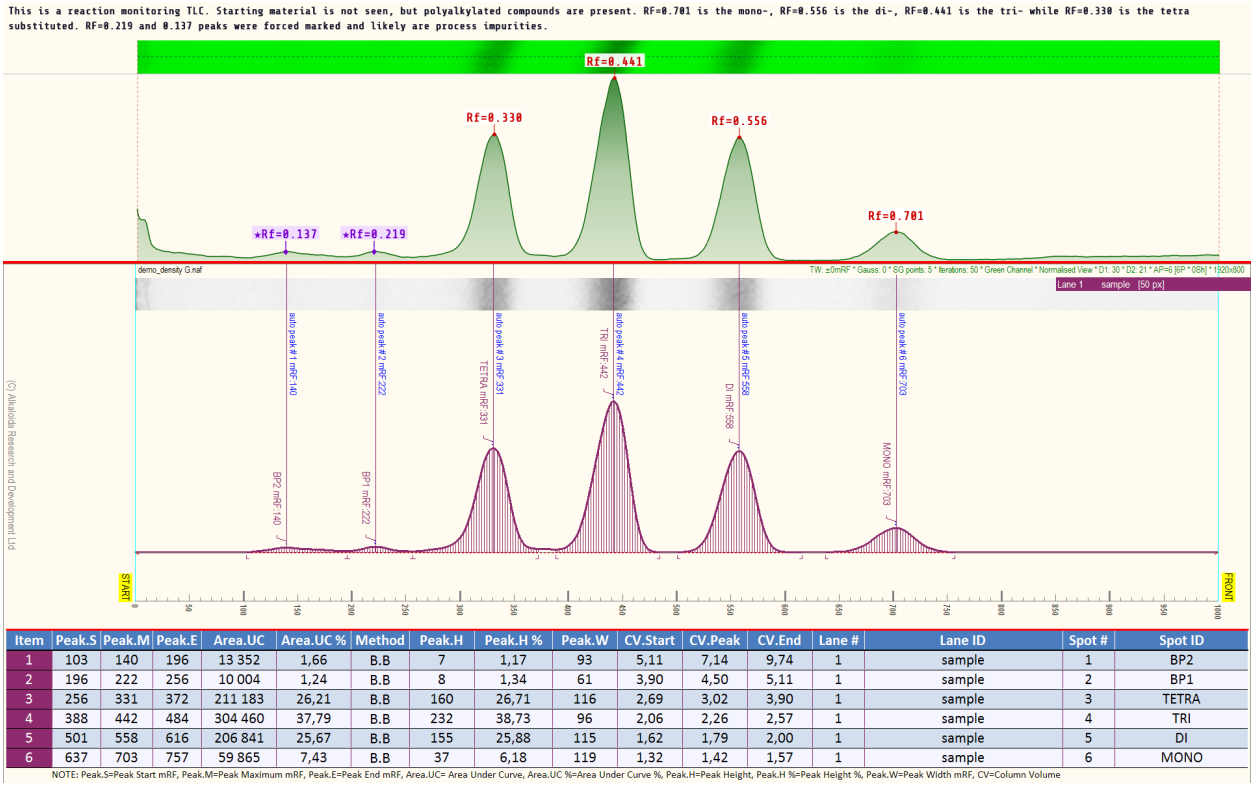
# TLC RF READER

## User Manual

Version 1.0

*Interactive TLC plate image analysis with Rf measurement, intensity profiling, and peak detection.*




| Item | Peak.S | Peak.M | Peak.E | Area.UC | Area.UC % | Method | Peak.H | Peak.H % | Peak.W | CV.Start | CV.Peak | CV.End | Lane # | Lane ID | Spot # | Spot ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 103 | 140 | 196 | 13 352 | 1,66 | B.B | 7 | 1,17 | 93 | 5,11 | 7,14 | 9,74 | 1 | sample | 1 | BP2 |
| 2 | 196 | 222 | 256 | 10 004 | 1,24 | B.B | 8 | 1,34 | 61 | 3,90 | 4,50 | 5,11 | 1 | sample | 2 | BP1 |
| 3 | 256 | 331 | 372 | 211 183 | 26,21 | B.B | 160 | 26,71 | 116 | 2,69 | 3,02 | 3,90 | 1 | sample | 3 | TETRA |
| 4 | 388 | 442 | 484 | 304 460 | 37,79 | B.B | 232 | 38,73 | 96 | 2,06 | 2,26 | 2,57 | 1 | sample | 4 | TRI |
| 5 | 501 | 558 | 616 | 206 841 | 25,67 | B.B | 155 | 25,88 | 115 | 1,62 | 1,79 | 2,00 | 1 | sample | 5 | DI |
| 6 | 637 | 703 | 757 | 59 865 | 7,43 | B.B | 37 | 6,18 | 119 | 1,32 | 1,42 | 1,57 | 1 | sample | 6 | MONO |

NOTE: Peak.S=Peak Start mRF, Peak.M=Peak Maximum mRF, Peak.E=Peak End mRF, Area.UC= Area Under Curve, Area.UC %=Area Under Curve %, Peak.H=Peak Height, Peak.H %=Peak Height %, Peak.W=Peak Width mRF, CV=Column Volume

Upper image: current TLC RF Reader. Bottom image: advanced TLC Analyzer.

# 1. Overview

TLC RF Reader is a browser-based tool for analysing Thin-Layer Chromatography (TLC) plate images. It allows you to:

- Load a TLC plate photograph directly in the browser (no data is uploaded to any server).
- Define the baseline (START) and solvent front (FRONT) to calibrate the lane.
- Move the crosshair along the lane and read the Rf value at any position in real time.
- View the pixel intensity profile of the lane as an interactive plot.
- Detect peaks automatically and force-assign additional peaks by keyboard.
- Set a background reference row for background subtraction.
- Annotate the plot with free text and export the result as a PNG image.

> TLC RF Reader runs entirely in your web browser. No image data ever leaves your device.

# 2. Getting Started

## 2.1 Opening the Application

Open the file **TLC_RF_Reader.html** in a modern browser (Chrome, Edge, Firefox, or Safari). No installation or internet connection is required.

## 2.2 Loading an Image

There are two ways to load a TLC plate image:

- Click the **OPEN IMAGE** button in the header, or press O.
- Drag and drop an image file anywhere onto the browser window.

Supported formats include JPEG, PNG, TIFF, WebP, and all other formats natively supported by the browser.

> When a new image is opened, the plot area, text annotation, and all markers are automatically cleared and reset to a blank state.

## 2.3 Rotating the Image

Images are automatically rotated 90° clockwise on load to display TLC lanes in the correct vertical orientation. You can fine-tune the rotation:

- → (Right Arrow) — rotate 90° clockwise
- ← (Left Arrow) — rotate 90° counter-clockwise

Rotation resets the START and FRONT markers and clears the plot.

# 3. Setting Up the Lane

## 3.1 Setting the START Line

After an image is loaded, the crosshair turns red and prompts you to set the start line.

Click on the image at the position of the **baseline (origin)** of the lane — the point from which spots migrate.

A dashed red vertical line labelled **START** is drawn on the image at this position.

## 3.2 Setting the FRONT Line

After setting START, click a second time to the right of the start line at the position of the **solvent front**.

A second dashed red line labelled **FRONT** is drawn. The Rf reader is now active.

> The FRONT line must be placed to the right of the START line. Clicking to the left of START is ignored.

## 3.3 Resetting Markers

To reposition the START and FRONT lines, click the ↺ **RESET MARKERS** button in the header. The markers, plot, background reference, and forced peaks are all cleared.

# 4. Reading Rf Values

Once both lines are set, move the mouse across the image. While the crosshair is between the START and FRONT lines:

- A blue crosshair tracks your cursor.
- The current **Rf value** is displayed next to the crosshair in real time:

  ```
  Rf = (cursor position − START) / (FRONT − START)
  ```

- The **intensity plot** at the bottom of the screen updates continuously, showing the pixel intensity profile along the sampled horizontal row.

Scrolling the page updates both the crosshair label and the plot to reflect the new vertical position of the crosshair.

# 5. The Intensity Plot

## 5.1 What the Plot Shows

The lower third of the screen is the intensity plot panel. It contains:

- A **thumbnail strip** of the sampled pixel rows from the image, shown at the top of the plot.

- An **intensity curve** with a gradient fill showing pixel brightness from START to FRONT.
- Automatic **peak markers** (red circles with Rf labels) for detected local maxima.
- User-forced **peak markers** (purple diamonds with ★Rf labels) plac
  `F` key.

## 5.2 Sample Width

Intensity at each x-position is calculated by averaging pixels over a vertical strip of ±25 image pixels (51 rows total). This smooths out noise.

- `L` — widen the sample strip by 25 pixels
- `Ctrl+L` — narrow the sample strip by 25 pixels

The current sample width is shown in the status bar when changed.

## 5.3 Channel Selection

By default, intensity is averaged across the Red, Green, and Blue channels. You can switch to a single channel:

- `R` — toggle Red channel (press again to return to average)
- `G` — toggle Green channel
- `B` — toggle Blue channel

Only one channel is active at a time. Switching channels clears any stored background.

## 5.4 Invert and Normalise

- `I` — invert the intensity values (useful for UV-active plates where spots appear as dark dips)
- `N` — normalise the plot so the tallest peak fills the full plot height

## 5.5 Peak Detection

Peaks are detected automatically using a local-maximum algorithm with the following criteria:

- A peak must be strictly higher than all neighbours within a window of ±8 data points.
- The peak must have a prominence of at least 6% of the total data range.
- Peaks closer than 4% of the lane width are merged (the taller one is kept).
- Up to 10 peaks are displayed, chosen by descending intensity.

When labels from closely spaced peaks would overlap, the layout algorithm automatically shifts them apart horizontally and draws a dashed leader line to each peak marker, so you can always tell which label belongs to which peak.

# 6. Peak Operations

## 6.1 Forcing a Peak [F]

Sometimes a real spot may not meet the automatic detection thresholds. You can force-assign a peak at any position:

1. **Position the crosshair between START and FRONT at the Rf value of interest.**
2. **Press `F`.**

A purple diamond marker labelled `*Rf=x.xxx` appears on the plot at that position. The status bar confirms the Rf value and total count of forced peaks.

> Forced peaks are color-coded purple to distinguish them from auto-detected peaks (red). Duplicate positions within ±0.005 Rf are ignored.

## 6.2 Clearing All Forced Peaks [Ctrl+F]

Press `Ctrl+F` to remove all forced peak markers. The plot is redrawn immediately.

# 7. Background Subtraction

## 7.1 Setting the Background [H]

To remove a non-uniform background from the intensity curve:

1. Move the crosshair to a row of the image that contains **only background** (no spots).
2. Press `H`.
3. A dialog appears asking whether the background is **LIGHT** or **DARK**:
    - Choose ☀ `LIGHT` for bright plates (e.g., UV254 silica gel: spots appear dark on a bright background).
    - Choose ◑ DARK for dark plates (spots appear bright on a dark background).

The background intensity is sampled from the current crosshair row and subtracted from all subsequent plots. A dashed orange zero-baseline and a "BG ▸ SUBTRACTE on the plot to confirm.

## 7.2 Clearing the Background [Ctrl+H]

Press `Ctrl+H` to remove the stored background reference. The plot reverts to raw intensity values.

> Switching colour channel (R / G / B) automatically clears the background because the sampled values are channel-specific.

# 8. Text Annotation

## 8.1 Adding Text [T]

You can add a free-text label that appears above the intensity plot (and is included in exported images):

1. Press `T`. A dialog asks: *"Would you like to add some text?"*
2. Press `Y` (or click **Yes [Y]**) to open the text input area. Press `N` (or click **No [N]**) to cancel.
3. Type your annotation in the text box. Press `Ctrl+Enter` to render it, or press `T` again.

The text banner appears between the image and the plot. Long text wraps automatically and the banner expands to fit.

## 8.2 Clearing Text [Ctrl+T]

Press `Ctrl+T` at any time to remove the text annotation and collapse the banner.

> Opening a new image automatically clears any existing text annotation.

# 9. Exporting

## 9.1 Export as PNG [C]

Press `C` to save the current plot (including any text banner above it) as a PNG image file named `TLC_plot.png`.

The exported image is a composite of:

- The text banner (if text has been added).
- The intensity plot with the colour strip, curve, peak labels, and background indicators.

The image area above the plot is **not** included in the export — only the plot panel.

# 10. Closing the Application

Press `Ctrl+X` to close the browser window/tab running TLC RF Reader.

> Ctrl+X calls window.close(). Depending on your browser security settings, this may only work when the tab was opened programmatically (e.g., from a script or another page). If nothing happens, simply close the tab manually.

# 11. Keyboard Shortcut Reference

All shortcuts are case-insensitive. Shortcuts marked Ctrl also accept Cmd on macOS.

## Image & Navigation

| Key / Shortcut | Action |
|---|---|
| O | Open image file (file picker) |
| ← / → | Rotate image 90° counter-clockwise / clockwise |

## Lane Calibration

| Key / Shortcut | Action |
|---|---|
| Click (phase 1) | Set the START (baseline) line |
| Click (phase 2) | Set the FRONT (solvent front) line |

## Plot Options

| Key / Shortcut | Action |
|---|---|
| R | Toggle Red channel (press again for average) |
| G | Toggle Green channel |
| B | Toggle Blue channel |
| I | Invert intensity values |
| N | Normalise plot to tallest peak |
| L | Widen sample strip by 25 px |
| Ctrl+L | Narrow sample strip by 25 px |
| C | Export plot as PNG |

## Background Subtraction

| Key / Shortcut | Action |
|---|---|
| H | Set background reference at current crosshair row |
| Ctrl+H | Clear background reference |

## Peak Operations

| Key / Shortcut | Action |
|---|---|
| F | Force-assign a peak at the current crosshair Rf value |
| Ctrl+F | Clear all forced peaks |

## Text Annotation

| Key / Shortcut | Action |
|---|---|
| T | Open the Add Text dialog |
| Y | Answer Yes in the text dialog (open text input) |
| N | Answer No in the text dialog (cancel) |
| Ctrl+Enter | Commit typed text and render banner (inside text box) |
| T | Commit text and render banner (second press while text box open) |
| Ctrl+T | Clear text annotation |

## Application

| Key / Shortcut | Action |
|---|---|
| Ctrl+X | Close the application window |

# 12. Step-by-Step Workflow Example

The following example shows a complete analysis session from image load to export.

## Step 1 — Load the image

- Press `O` and select your TLC photograph, or drag it onto the window.
- If the orientation is wrong, press → or ← to rotate.

## Step 2 — Set the lane boundaries

- Click on the baseline (origin) of the lane. The **START** line appears.
- Click on the solvent front. The **FRONT** line appears and the RF reader activates.

## Step 3 — Set the background (optional)

- Move the crosshair to an empty row of the plate (above the spots or in the margin).
- Press `H` and select ☀ `LIGHT` or ◑ DARK as appropriate.

## Step 4 — Read Rf values

- Move the cursor across the lane. The Rf label updates in real time.
- The intensity plot shows the profile at the current crosshair row.
- Automatic peak markers (red) identify detected spots. Read their Rf values from the labels.

## Step 5 — Force additional peaks if needed

- Position the crosshair over a spot that was not automatically detected.
- Press `F`. A purple ★ marker is added to the plot.

## Step 6 — Add a text annotation (optional)

- Press `T`, then `Y` to open the text box.
- Type your label (e.g., sample name, solvent system, date).
- Press `Ctrl+Enter` to render.

## Step 7 — Export

- Press `C` to save `TLC_plot.png`.

> To analyse a different lane in the same image, press the ↺ Reset Markers button from Step 2.

# 13. Tips and Troubleshooting

### Plot shows no peaks / all peaks are missing

The automatic detector requires a minimum prominence of 6% of the data range. Try:

- Switching colour channel (`R`, `G` `B`) to one that gives stronger contrast for your staining method.
- Setting a background reference (`H`) to flatten the baseline before detection.
- Using `F` to force-assign peaks that the detector misses.

### Labels overlap even after layout adjustment

The overlap resolver shifts labels apart horizontally. For very dense spots, some crowding may remain. Consider:

- *Zooming the browser window (*`Ctrl+` / `Ctrl-`) to spread the image and lane wider.
- Narrowing the lane (reposition START or FRONT closer together) to isolate a region of interest.

### Background subtraction makes the curve flat

The sampled background row may contain spot signal. Choose a row that is truly free of spots. Press `Ctrl+H` to clear the background and try a different row.

### Rf value reads incorrectly

Verify the START and FRONT lines are placed precisely. Small errors in line placement will shift all Rf values uniformly. Use Reset Markers and re-click to recalibrate.

### Ctrl+X does not close the window

Some browsers block `window.close()` if the tab was opened by the user directly (not by a script). In this case, simply close the tab manually using the browser's close button or `Ctrl+W`.