Optical coherence tomography to assess neurodegeneration in multiple sclerosis

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Abstract

Retinal spectral domain optical coherence tomography (OCT) has emerged as a clinical and research tool in multiple sclerosis (MS) and optic neuritis (ON). This chapter summarises a short OCT protocol as included in international consensus guidelines. The protocol was written for hands–on such that both clinicians and OCT technicians can make use of it. The protocol is suitable for imaging of the optic nerve head and macular regions as a baseline for follow–up investigations, individual layer segmentation and diagnostic assessment.

Running head: OCT in MS.

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1 Introduction

Time domain optical coherence tomography (OCT) assessment of the retina has made a tremendous impact in the field of multiple sclerosis (MS) [**Fro2008˙664**, **Pet2010˙921**]. State of the art spectral domain OCT now permits for sufficient axial resolution of individual retinal to enable reliable automated layer segmentation. The method stands at the brink of making the transition from a research tool in MS to standard clinical care [**Pet2014˙nnr**].

There is clear evidence in MS that degeneration affects the inner retinal layers al the way down to the inner nuclear layer (INL) [**Bal2014˙a**]. Inner retinal layer atrophy can be seen early in the disease course [**Obe2013˙1887**, **You2013˙803**, **Gel2012˙36847**]. Inner retinal layer atrophy correlates with other structural imaging markers for neurodegeneration such as magnetic resonance imaging (MRI) [**Sai2012˙1**, **You2013˙803**]. The cause for correlated atrophy of the inner retinal layer atrophy, the visual pathways and the primary visual cortex (V1) are anterograde and retrograde trans–synaptic axonal degeneration [**Pet2010˙921**, **Gab2014˙98**, **Kli2014˙**]. Logically, axonotmesis at any position in the hard–wired visual pathways will always result in bi–directional trans-synaptic axonal degeneration [**Pet2014˙227**]. This concept is illustrated in Figure 1. In case of the retina the first order neuron is the retinal ganglion cell (RGC), the second order neuron within the lateral geniculate nucleus (LGN) and the third order neuron in the visual cortex (V1) [**Pet2010˙921**]. In the retina presence and thickness of retinal layers varies according to location. The most frequently scanned areas by OCT are the peripapil-

lary region (Figure 2A) and macula (Figure 2B).

In addition to quantitative OCT data, qualitative assessment has become important [**Gel2012˙1786**]. Microcystic changes present in the INL have been called microcystic macular oedema (MMO) according to British and edema (MME) according to US spelling (Figure 3). Whilst these changes are not specific for MS [**Bal2012˙226**, **Bur2014˙952**], they are of potential prognostic value in MS [**Sai2012˙963**, **Pet2012˙933**]. Therefore retrogarde maculopathy should be the preferred terminology [**Abe2014˙142**]. This chapter describes a protocol for retinal OCT of the peripapillary and macular regions useful for the assessment of neurodegeneration in MS.

2 Materials

2.1 Hardware

A retinal OCT device. At time of writing this should be a spectral domain OCT device, ideally with an eye tracking function for optimal reproducibility [**Bal2013˙3045**, **Wol2009˙3432**]. An updated list of the United Stated Food and Drug Administration (FDA) approved devices can be found on: www.fda.gov/MedicalDevices. Please consult the manual of your device for specific instructions and safety regulations.

This protocol was written for a Heidelberg Spectralis OCT and Spectralis HRA+OCT. The patient interface for all Spectralis systems is shown in Figure 4.

2.2 Software

The software version at the time of writing is 5.4.8.0 for the Acquisition Module and 5.4.6.0 for the viewing module. All data are stored in the "Heidelberg Eye Explorer" database version 1.7.0.0. Please take a second to check which software versions your system has. To do so click on "Help" at the menu bar and then click on "About...". The new window will list all software versions. Please note that automated layer segmentation may not be available for older software versions.

3 Methods

3.1 Activating the OCT device

1. Prepare the device: (a) pull the camera all the way back, (b) clean forehead rest and chin rest, (c) check that the lens is clear, (d) adjust table height and chin rest so that the canthus marker (see Figure 4) on the right is at the patient's eye hight and that the patient feels comfortable.

2. Prepare the patient and inform him/her that there will be a high-pitched noise during the operation. Offer ear plugs in case the patient is phonophobic and cannot tolerate the noise.

3. Start the software, which will open the database on the screen. Create a new patient file. Alternatively open an old patient file if you have already examined this patient before. Start a new examination. You will be guided

to a number of windows allowing you to enter the patient's demographic information. Additional information may be added if required.

4. Select the acquisition mode. You will note that the square on the bottom right of the screen turns yellow. It will take a few seconds before the computer has calibrated and started up the OCT camera. The OCT camera is working when the high-pitched sound starts. The sound is caused by the fast–turning motor that is needed to rotate the mirrors that reflect the light needed for OCT image acquisition. You will see immediately the fundus image in the large window on the left of the screen. It takes another few seconds before the turning mirror wheel provides enough light information to create an OCT image signal, which will appear in the window on the right side of the screen.

5. Explain to the patient what will happen (see Table 1). Next, select a fixation target. There are two options: (a) an internal fixation target is selected by clicking on the point raster (nine points) on the bottom of the screen. The easiest option is to select the point in the middle of the raster by clicking on it. The point will change colour from black to blue, indicating that it is working. Some patients may have difficulties seeing the BLUE internal fixation target either because vision in the eye to be examined is poor (e.g. optic neuritis) or for other reasons. In this case (b) an external fixation target is needed (see figure 4). Needless to say that the patient will look at the external fixation target with the eye that is not being examined by OCT. In

some cases a moving finger tip may be easier for a patient than the external fixation lamp. In very few cases with extremely poor vision an imaginary target will be needed and you will need to instruct the patient to look a tiny bit more to the left/right/up/down.

6. Now ask the patient to sit forward and rest his/her chin on the chin rest and lean his/her forehead against the headrest. Adjust the height of the patient's head such that the canthus of the eye (lateral line between upper and lower lid) is approximately in line with the canthus mark on the metal rod to the right of the camera. Ask the patient if he/she can see the blue light in the camera (internal fixation target). If not, try to provide an alternative internal fixation target or switch to an external fixation target. Good visual fixation is not mandatory but will facilitate and speed-up retinal OCT image acquisition. Encourage patients to blink in between for their own comfort.

7. After selecting the category "Axonal" in the "Application" section, a specific set of "Preset" buttons becomes available. The axonal setting was developed specifically for neurological conditions. The orientation of the OCT B–scans is vertical. This is because all axons projecting from the macular to the optic nerve head will be captured. In contrast the slightly faster horizontally orientated B–scans need to interpolate in between axonal projections with less accurate volumetric information.

8. As a minimal OCT scan protocol a peripapillary ring scan (Figure 2A) and a macular volume scan (Figure 2B) need to be performed. There may be

additional preset or custom scan protocols which are beyond the scope of this short protocol.

3.2 Acquisition of the peripapillary ring scan

1. Chose the peripapillary ring scan from the preset buttons. Set resolution and averaging (ART) as wanted. For imaging of MMO an ART of 3-9 may be advantageous. For good contrast images best suited for segmentation a higher ART is better. There is no golden rule to this. In a patient who can fixate well you will get a suitable image with a low ART. In a patient with poor fixation or opacifications in the media you may need more. Remember the higher the ART, the higher the scanning time. Not all patients may easily tolerate a long scanning time.

2. Activate the "Eye-Tracker" by pressing the button on the joystick for about 2 seconds.

3. Position the Ring Scan well centred around the ONH using drag and drop with the mouse. Note that a poorly centred ring scan is a rejection criterion for OCT quality control (QC)[**Tew2012˙34823**, **Sch2014˙**].

4. Define the exact position of the Fovea and mark this position (blue line in the SLO image in Figure 2A).

5. Make sure that the OCT live image gives a good signal, is as horizontally aligned as possible and the chosen number number of OCT scan averages

(ART) is filled prior to capturing the image with a short click on the joystick button (Figure 4).

3.3   Acquisition of the macular volume scan

1. Chose a macular volume scan from the preset buttons. Set resolution and averaging (ART) as wanted. When setting ART for a volume scan please remember that this will take longer compared to the pRNFL scan explained in the previous section.

2. Activate the "Eye-Tracker" by pressing the button on the joystick for about 2 seconds.

3. Position the volume scan well centred over the foveola using drag and drop with the mouse.

4. Make sure that the OCT live image gives a good signal, is as horizontally aligned as possible and the chosen number number of OCT scan averages (ART) is filled prior to capturing the image with a short click on the joystick button (Figure 4). Always observe the live window to the bottom of the screen to ensure that subsequent B–scans of the volume scan remain of good quality. This will frequently require some minor readjustment of the live image with the joystick during acquisition.

3.4 Post acquisition procedures

Quality control All retinal OCT scans should be visually inspected during and after acquisition to determine whether the QC criteria will be met [**Tew2012˙34823**]. These criteria are summarised in Table 2.

Reference scan Save the images and leave the acquisition window. All SLO images will be displayed as individual image thumbnails. Please only select those images as reference which have passed your own quality control.

If you are happy with QC, mark the thumbnails and set the reference for the "Follow-Up" scan. You can either individually right click on the scans or keep the mouse button pressed and drag a square over all scans together. This allows to set all scans at once with a right click as "Follow-Up".

Automated segmentation At time of writing experience with automated segmentation is undergoing rapid development. A review of the methods available is beyond the scope of this chapter. There are proprietary algorithms which give good results for high quality images and in absence of any major retinal pathology. It is recommended to always visually revise segmented scans and double check for algorithm failures.

4 Notes

1. Before starting a scan please make certain that (1) the patient us comfortable. (2) That you have prepared everything you need to have at hand for the scans. (3) This includes having selected and marked baseline scans as reference if you want to acquire follow-up scans.

2. Can the patient fixate? If not, please have a look at the item 5 of the acquisition protocol and Table **??**.

3. Remember the most common do's and don't's (Table 3).

4. Prioritise your needs. Start with the pRNFL scan. This is the quickest scan which already gives a wealth of information. If a patient cannot tolerate longer scanning you can skip the more time consuming volume scans.

5. In addition to the scans discussed here there are scan patterns addressing specific aspects of the retinal anatomy. Each of these has their respective advantages, but will prolong the total imaging time.

6. Remind the patient to blink during the examination if the eyes get dry.

7. Remind the patient to look at the fixation target and not to get distracted by the moving scan lines.

8. Take care that the patient does not speak during the examination. The head will bounce on the chin–rest and this will affect image quality.

9. Remember some patients will have make-up. Always check that the lens is clean prior to scanning.

10. Patients with cognitive disabilities, mental disorders (including dementia), movement disorders or tremor may require you to have an assistant which will hold the head of the patient from the back during scanning.

11. Be systematic but not overly dogmatic. If the composite image looks fine after averaging 40 images and the patient becomes restless, there is no need to wait for averaging of 100 images.

12. Do you have a policy in place in case of accidental findings?

13. If the answer to above is no seek advice from your colleagues and/or the ethics committee.

14. Remember that the "penalty" for over reporting findings you are not entirely sure about is likely to be greater than the that of under reporting.

15. Do make the safety and comfort of the patient your primary concern throughout the examination and afterwards.

16. Under certain conditions you may consider adding extra scans to your protocol. Four examples are discussed below, but there are many more options and selecting the right one will best be guided by common sense.

17. Volume scan of the ONH. This scan gives a more detailed view on the ONH which permits to analyse details around Bruch's membrane [**Kup2011˙6558**,

Kau2012˙36965]. In addition, spectral domain OCT permits in some cases to investigate the lamina cibrosa at which point myelination of the optic nerve starts. For better quality some vendors provide enhanced depth imaging. Future devices may incorporate swept source imaging [**Wan2014˙95526**].

18. Volume scan of the papillomacular bundle (PMB). The PMB has long been recognised as a particular vulnerable bundle of retinal nerve fibres [**Uht1886˙95**]. Anatomically this is due to the small fibre diameter in the PMB of $\approx$0.4 μm. The PMB lacks the larger 2.5 μm fibres seen elsewhere in the retina [**Ogd1984˙19**]. Therefore a volume scan of the PMB will permit analyse into greater detail localised atrophy and test related hypotheses on neurodegeneration of this particularly vulnerable structure.

19. Ring–scans of increasing eccentricity. Considerable anatomical variability exists for the ONH between individuals. The disadvantage of a single sized ring scan is that retinal layer thickness will vary considerably between individuals with a small or large ONH. Therefore a set of several ring-scans of increasing diameter (as more rapid scan protocol compared to an ONH volume scan) may provide more data, which is likely to be of additional use in the presence of papilloedema/pseudo-papilloedema [**Far2014˙**].

20. Ring-scans localised to the opening of Bruch's membrane. Above mentioned inter-individual anatomical variations of the ONH make it sometime difficult to consistently centre a ring scan. An elegant solution to this prob-

lem is to centre the ring-scan according to the opening of Bruch's membrane. A semi-automated protocol to this technique does exist for some vendors.

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Table 1: *Fixation guidelines.*

Always explain to the patient what he/she will see during the OCT examination during which his/her eye is investigated.
The patient will see either a red circle (in the case of pRNFL) or a set of red parallel lines (in the case of the macular scan). As the OCT images are taken, the red light will move. This is particular noticeable for the red lines. Tell the patient that the eye always reacts sensitively to movement and that this is normal that he/she will eventually look at the moving target. When he/she does so, the OCT measurement will stop (fixation loss) and start again when he looks back at the fixation target. Tell the patient that the same will happen if he/she blinks or sits back for a moment. This is not a problem and will only slightly prolong the time of the OCT image acquisition.
There are a few patients for whom maintaining fixation will be difficult because of the underlying neurological disease (e.g. nystagmus, oscillopsia). In these patients it is reasonable to turn the automated retina tracking function (ART) off in order to shorten the image acquisition time. Please make a note if you turn ART off. If ART is turned off, this information will automatically be added by the software to the datafile.

Table 2: *The OSCAR-IB quality control criteria for retinal OCT scans. Table modified with permission from reference [Tew2012˙34823].*

Item	criteria
O	Obvious problems (e.g. unfocused image, wrong scan protocol, etc).
S	Is the OCT signal sufficient? Signal strength > 15, appropriate B–scan averaging (ART function activated).
C	Is the scan correctly centred?
A	Is there an algorithm failure?
R	Is there visible retinal pathology which may potentially impair the RNFL reading? (minor floaters casting a shadow are not relevant)
I	Is the fundus well illuminated?
B	Is the measurement beam placed centrally?

Table 3: .

Do's	Don't's
Keep the ring-scan still whilst the averaging (ART) is ongoing and only press the button to acquire the image after averaging is complete (horizontal bar under ART).	Do not be trigger active. Premature pressure of the acquisition button will result in low quality images.
Most people are good in getting the first scan well aligned. But please keep an eye on the live imaging window during acquisition of the volume scan. A volume scan is composed of a number of B-scans. Make sure that you do not inadvertently "cut-off" subsequent B-scans by either moving the camera too much, or not at all.	Never ever keep scans which are "flipped over" during acquisition. Repeat them.
Keep the scan horizontally aligned in the live image window. If this should not be possible, please make sure that you keep the direction of the tilt on subsequent scans. This is particularly relevant for the ring-scans.	If there is no chance to get a horizontally aligned scan, remember to tilt it the same way at followup.

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Figure 1: *Bi–directional trans-synaptic axonal degeneration in a hard–wired pathway.* **(A)** *Normal situation.* **(B)** *Axonotemesis of the first axon results in anterograde axonal degeneration (red arrow to the right). This process can pass on to the second axons trans–synaptically.* **(C)** *Likewise, axonotemesis of the second axon results in retrograde degeneration. Again this process can pass on the first axon trans–synaptically. Logically, the process will always be bi–directional.*

Figures

All Figures will be submitted as separate files and are shown here only for illustrative purposes.

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Figure 2: *SLO images (left) and corresponding (green lines) OCT B–scans (right).* *(A) Optic nerve head. Segmented retinal layers are shown for a peripapillary ring scan (green circle in SLO image to the left). Note the almost complete absence of the GCL close the optic nerve head explaining why here the combined GCL-IPL (also called ganglion cell complex, GCC) were shown for segmentation. The blue line in the SLO images indicates a physiological tilt of 7 degrees of the foveola with respect to the optic disc. (B) Macular region. Segmented layers are shown for a B-scans crossing the foveola (green vertical line in SLO image) constituting the macular volume scan. Note the absence of inner retinal layers from the foveola. RNFL = retinal nerve fibre layer, GCL = ganglion cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer nuclear layer. Figure reprinted with permission from [**Bal2014˙165**].*

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Figure 3: *Microcystic macular oedema (MMO) of the INL in a patient with RRMS who had experienced optic neuritis in this eye [*Bal2012˙226 *]. The INL is thickened (72 μm) at site of MMO compared to the opposite site (52 μm). The vertical green line in the SLO image (left) indicates placement of the OCT B–scan shown to the right.*

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Figure 4: *Photograph of the Heidelberg Spectralis OCT. The magenta arrows identify those parts which are discussed in the Methods section.*