CHEMUNEX
SCAN CYTOMETRY SOLUTIONS

PIONEERING DIAGNOSTICS

BIOMÉRIEUX
INDUSTRY

Menu

- Advantage of the Real Time Microbiology Testing
- Chemunex product range
- Scan*RDI*® analysis
- Applications
- Conclusion

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- Conclusion

Advantage of the Real Time Microbiology Testing

- The challenge
- The traditional microbiology
- Advantage of the Real Time Microbiology Testing

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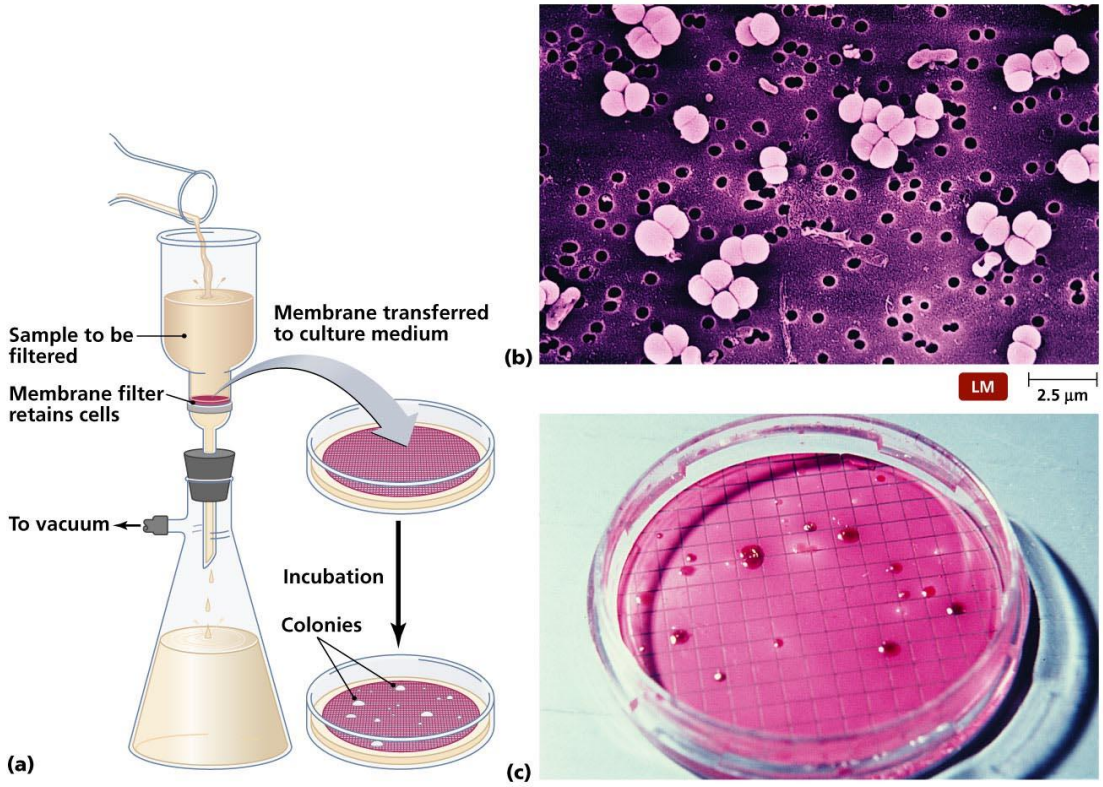
The challenge

- Microbial contamination is a prime concern ➔ Crucial for industries such as pharmaceutical, biotechnology, personal care, drinking water distribution, breweries…
- Better control on finished product if there is a comprehensive in-process testing at every crucial stage of production
- Delays in microbial testing can directly impact effective consumer protection

The traditional microbiology (1/2)

The traditional microbiology : Growth based, agar plate method

Introduced by Robert Koch (1843-1910) and Julius Richard Petri (1852-1921)



⇨ **75 % of all microbial testing use the ~130 year old method**

The traditional microbiology (2/2)

The traditional microbiology : Growth based, agar plate method

Spores and stressed cells require extended growth period

Products need to be kept in quarantine until micro results are available

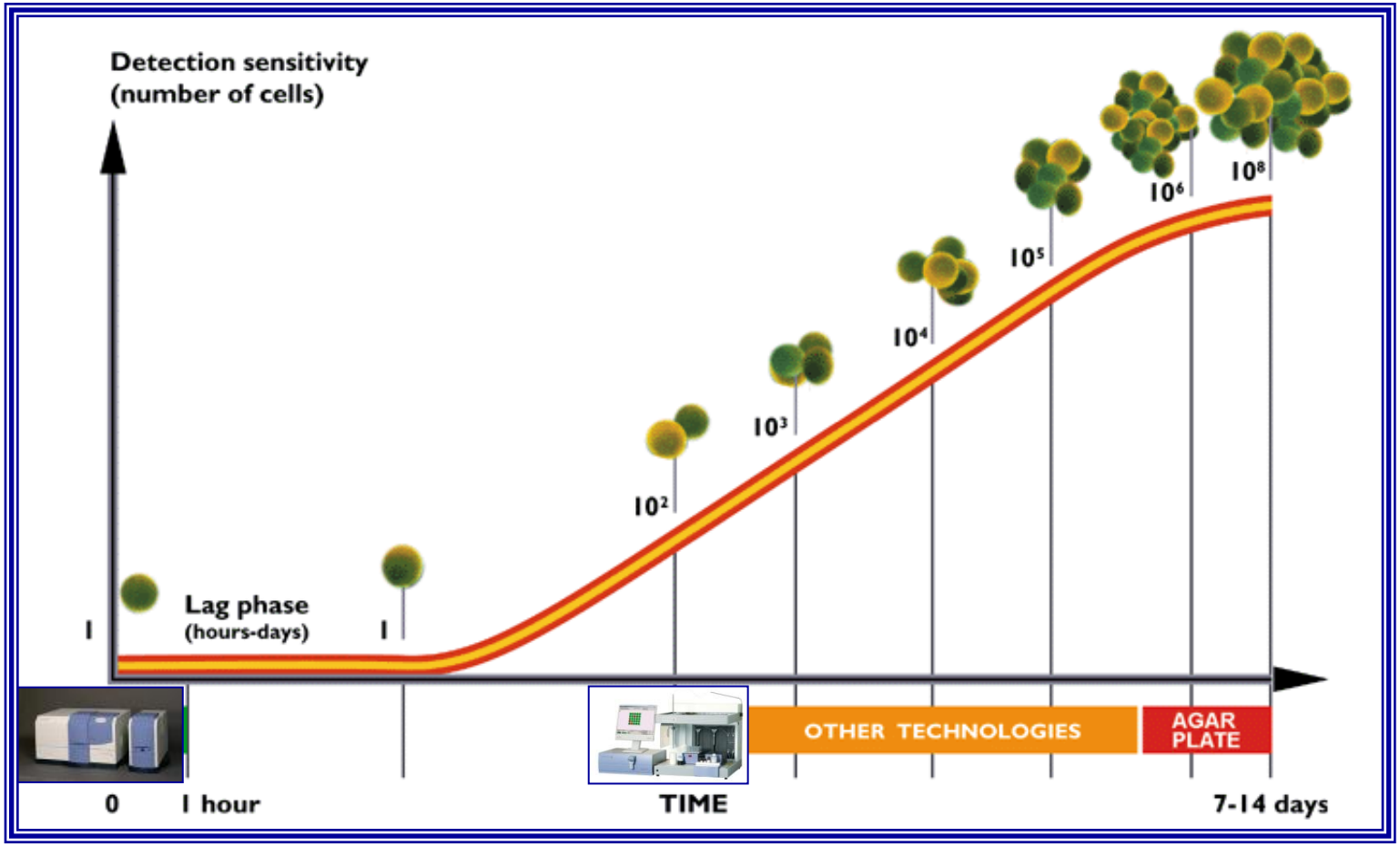
Raw material can not be used for production before micro lab delivers results

Results vary with microbial population, media and conditions

Have an effect on production schedules

➔ The traditional method is time consuming

Advantage of the Real Time Microbiology Testing (1/2)



Advantage of the Real Time Microbiology Testing (2/2)

- Avoid the requirement for any cell multiplication
- Potential contamination can be detected immediately
- Storage can be dramatically reduced
- Remedial action can be taken before processes drift out of specifications
- Cleaning actions can be validated immediately
- Process improvments can be evaluated on-line

Chemunex product range

- Flow cytometry
- Solid phase cytometry
- Why the Scan*RDI*® ?

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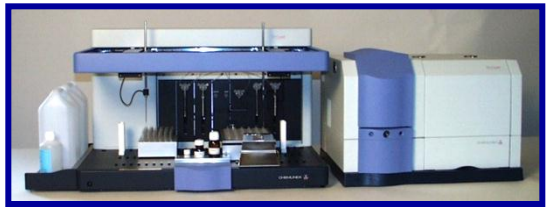
Chemunex product range: Flow cytometry



D-*Count*

- Fully automated flow cytometer
- Up to 350 samples per working shift
- Throughput 50 samples/h (application dependent)
- Capacity up to 64 samples/batch (configuration dependent)

Bacti*Flow ALS*

- Fully automated flow cytometer
- Up to 150 samples per working shift
- Throughput 20 samples/h (application dependent)
- Capacity 1-25 samples/batch

BactiFlow

- Table top flow cytometer for manual use
- Up to 50 samples per day

Chemunex product range : Solid phase cytometry (1/2)

Chem*Scan* ® *RDI* / Scan*RDI*®

The only system allowing

real time

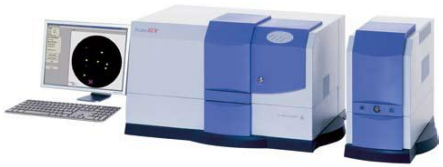
detection & enumeration

of micro-organisms in filterable

samples with a sensitivity

down to one cell

Chemunex product range: Solid phase cytometry (2/2)



ChemScan®RDI
ScanRDI®

- First generation of ChemScan RDI
- Argon Ion Laser not integrated
- Software 3.4.11

ChemScan®RDI SSL
ScanRDI® SSL

- Solid state laser (SSL) integrated into the analytical module
- Software 3.4.11

CHEMUNEX ScanRDI

- Second generation of Scan*RDI*®
- Solid state laser (SSL) integrated into the analytical module
- One holder for CB0.4 and FIFU
- Software 3.4.18

Why the *ScanRDI*® ?

Scan*RDI*®: The ultimate combination of speed and sensitivity

- 1 protocol for all compatible matrices
- Detection
  - Direct detection of bacteria, yeast, molds and spores
  - Linear response from 1 to 10^5 cells for bacteria and 1 to 10^4 for yeast and molds
  - Spores, stressed cells and fastidious microorganisms detected within minutes
  - No multiplication required
- Sensitivity
  - down to one microbial cell in a sample
  - independent from the volume filtered (large volume can be tested)
- Non-destructive test protocol permits microscopic confirmation
- Robust and easy to use
- 21 CFR 11 compliant
- Audit trail

Scan*RDI*® analysis

- A simple three steps procedure
- Sample preparation
- The Scan*RDI*® analysis
- Data treatment
- Results display
- Microscope validation

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Scan*RDI*® analysis : A simple three steps procedure

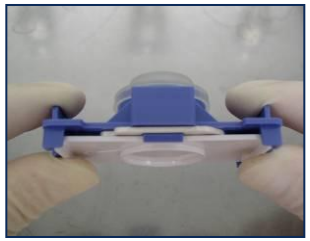
Filtration

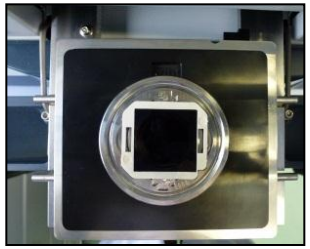
↓

Cell labelling

↓

Scan*RDI*® analysis





Sample preparation : The filtration (1/2)

Filtration

↓

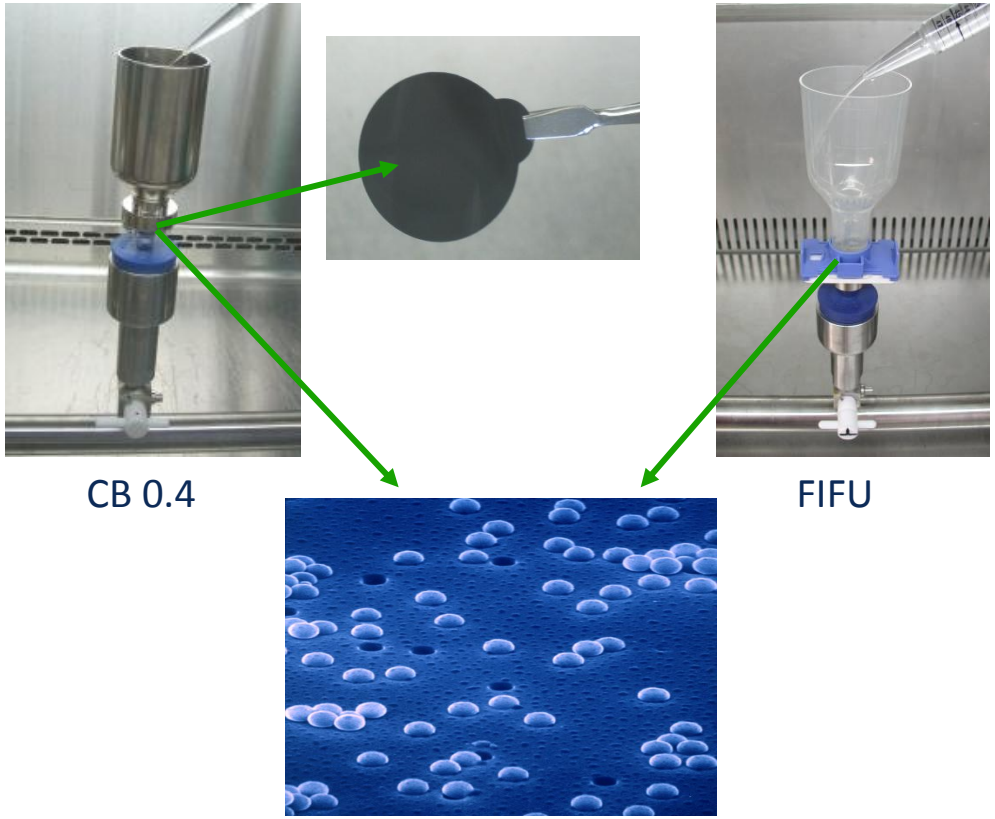
Cell labelling

↓

Scan*RDI*® analysis

Sample filtration

Large volumes can be tested using :

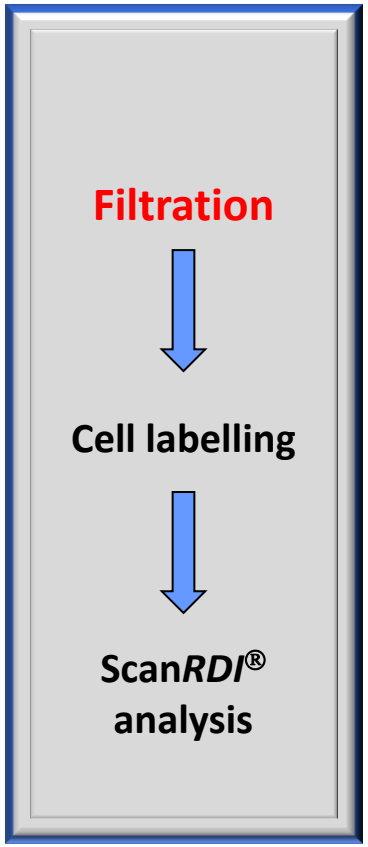


CB 0.4

FIFU

0.4µm polyester track-etched membranes (= ChemFilter)

Sample preparation : The filtration (2/2)



The membrane

- **ChemFilter CB 0.4 membrane = A true membrane filter and not a depth filter should be used**
  - 25 mm diameter black track etched polyester membrane
  - 23 µm thick and has a pore specification of 0.36 µm to retain MO on the surface of the filter
  - Pores of accurately controlled dimension are created by ion bombardment of a plastic film followed by controlled chemical etching
  - A tab to facilitate handling to ensure no damage to the membrane during manipulation
- **FIFU : Fluorassure Integral Filtration Unit**
  - Translucent funnel + Blue membrane carrier + Membrane CB04 (0.4µm black membrane) inserted in square white support + white filtration support + translucent cap
  - 2 white pad supports with one labelling pad each.

Sample preparation : The cell labelling (1/3)

Filtration

Cell labelling

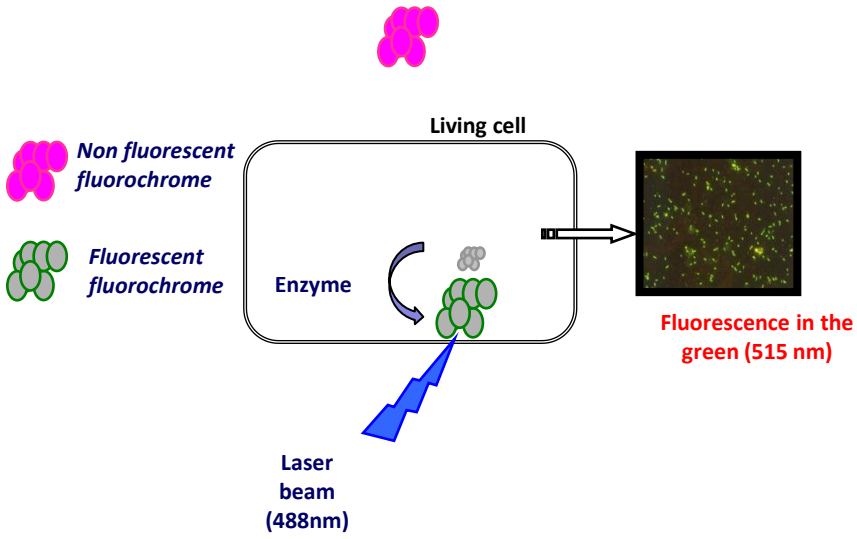
Scan*RDI*® analysis

The principle of the cell labelling : Fluorassure viability markers

❶ Viability substrate enters into the cell

❷ Enzymes cleave the viability substrates and release fluorochromes
Enzyme activity

❸ Fluorochromes accumulate into the cell cytoplasm
Membrane integrity



The Fluorassure viability marker will label all the viable micro-organisms and it is a non destructive method

Sample preparation : The cell labelling (2/3)

Cell labelling – ChemFilter CB0.4

Filtration

↓

Cell labelling

↓

Scan*RDI*® analysis

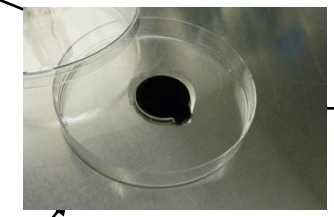
1. FILTRATION	2. LABELLING	3. LASER SCANNING

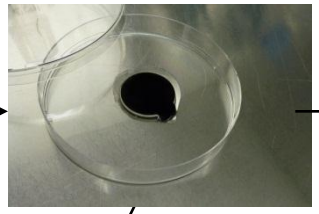
Sample

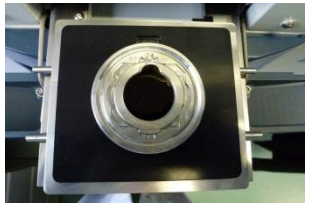
Filtration

Activation

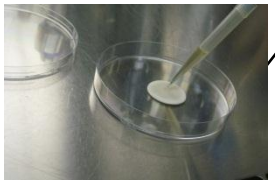
Labelling







Scan*RDI*® analysis



Pad saturated with activation solution

Pad saturated with labelling solution

Sample preparation : The cell labelling (3/3)

Cell labelling – ChemFilter FIFU

Filtration

↓

Cell labelling

↓

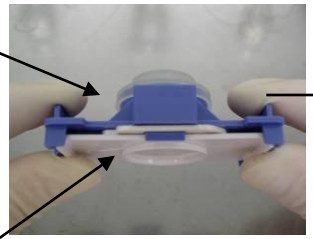
Scan*RDI*® analysis

1. FILTRATION	2. LABELLING	3. LASER SCANNING

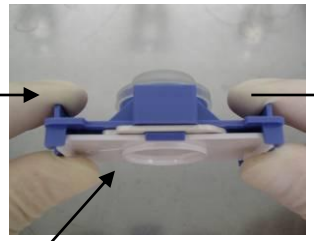
Sample

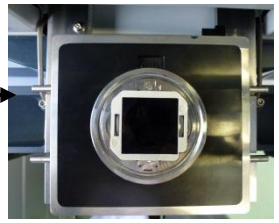
Filtration

Activation

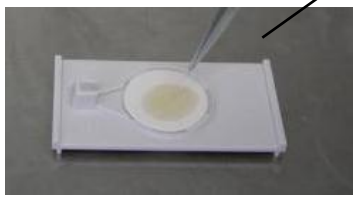


Labelling

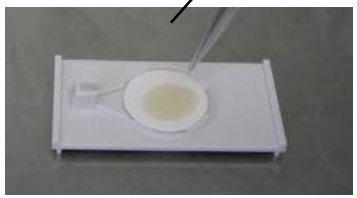




Scan*RDI*® analysis



Pad saturated with activation solution



Pad saturated with labelling solution

The Scan*RDI*® analysis (1/4)

- After labeling, the filter is simply placed into the Scan*RDI*® analyzer and the scan is automatically initiated. All viable microorganisms present are individually detected and counted.

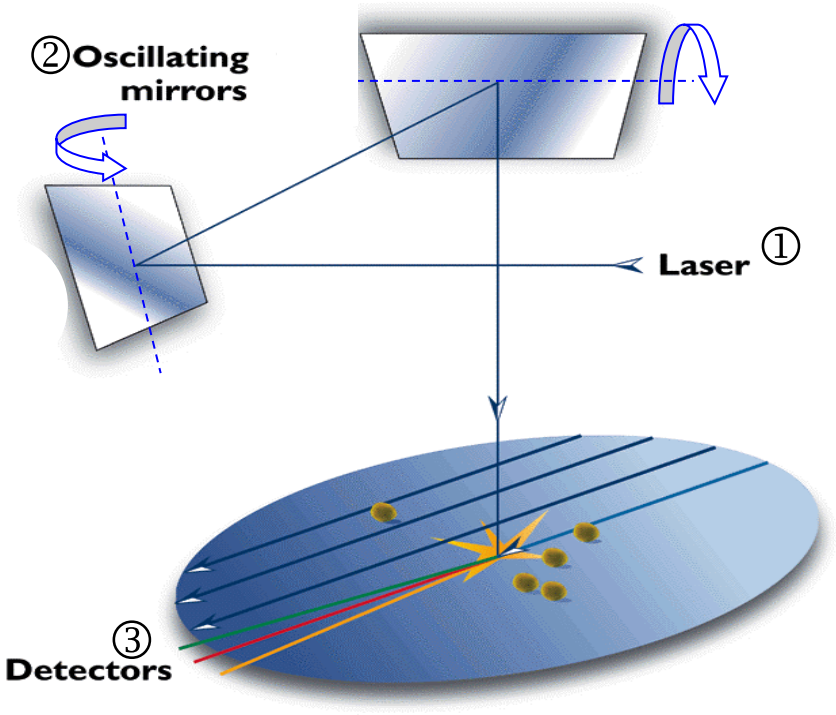
Filtration

↓

Cell labelling

↓

Scan*RDI*® analysis



The laser beam (488nm emission wavelength) is guided via a fiber optic cable to a two-axis scanning module which moves the focused laser beam across the entire surface of the membrane.

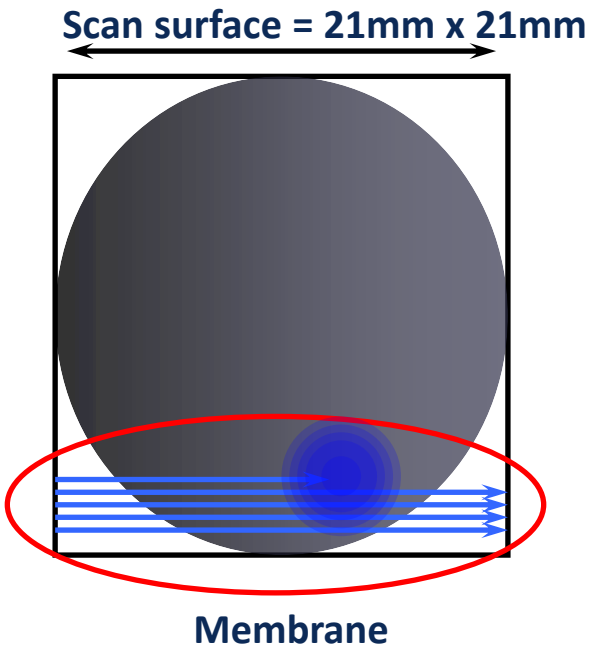
⇨ A laser scans the entire surface of 25 mm diameter membrane in 3 to 5 min

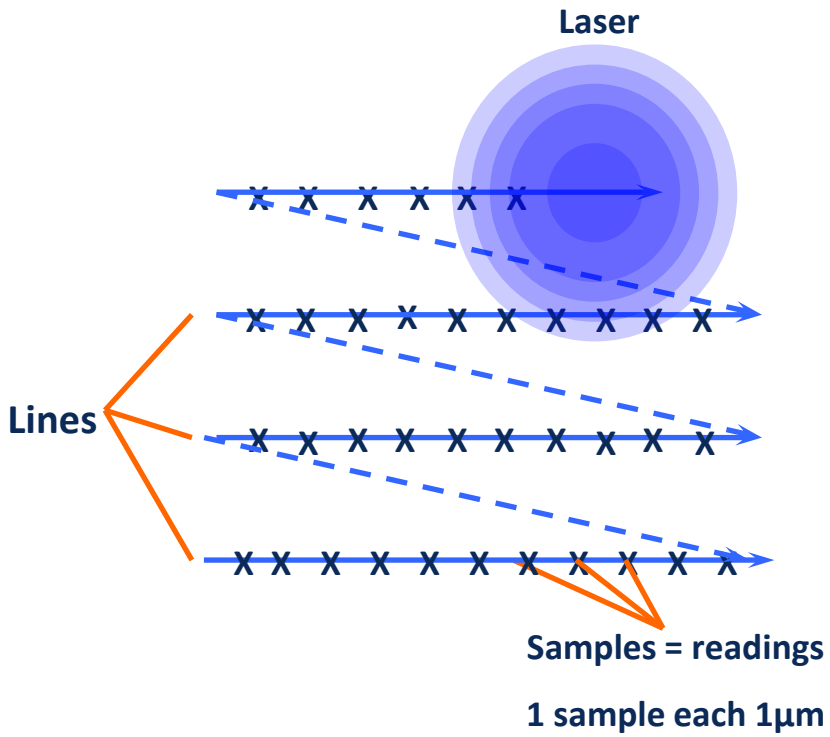
The Scan*RDI*® analysis (2/4)

Filtration

Cell labelling

Scan*RDI*® analysis





➔ The laser scans the membrane at a speed of 2 meter/sec

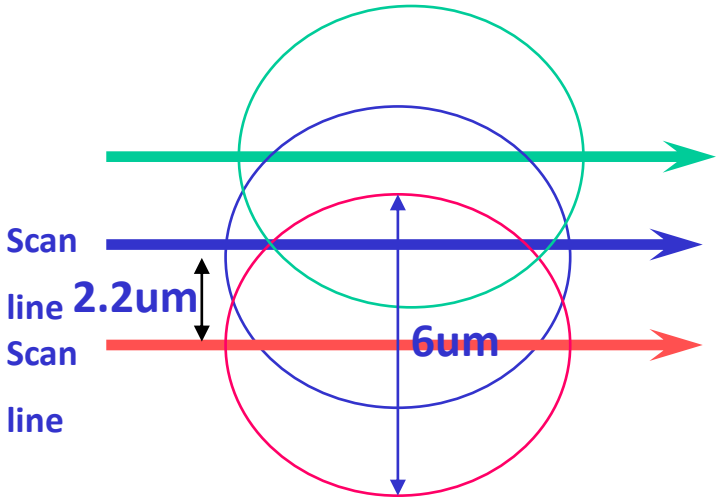
➔ Laser makes 9 545 lines on the membrane

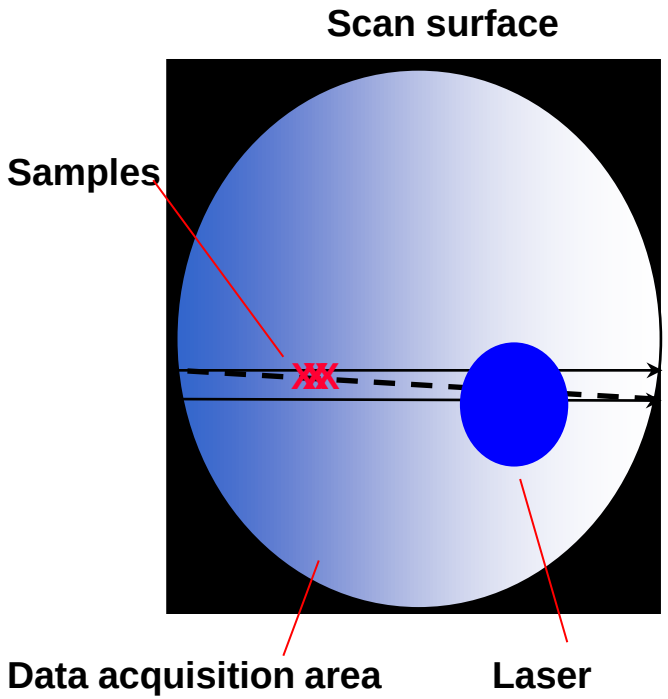
The Scan*RDI*® analysis (3/4)

Filtration

Cell labelling

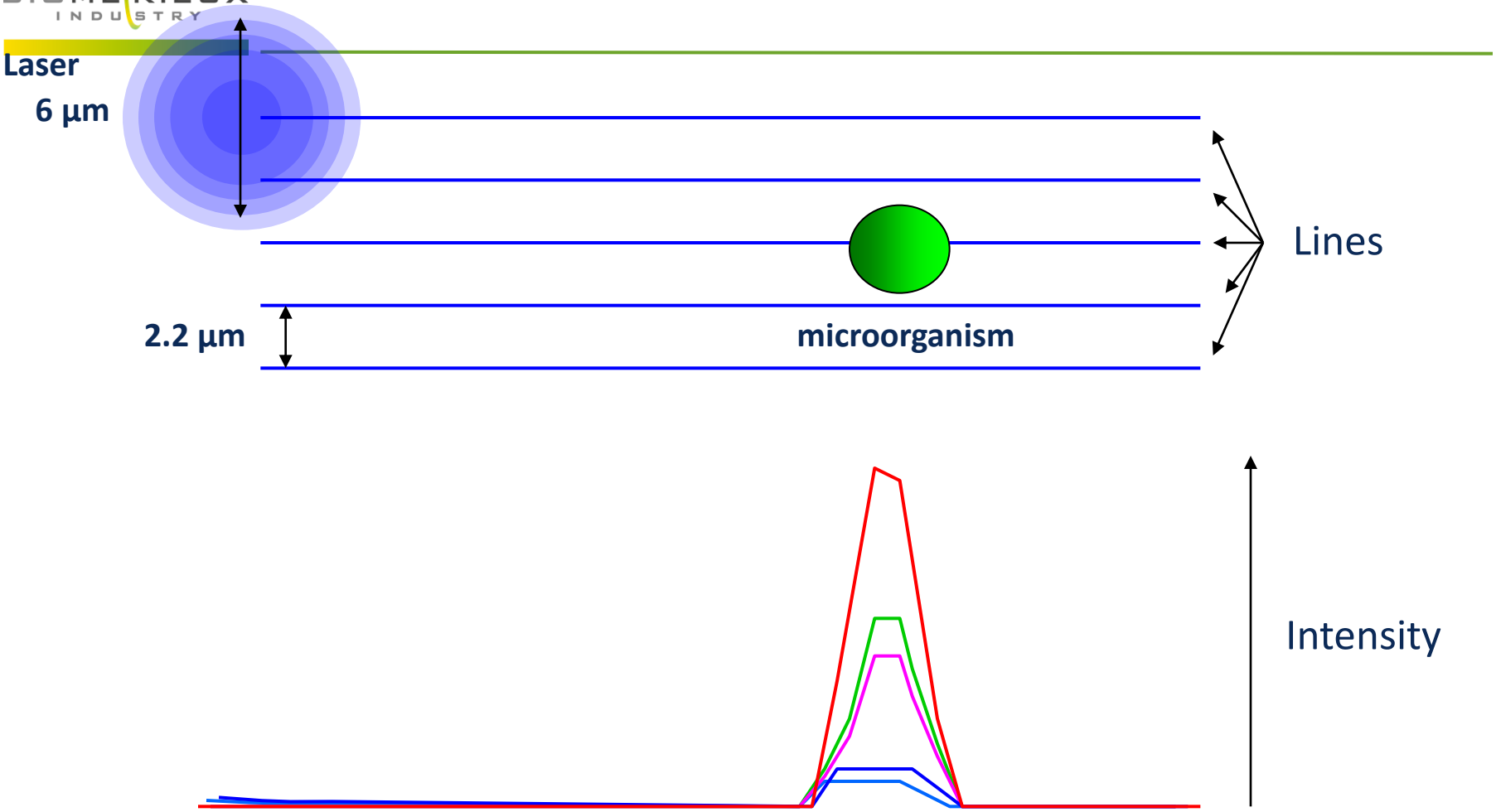
Scan*RDI*® analysis



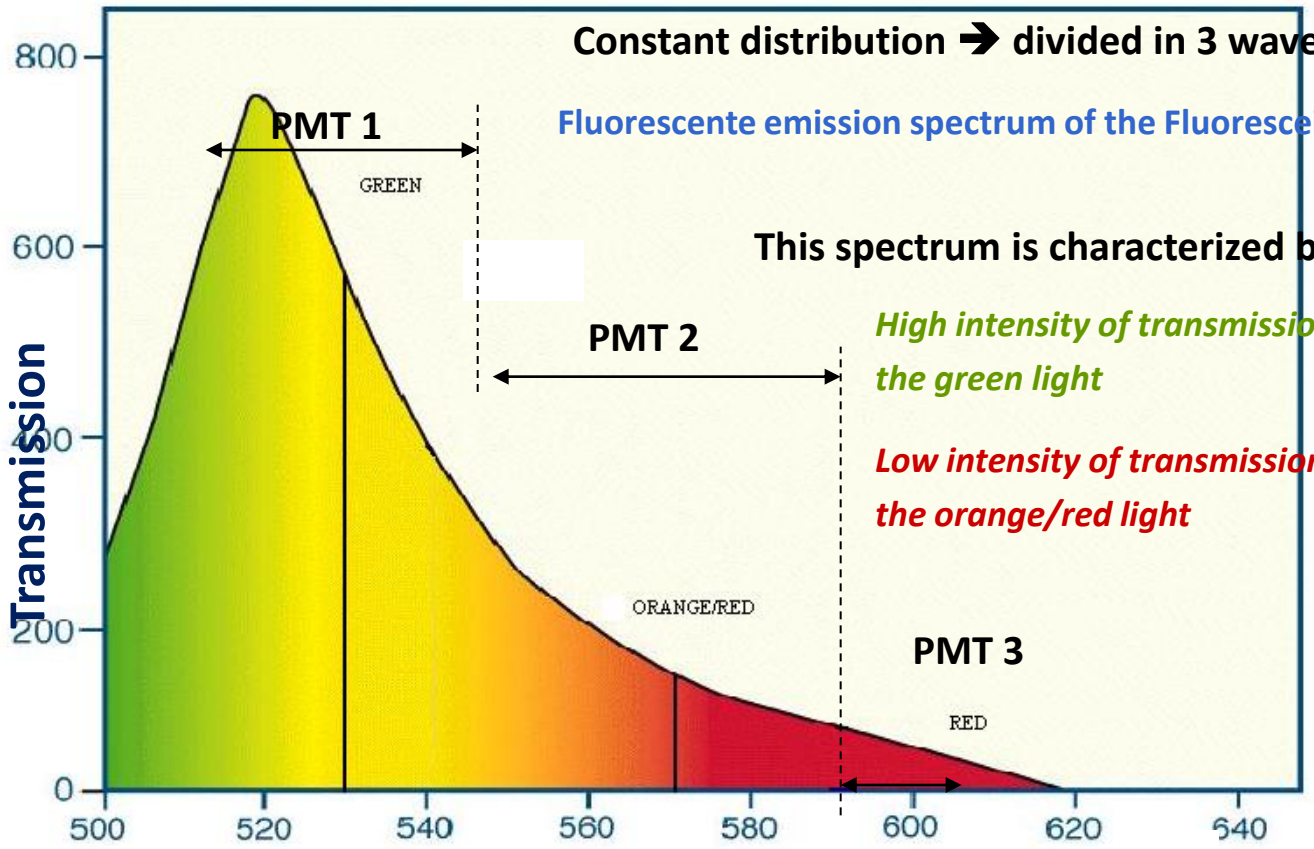


Scanning is fully overlapping ➔ Covering event

The Scan*RDI*® analysis (4/4)



The emission spectrum of the fluorochrome



Constant distribution ➔ divided in 3 wavelength sections

Fluorescente emission spectrum of the Fluorescein

This spectrum is characterized by :

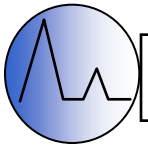
High intensity of transmission in the green light

Low intensity of transmission in the orange/red light

Wavelength (nm)

Datas treatment : Datas processing

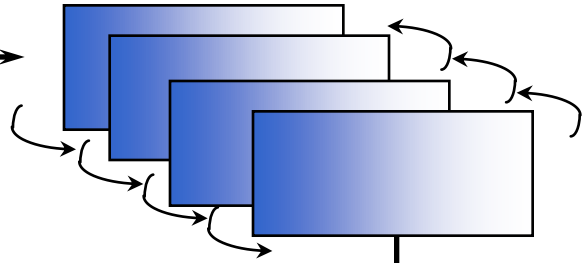
Datas collected on the membrane



PMT Detector

Electronic signal

Discrimination : 20 different criteria



- Colour discrimination
- Light intensity
- Signal shape

Rejected

Background

Results

The aim of the discriminated setting :

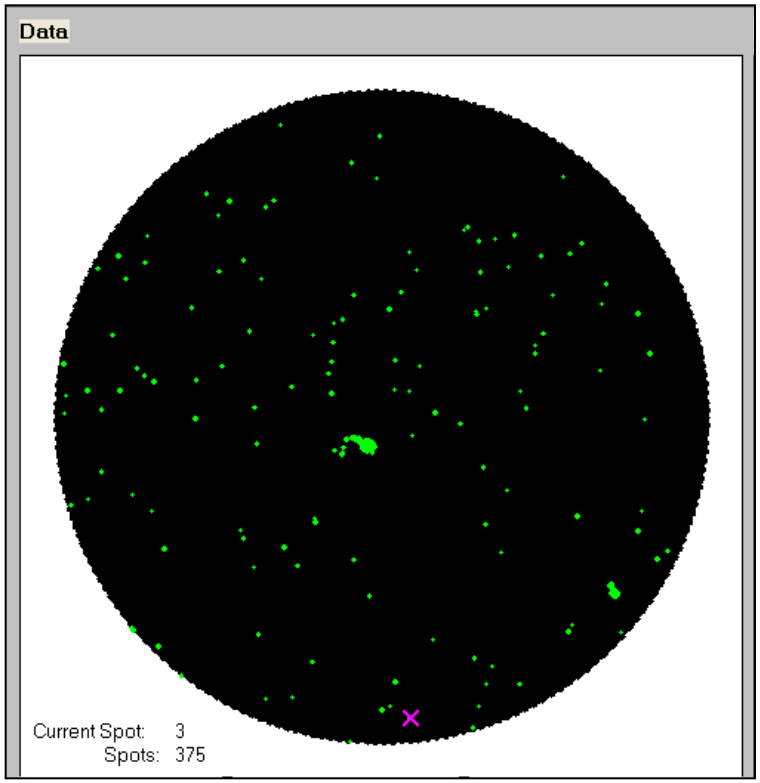
This discrimination allows to separate the microorganisms from the background

- optimise the recovery of organisms

- minimise the possibility of false negative result

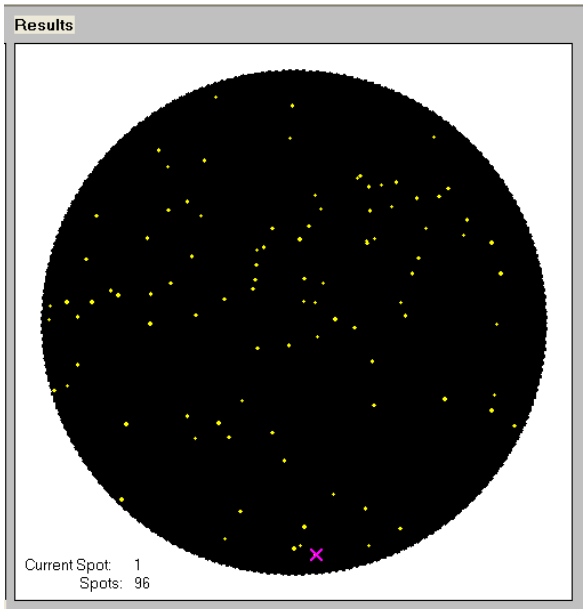
Datas treatment

Data Map = Total count



Results Map

= labelled microorganisms

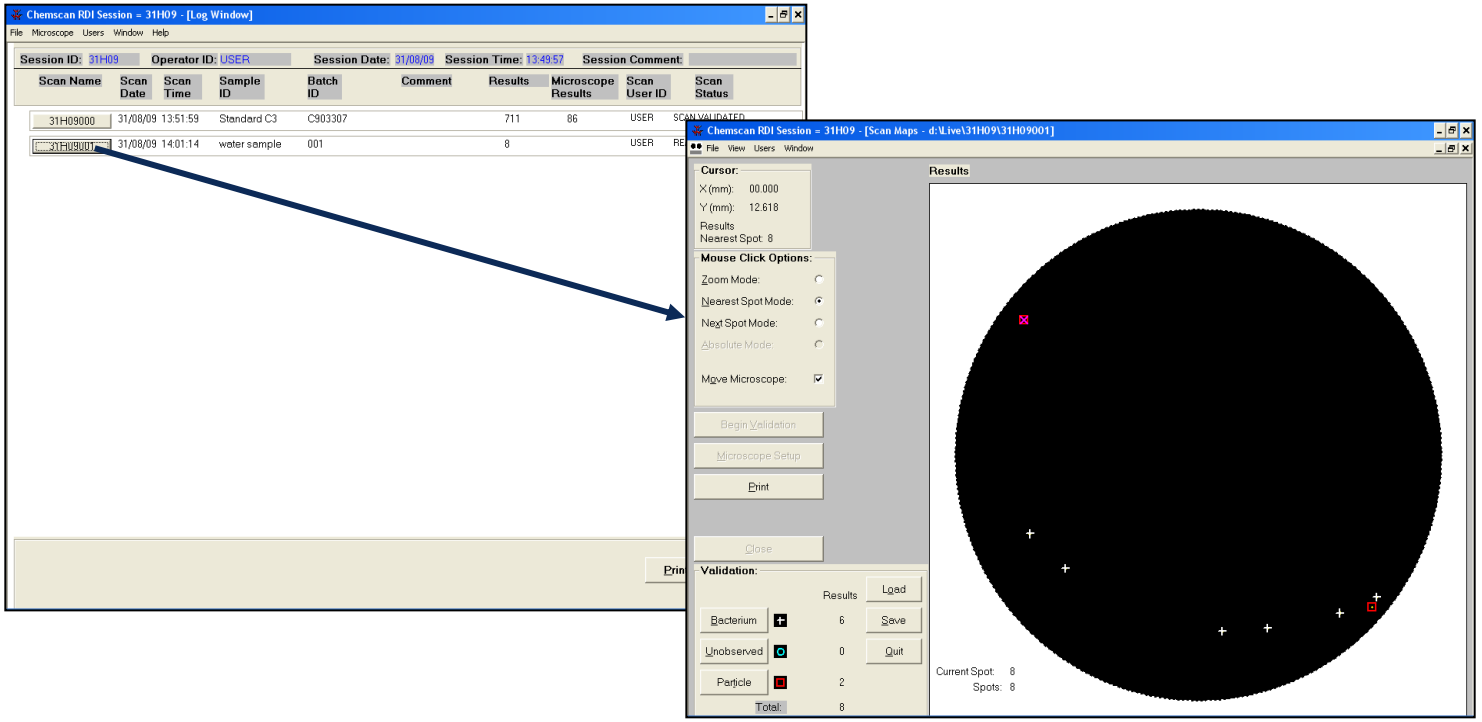


Rejected Background

- Autofluorescent Particles
- Membrane Fluorescence
- Electronic Noise

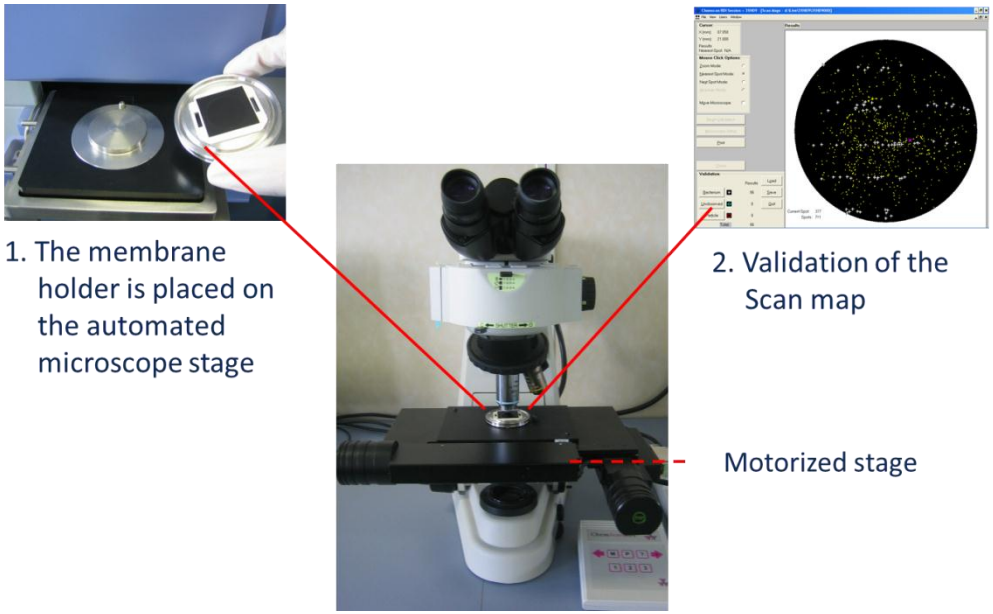
Results Display

- Results are immediately displayed as direct viable cell counts requiring no operator interpretation.
- In addition, a scan map display shows the precise location of each detected microorganism at the surface of the membrane.
- This enables fast visual result confirmation using an optional microscope.

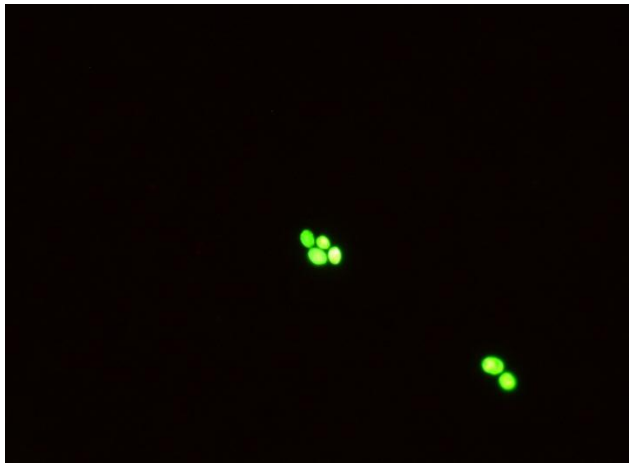


Microscope Validation (1/2)

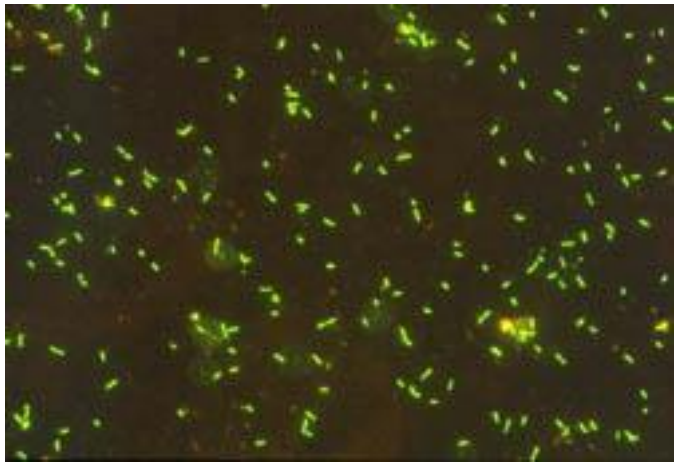
- Data on microorganism counts obtained using the Scan*RDI*® have been through the multi-level discrimination process described above.
- It is possible to confirm that the 'spot' detected is a true organism
- To enable users to perform this task the Cytometer has an epifluorescence microscope with a motorized stage that is directly linked to the Cytometer database.
- The Cytometer can drive the motorized stage to any selected point on the membrane to allow the visual confirmation that the detected event is in fact a microorganism.



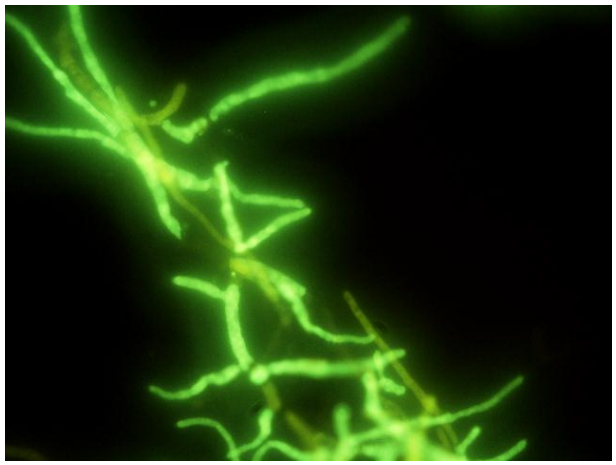
Microscope Validation (2/2)



Candida



Bacillus



Mould

Direct Viability Labelling Demonstrated With Wide Range of Microorganisms

Bacteria Gram -

Achromobacter xylosoxydans
Aeromonas hydrophila
Agrobacterium radiobacter
Alcaligenes eutrophus
Alcaligenes faecalis
Burkholderia cepacia
Burkholderia diminuta
Burkholderia pickettii
Caulobacter sp.
Cedecea lapagei
Citrobacter diversus
Citrobacter freundii
Comamonas terrigena
Edwardsiella hoshinae
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter cloacae
Enterobacter gergoviae
Enterobacter sakasakii
Enterobacter intermedium
Erwinia Sp.
Escherichia coli
Escherichia coli HB 101
Escherichia coli 0126 :B16
Flavobacterium Sp.
Klebsiella oxytoca
Klebsiella planticola
Klebsiella planticola
Klebsiella pneumoniae
Klebsiella terrigena
Kluyvera Sp.
Moraxella sp.
Pasteurella aerogenes
Proteus mirabilis
Pseudomonas diminuta
Pseudomonas aeruginosa
Pseudomonas alkagenèse
Pseudomonas mesophilica
Pseudomonas putida
Pseudomonas fluorescens
Pseudomonas stutzeri
Salmonellac holeraesuis
Salmonella indiana
Salmonella typhimurium
Salmonella eboni
Salmonella sp.
Salmonella virchow
Serratia marcescens
Shigella sonnei
Xanthomonas maltophilia
Yersinia enterocolitica

Bacteria Gram +

Aerococcus viridans
Bacillus anthracis
Bacillus amyloliquefaciens
Bacillus cereus
Bacillus circulans
Bacillus coagulans
Bacillus globigii
Bacillus lentus
Bacillus licheniformis
Bacillus megaterium
Bacillus mycoides
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilus
Bacillus subtilis
Bacillus thuringiensis
Bacteroides fragilis
Bacteroides thetaiotamicron
Bacteroides vulgatus
Clostridium acetobutylicum
Clostridium bifermentans
Clostridium butyricum
Clostridium perfringens
Clostridium sporogenes
Clostridium tyrobutyricum
Corynebacterium aquaticum
Corynebacterium pseudodiphtheriticum
Enterococcus faecium
Enterococcus faecalis
Fusobacterium nucleatum
Lactobacillus acidophilus
Lactobacillus brevis
Lactobacillus buchneri
Lactobacillus bulgaricus
Lactobacillus casei casei
Lactobacillus casei
Lactobacillus cellobiosus
Lactobacillus curvatus
Lactobacillus delbrueckii
Lactobacillus fermentum
Lactobacillus leichmannii
Lactobacillus plantarum
Lactobacillus lactis
Lactobacillus sake
Lactobacillus sp.
Leuconostoc oenos
Leuconostoc Sp.
Listeria innocua
Listeria monocytogenes
Micrococcus luteus
Mycobacterium bovis
Mycobacterium parafortuitum
Mycobacteriums megmatis
Mycobacterium tuberculosis
oerskovia sp.
Pediococcus damnosus
Pediococcus pentosaceus
Porphyromonas canoris
Porphyromonas gingivalis
Propionibacterium acnes
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus hominis
Staphylococcus warneri
Staphylococcus xylosus
Streptococcus faecalis
Streptococcus salivarius
Streptococcus thermophilus
Streptococcus viridans
Thiobacillus ferrooxidan

Direct Viability Labelling Demonstrated With Wide Range of Microorganisms

Yeast

Acremonium kiliense
Candida albicans
Candida ciferii
Candida colliculosa
Candida famata
Candida famata
Candida fumentans
Candida humicola
Candida humicola
Candida krusei
Candida luxitaniae
Candida magnolia
Candida parapsilosis
Candida pelliculosa
Candida tropicalis
Cryptococcus albidus
Debaryomyces hanseni
Galactomyces geotrichum Geotrichum candidum
Hansenulaspora uvarum
Hansenula anomala
Kloechera japonica
Kloechera Apis apiculata
Pichia anomala
Pichia guillermondii
Pichia menbrena faciens
Rhodotorula rubra
Saccharomyces bailli
Saccharomyces bisparus
Saccharomyces cerevisiae
Saccharomyces rosei
Torulopsis candida
Torulopsisi inconspicua
Torulopsis maris
Torulospora delbrueckii
Zygosaccharomyces bailli
Zygosaccharomyces rouxii

Mould

Acremonium Sp.
Aspergillus versicolor
Aspergillus versicolor
Aspergillus fumigatus
Aspergillus niger
Basydiomycetes Sp.
Bassochlamis fulva
Byssochlamys Sp.
Cladosporium cladosporioides Epicocum nigrum ou altenaria Fusarium oxysporum Fusarium oxysporum
Fusarium gramineatium roseum Humicola fuscoatra
Mucor circinelloides
Mucor plumbeus
Mucor racemosus
Mucor Sp.
Neosartoeya Sp.
Penicillium decumbens
Penicillium expansum
Penicillium frequentans
Penicillium roquefortii
Rhizopus Sp.
Rhodoturola rubra
Rhizopusoligosporus
Scopulariopsis candida
Trichoderma Sp.

Pharmaceutical applications

Exemple of applications in the field:	Time to results
In-process analysis	
TVC Bioburden for in-process	90 min
TVC Bioburden for raw material	90 min
Environmental controls	
TVC Bioburden for pharmaceutical water	90 min
Air monitoring using Coriolis	< 3 hours
Surface monitoring using ChemSwab	< 3 hours
Biotechnology	
Contaminations of cell cultures	< 2 hours
Control of fermentations	90 min
Finish product testing	
Scan Bio II for sterility test	< 3 hours

Conclusion

- One protocol (Scan Bio II) for all compatible matrices

- **Improve productivity and maximize yields**
  - Real-time detection and correction of contamination problems
  - Minimize plant down time for decontamination/cleaning
  - Immediate cleaning validation
  - Reduce stock ➔ Save on warehouse costs

- **Guarantee quality**
  - Reduce the risk for contamination ➔ Increase consumer protection
  - Decrease the likelihood for recall
  - Rapidly test new product developments