

# SLIX: A Python package for fully automated evaluation of Scattered Light Imaging measurements on brain tissue

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## Software

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## Summary

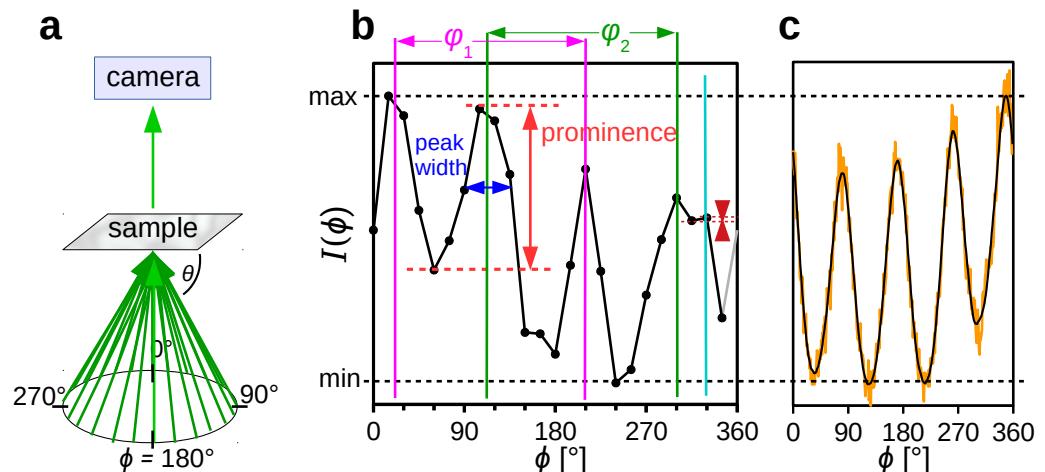
To study the structure and function of the brain, a detailed reconstruction of the brain's nerve fiber architecture is required. However, many neuroimaging techniques, such as 3D-Polarized Light Imaging (M. Axer, Amunts, et al., 2011; M. Axer, Grässel, et al., 2011) struggle with the correct reconstruction of crossing nerve fibers. The recently developed technique *Scattered Light Imaging (SLI)* uses light in the optical range to explore the substructure of (crossing) nerve fibers in whole brain sections with micrometer resolution, providing crucial information such as the orientations of crossing nerve fibers in a measured image pixel (Menzel, Reuter, et al., 2020). The measurement principle was first introduced by Menzel, Axer, et al. (2020). Unstained histological brain sections are illuminated from different angles and the transmitted (scattered) light behind the sample is measured under normal incidence (see Figure 1a). Every pixel in the resulting SLI image series contains a light intensity profile (*SLI profile I(ϕ)*, cf. Figure 1b-c) which is characteristic for the brain tissue structure at this point. The number and position of significant peaks in the SLI profiles can be used, for example, to determine the individual orientations of (crossing) nerve fibers in each image pixel.

Here, we present the *Scattered Light Imaging ToolboX (SLIX)* – an open-source Python package that allows for the first time a fully automated evaluation of SLI measurements and the generation of different parameter maps (see Figure 2a-h) which reflect different characteristics of the SLI profiles: the average, the number of peaks, the average peak prominence, the average peak width, the distance between peaks, and the position of the peaks from which the in-plane direction angles of the nerve fibers are computed in regions with up to three crossing nerve fiber bundles. The user can choose which parameter maps are generated by using specific command line parameters. The resulting direction maps can be used to visualize the individual orientations of in-plane (crossing) nerve fibers, as shown in Figure 2i. The purpose of SLIX is to process the SLI raw data and to provide interpretable parameter maps that can easily be processed and analyzed by other researchers. As SLI is a completely new technique and there exist no comparable methods to our knowledge, SLIX is the first software of its kind and a necessary step for processing scattered light imaging data.

Depending on the number of illumination angles used in the SLI measurement, the line profiles are discretized, typically in steps between 1° and 22.5°. For small step sizes, the line profiles show a zig-zag line with many minor peaks which are not of interest (cf. Figure 1c, in orange). Therefore, the software offers smoothing to suppress these undesired features and study the overall structure of the peaks (Figure 1c, in black). For large step sizes, the line profiles are strongly discretized (cf. Figure 1b) so that the position of the peaks does not necessarily correspond to the position of the peaks in the non-discretized profiles. The software improves the accuracy of the determined peak positions by taking the centroid of the peak tips into account (see Menzel, Reuter, et al. (2020) for more details).

SLIX has already been used to study nerve fiber architectures in human, monkey, and rodent brain tissue samples, and to unravel complex arrangements of crossing nerve fibers like the corona radiata of the vervet monkey brain (Menzel, Reuter, et al., 2020).

For a full documentation of SLIX, the reader is referred to our [GitHub page](#).



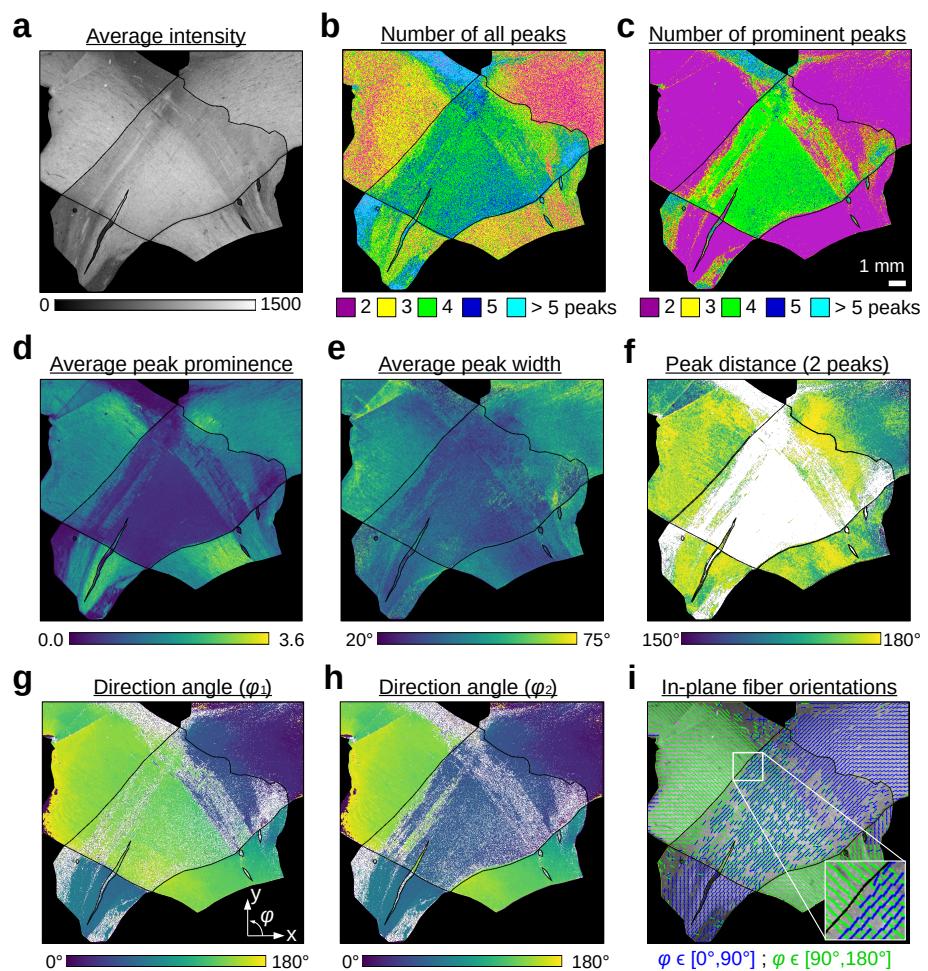
**Figure 1:** (a) Schematic drawing of the SLI measurement: The sample is illuminated from different angles (with constant polar angle  $\theta$  and different equidistant azimuthal angles  $\phi$ ) and the transmitted light behind the sample is measured under normal incidence. Each pixel in the resulting image series contains a characteristic light intensity profile (SLI profile  $I(\phi)$ ). (b) SLI profile measured in steps of  $15^\circ$ . The prominence of the peaks (in red) is computed by the difference between the top of the peak and the highest of the two neighboring minima. The peak width (dark blue) is determined as the full width of the peak at a height corresponding to the peak height minus half of the peak prominence. The determined positions of the peaks (vertical lines) have been slightly corrected by computing the centroid of the peak tip as described in Menzel, Reuter, et al. (2020), Appx. B to account for discretization artifacts. Only *prominent* peaks, i.e. peaks with a prominence above 8% of the total signal amplitude ( $\max - \min$ ), are used for further evaluation (green/magenta lines). Peaks with lower prominences (cyan line) are expected to be caused by noise or details in the fiber structure that are not of interest (for derivation see Menzel, Reuter, et al. (2020), Appx. A). The fiber direction angles  $\varphi_1$  and  $\varphi_2$  are computed from the mid positions of prominent peak pairs with a pair-wise distance of  $(180 \pm 35)^\circ$ . (c) SLI profile measured in steps of  $1^\circ$  (orange: original line profile, black: smoothed line profile).

## Statement of Need

Scattered Light Imaging (SLI) is a promising new imaging technique, providing crucial information about the organization of crossing nerve fibers in the brain. So far, the SLI measurements had to be evaluated manually, making the analysis of whole brain tissue samples and processing of the data difficult. The presented software SLIX (Scattered Light Imaging ToolboX) allows for the first time to evaluate the SLI measurements in a fully automated way and to study and interpret the scattering signals for whole measured brain tissue samples. The SLI raw data is very complex and not easily interpretable. The purpose of SLIX is to process the raw data further, e.g. to extract the in-plane (crossing) nerve fiber orientations from the measured signals and to provide interpretable parameter maps that can be easily processed/analyzed further by other researchers. Currently, there exists no comparable imaging technique that uses light scattering in the optical range to reveal neural organization in the brain. SLIX is therefore the first tool of its kind and of particular need for this new emerging field of Scattered Light Imaging.

## Acknowledgements

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**Figure 2:** Parameter maps generated with SLIX, shown exemplary for two artificially crossing sections of human optic tracts: (a) average intensity in the SLI profiles; (b/c) number of all/prominent peaks in the SLI profiles; (d) average prominence of the peaks in the SLI profiles, normalized by the average of the profile; (e) average width of all prominent peaks in the SLI profiles; (f) distance between two prominent peaks; (g/h) in-plane direction angles of the nerve fibers (two out of three possible directions); (i) visualization of direction angles in g and h, showing the crossing nerve fibers in the center. This figure has been adapted from Menzel, Reuter, et al. (2020), Figure 8. Subfigures a-h were generated with SLIX, using a viridis color map to display the results and manually masking the tissue regions. Subfigure b shows the number of all peaks, i.e. the sum of low and high prominence peaks. Subfigure i was generated from the results in g,h by representing the direction angles of 24x24 pixels by a line with the respective polar/direction angle (not part of the software).

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