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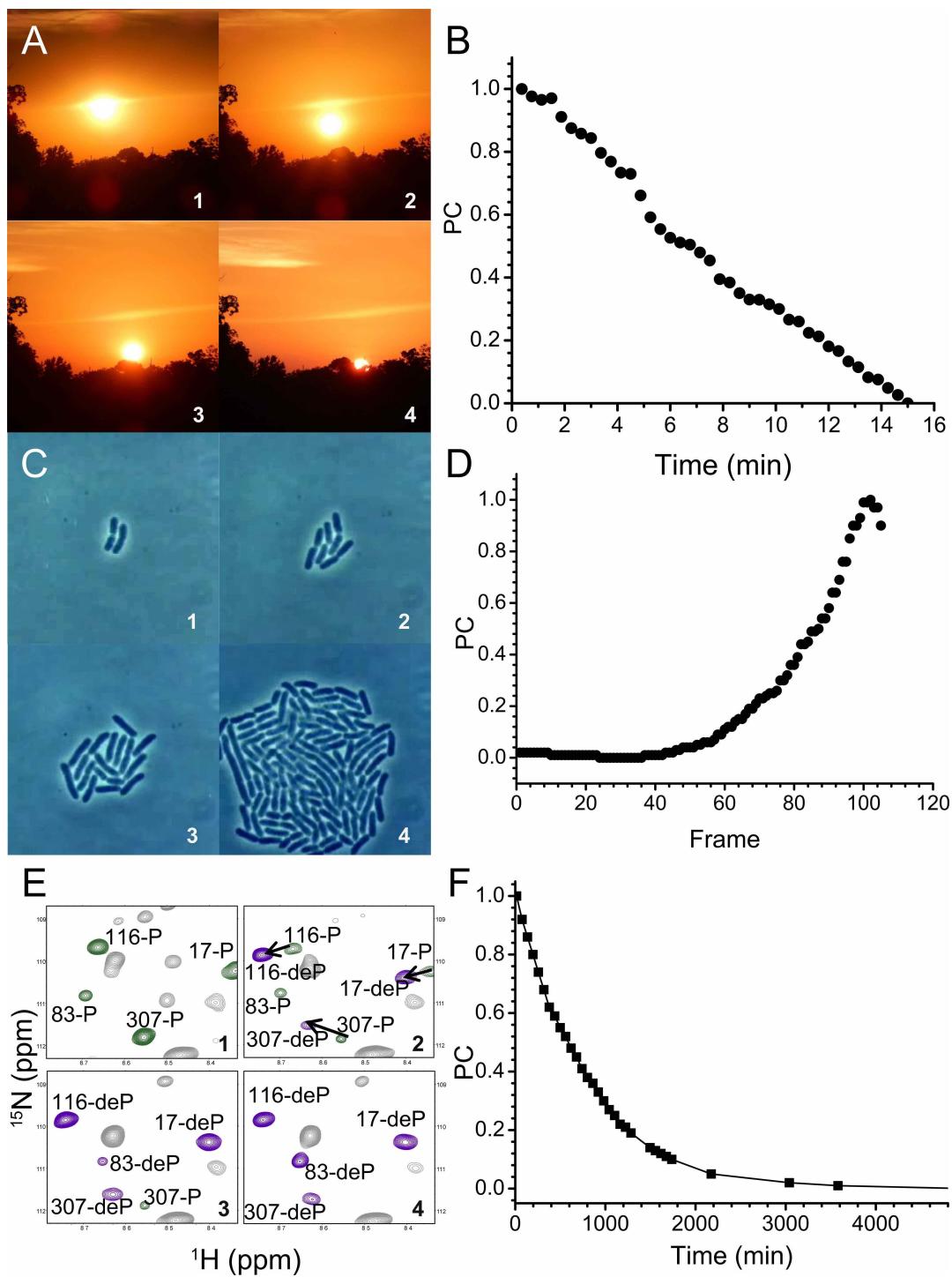
TREND: TRack Equilibrium or Non-equilibrium shifts in Data

Resolve Trends such as Binding Isotherms and Time Courses Directly from Spectra, Imaging, or Lists

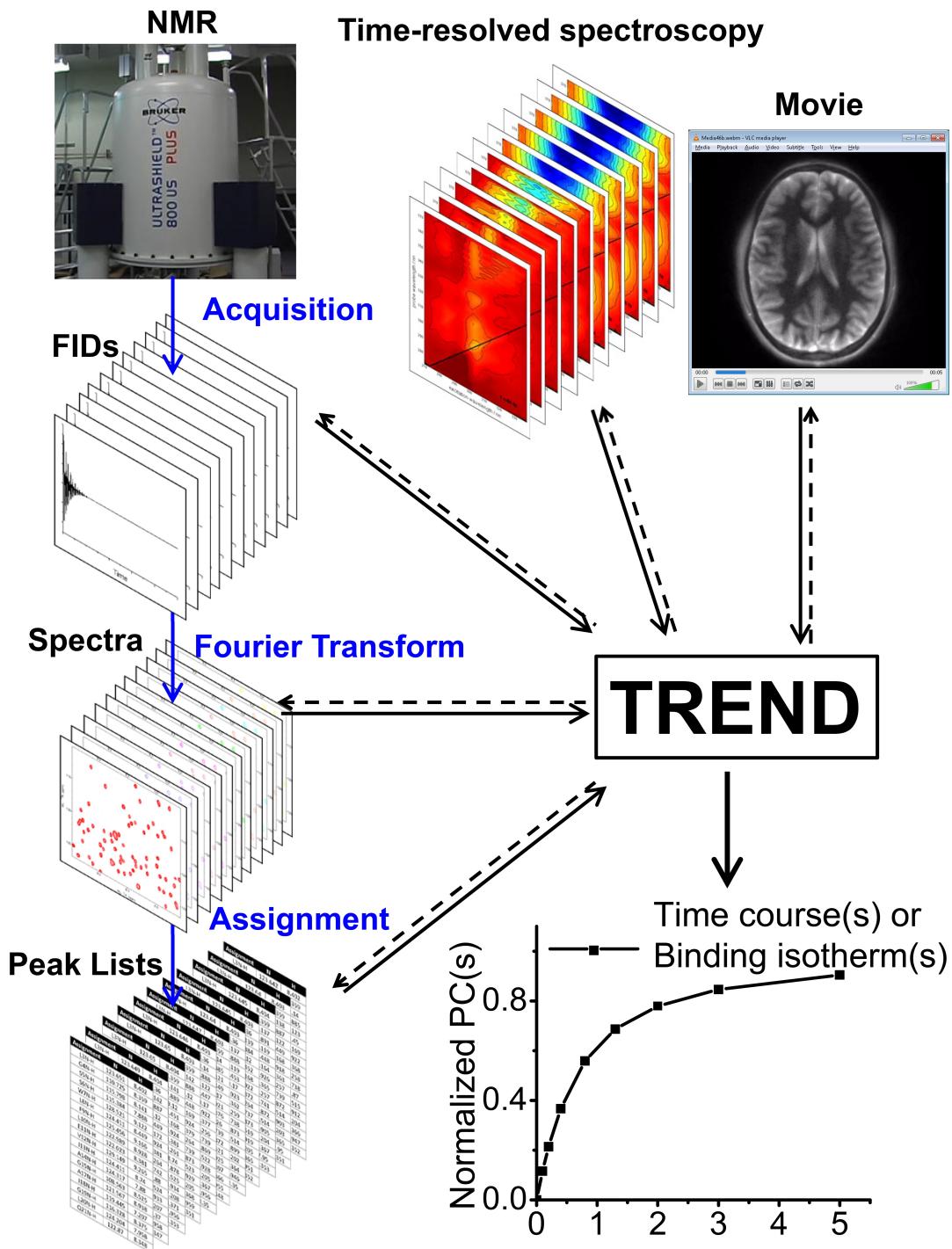
Current version 0.6.9.5 , new version is coming next week

Introduction:

TREND is a package that determines the main trend(s) of change across a series of 2D digital measurements, such as imaging and NMR spectra. TREND works by applying principal component analysis to non-interpreted 2D digital measurements in multiple formats including NMR spectra, raw NMR data, images, movies, lists, and spreadsheet files. The principal components extracted from them by TREND represent the main changes among the data frames. These could be binding isotherms, time courses, or a variety of population shifts. The main requirement is that the data frames be measured consistently (with the same parameters) to support quantitative comparison. Series of 2D measurements in an unsupported format may be converted into a text file or spreadsheet in order to be read by TREND for analysis. TREND is written as a set of Python scripts. These were packaged using PyInstaller for ease of installation under Windows, Mac OS X, and Ubuntu and Fedora versions of Linux. TREND (sponsored by NSF) is free for academic users.



TREND captures main time courses of change of movies or NMR spectra



Work Flows of TREND

[Academic Software License Request](#)

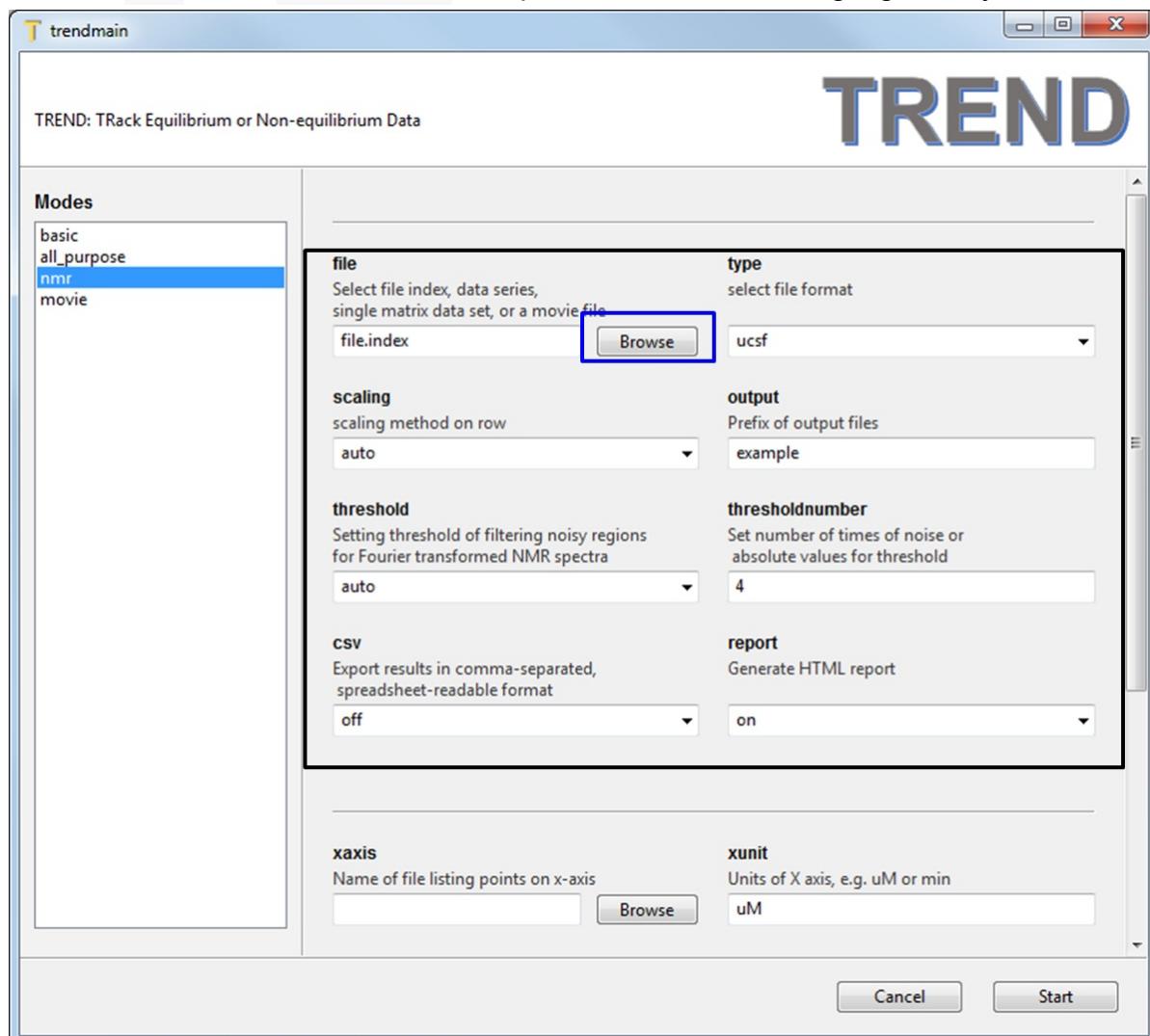
[Non-Academic/Commerical Software License Request](#)

[TREND Academic Software License Agreement \[PDF\]](#)

Simple Example: Determine binding isotherm from NMR spectra in UCSF format

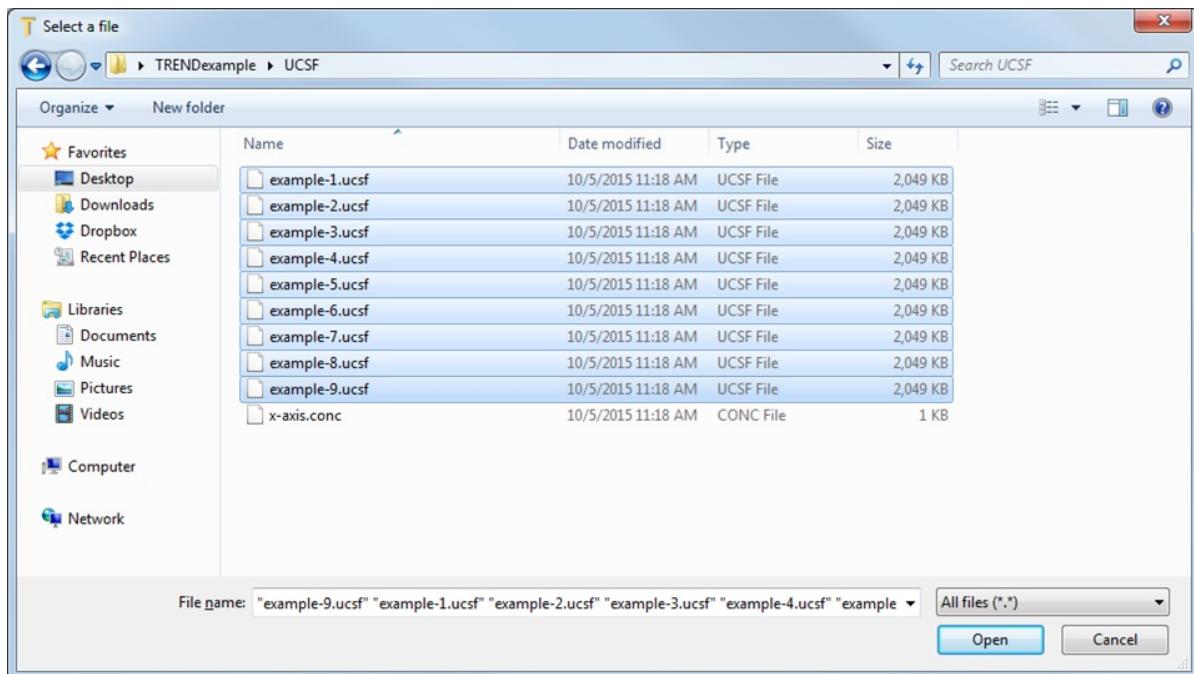
GUI USAGE

1. Choose `nmr` tab of `trendmaingui` and press browse button highlighted by the box.



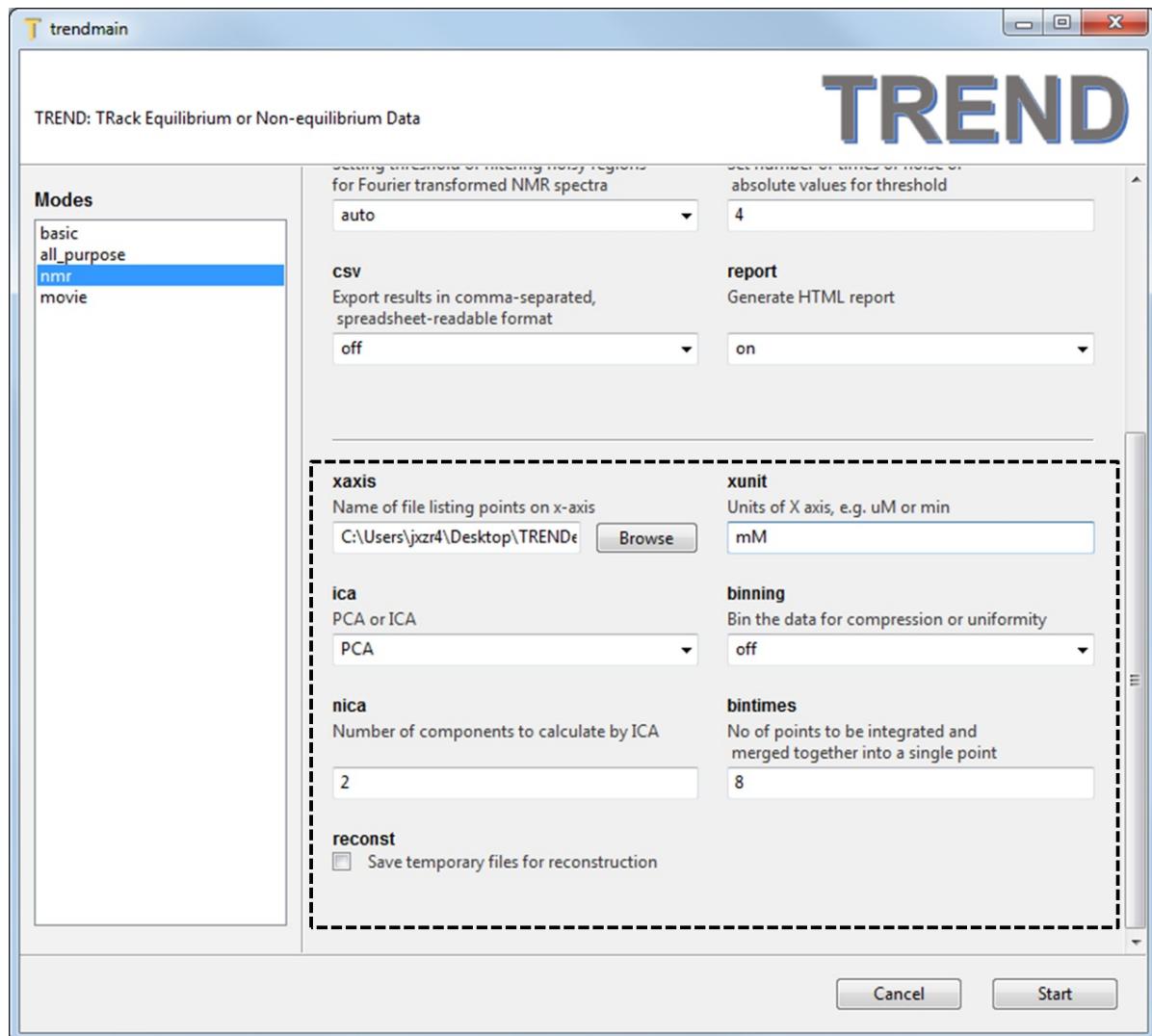
Choose a series of Sparky format spectra (.UCSF files) and TREND will sort them numerically.

A simple example



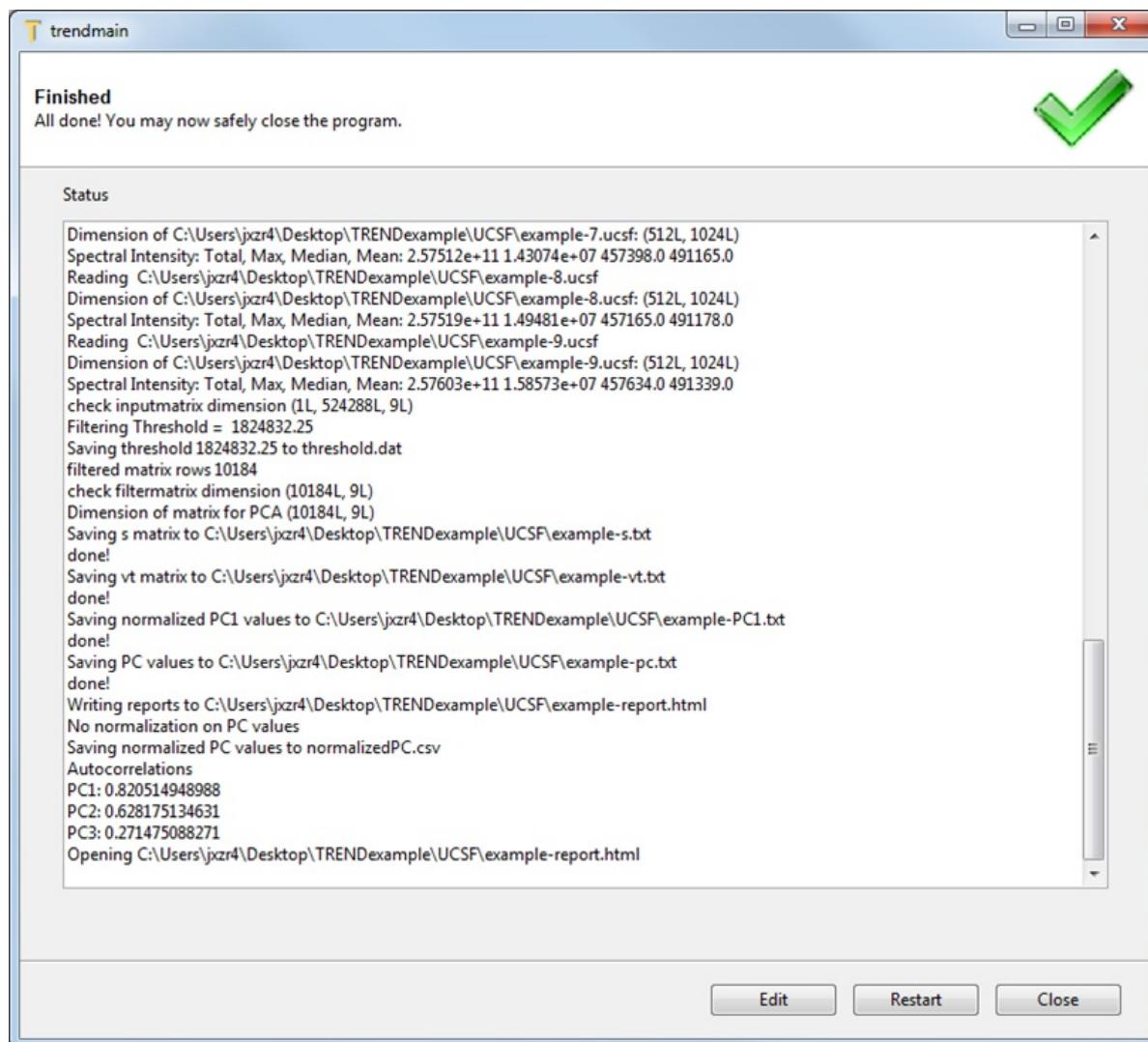
2. Specify required arguments including file format, method of scaling rows, the prefix of output files, threshold for smallest signals to be retained (x-fold the noise), whether to export CSV-format spreadsheet files, and whether to generate an HTML report. The default settings are recommended.
3. Optional arguments are highlighted within the box with dashed line. See the manual for details. Here we specify `xaxis` and `xunits`

A simple example



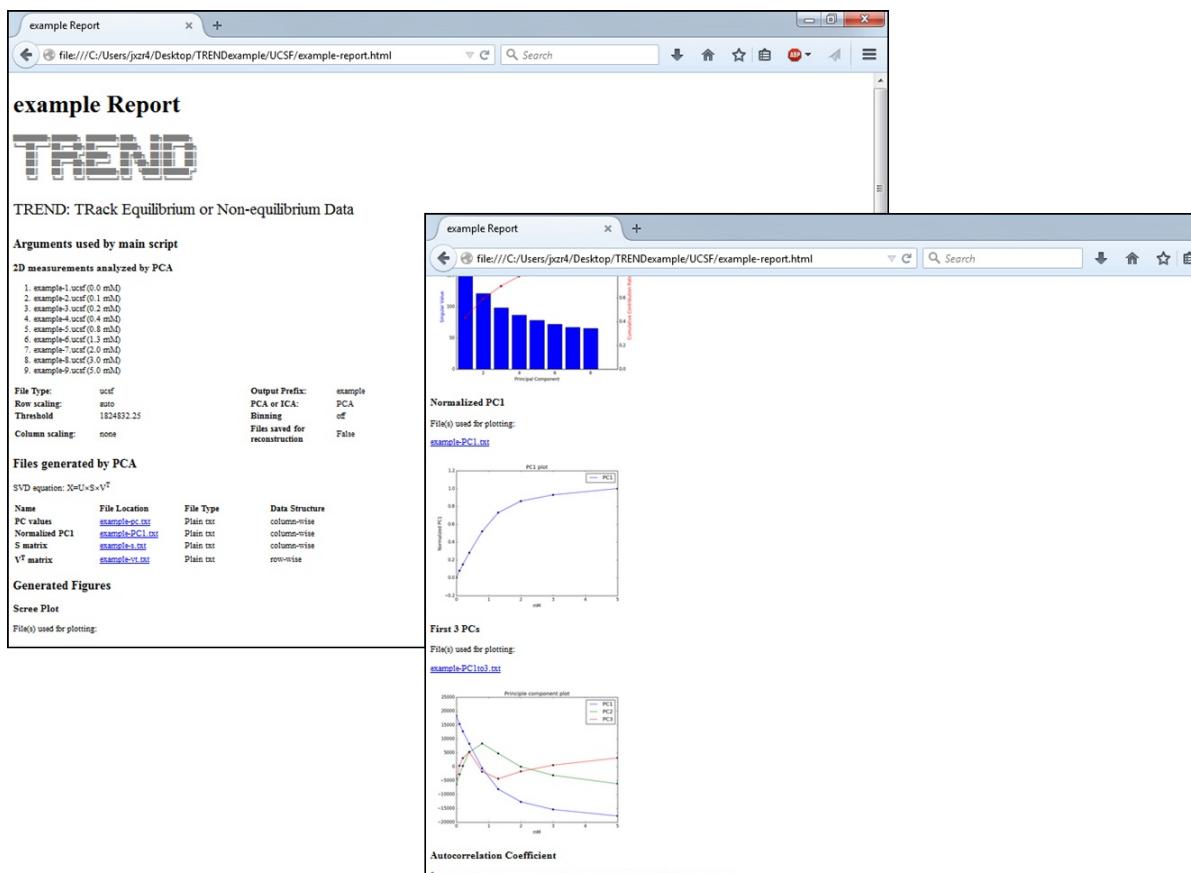
4. Press start button, TREND will run for a while and finish.

A simple example



5. After `trendmain` finishes, an HTML report is generated. It includes the arguments just used, scree plots, and plots of principal components, etc. Please see the manual for details.

A simple example



Corresponding shell command

```
trendmain.exe -f file.index -t ucsf -s auto -o example -r auto --report
```

Citation

If you use TREND in your research, please cite these references in resulting publications:

Jia Xu and Steven R. Van Doren, Binding Isotherms and Time Courses Readily from Magnetic Resonance. *Anal. Chem.* 2016, 88 (16), pp 8172-8178

and

Jia Xu and Steven R. Van Doren, Tracking Equilibrium and Non-equilibrium Shifts in Data with TREND. Submitted.

Installing the TREND package under Windows, OS X or Linux

To obtain access to TREND, go to <http://biochem.missouri.edu/trend/>

Submit either an academic license agreement or request for commercial licensing, as well as the contact information for you and your laboratory. Await approval and an email message providing download access to the TREND distributions.

Though written in Python, TREND does not depend on a Python environment. The simple installation process merely creates shortcuts on the desktop and helps set environment variables of for the text shell window that will make TREND easy to use. The installation script places on the Desktop a directory containing symbolic links to the executable files with GUI.

The OS X and Linux installation script change the PATH environment variable. The Windows script `TRENDterminal.bat` opens a command shell in which all executable files of TREND (with suffix of `.exe`) can be invoked, both the versions operated by GUI and at the command line. Note that the installation process is not completed until the first calculations using `trendmain` and `trendreconstruct` trigger the download and installation of two pieces of software from the public domain for handling movies and images. This download requires both a reliable internet connection and access to github. If this access is lacking upon initial usage, please see the document [no_internet_connection.pdf](#)

Windows 7 or above

- Unzip the `TREND-Win7-0.7.8.3.7z` by [7zip](#).
- In the unzipped folder, double clicking `install.bat` will create a file named `TRENDterminal` and a folder on the desktop and start menu named `TREND-GUI` containing `trendmaingui.exe`, `trendplotgui.exe`, and `trendreconstructgui.exe`. Refresh the desktop by right-clicking in an open area and select `Refresh`.
- Invoke any of these GUI versions of the executable files by double-clicking on them within the `TREND-GUI` folder. If TREND was previously installed, the old version will be uninstalled before new version is installed.
- An alternative way to run either the GUI or command-line versions of the executable files involves first double clicking on `TRENDterminal.bat`. This will open a Windows command shell in which you can `cd` to your data folder and invoke the TREND executable files. This does not change your path environment permanently and should be run every time you need a command shell window for TREND.

- Note the `.7z` tarball can be deleted but the unzipped folder should not be removed or deleted, since the installation step just creates shortcuts.

Mac OS X 10.7.5 and later

- Unzip the `TREND-OSX-Lion-0.7.8.3.tar.gz` by double clicking the file displayed in the OS X GUI. Alternatively, run the command `tar -xzvf TREND-OSX-Lion-0.7.8.3.tar.gz` within a terminal shell.
- Open a terminal shell and `cd` to the unzipped folder named `TREND-0.7.8.3-OSX`, enter the command `./install.script` to create a `TREND-GUI` folder on the desktop, which contains `trendmaingui.app`, `trendplotgui.app`, and `trendreconstructgui.app`. The installation scripts can only be executed within the terminal shell. (Do not run this script directly from OS X desktop GUI by clicking because the installation script will not work properly).
- Three icons, `trendmaingui.app`, `trendplotgui.app`, and `trendreconstructgui.app` will also be added to `/Applications/TREND`. These icons should be visible in Launchpad and the Applications folder in Finder. (Refreshing Launchpad may be needed).
- The current TREND directory is also added to the `PATH` environment variable. Three lines will be added to the file `~/.bash_profile`. The lines begin with `#TREND path environment`
- Open a new terminal or type and enter the command `source ~/.bash_profile` within a terminal shell. Now all TREND commands can be launched in a terminal.
- If TREND was previously installed, the old version will be uninstalled before the new installation.
- `sudo` may be necessary if permission is required.
An example: Download `TREND-OSX-Lion-0.7.8.3.tar.gz` to `~/Downloads/`
- If you are comfortable with shell terminal, you can just enter `./change_path_env.script` to add the current TREND directory to `PATH` environment variable. No permission is required.

```
tar -xzvf TREND-OSX-Lion-0.7.8.3.tar.gz
cd ~/Downloads/TREND-OSX-Lion-0.7.8.3
./install.script
(or sudo ./install.script if permission is required)
```

Linux 64-bit, such as recent Ubuntu 14.04, Fedora 23 and later

- Unzip the `TREND-Ubuntu14.04-0.7.8.3.tar.gz` by entering the command `tar -xzvf`

`TREND-Ubuntu14.04-0.7.8.3.tar.gz` within a terminal shell.

- Enter `./install.script` within the terminal shell to add the current TREND directory to the `PATH` environment variable. Three lines will be added to `~/.bashrc`, starting with `#TREND path environment`.
- Open a new terminal or type `source ~/.bashrc`. Now all TREND commands can be launched in terminal.
- Installation in Linux is similar to OS X, as the `install.script` is written for `bash`, a shell that is the default in Mac OS X and many Linux distributions. However, a Linux computer may run without a desktop and may use a shell other than `bash`. In this case, you need to set the path environment variables yourself. e.g., modifying a shell configuration file such as `.bashrc`, `.cshrc`, `.tcshrc`

Uninstalling or Moving

- Run `uninstall.bat` in Windows or `uninstall.script` in OS X and Linux.
- Then delete the `TREND-Win7-0.7.8.3`, `TREND-OSX-Lion-0.7.8.3`, or `TREND-Ubuntu14.04-0.7.8.3` folder.
- To move the TREND folder to a new location, first uninstall it, move the TREND folder to the new location, and install it again, thereby overwriting the previously installed TREND executable files.

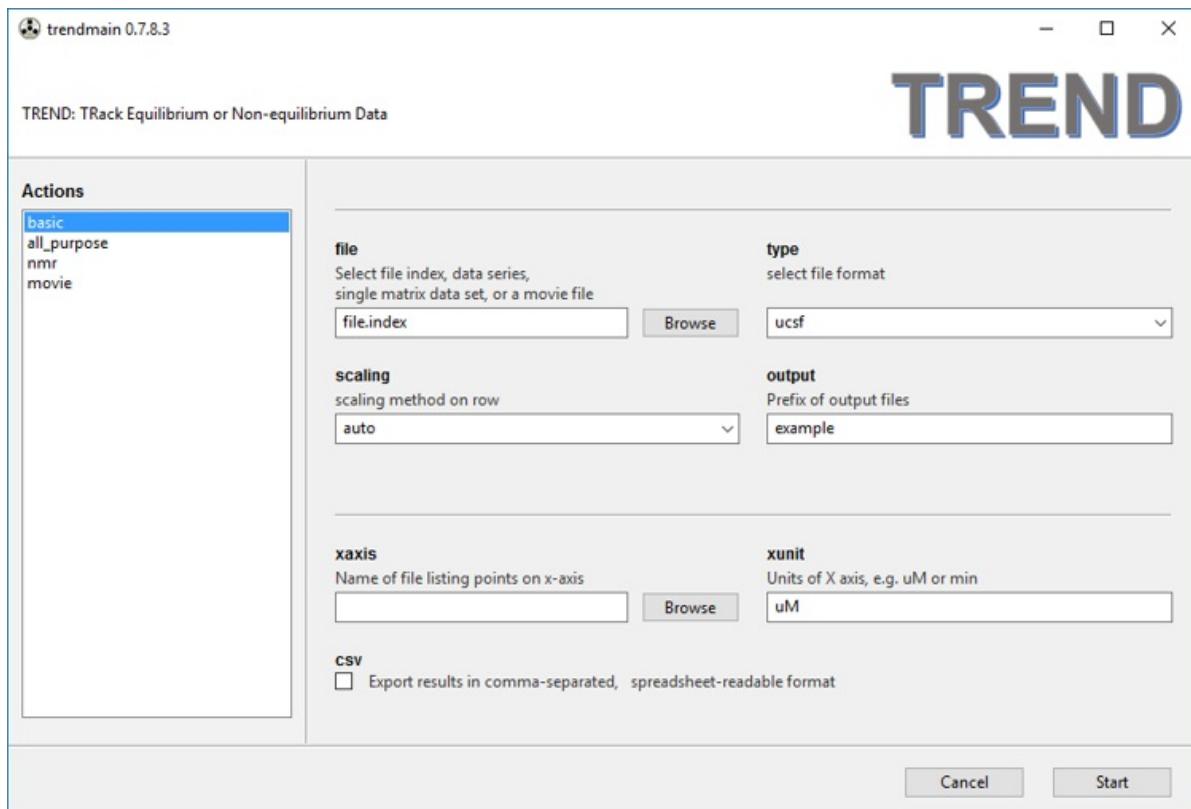
Introduction to Graphic User Interface of TREND

Description:

Both GUI and command-line versions of TREND are available. The arguments and usages of GUI panels are briefly introduced below. Detailed explanations of choices of arguments and parameters are provided in the manual for command-line versions.

Trendmain - Obtain Principal Components from a series of 2D Measurements

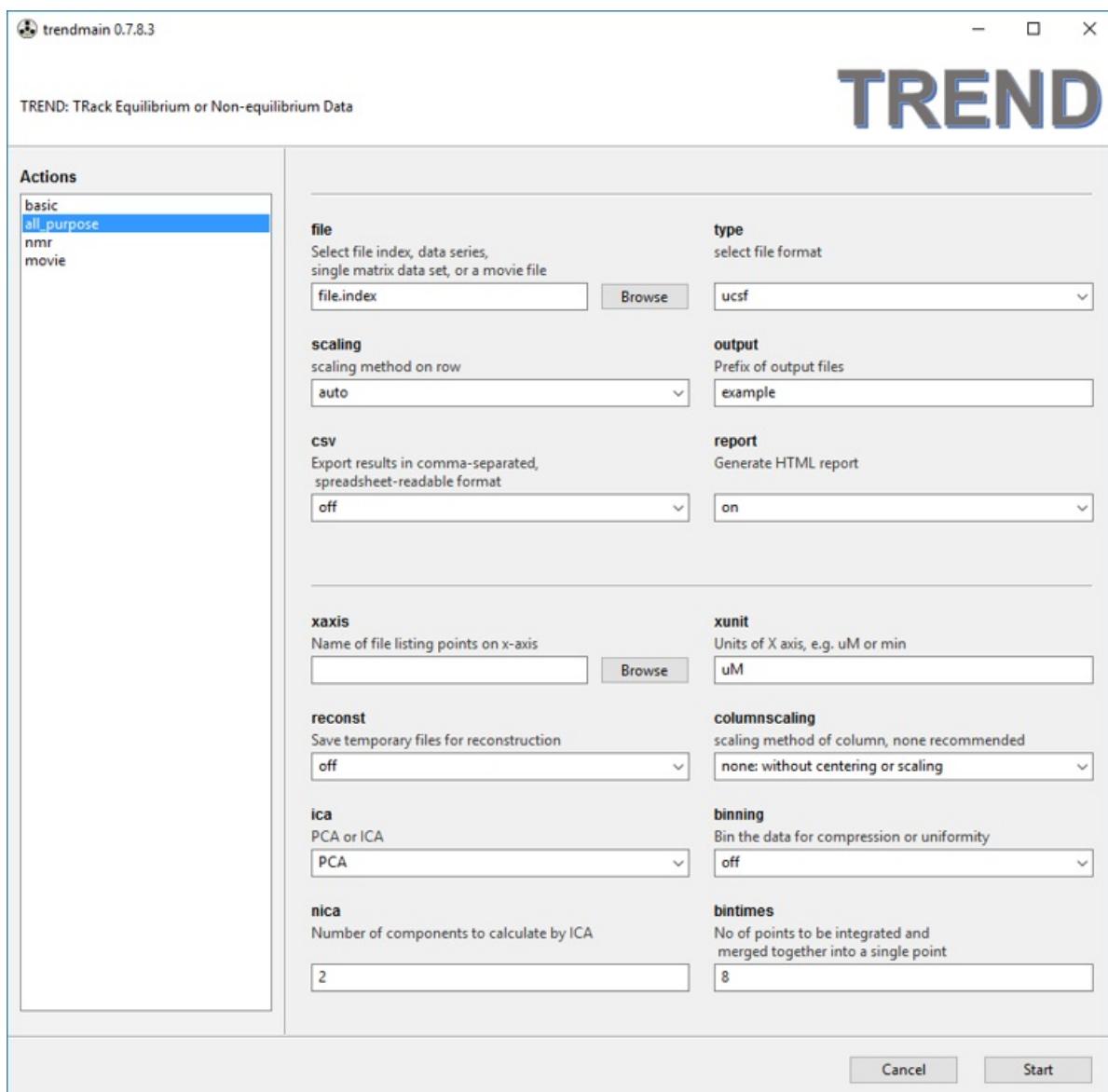
- Launch using `trendmaingui.exe` or `trendmaingui.app`. In the left side bar, there are four different modes to choose from: `basic`, `all_purpose`, `nmr`, and `movie`. They function similarly with minor differences.



- The `basic` menu can be used for simple PCA work. A series of 2D data files (or an index file listing the series of data files) can be read by the `file` File Chooser widget. Note the `file` File Chooser can do two things: If input file names contain numbers, TREND can extract numbers from the file names and sort these names numerically. A corresponding file index (`file.index`) will be generated automatically.

Or you can make a file index manually with a text editor according to the format described in the manual.

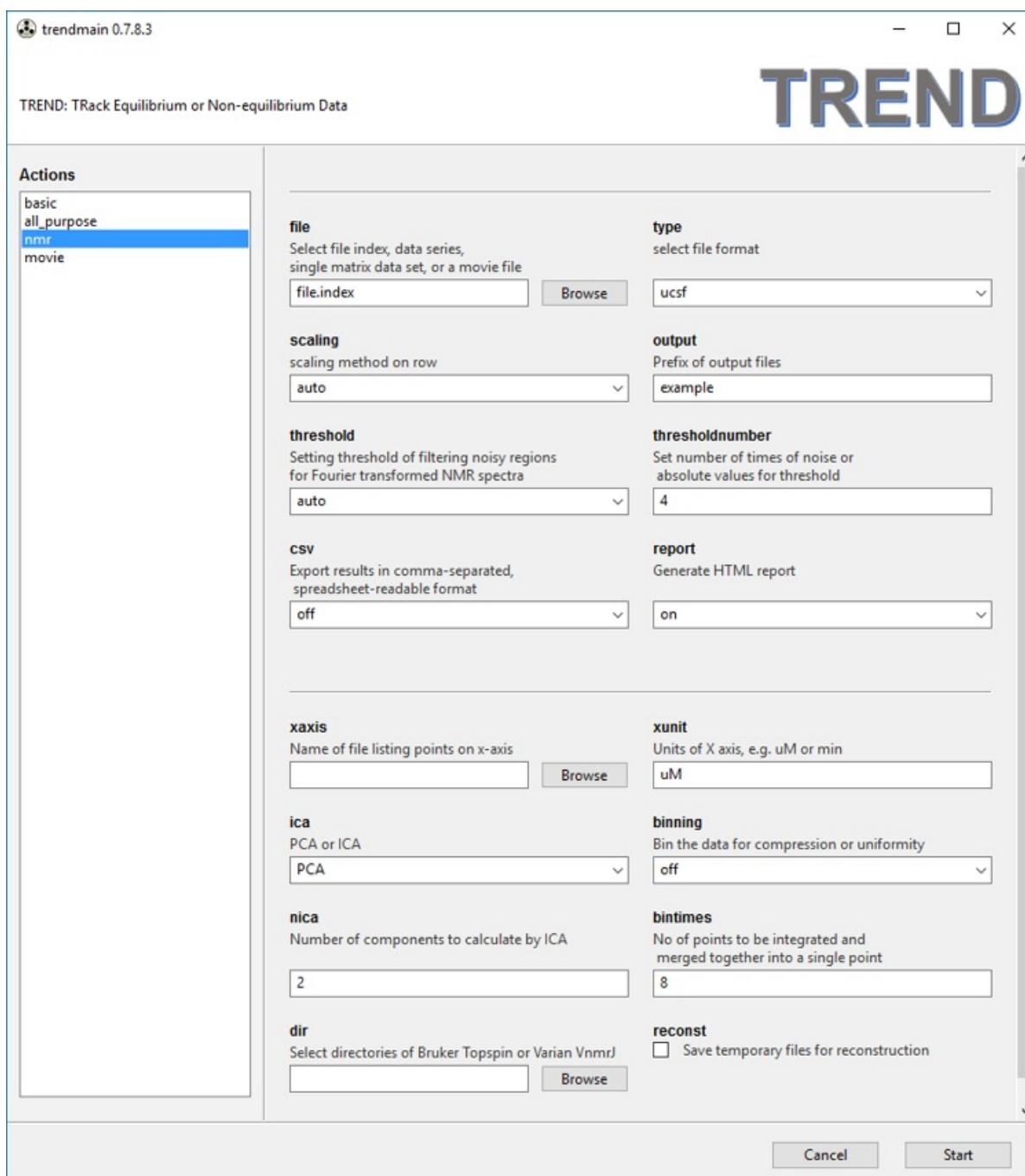
- File format, scaling methods (on row), prefix of output file names can also be specified. The lower half of the panel shows optional arguments which can be left blank. A file list the tick marks for the x-axis can be selected by the `xaxis` File Chooser. The units of the x axis can be also specified and will take effect if a file is selected in `xaxis`. When the `csv` checkbox is turned on, the PCA results will be exported in comma-separated values (CSV) format that can be read by spreadsheets such as Excel or OpenOffice. The `report` checkbox is on by default. This generates an HTML report of arguments used by main script, links to files generated by PCA (PC values, normalized PC1, other files for plots, the S matrix, etc), and illustrations of the scree plot, normalized PC1, first 3 PCs, and autocorrelation coefficients.



- The `all_purpose` menu provides additional options. When the `reconst` option is

turned on, temporary files are saved for later use in reconstruction of the data from the components specified. Scaling of columns can be specified, but should not be necessary if the rows are scaled. If the input file type is Fourier-Transformed NMR spectrum (NMRPipe `ft2` format or Sparky `ucsf` format), the spectra can be "binned" for compression or uniformity. The `bintimes` parameter for this can be specified. Detail descriptions can be found in the manual. In the `all_purpose` menu, ICA (independent component analysis) can be selected instead of PCA. When `ICA` is chosen, the number of independent components to be calculated should be specified (Xu and Van Doren, submitted).

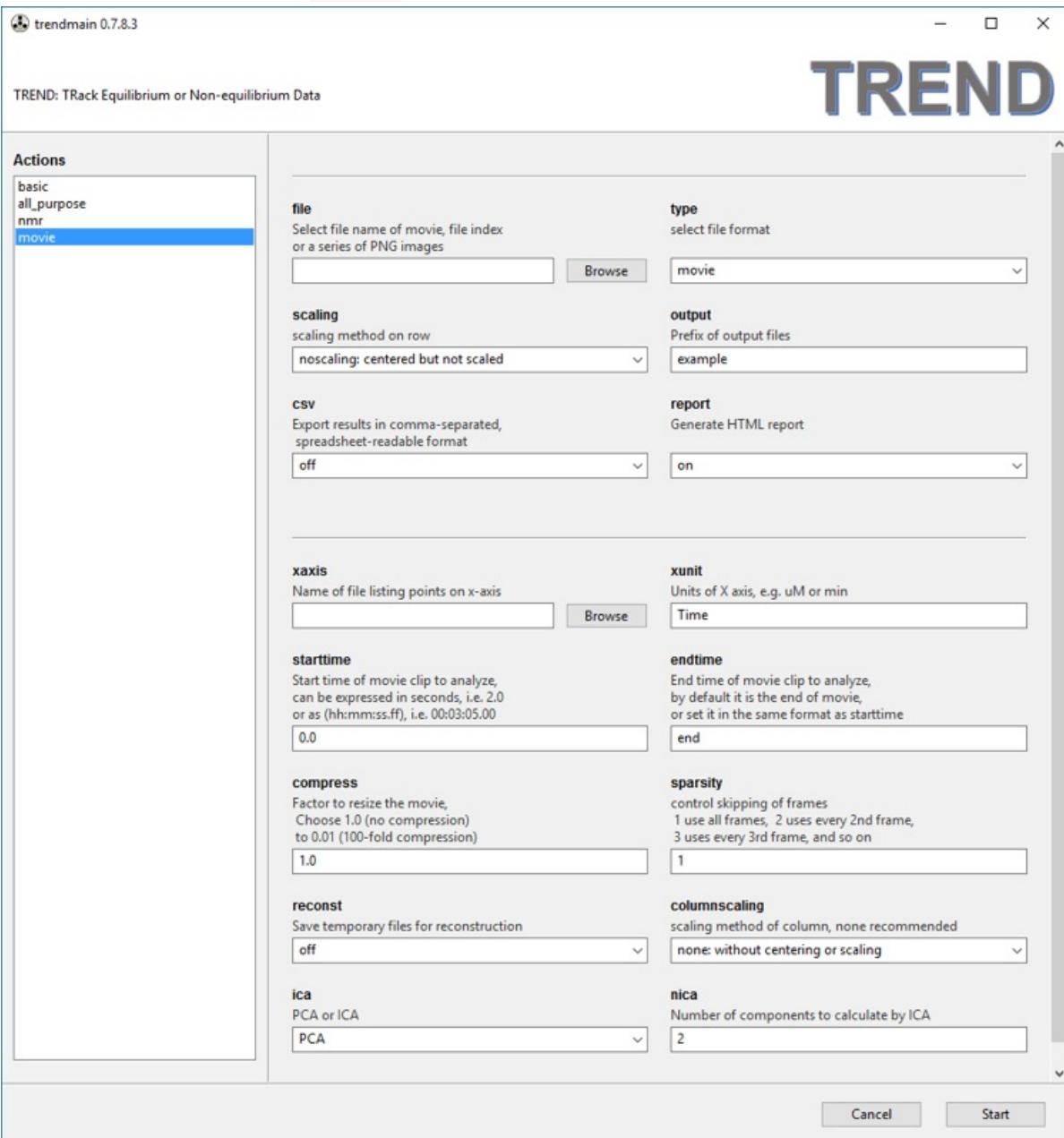
- The `nmr` menu is for analysis of NMR spectra. It is similar to the `all_purpose` menu but only offers selection of data only in these formats: **JCAMP-DX format**, NMRPipe **fid** and **ft2**, Sparky **ucsf** and Sparky peak list as **sparkylist**, Bruker Topspin FID and spectra as **brukerfid** and **brukerspectra**, and Agilent (Varian) VnmrJ FID and spectra as **agilentfid** and **agilentspectra**. The Topspin and VnmrJ formats are saved as directories, contrasting them from the other formats that save data as individual files. Therefore a `dir` Directory Chooser widget is provided for the Topspin and VnmrJ data directories. Its usage is similar to the `file` File Chooser widget described previously. A manually made directory index, which is in the same form of `file.index` can still be read by the `file` File Chooser for parsing Topspin and VnmrJ directories. See manual for details.



The default scaling method of rows of the data matrix is **auto**, which is recommended for series of NMR spectra in fast or slow exchange regimes. **pareto** is recommended for spectra with intermediate exchange. **noscaling** should be acceptable for all exchange regimes. Details of choosing scaling method and setting the threshold to filter out noise are given in the manual and [Jia Xu and Steven R. Van Doren, Binding Isotherms and Time Courses Readily from Magnetic Resonance. Anal. Chem. 2016, 88 \(16\), pp 8172-8178](#)

`threshold` and `thresholdnumber` can be set for filtering noise out of NMR spectra. The three ways to set the `threshold` are **auto**, **absolute**, and **the number of times the noise level**. In **auto** mode, the program determines noise from the first spectrum and sets the threshold as 4-fold the noise level for `autoscaling` and 0.5

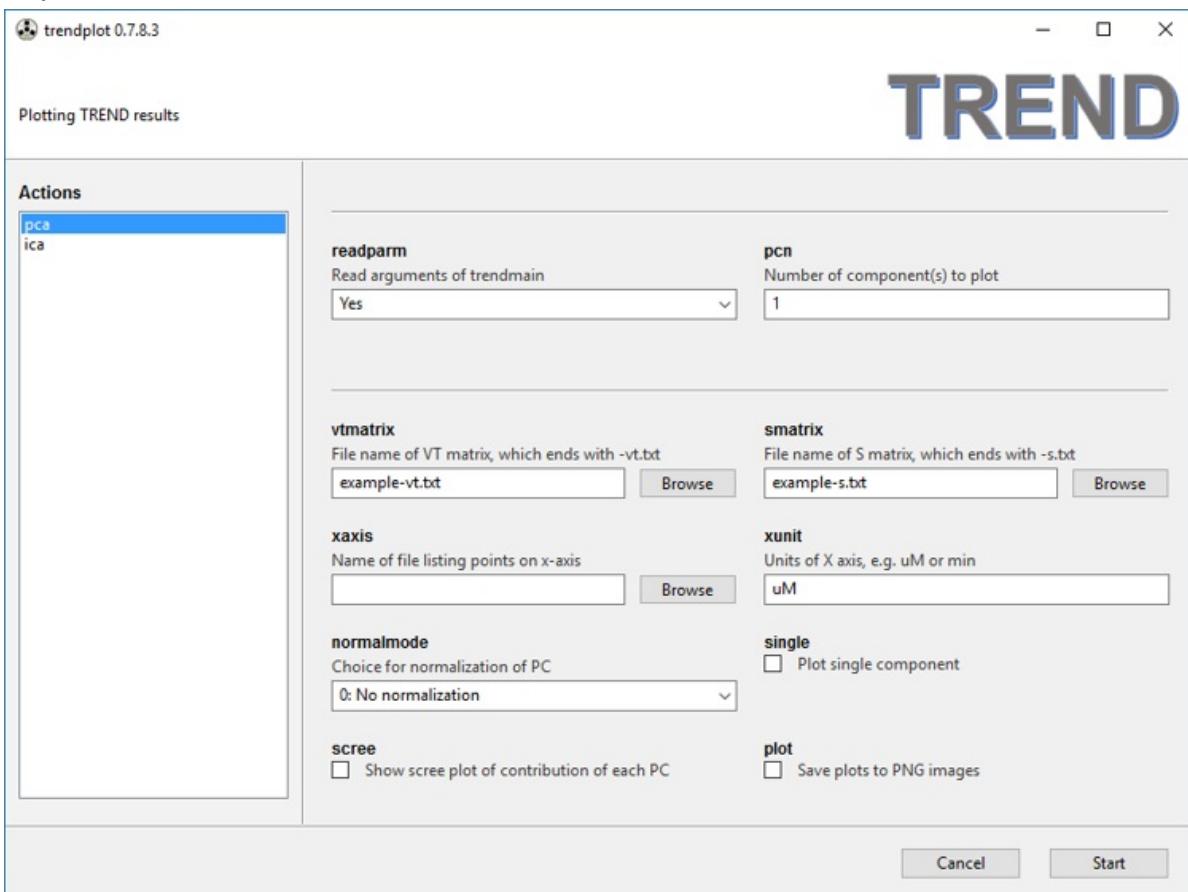
times the noise level for `Pareto` scaling.



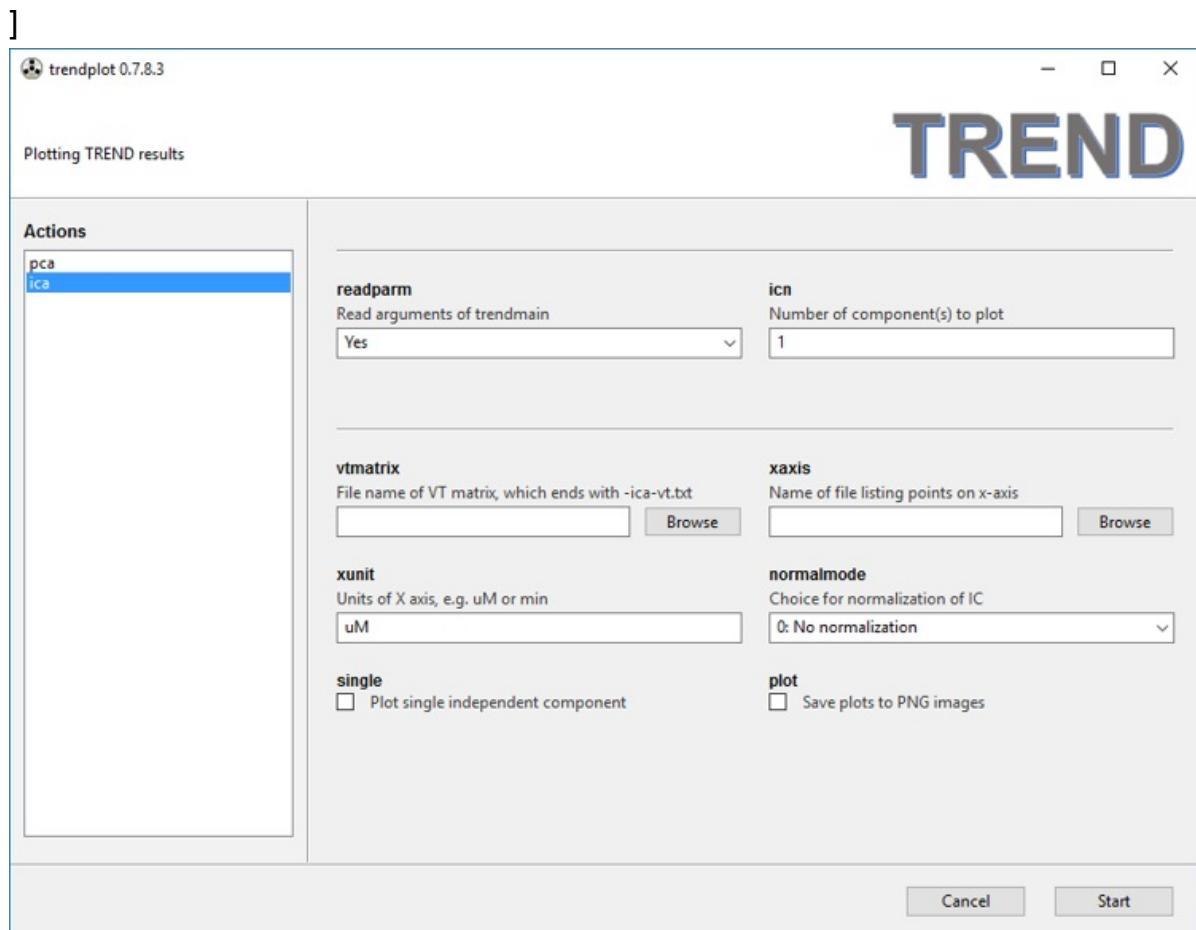
- The `movie` menu is for processing movies or time-dependent PNG image series. The recommended scaling method of rows is **noscaling**. A file listing tick marks for the x-axis can be specified. When processing a movie, time will be automatically extracted from the movie and saved as `movie_time.txt` for use as x-axis. The movie (but not series of PNG images) can be resized to make it smaller by skipping frames of the movie and setting the parameters `compress` and `sparsity` according to the manual. TREND can select a certain portion of a movie by setting `starttime` and `endtime` parameters. The number format for setting start and end time can either be floating point numbers of seconds (e.g. `0.2`), or `hh:mm:ss.ff`, such as `00:03:05.00`. The default values for the `starttime` and `endtime` are `0.0` and `end`, which do not trim the movie.

Trendplot -- display the principal components identified by the main script

- The program for plotting with GUI is `trendplotgui.exe` in Windows and Linux, or `trendplotgui.app` in OS X. The `report` option of the main script generates an HTML report including several figures including the first 3 PCs and scree plot, `trendplotgui` non-essential for visualizing results. However, `trendplotgui.exe` provides more control of plotting, such as choices of kinds of normalization and single components to plot.



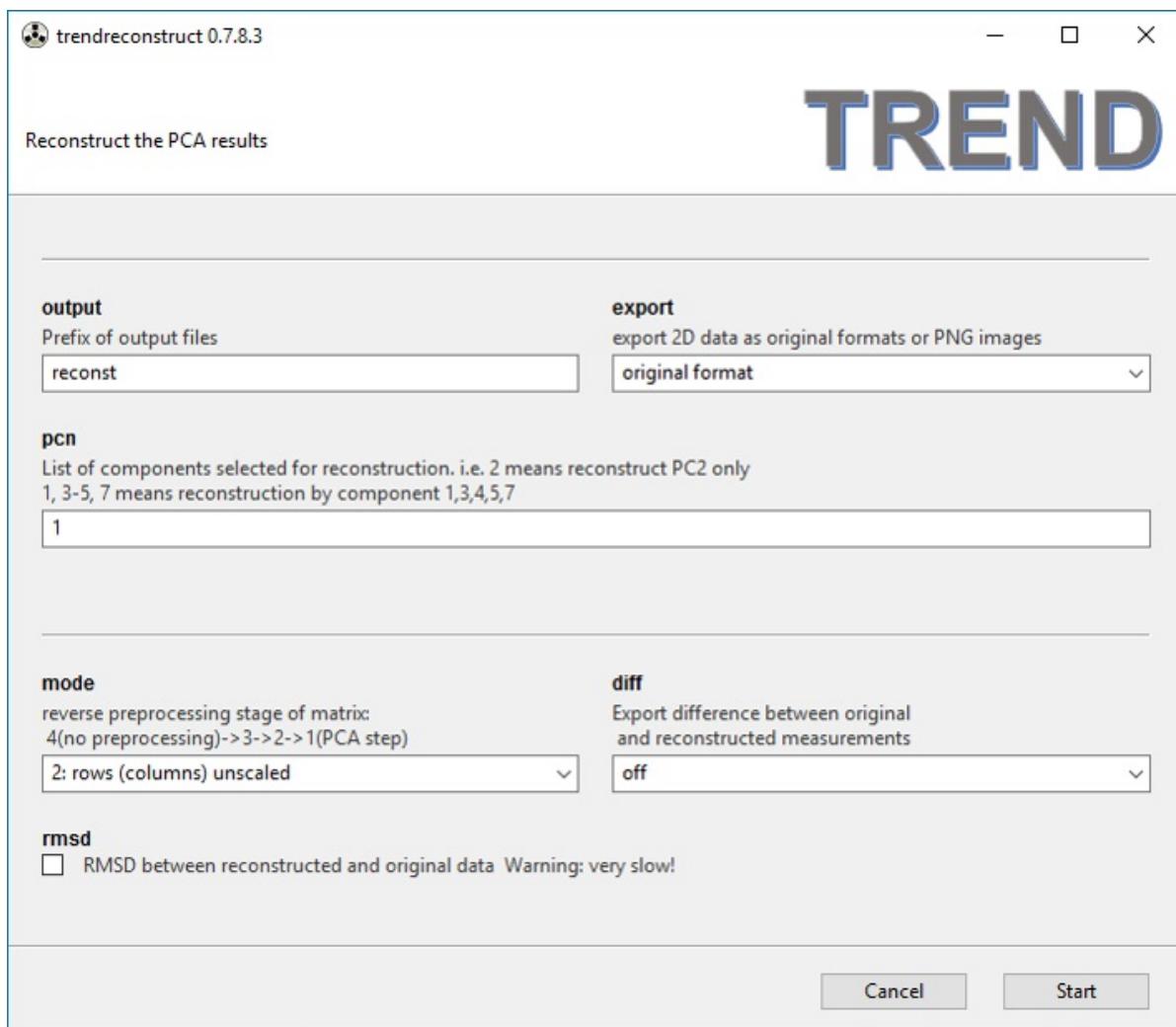
- There are two modes `trendplotgui` : PCA and ICA.. It is critical to select the appropriate mode.
- `pca` mode: The Vt matrix and S matrix are needed for plotting PCA results. These matrices can be selected using the `vtmatrix` and `smatrix` file choosers. However, there is an easier way: Setting `readparm` to `Yes` will read the arguments that `trendmain` used and automatically locate the matrices as well as the choices of `xaxis` and `xunit`. `pcn` sets the first N PCs to be plotted. When the `single` checkbox is turned on, `pcn` just plot the Nth PC. The type of normalization can be selected by setting `normalmode` according to Table 1 of the manual.
- `ica` mode is very similar to `pca` mode, but does not read `smatrix` or show a scree plot.



Trendreconstruct - Use the PCA calculations to reconstruct features

in the series of 2D measurements

`trendreconstructgui.exe` in Windows and Linux or `trendreconstruct.app` in OS X is the program with GUI that uses the results from `trendmaingui.exe` to reconstruct single or multiple PC representations of the original series of 2D measurements. It is quite self-explanatory. The manual provides more details.



`trendreconstructgui` reads parameters and results from `trendmain` OR `trendmaingui`. A PCA calculation must be carried out by `trendmain` with the `reconstruction` option turned on. When performing reconstruction of a PNG image series, choosing to export the `original format` will actually generate an `mp4` movie clip. Choosing the `PNG` option instead plots reconstructed images.

Main Script - Obtain Principal Components from a series of 2D Measurements

- Both GUI (Graphic User Interface) and CLI (Command Line Interface) programs

GUI: `trendmaingui.exe` in Windows and Linux, `trendmaingui.app` in OS X

CLI: `trendmain.exe` on all platforms

- Synopsis

```
trendmain.exe -t [ucsf/fid/ft2/png/movie/txt/sparky/csv/excel/...] -f [file index] -r  
[threshold] -o [output prefix name] -s [scaling methods] [-x [xaxis file]] [-u [Unit]] [--  
report]
```

- Description

`trendmain.exe` reads a series of NMR spectra, FIDs, png images, the Joint Committee on Atomic and Molecular Physical data - Data eXchange (JCAMP-DX) format, plain text files, comma-separated (CSV) or Excel spreadsheets listed in the index file.

`trendmain.exe` also reads and process single file, including movie, CSV or Excel spreadsheet, or text file. TREND preprocesses them, applies singular value decomposition to provide U, s, and V matrices (Xu and Van Doren, submitted). Its normal mode is to provide principal components (PCs) from the first few rows of the V matrix. TREND also has options to perform PCA reconstructions of the original data series or to perform independent components analysis. s, V, and normalized PC1 values are output as text files in the names of `prefix-s.txt`, `prefix-vt.txt`, and `prefix-pc1.txt`, which reports the binding isotherm. Additional PCs can be requested.

- Typical usage with NMR spectra:

```
trendmain.exe -t ucsf -f file.index -r auto -o outfile -s auto --report
```

- Options

- -t [ucsf/fid/ft2/png/movie/txt/complextxt/csv/complexcsv/singlematrix]

-t specifies the input file format, the default format is .ucsf for NMR spectra.

The options for input file formats are:

- **ucsf** Sparky ucsf format (default)
- **fid** NMRPipe fid format
- **ft2** NMRPipe ft2 format
- **png** image format (needs Scipy module)
- **bukerfid** Bruker Topspin FID format (`fid` or `ser` file in the experimental directory)
- **bukerspectra** Bruker Topspin processed spectra (`1r` (1D) or `2rr` (2D) in the `1/pdata/` subdirectory in the experimental directory . Note currently the processed spectra must be saved by setting processing number as 1 in the Topspin experimental directory)
- **agilentfid** Agilent VnmrJ (OpenVnmrJ) fid. (`fid` file in the data directory)
- **agilentspectra** Agilent (Varian) VnmrJ (OpenVnmrj) fid. (`phasefile` in the `datdir` subdirectory. Note in order to make `phasefile` readable by third-party software including TREND, set `trace='f1'`, display the full spectrum, and use the VnmrJ `flush` command are required. Otherwise the `phasefile` will be all zero values. See the [Sparky manual](#) for details.

Note TREND supports on-the-fly analysis on Bruker and Agilent spectrometers. However to avoid effect of non-uniformity or outliers, all experimental data analyzed by TREND are preferably to be collected and processed in identical conditions. Therefore, we highly recommend using the same set of `NMRPipe` scripts to convert Bruker or Agilent data into NMRPipe **fid** and **ft2** formats.

- **txt** Data stored in plain text format delimited by spaces, this option could be used for other spectroscopy methods such as time- dependent 2D-IR.
- **csv** csv format is supported by Excel, OpenOffice and many software for spectroscopy instruments.
- **complextxt** Similar to plain text format (`txt`) but using complex number in the form of `0.00+0.00j` .
- **complexcsv** Similar to csv format. However, each cell should be in the format of `0.00+0.00j` .
- **excel** Microsoft Excel format (`xlsx` is preferred, the older `xls` format is also accepted). All cells must be float or complex numbers. The complex number should be in the form of `0.00+0.00j` . By default an Excel file contains three sheets, only the first sheet is analyzed. Excel with multi-

- sheets can be processed as `singleexcel` option.
 - **sparky** or **sparkylist** Sparky list format. The 1st column lists any residue assignments. The 2nd and 3rd columns are F1 (often 15N) and F2 (often 1H) chemical shifts, respectively.
 - **singlématrix** This mode serves as a general PCA tool to process a single matrix in the text format delimited with spaces.
 - **singlecsv** Similar to `singlématrix` format but in `csv` format
 - **singleexcel** Similar to `singlématrix` and `singlecsv` formats but in the `xlsx` or `xls` formats. Besides, a series of 1D or 2D dataset can be stored in a single Excel file containing different, sequentially-ordered sheets.
 - **movie** Common video formats, such as `.ogv`, `.mp4`, `.mpeg`, `.avi`, `.mov`, `.webm`
 - **jcamp** JCAMP-DX is a general format for exchanging and archiving data from many instruments, including but not limited to IR, Raman, Uv-Vis, Fluorescence, NMR, and EMR. The data stored in JCAMP-DX files can be spectral plots, contours, or peak tables. JCAMP-DX is very flexible in order to support most kinds of computerized instruments. As a result, it is impractical to support all existing JCAMP-DX variants. However, TREND supports most common JCAMP-DX formats. The digital data in JCAMP-DX can be **AFFN** (ASCII FREE FORMAT NUMERIC) form or **ASDF** (ASCII SQUEEZED DIFFERENCE FORM). TREND supports decoding compressed data, including `PAC`, `SQZ`, `DIF`, `SQZDUP`, and `DIFDUP`. Two most common tabular data form, `(x++(Y..Y))` and `(XY..XY)` are supported. TREND reads a series of JCAMP-DX files, or a single JCAMP-DX file with one or multiple blocks. TREND supports `NTUPLE` format (introduced by JCAMP-DX 5.0 standard), which is designed for multi-dimensional techniques with data sets of multiple variable. For example, JCAMP-DX NMR uses `NTUPLE` to show mixed real/imaginary FID data sets. See <http://www.jcamp-dx.org/>, https://badc.nerc.ac.uk/help/formats/jcamp_dx/ and <http://wwwchem.uwimona.edu.jm:1104/spectra/testdata/index.html> for details of JCAMP-DX formats.
- `-f [fileindex/filename]`
 - `-f` reads file index and is required
 - `[fileindex]` is a text format index referring to the series of NMR spectra, images, lists, or directories (for Bruker Topspin and Agilent VnmrJ data). The default file index name is `file.index`. An example is a series of five `.ucsf` files named numerically. The index file can be named as `index.ucsf`

and includes the following lines in the example:

![file.index.png] (<https://bitbucket.org/repo/bboE8M/images/1070799385-file.index.png>)

Another example is a series of Bruker Topspin directories on a Windows platform. Absolute paths are supported as well as relative path, which is shown in the `UCSF` example. This "directory list" can be used for both **brukerfid** and **brukerspectra**. For **brukerfid** data, TREND will read `fid` or `ser` data from the listed directories. For **brukerspectra** data, TREND will read `1r` or `2rr` files from the `pdata1` subdirectories. Therefore, the processing number must always be set as `1`.

```
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\13
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\15
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\17
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\19
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\21
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\23
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\25
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\27
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\29
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\31
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\33
D:\Bruker\TopSpin3.5pl6\examdata\GCDA\35
```

`file.index` for Agilent VnmrJ could be used in a similar manner, except its spectra are saved in the `datdir` subdirectories.

- [filename] can be the name of a singlematrix data set or a movie file for processing.
 - `-s` [none/noscaling/auto/pareto/]
 - `s` specifies scaling method applied on rows of the data matrix.
 - **none** means skip centering and scaling of the rows of the data matrix.
 - **noscaling** means do data centering without scaling. It should be acceptable in most conditions.
 - **auto** means Autoscaling. It treats all peaks or features as equally important but inflates the measurement errors. It is recommended for NMR spectra in fast and slow exchange regimes.
 - **pareto** means Pareto scaling. It reduces the importance of large peaks but enhances the low, broader peaks. It is recommended for NMR spectra in intermediate exchange.
 - Definitions of the scaling methods are given in: *J. BMC Genomics 2006, 7, 142. Other scaling options mentioned in this paper, such as `vast`_scaling, `range` scaling, `level` scaling are also provided.*
 - `-r` [auto/1e5/3T/5t]

-r sets the threshold for filtering low intensity regions. The threshold is meaningful when processing 2D NMR spectra (ucsf, ft2), but not for FIDs or images in png format. There are three ways to set the threshold: auto, absolute, and manual (set the number of times the noise level).

- **auto** In **auto** mode the program determines noise from the first spectrum and set threshold as 4 times of the noise level for autoscaling and 0.5 times the noise level for Pareto scaling.
 - **1e5**: When the arguments are set as integral numbers or floating point numbers , such as `1e5` or `100000` . This is used to specify the threshold value using the numbering scale of the measurement.
 - **3T or 5t**: Numbers with suffix `T` or `t` annotates times of noise level of the first spectrum. In this mode threshold is set as 3 or 5 times of noise level of the first spectrum. Three to seven-fold (3T to 7T) is the recommended range for NMR spectra.
- -o [output prefix name]
 - o specifies the prefix of output files `prefix-s.txt` , `prefix-vt.txt` , `prefix-pc.txt` , and `prefix-PC1.txt` from PCA. `prefix-pc.txt` reports all principle components as successive vertical columns. `prefix-PC1.txt` reports the first principal component as a single vertical column, which in a titration is the binding isotherm.

For example, the option `-o test` generates output files named as `test-s.txt` , `test-vt.txt` , `test-PC.txt` , `test-PC1.txt`

`prefix-s.txt` and `prefix-vt.txt` save S and VT matrices in the SVD calculation. U matrix is by default omitted because its usually big size.

When ICA is used instead of PCA (`-i` option is on), the prefix includes `prefix-vt.txt` , which is unmixing matrix, and `prefix-IC.txt` , which reports normalized independent components as successive vertical columns.

- --csv When `--csv` is turned on, data in `prefix-s.txt` , `prefix-pc.txt` , `prefix-PC1.txt` , or `prefix-IC.txt` are generated as CSV files, which can be read by software such as Excel or OpenOffice. The corresponding file names are `prefix-s.csv` , `prefix-pc.csv` , `prefix-PC1.csv` , `prefix-IC.csv` . In csv files (except `prefix-s.csv`), data is organized as sucessive vertical columns, where the first column represents component number. A table header is added, such as `# Component, PC1, PC2, PC3 ...` .

- `-b`
Traditional uniform binning is applied for NMR data when `-b` option is turned on, it does not improve accuracy but may be useful when the input dataset is too large. By default `-b` option is off. `bintimes` option needs to be set in this condition.
- `--bintimes [binning time]`
Number of points to be integrated and merged together into a single point. The default is 8. For example, if the size of a 2D spectra is 1024*2048, setting `--bintimes 8` causes size of binned spectra to become $1024/8 \ 2048/8 = 128 \ 256$
- `--compress [compress factor]`
When processing **movie** the video can be resized by [compress factor] to reduce computational cost. For example, 0.8 means the video size will be resized to 80%. [compress factor] can be chosen from the interval (0, 1.0]. By default [compress factor] is set as 1.0, which means use all the data, i.e. don't compress the data.
- `--sparsity [sparsity factor]`
This option controls skipping frames of the input video file by picking up every n-th frame from the video, where n is specified by [sparsity factor]. For example, a [sparsity factor] of 1 means all frames will be used. 2 uses every second frame, 3 uses every third frame, and so on.
- `--starttime [start time]`
TREND supports making a subclip of input movie by setting start and end time. The default value for `--starttime` is 0.0. The number format for setting start and end time could either be floating point numbers of seconds (e.g. `0.2` stands for 0.2 s), or `hh:mm:ss.ff`, such as `00:03:05.00`, which means 3 minutes and 5 seconds.
- `--endtime [end time]`
This option sets the end time for the subclip of input movie. By default it is set as `end`. When `--starttime` and `--endtime` are set as default, (`0.0` and `end`, respectively), the whole input video will be analyzed. Otherwise a subclip video will be analyzed and exported as `from-starttime-to-endtime_movieclip.mp4`, Or `from-starttime_movieclip.mp4` if the `--endtime` is set as the default `end`.
- `--columnscaling [none/noscaling/auto/pareto]`
`--columnscaling` specifies the scaling method applied on columns of the data matrix. Options and meanings of scaling methods are defined in the `-s` option. By default `--columnscaling` is set as [none].
- `-i` OR `--ica`
This option uses the independent component analysis (ICA) module instead of

PCA.

- `--nica`

When ICA is used, the number (n) of components to be calculated must be set as `--nica n`. The default n is 2.

- `--reconst`

`--reconst` is an option for reconstruction. It has no effect on the calculation, but dumps necessary files for reconstruction in the future

- `--keepfiltered` is an option for 2D NMR spectra reconstruction when `--reconst` is on. When the input files are 2D NMR data such as ucsf or ft2, a threshold can be set using the `-r` option. All data points below the threshold are filtered out. `--keepfiltered` dumps the filtered data points to a file for reconstruction purpose. This file is not required because reconstruction of PCA can be done without the filtered data points.

- `-x [xaxisfile]`

`-x` specifies the name of file containing the list of tick marks to be used for the x-axis. It is optional. By default this option is off and x-axis ticks are integers.

When `xaxisfile` is specified, numbers within it will form the x-axis. The number of rows in `xaxisfile` should be identical to `index` file specified by the `-f [fileindex]` option of `trendmain.exe`

For example, `concentration.txt` lists the five concentrations of a titration, one per line:



- `-u [Unit]`

`-u` specifies label placed on the X-axis for plotting. [Unit] can be any string.

- `--report`

`--report` generates an HTML file named as `prefix-report.html` that reports the results. The report lists arguments used for calculation and files created. It plots the first 3 PCs or ICs without normalization. It also plots scree plot as well as autocorrelation coefficients that represent the smoothness of component curves. For more control of plotting of principal or independent components display, please use `trendplot.exe`.

Plotting script -- display the principal components identified by the main script

- **GUI and CLI programs**

GUI: `trendplotgui.exe` in Windows and Linux, `trendplotgui.app` in OS X

CLI: `trendplot.exe` in all platforms

• Synopsis

```
trendplot.exe [-r] [-f] [prefix] [-x [xaxisfile]] [-u [Unit]] [-n [the first N components]] [-p] [-e] -m [0/1/2/3/4/5] --plot
```

• Description

`trendplot.exe` plots the output of `trendmain.exe`. It is normally used to plot first principal component (PC1) or n principal components. It can also graph a scree plot of the sizes of the PCs. It uses matplotlib.

• Options

- `-r`
`-r` reads all arguments used by `trendmain.exe`. Therefore when `-r` is used all `trendplot.exe` options related to `trendmain.exe` program, such as `-f` and `--ica` options can be omitted.
- `-f [input prefix name]`
`-f` specifies prefix of input files. This needs to be the same as specified with the `-o` option when using the `trendmain.exe` program. e.g. uM or time (min)
- `-x [xaxisfile]`
The usage is identical to the `-x` option of `trendmain.exe`.
- `-u [Unit]`
The usage is identical to the `-u` option of `trendmain.exe`.
- `-n [integral number N]`
`-n` specifies the number of principal components to plot. For example, `-n 3` plots the first 3 principal components.
- `--single`
`--single` plots the component number `N` specified by `-n` option. For example, `-n 3 --single` plots the third principal component only.
- `-p`
`-p` plots PC1 values with normalization. This is normally the most important trend of change in an experiment. It is used without argument.
- `-e`
`-e` plots the scree plot, i.e. the size of each of the principal components

- `-m [0/1/2/3/4/5]`

`-m` turns on normalization of the `n` principle components plotted using the `-n` option. There are several modes of normalization to choose among; see the table below for the choices. When `-m` option is turned on, the normalized PC values will be saved to `normalizedPC.csv` file.

Normalization mode	meaning
0	No normalization on PC values
1	Normalize all PC values to 1.0, with PC1 increasing (typical for a ligand binding isotherm)
2	Normalize all PC values to 1.0, regardless of the direction
3	Normalize PC1 to 1.0 and set it to increase. Normalize all other components to PC1
4	Normalize PC1 to 1.0 without changing its orientation. Normalize all other components to PC1
5*	Normalize PC values to the sum of PC1+PC2
6\$	Normalize 2 phase binding isotherms into monotonic increasing, reverse turning point

* This mode was used for two sequential binding events

\$ This option might be used for binding isotherms of titrations where spectra in the middle of the titration are severely perturbed and the trajectory of change is V-shaped.

- `--biplot`

This option shows the "bi-plot" of the first two principal components in matrix VT. (A bi-plot is the equivalent of a score plot in the nomenclature of the metabolomics field).

- `--ica`

This option is required for plotting ICA results. All *principal components* mentioned above will be replaced by *independent components* when `--ica` is turned on.

- `--plot`

`--plot` is optional. When it is on, all figures will be saved as PNG images.

Reconstruction script - Use the PCA results from the Main script to model the 2D measurements

- **GUI and CLI programs**

GUI: `trendreconstructgui.exe` in Windows and Linux, `trendreconstructgui.app` in OS X

CLI: `trendreconstruct.exe` in all platforms

- **Synopsis**

```
trendreconstruct.exe -n [components] -m [1/2/3/4] [-e] [-p] [-r] [-z]  
[-d] [--rmsd] [--gif] [--dpi integral for dpi of output PNG files]
```

- **Description**

`trendreconstruct.exe` rebuilds the original 2D measurements using the first N principal components or the Nth principal component obtained from the main script. The script `trendreconstruct.exe` requires that the `--reconstruct` option be turned on when using `trendmain.exe`. Normally `trendmain.exe` will not save the files needed for PCA reconstruction unless the `--reconst` option is used.

- **Options**

- `-n component(s) selected for reconstruction`
`-n` specifies principal components selected to use in rebuilding the original measurements. For example, `-n 1` means reconstruct the 2D measurements using only the first component, while `-n 3` uses the third components. To reconstruct individual, non-consecutive components, you can separate the page numbers with commas: e.g. `-n 2,4,6` would reconstruct using the second, fourth, and sixth components. To reconstruct the range of components 1 to 3, use `-n 1-3`. Range and individual components can be selected together, e.g. `-n 1,3-5,7` means reconstruction by components 1, 3, 4, 5, 7. Note space is **not** allowed between selected components unless quotation marks are used. i.e. `-n '1, 3-5, 7'`.
- `-m [1/2/3/4]`

Background: `trendmain.exe` reorganizes the measurements listed in `file.index` as a large 2D matrix, filters them, and scales them for PCA. The `-m` option specifies which stage of the process should be reconstructed. `-m` specifies which matrix will be reconstructed. The choices are:

matrix mode	meaning
1	This recovers the state right before doing PCA. The large 2D matrix is row (column) scaled, with unchanged rows and noisy regions filtered out
2	This differs from matrix mode 1 in that the rows (columns) are unscaled
3	This option also recovers the filtered, unchanged rows from matrix mode 2
4*	This option differs from matrix mode 3 in also recovering the low intensity regions filtered out from being below the threshold

* mode 4 is for **Fourier-Transformed 2D NMR spectra** where `-r` option was used in `trendmain.exe` to set the threshold for filtering low intensity regions.

- `-e`

`-e` is optional. When it is on, the reconstructions of the measurements are exported in the same format as the original measurements read into `trendmain.exe`. If input format is `movie` or `png`, the output format will be mp4 format movie clip. mp4 movie reconstructed from `movie` shares the same time length as the input movie. mp4 movie reconstructed from a series of PNG images is set as 10 second long.
- `-p`

`-p` is optional. When it is on, the reconstructed images are exported as images in PNG format.
- `--gif`

`--gif` is optional. When it is on, the reconstructed movies are exported as GIF animation just in case there is no appropriate media player to play `.mp4` movie clip generated by `-e`. GIF animation is more compatible since most web browsers can play it, although it has larger size than mp4.
- `--dpi`

When `-p` option is turned on, `--dpi` specifies the dots-per-inch resolution of the

png files to be reconstructed. By default, this is set to 96. This option is not required.

- `-z`
`-z` option is used to recover rows that do not change and hence was filtered out before doing PCA.
- `-r`
`-r` is used only when reconstructing 2D Fourier-transformed NMR spectra. When doing PCA on 2D NMR spectra, a threshold can be set to filter out noisy regions using the `-r` option for `trendmain.exe`. The `-r` option can be used in reconstruction to recover those noisy regions in order to fully recover the original spectra. It is not required and is usually not necessary.
- `-d` or `--diff`
`-d` turns on the `diff` mode, exporting difference spectra instead of reconstructed spectra. It is used to visualize the difference between original and reconstructed spectra.
- `--rmsd`
`--rmsd` calculates the RMSD between the reconstructed and original data for all principal components. A file with suffix name as `-rmsd.txt` and prefix name specified by the `-o` option of `trendmain.exe` will be stored. For example, if `trendmain.exe` was used with the `-o example` option, `--rmsd` option generates a file named as `prefix-rmsd.txt`.
- `-o [output prefix name]`
`-o` specifies the prefix of reconstructed files or images

SVD test script

- **CLI program**

`trendtest.exe` in all platforms

- **Synopsis**

```
trendtest.exe [-r] [-f [outname prefix of trendmain.exe]]
```

- **Description**

`trendtest.exe` analyzes PCA results generated by `trendmain.exe` and `reconst.py`. It saves a table of attributes for evaluating the SVD outcome in the `prefix-svdtest.csv` file. Columns of the table are: Component, Size of singular value, cumulative contribution ratio, auto correlation coefficient, RMSD, and Normalized RMSD, respectively. The last two columns are optional and depend on whether `trendreconstruct.exe --rmsd` was executed.

- **Options**

- `-r`
`-r` reads all arguments used by `trendmain.exe`. When `-r` is used `-f` is not necessary
- `-f [input prefix name]`
`-f` specifies the prefix of input files. This needs to be the same as specified with the `-o` option when using the `trendmain.exe` program

Report script

- **CLI program**

`trendplot.exe` in all platforms.

- **Synopsis**

`trendreport.exe`

- **Description**

`trendreport.exe` generates an HTML report just in case the `trendmain` was executed without turning on `--report` option.

Known limitations by operating system

Fedora 23

- `trendmain` and `trendreport` are not able to launch web browser to open `-report.html` automatically

OS X 10.7 to 10.8

- `GIF movie` option of `trendreconstruct` is not supported

OS X 10.11

- The strict safety precautions of El Capitan prevent double clicking or use of the `open` command in terminal from launching TREND apps. The TREND apps may nonetheless be launched in a terminal (text) window. To enable opening of the apps by double clicking, enter the system preferences menu and go to Security & Privacy and select Allow app downloaded from anywhere, at least temporarily until you have run each of the apps.

Initial uses of TREND without internet connection

`trendmain` and `trendreconstruct` depend upon `moviepy` and `imageio` to read, process, and write movies. During first uses with a fully functional internet connection, `imageio` automatically downloads and installs `ffmpeg` and `freeimage` to the `appdata` directory. However, absence of a stable internet connection may prevent these downloads and prevent TREND from processing and exporting movies. Running TREND later with an internet connection should rectify this. Alternatively, `ffmpeg` and `freeimage` should be downloaded manually and placed in the `appdata` directory.

Windows

- Download and unzip [no_internet_patch_Windows.7z](#)
- Run `install_imageio_thirdparty.bat` or manually copy `imageio` folder to the `appdata` directory: `C:\Users\your_user_name\AppData\Local`

OS X 10.7, 10.8

- Download and unzip [no_internet_patch OSX_10.7_10.8.tar.gz](#)

OS X 10.9 or later

- Download and unzip [no_internet_patch OSX.tar.gz](#)
- Run `./install_imageio_thirdparty.script` in a terminal or copy the `imageio` folder to the `appdata` directory by typing this command in a terminal:
`cp -r imageio /Users/your_user_name/Library/Application Support/`
- Note the `appdata` directory `/Users/your_user_name/Library/Application Support/` is hidden in Finder.

Linux (Ubuntu 14.04, Fedora 23 or later)

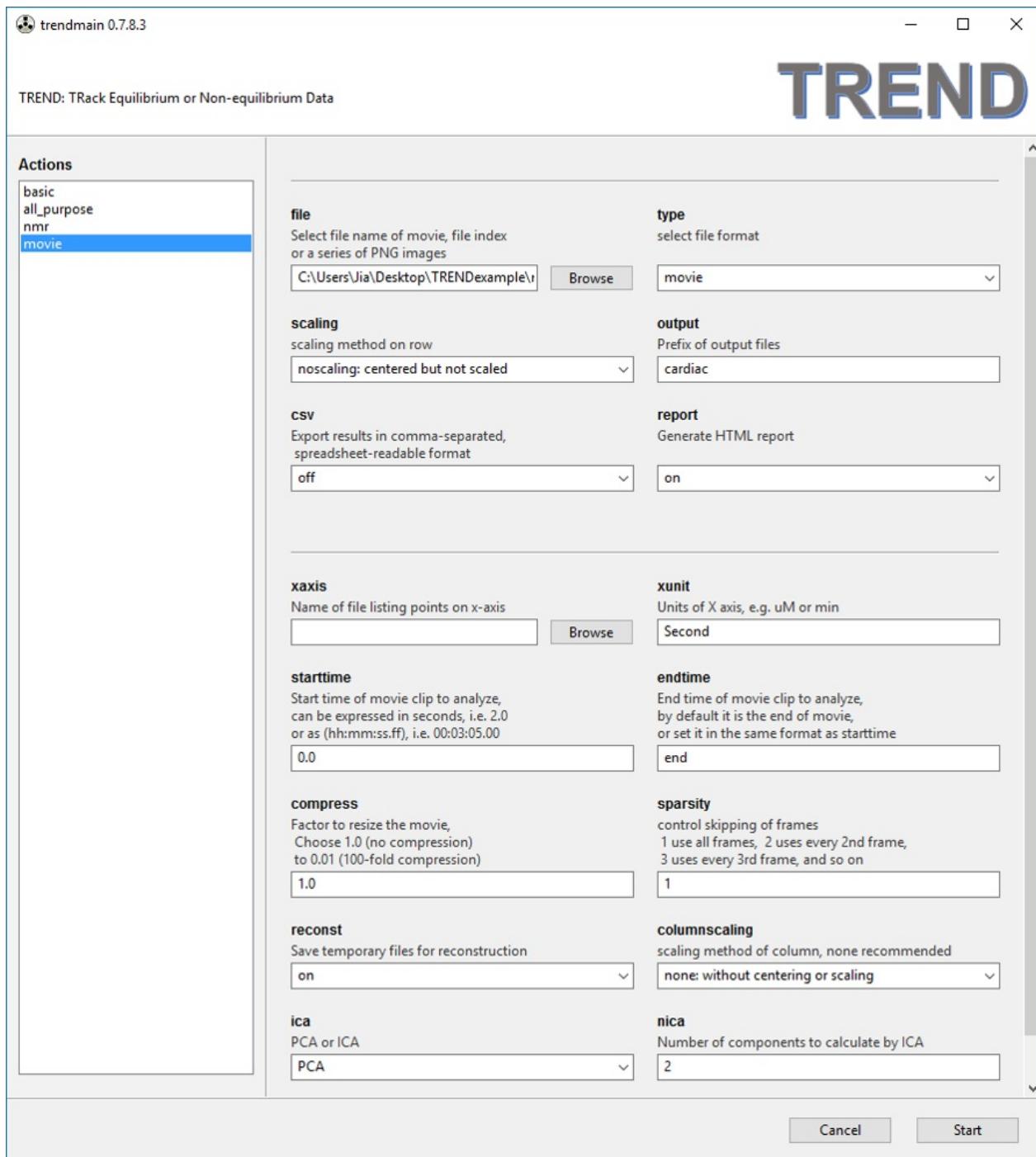
- Download and unzip [no_internet_patch_Linux.tar.gz](#)
- Run `./install_imageio_thirdparty.script` in a terminal or copy `imageio` folder to the `appdata` directory by typing command within terminal shell:
`cp -r imageio ~/.imageio`
- Note the `appdata` directory `~/.imageio` is hidden in either desktop or terminal shell except using command `ls -a .imageio`

Tutorial: Extract the time courses of respiration and heart contraction

(The MRI movie is courtesy of Jens Frahm and coworkers at Max-Planck-Institut für Biophysikalische Chemie in Göttingen and is available at:
http://www.biomednmr.mpg.de/index.php?option=com_content&task=view&id=132&Itemid=38#Cardio

1. Do PCA on the movie

Select the movie named as `cardiac-rtMRI.avi` in the unzipped `TRENDexample` folder. Run PCA using `trendmaingui` with row scaling method set as `noscaling`, and `reconstruct` option turned on to save files for reconstruction.



To do this at the command line, `cd` to the directory where the `cardiac-rtMRI.avi` is, and run:

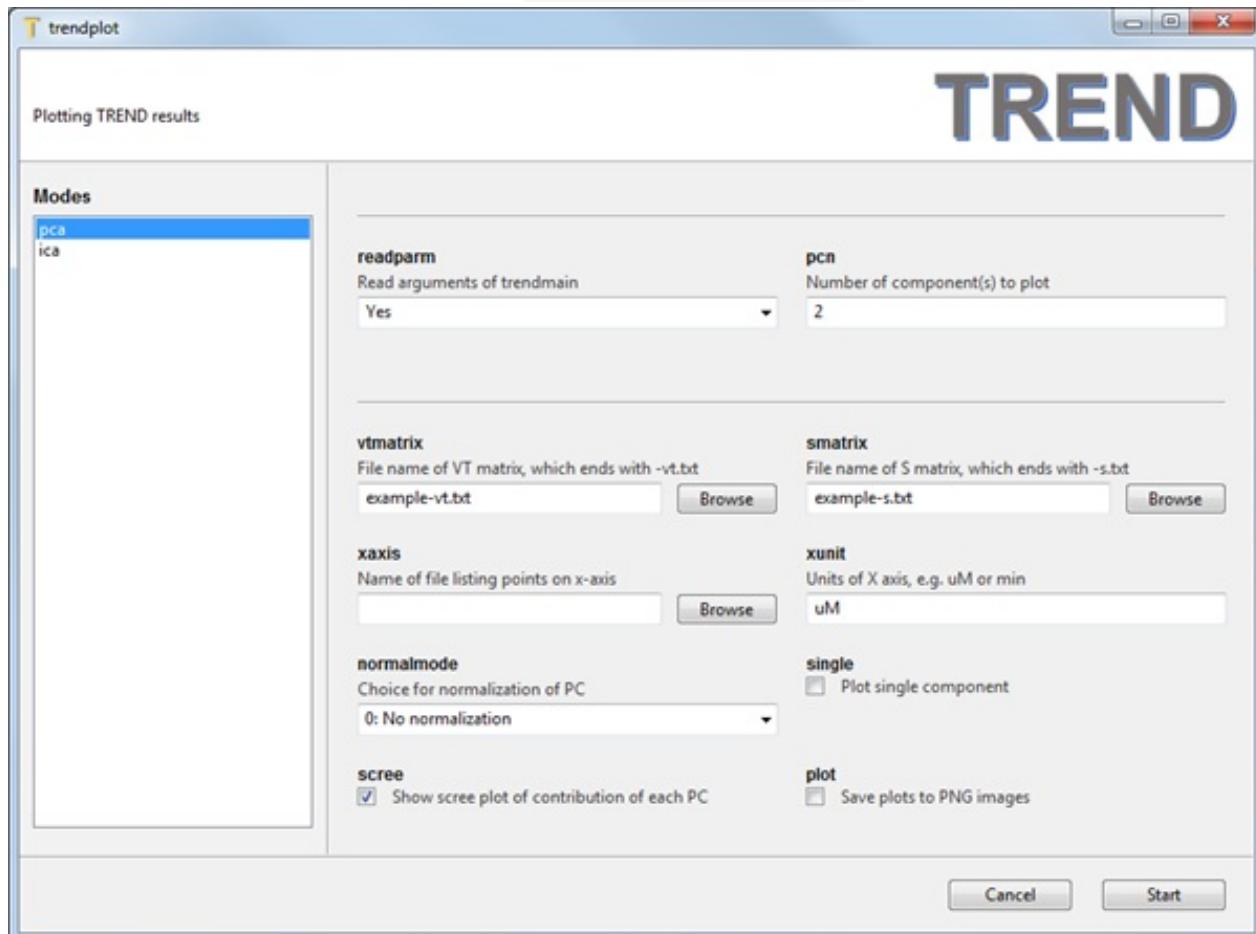
```
trendmain.exe -f cardiac-rtMRI.avi -t movie -s noscaling --reconst --report
```

2. Visualize PCA results

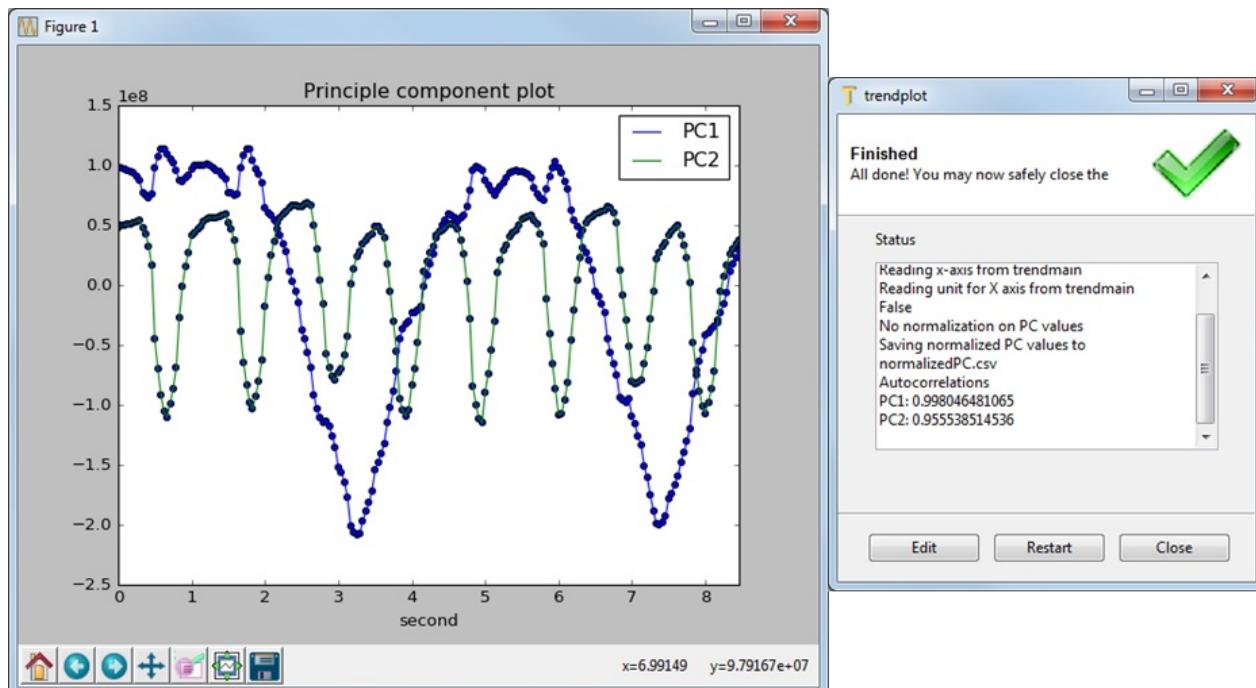
When the PCA calculation finishes, run `trendplotgui` with `readparm` option set as `yes` to read arguments from the just finished `trendmaingui`. Set `pcn` as 2 to show the first two components as well as scree plot. To visualize results obtained from PCA on movie, TREND will extract time of frames and use it as ticks in X-axis. If not specified, the default units will be seconds.

Extract the time courses of respiration and heart contraction

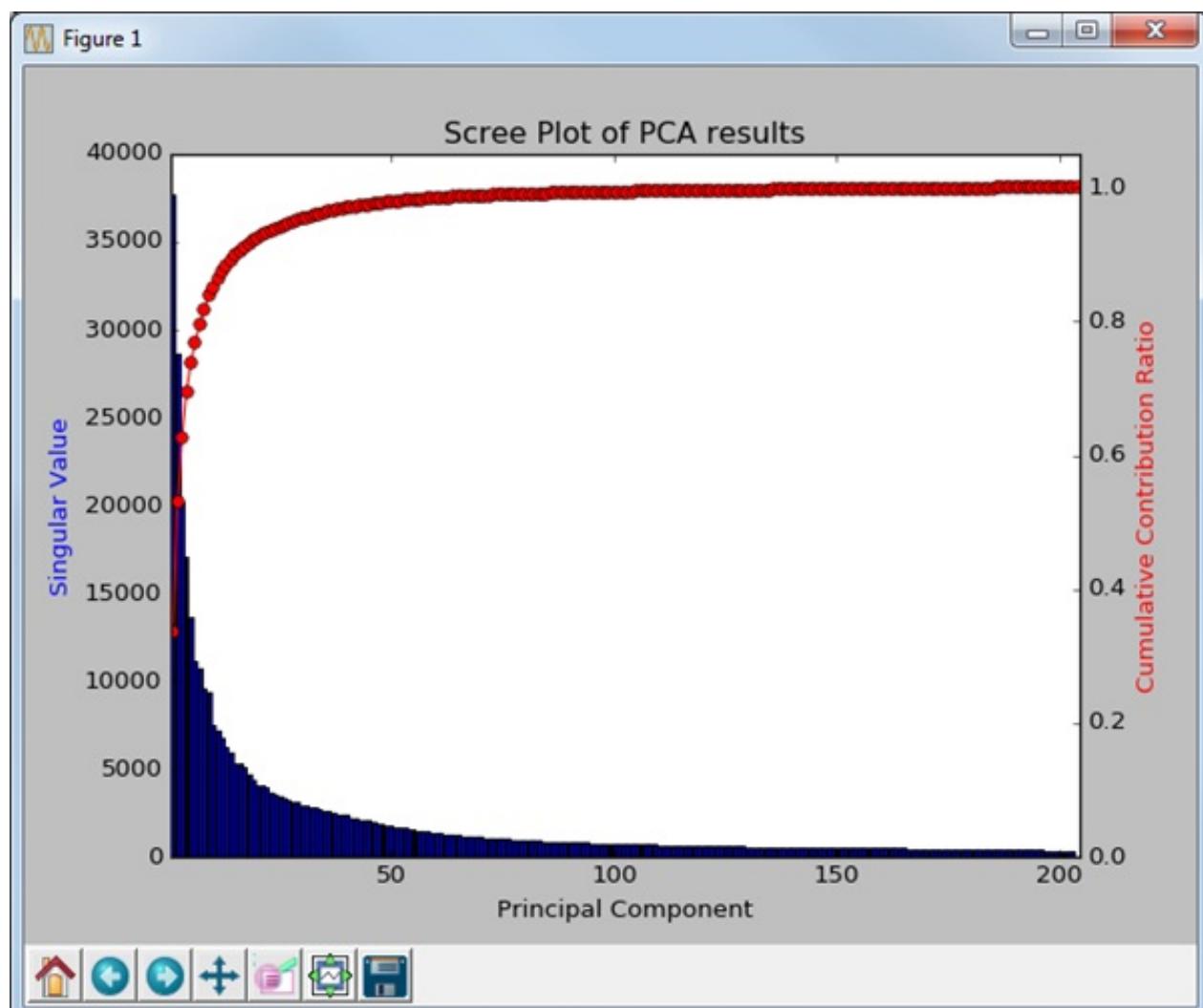
To do this at the command line, use `trendplot.exe -r -e -n 2`



When `trendplotgui` finishes, PC1 and PC2 are plotted. It is clear that PC1 represents time course of two breaths and that PC2 tracks the cardiac oscillation between diastole and systole.



Extract the time courses of respiration and heart contraction

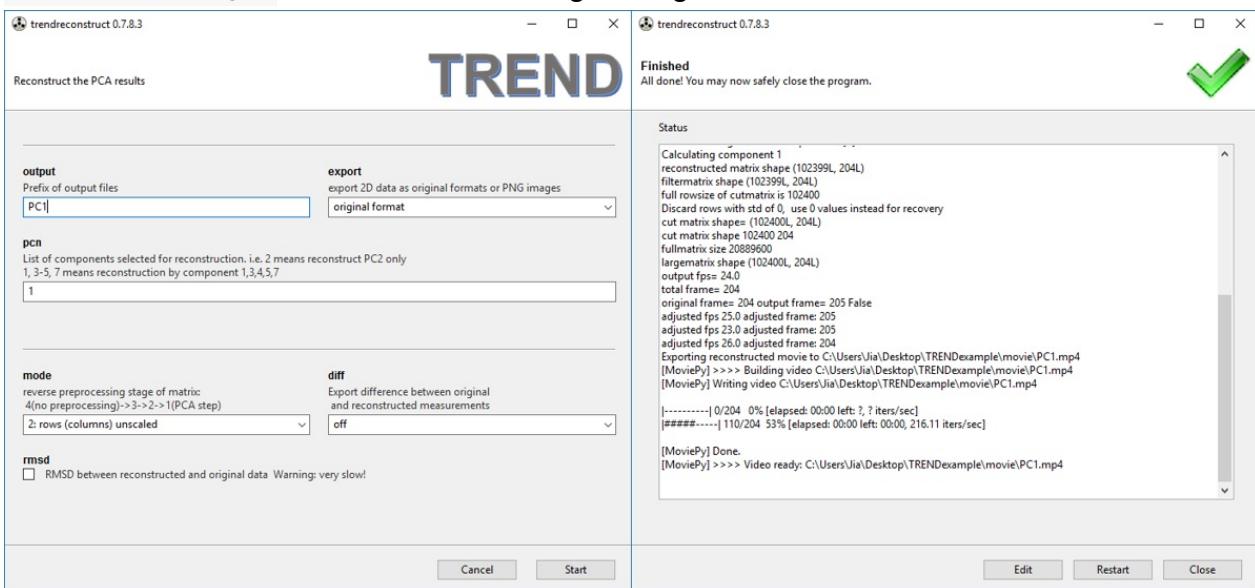


From the scree plot we can find that PC1 and PC2 contribute to ~50% of the total variance.

3. Reconstructing Movies from only PC1 and PC2

Extract the time courses of respiration and heart contraction

It will be interesting to see movies reconstructed by PC1 and PC2. Run `trendreconstructgui` and use the following settings.

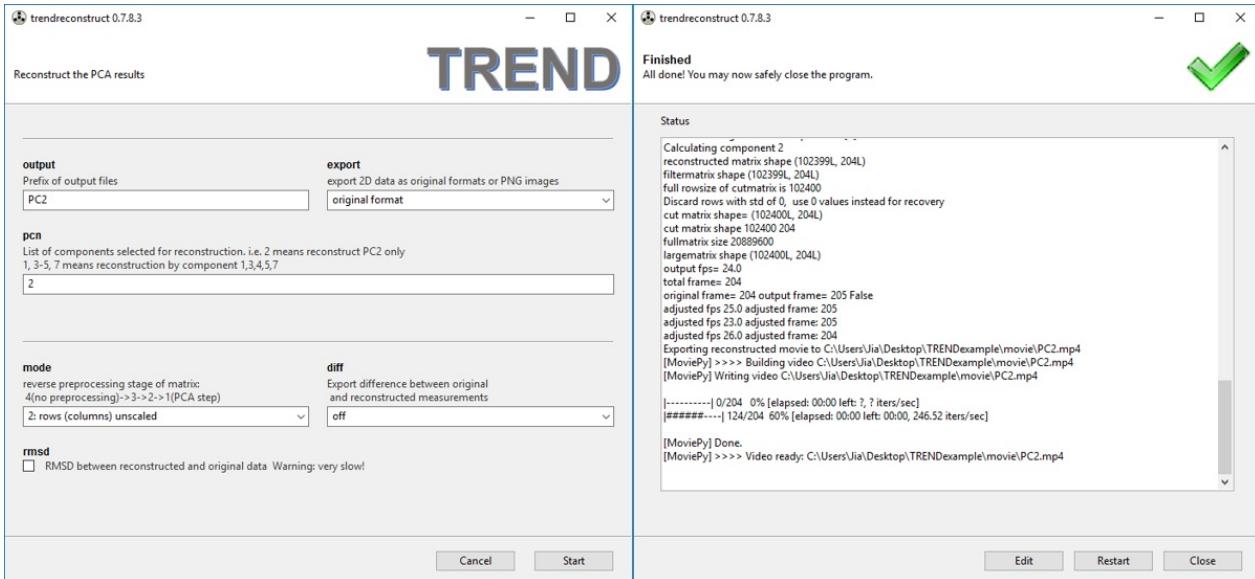


Extract the time courses of respiration and heart contraction

Leaving most settings at the default. Set the `prefix for the output` to `PC1`. It can be seen that a `PC1.mp4` is generated once `trendreconstructgui` finishes.

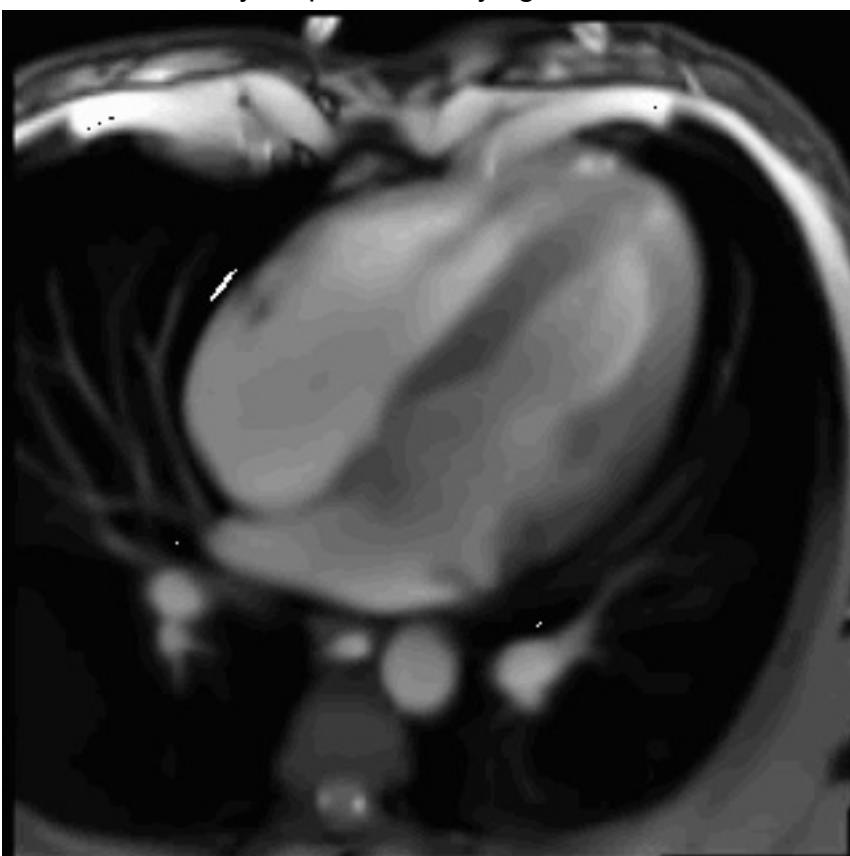
The command line usage is: `trendreconstruct.exe -r -n 1 -e -o PC1`

A movie clip `PC2.mp4` reconstructed from PC2 is generated in the same way:



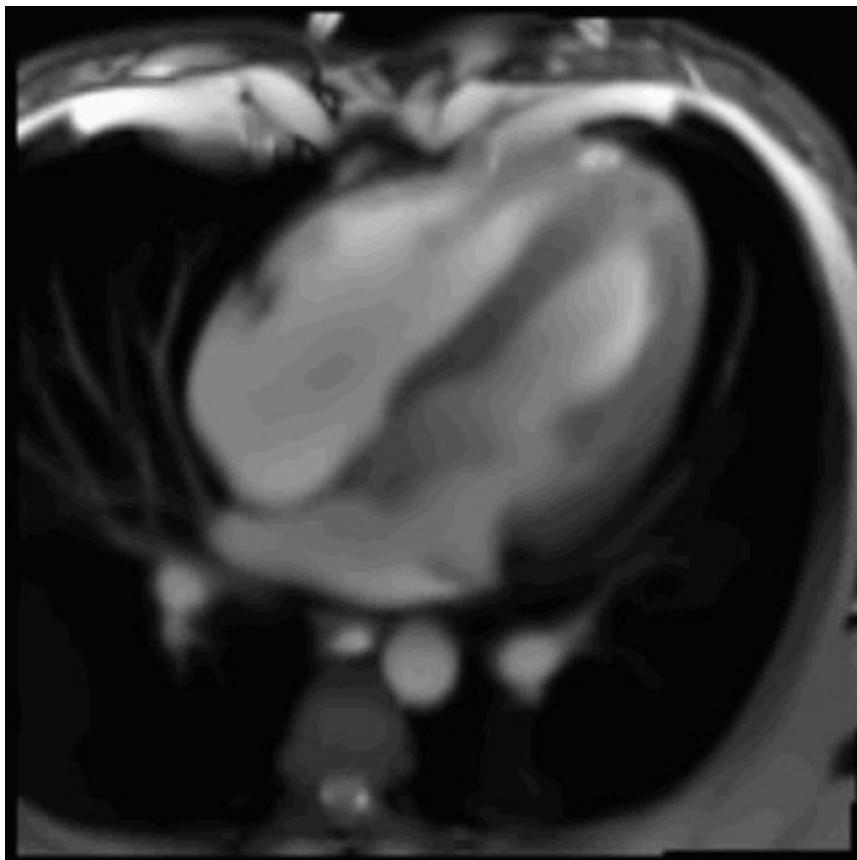
Its command line usage is: `trendreconstruct.exe -r -n 2 -e -o PC2`

The two reconstructed movies to result are `PC1.mp4` and `PC2.mp4`. Playing the `PC1` movie shows only respiration. Playing the `PC2` movie shows only the heart beating.



Above is `PC1.mp4`

Extract the time courses of respiration and heart contraction



Above is PC2.mp4

If TREND is used in your research, please cite the references

Jia Xu and Steven R. Van Doren, Binding Isotherms and Time Courses Readily from Magnetic Resonance. *Anal. Chem.* 2016, 88 (16), pp 8172-8178

Jia Xu and Steven R. Van Doren, Tracking Equilibrium and Non-equilibrium Shifts in Data with TREND. Submitted.

Coming soon (2016-11-21)

trendmaingui and trendmain

- Supports Bruker Topspin FID/spectra, Agilent(Varian) FID/Spectra
- Supports various [JCAMP-DX](#) formats
- Enables clipping of input video by setting `starttime` and `endtime`

trendreconstructgui and trendreconstruct

- Supports reconstruction of NMRPipe FT2 format
- Supports reconstruction of Topspin FID/Spectra, Agilent FID