Rametrix™ LITE Toolbox v1.1 Tutorial
With additional instructions and frequently asked questions

Last Updated: October 18, 2018

Introduction

The Rametrix™ LITE Toolbox is for use with MATLAB®(version R2016a or later) and can be downloaded from GitHub at https://github.com/SengerLab/RametrixLITEToolbox

The Rametrix™ LITE Toolbox is subject to copyright and the MIT License agreement located on the GitHub site and in the "Terms of Use" tab in the Rametrix™ LITE Toolbox, itself.

Version Updates

Substantial changes have been implemented between the initial version (v1.0) and the current version (v1.1) of the Rametrix™ LITE Toolbox. The following are a summary of changes since the last version:

- "Field" values are no longer stored in the filename of spectral files (separated by underscores). They are now stored in a new "Fields File," which is either a Google Spreadsheet (GSHEET) or comma-separated value (.csv) file.
- Spectral files are now named according to a barcode value, which also appears in the Fields File.
- All spectral files (from multiple experiments) can now be stored in a common spectral database folder. Individual folders for separate experiments are no longer needed.
- After baselining, spectra can now be "trimmed" on either end to enhance the baseline fit, if necessary.
- The spectra averaging function is more efficient, and an "un-averaging" function has been included.
- Several bug-fixes and viewing updates have been included to enhance the overall experience of using the Rametrix™ LITE Toolbox.

Tutorial

This tutorial makes use of a dataset of Raman scans of different concentrations of 2-Nitrophenol.

Obtaining, Installing, and Starting the Rametrix™ LITE Toolbox GUI

1. Download the "Rametrix™ LITE Toolbox v1.1.mltbx" file from GitHub (at this link).

2. Double-click the MATLAB Toolbox .mltbx file to start MATLAB and open the install dialog box. Click "Install."

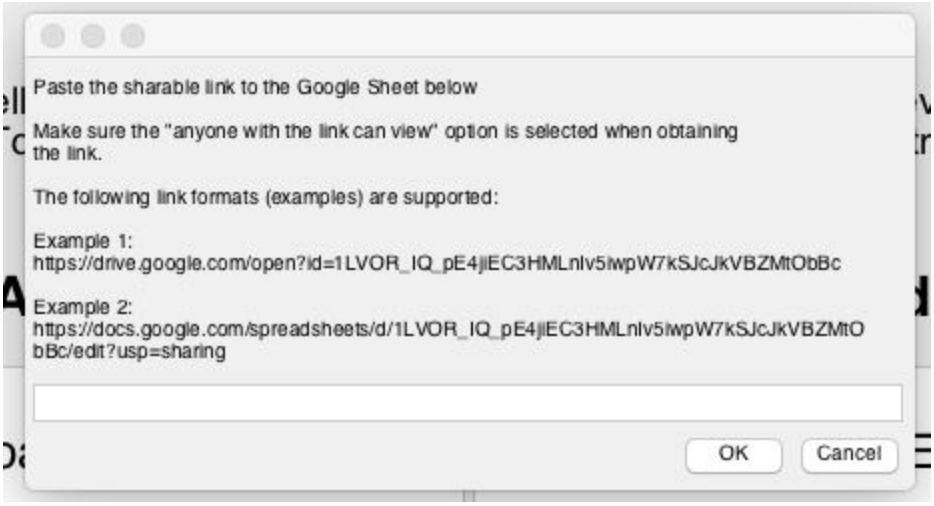
3. Run the Rametrix™ LITE Toolbox by typing "Rametrix" in the MATLAB Command Window. From here, everything runs through the Rametrix™ GUI.

Loading Spectral Files

4. Download the 2-Nitrophenol Calibration Curve Dataset from GitHub (zipped file at this link). Unzip the folder and place in a convenient location.

   Note: Spectral files can be in .SPC, .TXT, or .CSV format. However, files must be named accordingly. In the 2-Nitrophenol Calibration Curve Dataset, the first spectral file included is "NPC1_0001.spc". The portion of the file name "NPC1" is the "barcode," and "_0001" is the scan replicate. The barcode is used to associate other experimental conditions (called "fields" or "factors") with the sample. Often, up to 10 scan replicates are obtained per sample. This produces one spectral file per scan. The Rametrix™ LITE Toolbox has the option to average all scan replicates, so it is important this file naming convention is used. All spectral files must be names with a barcode followed by an underscore and a 4-digit scan replicate value.

5. In the Rametrix™ LITE GUI, Click the “Load Fields (GSHEET)” button in the “Start” tab. This displays the following input box:



6. Copy and paste the following address in this input box and click “OK”: https://docs.google.com/spreadsheets/d/1NRDzVOBL1AeP8cl-pw8ci0R5Xrhkp1lzDWHgWQMIhdg/edit?usp=sharing

7. After seeing the “Success!” notification, Click the “Load Spectra (.SPC)” button. Navigate to the downloaded “2-Nitrophenol calibration curve dataset” folder, and select “Open.” The files will load and another “Success!” dialog box will be displayed. Click “OK.”

Creating and Editing Fields Files

Note: Fields files contain all experimental information about a sample. The example for the 2-Nitrophenol calibration curve dataset can be viewed by copying/pasting the address above (in item #6) into a browser window. This is shown in the figure below.

Note: To use Google Sheets Fields Files in Rametrix™, specific file sharing accesses must be granted. These are discussed in the *Fields Files Sharing* section below.

NPC - 2-Nitrophenol Calibration Curve Log

File Edit View Insert Format Data Tools Add-ons Help All changes saved in Drive

	A	B	C	D	E	F	G	H	I	J	K
1	Index	Barcode	Project	Instrument	Scan Date	Scanned By	Collection Date	Concentration (mM)	Vial	Scan Replicate	
2	1	NPC1	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	0.1	1	1	
3	2	NPC1	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	0.1	1	2	
4	3	NPC2	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	0.1	2	1	
5	4	NPC2	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	0.1	2	2	
6	5	NPC3	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	0.5	1	1	
7	6	NPC3	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	0.5	1	2	
8	7	NPC4	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	0.5	2	1	
9	8	NPC4	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	0.5	2	2	
10	9	NPC5	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	1	1	1	
11	10	NPC5	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	1	1	2	
12	11	NPC6	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	1	2	1	
13	12	NPC6	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	1	2	2	
14	13	NPC7	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	5	1	1	
15	14	NPC7	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	5	1	2	
16	15	NPC8	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	5	2	1	
17	16	NPC8	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	5	2	2	
18	17	NPC9	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	10	1	1	
19	18	NPC9	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	10	1	2	
20	19	NPC10	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	10	2	1	
21	20	NPC10	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	10	2	2	
22	21	NPC11	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	20	1	1	
23	22	NPC11	2-Nitrophenol calibration curve	Alpha	20161212	Amanda Fisher	20161212	20	1	2	

<u>The following columns are contained in the Fields File:</u>

Column A: **Index** - numerical counting so the list can be easily sorted
Column B: **Barcode** - this is the same as the barcode in the spectral file name
Column C: **Project** - the project identifier
Column D: **Instrument** - the name of the Raman instrument used to perform the scan
Column E: **Scan Date** - the date the scan was obtained
Column F: **Scanned By** - the name of the researcher who performed the Raman scan
Column G: **Collection Date** - the date the sample was obtained or prepared (can differ from scan date)
Column H: **Concentration (mM)** - the concentration of 2-nitrophenol in the vial
Column I: **Vial** - the replicate vial number
Column J: **Scan Replicate** - the Raman scan replicate. This is the same as the scan replicate number in the spectral file name.

<u>For new Fields Files, Columns A through G are always required as-is. The next columns are dedicated to experimental parameters of the given experiment (any number can be included). The final column is reserved for the scan replicate. This is also a required column in the Fields File.</u>

This setup allows the user to add, edit, and delete fields in the experiment easily without altering or renaming spectral files.

An added benefit is that rows can easily be added to this Fields File (including barcodes from other experiments). Specific rows can also be copied and added into other Google Sheets. Users may find it useful to sort this file be one or several columns to group rows by the value in a specific column. This often allows for easy copying and pasting into another sheet. For example, for the 2-Nitrophenol Calibration Curve Log (shown above), highlight cell I2. Then, select the "Data" drop-down menu in the toolbar above. Then select "Sort sheet by column I, A→Z." This allows the user to select/copy all entries in either Vial 1 or Vial 2 easily. This can be undone by highlighting cell A2 and repeating the sorting operation.

How To Choose a Barcode

Barcodes must consist of a 3-letter capital-letter prefix, followed by a number. The prefix often describes the project (e.g., NPC for 2-nitrophenol calibration). The numbering always starts at "1" and increments for each sample.

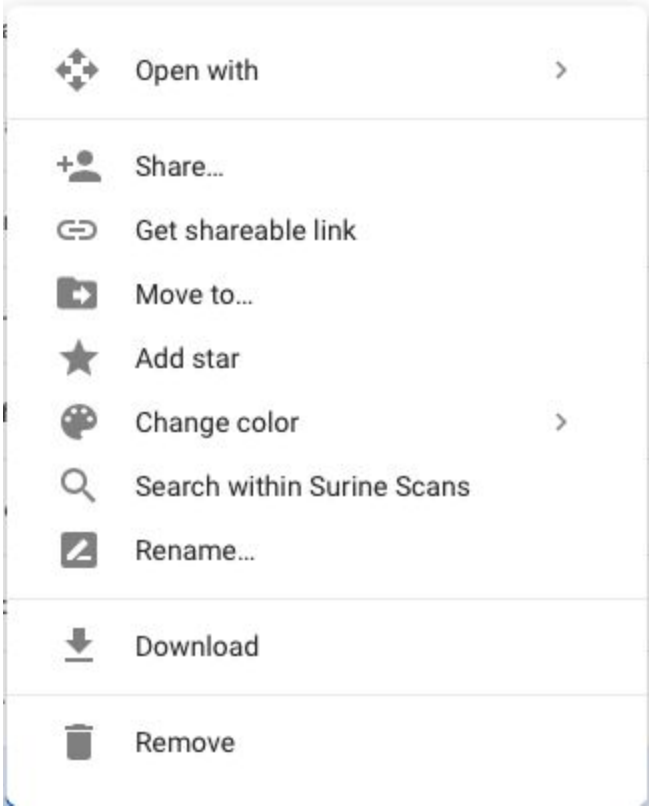
Types of Fields Files

It has been found beneficial to create Fields Files as Google Sheets. However, they can be prepared using any spreadsheet program, including Microsoft Excel. In these cases, the spreadsheet must be exported in comma separated value format (.CSV). This is usually an option under "Save As." Then, the Fields File is read into Rametrix™ using the "Load Fields (.CSV)" button.

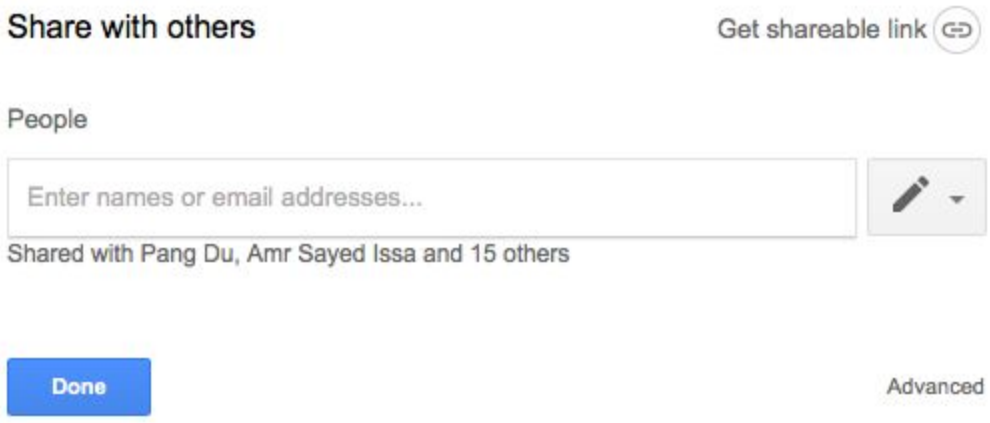
Fields Files Sharing (Required to Use Google Sheets with Rametrix™)

Fields Files must be shared so that "anyone who has the link" can access the file. This is done with the following steps. These instructions apply for an individual Google Drive. Google Team Drives are a bit different but follow the same concept. The goal is to allow access to the file to "anyone who has the link."

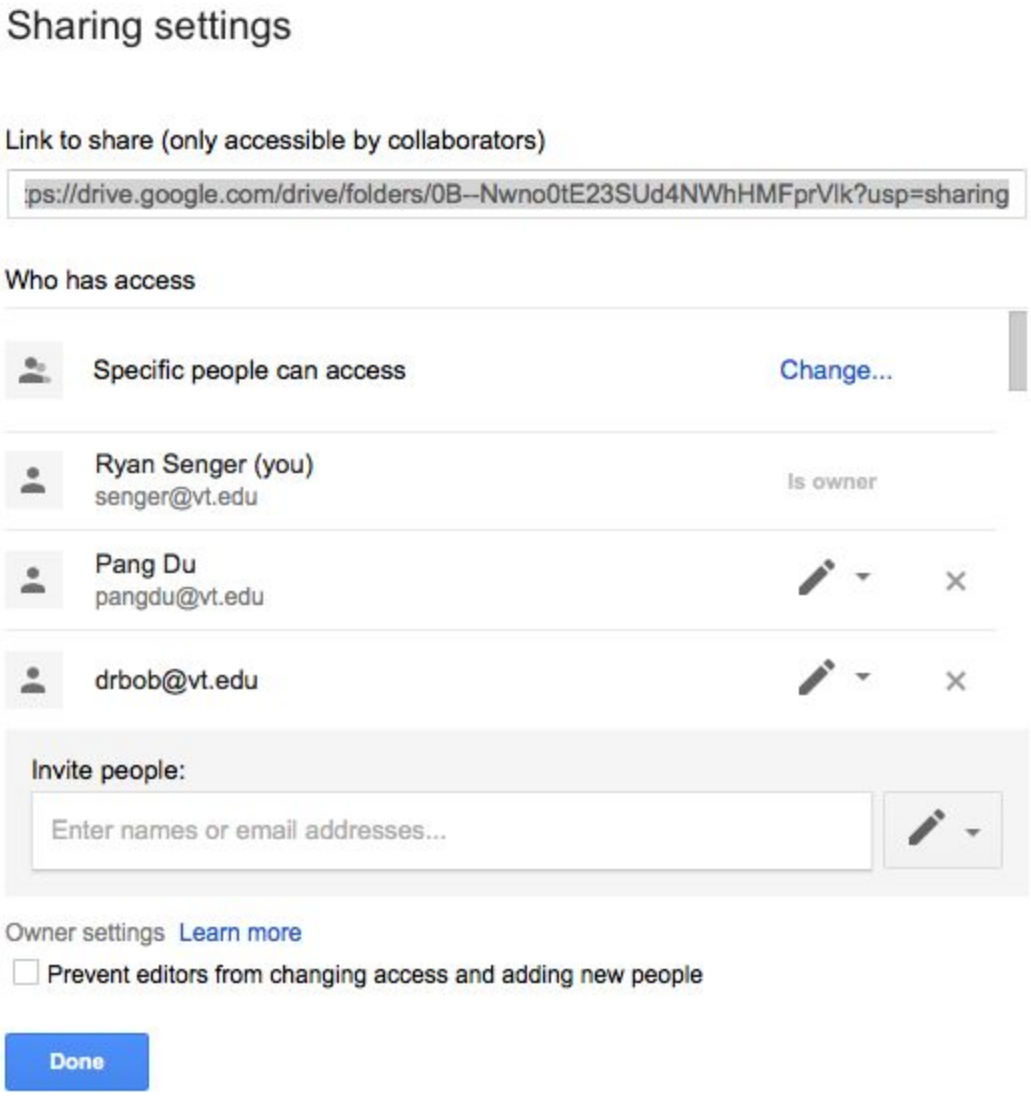
A. Right click on the folder containing the Fields File and select "Share…" as shown in the menu below.



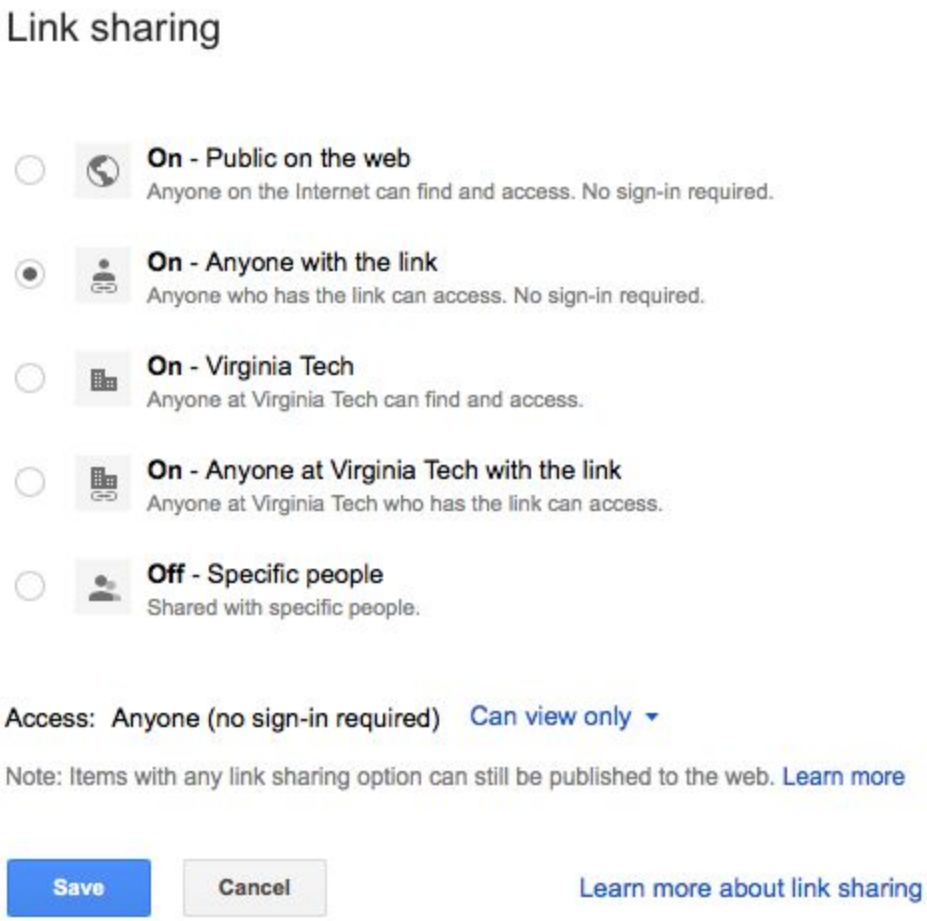
B. Next, click the “Advanced” link in the menu shown below.



C. Click the “Change…” link in the Sharing Settings menu, as shown below.



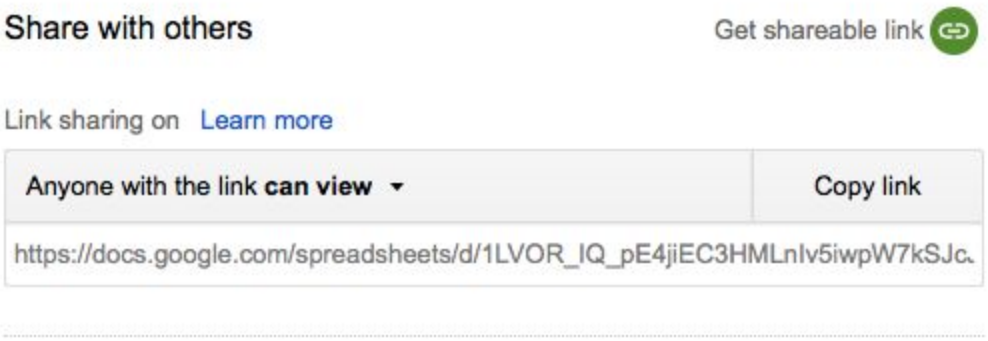
D. Select the “Anyone with the Link” option, and click “Save.”



With these steps, all Fields Files in the shared folder will be accessible to anyone with the link, which is required for Rametrix™ to read the file.

E. Next, open the Fields File to be read into Rametrix™, and select the “SHARE” button on the top right, as shown below.

F. The select the “Copy Link” button. This link will be pasted into Rametrix™ (as shown above in the Tutorial item #6).



Note: Be sure the “Anyone with the link can view” option is selected. If it is not, this can be done from the dropdown menu.

The Explore Tab of the Rametrix™ GUI

The following steps resume with the 2-Nitrophenol Calibration Curve tutorial.

8. After loading the 2-Nitrophenol Calibration Curve Dataset into the Rametrix™ GUI, select the “Explore” tab at the top.

The remaining sections of this tutorial are under construction and will be completed before November 1, 2018. Please check back for the updated version.