Environmental monitoring using a rapid non-destructive automated compendial method

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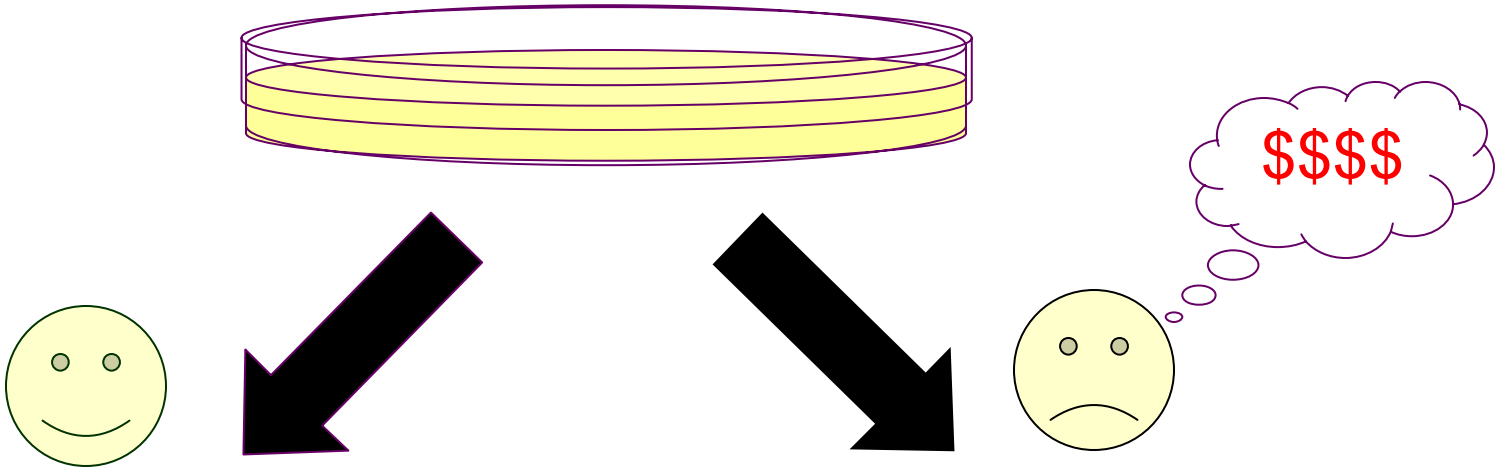
16 May, 2008

Overview

- overview of the automated compendial rapid microbial enumeration technology- the Growth Direct system

- application to environmental testing in manufacturing facilities:
  - water
  - air
  - surface

*The **business problem**: **high cost** of culture-based QC microbiological testing in pharmaceutical manufacturing*



↓cost of materials
↓regulatory risk: “gold standard”
↓skills required
↑sensitivity (for culturable bugs)

↑ time to results
↑ cost of labor
↑ cost of held inventory
↑ cost of product scrap
↑ cost of plant downtime
↑ cost of cleanup

Goals in automating the compendial method

- Improve accuracy & decrease time-to-results
  - replace human eye with digital imaging
- facilitate system validation
  - use same procedures and method principles as traditional culture
- save labor & improve compliance
  - automate analysis and documentation

Automating the compendial method by replacing the human eye with sensitive digital imaging- a better set of eyes

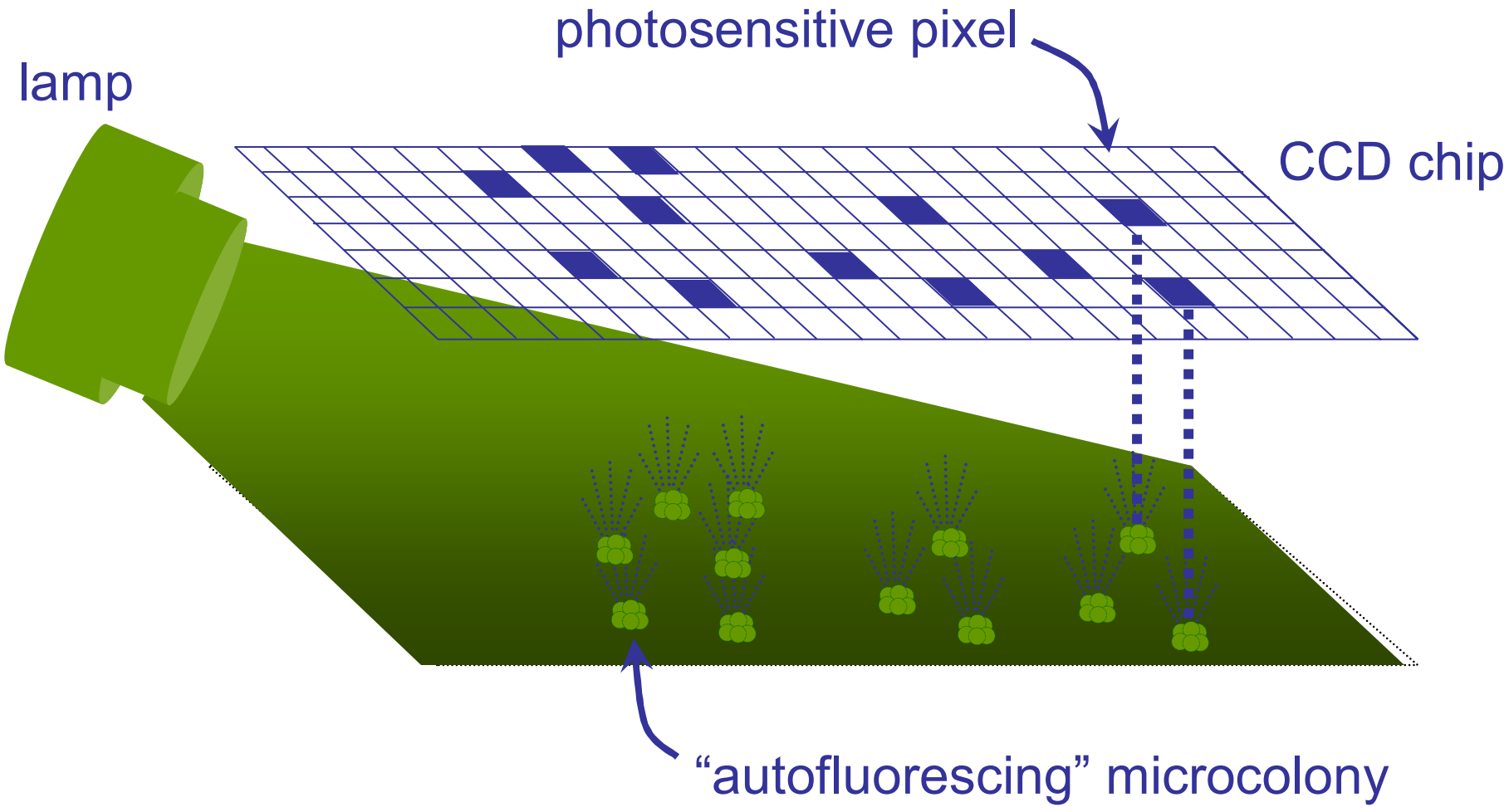
Growth Direct system

visual plate counting

~100 cells

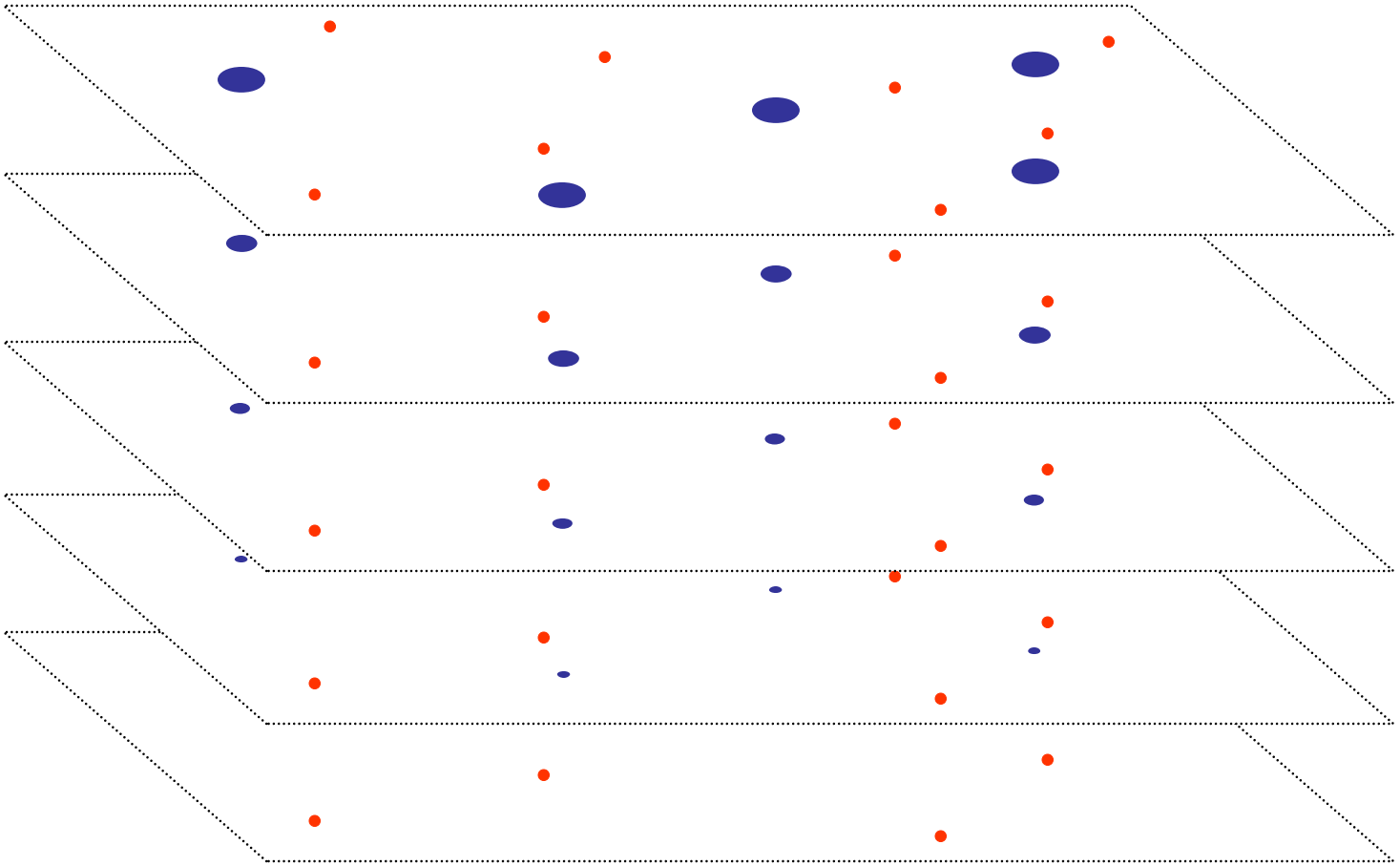
~5x10^6 cells

Using large area non-magnified digital imaging to detect microscopic microcolonies



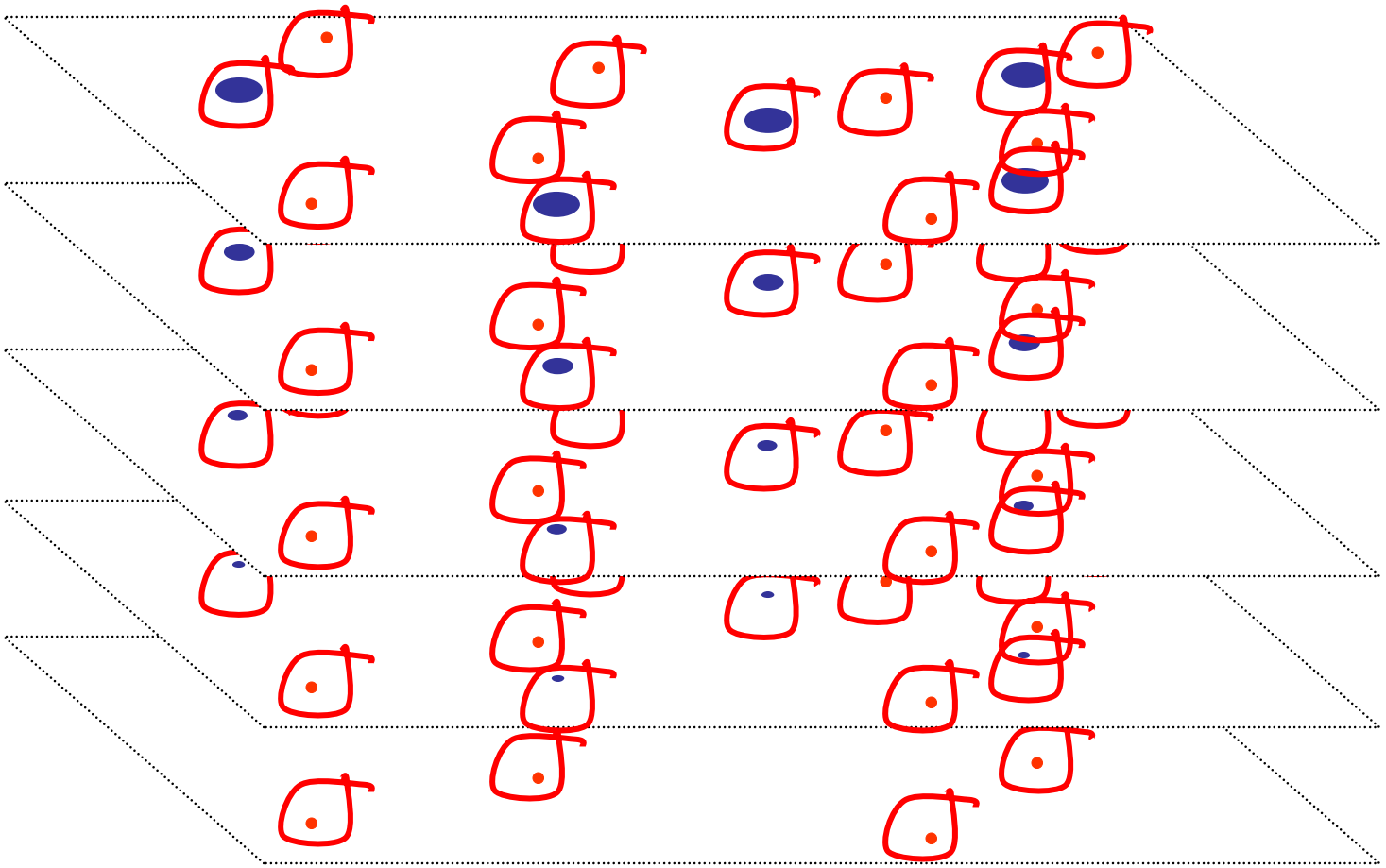
How the image analysis software enumerates growing microbes

Image analysis using Growth Direct software



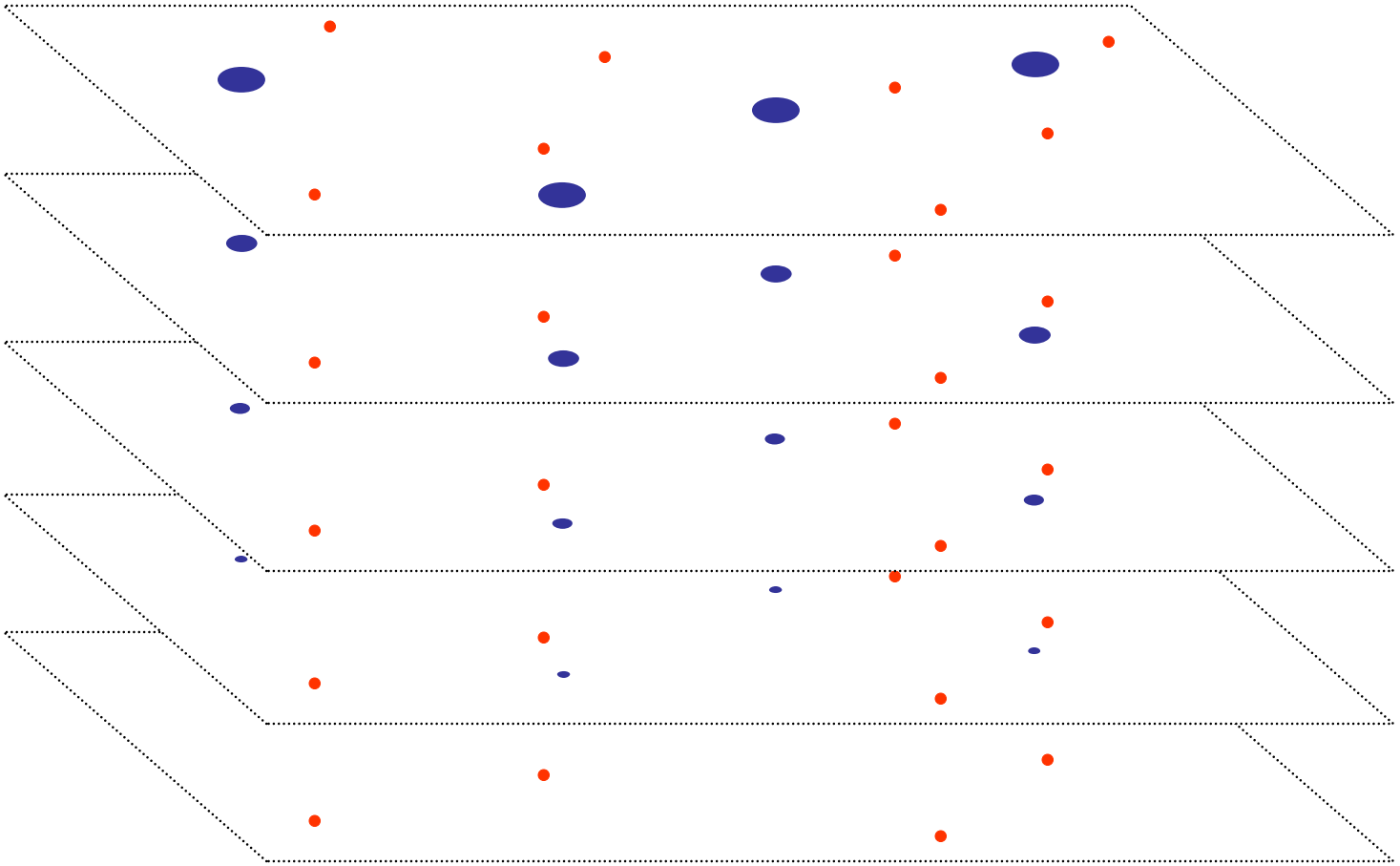
make a stack of images from the various time points

Image analysis using Growth Direct™ software



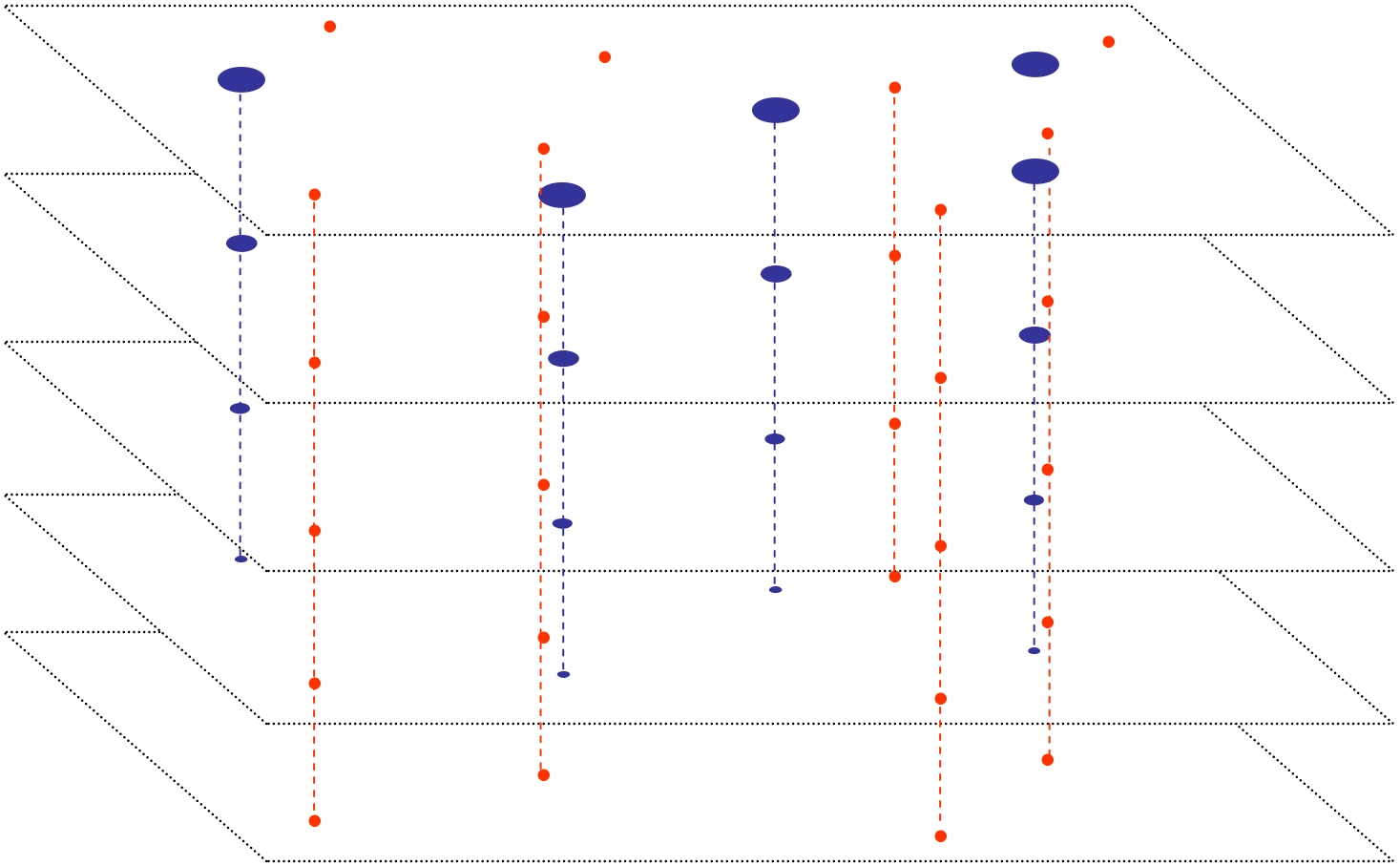
find objects on each image using image analysis software

Image analysis using Growth Direct™ software



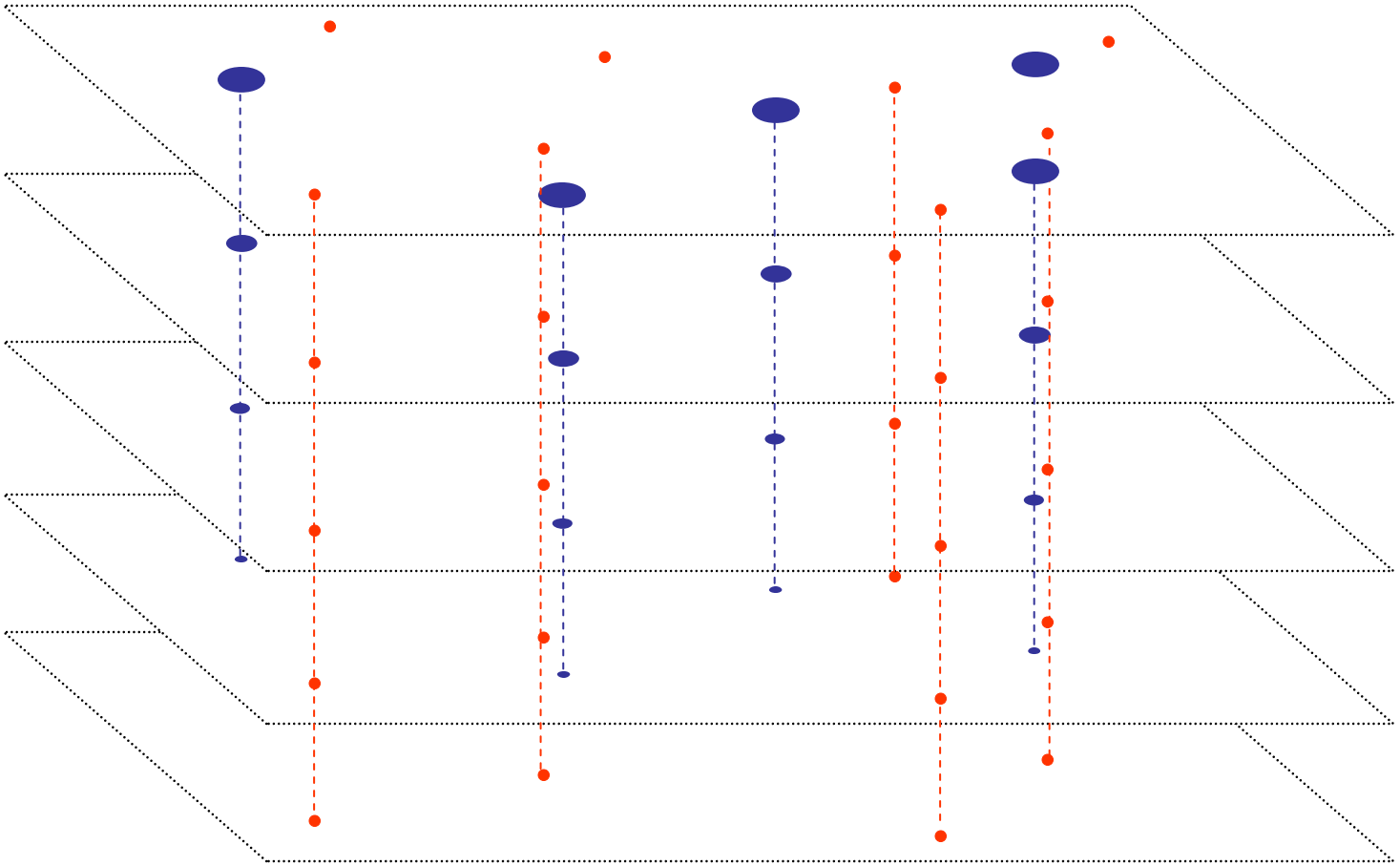
align images

Image analysis using Growth Direct™ software



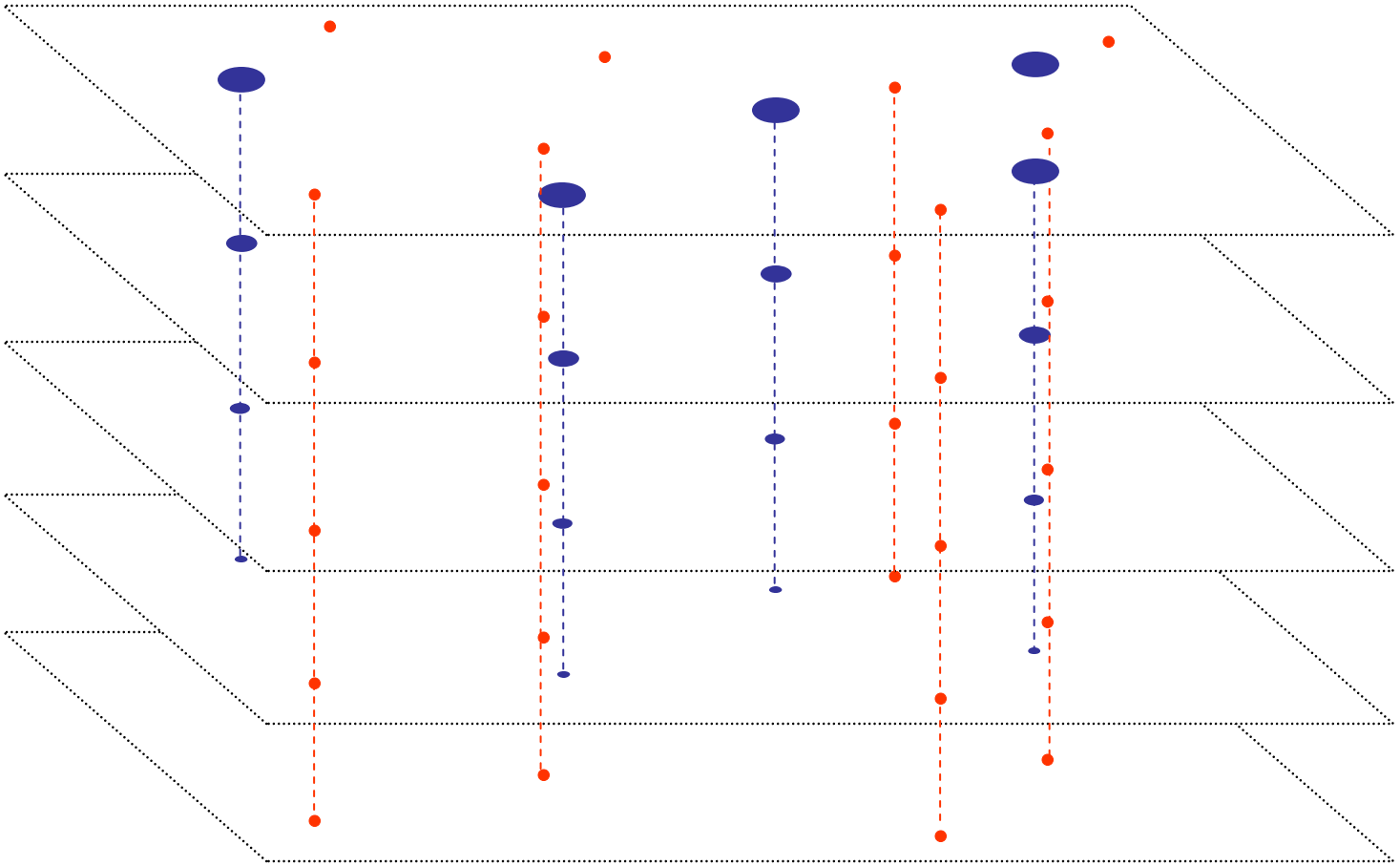
trace all objects backwards through time

Image analysis using Growth Direct™ software



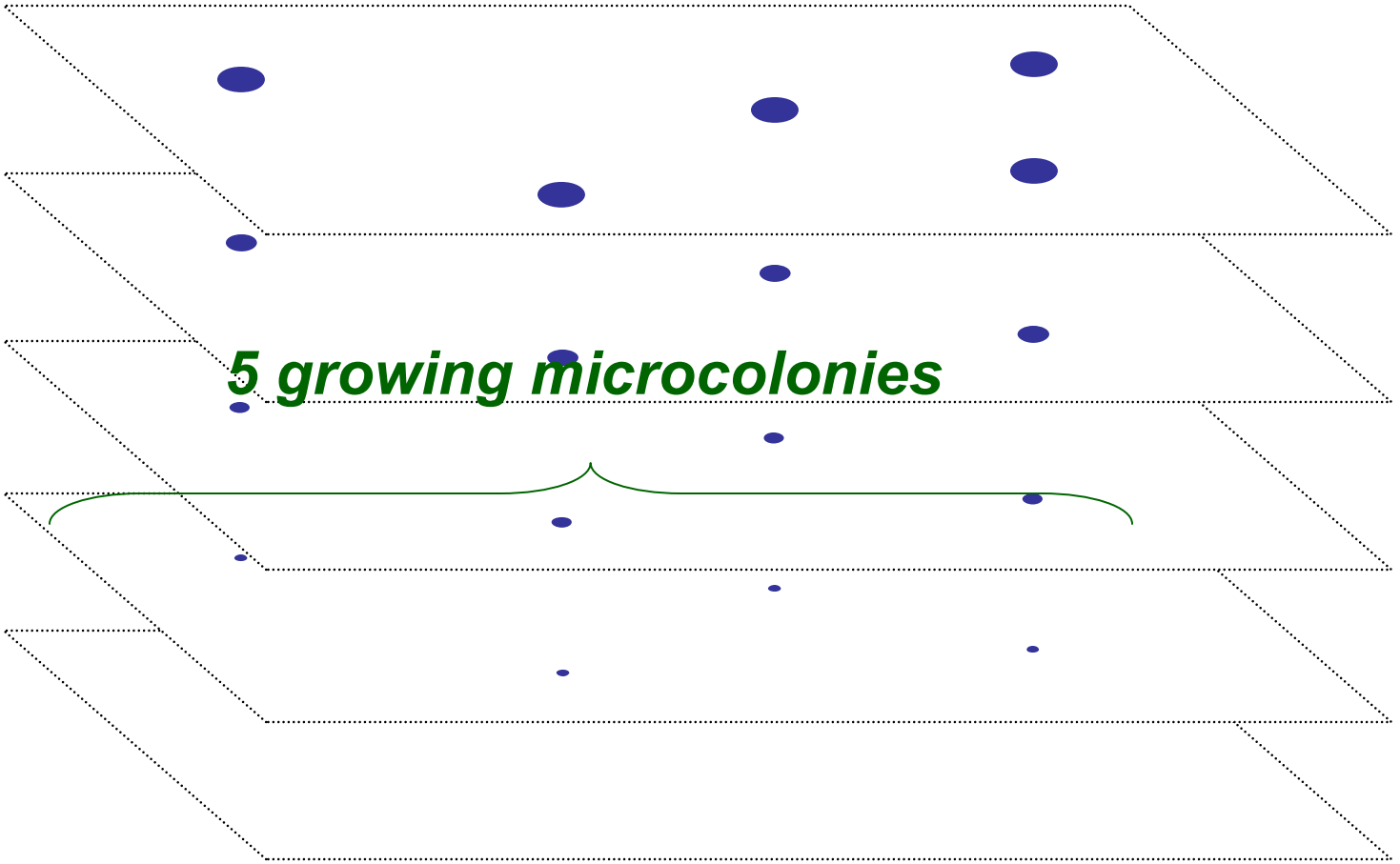
identify growing objects (intensity increases over time)

Image analysis using Growth Direct™ software



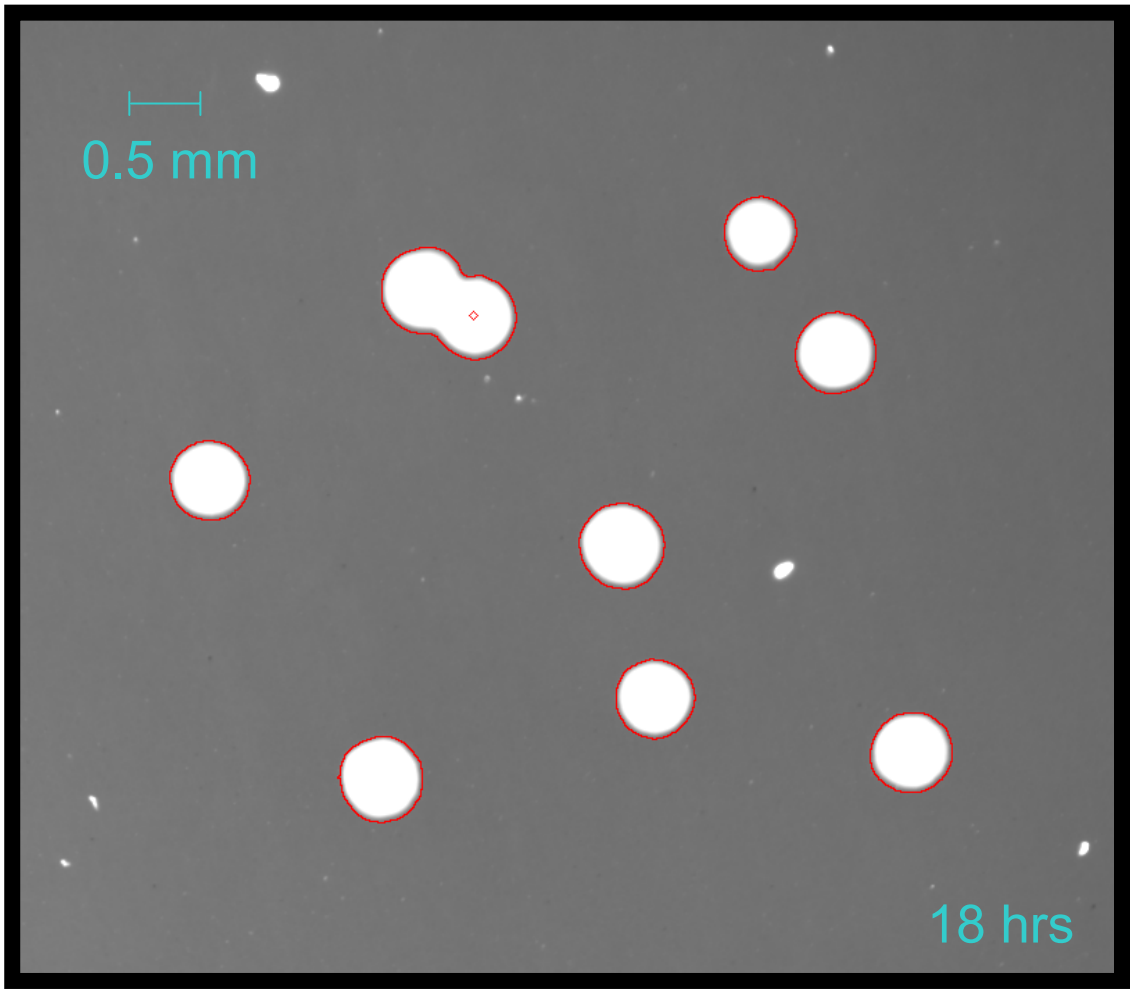
ignore debris (objects that do not grow over time)

Image analysis using Growth Direct™ software



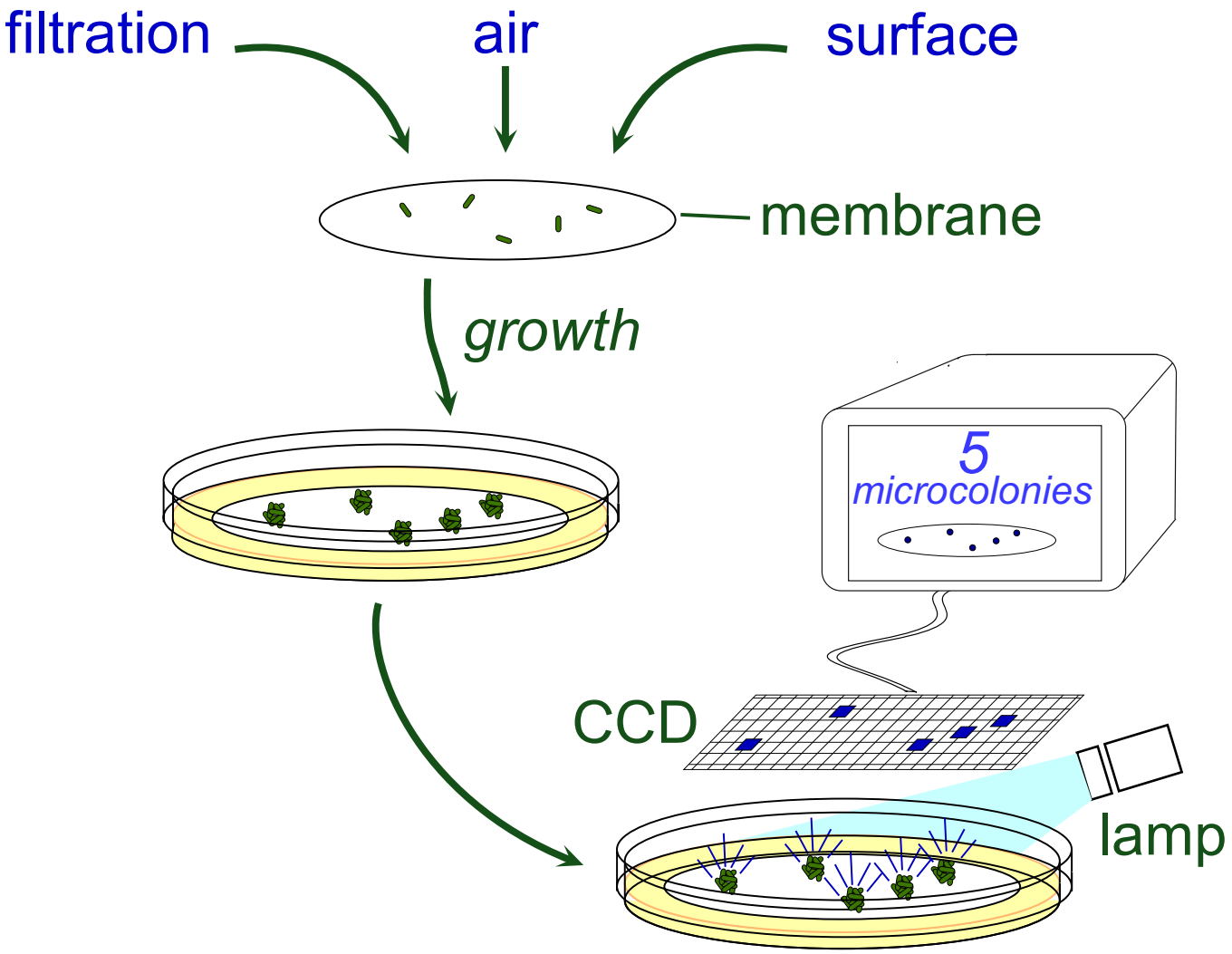
report number of growing objects

Accuracy: by analyzing image time series system counts growing colonies and ignores inanimate fluorescent debris



P. aeruginosa

The work flow of the automated compendial test



Labor savings and improved compliance from an automated compendial test

- labor savings
  - data acquisition is automated
  - documentation is electronic, and easily transferred to data management systems

- increased compliance
  - fewer data management errors
  - greater reproducibility

Automating the compendial test preserves its advantages while addressing its weaknesses

- captures the positive features of the compendial tests
  - non-destructive
  - ultra-sensitive (1 CFU)
  - breadth of testing applications
  - enumerates replicating cells
  - high throughput
  - no added reagents
  - industry standard media, membranes

- addresses the limitations of the compendial tests
  - automation: ↓labor, ↑compliance, ↑reproducibility
  - speed: saves days, generally ~50% faster

Bacteria detected by cellular autofluorescence

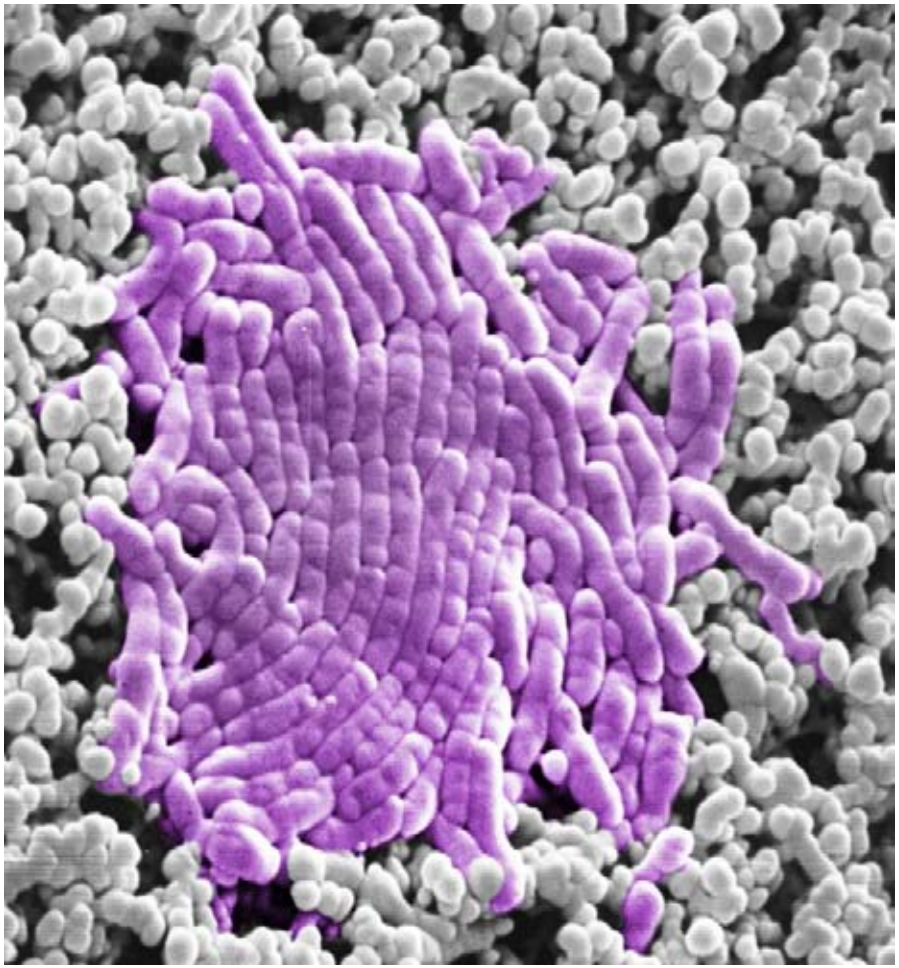
Acidovorax delafieldii
Acidovorax sp.
Acidovorax temperans
Acinetobacter junii
Afipia broomeae
Arthrobacter sp.
Bacillus cereus
Bacillus clausii
Bacillus fusiformis
Bacillus gibsonii
Bacillus licheniformis
Bacillus megaterium
Bacillus pumilus
Bacillus sp.
Bacillus subtilis
Bacillus vortex
Bacteriodes fragilis
Brachybacterium sp.
Bradyrhizobium spp.
Brevibacterium sp.
Brevundimonas diminuta
Burkholderia cepacia
Caulobacter leidyii
Cellulomas sp.
Chromobacterium violaceum
Clostridium sporogenes
Corynebacterium sp.
Corynebacterium xerosis
Corynebacterium pseudodiptheriticum
Curtobacterium sp.
Deinococcus proteolyticus
Dermacoccus nishinomiyaensis
Enterococcus faecalis
Escherichia coli
Geobacillus stearothermophilus
Hydrogenophagea sp.
Hyphomicrobium sp.
Kocuria kristinae
Kocuria rhizophila
Kytococcus sedentarius
Macrococcus caseolyticus
Methylobacterium extorquens
Methylobacterium radiotolerans
Microbacterium luteolum
Microbacterium maritypicum
Microbacterium sp.
Micrococcus luteus
Moraxella osloensis
Myxococcus xanthus
Neisseria sp.
Paenibacillus lautus
Paenibacillus sp.
Pantoea agglomerans
Paracoccus sp.
Porphyromonas gingivalis
Prevotella melaninogenica
Propionibacterium acnes
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Ralstonia pickettii
Rhodococcus erythropolis
Roseomonas gilardii
Roseomonas sp.
Salmonella enterica
Serratia marcesens
Sphingomonas paucimobilis
Sphingomonas spp.
Sphingomonas terrae
Staphylococcus aureus
Staphylococcus capitis
Staphylococcus epidermidis
Staphylococcus equorum
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus saccharolyticus
Staphylococcus sp.
Staphylococcus warneri
Streptococcus sp.
Streptomyces chrysolmalus complex
Streptomyces coelicolor
Streptomyces sp.
Vibrio natriegens

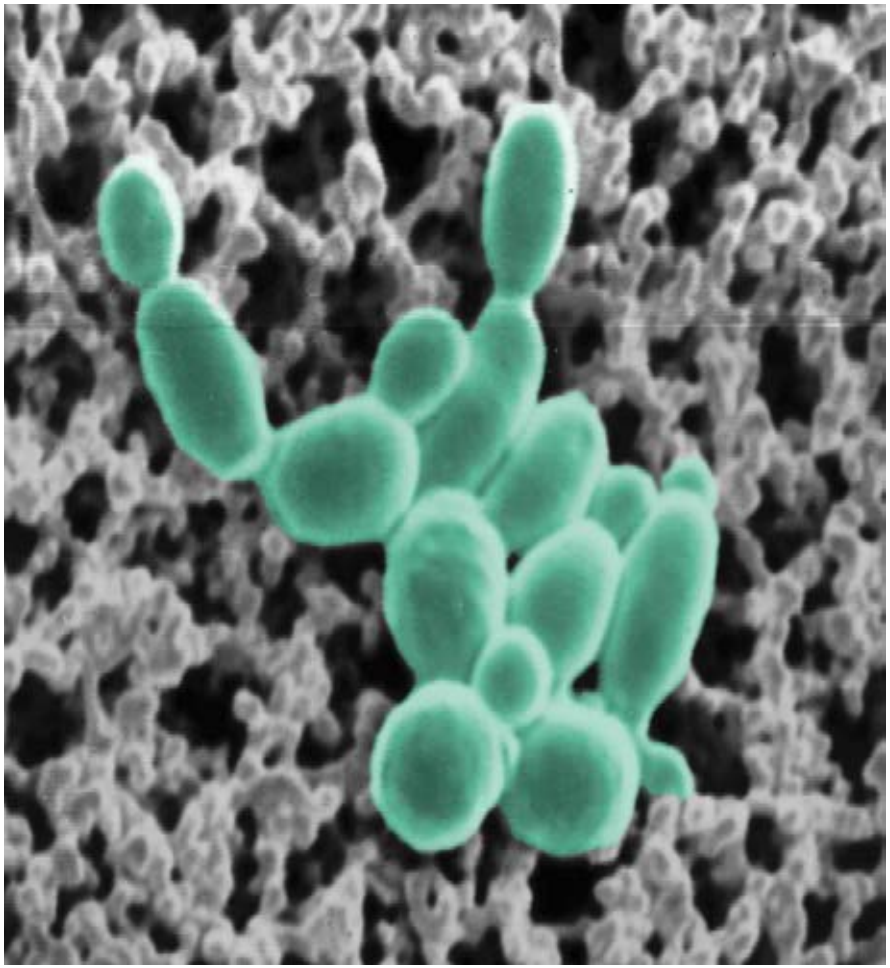
Fungi detected by cellular autofluorescence

Alternaria alternata
Alternaria geophila
Arthrinium sacchari
Aspergillus flavus
Aspergillus fumigatus
Aspergillus niger
Aspergillus sp.
Aspergillus versicolor
Aureobasidium pullulans
Candida albicans
Candida parapsilosis
Chaetomium globosum
Cladosporium herbarum
Epicoccum nigrum
Fusarium solani
Penicillium camemberti
Penicillium chrysogeneum
Penicillium corylophylum
Penicillium notatum
Penicillium roquefortii
Rhizopus oligosporus
Saccharomyces cerevisiae
Schizophyllum commune
Schizophyllum fasciatum
Schizosaccharomyces pombe
Sporidiobolus johnsonii
Sporotrichum pruinosum
Trichoderma asperellum
Zygosaccharomyces rouxii

Time savings: the system detects microscopic microcolonies (scanning EM images)

1 micron





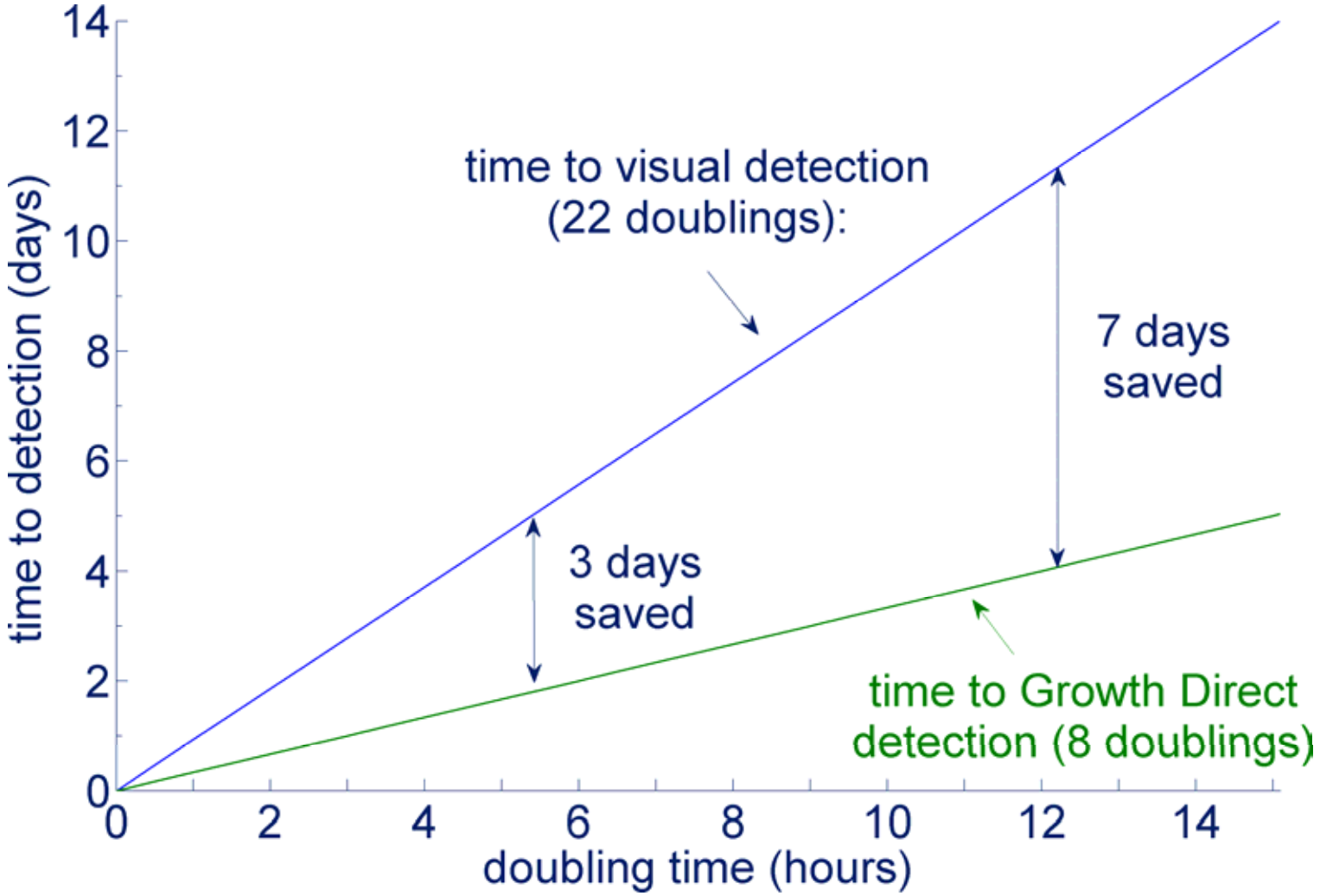
Escherichia coli (~120 cells)

Candida albicans (~12 cells)

*The automated compendial method saves **days** for **slow growing** strains*

	Growth Direct (days)	**Visual** (days)	**Days saved**
Methylobacterium extorquens	2.6	17.2	14.6
Bacteroides vulgatis	0.9	7	6.1
Mycobacterium chelonae	1.9	6.7	4.8
Proionibacterium acnes	0.9	3.6	2.7
Deinococcus proteolyticus	1.6	4	2.4
Mycoplasma bovis	1.3	3.7	2.4
Aspergillus versicolor	1.5	3.6	2.1
Ralstonia picketii	1.1	3	1.9
Aspergillus niger	0.8	2.4	1.6
Clostridium sporogenes	0.6	1.8	1.2

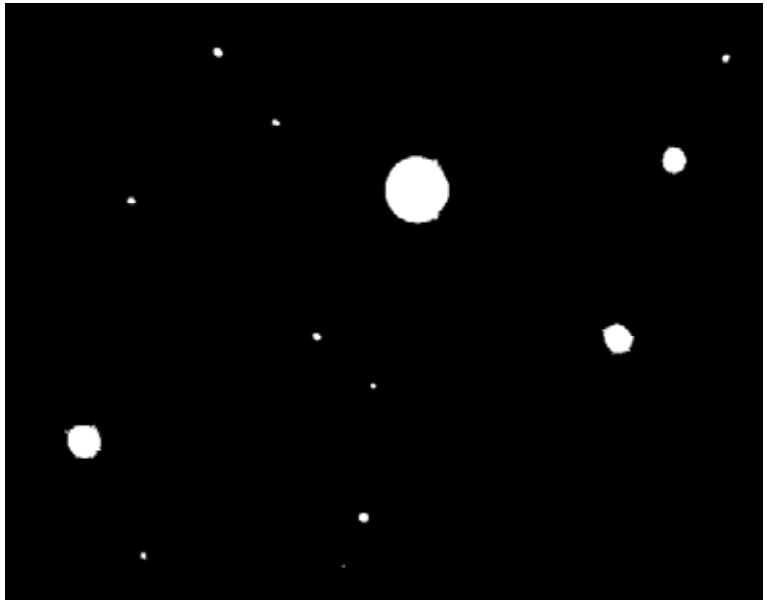
Time savings is greatest for slow growing microbes



Water testing

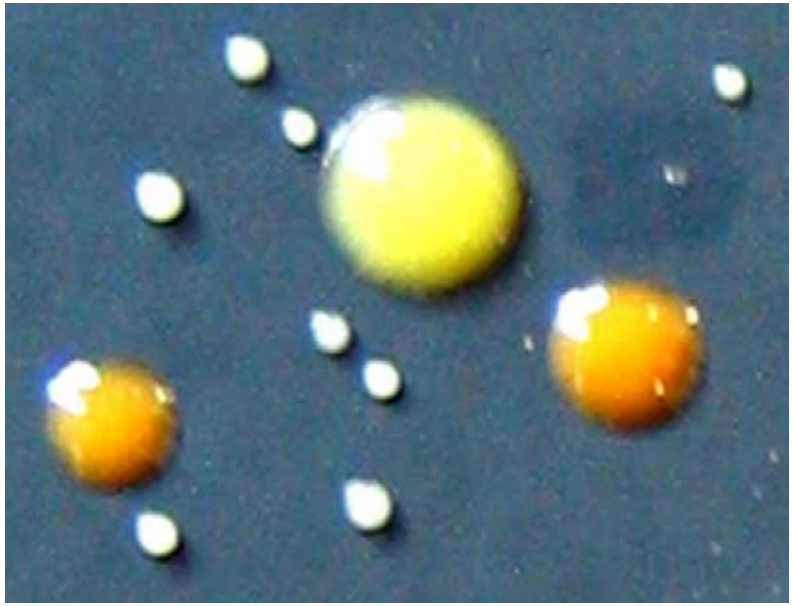
Rapid detection of water microbes: autofluorescent detection detects the same colonies that later become visible by eye

Growth Direct microcolonies



2.5 days

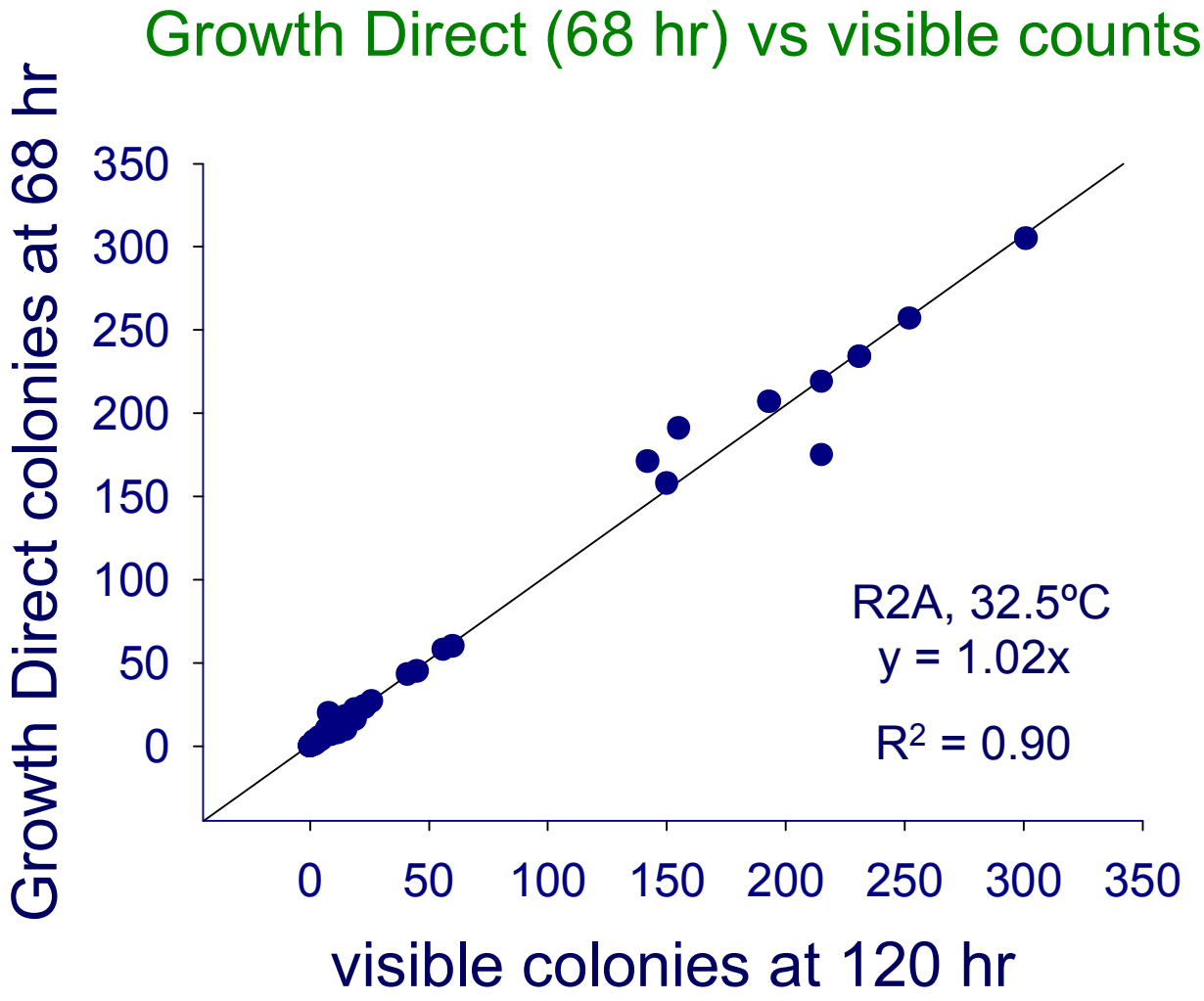
visual plate counting



5 days

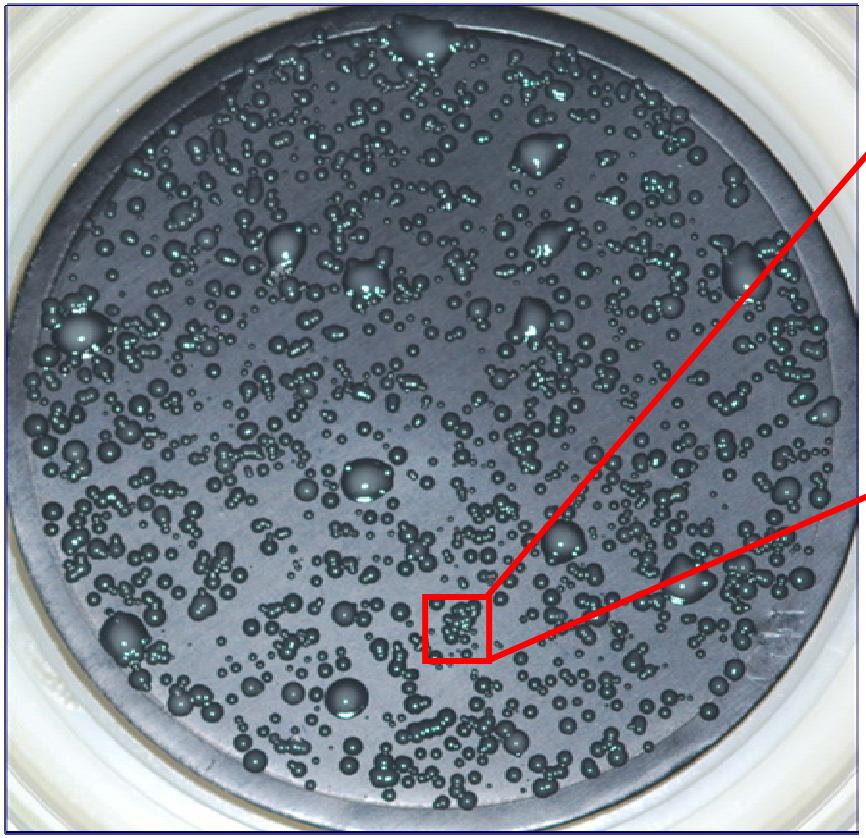
sample: purified water from a pharmaceutical facility

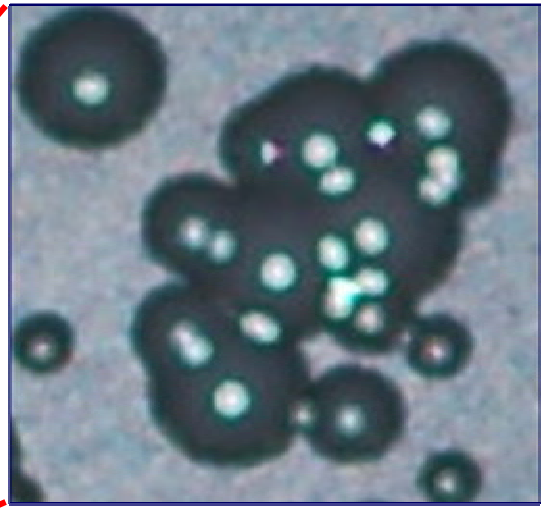
Correlation of Growth Direct and visible counts in pharma water samples



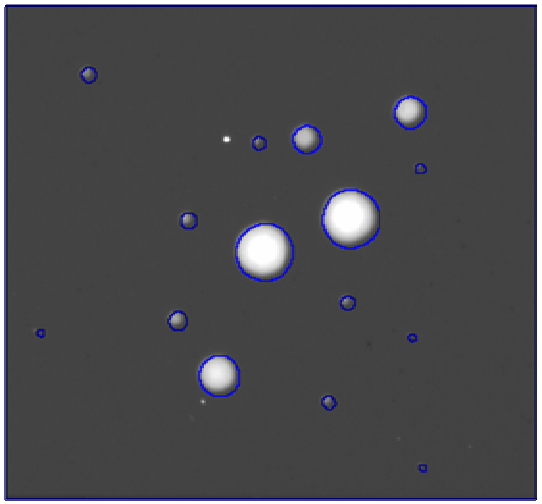
Accuracy: resolving at the microcolony stage colonies that are uncountable by traditional visible plate

visual plate counting (5 days)





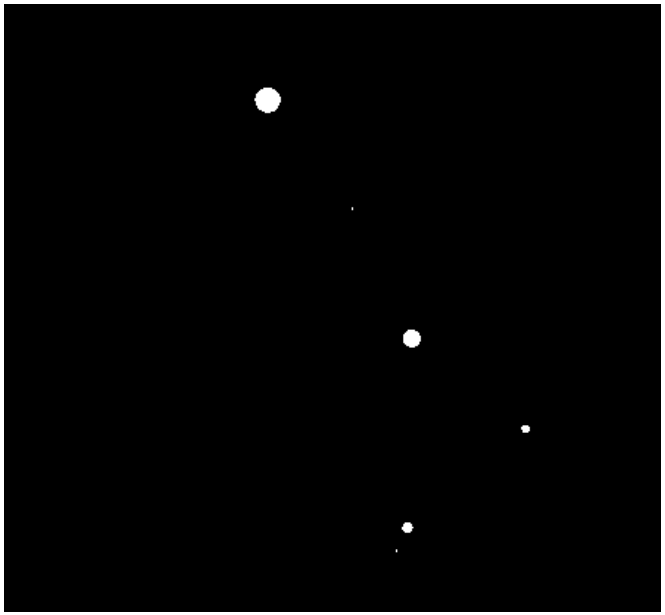
Growth Direct (1.5 days)



Air monitoring

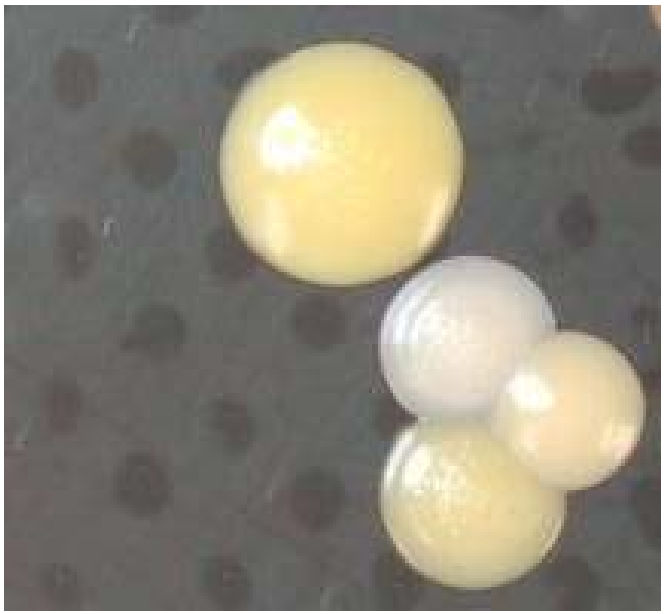
Rapid detection of airborne microbes at a pharma plant

Growth Direct microcolonies



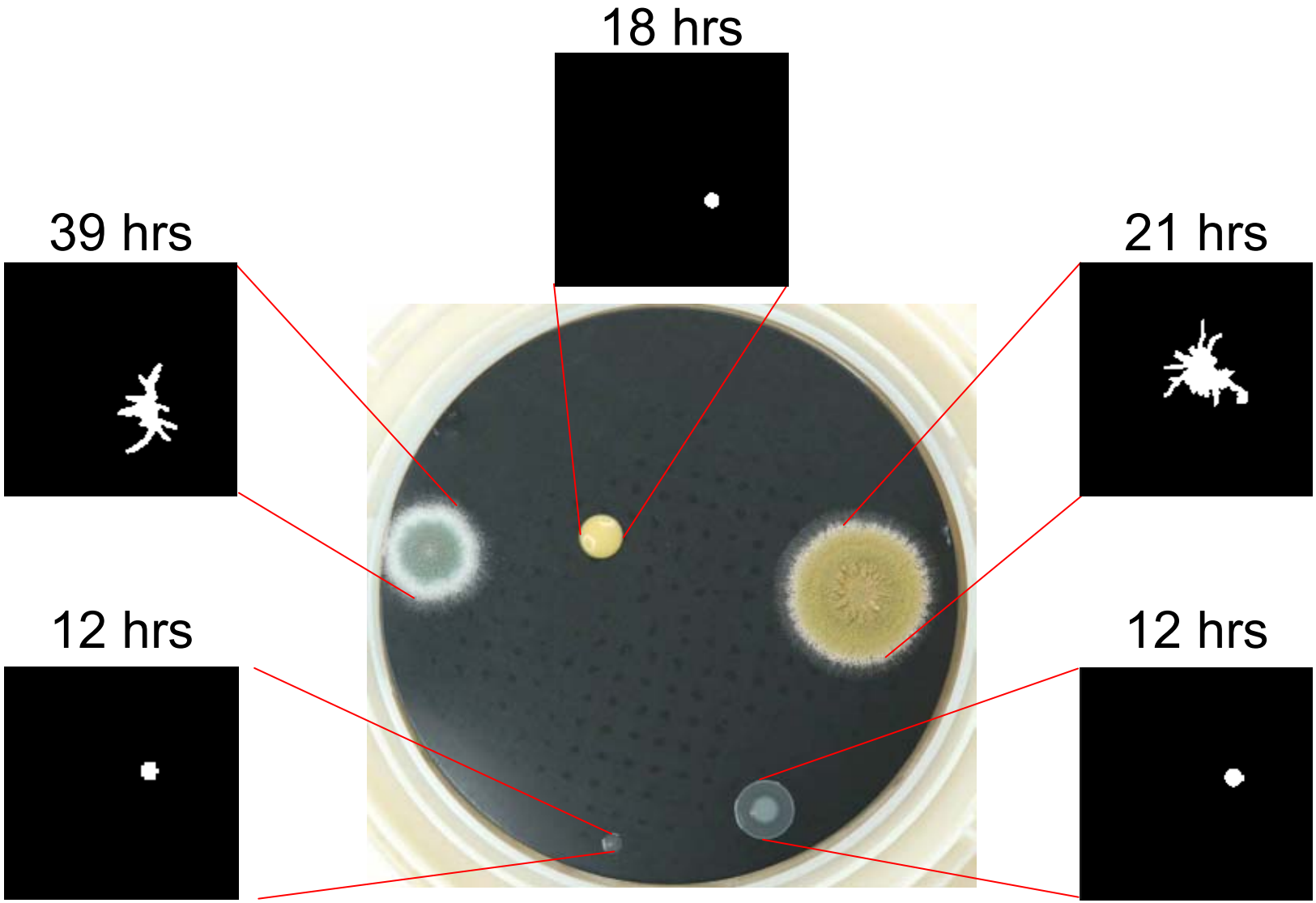
20 hr

visual plate counting

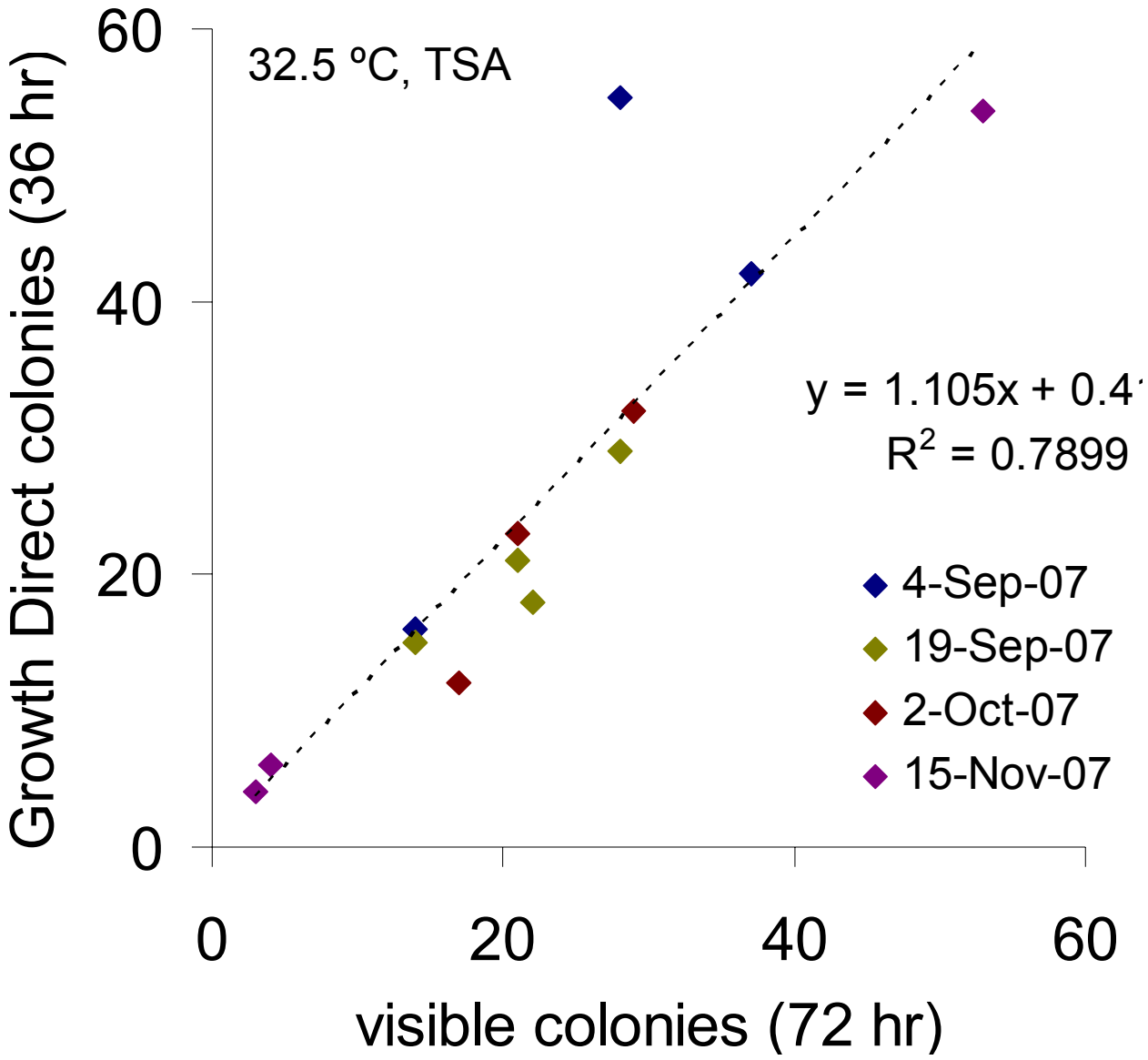


72 hr

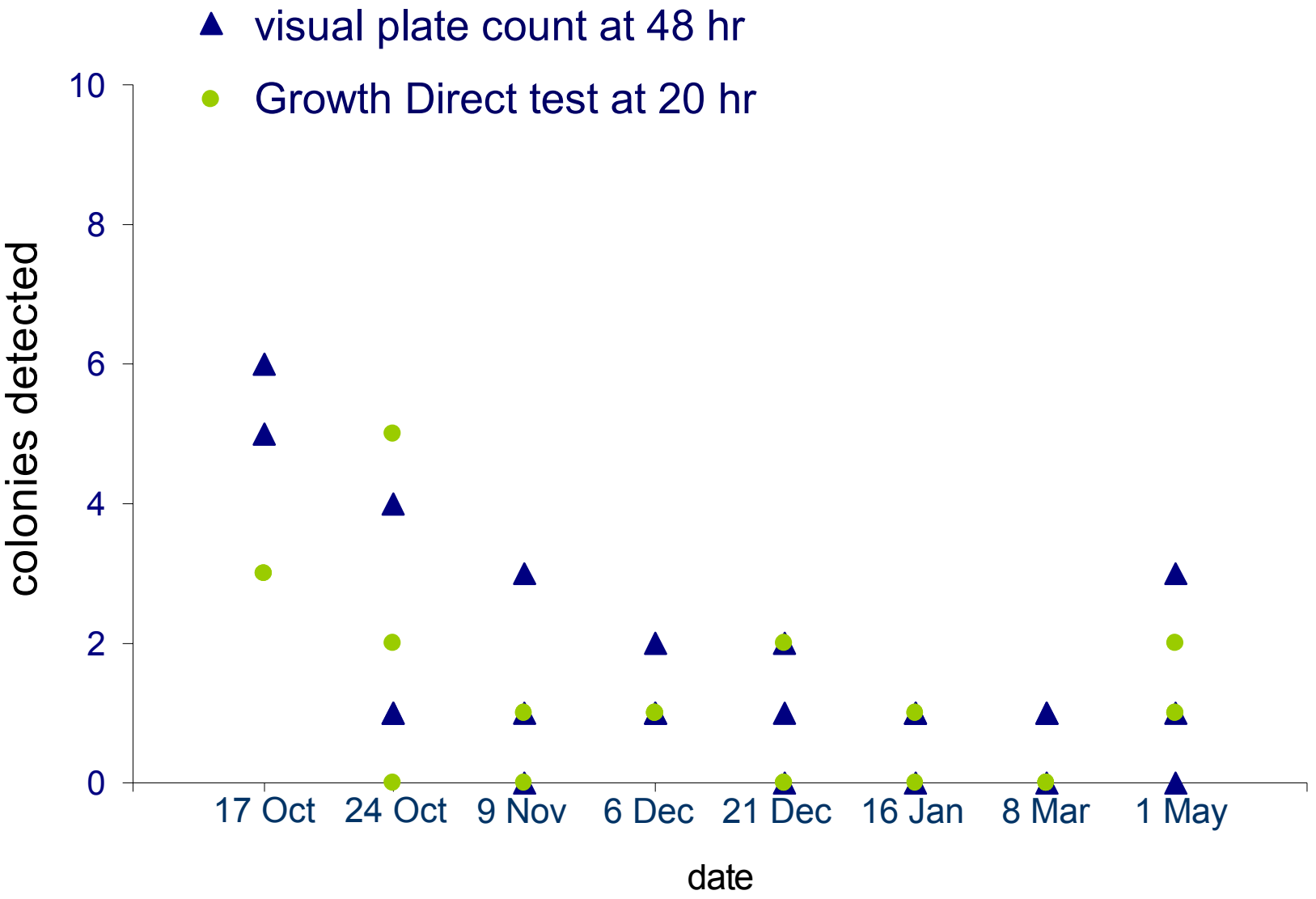
Rapid detection of diverse airborne microbes at a pharma facility



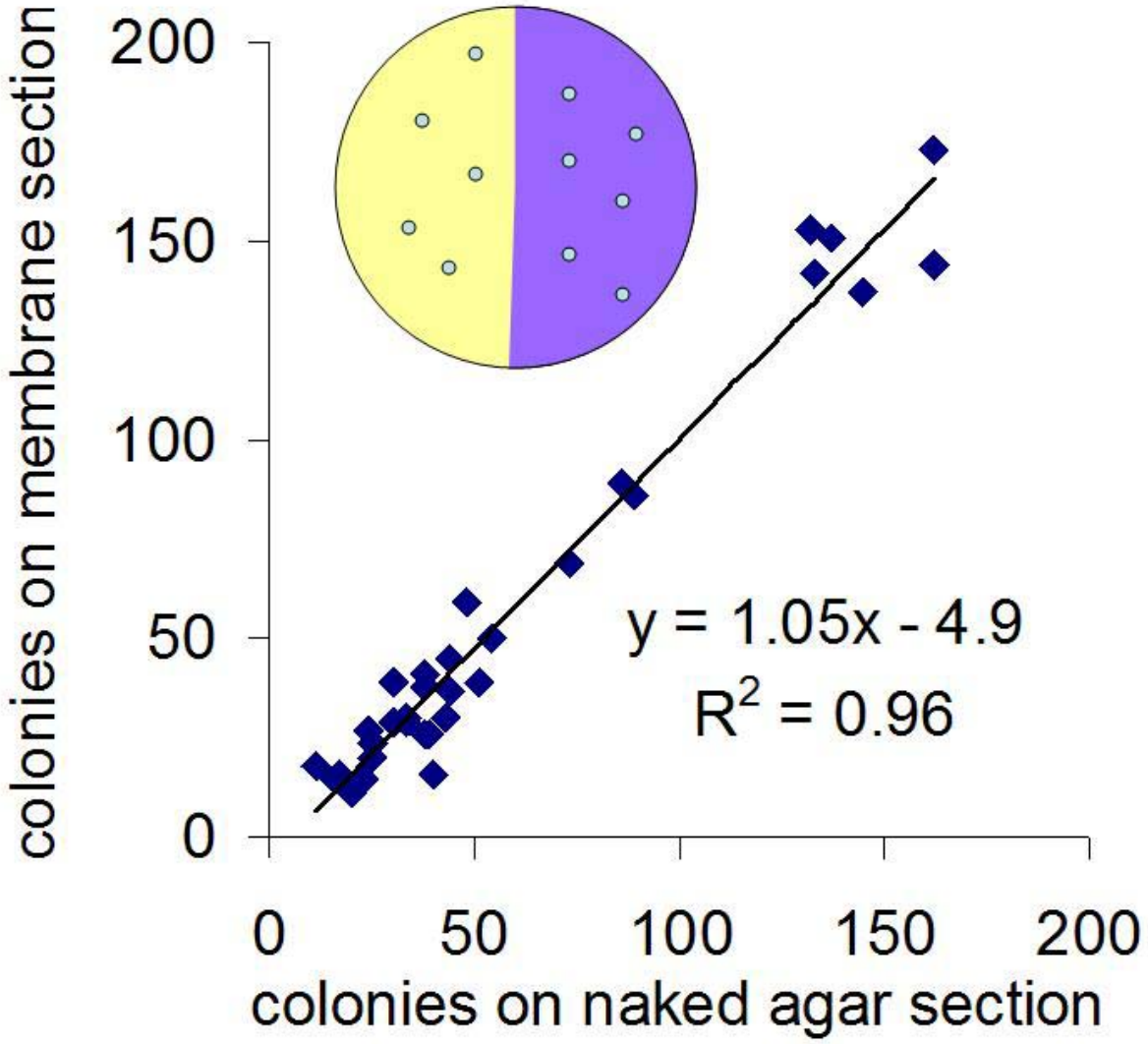
Air monitoring: co-trending of rapid (1.5 day) and traditional (3 day) tests at a pharma facility



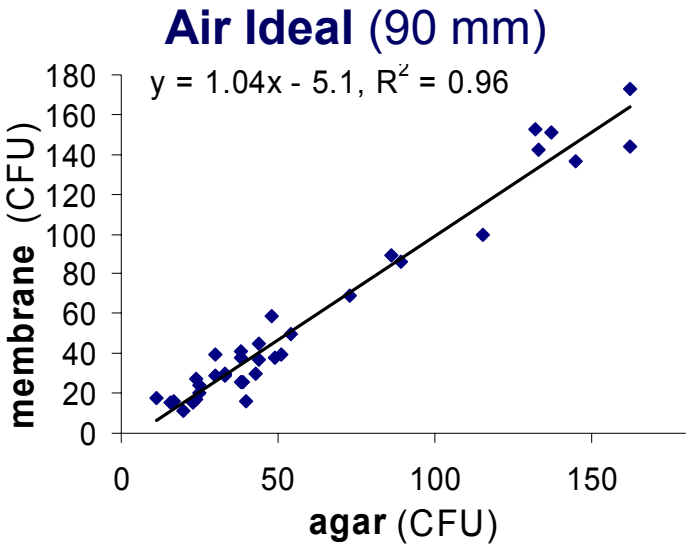
Air monitoring: co-trending of rapid (1 day) and traditional (2 day) tests at a pharma facility

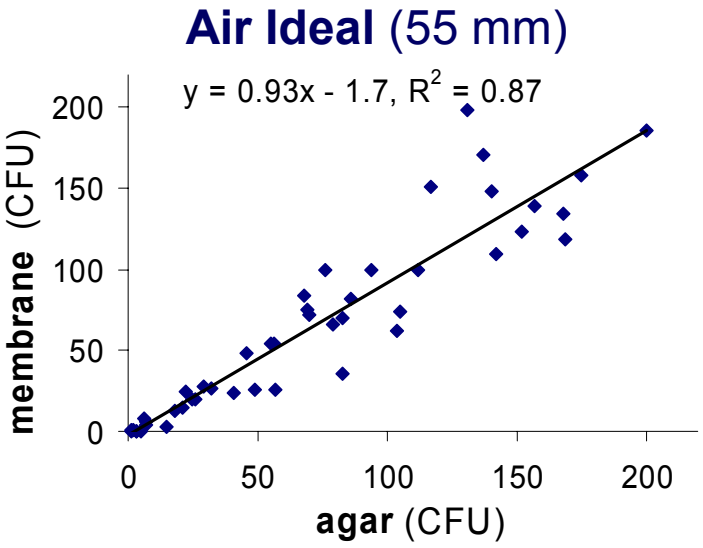


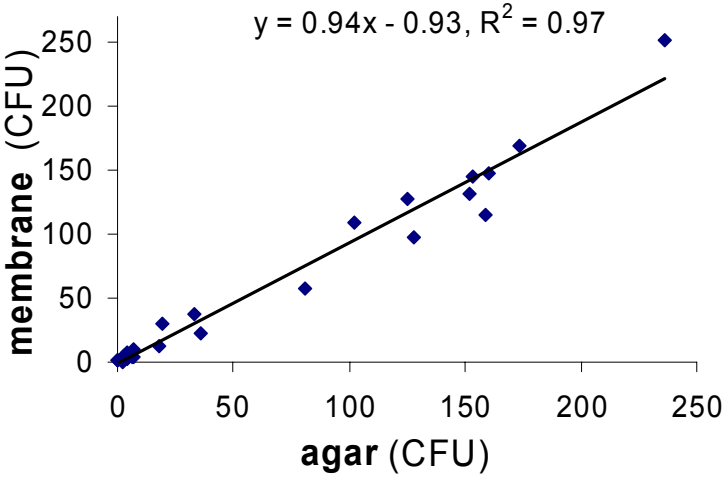
Comparing recovery on membranes and agar in air testing using a "half membrane" strategy

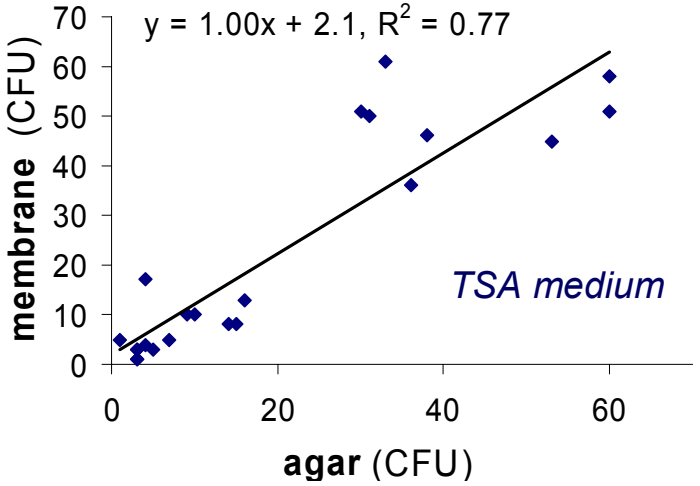


Various air samplers, "half-membrane" experiments show equivalent recovery on membrane and agar





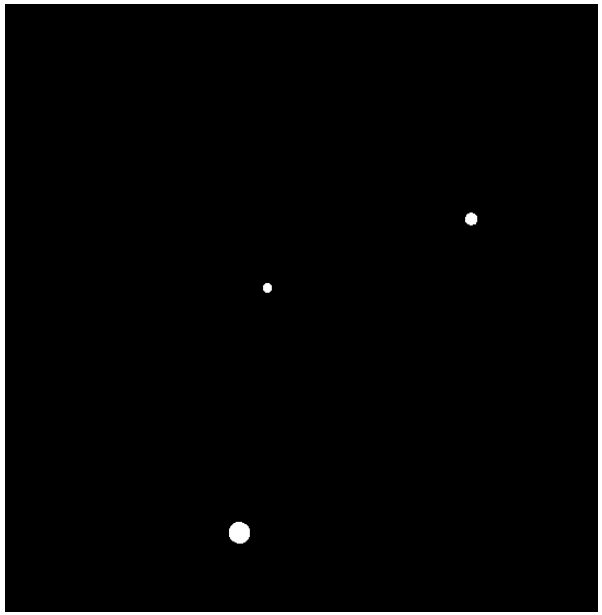




Surface monitoring

Rapid detection of microbes on surfaces at a pharma site

Growth Direct microcolonies

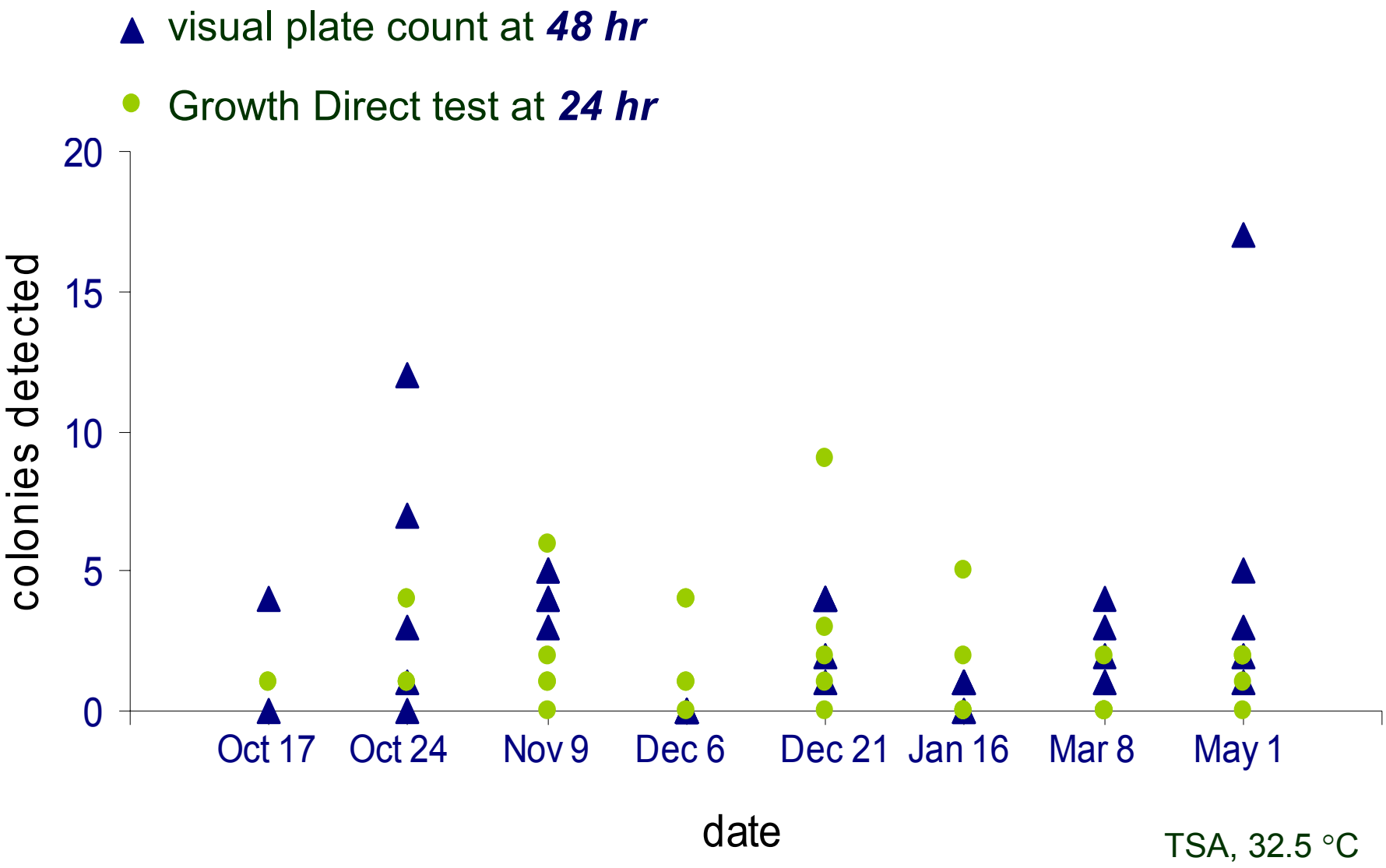


15 hr

visual plate counting

72 hr

Surface testing: co-trending of rapid (1 day) and traditional (2 day) tests at a pharma facility



*Comparing recovery on membranes and agar in surface testing using capture efficiency (*Whyte et al, 1989)

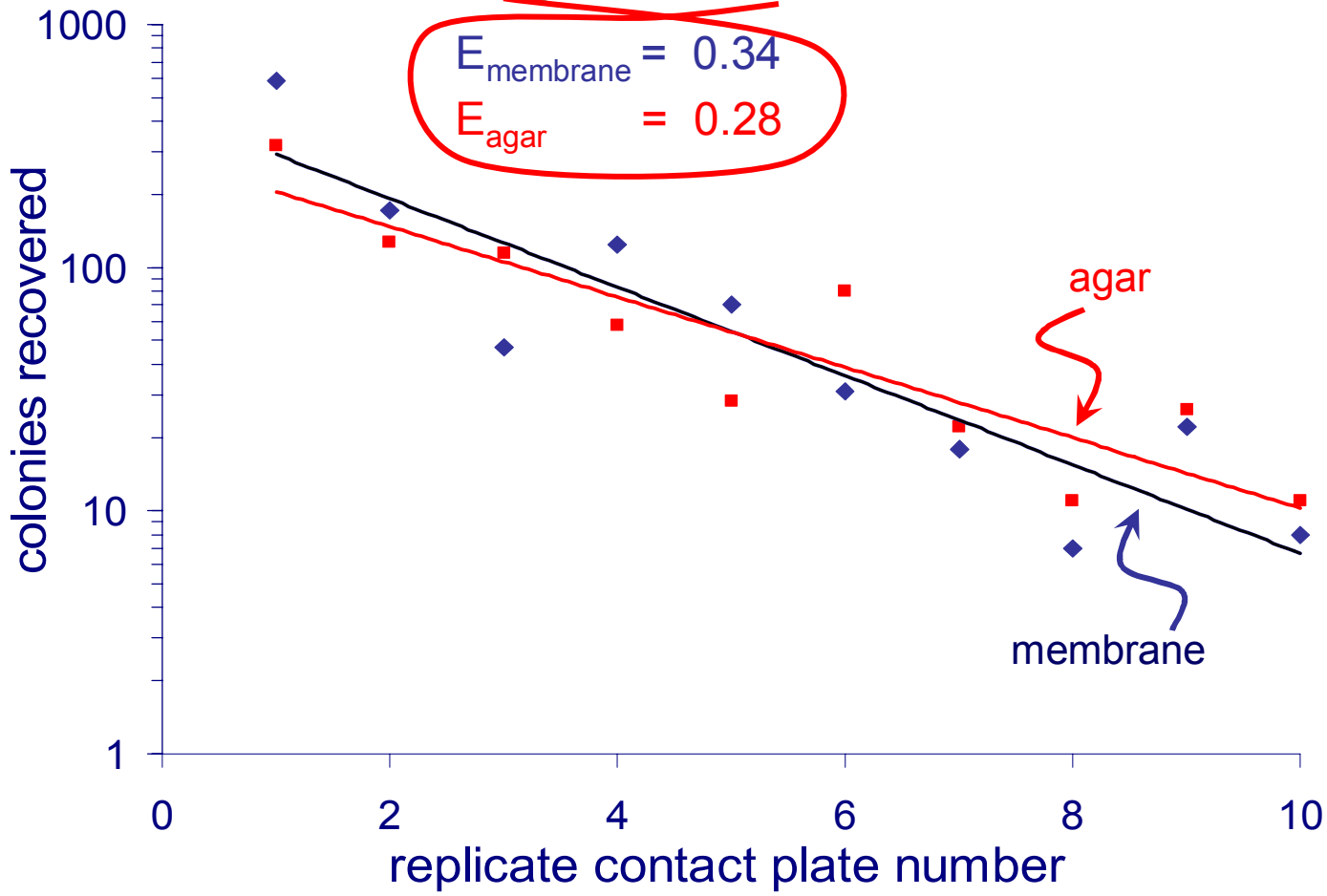
Efficiency (E) = fraction recovered of total microbes/ replicate

- Sample multiple times on same location (e.g. 5 replicates)
- Incubate
- Count each plate

Comparing efficiency of recovery for surface contact plates: membrane Vs agar

Efficiency (E) = fraction recovered of total microbes/ replicate

E = [1 − log (slope)]



Whyte et al, 1989. J. Hosp. Inf., 13: 33-41

Surface contact testing: equivalent capture efficiencies on membranes vs. agar

	average capture efficiency	
Surface	**membrane**	**agar**
stainless steel 12 sites	0.40 ± 0.13	0.38 ± 0.11
glass 10 sites	0.38 ± 0.12	0.49 ± 0.07
tyvek 8 sites	0.32 ± 0.13	0.31 ± 0.10
plexiglass 10 sites	0.40 ± 0.08	0.40 ± 0.09
latex gloves 9 “thumbs”	0.26 ± 0.09	0.32 ± 0.10

Validation Question - Growth Direct System, New Technology?

- Growth Direct is not an alternative technology
  - it is based on standard USP growth based membrane filtration methods
  - the results are given as CFU's.

- The "novel" Growth Direct is an automated compendial method:
  - **the system is an "Automated" colony counter and can be linked to the USP Chapter <16> Automated Methods of Analysis.**
  - validation requires proof that the camera sees as many micro-colonies as the eye would see colonies on the membrane surface.
  - Performance Qualification would follow standard requirements in chapter <1227>, <61> etc.
  - other validation components are standard incubator and software validation protocols.

Summing up

- Advantages of the an automated compendial enumeration method:
  - addresses same broad spectrum of QC applications as the compendial method
  - sensitive digital imaging detects microcolonies
  - non-destructive, compatible with microbial ID
  - equivalent counts to current method
- Autofluorescence-based detection offers equivalent results with substantial time savings for environmental applications:
  - water
  - air
  - surfaces

Environmental monitoring using a rapid non-destructive automated compendial method

Rapidmicro biosystems