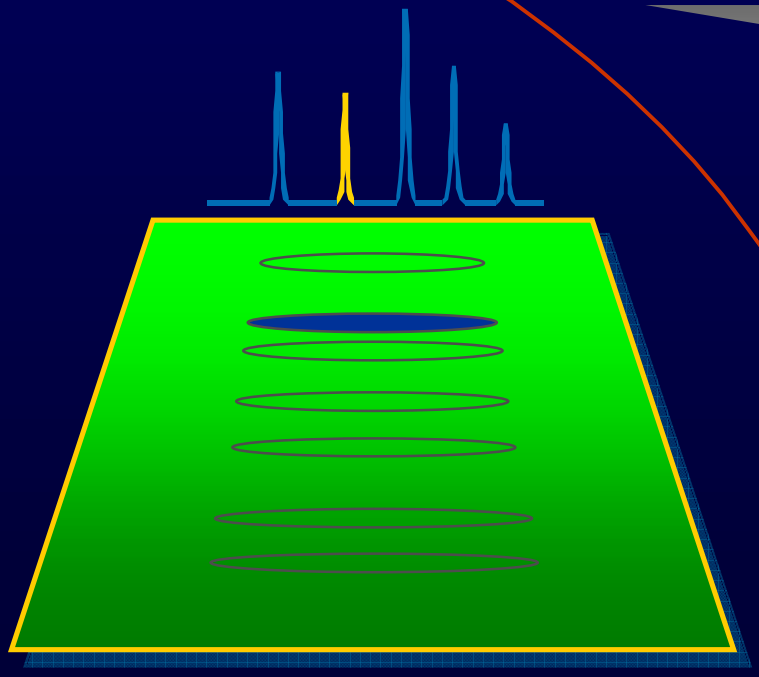
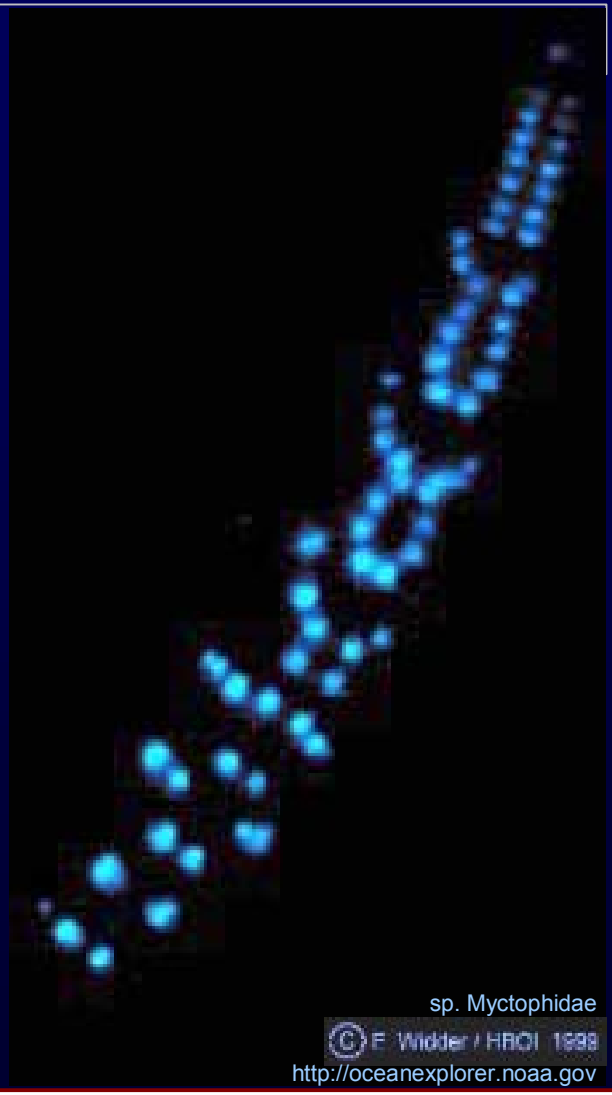
Bioluminescence for TLC Detection



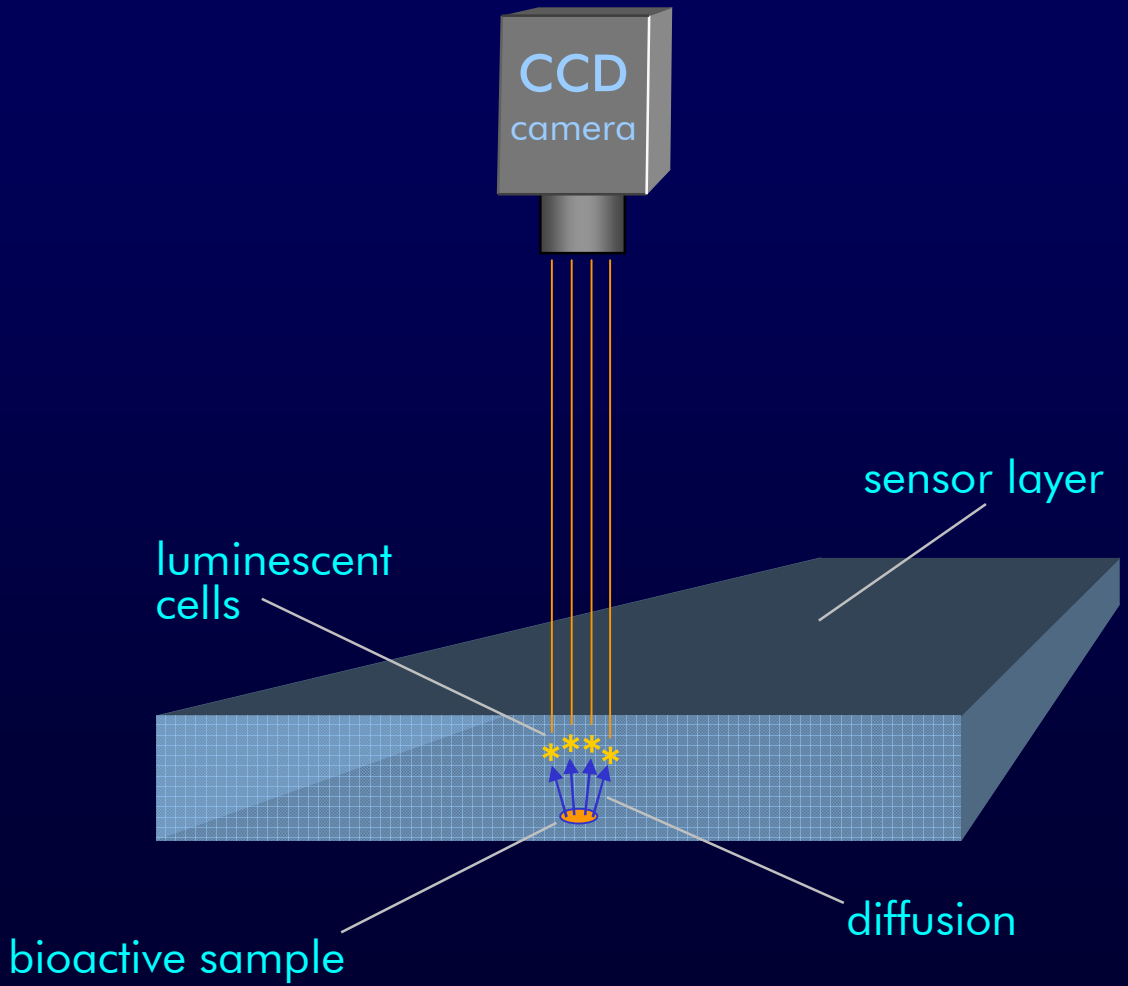
- bioactivity in mixtures
- toxicity screening
- drug discovery

Bioluminescence and TLC

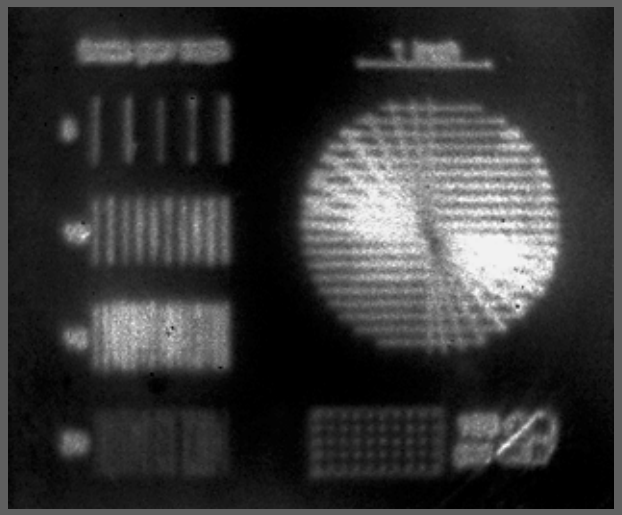
- imaging of bioactivity on planar surfaces
- bioluminescence for TLC detection
- detection process
- application examples
- luminescent reporter gene cells
- bioluminescence coupling - TLC or HPLC
- benefits, limitations and current status



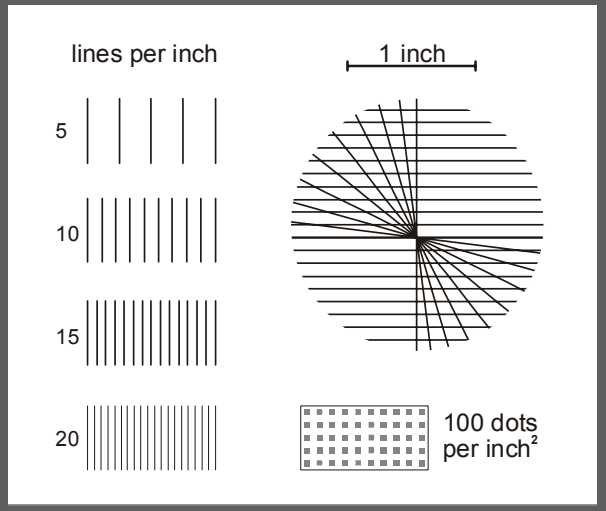
Imaging of Biological Activity



Spatial Resolution

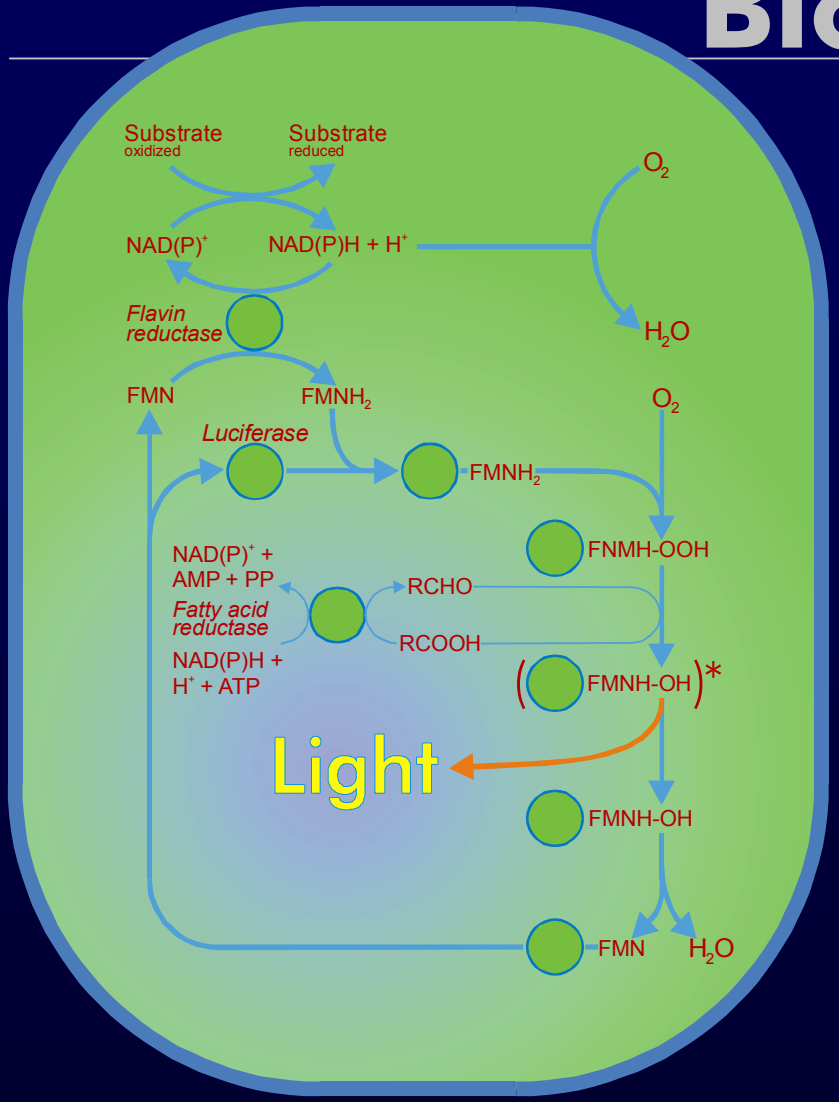


bioactivity image
from inkjet print



graphics for
resolution testing

Bioluminescence



coupling of bioluminescence to respiratory chain and ATP pool

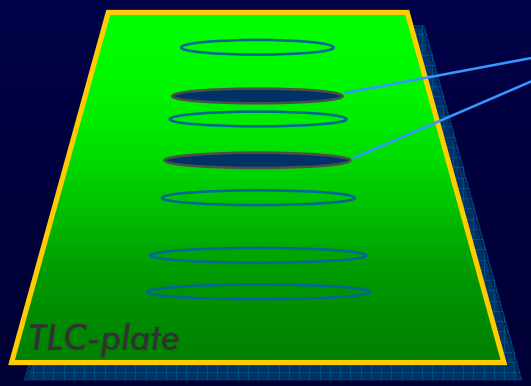
close link to the metabolic status of the cell

luminescent cells as biosensors

Principle of the BioTLC

- separate compound mixture by TLC
- evaporate solvent
- dip into bioluminescence kit
- evaluate by videoimaging

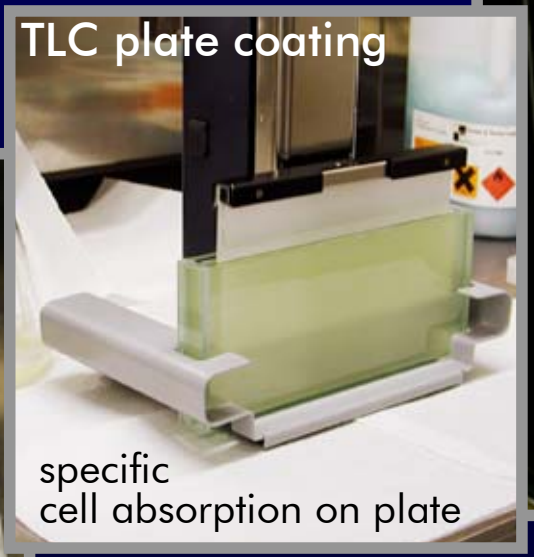
results:

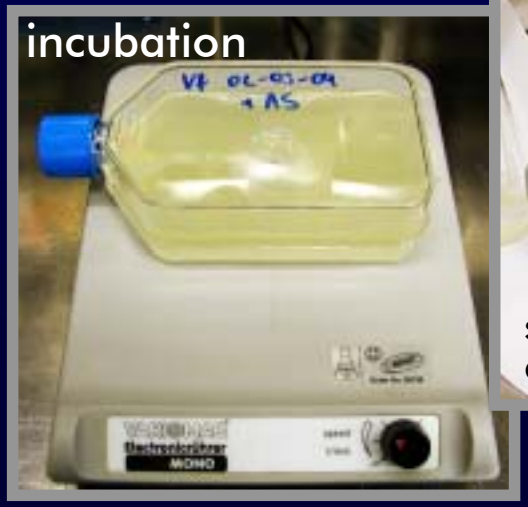


120 sec for detection

BioTLC - Process

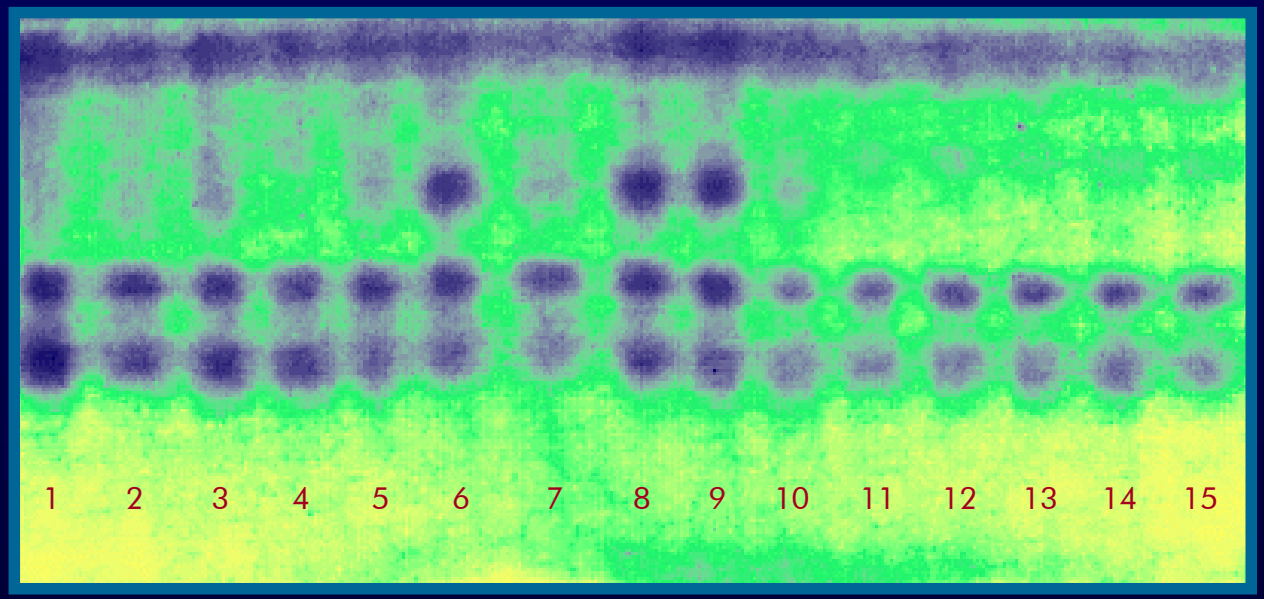
3 step procedure





Toxicity Profiling of Waste Water

TLC-Bioluminescence screening - detection: *Vibrio fischeri*

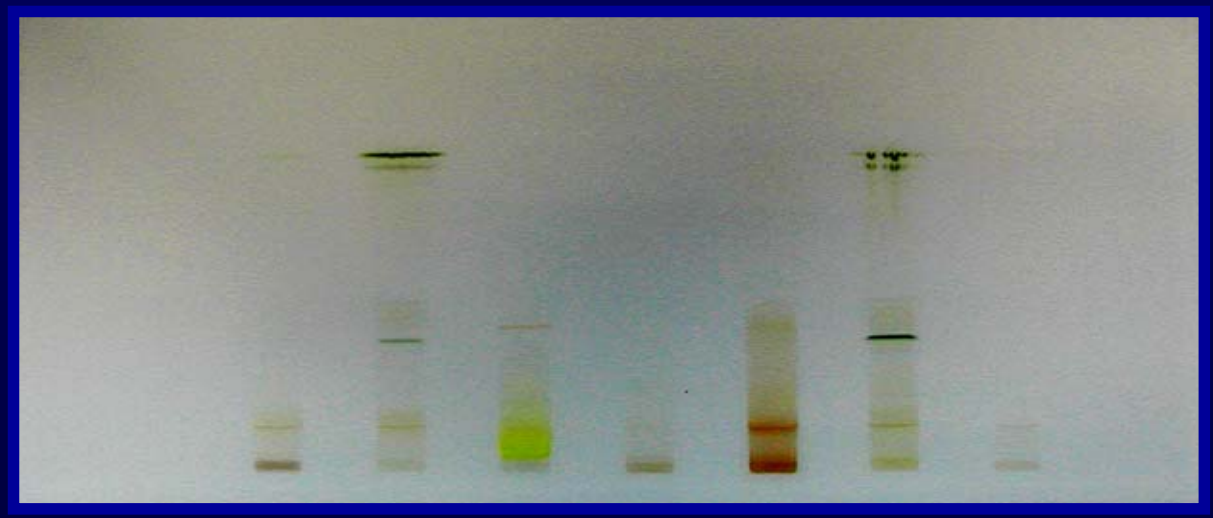


decreased bioluminescence indicates toxic substance zones

samples from 15 days in succession

Natural Products Extracts

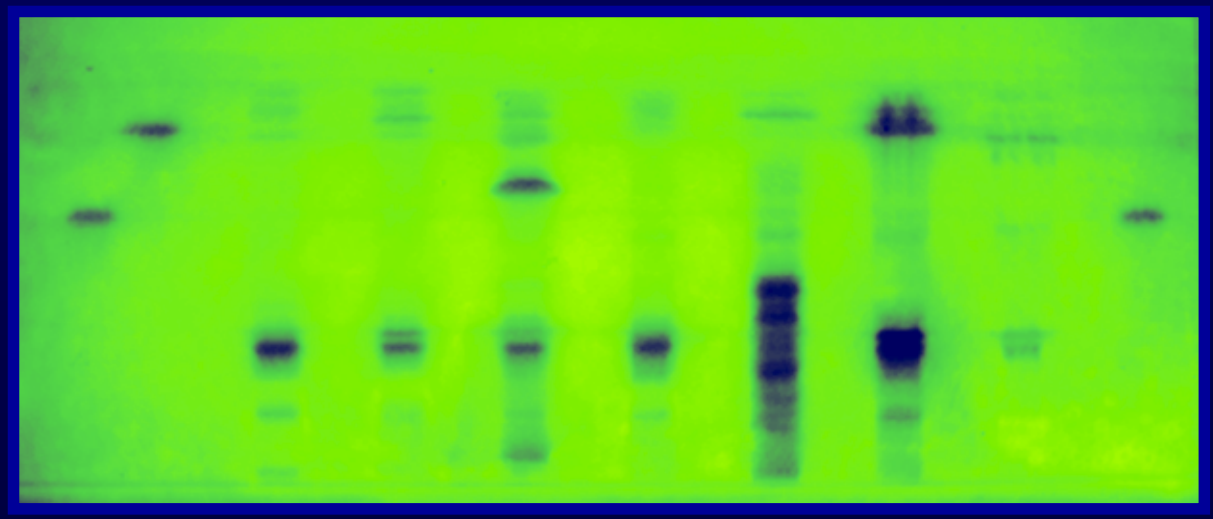
TLC-technique: AMD detection: visual



A - G : 100 µg natural products extracts
np : 200 ng 4-nitrophenol (tox-reference 1)
bp : 20 ng 4-tert.-butylphenol (tox-reference 2)

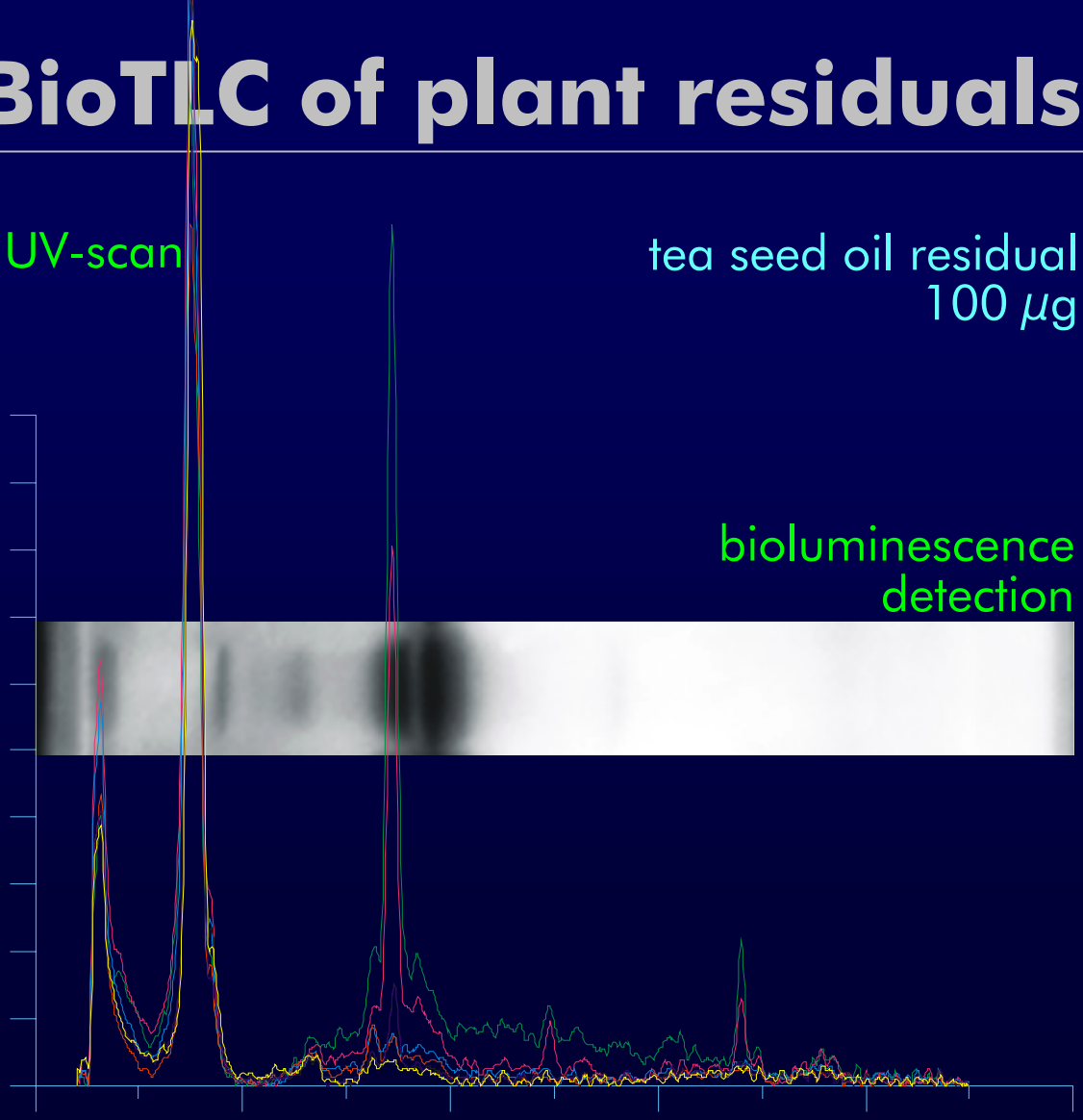
Natural Products Extracts

BIO-TLC detection: *Vibrio fischeri*



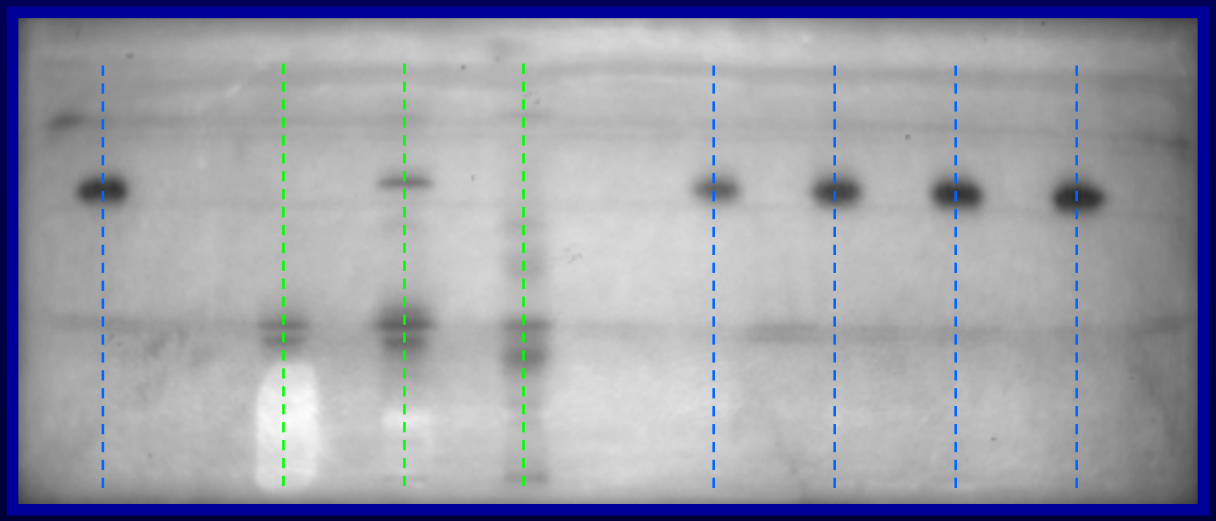
A - G : 100 µg natural products extracts
np : 200 ng 4-nitrophenol (tox-reference 1)
bp : 20 ng 4-tert.-butylphenol (tox-reference 2)

BioTLC of plant residuals

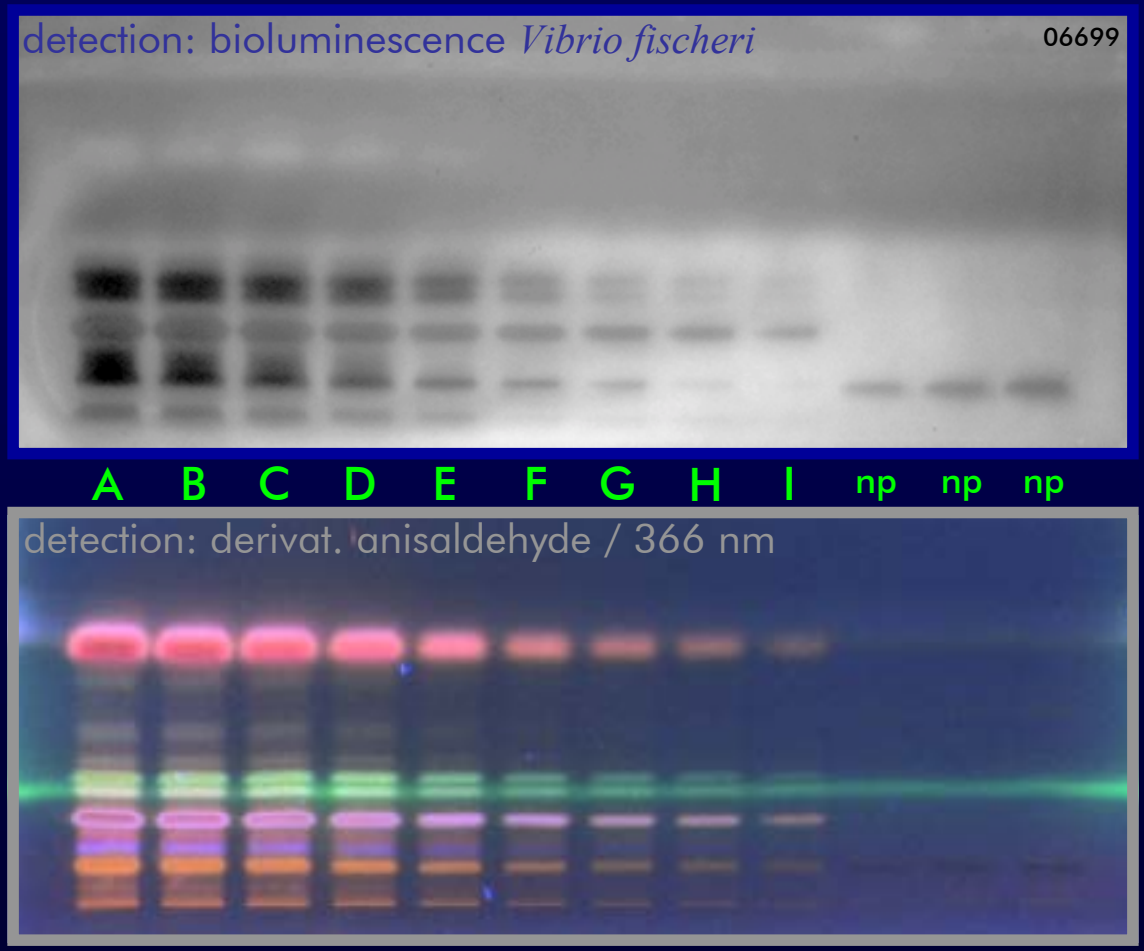


Quantitation of Toxicity

quantitation by toxicity equivalents
based on calibration with 4-nitrophenol (np)



BioTLC for USP-Methods

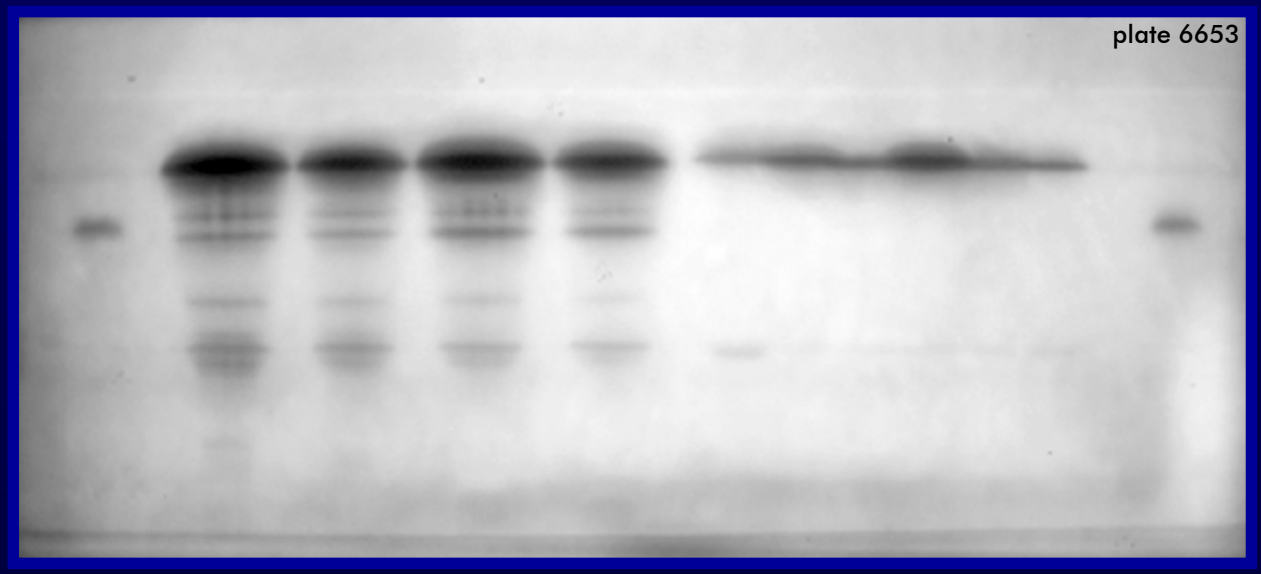


TLC by CAMAG

A - I : *Matricaria chamomilla* np : 4-nitrophenol

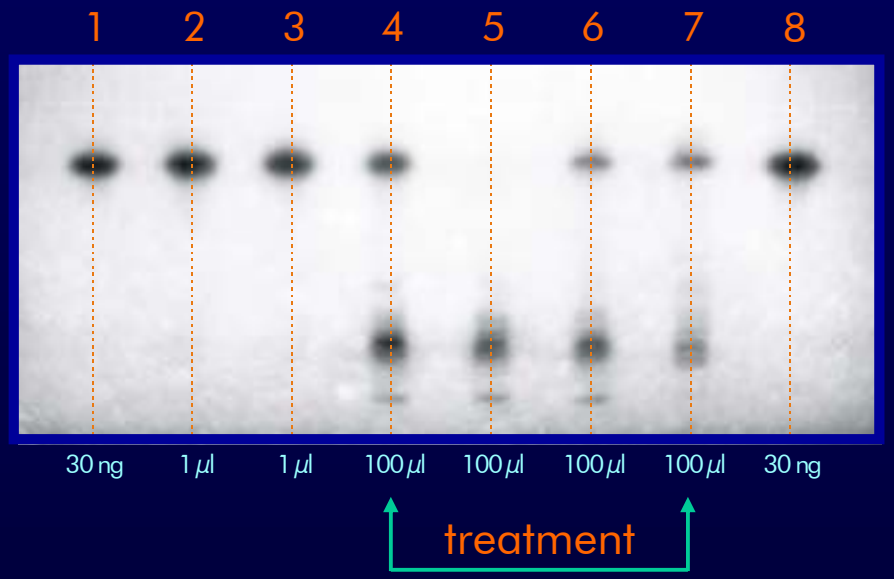
BioTLC of Kava Kava

detection: *Vibrio fischeri*



A, B : Kava Kava sample 1
C, D: Kava Kava sample 2
1 - 6: references; np = 4-nitrophenol

Toxicity in Process Water



TLC-Bioluminescence - detection: *Vibrio fischeri*

Current state of BioTLC

- mature technique (*Vibrio fischeri*)
- sensor cells in research applications
- very fast bioassay (*Vibrio fischeri*)
- excellent fit with AMD technique
- high compatibility to standard TLC
- TLC kit available (BioLuminex / ChromaDex)
- detection equipment available (CAMAG)

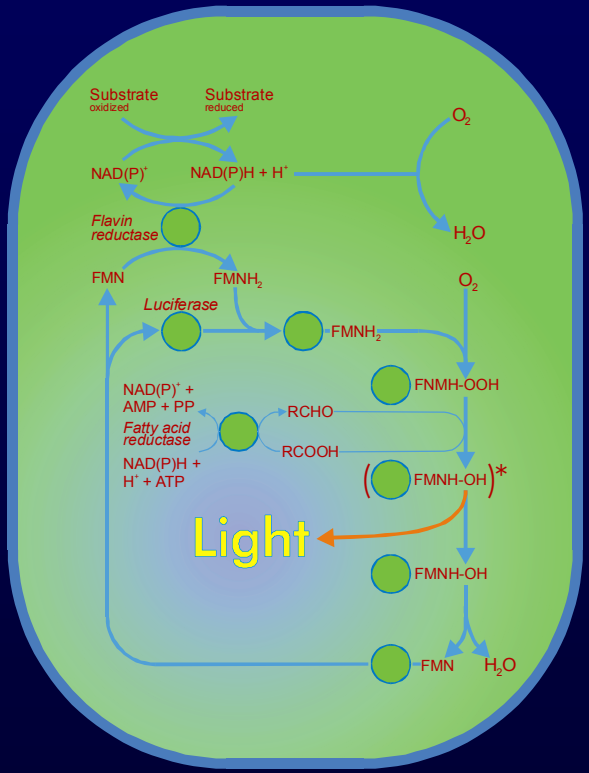
Bioluminescence: TLC or HPLC ?

pro TLC

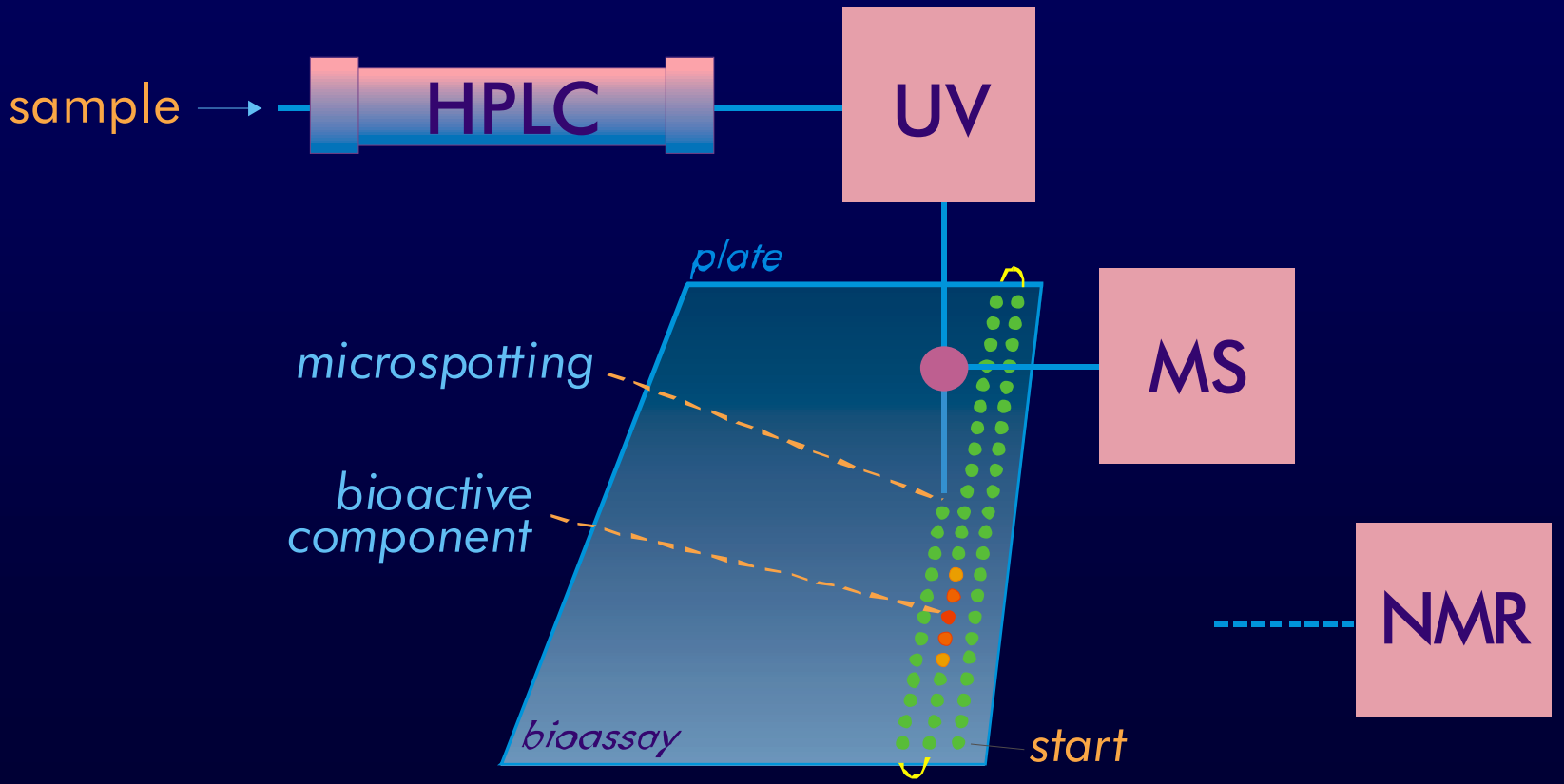
- single use separation system
- simultaneous parallel detection
- high productivity technology

pro HPLC

- efficient correlation with MS data
- quantitation
- mainstream technology



HPLC-Bioluminescence-coupling



Benefits of BioTLC

- **rapid toxicity profiling**
  successful assignment of toxicity in compound mixtures

- **versatile application**
  waste water analysis, food industries, regulatory affairs

- **relevant results**
  BioTLC employs accepted bioluminescence principles

- **advanced technology**
  high productivity technique with easy set up

Options for the BioTLC

- detection of specific bioactivity (specific cell types)
- luminescent cells e.g. yeast, insects, algae, ...
- multiplex detection (novel sensor cells or mixtures)
- regulation of bioluminescence (e.g. autoinducer)
- time dependent detection (fast/slow activity)
- standardization (kits, methods, camera systems)

Future developments

TLC-detection by reporter gene systems

1: bioactivity reference; 2 and 3: natural products extracts

Acknowledgements

Microbiology

Eberz

Bioluminescence Detection

Weisemann

AMD-TLC

Kinast

Verbitsky

CAMAG

Reich