Chemiluminescence in Thin-Layer Chromatography for the Quantification of Polyaromatic Hydrocarbons

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Objectives:

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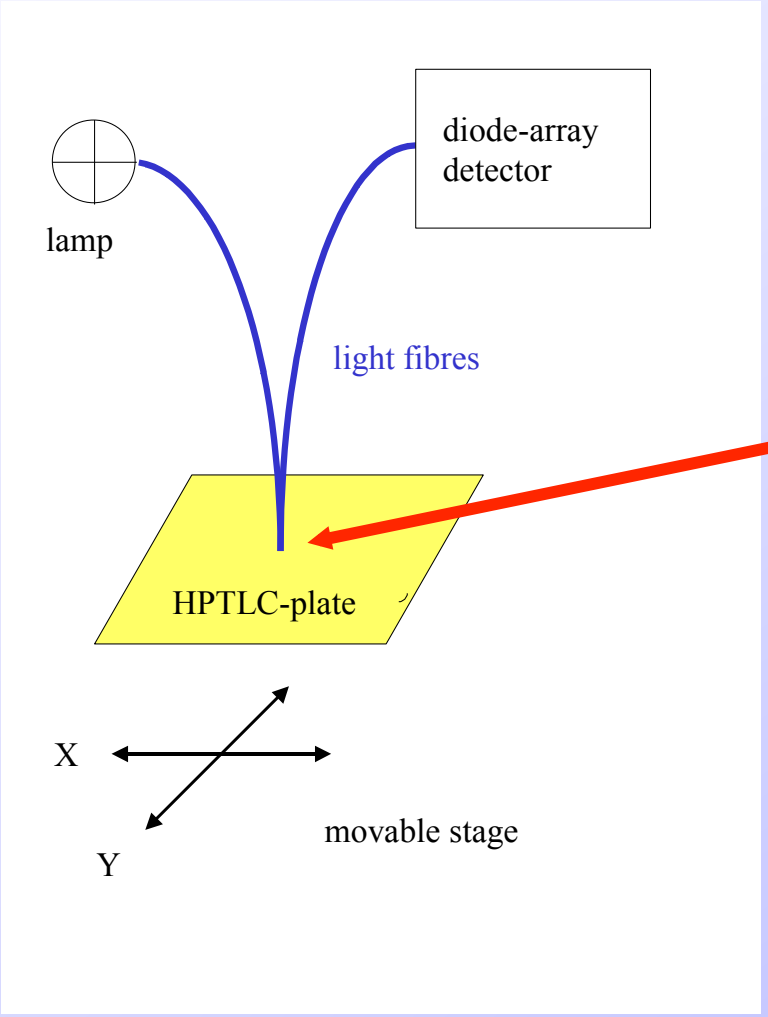
1. TLC separation of 16 PAHs (EPA-standard)

Acenaphthene 1000 ng/µl
Acenaphthylene 2000 ng/µl
Anthracene 100 ng/µl
Benzo(a)anthracene 100 ng/µl
Benzo(a)pyrene 100 ng/µl
Benzo(b)fl uoranthene 200 ng/µl
Benzo(g,h,i)perylene 200 ng/µl
Benzo(k)fluoranthene 100 ng/µl
Chrysene 100 ng/µl
Dibenzo(a,h)anthracene 200 ng/µl
Fluoranthene 200 ng/µl
Fluorene 200 ng/µl
Indeno(1,2,3-cd)pyrene 100 ng/µl
Naphthalene 1000 ng/µl
Phenanthrene 100 ng/µl
Pyrene 100 ng/µl

2. Quantification of PAHs by chemiluminescence

Principle of a modern diode-array scanner



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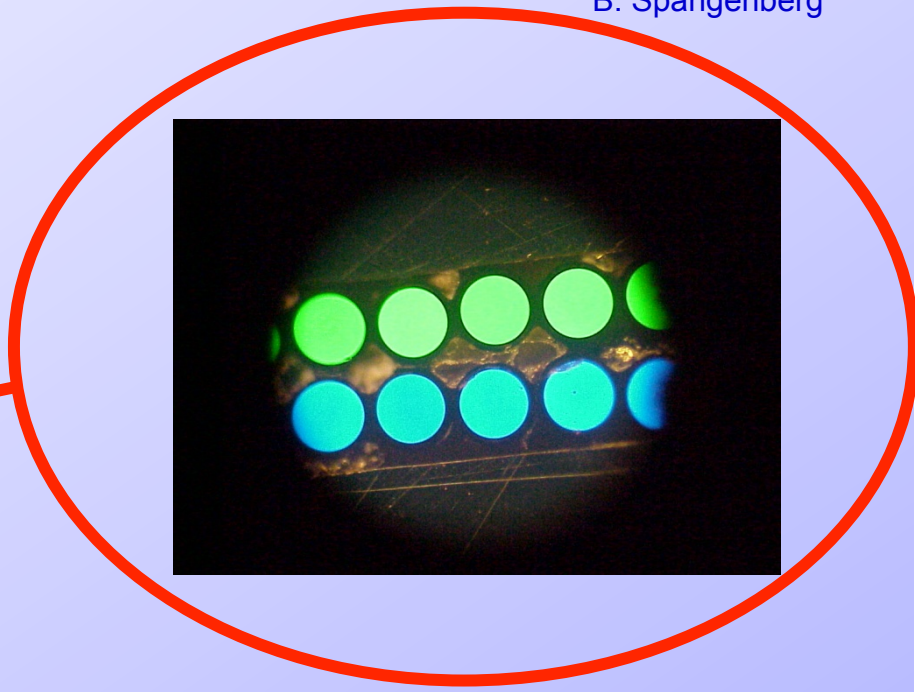

S. Bayerbach, G. Gauglitz,

Z. Anal. Chem. **335** (1989), 370-374

B. Spangenberg, K.-F. Klein J. Chromatogr., **898** (2000), 265-269

A modern diode-array scanner for TLC-plates

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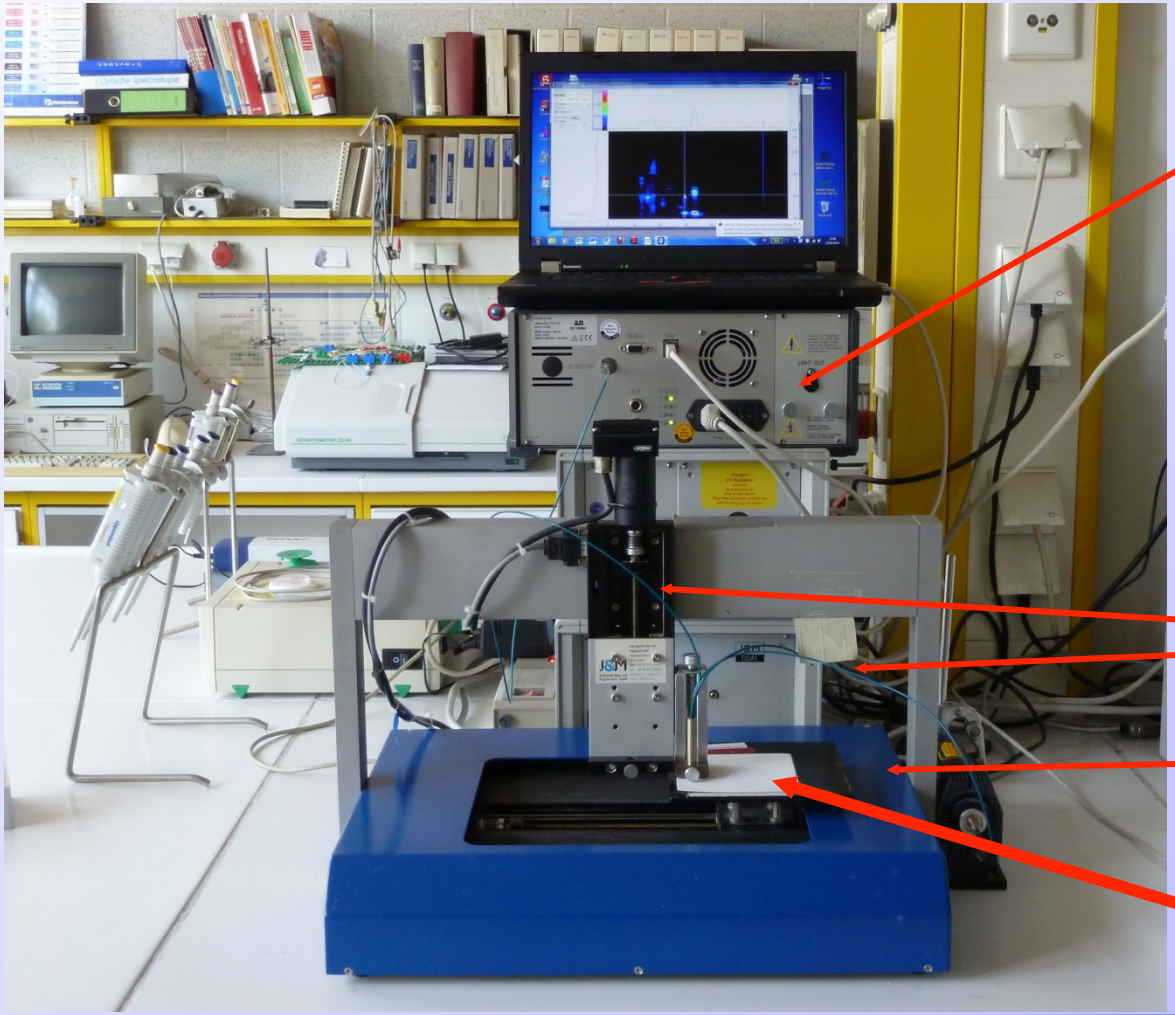

Diode-array detector (DAD)

Range: 190 – 1000 nm

Optical resolution: <1 nm

Spatial resolution: < 100 µm

Light fibers

X/Y-stage

TLC-plate

Kubelka-Munk theory for densitometric evaluations

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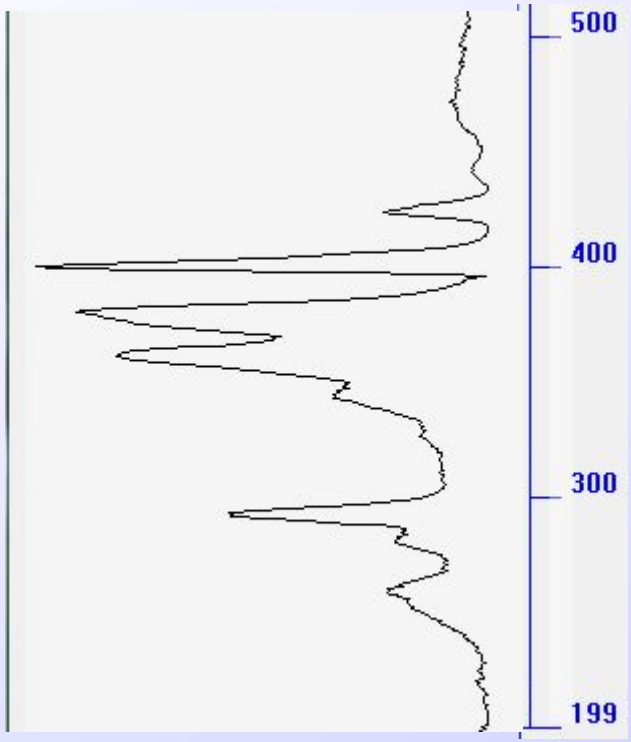

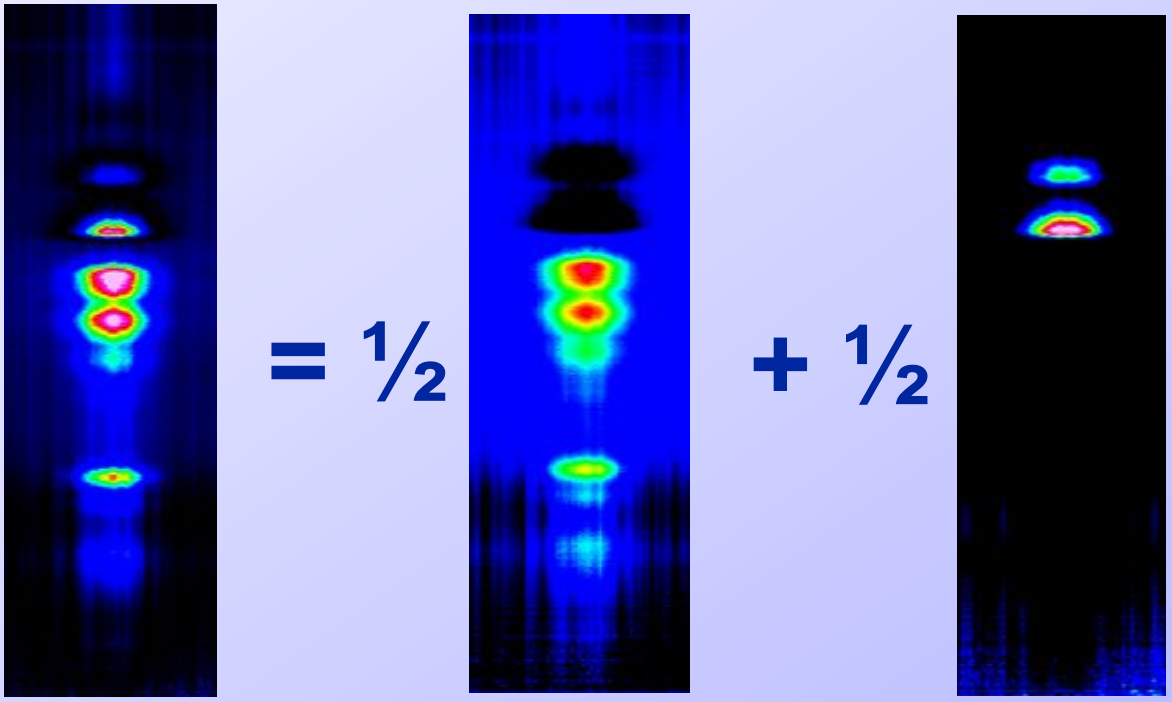

= ½ + ½

$$R(\lambda) = \frac{J_{sample}(\lambda)}{J_{reference}(\lambda)} = \frac{J(\lambda)}{J_0(\lambda)} \quad \frac{(1-R(\lambda))^2}{2R(\lambda)} = \frac{1}{2}\left[\frac{1}{R(\lambda)} - 1\right] + \frac{1}{2}\left[R(\lambda) - 1\right]$$

B. Spangenberg, C. Poole, Ch. Weins, Springer 2011

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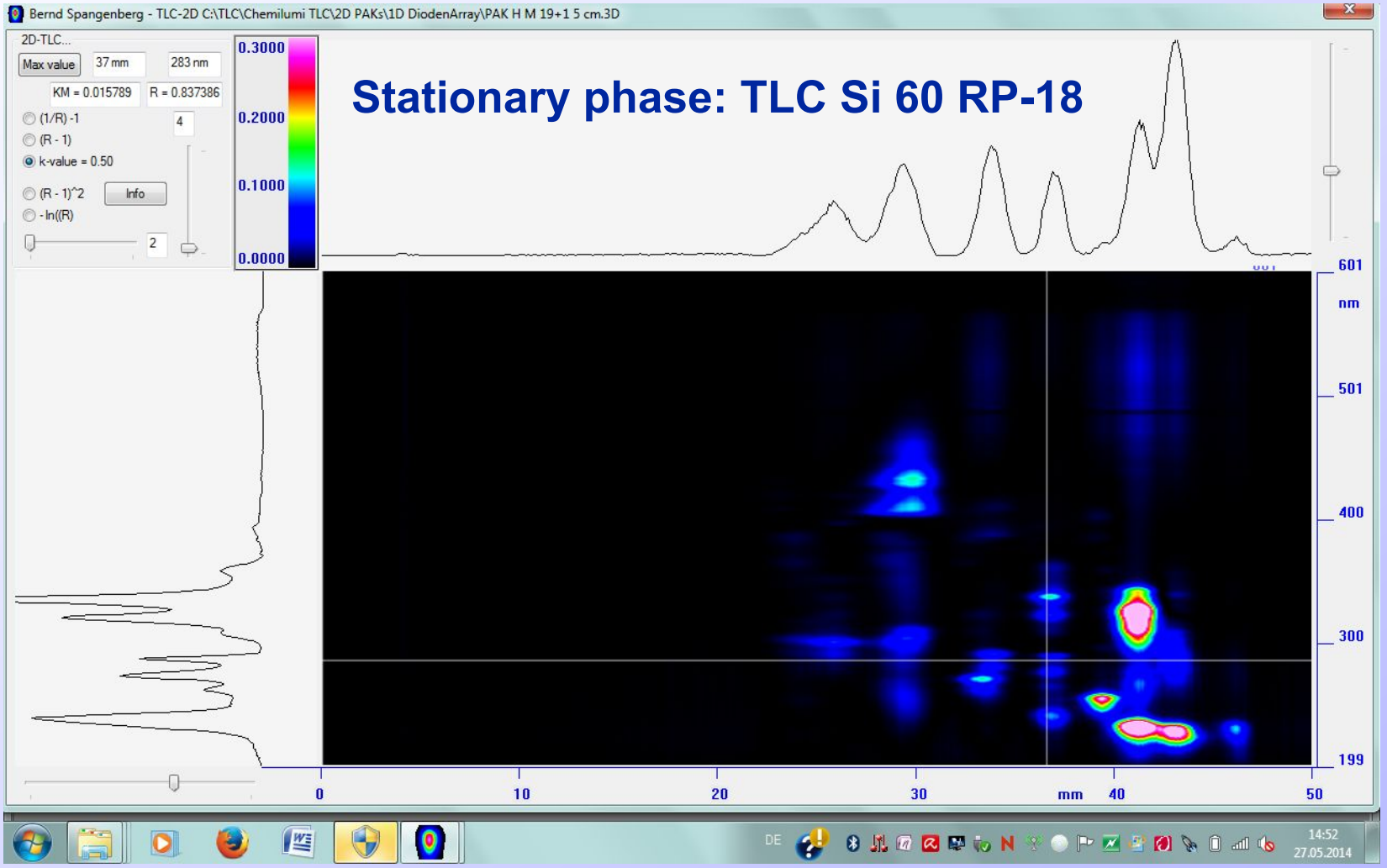
Separation in the first direction



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N-pentane, methyl-tert. butyl ether (19+1, v/v) at -20°C,

Separation distance:

5 cm,

Separation time:

5 minutes

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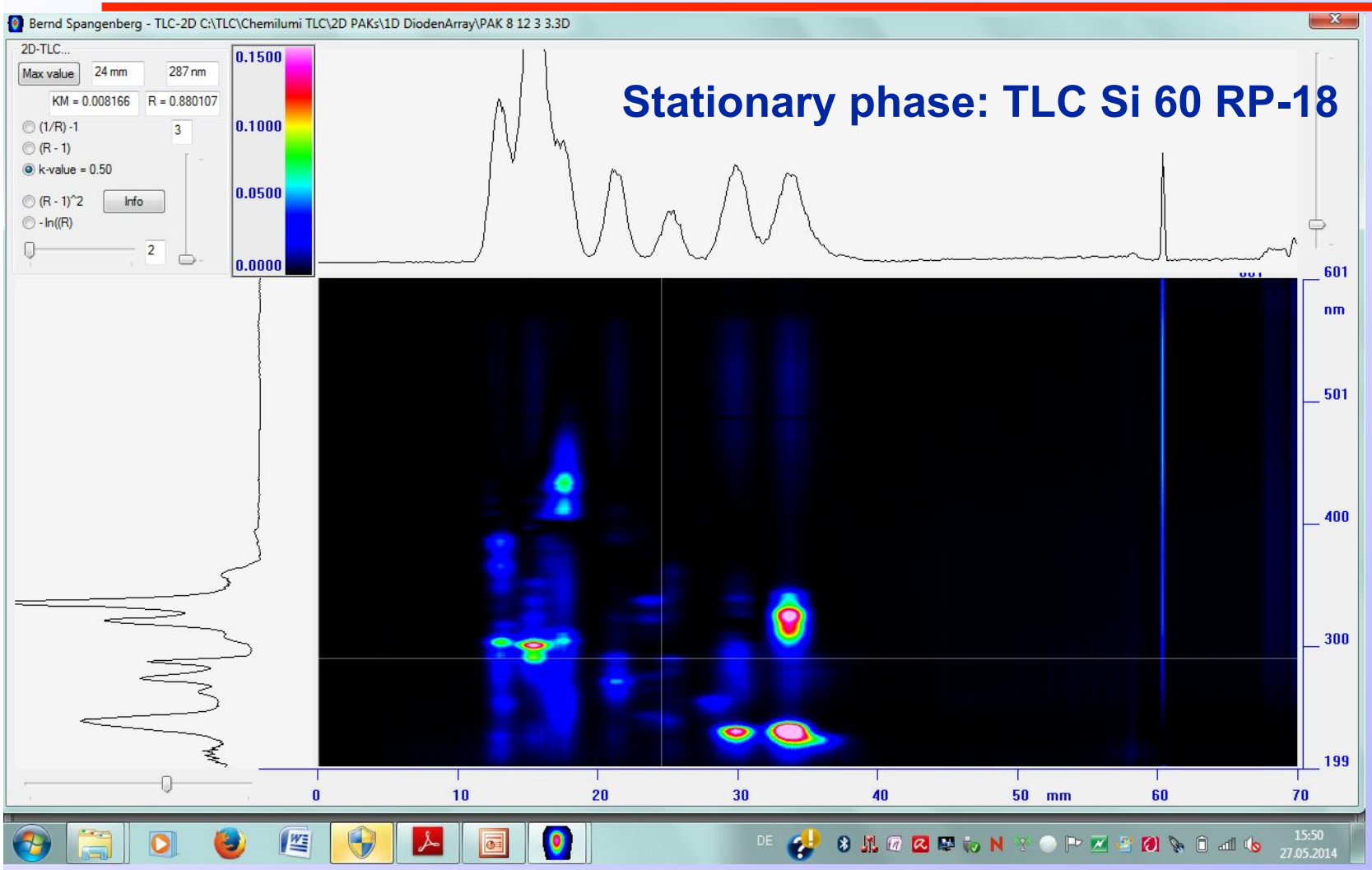
Separation in the second direction



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Methanol, CH_3CN, acetone, water (8+12+3+3, v/v)

Separation distance:

5-7cm

Separation time:

6,5 minutes

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2-D separation on RP-18 plate (5 x 5 cm)

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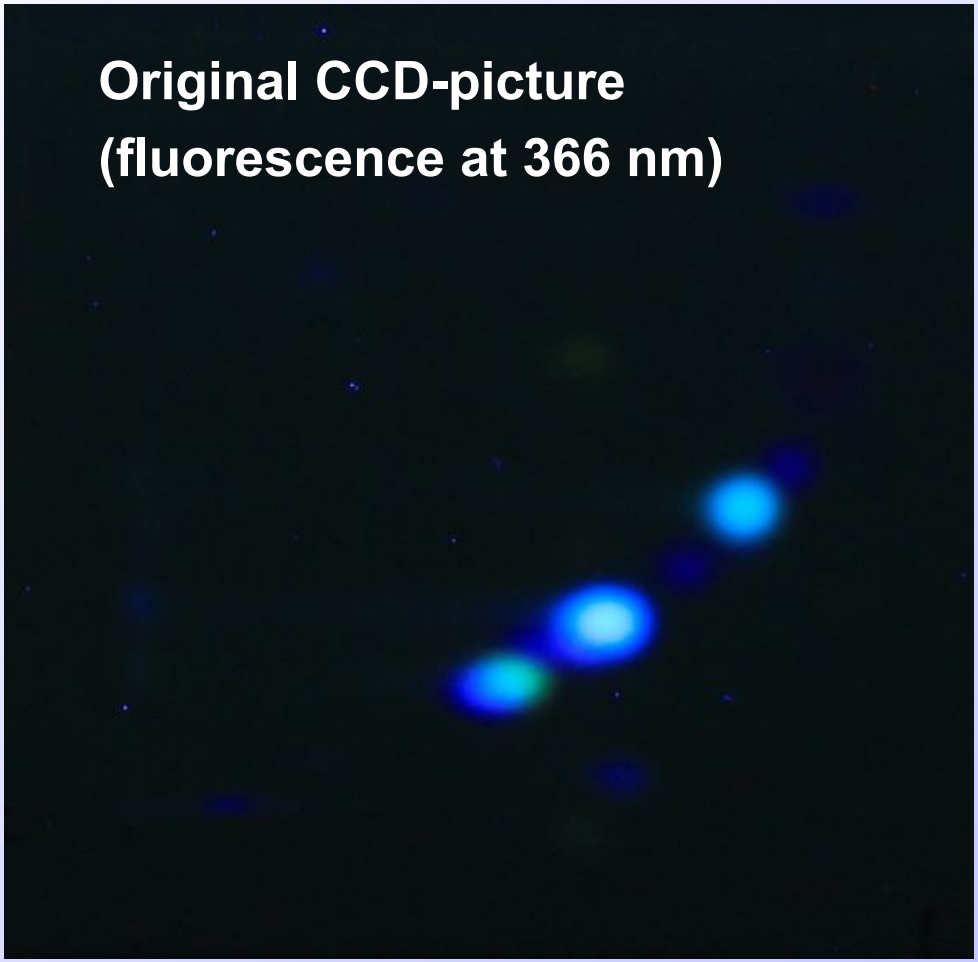
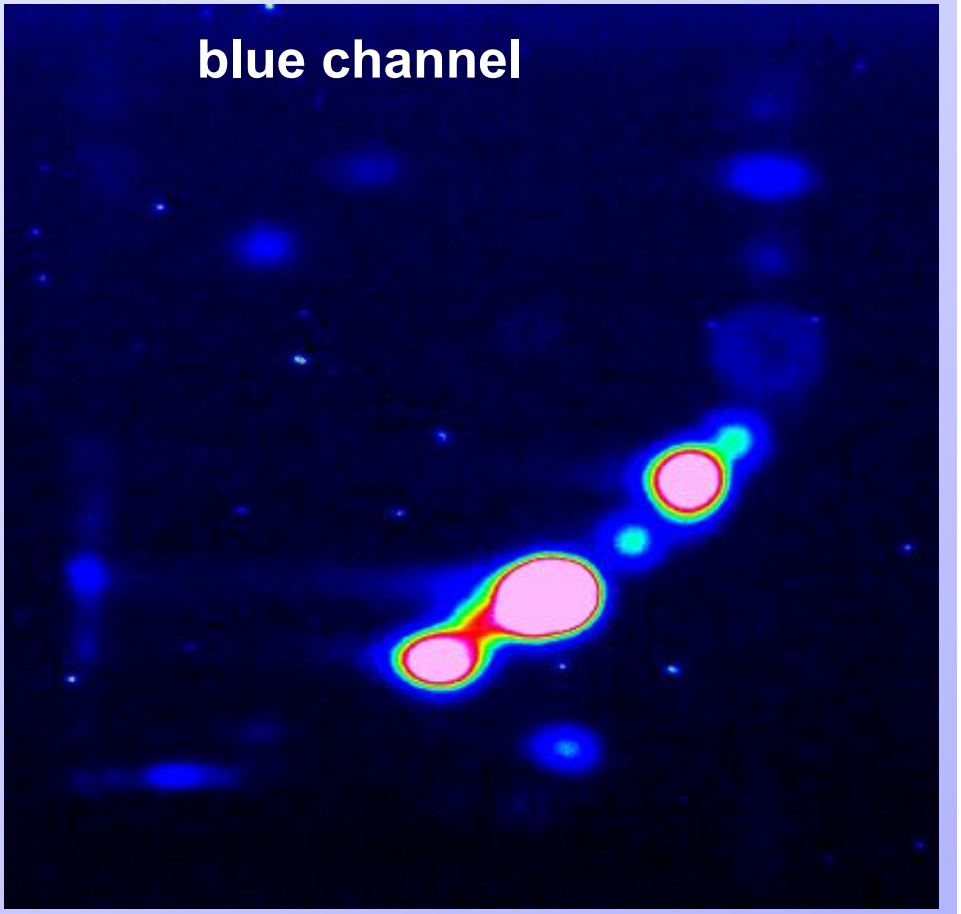
2. D.





1. Dimension

Reference about chemiluminescence in TLC

Published are six papers only:

[1] T. G. Curtis, W. R. Seitz, *Chemiluminescence: A new method for detecting fluorescent compounds separated by thin-layer chromatography*, J. Chromatogr. 134 (1977), 343 – 350

[2] T. G. Curtis, W. R. Seitz, *Coupling of chemiluminescence thin-layer chromatographic detection to a vidicon rapid scanning detector*, J. Chromatogr. 134 (1977), 513 – 516

[3] N. Wu, C.W. Huie, *Synchronization of timing in chemiluminescence thin-layer chromatographic system by coupling pneumatic nebulisation with optical fiber-based detection*, Anal. Chem. 64 (1992) 2465 - 2468.

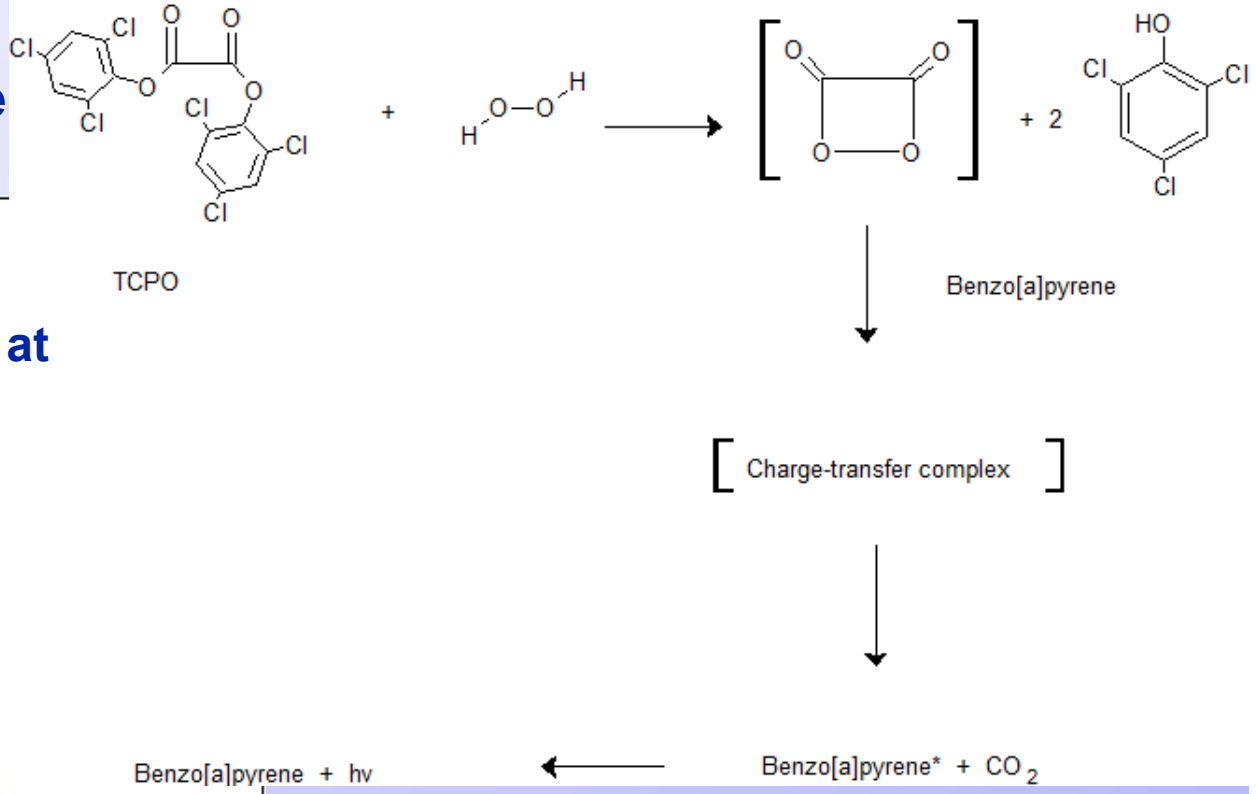
[4] N. Wu, C.W. Huie, *Factors influencing peroxyoxalate chemiluminescence detection in thin-layer chromatography* J. Planar Chromatogr. 7 (1994), 88 - 94

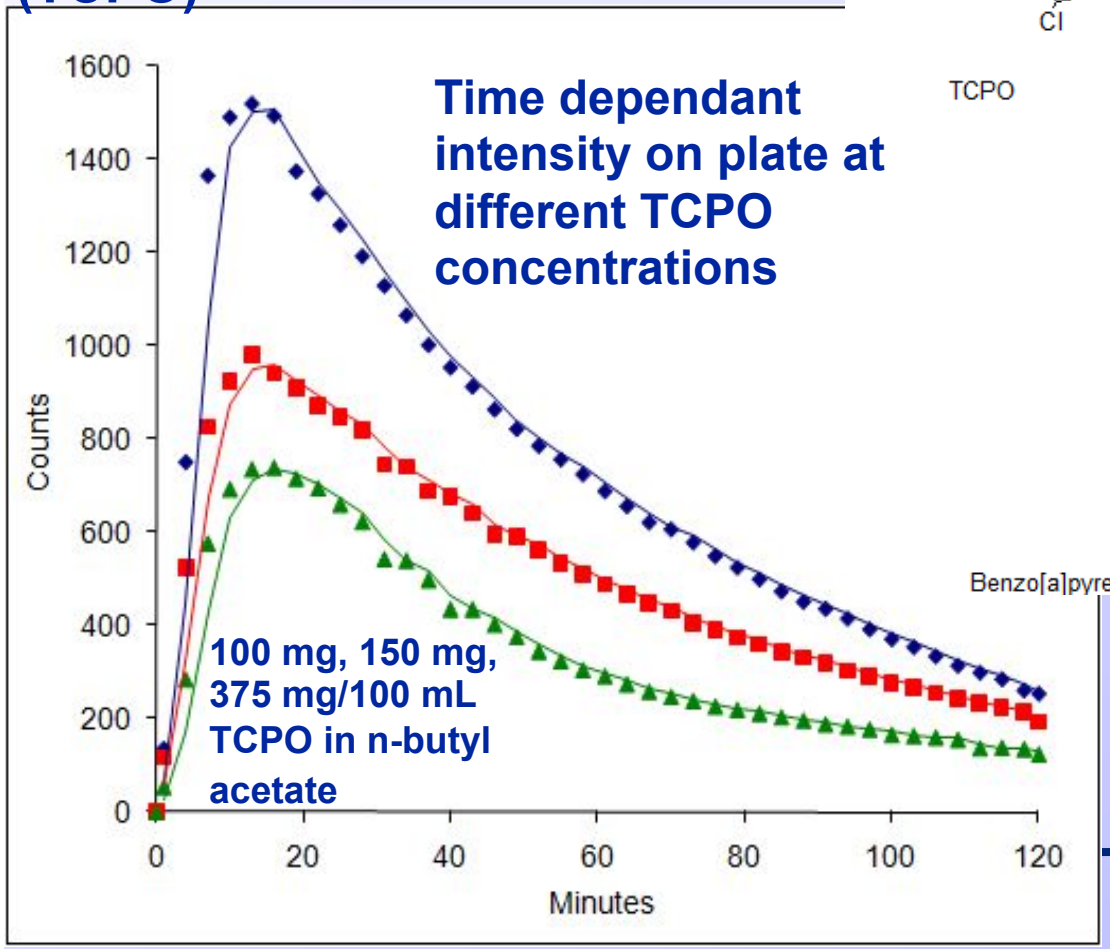
[5] A. N. Díaz, F. G. Sánchez, J. A. Gonzáles García, *Thin-layer chromatography with chemiluminescent detection of enhancers of the luminol-H_2O_2-peroxidase system*, J. Chromatogr. A 725 (1996), 411 - 415

[6] Bernd Spangenberg, *A new way of using chemiluminescence in thin-layer chromatography,* J. Planar Chromatogr. 24 (2011), 357 – 359

Chemiluminescence using H_2O_2 und TCPO

Bis(2,4,6-trichlorophenyl)oxalate (TCPO)





Bernd Spangenberg, „ A new way of using chemiluminescence in thin-layer chromatography", Journal of Planar Chromatography **24** (2011), 357 – 359

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Chemiluminescence measurements

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A solution of 250 mg TCPO (Fluka, Buchs, Switzerland) in 40 mL n-butyl acetate (Merck) was prepared. The amount of 500 µL H_2O_2 (35%, Fluka) was vigorously shaken with this solution for 20 min. This mixture is suitable for chemiluminescence measurements within a period of 6 hours. Best results are observed when the plate is dipped for one second in this solution. The wet plate is dried until no light reflection can be seen on the surface. Then the TLC plate is covered by a glass plate and measured for 1 minutes using a light sensitive CCD-camera.

Time dependant activity of the reaction mixture

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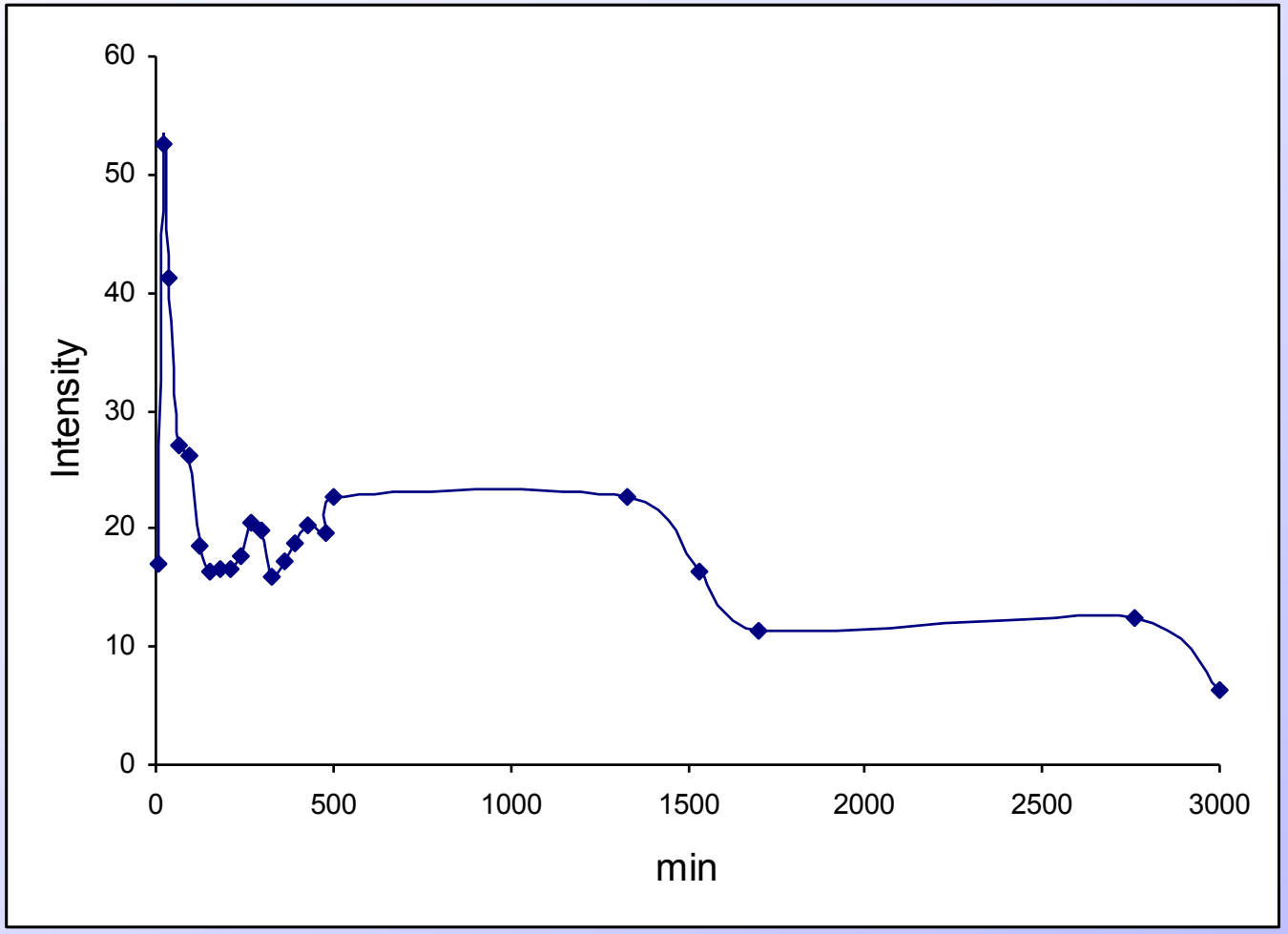

The reaction mixture is functioning for 50 hours (3000 min)!

Celvin® S

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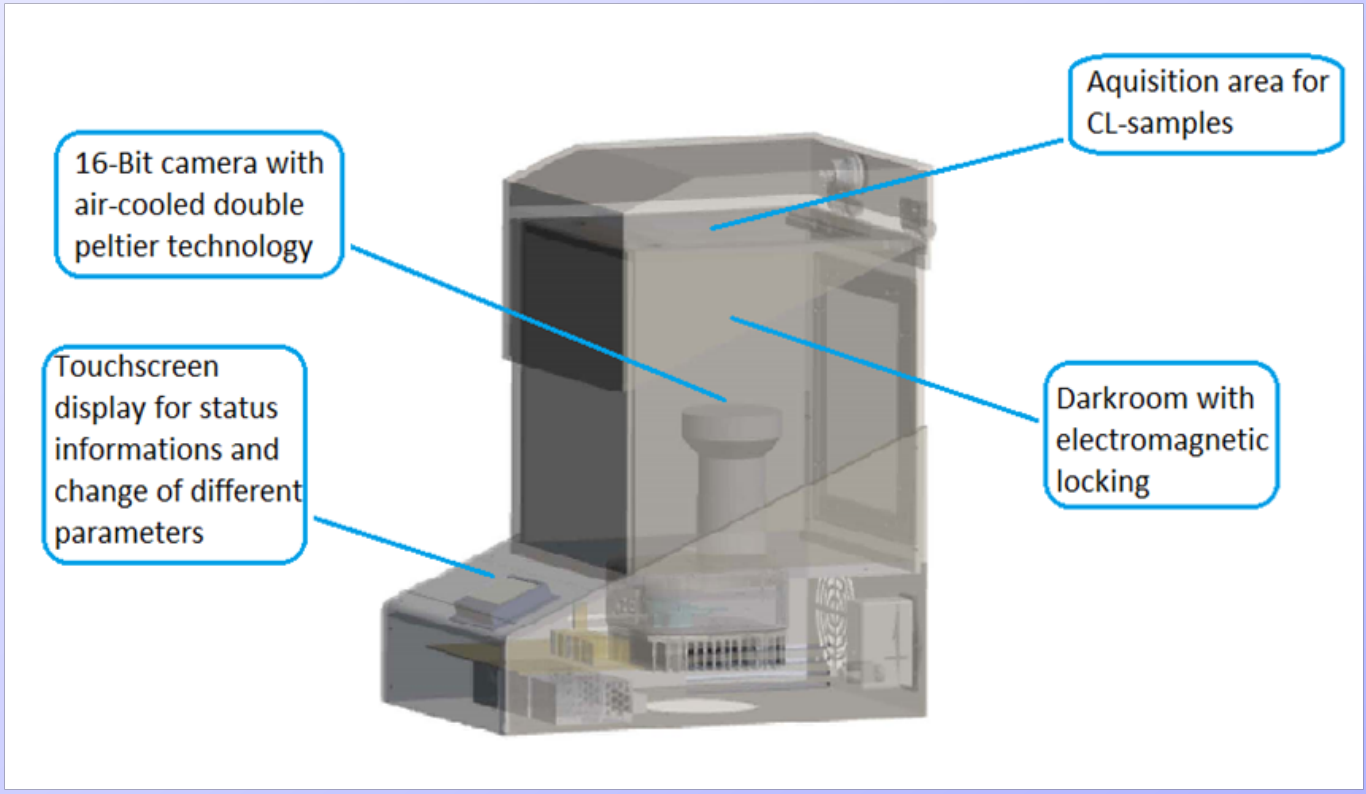
The light sensitive CCD-camera, used



2-D separation on RP-18 plate (5 x 5 cm)

2. D.

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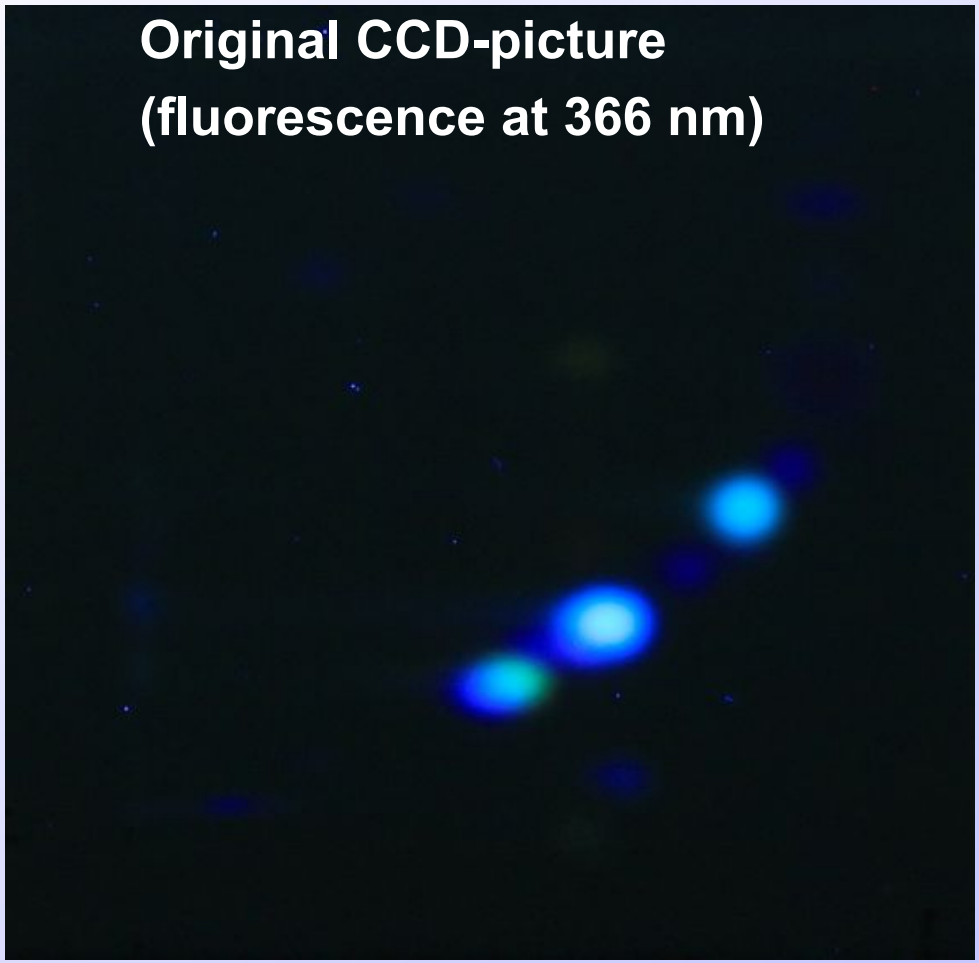

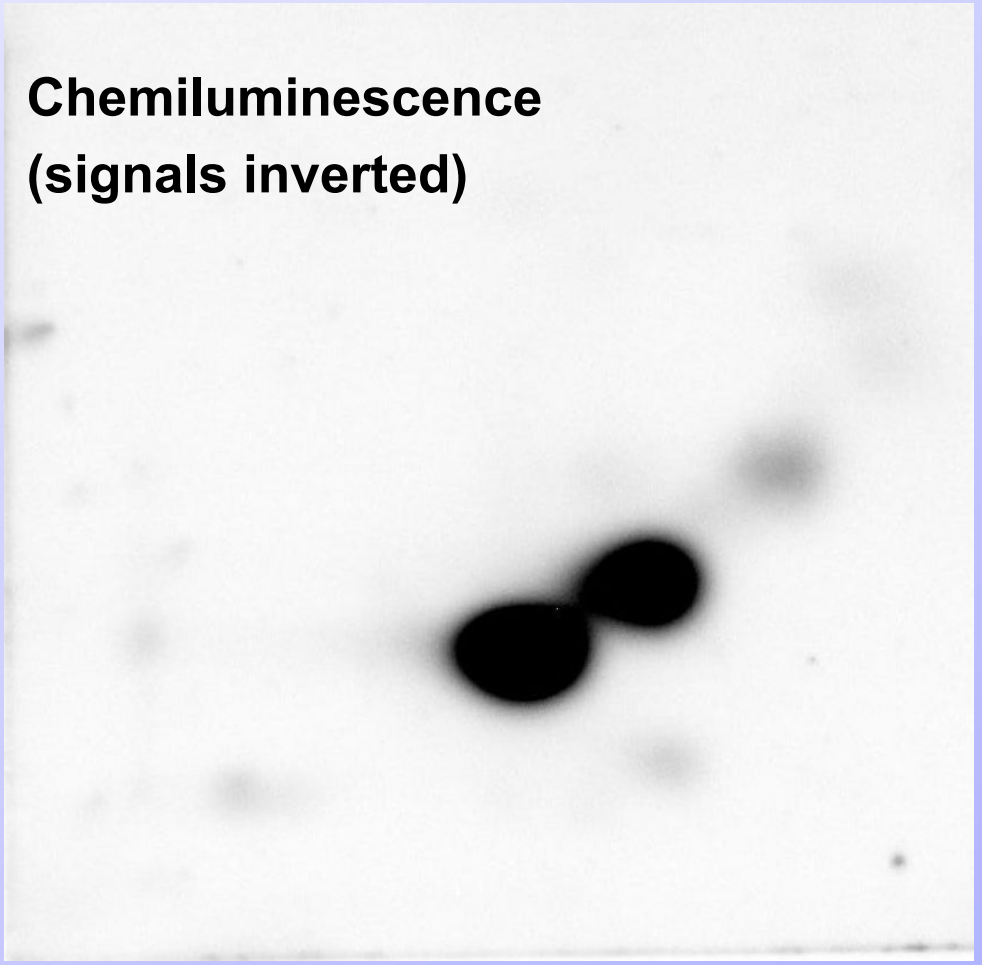

1. Dimension

Two PAHs show a strong chemiluminescence

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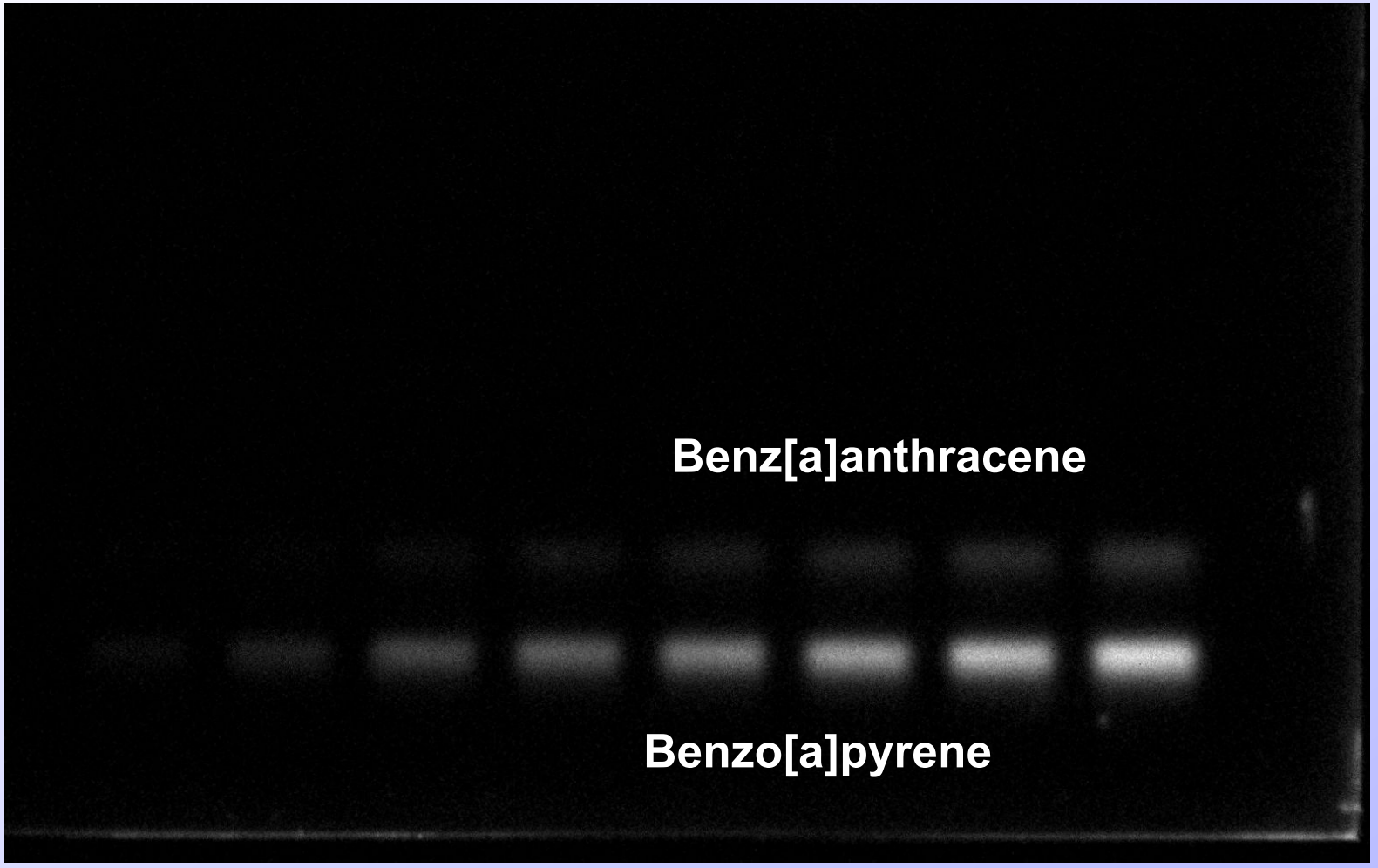
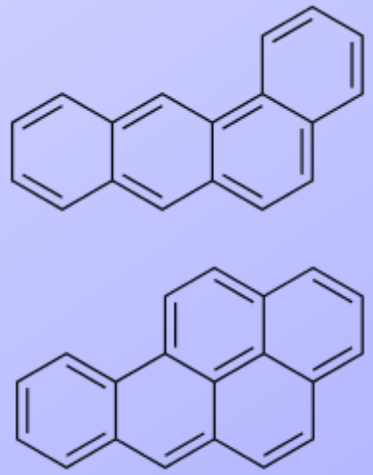
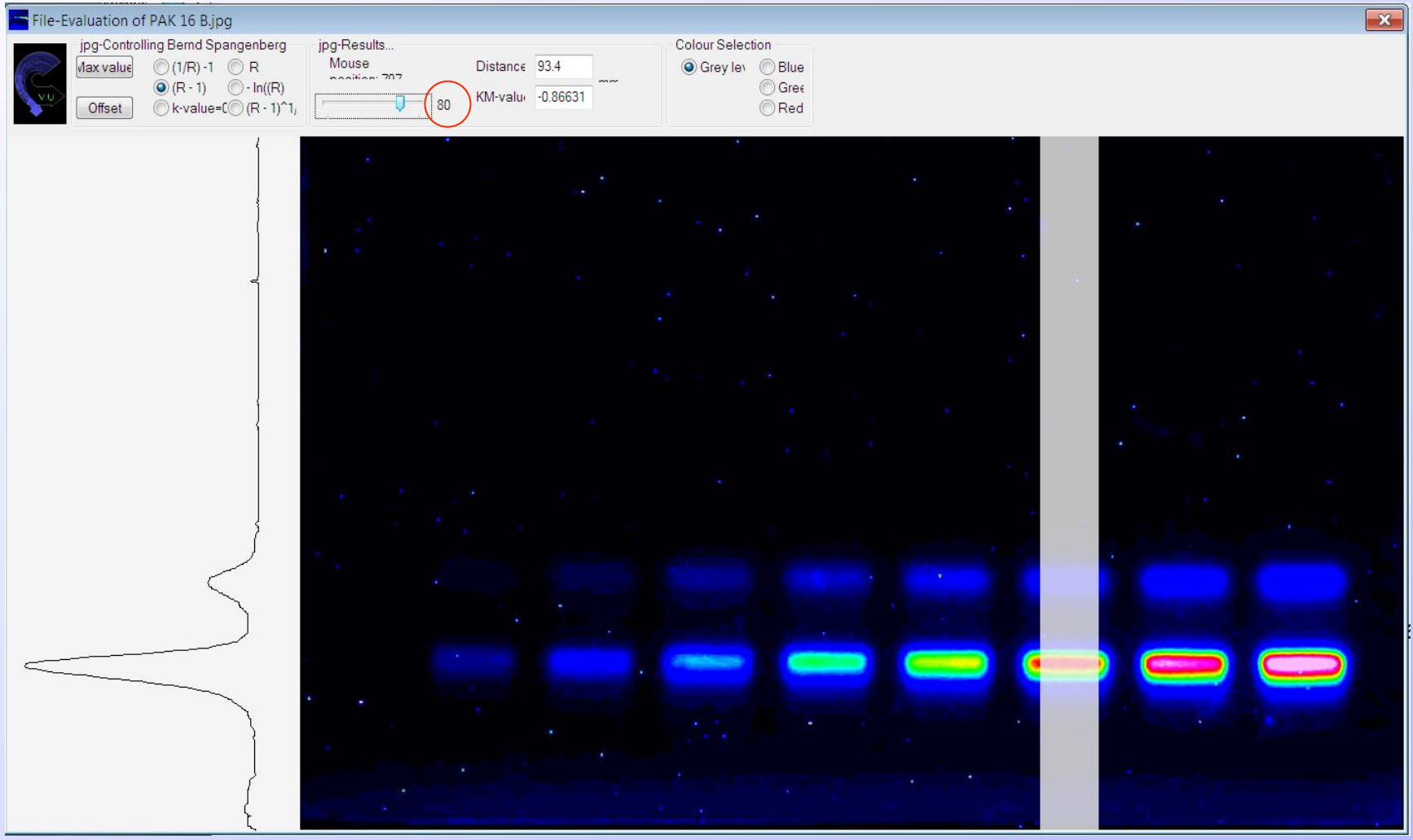




Evaluation of benzo[a]pyrene and benz[a]anthracene



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80 diodes were bundled and evaluated using the fluorescence expression (R-1)!

Evaluation of benzo[a]pyrene and benz[a]anthracene

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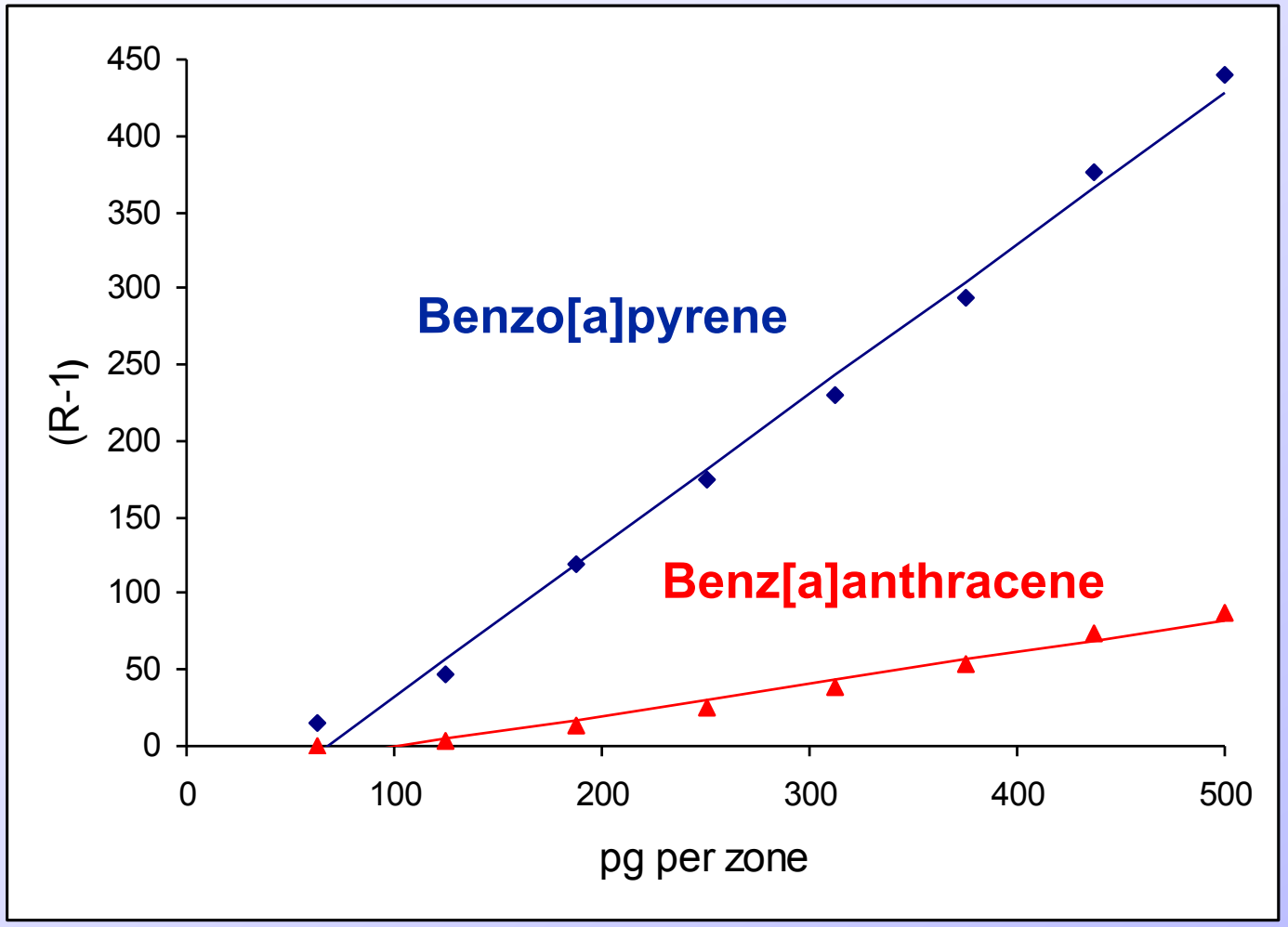

$LOD_{Benzo[a]pyrene}$ = 50 pg

$LOD_{Benz[a]anthracene}$ = 120 pg

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