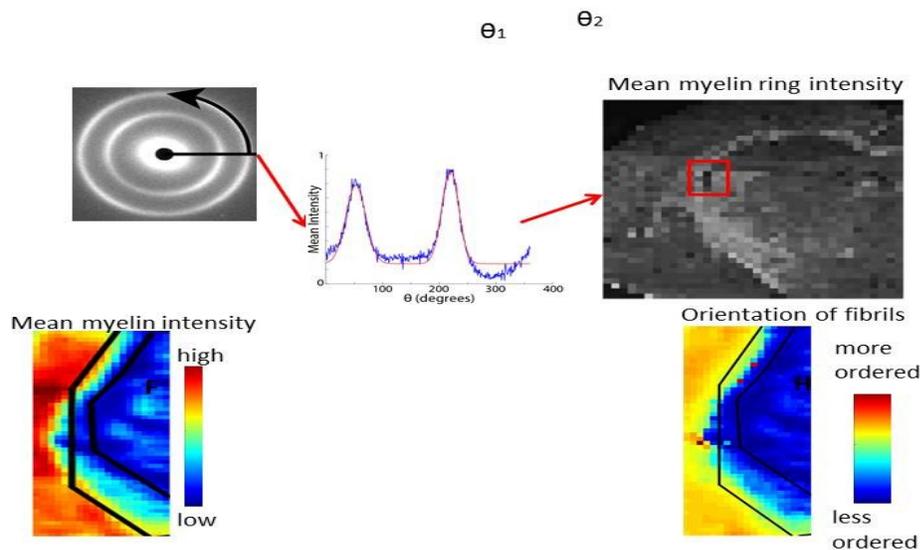


## Fiber diffraction image processing to do list

1. Subtract collected background image from all images in data set.
2. Define beam center (either calculate using Fit2D or create calibration option, which will define beam center based on image symmetry and calibrate it based on detector pixel size and sample-detector distance)
3. Define diffraction feature to work with.
  - 5-th order for collagen pattern ( $\sim 135 \text{ \AA}$ ), 3<sup>rd</sup> order myelin ring ( $\sim 54 \text{ \AA}$ ) (top left), cross-beta signal from amyloid ( $2\text{-}4 \text{ \AA}$ ), muscle 1.0 and 1.1 equatorials.
4. For orientation mapping – define angle of main feature.
  - Similar approach before straightening image in muscle data processing
  - Build 1D map of intensity distribution through defined ring (need to know center (2), inner and outer radius for diffraction feature). Tighter ring  $\rightarrow$  less background picked up.
  - 1D map – averaged intensities from inner to outer radius for every angle (top middle)
5. We can extract 3 values from this map: 1) average intensity (top right, bottom left) 2) find if there is preferred orientation (2 defined peaks) and if there is – measure angle of fiber orientation, 3) define if fibers are less or more ordered (from peak height compared with some background level) (bottom right).



6. Fiber orientation mapping. For every pixel define angle – from position of 2 peaks of 1D map above. Do it for every pixel. Plot distribution (x and y position of each pixel are in hdf file format). If there is no preferred orientation – we just have block from  $0^\circ$  to  $360^\circ$ .
7. Degree of orientation (less ordered vs. more ordered). For not ordered 1D map (even block from 0 to 360) define intensity value, can average it for several not-ordered pixels. It will be 0. Most ordered system will have narrowest and tallest peaks. Less ordered system will have shorter and wider peaks. Same if you don't have enough tension to straighten up fibers, some slack. If there are more than one ordered fiber populations we will have more than 1 pair of reflections, more than 1 pair of peaks.

Please note that all preliminary work had been done by Brendan Sullivan from Yulia Pushkar group (his PhD thesis) and Olga Antipova from BioCAT.