Documentation/ Image Acquisition

For electronic image acquisition visible polychromatic light is captured. When long-wave UV light is used for object illumination, the camera captures the light emitted by fluorescent substances. When short-wave UV light is used for illumination of a chromatogram layer containing fluorescence indicator, the camera – like the human eye – registers the visible light emitted from the layer background.

The quantification of image data is possible via the so called grey scale. Spectral selectivity is restricted to the colors of fluorescence.

The strength of the electronic image acquisition is the overview of the complete chromatogram.

Chromatogram under white light

Chromatogram under UV 254 nm

Chromatogram under UV 366 nm
CAMAG TLC Visualizer

Digital image acquisition opens a new dimension for HPTLC, particularly in the field of sophisticated qualitative analysis.

The complete system TLC Visualizer is comprised of the illumination unit and a digital camera. It features ergonomic design and easy, intuitive handling with the relevant software.

The design of the illumination unit ensures homogeneity of the captured image under UV 254 nm, UV 366 nm and white light, the latter in direct mode, transmitted mode or combined. The light tubes are operated by 65 KHz high frequency in order to avoid synchronization problems with the digital camera. An automatic door control protects the operator against UV irradiation.

The powerful 12 bit camera combines high resolution (0.1 mm) with excellent color fidelity and low noise level. It features high reproducibility due to its automatic image optimization and fixed capturing parameters for all illumination modes.

The software offers easy and intuitive handling and automatic background correction with a fast data transfer. Archiving of all images from one plate together with all operational data of the analysis is effected in one file.

The software module “Professional Image Enhancement” provides additional optimization options such as the use of color profiles, layer structure subtraction and the possibility of serial shots.

The module “Image Comparison Viewer” serves for the comparison of tracks from multiple images or plates on the same screen.

Image Comparison

Image Comparison View: Selected tracks of images taken of the same plate under UV 254 nm (20; 25), white light (30, 40) and UV 366 nm (all other tracks) are compared

Example for white light

No Correction Standard Correction Individual Correction with “Professional Image Enhancement”
**VideoScan**

The VideoScan software allows evaluation of stored data captured with the TLC Visualizer. The program is easy to use and rapid. Flexible applications such as profile comparison of tracks from several chromatograms, evaluation of tracks with variable distances, distorted tracks, etc. are provided. Quantitative evaluation can be done at any time, even years after capture.

Quantification is possible via peak area and/or peak height, using single or multi level calibration, linear or polynomial.

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**Note**

The TLC Visualizer with winCATS meets all the requirements of GMP/GLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the option 21 CFR Part 11 “compliance ready” is required for each winCATS workstation.

**Ordering information**

- **022.9780** CAMAG TLC Visualizer, complete with high-resolution 12 bit CCD camera with 12 mm objective (for 20 x 20 cm plates), with Equilink, without software → **visionCATS**
- **022.9781** CAMAG TLC Visualizer, complete with high-resolution 12 bit CCD camera with 16 mm objective (for 10 x 10 and 20 x 10 cm plates), with Equilink, without software → **visionCATS**
- **022.9579** CAMAG VideoScan Chromatogram Evaluation Software

Further Information can be found in the special brochure “CAMAG TLC Visualizer” and under www.camag.com/tlcvisualizer
Selective Detection of Bioactive Compounds

CAMAG BioLuminizer®

The BioLuminizer system is comprised of a compartment excluding any extraneous light, climate controlled for extended stability of the plate, and a 16 bit CCD digital camera of high resolution and high quantum efficiency. It features ergonomic design and easy, intuitive handling in stand-alone mode using the special software. With the BioLuminizer bioactivity can be detected and registered economically and with short response time. Special antibacterial protection measures are not necessary, as the bioluminescent bacteria Vibrio fischeri are atoxic for humans.

- Cooled 16 bit CCD camera with high resolution and high quantum efficiency
- Climate controlled compartment for prolonged stability of the plate
- User friendly compact design, easy to clean

Hyphenating TLC/HPTLC and bioassay is an excellent tool for identification of single toxic compounds in complex sample matrices.

The method is suitable for the detection of toxins in foodstuff, beverages, cosmetics, waste water, drinking water and for the detection of bioactivity in natural products.

After chromatographic separation of the complex sample the plate is immersed in a suspension of bioluminescent bacteria Vibrio fischeri. The reaction takes place within a very short time. All zones with inhibitory or toxic effects appear as dark zones on the luminescent plate background.

Example

Processed waste water containing X-ray contrast media is frequently irradiated with UV light. The HPTLC bioluminescence image shows the bioactive effect of degradation products. As can be seen, an increase of the irradiation time generates substances with a distinctively inhibitory effect on the bioluminescent bacteria. In a cuvette test, this inhibitory effect would have been masked by degradation products.

The example is taken from an internship report at the “Zweckverband Landeswasserversorgung” in Langenau, Germany.

Ordering information

022.9750 CAMAG BioLuminizer® for detection of bioluminescence patterns on HPTLC plates, including special software

Further information can be found in the special brochure "CAMAG BioLuminizer®" and under www.camag.com/bioluminizer
Classical Densitometry

In classical densitometry the tracks of the chromatogram are scanned with monochromatic light in the form of a slit selectable in length and width. The spectral range of the CAMAG TLC Scanner 4 is 190–900 nm. Reflected light is measured either in the absorbance or in the fluorescence mode. From the acquired data quantitative results are computed with high precision and spectral selectivity.

With the TLC Scanner 4 absorption and fluorescence excitation spectra can be recorded. The strengths of classical densitometry as compared with image evaluation are spectral selectivity and the higher precision of quantitative determinations.

Recommendations
Applying samples in the form of narrow bands allows densitometric evaluation by aliquot scanning, i.e. scanning with a slit about 1/2 of the track width. This improves reproducibility as the center portion of the sample zone is homogeneous and positioning errors, which can occur with samples applied as spots, are avoided.

For quantification sample zones should always be scanned with the wavelength of maximum absorbance which can be determined by spectra recording or by multi-wavelength scanning.

For further recommendations reference the TLC Scanner 4 instruction manual.
CAMAG TLC Scanner 4

The TLC Scanner 4 is the most advanced workstation for densitometric evaluation of TLC/HPTLC chromatograms and other planar objects. All functions of the scanner are controlled by the winCATS software. Only positioning of the object to be measured is performed manually and, if desired, switching on the internal illumination to assist correct positioning. Optimal settings of the electronic amplification are automatically selected for scanning in absorbance or fluorescence mode respectively. The 16 bit A/D converter ensures optimally adapted resolution of the measurement signal.

Key features

- Measurement of reflected light, either in absorbance or fluorescence mode
- Object formats up to 20 x 20 cm
- Spectral range from 190 to 900 nm
- Automatic start of all lamps: deuterium, halogen-tungsten, and high pressure mercury lamp
- Data step resolution 25–200 μm
- Scanning speed 1–100 mm/s
- Spectra recording with a speed up to 100 mm/s
- Automatic adjustment of the analog system
- Rapid data transfer

The object, here a 20 x 10 cm HPLC plate, is conveniently positioned on the scanning table which then automatically moves to the start position. The coordinates are displayed during manual positioning and can be transferred into the program by mouse click.
Perfect evaluation with winCATS

The Planar Chromatography Manager winCATS organizes all steps of instrumental thin-layer chromatography from sample application through quantitative evaluation.

The well structured and easy to use software controls all functions of the TLC Scanner 4 and processes all measuring data up to the final result. The winCATS standard program for TLC Scanner 4 comprises scanning the chromatogram with subsequent integration, computing results, and printout of the analysis protocol with details in the form of colored graphics as required by the user. Also spectra recording is included in the standard program.

A number of winCATS program options allow the user to adapt the evaluation system to his needs.

The following options are available:

- Quantitative evaluation supports: Single level calibration and multi level calibration with linear or nonlinear regression using internal or external standards. Statistics as relative standard deviation (cv) or confidence interval (CI). Subcomponent evaluation supporting quantitation of unknown peaks by relating them to the main component as is prescribed by European or US pharmacopoeias ("Related Compounds").

- Dual-wavelength scan: The chromatogram is scanned at two freely selectable wavelengths. During integration the signal from the second wavelength is subtracted from that of the first wavelength to eliminate matrix effects. Dual-wavelength scanning is also useful for the quantitation of incompletely resolved peaks.

- Multi-wavelength scan: The chromatogram can be scanned automatically with up to 36 selectable wavelengths between 190 and 900 nm. For quantitation, data from the scan at the optimum wavelength for each compound is used. This winCATS function is unique to thin-layer chromatography!

- Track optimization: Each track of the chromatogram is scanned several times with a small lateral offset. From this data the optimum virtual track following the peak maxima is calculated and used for quantitation.

- Scanner qualification (SelfTest): This option offers automatic monitoring of the mechanical, optical and electronic functions of the scanner. Results are evaluated, documented and stored. When appropriate, lamp positions and monochromator alignment are automatically adjusted.

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Note

The TLC Scanner 4 with winCATS meets all the requirements of GMP/GLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the option 21 CFR Part 11 “compliance ready” is required for each winCATS workstation.

Ordering information

027.6200 CAMAG TLC Scanner 4 complete with Equilink, without software

Further information can be found in the special brochure “CAMAG TLC Scanner 4” or under www.camag.com/tlc-scanner
TLC-MS Coupling

CAMAG TLC-MS Interface

Hyphenating TLC and mass spectrometry opens new application areas for Planar Chromatography.

Not all samples are suitable to be processed by HPLC-MS or HPLC-DAD systems. This can be due to no or low UV response of the compounds or their impurities, to heavy matrix load or to incompatibility of the HPLC mobile phase with MS detection. In these cases HPTLC is an efficient alternative.

Hitherto zones with unknown substances had to be scraped off from the TLC/HPTLC plate, extracted in a tube and transferred into the MS. Now a very convenient and universal TLC-MS Interface is available which elutes zones of interest semi-automatically and feeds them online into the HPLC-MS system. This can be of various brands and techniques (APCI-MS, APPI-MS or EI-MS). Only the zones of interest need to be extracted and are MS analyzed within minutes.

The chromatogram zones are eluted from the HPTLC plate with methanol or another suitable solvent using the round or oval elution head with a flow speed appropriate for the HPLC-MS system, e.g., 0.2 ml/min. Positioning of the elution head is done semi-automatically with the help of the laser pointing device incorporated in the Interface or according to the coordinates determined by the TLC Scanner or the TLC Visualizer. After each circle the elution head is cleaned automatically.

The oval elution head is preferable for narrow, closely neighboured zones.

Ordering information
022.8406 CAMAG TLC-MS Interface for online coupling HPTLC with HPLC/MS including two elution heads, one round 4 mm and one oval 2 x 4 mm

Further information can be found in the special brochure “CAMAG TLC-MS Interface” or under www.camag.com/tlcms