Quantitative TLC with CCD detection

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Overview

- Why CCD detection
- Chromatography
- Imaging
- Signal referencing
- Image processing
- Results

Why CCD Detection?

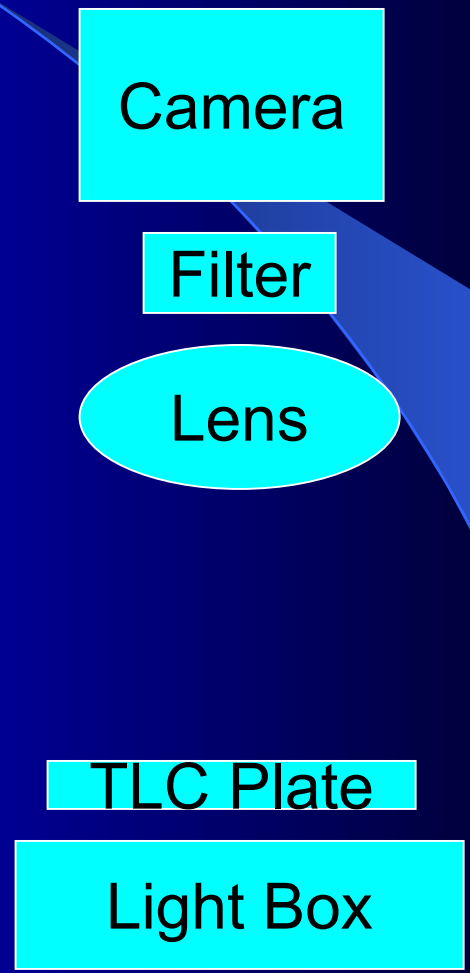
- Two dimensional array detector
- Evaluates whole plate in one exposure
- Fast

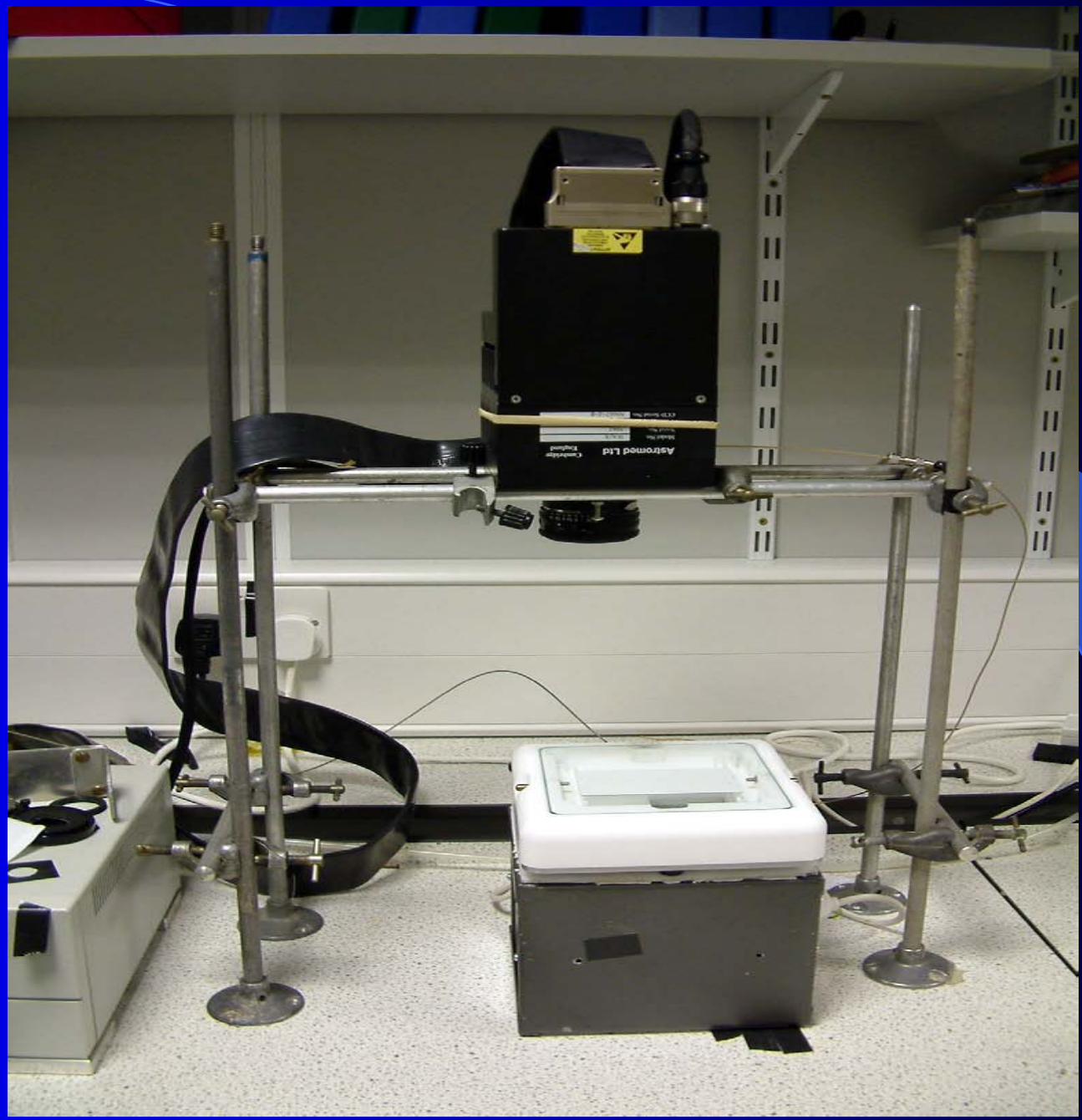
Chromatography

- Normal phase glass backed silica gel 60 F_{254} plates
- Sudan dyes dissolved in hexane
- Samples applied by glass capillary
- Sample zones focused with acetonitrile
- Horizontal development with dichloromethane for 5-6 cm

Imaging

- Astromed TE4/A CCD camera used
- Camera lens – 50 mm focal length
- Filter behind lens
- Laboratory lights used in reflectance
- 11W light box used in transmission

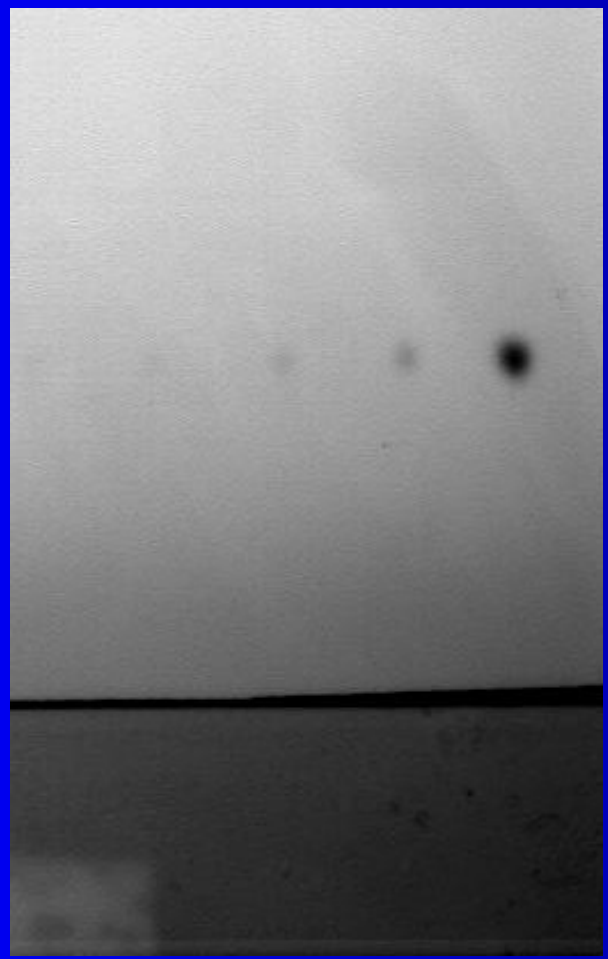



Astromed Ltd

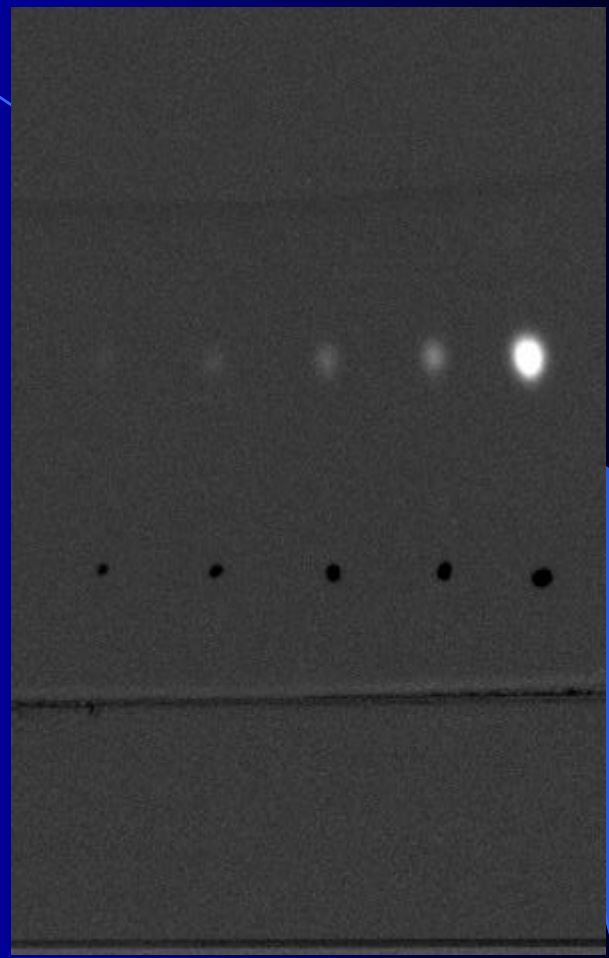
Signal Referencing

- The CCD output is a series of voltages corresponding to a greyscale for each pixel
- Two images taken – one after spotting and one after chromatography and drying
- The two sets of data are ratioed pixel by pixel
- Logarithm taken of each ratio

Signal Referencing



No referencing



With referencing

Signal Referencing

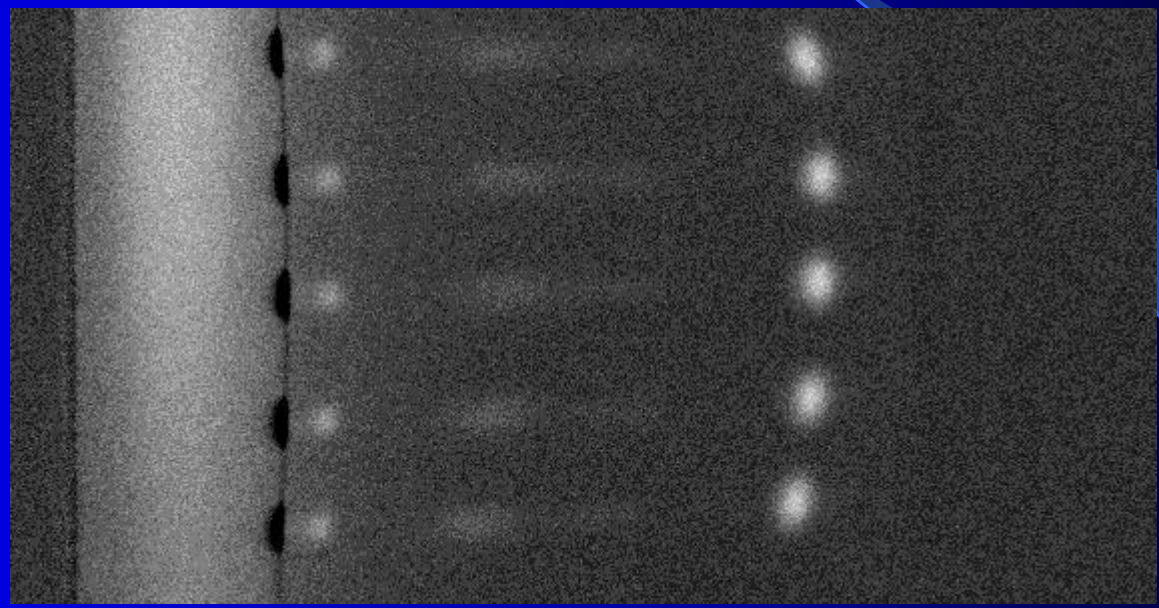
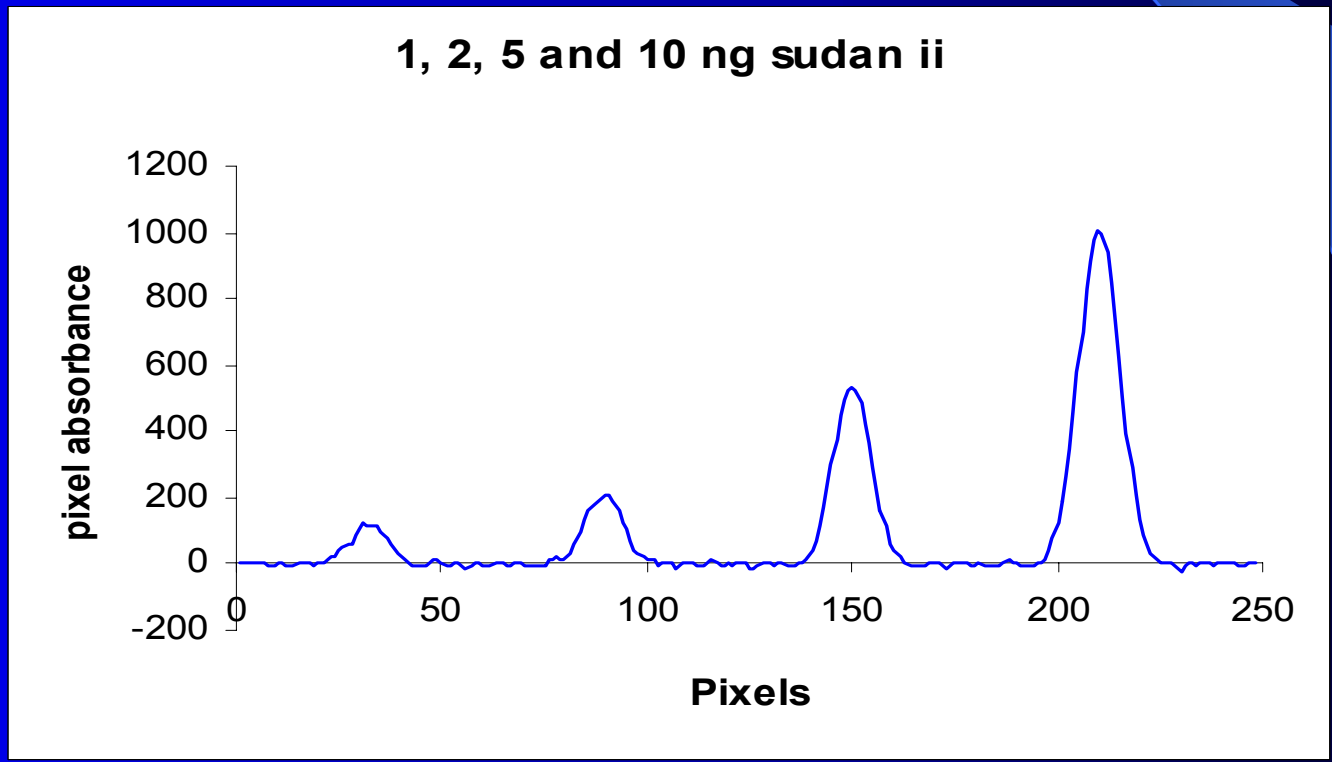


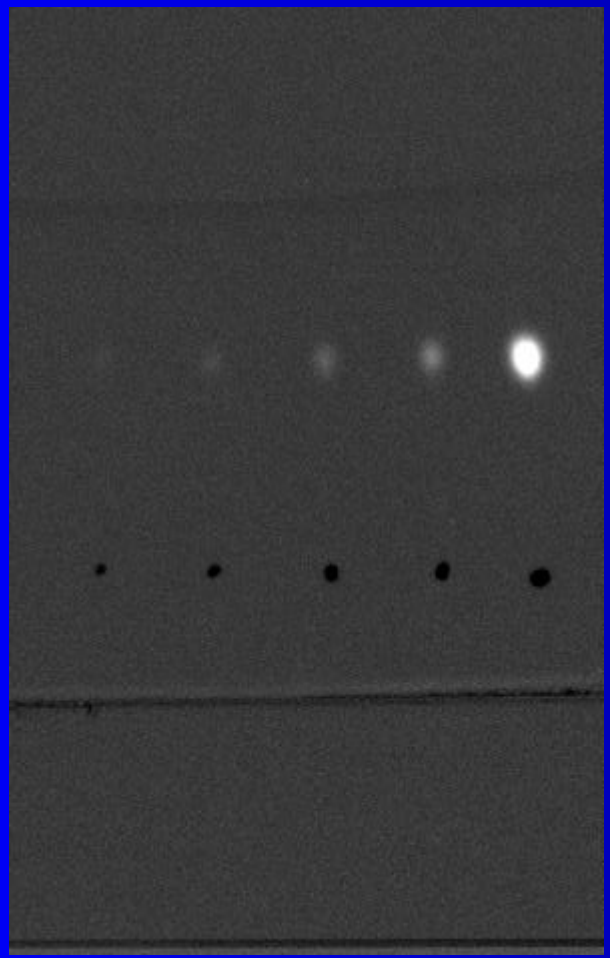
Image Processing

- Beer Lambert law $\log (I_o / I) = A = \varepsilon bc$
- Absorbance peak area proportional to amount in spot measured
- Linearity limited – model meant for non scattering medium

Image Processing

- Scion image beta 4.02 software used to integrate in one direction





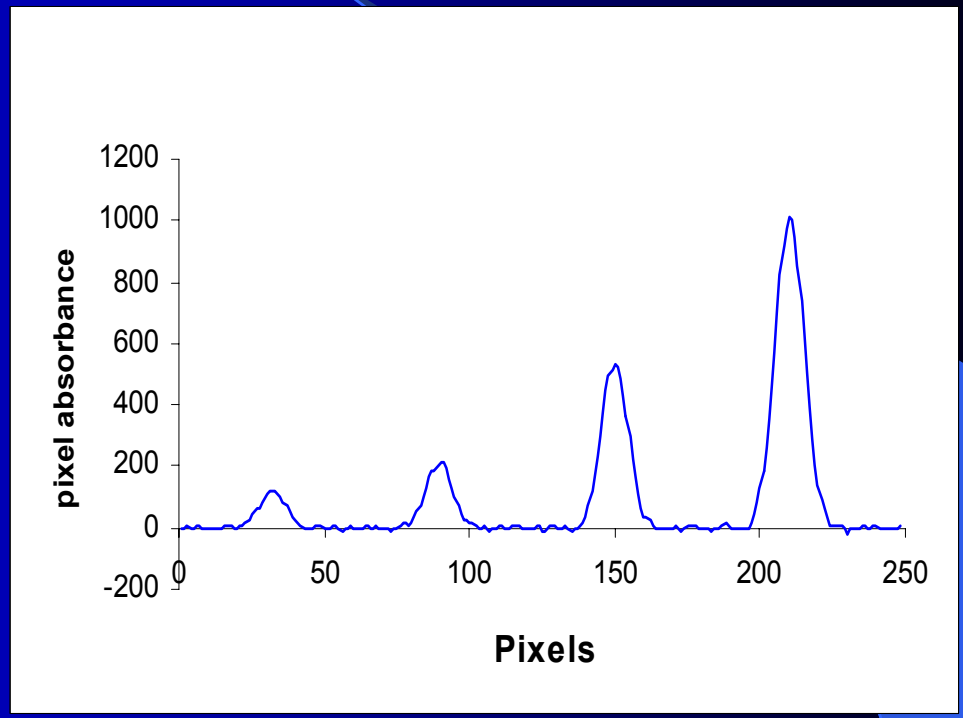
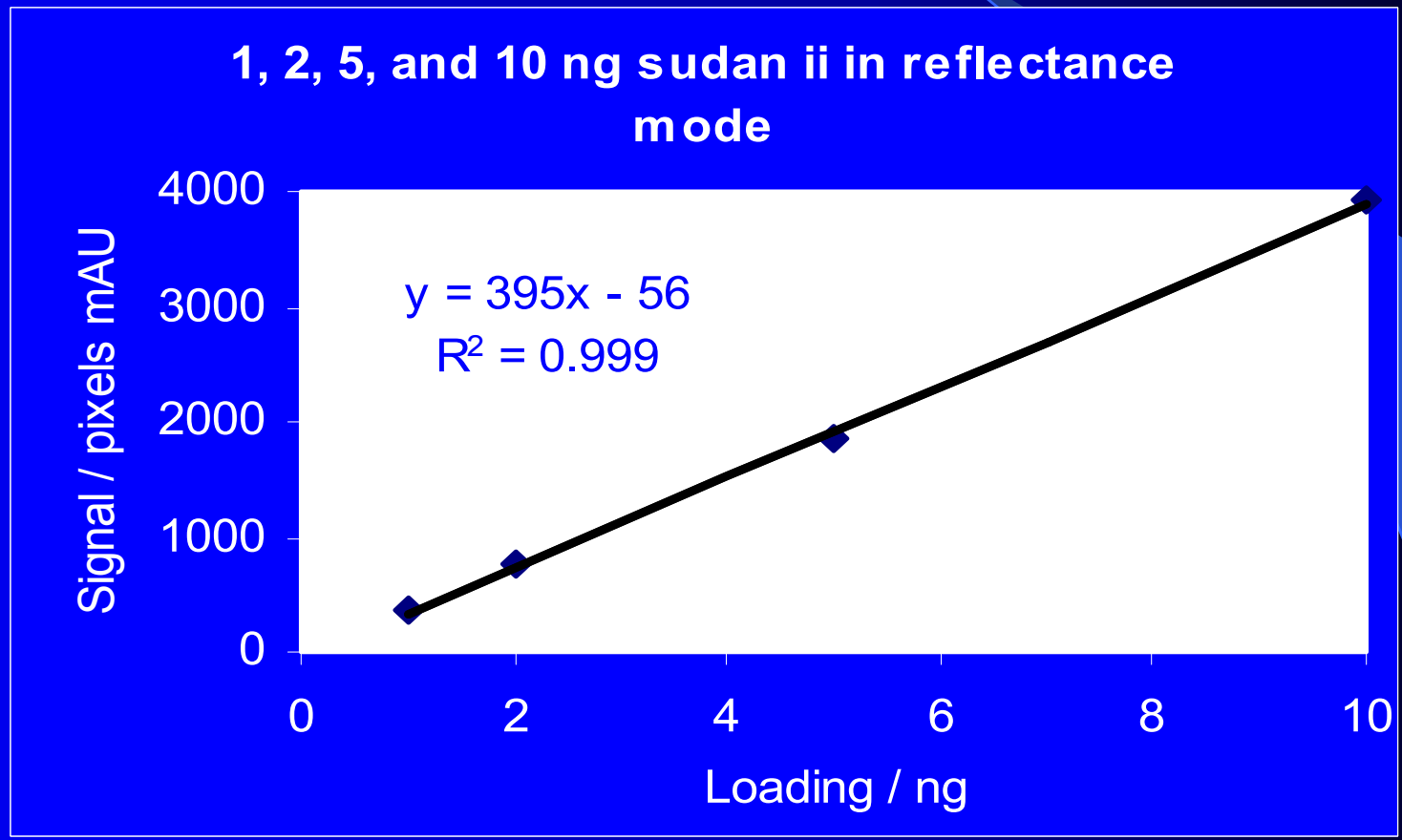

1200
1000
800
600
400
200
0
-200
pixel absorbance
0
50
100
150
200
250
Pixels

Image Processing

- Data smoothing carried out on output from Scion image using peak fitting software
- Data integrated for a second time and peak areas quantified

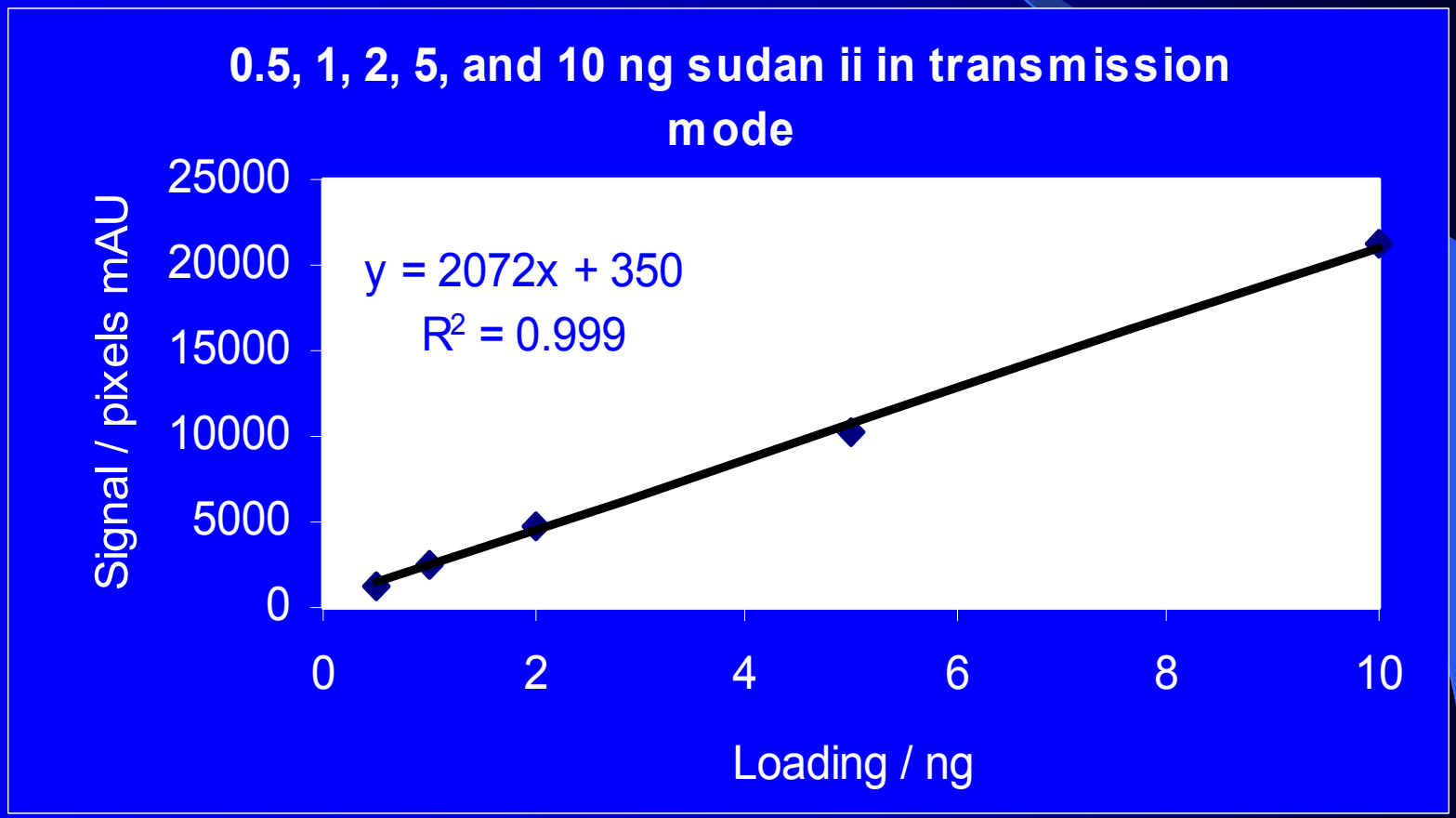
Reflectance - Linearity



Reflectance – Precision, Range and LOD

- Five 10 ng spots on the same plate gave a RSD of <2%
- Linear range from 1 – 10 ng
- LOD – 0.5 ng could be seen

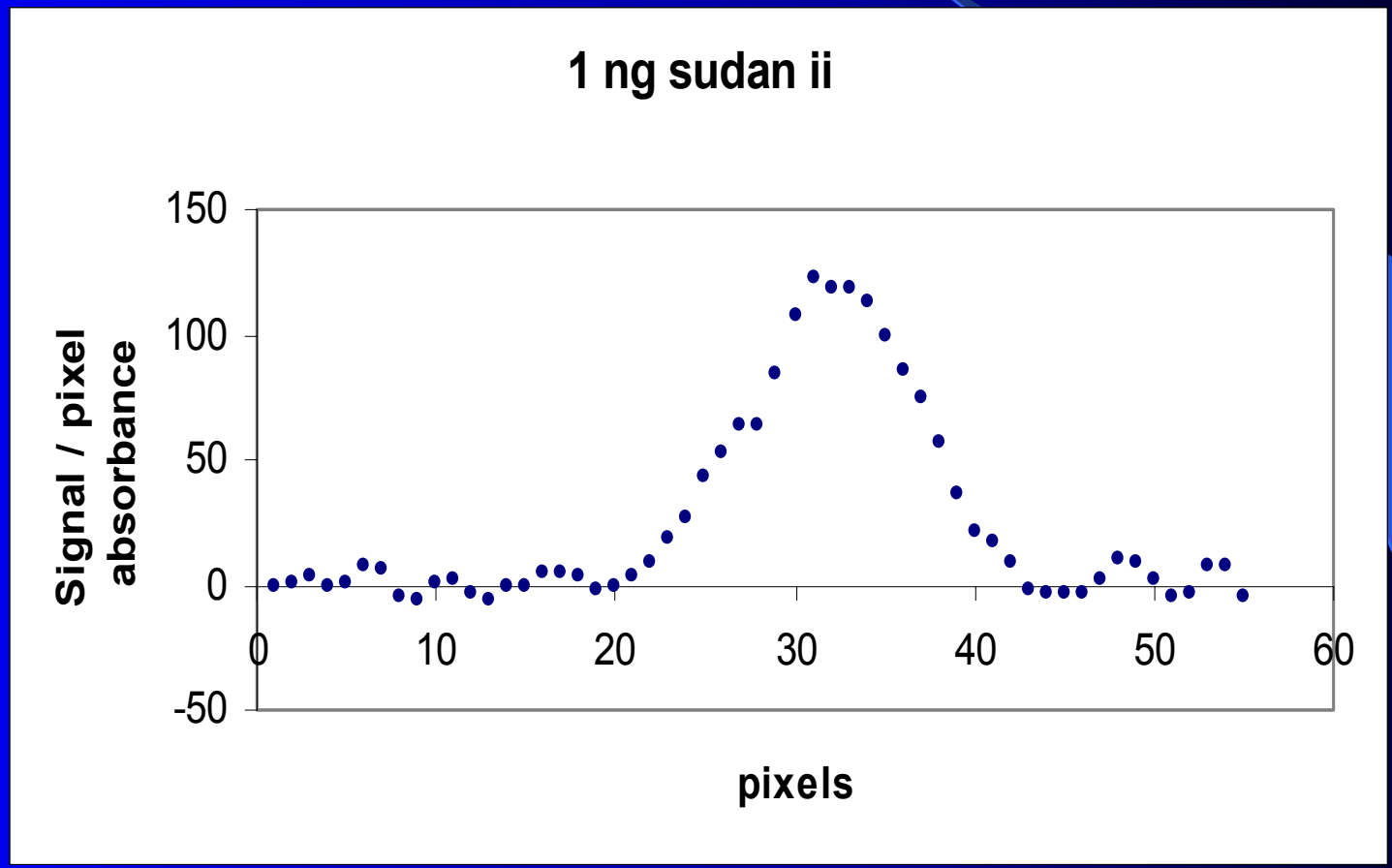
Transmission - Linearity



Transmission – Precision, Range and LOD

- Five 5 ng spots gave a RSD of 1.7%
- Linear range from 0.5 – 10 ng
- LOD - 0.2 ng can be seen

Transmission - S/N



Summary

- Fast method for quantitative TLC
- Linear over one order of magnitude
- Good Reproducibility
- Good LOD

Future Work

- Extend in to UV
- Real Time
- Forced Flow

Acknowledgment

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