

Improving HPTLC Results: Standardization & New Instruments



Standardization & new instruments

- Aim of standardization
- Optimizing the reproducibility
- New developing chamber ADC 2
- New documentation system Digistore2
- New *smart*ACCESSORIES for daily use
- Outlook

Standardization aim → reproducibility

■ Standardization

- Same “settings” (manually, set by SW...)
- Reproducibility (instrument, ...)

■ Reproducibility

- Within the lab
- Between labs
- Daily, weekly, monthly, seasonal, annual ...
- Independent of staff / human factor

Optimizing the reproducibility

- Parameters \leftrightarrow reproducibility
 - Internal: caused by the „instrument“
 - External: „environmental conditions“
 - Staff: different handling

Aim:

- Reduce uncertainties of instruments
- Transfer „external“ & „staff“ parameters to internal ones which can be controlled

New developing chamber, ADC 2

- **Standardization**
 - Same settings, additionally for the most critical environmental condition

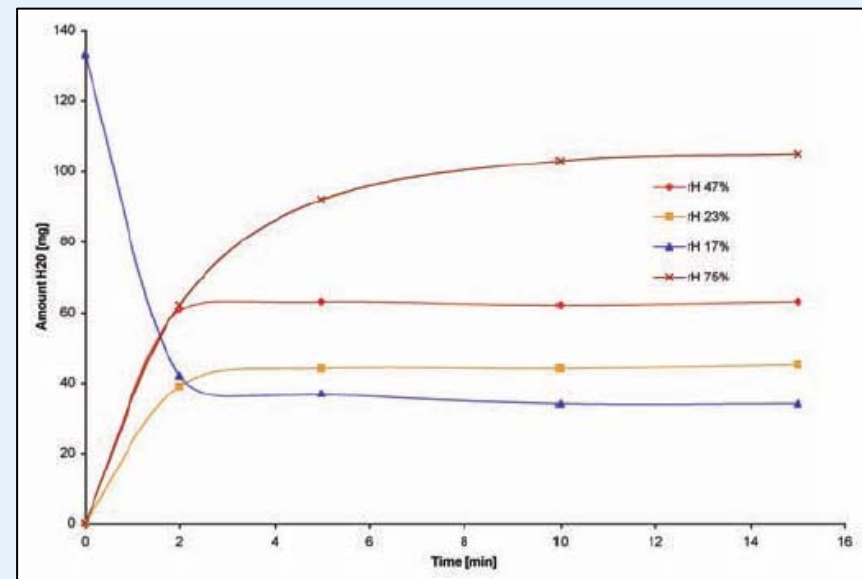
- **Reproducibility**
 - RH → internal
 - Staff → internal (MD, drying, disturbances, saturation, all times → automation)



ADC 2: Relative humidity

- First test results of closed loop conditioning

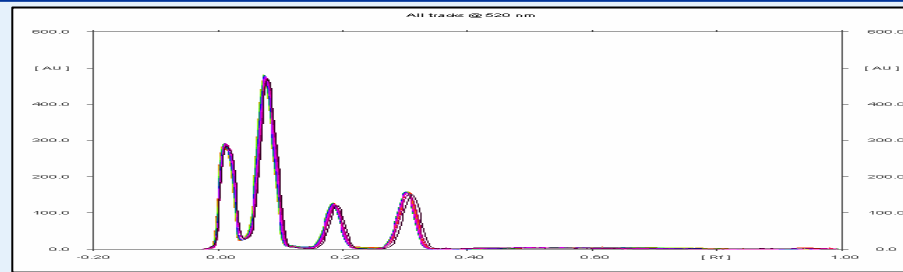
- Fast equilibrium time
- Works bi-directional



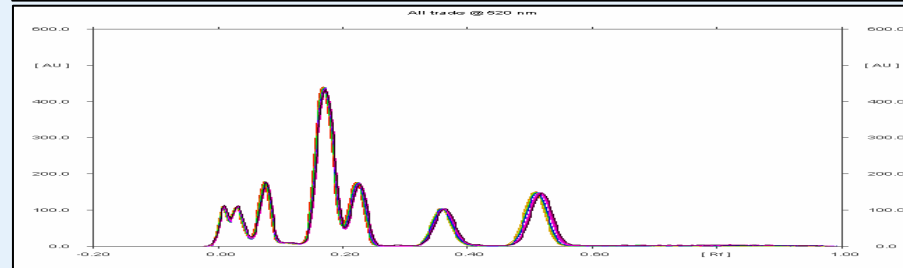
→ Significantly improved reproducibility

ADC 2: Relative humidity

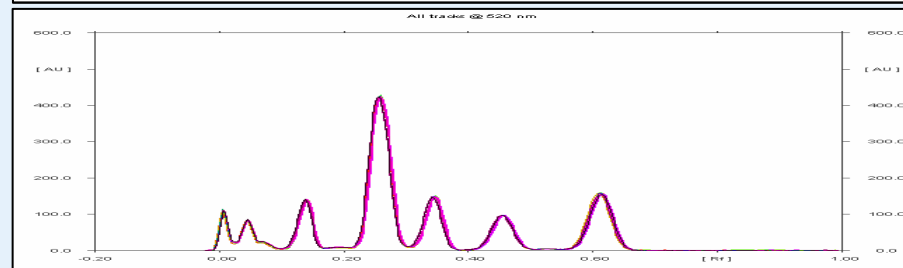
- 17% RH
all 18 tracks



- 47% RH
all 18 tracks



- 75% RH
all 18 tracks



→ RH: Internal parameter therefore reproducible

→ Standardization to a fixed value possible

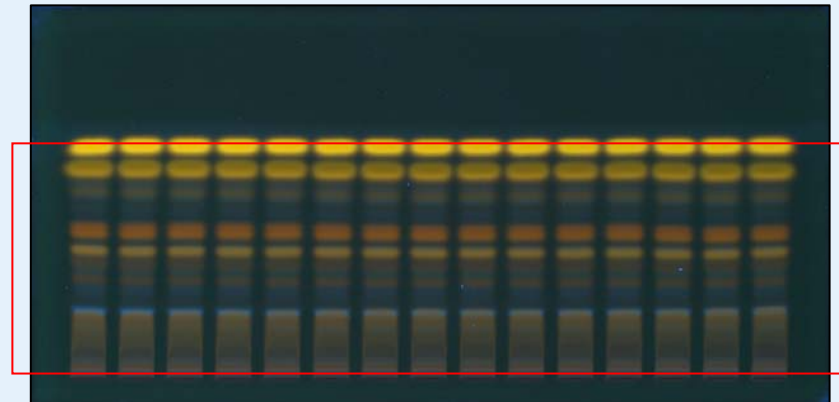
ADC 2: Staff / human factor

■ Twin Trough Chamber

- Standardized tank but manual operation (disturbances... depending on operator)
- Environmental conditions not controlled

■ Reproducibility

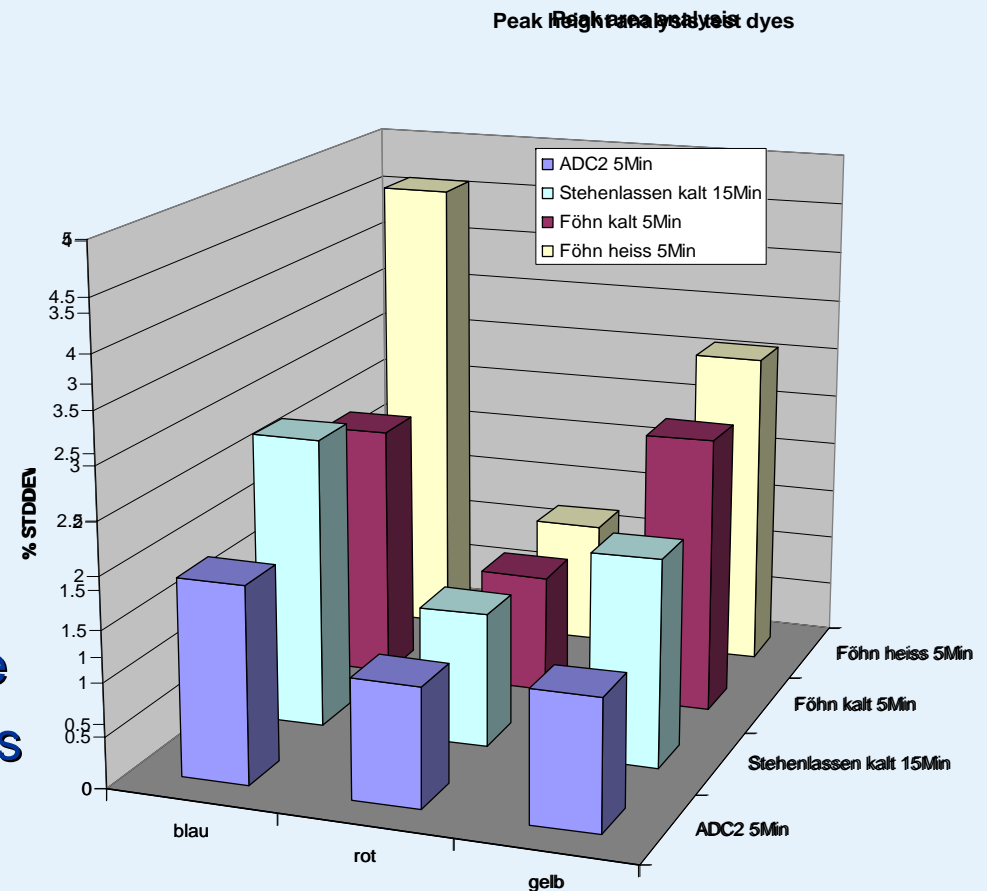
- Migration Distance...
- Disturbances...
- Automation



→ MD & R_F constant for different tracks / plates

ADC 2: Drying process studies

- ADC 2 development +
 - Cold air, high speed
 - Waiting 15 minutes
 - Cold air hair dryer
 - Hot air hair dryer
- Substance dependent!
- Reasons?
 - Depth distribution change
 - Heat sensitive substances



ADC 2: Summary

- **Reproducible results regardless of environment, staff / handling**
- **Standardization now possible**

- **Community has to agree on certain parameters**
- **Comparison between chromatograms will be improved**

New documentation system, DigiStore 2

- **Standardization**
 - Preset parameters
 - Automatic performance

- **Reproducibility**
 - Intensity (overall)
 - Scaling & color balance
 - Amplitude (res. & linearity)
 - Homogeneity (spatial)
 - Clean plate compensation



Digistore 2: Standardization

- Many fix parameters (factory preset)
 - Entire optics setup!
→ **Reproducibility**

- Automatic exposure optimization algorithms
 - Different standardized algorithms for
 - UV 254nm (quenching of indicator)
 - UV 366nm (fluorescence)
 - White light
 - e.g. white plate at 90% digital level ($< \pm 1\%$)

- Manual high precision mode

Digistore 2: Reproducibility

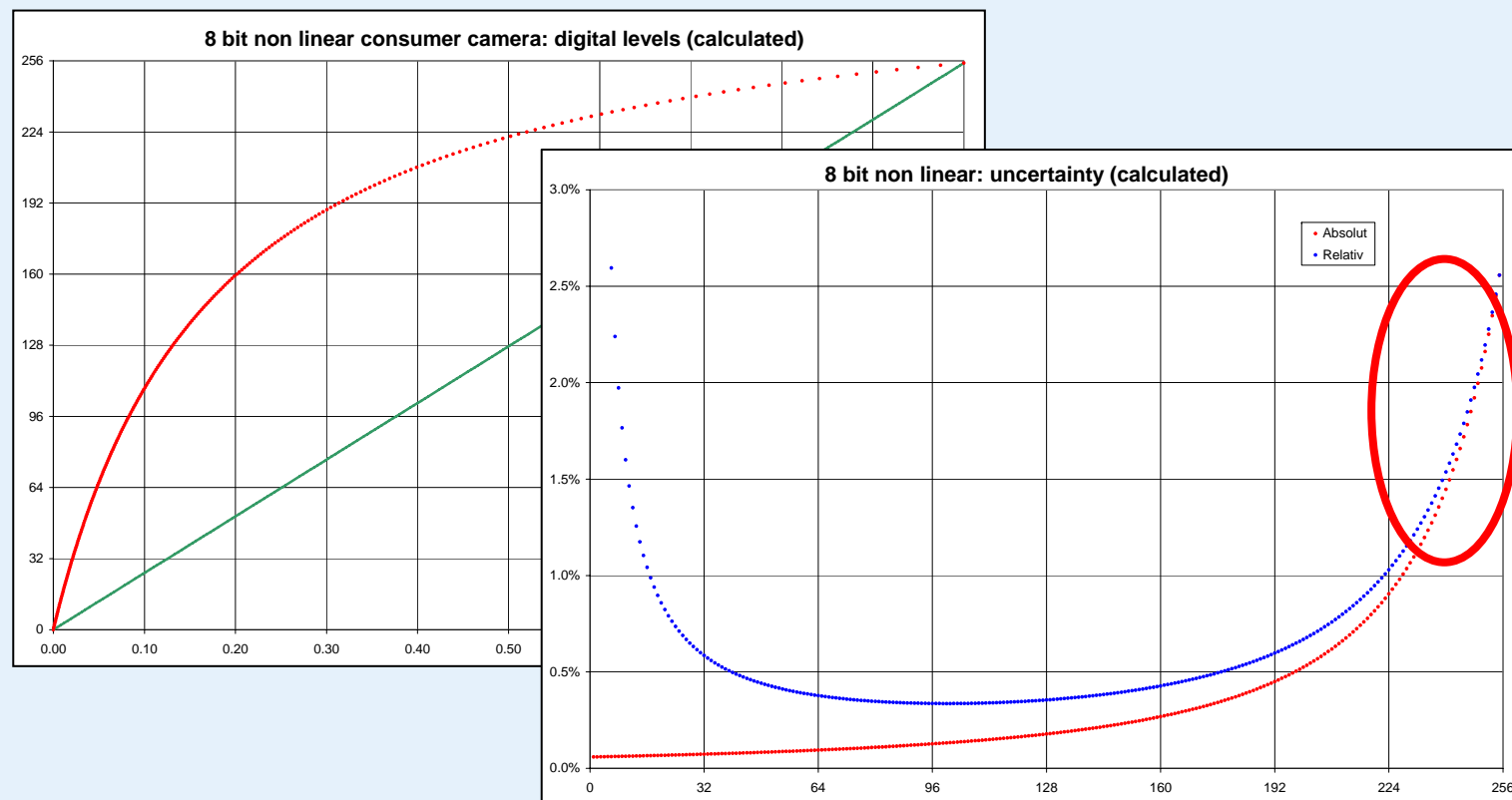
- **Intensity (overall exposure)**
 - Automatic exposure algorithm
(remaining uncertainty: illumination drift)
 - Final uncertainty <1%

- **Scaling calibration**
 - Fix focal length → fix scale

- **Color balance**
 - Kept constant

Digistore 2: Amplitude measurement

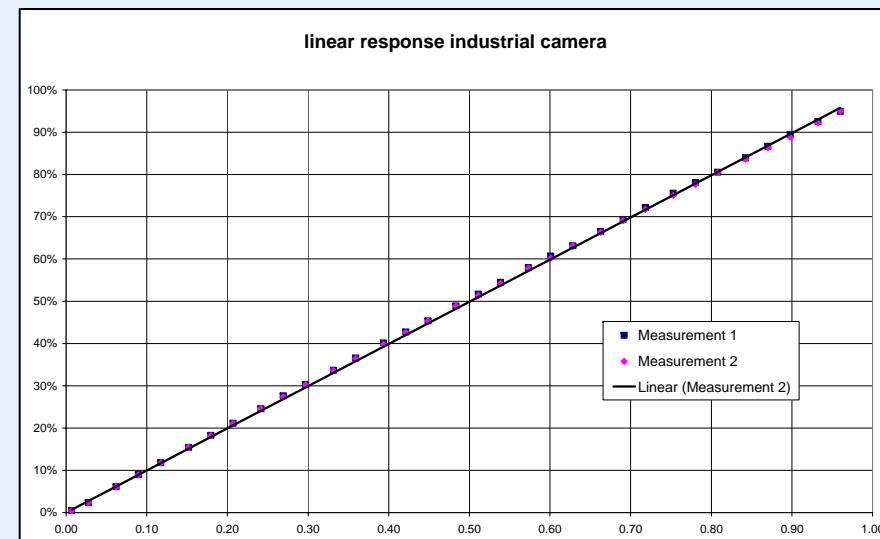
- 8 bit → 12 bit resolution, non linear → linear



Digistore 2: Amplitude measurement

- Industrial 12 bit camera
 - 4095 levels or 0.025% steps
 - very linear

- Reproducible images
amplitude & colors
- Very realistic images
- Smaller fractions
detectable



- Extensive comparisons... quantification possible

Digistore 2: Homogeneity

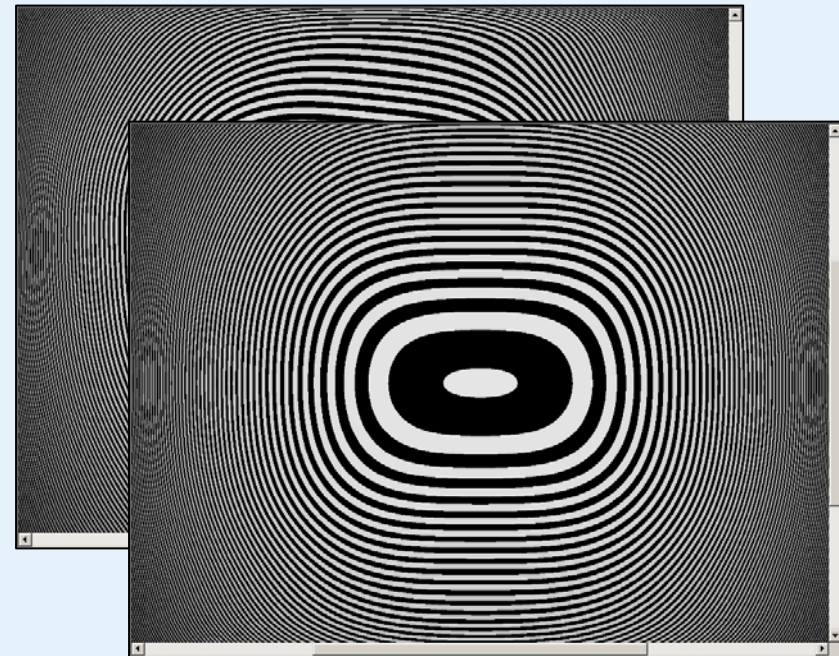
■ Homogeneity (spatial)

- Illumination
- Lens
- Angle on chip

→ Fix focal length

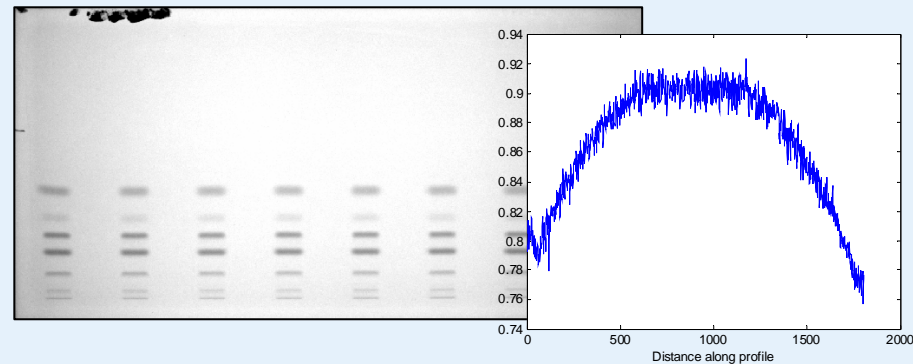
→ Fix opening (aperture)

→ Flat field correction reliable
(no zoom, no opening, no speed uncertainties & hyst.)

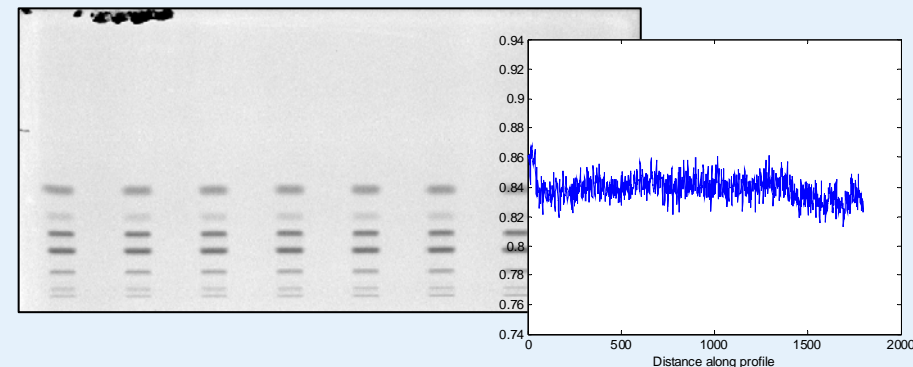


Digistore 2: Homogeneity correction

- **Uncorrected image**
 - >10% intensity variations



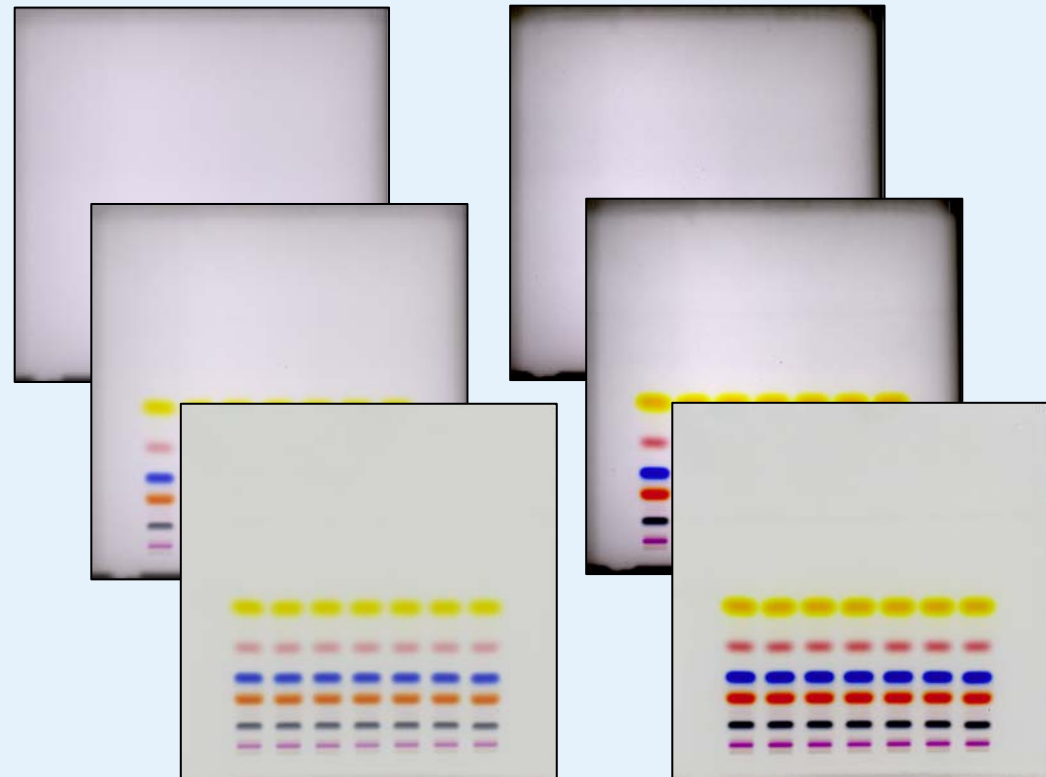
- **“Flatfield correction”**
 - Individually <2% possible



→ Accuracy of correction:
 “General” → “per device” → finally “per plate”

Digistore 2: TD analysis, vis. trans.

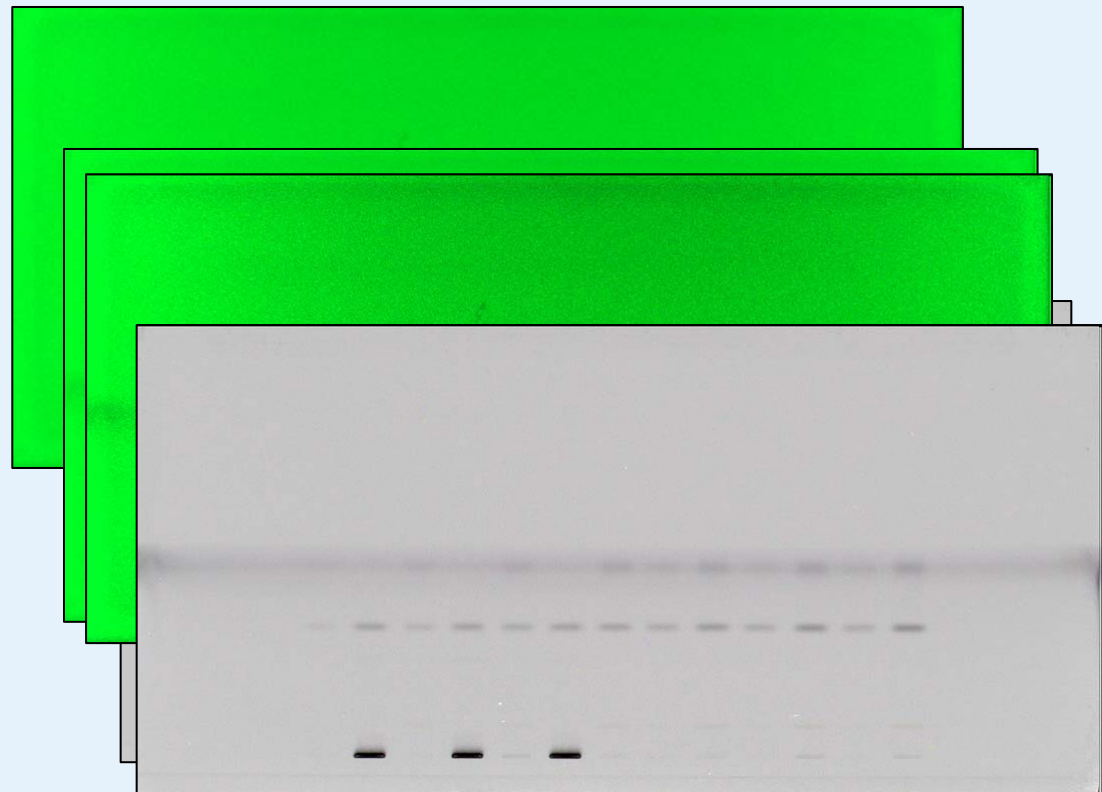
- Test dyes (reproducibility)
- Clean plate
- Measured plate
- Compensated result



→ Excellent base for quantitative analysis

Digistore 2: CIT analysis, 254nm

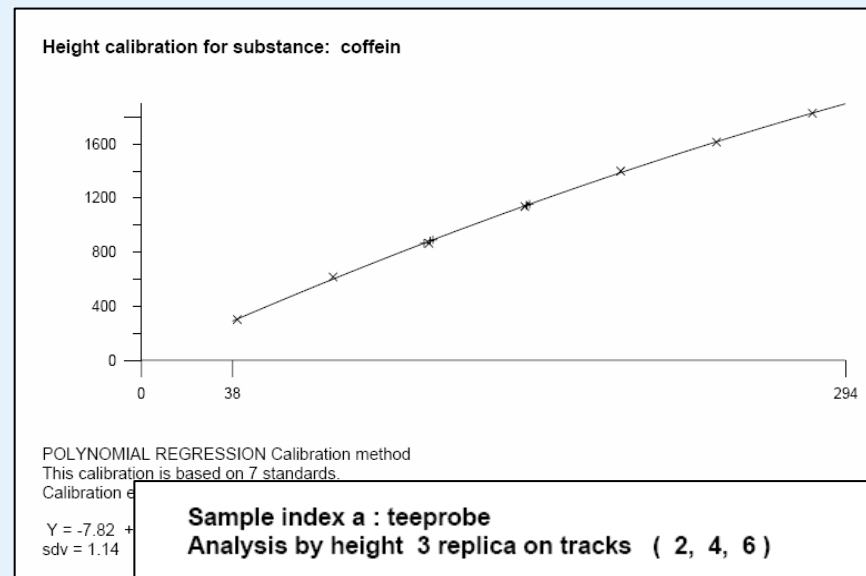
- Caffeine in tea
(3 ctrl.std. + 7pt.cal.)
- Clean plate
- Measured plate
- Compensated result



→ Excellent results if spots are visible to the camera

Digistore 2: Quantitative analysis, 254nm

- Using CAMAG VideoScan



Sample index a : teeprobe
Analysis by height 3 replica on tracks (2, 4, 6)

Sample index b : control
Analysis by height 3 replica on tracks (8, 10, 12)

Substance	Rf	Mean	CV (%)	n	Method	Comment
coffein	0.395	120.02 ng	1.6	3		

→ Excellent results if spots are visible to the camera

Digistore 2: Summary

- **Reproducible results (fully controlled industrial camera)**
 - **Fix optical system & linear digitization with 12 bit allow optimal reproducibility**

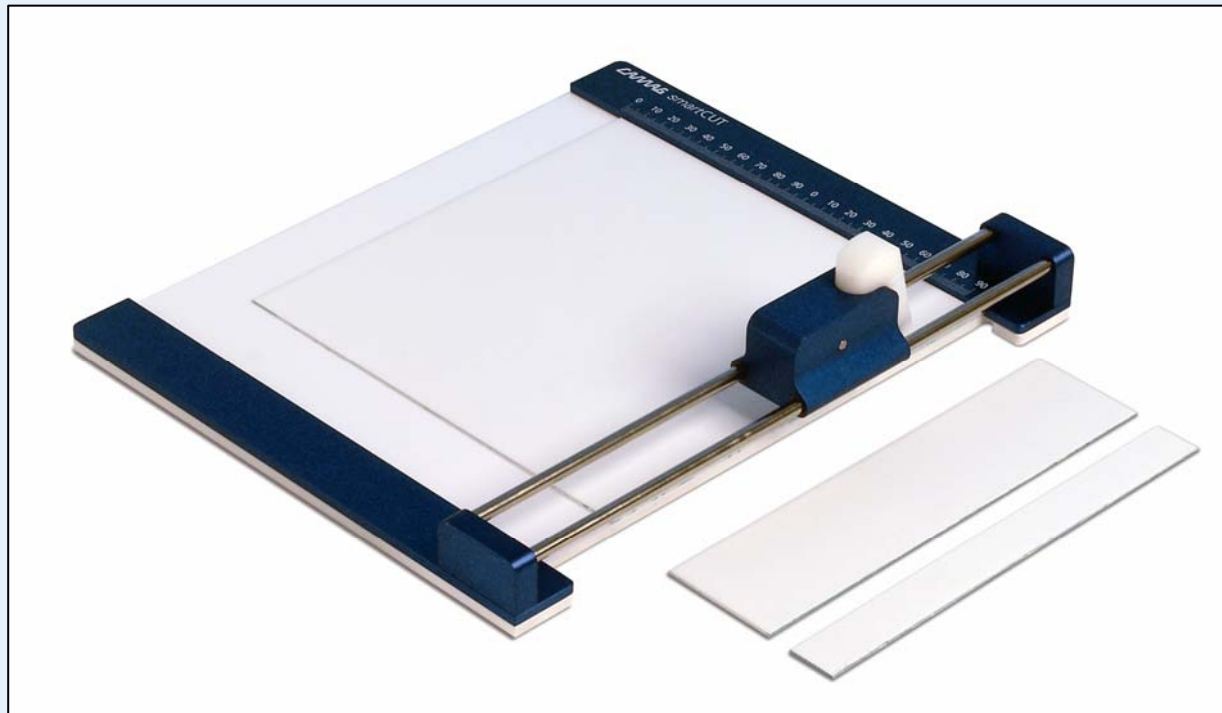
- **Standardization due to**
 - **Fix parameters / accurate optimization algorithms**

- **Base for quantitative image analysis & color comparison is set!**

*smart*ACCESSORIES

- *smart*CUT

For exact cutting of TLC/HPTLC plates.



Demo *smartALERT*

- *smartALERT*

With *smartALERT* you'll never 'forget' your TLC plate in the developing chamber anymore.



Outlook

- **ADC 2 & Digistore 2**
 - **Improve the reproducibility of TLC**
 - **Standardization to a higher level possible**
→ **Allows comparisons between any labs**

- **smartACCESSORIES**
 - **Making life easier for basic TLC tasks**

- **Possibilities for the future**
 - **Image analysis (qualitative & quantitative)**
 - **Pattern recognition**

**Thank you for your
attention!**

Questions?

CAMMAG
