

# Readme file

Accompanies code and demo dataset for myelin-specific signal extraction, pertaining to manuscript “Nanostructure-specific X-ray tomography reveals myelin levels, integrity and axon orientations”, by Georgiadis *et al.*, 2021, Nature Communications

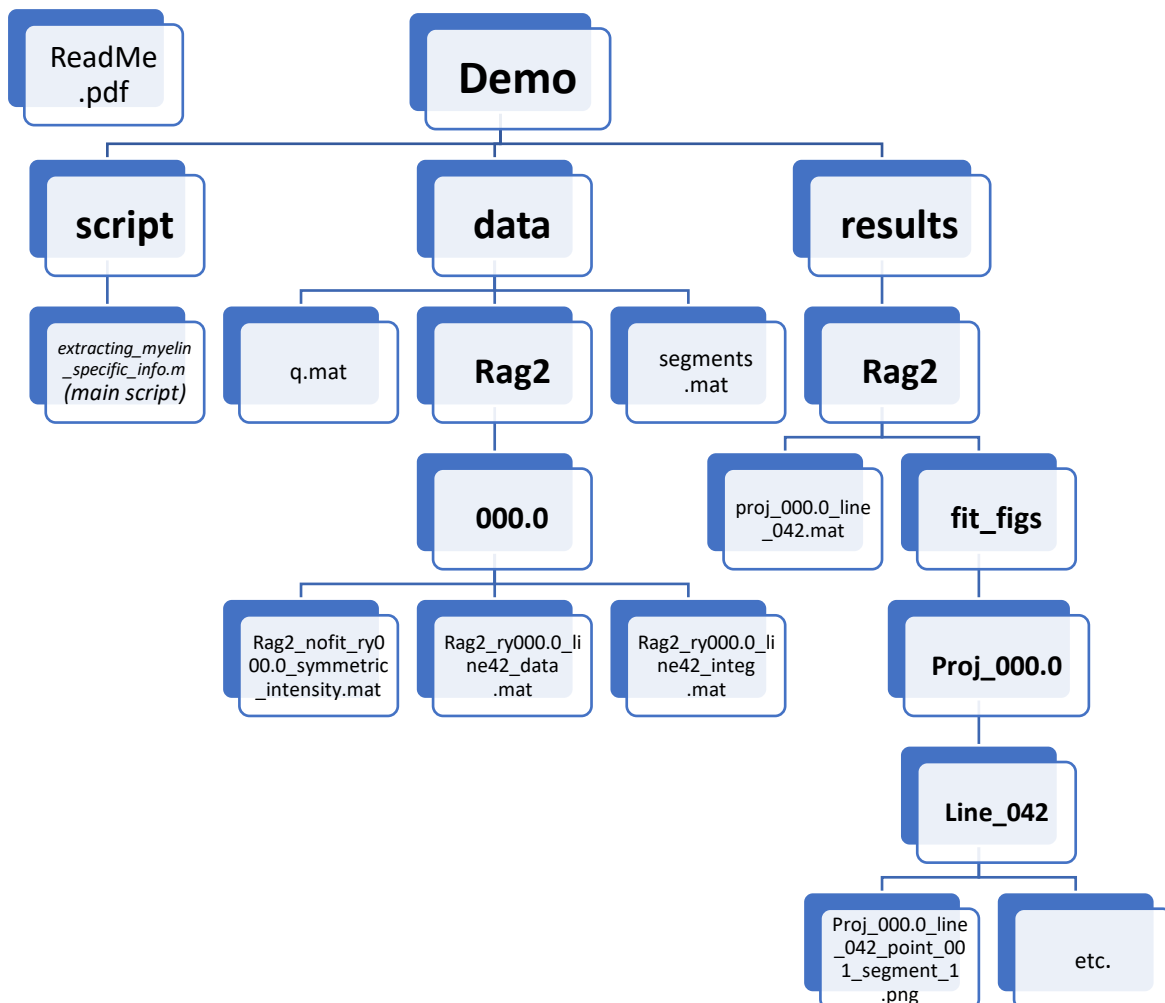
## Introduction

This demo is intended to demonstrate a central, non-documented feature of the study, namely the myelin-specific signal extraction from the semi-raw data -that is, after the scattering pattern for each point has been turned into *q*-plots for the different azimuthal segments (see Fig. 1a,b in the manuscript). The file structure of the “Demo” folder is explained below, instructions for running the code, requirements and dependencies are in page 3, and an example output figure with its subparts explained is in page 4.

For questions, please contact Marios Georgiadis at: [mariosg@stanford.edu](mailto:mariosg@stanford.edu) or [mariosg.gr@gmail.com](mailto:mariosg.gr@gmail.com).

## Content

The file structure of the folder “**Demo**” containing all the materials is demonstrated in the following graph:



The folder “**Demo**” consists of:

- **script** folder  
contains the “*extracting\_myelin\_specific\_info.m*” Matlab script, that performs the myelin-specific signal extraction, and visualizes the process
- **data** folder  
contains the demo dataset and necessary accompanying files to run the script
  - **q.mat** file  
contains the vector of momentum transfer  $q$ , corresponding to the acquired data
  - **segments.mat** file  
contains information about the different angular segments in the scattering patterns, and is used for visualizing the patterns in the figures generated by the “*extracting\_myelin\_specific\_info.m*” script
  - **Rag2** folder  
contains information specific to sample (mouse brain) Rag2<sup>-/-</sup> in the manuscript. This further includes:
    - **000.0** folder  
refers to the very first projection angle, at 0 degrees. In a complete dataset, the **Rag2** folder contains subfolders for all projection angles, as described in the manuscript. **000.0** contains:
      - **Rag2\_nofit\_ry000.0\_symmetric\_intensity.mat** file  
2D projection of the mouse brain at 0 degrees, used to visualize the global position of the analyzed sample point at any time. The figure depicts the azimuthally integrated intensity of the scattering pattern of each point, at the  $q$ -value corresponding to the myelin peak.
      - **Rag2\_ry000.0\_line42\_data.mat** file  
contains the raw data (scattering patterns) for all 133 points in line 42. Line 42 crosses the mouse brain from the spinal cord to olfactory bulbs in a top view of the brain, close to the mid-sagittal plane.
      - **Rag2\_ry000.0\_line42\_integ.mat** file  
contains the radial integration of the scattering pattern data for all 133 points in line 42. The scattering pattern has been split in 16 azimuthal segments (see Fig. 1a,b in the manuscript), and the scattered photons for all radii from the center of the pattern to the edges for each segment have been calculated. This information is used to extract the myelin-specific part of the signal in the script, and displayed in the produced figures.
- **Results** folder  
contains the outputs of the script:
  - **proj\_000.0\_line\_042.mat** file  
contains the extracted myelin-specific signal values for each segment of each point
  - **fit\_figs** folder  
contains figures generated by the script, depicting the myelin-specific signal extraction. The folder is split in subfolders for each projection (only the one at zero degrees contained in this demo dataset), and each subfolder further contains subfolders for each line of the scan (here only the **Line\_042** folder, for line 42 -out of 91 of the projection-, near the mid-sagittal plane). The output figure files are of the filename form **Proj\_000.0\_line\_042\_point\_001\_segment\_1.png**, denoting the projection angle, the line, the point and the segment the analysis of which they depict.

## How to run the code

### 1. System requirements

The demo script (*extracting\_myelin\_specific\_info.m*), located in the folder “Demo/script” is to be run in Matlab. It is compatible with Matlab versions from 2015 onwards (possibly previous ones too, but has not been tested). No other software is needed, but a few Matlab toolboxes are required, see below.

### 2. Installation guide

Matlab can be downloaded here: <https://www.mathworks.com/downloads/>. It is a licensed software, and academic institutions typically have subscription for individual or group licenses.

Important: The following toolboxes are required for the demo and need to be selected during Matlab installation: *signal processing*, *curve fitting*, *image processing*, *statistics*. For existing Matlab instances, where the toolboxes are not already installed, instructions for adding them can be found [here](#). After installation, or if Matlab and toolboxes are available, the script can be run without other dependencies.

### 3. Demo

The script can be run by simply loading it in the Matlab editor, and clicking on “Run”, or pressing F5.

Before starting, the user can set several parameters, affecting the plotting and output of the script:

```
%% User input needed

% set to 0 if functionality is unwanted
to_plot=1;      % =1: plot the fit (and fitted point and scattering pattern)
save_figure=1;  % =1: save the fit figure
save_fit=1;     % =1: save the output
plot_q=1;       % =1: plot with momentum transfer or pixels in x-axis
run_parallel=0; % =1: run in parallel -suggested unless explicit fit visualization is needed
```

These control whether the scripts shows a figure displaying the myelin-specific signal extraction for each scattering pattern segment, of each point (to\_plot), whether these figures will be saved (save\_figure), whether the fit results will be saved (save\_fit), the x-axis of the plots visualizing myelin-specific signal extraction (plot\_q=1 has momentum transfer values in x-axis, otherwise it is in pixels), and whether the script should run in parallel (run\_parallel=1, runs much faster).

If the user wants to see the figures “online” on the screen as they are being generated by the script, one should choose “to\_plot=1”, and “run\_parallel=0” (default setting). Alternatively, the user can choose to run in parallel (run\_parallel=1), which is much faster, and have all figures saved in the “Demo/Results/fit\_figs/Proj\_000.0/Line\_42” folder, where they can be viewed offline.

Once the script is started, a window appears, where the main folder “**Demo**” has to be selected.

### 4. Instructions for use

1. Download “Demo.zip” file
2. Unzip it using an unzipping software (there are multiple free for different platforms)
3. Open Matlab
4. Load the main demo script found in the folder “Demo/script”.
5. Set parameters values (see above, default parameters display the myelin-signal extraction online)
6. Run, and select the location of the “Demo” folder when prompted.
7. If “save\_figure=1”, see figures in “Demo/Results/fit\_figs/Proj\_000.0/Line\_42” folder.

## Example output figure:

