

# *LabChrom-2 & TR-542a*

Chemotron Instruments

1991-2011

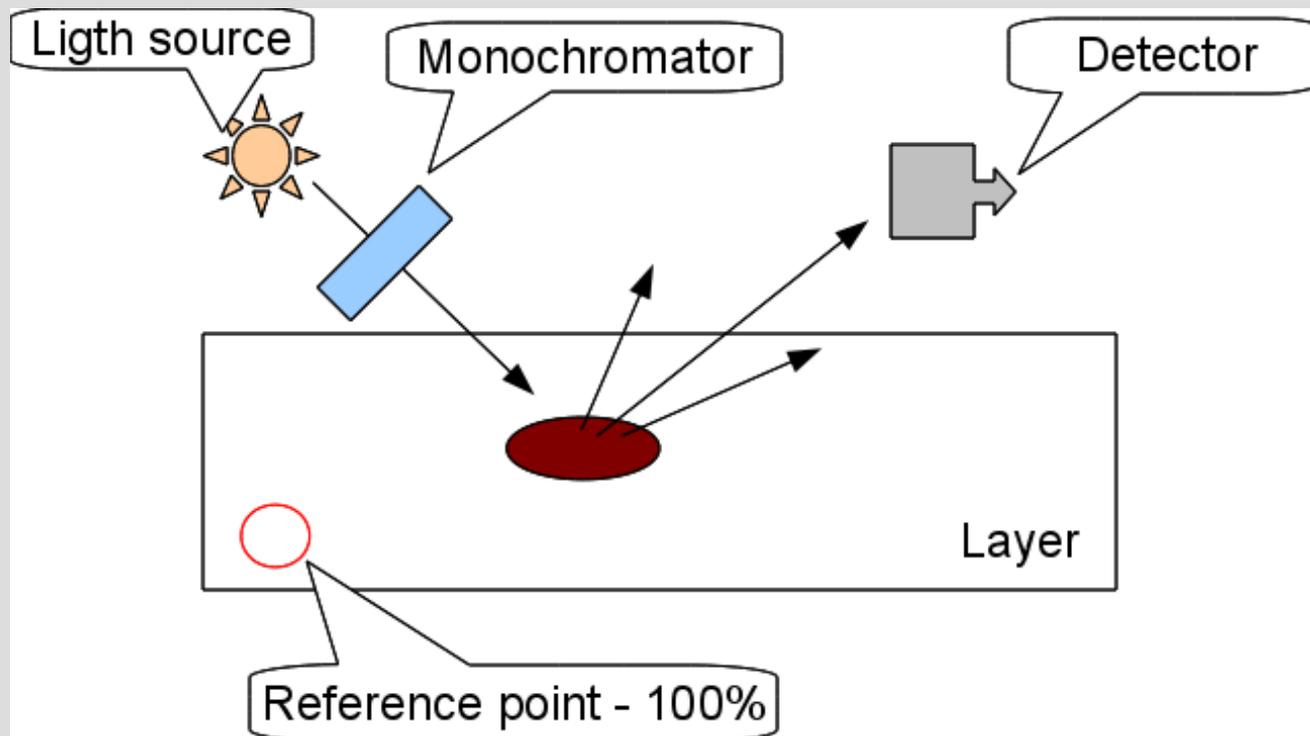
# *Measurement basics*

Three types of measuring methods, by interaction of light

- ◆ Reflectance, TLC
- ◆ Fluorescence, mainly TLC
- ◆ Transmission, gel

# Measurement basics

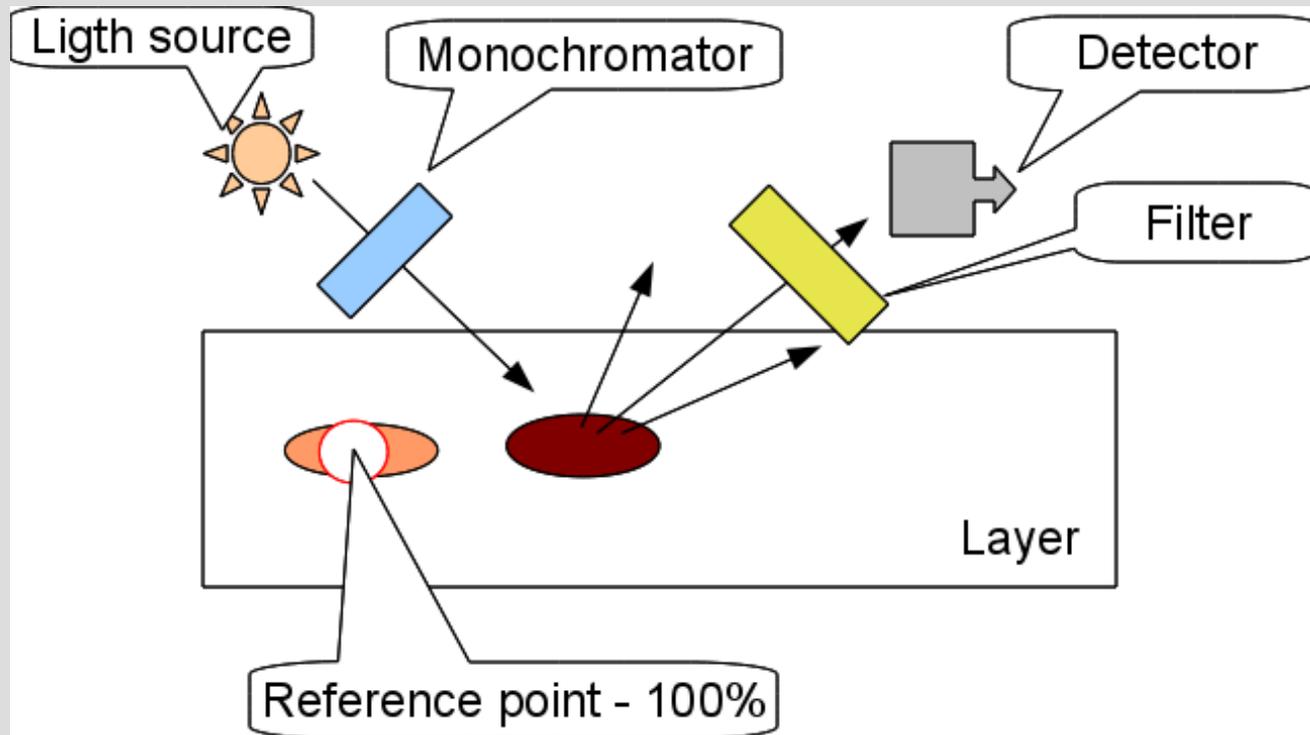
## ◆ Reflectance



The reference point is the „whitest” (lightest) area

# Measurement basics

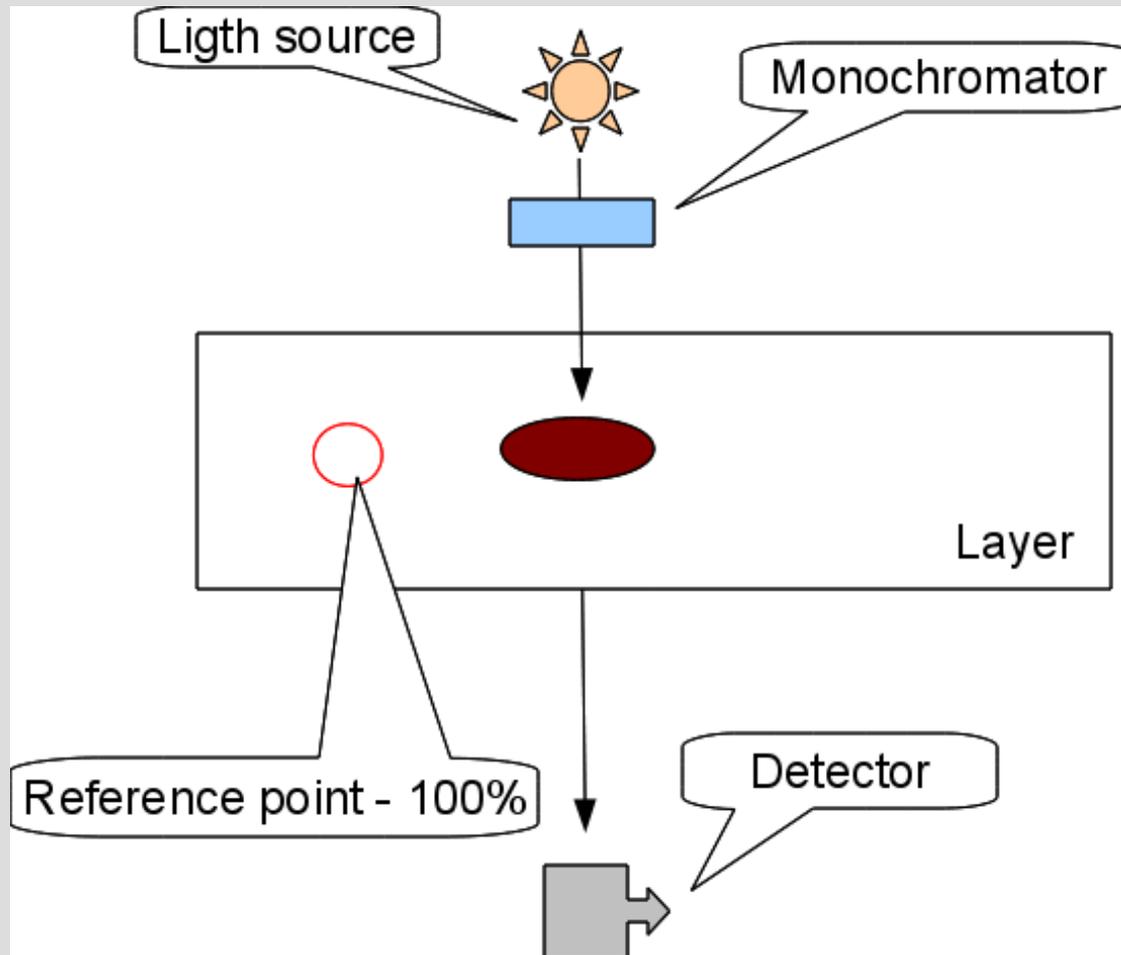
## ◆ Fluorescence



The reference point is the lightest spot on the layer

# Measurement basics

## ◆ Transmission



# *Measurement settings*

- ◆ Make a *Method* file
- ◆ Save it
- ◆ Set in *Preferences*
- ◆ Start measure
- ◆ Make modifications if necessary

# Measurement settings - Make a Method file

## ◆ Main window → Method

Instrument Par Data Proc Instrument Column/Layer

Short ViewScan (100 mm)

Scan limits

Norm X 10 Norm Y 10

X start 1 X stop 1

Y start 1 Y stop 1

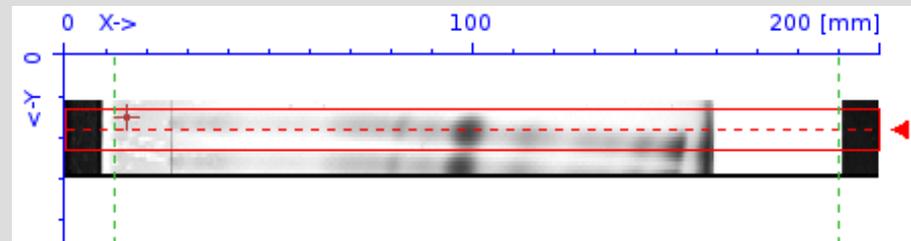
Scan parameters

Wavelength 614 nm

Fluorescence

Reflectance  Transmittance

- Short ViewScan – for 100 mm wide layers
- Norm X,Y – coordinates for reference point, designated by a light red cross, usually in upper left corner
- X start, stop – chromatogram recording start-stop retentions (in mm unit)
- Y start, stop – upper and lower track limits



## *Measurement – The ViewScan*

The ViewScan is a full resolution layer scan, between Y start/stop limits and in 200 mm (or 100 mm in case of short view) length of the layer.

The purpose: to get the whole layer data and in the next step you can easily select the desired tracks.

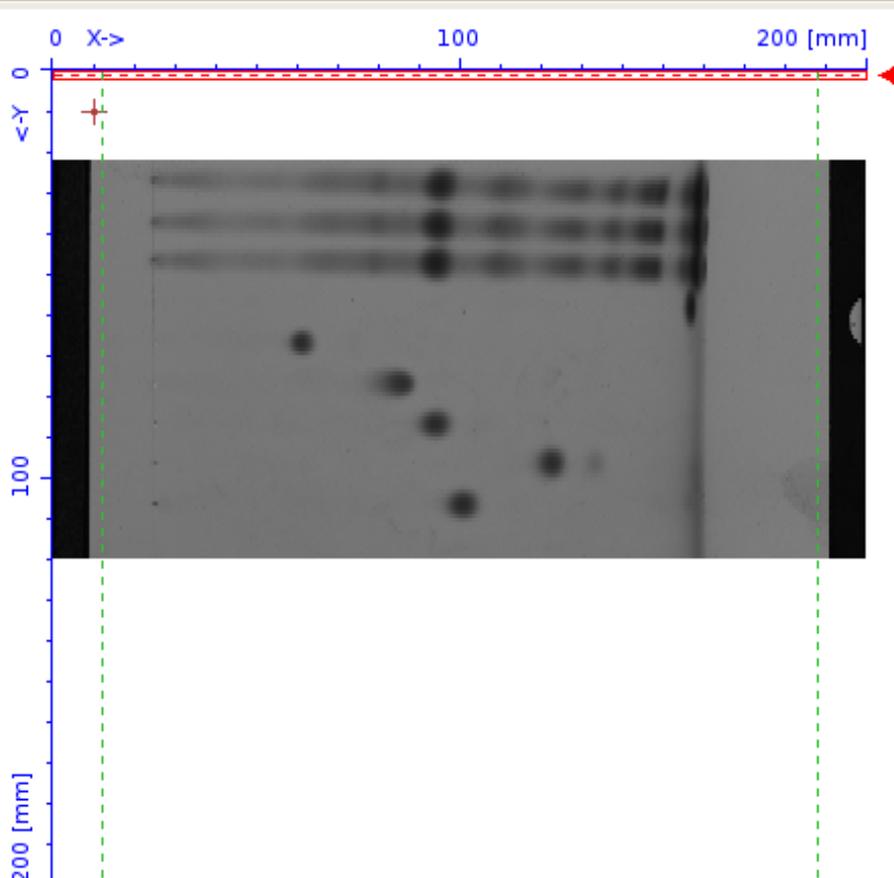
All the data is available now.

# The ViewScan

Control panel for the ViewScan interface:

- Add** **Remove** Selected track: 1
- Up** **Down** Track position: 1
- Expand** **Shrink** Track width: 2

View area (X and Y axes in mm):

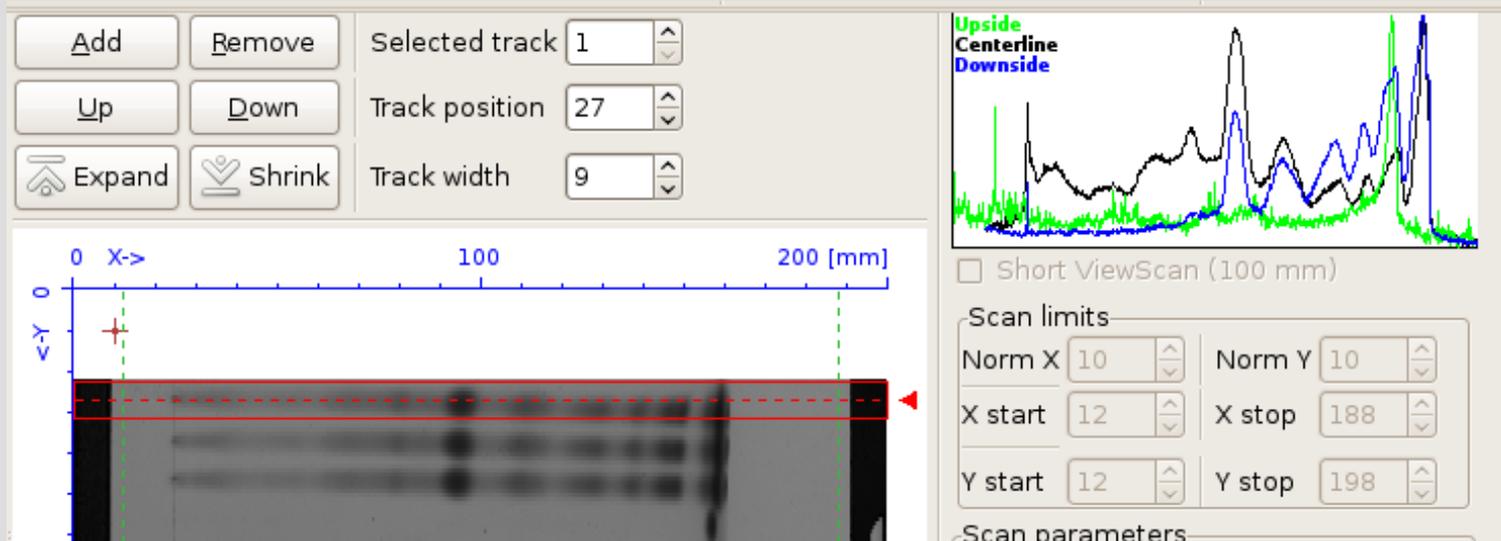


Right-side configuration panel:

- Orientation: **Upside** (green), **Centerline** (black), **Downside** (blue)
- Short ViewScan (100 mm)
- Scan limits:**
  - Norm X: 10, Norm Y: 10
  - X start: 12, X stop: 188
  - Y start: 12, Y stop: 198
- Scan parameters:**
  - Wavelength: 548 nm
  - Fluorescence
  - Reflectance  Transmittance
  - for upper scale value: 1000
  - for lower scale value: 0
- Autoscale** **Refresh**

# Track handling

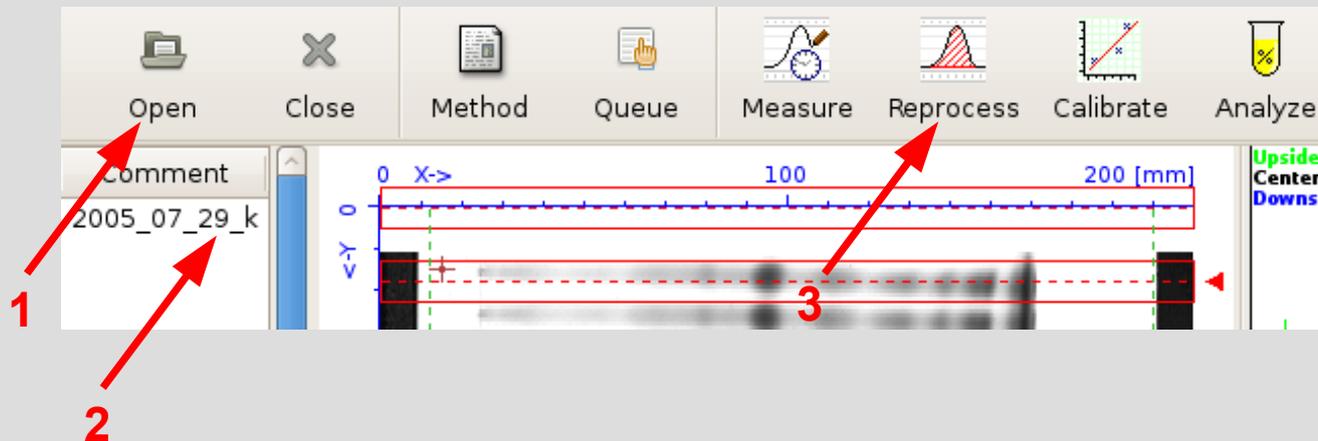
- ◆ After the ViewScan executing we can handle tracks:



- Add, remove tracks
- Modify their positions
- Modify the track width
- Visually detect the track side, if their width is too wide or too narrow

# *Main window - Open an existing layer*

- ◆ Click on the **Open** button and select the „.lay” file which you need (1)
- ◆ Select the track from the list that you want to process (2)
- ◆ Go to **Reprocess** window (3)

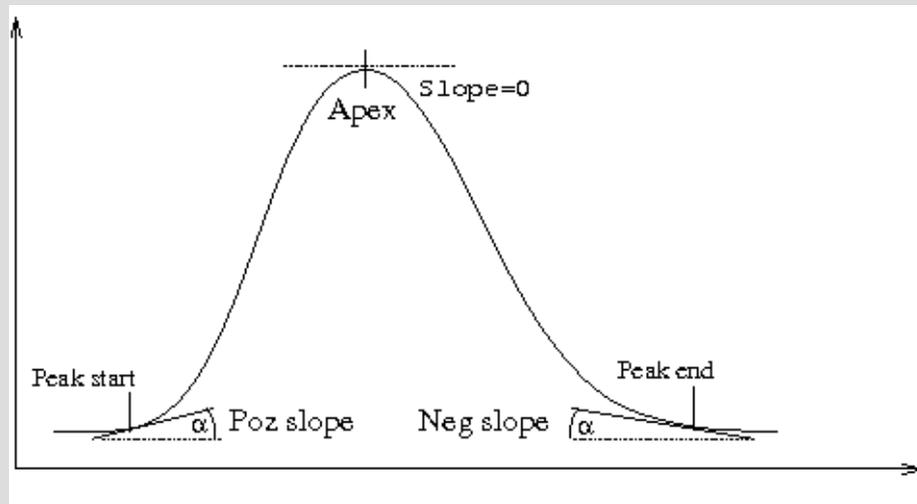


# *The Reprocess window*

- ◆ Reintegrate
  - ◆ Compare / overlay
  - ◆ Save
  - ◆ Export
  - ◆ Print
- the chromatograms.  
The integration can be
- ◆ automatic (by parameter set)
  - ◆ manual

# Integration basics

## ◆ How to detect a peak?

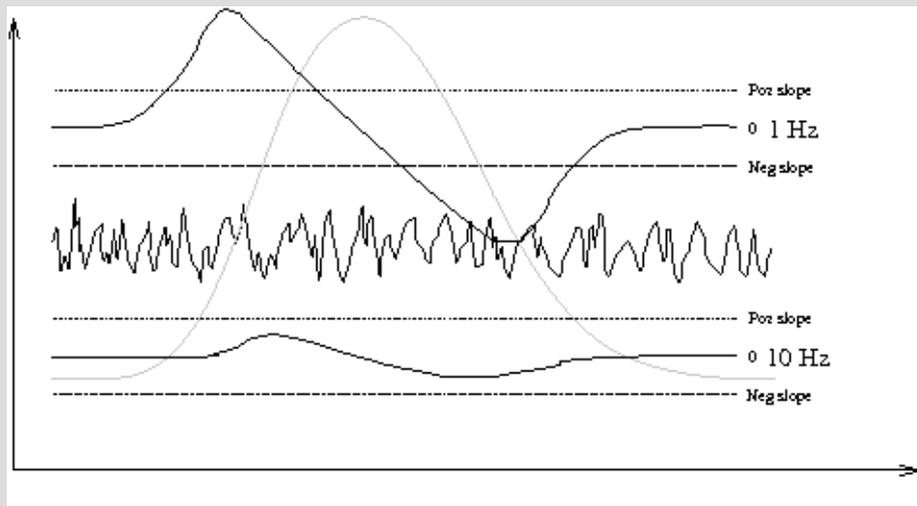


The software continuously calculates the derivative values of data stream. When the derivative value is higher than a previously set threshold value it detects as a peak start.

When this value's sign turns from positive to negative, this signals it is a peak apex. The peak end is detected by a negative slope threshold, similar to the peak start.

# Peak detection - derivation

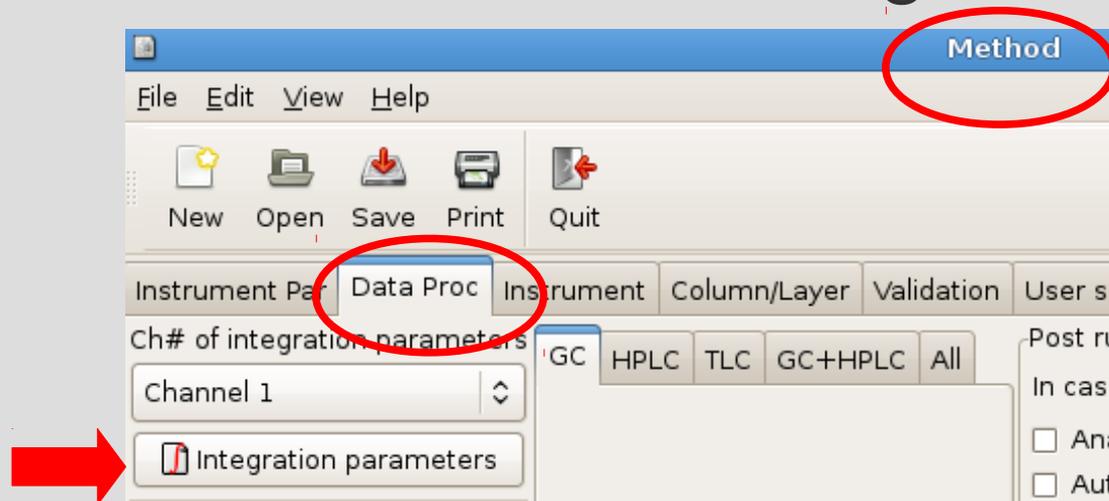
## ◆ Look, how does it work



When the sampling rate is not in concerning with peak width, the derivative data values can be too low, the peaks can not be detected.  
In TLC scanning the sampling rate therefore is 0.2 mm, that give detectable peaks.

# Integration parameters

- ◆ Select the integration button to set the parameters. 
- ◆ If the parameters give acceptable result, then you can store these parameters in the **Method** file and the next measure process will be finished with good automatic results



# Integration parameter settings

## ◆ The detection parameters: slope and threshold

The screenshot shows a software window with the 'Detection' tab selected. The window contains several sections of parameters, each with a title and three spinners (up, down, and a central value):

- Detection parameters:**
  - Up slope: 10.000
  - Down slope: -10.000
- Thresholds:**
  - Area: 10000.000
  - Height: 1000.000
  - Width: 0.100
- Solvent peak detection parameters:**
  - Slope: 0.000
  - Area: 0.000
  - Height: 0.000
  - Width: 0.000
- Followers:**
  - Upside: 1
  - Apex: 1
  - Downside: 1

On the right side of the window, there are four buttons: OK, Cancel, Help, and Apply.

- Slope: as shown on *Integration basics* slide
- Thresholds: **All** the three thresholds values have to be override to detect a peak. They have logical *AND* connection.
- Solvent: Do not care in TLC scanning, it is a GC speciality
- Followers: If the signal is too noisy, the peak detection can be unstable, try to increase the appropriate values to 2 or 3. Those are calculated in data points unit.

# Integration parameter settings

## ◆ The lock table

Detection Advanced **Lock** Merge Timed events

Start lock: 0.000  Drop truncated peaks

#	Lock ON	Lock OFF
1	1.000	46.000

1 Lock ON: 1.000 Lock OFF: 46.000

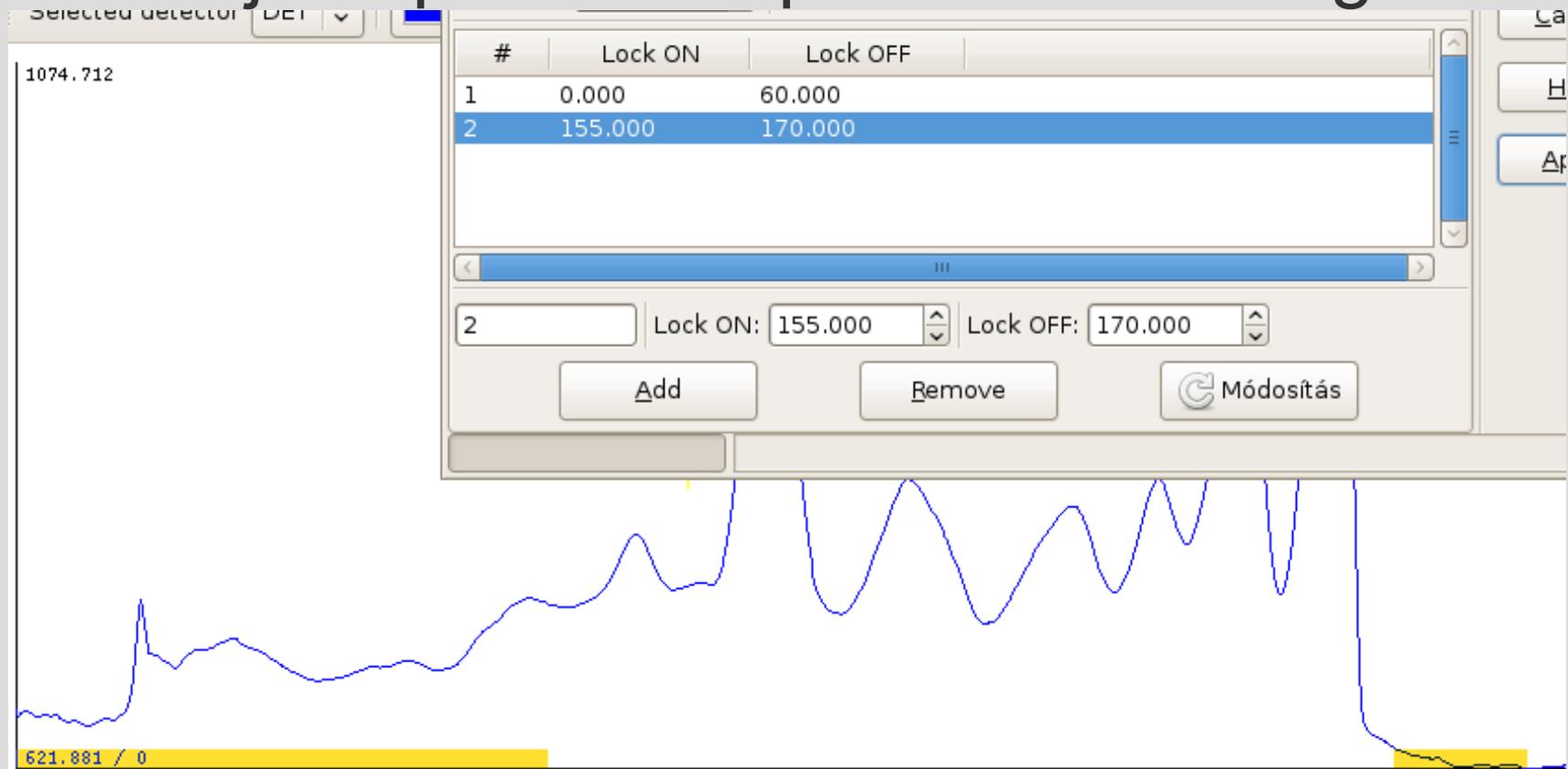
Add Remove Módosítás

OK Cancel Help Apply

You can add, remove and modify existing lock areas.

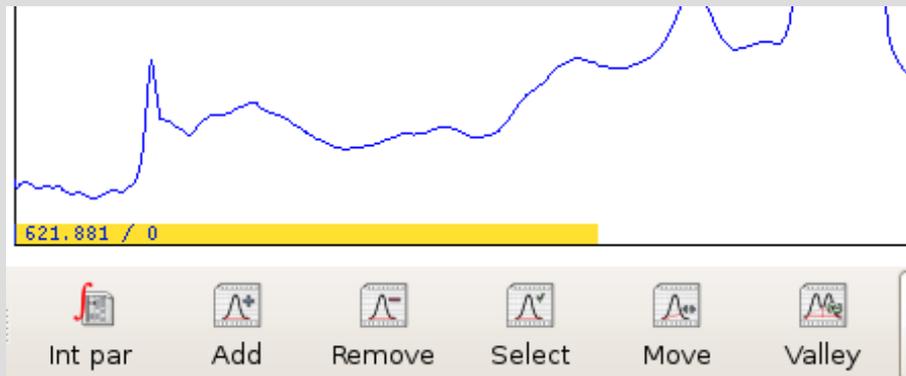
# How lock works?

- ◆ The locked area are excluded (shown with yellow bars) from integration, it is very useful when junk peaks are present in the signal.



# *Manual integration*

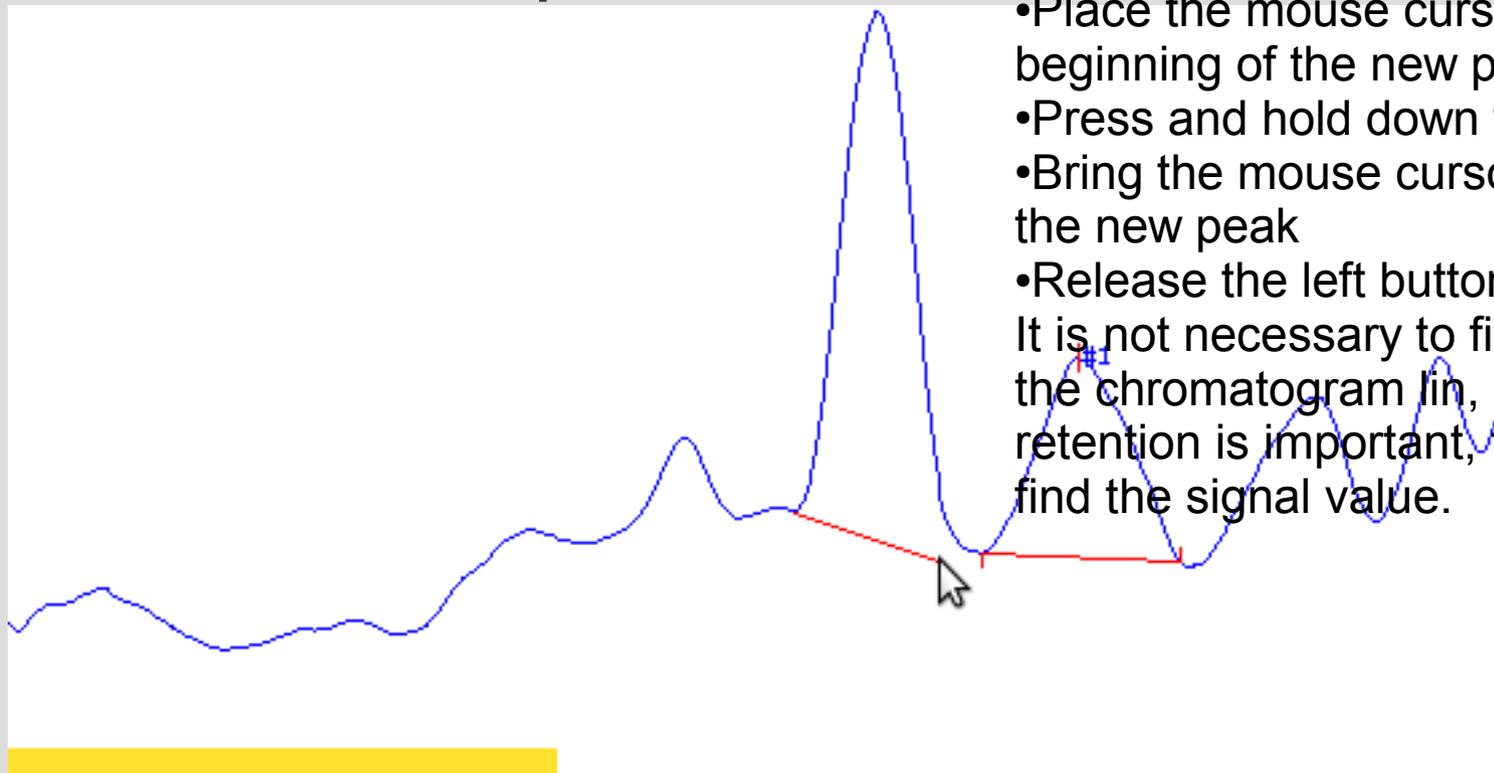
- ◆ You can add, remove, modify peaks manually



# Add peak

## ◆ How to add peaks?

- Press „**Add**” button
  - Place the mouse cursor to the beginning of the new peak
  - Press and hold down the left button
  - Bring the mouse cursor to the end of the new peak
  - Release the left button
- It is not necessary to fit the points to the chromatogram lin, only the retention is important, the software will find the signal value.



Add



Remove



Select



Move



Valley



Tangent



Set

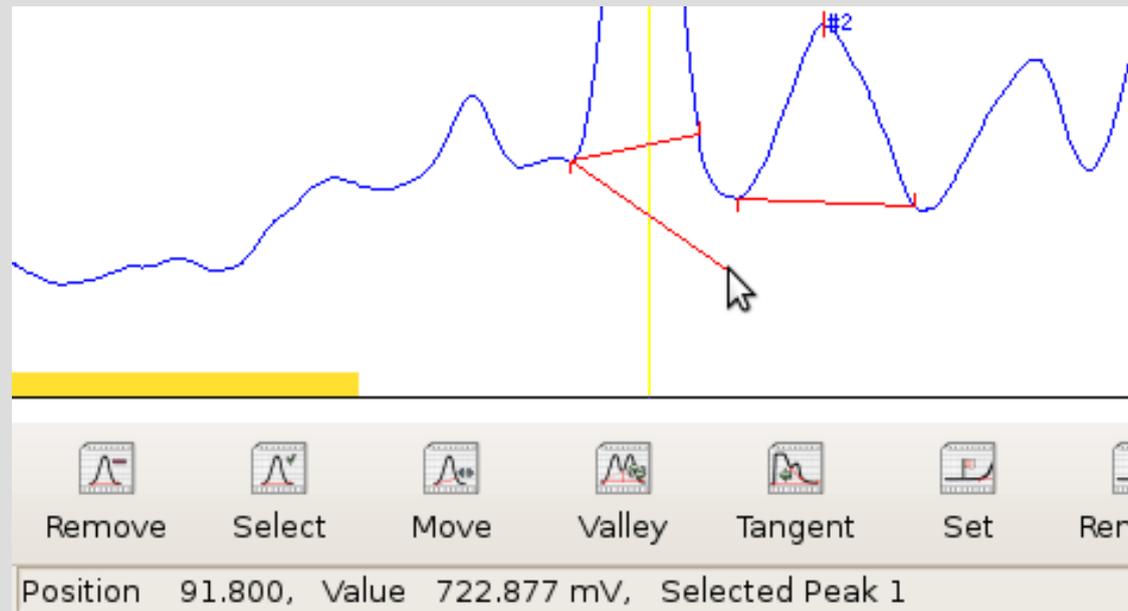


Remove

Position 89.400, Value 753.147 mV

# Delete - Move

- ◆ To delete a peak from the list, first click in the peak area, the yellow cursor line will be placed there. Then press **Remove** button.
- ◆ To move a basepoint, press **Move** button, drag the basepoint and drop to the new place.

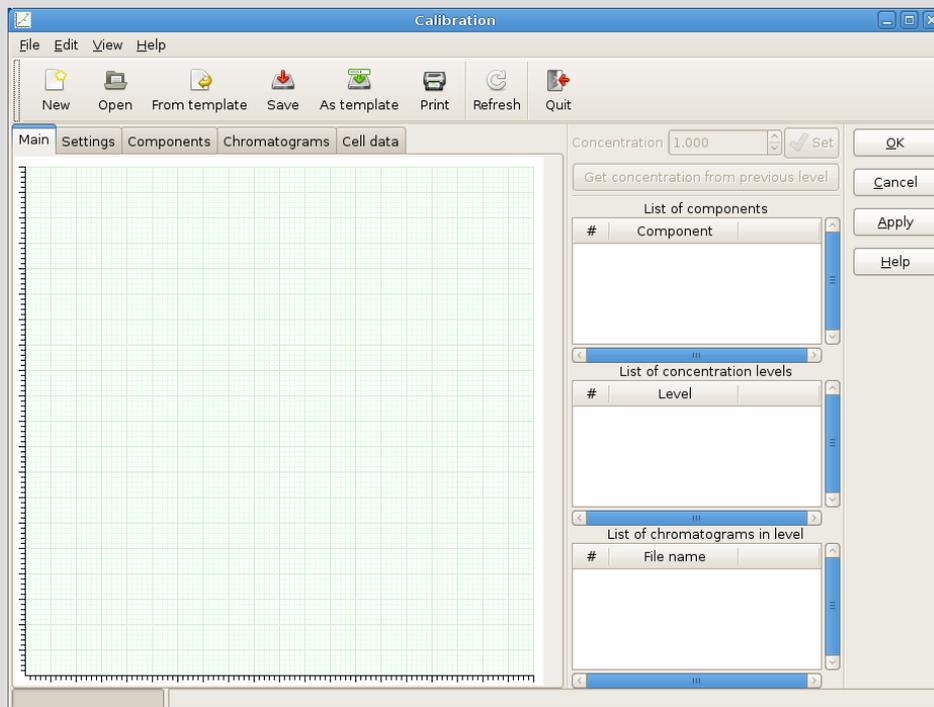


# *Print*

- ◆ To print the results press *Print* button.
- ◆ Select the printer.
- ◆ Press Print button.
- ◆ Before you print, in the menupoint „*Printer settings*” you can set the graphical parameters for the print.
- ◆ The report can be directly print to a PDF file.

# Calibration

- ◆ Multi component
- ◆ Multi level (concentrations levels)
- ◆ Multi chromatogram (parallel chromatograms inside in one level)



# Component settings

## ◆ Fill the component list

Concentration 1.000

Get concentration from previous level

List of components

#	Component
1	Comp-1
2	Comp-2

Main Settings **Components** Chromatograms Cell data

Template chromatogram to find easier the retentions

Name   Internal standard Detector:

ID num

ID

Detector

Retention

When you filled the fields, press **Add** button.

# Level settings

## ◆ Add level names

Main Settings Components **Chromatograms** Cell data

Add / Delete / Modify levels if necessary

Level name

Amount  Volume  Factor

Inj.vol.  IST conc

Temper.  Pressure

Add (Delete / Modify) chromatogram to the selected level if necessary

Concentration

List of components

#	Component
1	Comp-1
2	Comp-2
3	Comp-3

List of concentration levels

#	Level
1	Level-1

# Fill levels with chromatograms

- ◆ Select a level and **Select/Add** the chromatogram in to the level.

The screenshot displays a software interface for managing chromatograms. At the top, a text box contains the file path `/home/labor/LabChrom2/Data/2005/07/29/2005_07_29_kamilla K32.001` and a **Select** button. Below this are **Add**, **Remove**, and **Módosítás** buttons. A **Select chromatogram** dialog box is open, showing a table of available chromatograms:

#	Type	Name
1	Sample	2005_07_29_kamilla K32.001.chr
2	Sample	.
3	Sample	.
4	Sample	.
5	Sample	.

The main interface also features a **List of concentration levels** table with one entry:

#	Level
1	Level-1

Below this is a **List of chromatograms in level** section with a **File name** field containing `2005_07_29_kamil`. On the left, there are input fields for **Chromatogram name**, **Comment**, **Amount** (0.001), and **Inj.vol.** (0.001).

# *Set concentrations*

- ◆ Select a Component **and** a Level, now you can fill the Concentration field.

Concentration 0.001

List of components

#	Component
1	Comp-1
2	Comp-2
3	Comp-3

List of concentration levels

#	Level
1	Level-1

# Fill cells

- ◆ If you filled all the component names, all the level names, assigned all the chromatograms to their levels, you can fill the cells.

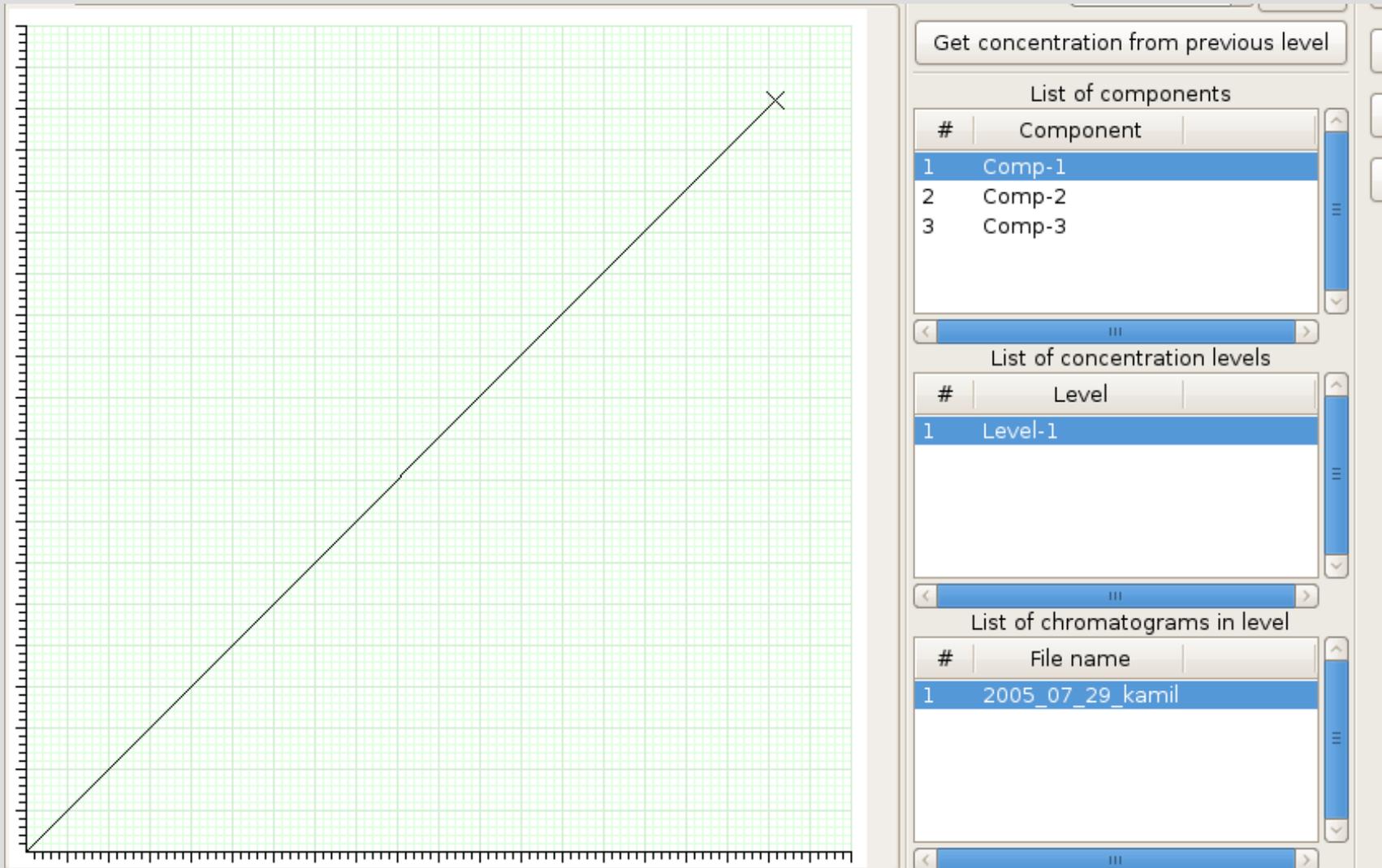
This filling means you must select the Component, the Level, the Chromatogram (each of them), the peak from the chromatogram (in the middle block) and pressing the button **Set to cell**. This process must be repeated to each of the components, levels, chromatograms.

The screenshot displays a software interface with several panels:

- Component status:** A table with columns for #, Status, Retention, and Name.
- Detector:** A dropdown menu currently set to 'DET'.
- Peak list of selected chromatogram:** A table with columns for #, Retention, and Area. The first row is highlighted: #1, Retention: 14.000, Area: 30854.000.
- Get concentration from previous level:** A button at the top of a panel.
- List of components:** A table with columns for # and Component. It lists three components: #1 Comp-1, #2 Comp-2, and #3 Comp-3.
- List of concentration levels:** A table with columns for # and Level. It lists one level: #1 Level-1.
- List of chromatograms in level:** A table with columns for # and File name. It lists one chromatogram: #1 2005\_07\_29\_kamil.
- Control buttons:** 'Set to cell', 'Identify', 'Find & set', 'All comp's', 'All levels', and 'All chrom's'.
- Unfilled components:** Radio buttons for 'Do nothing', 'Highlight them', and 'Show only them'.
- Unidentified peaks:** Radio buttons for 'Do nothing', 'Highlight them', and 'Show only them'.
- Selected peak's data:** A panel showing details for the selected peak: Retention: 14.000, Height: 3328.857, Area: 30854.000.
- Cell data at selection:** A panel showing: Cellidx: 0, Retention: 14.000, Height: 3328.857, Area: 30854.000.

# Curve

- ◆ Then you can see the calibration curve



# Analysis

- ◆ If you finished the calibration table, you can select the **Analysis** function.

The screenshot shows a dialog box titled "Analyze" with the following fields and options:

- Chromatogram:** /home/labor/LabChrom2/Data/2005/07/29/2005\_07\_29\_kamilla K32.001.chi
- Queue (sequence, layer):** /home/labor/LabChrom2/Data/Kerteszeti/29/2005\_07\_29\_kamilla K32.lay
- Calibration table:** (Empty field)
- Calculate on:**
  - Single chromatogram
  - Full sample queue

Buttons on the right: OK, Cancel, Apply, Help.

You can analyze either a single chromatogram or the full table. Analysis means the component identification and the calculation of the concentrations.

# *More information*

[www.chemotron.hu/service](http://www.chemotron.hu/service)

- ◆ New software versions
- ◆ Presentations
- ◆ Upgraded user manuals
- ◆ Application notes