OPTICAL COHERENCE TOMOGRAPHY Use for evaluating Macular Thickness

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Abstract

Background: The clinical macula is seen when viewed from the pupil, as in ophthalmoscopy or retinal photography. The term macula lutea comes from Latin macula, "spot", and lutea, "yellow. The macula lutea appears as a darkened region in the central retina and may seem to have a yellow hue because of the xanthophyll pigments, lutein, and zeaxanthin. These pigments are located throughout the retina, but the greatest concentration is in the macula. The newborn has little if any of these pigments, but they gradually accumulate from dietary sources. These pigments apparently act as filters, absorbing short wavelength visible light to reduce chromatic aberration but may also have an antioxidant effect, suggesting a protective role against UVR damage.

Background

Macular Regions:
The entire macular region consists of the fovea, the foveola, parafoveal, and perifoveal areas (both are annular regions) (Fig.1). (1)

- Fovea – 1.55 mm
- Foveal avascular zone (FAZ) – 0.5 to 0.6 mm
- Foveola – 0.35 mm
- Umbo – 0.15 mm

Fig. (1): Schematic showing regions of retina and corresponding histologic architecture (1).
1. Fovea (Fovea Centralis)

The shallow depression in the center of the macular region is the fovea, or central fovea of the retina (fovea centralis retinae). This depression is formed because the retinal neurons are displaced, leaving only photoreceptors in the center. The fovea has a horizontal diameter of approximately 1.5 mm. The curved wall of the depression is known as the clivus, which gradually slopes to the floor, the foveola. The fovea has the highest concentration of cones in the retina; estimates vary from 199,000 to 300,000 cones per square millimeter.

The number falls off rapidly as one moves away from the fovea in all directions. In this area of the retina, specialized for discrimination of detail and color vision, the ratio between cone cells and ganglion cells approaches 1:1.8. In more peripheral areas of the retina, which are sensitive to light detection but have poor form discrimination, there is a high ratio of rods to ganglion cells. Within the fovea is a capillary-free zone 0.4 to 0.5 mm in diameter (Fig. 2). The lack of blood vessels in this region allows light to pass unobstructed into the photoreceptor outer segment.

![Fig. (2): Capillary bed of macular region, with capillary-free zone (a) in its center (×42.5)](image)

Most of the other retinal elements are displaced, allowing light to reach the photoreceptors directly without interference of other retinal cells. The cells of the inner nuclear layer and ganglion cell layer are displaced laterally and accumulate on the walls of the fovea. The photoreceptor axons become longer as they deviate away from the center; these fibers are called Henle’s fibers. They must take an oblique course to reach the displaced bipolar and horizontal cells. This region of the OPL is known as Henle’s fiber layer.
Fig. (3): Light micrograph of the foveal region. The indentation caused by the absence of several retinal layers is evident (1).

Fig. (4): Light micrograph of foveal region. Layers present in the center of the foveal area are RPE, photoreceptor layer, external limiting membrane, outer nuclear layer, Henle fiber layer (note oblique orientation of fibers at heavy arrow), a few scattered nuclei from inner nuclear layer, internal limiting membrane. Light arrow shows middle limiting membrane within outer plexiform layer (1).

2. Foveola:
The diameter of the foveola is approximately 0.35 mm. The foveola contains the densest population of cones that have the smallest cross-sectional diameters of all the photoreceptors.

Foveolar Layers:
The layers present in the foveola are:
(1) RPE.
(2) Photoreceptor layer.
(3) External limiting membrane.
(4) ONL (which contains about 10 rows of cone nuclei).
(5) Henle’s fiber layer.
(6) The internal limiting membrane.
3. Parafoveal and Perifoveal Areas
The annular zone surrounding the fovea can be divided into an inner parafoveal area and an outer perifoveal area. The parafoveal area contains the largest accumulation of retinal bipolar and ganglion cells. The perifoveal area begins where the ganglion cell layer is four cells thick and ends where it is one cell thick. (3).

Histologic Structure:
1. Retinal Pigment Epithelium
The retinal pigment epithelium (RPE), the outermost retinal layer, is a single cell thick and consists of pigmented hexagonal cells. These cells are columnar in the area of the posterior pole and are even longer, narrower, and more densely pigmented in the macular area (4). The RPE cells contain numerous melanosomes, pigment granules, that extend from the apical area into the middle portion of the cell and somewhat obscure the nucleus, which is located in the basal region. Pigment density differs in various parts of the retina and in individual cells, which can give the fundus a mottled appearance when viewed with the ophthalmoscope. In the retina, melanin is densest in the RPE cells located in the macula and at the equator (5). Other pigmented bodies, lipofuscin granules, contain degradation products of phagocytosis, which increase in number with age. The cell cytoplasm also contains smooth and rough endoplasmic reticulum, Golgi apparatus, mitochondria, and numerous lysosomes (6). The apical portion of an RPE cell consists of microvilli that extend into the layer of photoreceptors, enveloping the specialized outer segment tips. However, no intercellular junctions connect the RPE and photoreceptor cells. A potential space separates the epithelial cell and the photoreceptor. This subretinal space is a remnant of the gap formed between the two layers of the optic cup after invagination of the optic vesicle (7).

2. Photoreceptor Cells
Photoreceptor cells, the rods and cones, are special sense cells containing photopigments that absorb photons of light. The cells originally were named for their shapes, Rods are more active in dim illumination, and cones are active in well-lit conditions. Visual pigments in the photoreceptors are activated on excitation by light (8).

3. Bipolar Cells:
The bipolar cell is the second-order neuron in the visual pathway. The nucleus of the bipolar cell is large and contains minimal cell body cytoplasm. Its dendrite synapses with photoreceptor and horizontal cells, and its axon synapses with ganglion and amacrine cells; glutamate is its neurotransmitter. Bipolar cells relay information from photoreceptors to horizontal, amacrine, and ganglion cells and receive extensive synaptic feedback from amacrine cells (9).

4. Ganglion Cells:
The next cell in the visual pathway, the third-order neuron, is the ganglion cell. Ganglion cells can be bipolar (e.g., a single axon and a single dendrite) or multipolar (a single axon and more than one dendrite) (10). Cell size varies greatly, with some large cell bodies measuring 28 to 36 μm(10). The various methods used to classify ganglion cells make classification rather confusing. An older, broad classification groups ganglion cells into three types. W cells project to the midbrain, carrying information for the pupillary response and reflexive eye movements. Y cells project to the lateral geniculate nucleus (LGN),
with some having collateral branches that travel to the midbrain, perhaps with pupillary information. X
cells primarily respond in visual discrimination and project to the LGN (5).

The **P1 ganglion cell**, also called the midget ganglion cell, is the most common P cell. This relatively
small cell has a single dendrite and can be differentiated into two types, according to the stratification of
the dendritic branching(10).

Certain **P1 midget cells** are connected to only one midget bipolar cell, invaginating or flat, which in turn
might be linked to a single cone receptor, providing a channel that processes high-contrast detail and
color resolution. This situation is likely to occur in the fovea. A convergent pathway occurs in some P1
cells that receive input from two bipolar axons(1).

The **P2 ganglion cell** also terminates in the parvocellular layers but has a densely branched, compact
dendritic tree that spreads horizontally. These cells can be differentiated into two types depending on the
location of the dendrite termination (11).

The **M-type ganglion cell** projects to the magnocellular layers of the LGN. The M cell has coarse
dendrites (because of its shape can also be called a parasol ganglion cell) with spiny features, and the
dendritic tree enlarges from central to peripheral retina(5).

Each ganglion cell has a single axon, which emerges from the cell body and turns to run parallel to the
inner surface of the retina; the axon releases glutamate at its synaptic cleft. The axons come together at
the optic disc and leave the eye as the optic nerve. The termination for approximately 90% of these
axons is the lateral geniculate nucleus; the other 10% project to subthalamic areas involved in processes
such as the pupillary reflexes and the circadian rhythm (5).

5. Horizontal Cells

The **horizontal cell** transfers information in a horizontal direction parallel to the retinal surface. It has
one long process, or axon, and several short dendrites with branching terminals; the processes spread out
parallel to the retinal surface, and all terminate in the outer plexiform layer. Horizontal cells synapse
with photoreceptors, bipolar cells, and other horizontal cells. Horizontal cells are joined to each other by
an extensive network of gap junctions. One type of horizontal cell synapses only within a cone pedicle
in the special triad junction. Horizontal cells can contact bipolar cells lying some distance from the
photoreceptor that activated the horizontal cell. Horizontal cells can affect an inhibitory response, thus
playing a role in the complex process of visual integration (12).

Horizontal cells provide inhibitory feedback to photoreceptors or inhibitory feed forward to bipolar
cells. Horizontal cells can modulate the cone response but are not thought to influence that of the rod
(13).

6. Amacrine Cells

The **amacrine cell** has a large cell body, a lobulated nucleus, and a single process with extensive
branches that extend into the inner plexiform layer. The process, which has both dendritic and axonal
characteristics and carries information horizontally, forms complex synapses with axons of bipolar cells,
dendrites and the soma of ganglion cells;

Most amacrine cells contain the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) or
glycine, and have both presynaptic and postsynaptic endings. Amacrine cells are joined to one another
via gap junctions, and some cells have been found to combine information from rod and cone pathways
before innervating a ganglion cell (14).
7. Neuroglial Cells
Neuroglial cells, although not actively involved in the transfer of neural signals, provide structure and support and have a role in the neural tissue reaction to injury or infection. Types of neuroglial cells found in the retina include Müller cells, microglial cells, and astrocytes (15).

OPTICAL COHERENCE TOMOGRAPHY

Background:
Optical coherence tomography (OCT) has been widely used for assessing retina and optic nerve by providing quantitative and qualitative assessment of macula and retinal nerve fiber layer (RNFL) in the last decade (16).

The key benefits of OCT are (16).

- Live sub-surface images at near-microscopic resolution
- Instant, direct imaging of tissue morphology
- No preparation of the sample or subject, no contact
- No ionizing radiation

OCT delivers high resolution because it is based on light, rather than sound or radio frequency. An optical beam is directed at the tissue, and a small portion of this light that reflects from sub-surface features is collected. Note that most light is not reflected but, rather, scatters off at large angles. In conventional imaging, this diffusely scattered light contributes background that obscures an image. However, in OCT, a technique called interferometry is used to record the optical path length of received photons allowing rejection of most photons that scatter multiple times before detection. Thus, OCT can build up clear 3D images of thick samples by rejecting background signal while collecting light directly reflected from surfaces of interest (16).

The technique is limited to imaging 1 to 2 mm below the surface in biological tissue, because at greater depths the proportion of light that escapes without scattering is too small to be detected. No special preparation of a biological specimen is required, and images can be obtained ‘non-contact’ or through a transparent window or membrane. It is also important to note that the laser output from the instruments is low – eye-safe near-infrared light is used – and no damage to the sample is therefore likely (16).

Definition:
OCT is an imaging technique that uses low-coherence light to capture micrometer-resolution, two- and three-dimensional images from within optical scattering media (e.g., biological tissue). Optical coherence tomography is based on low-coherence interferometry, typically employing near-infrared light. The use of relatively long wavelength light allows it to penetrate into the scattering medium (16).

History:
Starting from Adolf Fercher and colleagues’ work on low-, partial coherence or white-light interferometry for in vivo ocular eye measurements in Vienna in the 1980s, imaging of biological tissue, especially of the human eye, was investigated in parallel by multiple groups worldwide. A first two-dimensional in vivo depiction of a human eye fundus along a horizontal meridian based on white light interferometric depth scans (17).

Further developed in 1990 by (18), then a professor at Yamagata University it was referred to as heterodyne reflectance tomography, and in particular since 1991 by (19), in Prof. James Fujimoto laboratory at Massachusetts Institute of Technology, who successfully coined the term “optical coherence tomography”. Since then, OCT with micrometer resolution and cross-sectional imaging capabilities has become a prominent biomedical tissue-imaging technique that continuously picked up
new technical capabilities starting from early electronic signal detection, via utilization of broadband lasers and linear pixel arrays to ultrafast tuneable lasers to expand its performance and sensitivity envelope (20).

OCT is based on low-coherence interferometry. In conventional interferometry with long coherence length (i.e., laser interferometry), interference of light occurs over a distance of meters. In OCT, this interference is shortened to a distance of micrometers, owing to the use of broad-bandwidth light sources (i.e., sources that emit light over a broad range of frequencies). Light with broad bandwidths can be generated by using super-luminescent diodes or lasers with extremely short pulses (femtosecond lasers). White light is an example of a broadband source with lower power (16).

Light in an OCT system is broken into two arms – a sample arm (containing the item of interest) and a reference arm (usually a mirror). The combination of reflected light from the sample arm and reference light from the reference arm gives rise to an interference pattern, but only if light from both arms have traveled the "same" optical distance ("same" meaning a difference of less than a coherence length). By scanning the mirror in the reference arm, a reflectivity profile of the sample can be obtained (this is time domain OCT) (20).

**Theory:**
The principle of OCT is white light, or low coherence, interferometry. The optical setup typically consists of an interferometer with a low coherence, broad bandwidth light source. Light is split into and recombined from reference and sample arm, respectively (21).

**Fig. (5):** Full-field OCT optical setup. Components include: super-luminescent diode (SLD), convex lens (L1), 50/50 beam-splitter (BS), camera objective (CO), CMOS-DSP camera (CAM), reference (REF), and sample (SMP). The camera functions as a two-dimensional detector array, and with the OCT technique facilitating scanning in depth, a non-invasive three-dimensional imaging device is achieved (21).
Use in Ophthalmology

Ocular OCT is used heavily by ophthalmologists and optometrists to obtain high-resolution images of the retina and anterior segment. Owing to OCT's capability to show cross-sections of tissue layers with micrometer resolution, OCT provides a straightforward method of assessing cellular organization, photoreceptor integrity, and axonal thickness in glaucoma (22), macular degeneration, diabetic macular edema, and other eye diseases or systemic pathologies which have ocular signs (23).

Additionally, ophthalmologists leverage OCT to assess the vascular health of the retina via a technique called OCT angiography (OCTA). In ophthalmological surgery, especially retinal surgery, an OCT can be mounted on the microscope. Such a system is called an intraoperative OCT (iOCT) and provides support during the surgery with clinical benefits (23).

References


