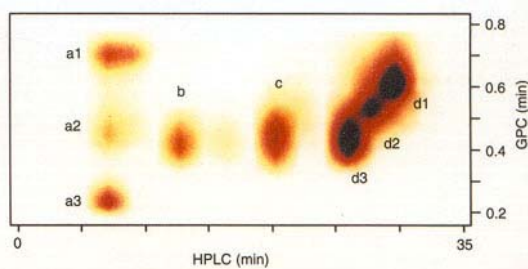


# LCLC Two-dimensional Chromatography Software from Kroungold Analytical, Inc.

Two-dimensional liquid chromatography (2DLC) offers many new capabilities for separation and analysis in fields as diverse as polymer characterization and proteomics research. The power of this method is just now being understood.

What the method does is periodically direct small aliquots of solute from the outlet of one column into the inlet of another column. The columns are chosen so that one retention mechanism in the first column (dimension) is vastly different than the retention mechanism in the second column (dimension). Examples typically include a reversed-phase (RP) column in the first dimension followed by a size-exclusion column in the second or an ion-exchange column in the first dimension followed by a RP column in the second dimension. Detection is typically a UV detector or evaporative light scattering detector. This technique becomes even more powerful when a UV diode-array detector and/or a mass spectrometer are used. The data are typically plotted as concentration contours as follows:



The 2DLC method has largely been practiced and developed by academic scientists who have looked at basic separations and have developed a number of protocols for use with this technique. The problem for increased utilization of this technique has been the lack of available turnkey solution software and hardware. These findings along with applications are summarized in a recent book on multidimensional chromatography (1).

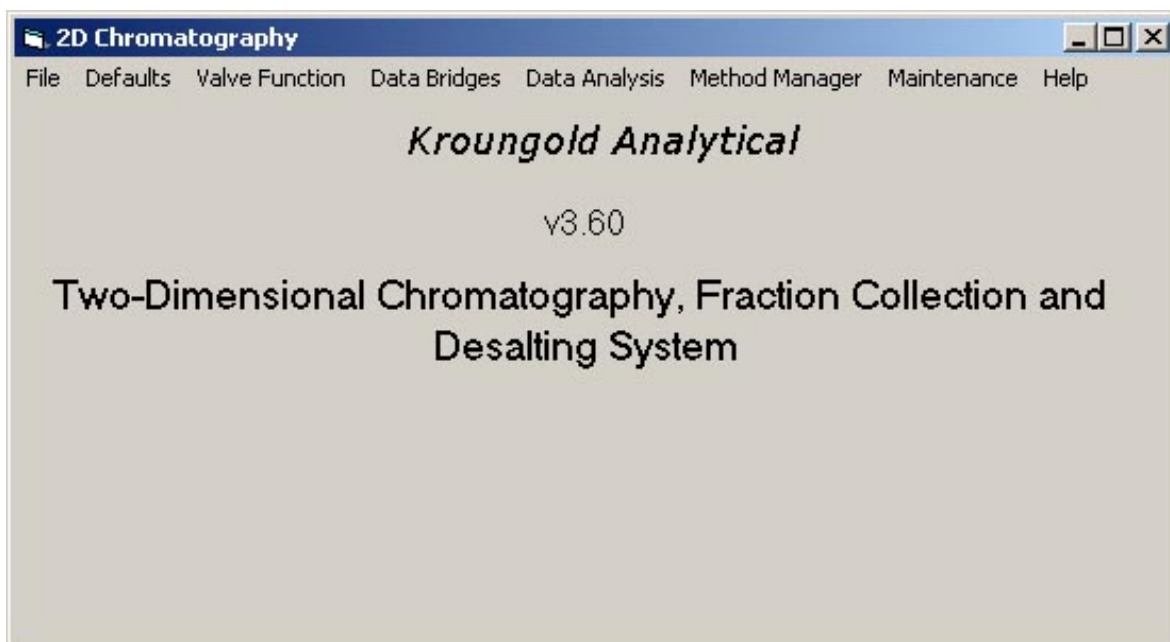
With LCLC from Kroungold Analytical, Inc., we introduce the key components solution to the implementation of multidimensional chromatography. This includes both software and hardware for valve control, data processing, data analysis, data presentation and methods development. Mass spectrometry data handling is also included as an integral part of the software offering. The hardware component includes start/stop sequencing and controlling external devices such as an autosampler which is directed from the LCLC software.

This allows one to assemble off-the-shelf components to form a total 2D LC system at a fraction of the cost that one might normally expect. For example, simply add one or two specified valves, add an extra HPLC pump and a second column and the 2DLC capability can be yours.

We will describe the hardware and software separately. Additional questions and price quotes can be obtained by looking at our website <http://www.kroungold.com> and e-mailing us at [kroungold.analytical@GMail.com](mailto:kroungold.analytical@GMail.com), respectively.

## Software

User Interface. The program, LCLC, is a standard windows-based program that loads from compact disk. All features are run from drop-down menus or command buttons. The software runs under Windows NT, Windows 2000, or Windows XP.



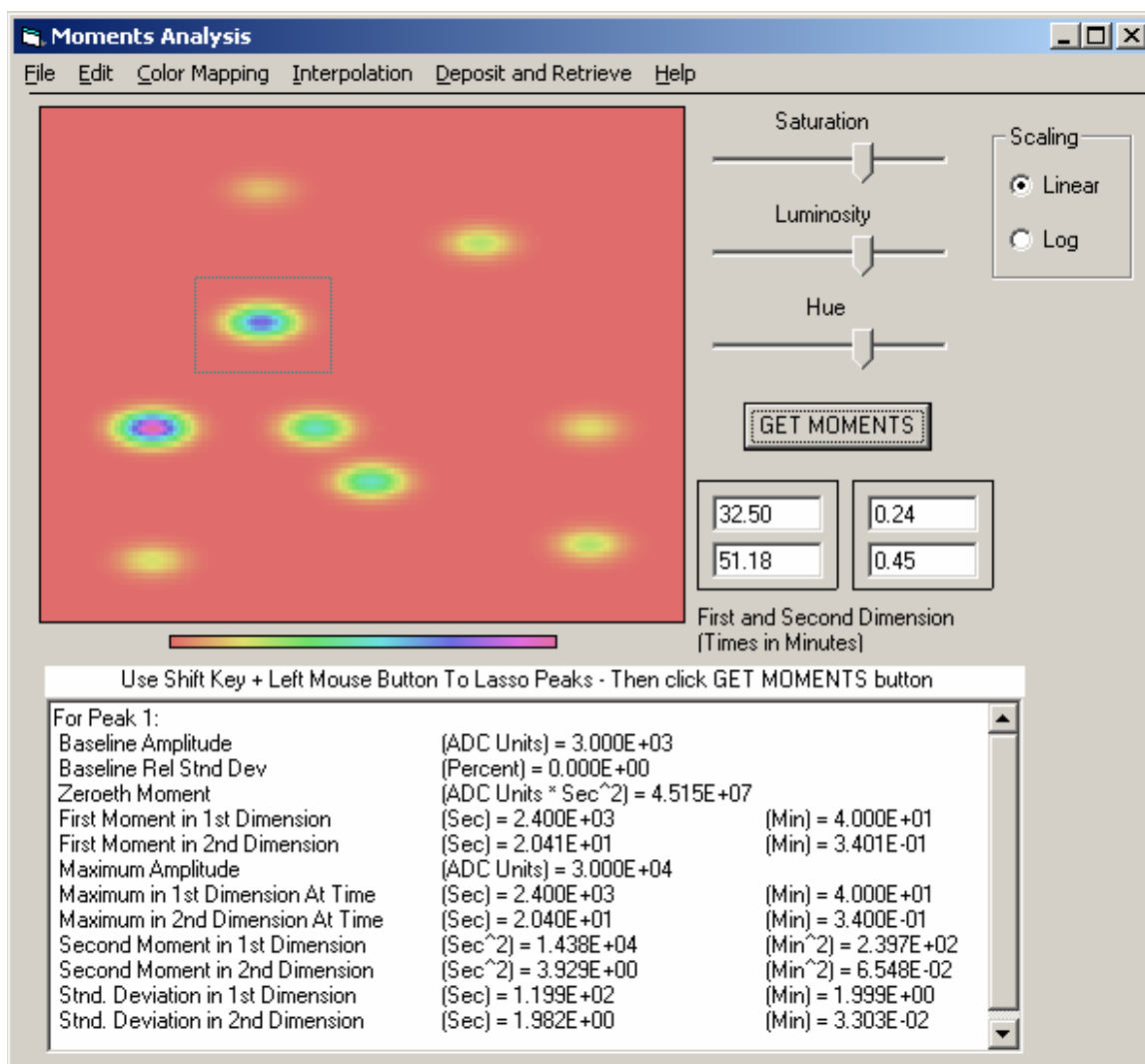
### Valve function methods:

- “Just” 2D chromatography. A two-position valve is used to switch between sample loops, no additional valves are needed.
- 2D Chromatography with 2D fraction collection. A second, multi-position valve, is utilized to collect fractions within specified rectangular times. This allows “spots” to be collected into tubes for sample prep for very difficult sample isolation problems. Automatic operation allows preparative sequences to be continued for repeated fractionation with an autosampler.
- 2D Chromatography with desalting. A second, multi-position valve, is utilized to divert a non-retained salt peak to waste. This is highly useful when using ion exchange columns and salt buffers which can cause problems when a mass spectrometer is used as a detector. Note that desalting and 2D fraction collection can be implemented easily using the 2D fraction collection mode.
- 1D fraction collector. This mode allows complex 1D fraction collection. With automatic mode, the fractionation can continue and be repeated.

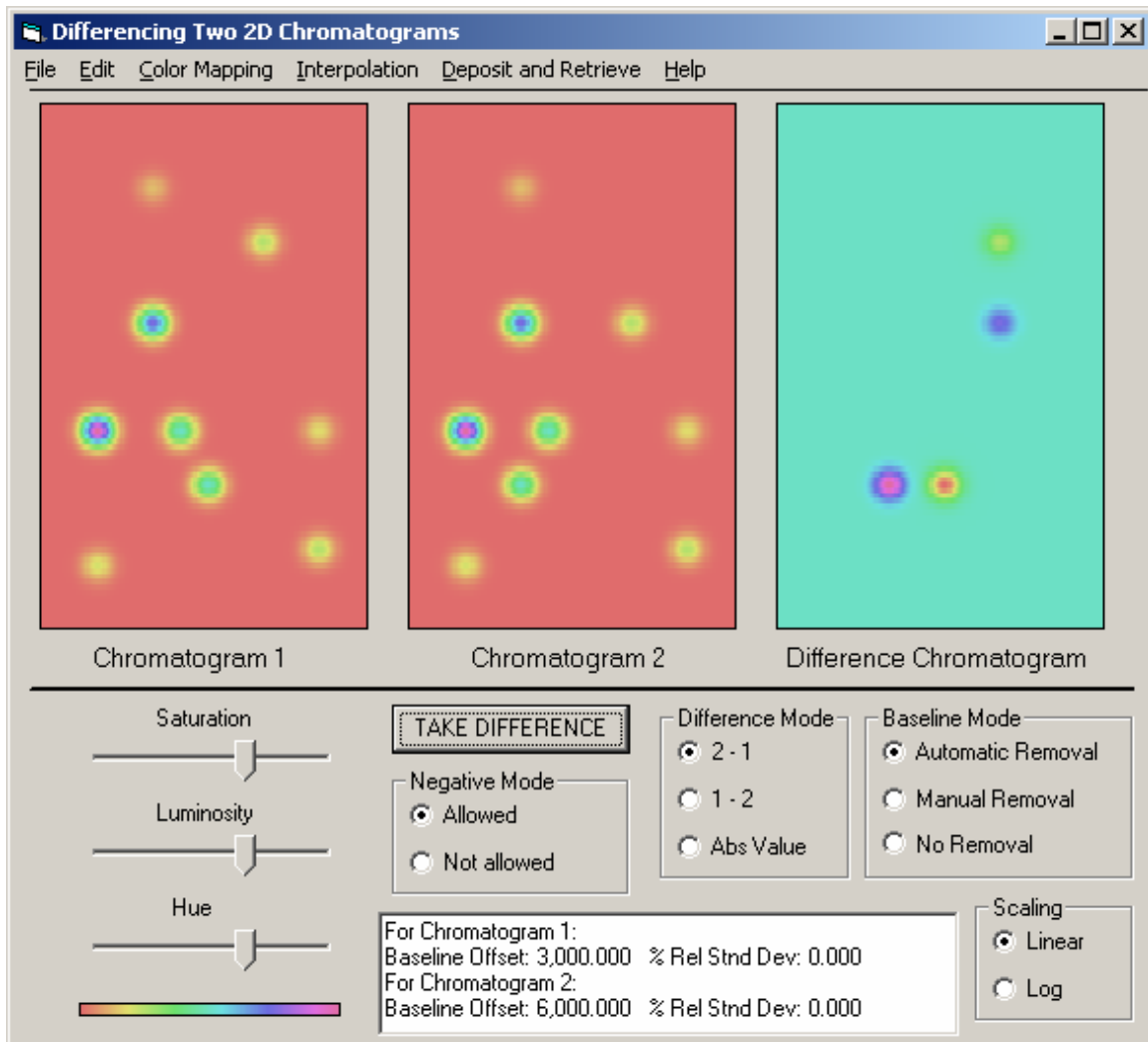
### Analysis modes:

There are two analysis modes: one for single channel detectors like UV or evaporative light scattering, useful for polymers, and multi-channel detectors such as mass spectrometry or diode array detection, typically used in biological applications. We will review the software for both modes.

Single channel analysis. In all cases 2D chromatography consists of a series of 1D data vectors. These data vectors are processed and turned into a 2D dataset in the LCLC software. One of the single channel analysis methods allows complete quantitation of retention times, statistical moments with 2D baseline subtraction, differencing between two 2D chromatograms and more. The moments analysis panel is shown here.



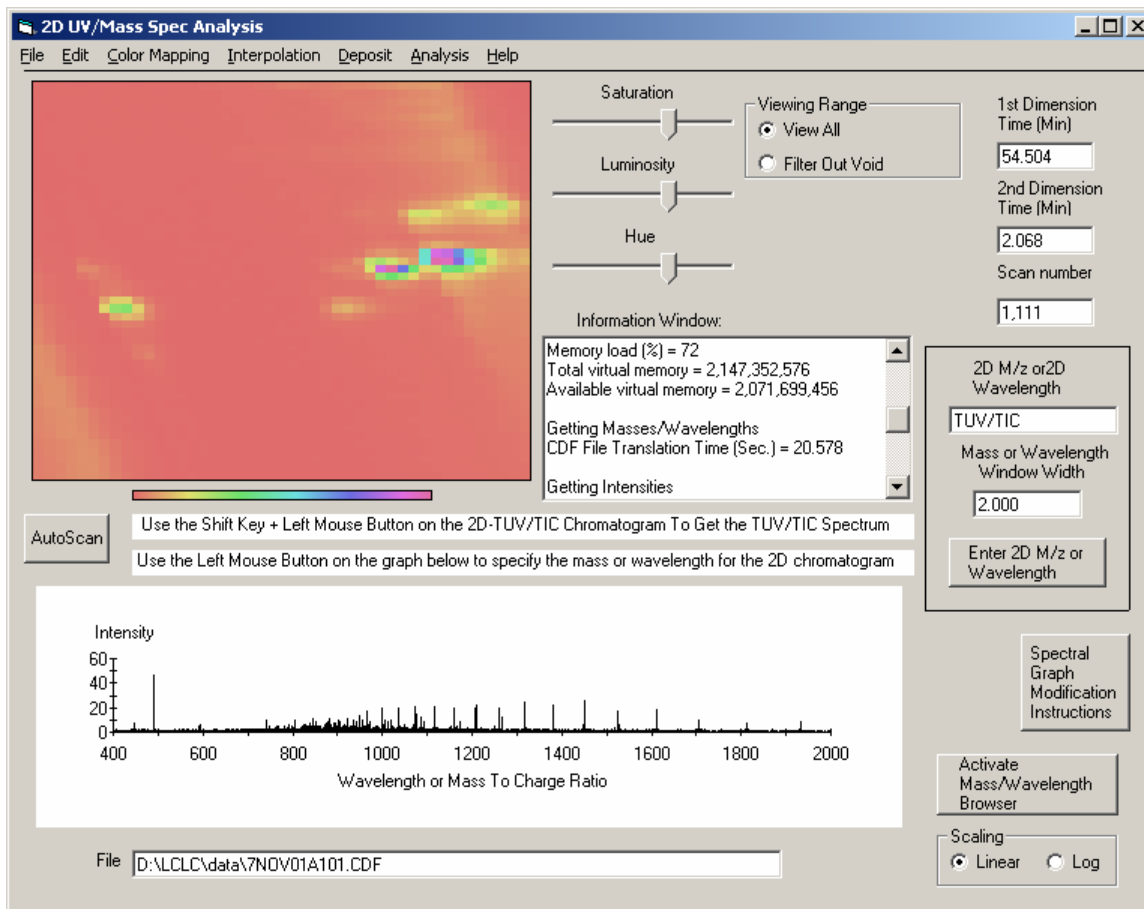
Even slices from the multi-channel analysis system can be stored in the data deposit facility and analyzed as a single channel system for moments. Differencing can also be conducted this way. Differencing of two single-channel 2D chromatograms is shown below here:



This can be very powerful for quality assurance applications as many well-known industrial firms have been exploiting 2D chromatogram differences to detect products that differ from batch to batch variation.

Multi-channel analysis. 2D liquid chromatography followed by mass spectrometry is an extremely powerful technique for complex analysis with applications from polymer analysis to complex proteomics and metabolomics analysis. For multi-channel analysis, the user is supplied at first with a total intensity (e.g. total ion current) 2D chromatogram map. This is shown below for a series of protein standards that could not be resolved with a 1D method. The user can then hold down the shift key and hit the left mouse button to point to parts of the chromatogram - this will reveal the mass spectrum of the specified peak. The user can click on any part of the mass spectrum and get the 2D chromatogram displayed using the selected m/z ratio. This “back and forth” analysis provides a key method to get at the underlying structure of the data. The scan number is listed as each location where the 2D chromatogram is clicked. This allows the user an easy way to export the mass spectra at parts of the 2D chromatogram from their native mass spec

software. A mass or wavelength-specific 2D chromatogram can be analyzed with the 1D tools such as moments and/or differencing.



All data, i.e. both single channel and multi-channel data are read in as NetCDF files. The NetCDF file system is an encapsulation system that packages the data and provides it in a universal package. Most all chromatographic and mass spectrometry data systems have NetCDF file capabilities. This allows a very convenient and efficient manner for data interchange.

## Hardware

The hardware interface allows the LCLC system to control the other chromatographic components or to act as a slave unit. The interface electrically can receive signals which are compatible with TTL levels (transistor-transistor logic) and with relay source and/or sinking. Two light emitting diodes give the status of the triggering conditions.

This interface offers excellent versatility as it can also control autosamplers for use with 2D fraction collection.

The hardware interface, the two-position valve controller (available from Valco, Inc.) and an optional multi-position valve for fraction collection (also from Valco, Inc.) share a common serial port. For computers which do not have serial ports, a USB to serial converter can be used easily.

The hardware interface, Model I, is shown here:



Two cables are supplied with twisted-pair wiring which can be used for relay sensing (which triggers the software) and relay closing (which is triggered by the software). The software has a number of output relay triggering schemes. These cables are terminated with a ¼" stereo phone plug and solder-tinned wiring leads.

References:

1. Multidimensional Liquid Chromatography: Theory, Instrumentation and Applications edited by S. A. Cohen and M. R. Schure, John Wiley and Sons, New York, 2008.