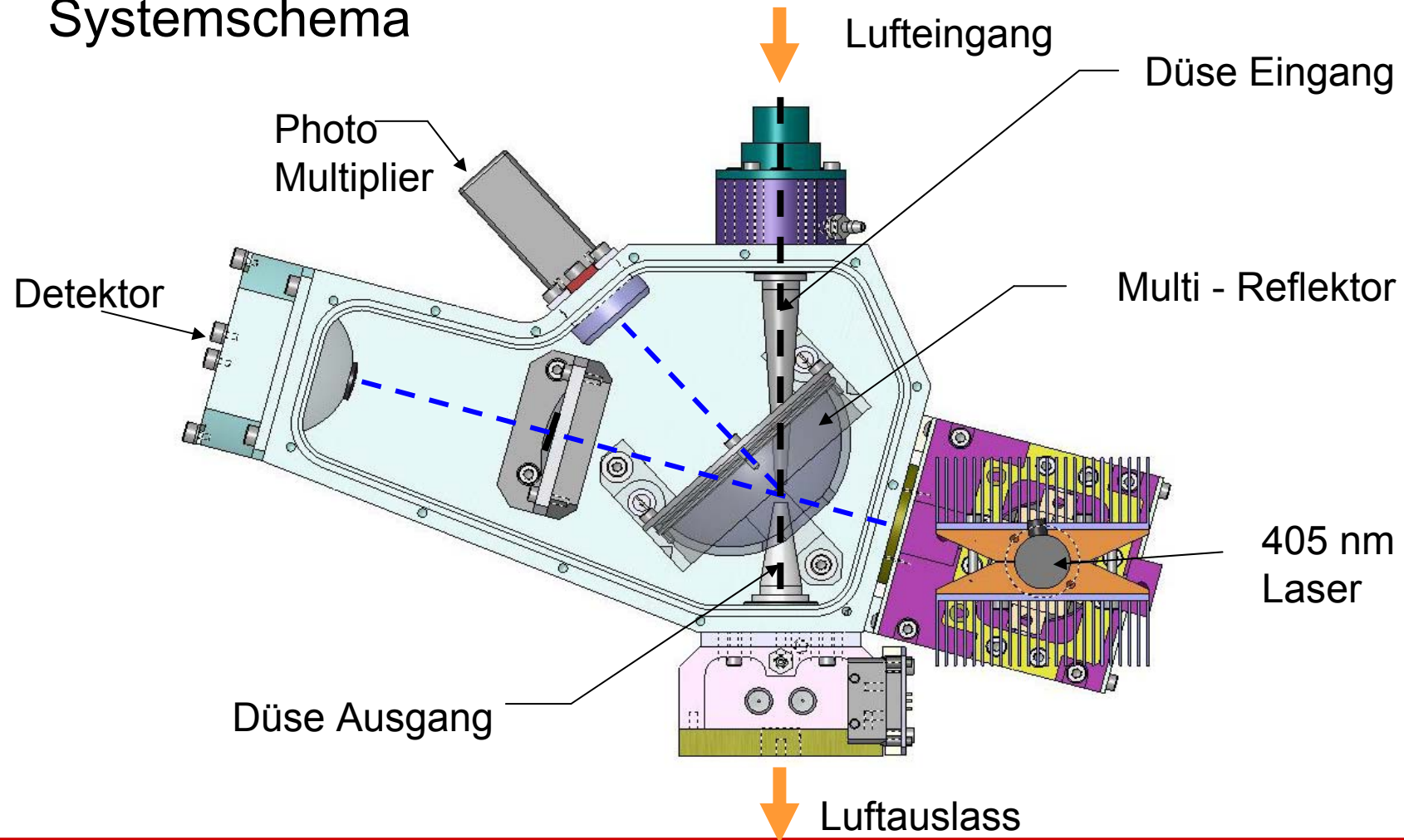


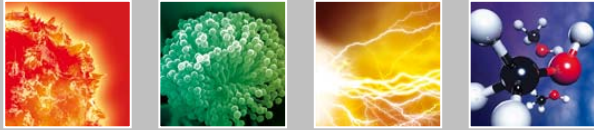
Autofluoreszenz bei 405 nm



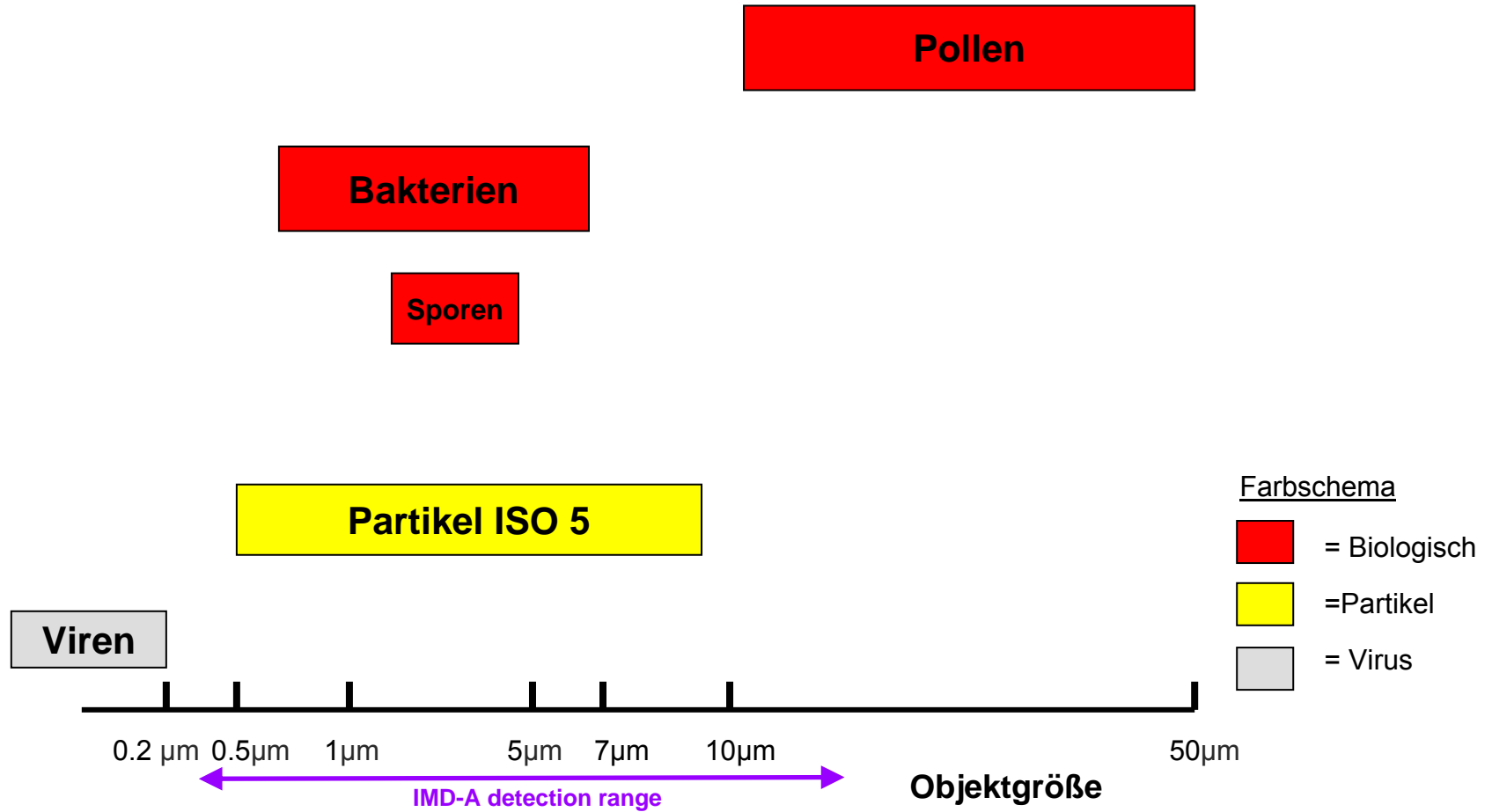


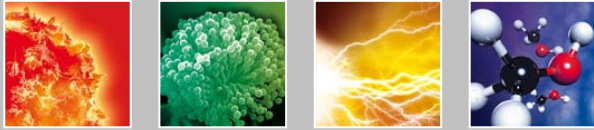
Systemschema



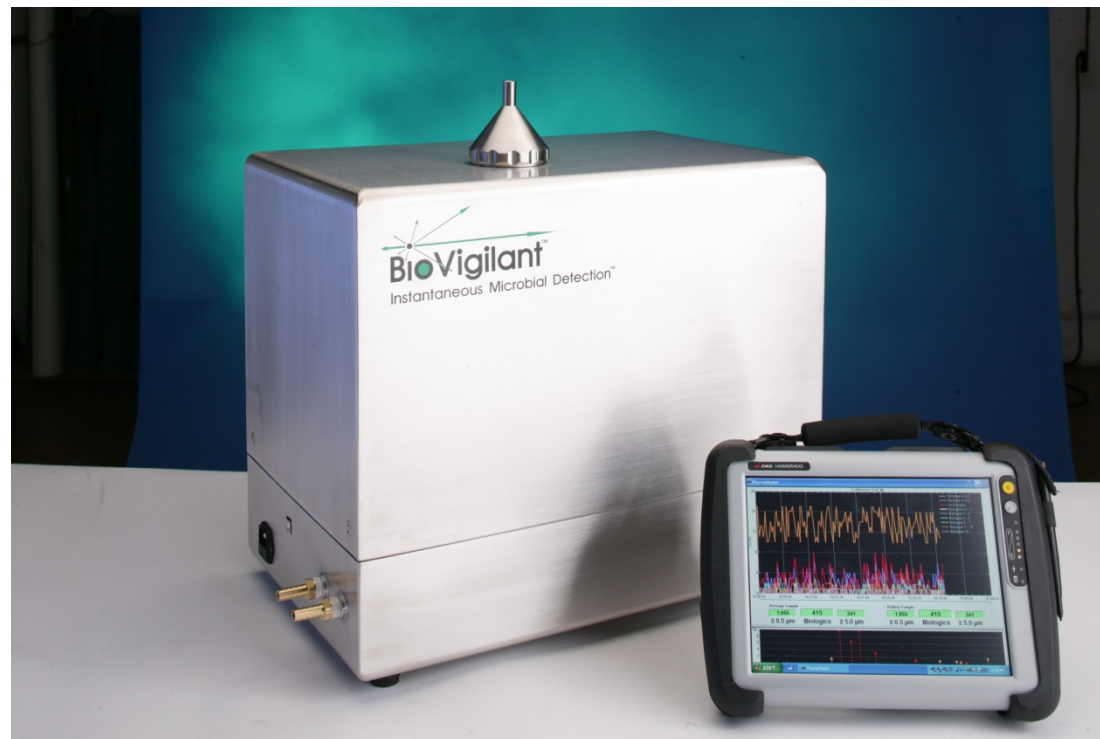


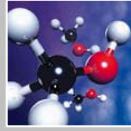
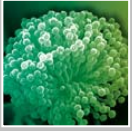
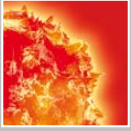
Auflösungsvermögen @ 405 nm





Systemaufbau Autofluoreszenz Keimzähler





Was “sieht” ein Autofluoreszenz Keimzähler ?

Luftkeime mit aktivem Stoffwechsel (CFU's)

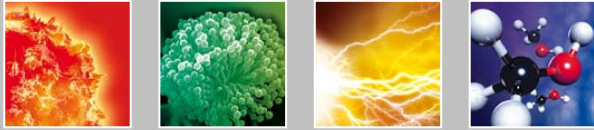
Luftkeime im Sporenstadium (potentielle CFU's)

Geschädigte Luftkeime (keine CFU's bildend)

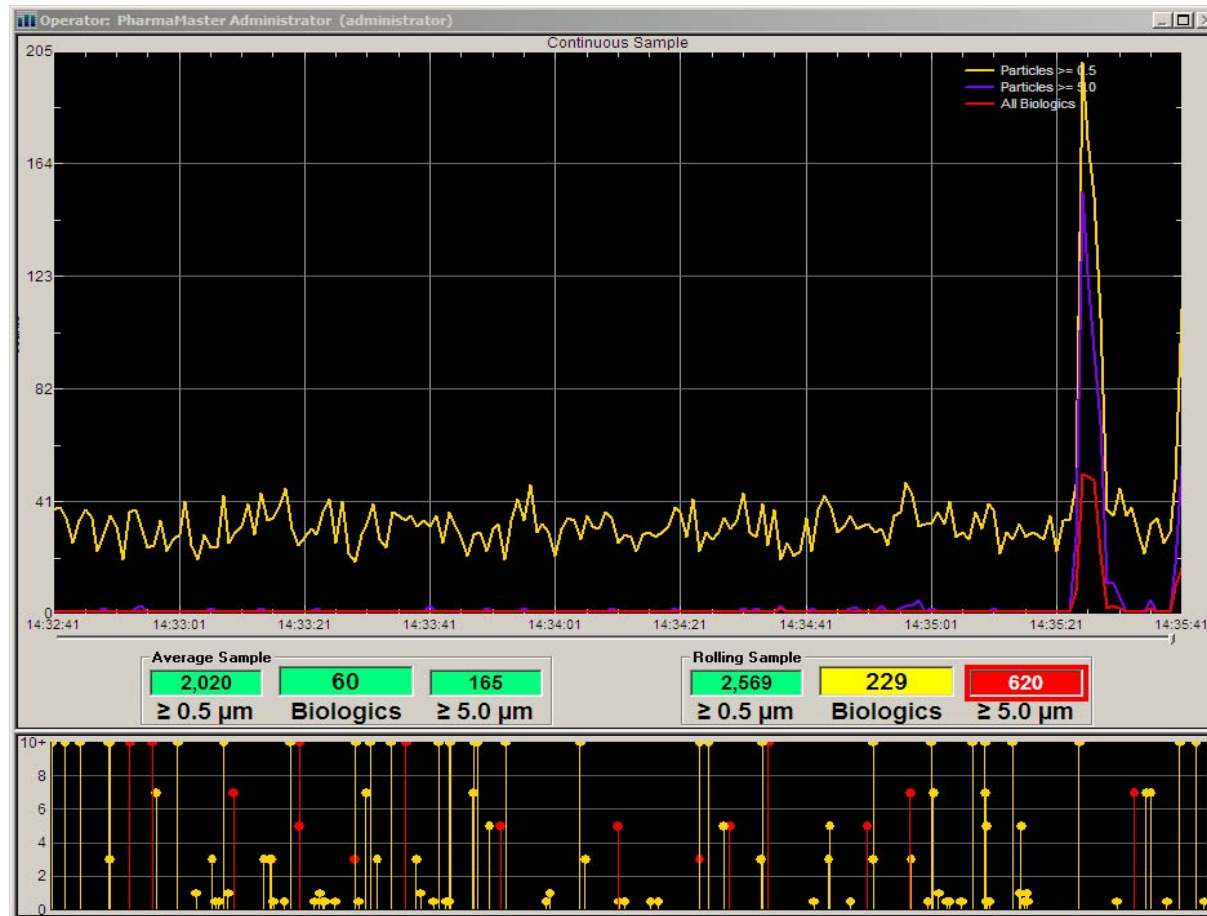
Abgetötete Luftkeime (keine CFU's bildend)

Plus

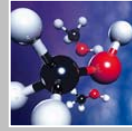
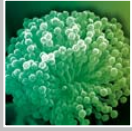
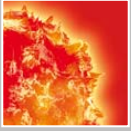
messtechnischer “OPC Nebeneffekt”: Summe viables + non viables



Wie "sieht" ein Autofluoreszenz Keimzähler ?

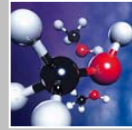
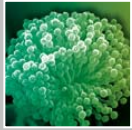
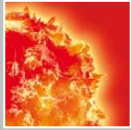


kontinuierlich !
&
zeitlich auflösend !



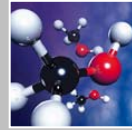
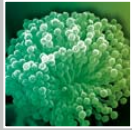
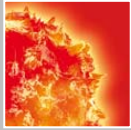
Praktische Anwendung





Messwerte Isolator

Isolator	Proben- volumen in m ³	Zeitinter- vall	Partikel pro m ³ ≥ 0,5 µm	Partikel pro m ³ ≥ 5,0 µm	Keime pro m ³
Transferbereich (3 Handschuhe)	28,00	16h 25m	231	8	<< 1 ^a
Transferbereich (8 Handschuhe)	28,37	16h 37m	241	6	<< 1 ^b
Füllbereich (12 Handschuhe)	29,00	16h 43m	2	0	<< 1 ^c

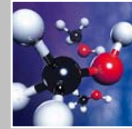
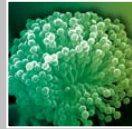
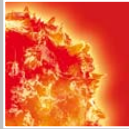


Strategiewechsel ?

Strategieänderung bei Betrachtung großer Volumina mit hoher statistischer Signifikanz.

Keine übermäßige Reaktion auf einzelne Zählungen von Luftkeimen.

Dagegen Reaktion auf Trend zu steigenden Zählraten.



Operator: PharmaMaster Administrator (administrator)

Continuous Sample

Legend:
— Particles ≥ 0.5
— Particles ≥ 5.0
— All Biologics

Sample Type	$\geq 0.5 \mu\text{m}$	Biologics	$\geq 5.0 \mu\text{m}$
Average Sample	75	7	1
Rolling Sample	75	7	1

Events: Apr 25 2008 10:41:31 (59 sec)

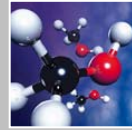
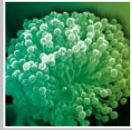
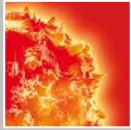
Air Sampled: 0.22 cubic meters % Bio: 9.3%

Marker: [dropdown]

Playback [x]

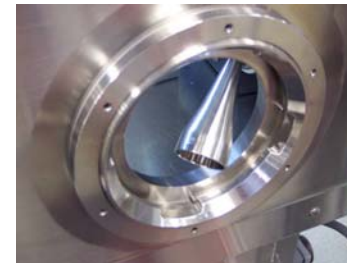
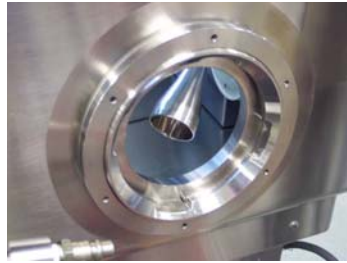
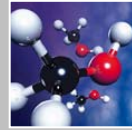
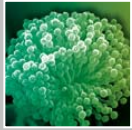
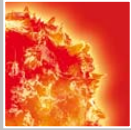
Linear Show Histogram Power Sample

Start | PharmaMaster | run 116 no glove 4.bmp ... | 12:34 PM



Simulation defekter Handschuhe

Aktivität	Proben- volumen in m ³	Zeitinter- vall	Partikel pro m ³ ≥ 0,5 µm	Partikel pro m ³ ≥ 5,0 µm	Keime pro m ³
Monitoring bei Loch von 75-100 µm	0,45	15m 35 s	7	0	0
Monitoring bei Loch von 200-250 µm	0,22	7m 51s	0	0	0
Monitoring bei abgeschnittener Fingerspitze	0,30	10m 5s	12	2	1
Monitoring mit offenem Handschuh (Foto)	0,23	8m 19s	75	1	7



Aktivität im mousehole Bereich	Proben- volumen in m ³	Zeitinter- vall	Partikel pro m ³ ≥ 0,5 µm	Partikel pro m ³ ≥ 5,0 µm	Keime pro m ³
ISP innerhalb Isolator	0,10 m ³	3m 24s	1	0	0
ISP im Grenzbereich Raum/Isolator	0,03 m ³	1m 01s	0	0	0
ISP ausserhalb Isolator	0,10 m ³	3m 37s	38	11	12