**This will become the README file for the Github repository containing the Python version of the isotopocule data corrections.**

**Configuring Python on your computer:**

1. Check that you have python3 installed on your computer. On a Mac, open a new Terminal window. Run:

colette$ python3 --version

1. This should output something like:

Python 3.9.2

*Note that the text before the dollar sign will vary based on the user and computer. If python3 is not yet installed on your computer, this is a useful guide for installing python as well as working with virtual environments: https://github.com/stanfordpython/python-handouts/blob/master/installing-python-macos.md*

1. Install the packages necessary to run pyIsotopomer. Run:

colette$ pip install --upgrade pip

colette$ pip install jupyter jupyterlab numpy scipy pandas

*This may take a while; pip (the python package manager) will check if these packages have already been installed, and if not, download from the cloud and install them on your machine.*

**Size correction:**

1. Open .xls file containing Isodat output. Note the two tabs at the bottom; one sheet contains raw data for each sample, the other contains raw data for the designated reference peak for each sample.
2. In the ‘sample’ tab, bring all fragment data in line, then delete extra rows. Do the same on ‘standard’ tab.
3. Open the excel data correction template “00\_excel\_template.xlsx”. Copy the raw sample data from columns A-O in the sample tab into columns A-O in the correction template. Copy the the ‘rR’ columns from the standards tab (columns M, N, O) into columns Q, R, S in the correction template. Save the correction template with a new name.
4. Check to make sure all references in columns Y, Z, and AA and AC, AD, and AE are correct.

*Note that the 31R, 45R, and 46R in columns V-X are specific to the Casciotti lab’s N2O reference gas. Note also that the size correction in columns AC-AE is specific to the linearity of the Casciotti Lab Delta V, as of February-March 2021.*

**Calibrating your instrument for scrambling with pyIsotopomer:**

*Here, two coefficients are used to describe scrambling at the ion source. This is described in further detail in Frame and Casciotti, 2010 and Kelly et al. (in prep). Below is a brief summary of the method for the determination of these scrambling coefficients.*

1. Run two reference gases with known 15R-alpha and 15R-beta, prepared in the same format as samples(i.e., some amount of N2O reference gas injected into a bottle of seawater or DI water that has been pre-purged with He or N2 gas). Process and size-correct these data in the excel correction spreadsheet, as in steps 1-4 above.
2. Open “00\_excel\_template.xlsx”. Copy and paste the size-corrected 31R, 45R, 46R (columns AG, AH, and AI) from the size\_correction tab into the scrambling\_input tab, columns C-E.
3. Re-organize the size corrected data into pairs of reference materials by copy-pasting into columns H-M of the scrambling input template. The columns should be in the following order: 31R, 45R, 46R for reference #1, then 31R, 45R, 46R for reference #2.
4. Copy-paste columns H-M in the scrambling input template into their own .csv file, with no heading. Save the .csv file into the pyIsotopomer directory.

*Note that it is important to save as the simple “comma-separated values (.csv)” file format. More complicated versions of the .csv format, such as CSV UTF-8, will not work.*

1. Open **constants.py** in a text editor. Note that we specify a dictionary of reference materials in lines 37-42. If the reference materials used to calibrate scrambling are not in this dictionary, add them, following the format of the existing lines. Save **constants.py**
2. Open a terminal window. Launch Jupyter Notebook:

colette$ jupyter notebook

1. This should open Jupyter in a new browser window. In Jupyter, navigate to the pyIsotopomer directory. Click on **run\_pyIsotopomer.ipynb** to open the Jupyter Notebook containing the code to run pyIsotopomer.
2. Follow the instructions in the Jupyter Notebook to run pyIsotopomer and obtain scrambling coefficients. Copy and paste these back into the correction template, columns P-Q.

**Correcting raw Isodat files for isotopomers with pyIsotopomer**

1. Size-correct your data, as above. Copy and paste columns AG, AH, and AI (size corrected R’s) into a separate spreadsheet, making sure to ‘paste special’, ‘values’. This will become your matlab input file. Make sure your data columns are in the following order: 31R, 45R, 46R, then remove the headings. Save input file in .csv format, e.g. **example\_input.csv**, into the pyIsotopomer directory.
2. Open a terminal window. Launch Jupyter Notebook:

colette$ jupyter notebook

1. This should open Jupyter in a new browser window. In Jupyter, navigate to the pyIsotopomer directory. Click on **run\_pyIsotopomer.ipynb** to open the Jupyter Notebook containing the code to run pyIsotopomer.
2. Follow the instructions in the Jupyter Notebook to run pyIsotopomer and obtain sample isotopocule values in delta notation. Copy and paste these back into the correction template, columns P-Q.
3. Çopy and paste output data back into working spreadsheet in olive-highlighted cells (columns AK-AI).
4. Note that a scale decompression may be applied after the isotopomer calculation. This is calculated in the “scale-decompression” tab of the excel worksheet, and is applied in columns AU-AZ of the size\_correction tab.

**Calculating concentrations of 44N2O, 45N2O-alpha, 45N2O-beta, and 46N2O**

1. Calculate the amount (nmol) of 44N2O in your sample from the ratio of mass 44 peak area to N2O (nmol/Vs) for your instrument
2. Use the weight difference of the bottle pre- and post-analysis to determine the volume of sample run, and thus the concentration of 44N2O
3. We calculate the *concentrations* of 45N2O-alpha, 45N2O-beta, and 46N2O (for tracer experiments) from the delta value and associated 15/14 ratios

**Calibrating your instrument for scrambling with pyIsotopomer, MATLAB-style**

*Two coefficients are defined to describe scrambling at the ion source. This is described in further detail in Frame and Casciotti, 2010 and Kelly et al. (in prep). Below is a brief summary of the method for the determination of these scrambling coefficients.*

1. Run two reference gases with known 15R-alpha and 15R-beta, prepared in the same format as samples(i.e., some amount of N2O reference gas injected into a bottle of seawater or DI water that has been pre-purged with He or N2 gas). Process and size-correct these data in the excel correction spreadsheet, as in steps 1-4 above.
2. Open “00\_excel\_template.xlsx”. Copy and paste the size-corrected 31R, 45R, 46R (columns AG, AH, and AI) from the size\_correction tab into the scrambling\_input tab, columns C-E.
3. Re-organize the size corrected data into pairs of reference materials by copy-pasting into columns H-M of the scrambling input template. The columns should be in the following order: 31R, 45R, 46R for reference #1, then 31R, 45R, 46R for reference #2.
4. Copy-paste columns H-M in the input template into their own .csv file, with no heading. Save the .csv file into the pyIsotopomer directory.

*Note that it is important to save as the simple “comma-separated values (.csv)” file format. More complicated versions of the .csv format, such as CSV UTF-8, will not work.*

1. Open **constants.py** in a text editor. Note that we specify a dictionary of reference materials in lines 37-42. If the reference materials used to calibrate scrambling are not in this dictionary, add them, following the format of the existing lines. Save **constants.py**
2. Open **automate\_gk\_setinputs.py** in a text editor. Change line 26 to reflect your input filename. This should read something like:

inputfilename= 'example\_scrambling\_input.csv'

1. Change line 31 to reflect what you would like the output filename to be. This should read something like:

outputfilename= 'example\_scrambling\_output.csv'

1. Change lines 35 and 36 to reflect the two reference materials you will use to calibrate scrambling. This should read something like:

ref1 = 'ATM'

ref2 = 'S2'

1. Save **automate\_gk\_setinputs.py**
2. Open a terminal window and navigate to the pyIsotopomer directory:

colette$ cd "/Users/colette/Box Sync/N2O Research/Spec Calibration Files/N2O\_isotopocule\_data\_corrections/Python/pyIsotopomer"

*Note that the text before “/N2O\_isotopocule\_data\_corrections/Python/pyIsotopomer” will depend on where you have saved these files.*

1. In the terminal, run **automate\_gk\_setinputs.py**:

colette$ python3 automate\_gk\_setinputs.py

1. Open your output file and copy-paste the two columns back into the scrambling input template. The first column will be the gamma values and the second column will be the kappa values for each pair of samples. These should all be quite similar.

**Correcting raw Isodat files for isotopomers, MATLAB-style**

1. Size-correct your data, as above. Copy and paste columns AG, AH, and AI (size corrected R’s) into a separate spreadsheet, making sure to ‘paste special’, ‘values’. This will become your matlab input file. Make sure your data columns are in the following order: 31R, 45R, 46R, then remove the headings. Save input file in .csv format, e.g. **example\_input.csv**, into the pyIsotopomer directory. **Note that it is important to save as the simple “comma-separated values (.csv)” file format. More complicated versions of the .csv format, such as CSV UTF-8, will not work.**
2. Open **N2OSPcalcs.py** in a text editor. Enter your input file name on line 26. It should read something like:

**inputfilename = 'example\_isotopomer\_input.csv'**

1. Enter your desired output file name on line 32. This will overwrite any existing file with the same name, so be sure to change the output file name each time. It should read something like:

**outputfilename = 'example\_isotopomer\_output.csv'**

1. Finally, modify line 37 to reflect the most appropriate scrambling coefficients for this set of samples (see below). It should read something like:

**gamma\_kappa = np.array([0.1722, 0.0794])**

1. Save **N2OSPcalcs.py**
2. Open a terminal window and navigate to the pyIsotopomer directory:

colette$ cd "/Users/colette/Box Sync/N2O Research/Spec Calibration Files/N2O\_isotopocule\_data\_corrections/Python/pyIsotopomer"

*Note that the text before “/N2O\_isotopocule\_data\_corrections/Python/pyIsotopomer” will depend on where you have saved these files.*

1. In the terminal, run **N2OSPcalcs.py:**

colette$ python3 N2OSPcalcs.py

1. Open your output file. Output data contains columns in the following order: d15Nalpha, d15Nbeta, 15N site pref, d15Nbulk, d17O, d18O. Çopy and paste output data back into working spreadsheet in olive-highlighted cells (columns AK-AI)
2. Note that a scale decompression may be applied after the isotopomer calculation. This is calculated in the “scale-decompression” tab of the excel worksheet, and is applied in columns AU-AZ of the size\_correction tab.