Frontiers and Controversies in Structural and Functional Ocular Imaging

A supplement to Ophthalmology Times Europe
Introduction

Heidelberg Engineering has designed, manufactured and distributed diagnostic instruments for eye care professionals for 20 years. In celebration of this milestone the company held a two-day symposium in Heidelberg, Germany, demonstrating the integral part the company has played and continues to play in worldwide research and clinical practice.

“Frontiers and controversies in structural and functional ocular imaging” featured a plethora of experts from all over the world discussing their research and results in imaging and diagnostics.

In this supplement Ophthalmology Times Europe and Heidelberg Engineering present the highlights of the 20th Anniversary symposium.

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In general, structure and function are related over the course of glaucoma. However, it is uncertain as to how structure and function measurements relate to each other in individual patients as they progress. According to Dr David Garway-Heath, IGA professor of ophthalmology at UCL, London, UK, “In viewing structural and functional parameters, each patient may behave differently and it is up to the surgeon to decide whether or not the changes seen will develop to potential blindness.”

**Rim area and disc size**

“There is a linear relationship between rim area and disc size, meaning that the larger the optic disc, the greater the rim area,” confirmed Dr Garway-Heath. From his previous work in optic disc photography, limits of normality were defined so that a rim area falling outside these disc-size related limits could be identified.¹ It was found that the majority of glaucomatous patients fell outside the lower level of prediction interval for rim area.

This principle was then applied to Heidelberg HRT measurements to develop the HRT ‘Moorfields regression analysis’ (MRA).²

In a study from the Manchester group the accuracy of classification of the MRA and the glaucoma probability score (GPS) were compared and both were found to offer similar levels of performance. “A key finding was that both the GPS and the Moorfields regression analysis are sensitive to the size of the optic disc,” Dr Garway-Heath said. For healthy subjects with large optic discs misclassification (as abnormal) is a possibility. To overcome the problem of false positives a new method for setting the prediction intervals using quantile regression has been suggested.³ This method accounts for the uneven spread in normal data so that the prediction intervals are more appropriate.

Other, currently unpublished, work by Haogang Zhu (supported by Vernon, Crabb and Artes), uses the shape of the optic nerve head (ONH) to increase the precision of the classification for HRT. This is a multidimensional model that enables software to determine types of optic discs which will be unreliably classified with the HRT. “This shows that we can use additional shape analysis to identify which optic discs can be reliably classified by the HRT algorithms,” Dr Garway-Heath added.

**Structural damage and visual functional damage**

The relationship between structural and visual functional damage has been considered for a long time and a classic...
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paper from 1974 by Read and Spaeth demonstrates the belief that structural change becomes apparent before visual field change.4

In a model developed by the San Diego group, it was proposed that structural parameters may be abnormal before pathway-specific functional parameters and before a white-on-white change. “However, I believe,” Dr Garway-Heath continued, “that this is not the true picture and actually structure and function damage probably proceeds in tandem; it’s simply the way that we think about it and measure structure and function that gives an apparent picture that structure and some forms of function may change first.”

“The key is understanding how we use reference standards for what is normal and what is abnormal,” he said. A typical approach is to use white-on-white perimetry as the reference standard for glaucoma suspects. With this approach, if a patient is found to have abnormal white-on-white they are labelled as having glaucoma. If their results are normal they are labelled either pre-perimetric glaucoma or normal. Once the white-on-white perimetry has been performed, the ‘normal’ labelled patients are given the new structural test or new vision function test to diagnose early glaucoma. “The problem with this is,” Dr Garway-Heath explained, “there is no opportunity for cases in which the other test (the structure test or the new vision function test) is ‘normal’ but the white-on-white is ‘abnormal’. So white-on-white will always seem less sensitive.” This emphasizes the importance of the choice of reference standard for what is glaucoma and what isn’t.

The San Diego group has achieved this by using an appropriate reference standard of abnormal structure to compare white-on-white perimetry, SWAP and FDT. It was determined from this study that the sensitivity of all three tests is similar in early glaucoma detection.

However, is it ever possible that structure becomes abnormal before function? In comparing two studies,5,6 Dr Garway-Heath found that structure was only slightly more sensitive than vision function for identification of early glaucoma.

Another factor to consider is the scale used to measure visual function. With a log plot of visual function (dB) versus linear plot of structure, it seems as though visual function doesn’t change a lot but the rim area does. However, in more advanced glaucoma, the picture is reversed. This can be attributed to the logarithmic scale used; if vision function is placed onto a linear scale for the exact same data, the relationship between structure and function appears to be much more linear.

Several linear models have been published,7–9 which indicates that people are now considering a continuous relationship between structure and function throughout the course of glaucoma.

“As either structure or function can become abnormal first, useful additional information may be gained from measuring both,” asserted Dr Garway-Heath.

Future possibilities
Currently, the usual practice in analysing both structure and function is to place the reports next to one another and use experience to put them together. The HRT and the Heidelberg Edge Perimeter (HEP) are capable of bringing together the data into one report. This displays the probability of being outside of normal limits for each test on the same map and so the relationship of structure and function can be seen (Figure 1).

“However, I think the really exciting potential way forward,” Dr Garway-Heath concluded, “is to actually combine the data from structure and function. Measure the nerve (neural rim or the nerve fibre layer) and put them together with measurements of the visual field.”10

References
Role of progression in the diagnosis of glaucoma

"Each optic disc has an individual footprint and each optic disc is absolutely unique to a person's eye," said Dr Balwantray Chauhan, professor of ophthalmology and visual sciences, research director and chair in vision research at Dalhousie University, Halifax, Canada.

This individuality can be useful in characterizing the specific point-to-point variability of each disc. Dr Chauhan explained, "This is really what the early phase of the Topographical Change Analysis (TCA) was trying to develop in both humans and animals."

In the current version of the TCA on the Heidelberg Retina Tomograph (HRT) it is possible to use a 'movie function' to check the alignment of the software and also the changes in the disc. "This is a very important functionality of the software and I suggest that you don't just rely on the print outs," urged Dr Chauhan. Clinicians can use this function to cycle between images easing determination of changes in the disc and mapping the probability levels of change.

Optic disc changes in relation to visual field

From previous research using the HRT, Dr Chauhan found that in patients with changes in both optic disc and visual field, standard automated perimetry (SAP) and HRT were equivalently sensitive in terms of which technique detected change first. However, the definition of progression is entirely dependent on criteria. Dr Chauhan added, "You can make as many of your glaucoma patients progress as you want or you can make none of them progress if you want. All you have to do is choose the right criteria. We did nothing in the study to control the false positive rate."

In later work, Dr Chauhan and colleagues chose to change criteria that classified the same number of patients progressing with SAP and HRT so that an overlap between the two could be observed. Unfortunately, the overlap was small and it reduced as the progression levels reduced. "What this data has shown us," Dr Chauhan continued, "is that visual field and optic disc progression within the time frame of a clinical study, tend to occur, primarily, independently of each other." This has been affirmed in other research.

New technology

Dr Chauhan performed a study to compare the observations of four glaucoma experts and the TCA from the HRT. "When we develop new technology we compare it to the current gold standard so, by definition, a new technology can never win," explained Dr Chauhan. "To solve this problem it is necessary to look at another approach," he continued. Dr Chauhan's team compared specificity-hit rate relationship for different progression criteria between the HRT/TCA and the experts. Dr Chauhan said, "I would argue that the HRT/TCA is at least as good if not better than a group of experts classifying optic disc images."

Dr Chauhan and colleagues then followed up with patients who only had changes in HRT over a period of eight years to determine if they were more likely to have subsequent visual field changes. They found that not only was this true but they were also more likely to progress earlier. "So to our satisfaction the changes that are being picked up by the HRT are real changes," emphasized Dr Chauhan.

Conclusion

"We need to do more analysis," concluded Dr Chauhan, "but, all the evidence we have thus far tends to suggest that changes we are detecting with the HRT are real and that they have functional consequences."
The Ocular Hypertension Treatment Study (OHTS) was designed to evaluate the safety and efficacy of topical ocular hypertension medication,” explained Dr Linda Zangwill, professor of ophthalmology and director of the Diagnostic Imaging Centre at the Hamilton Glaucoma Centre, Department of Ophthalmology at the University of California, San Diego, California, USA.

"What we already know from the OHTS is that treatment prevents or delays the onset of glaucoma and reduces the incidence of the disease in ocular hypertensive patients," she continued. However, another interesting finding was that baseline clinical and ocular factors can be used to predict glaucoma development. Among the ocular factors that predict the onset of glaucoma, stereophotograph based cup disc ratio measurements have an analogy with the HRT stereometric parameters.

CSLO ancillary study to the OHTS
Seven of the 22 OHTS study centres participated in the CSLO ancillary study to the OHTS. "We added annual HRT examinations from 1994 to 2008 to the OHTS protocol," Dr Zangwill said (Figure 1). Cornea curvature measurements were used to correct for magnification and each HRT image series was exported directly to the reading centre so image processing was completed centrally in a standardized manner.

The seven study centres examined over 441 patients during the 15-year study. It was found that in some patients the first detectable sign of glaucoma was
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structural change and in others it was functional change.

The team’s first paper was published in 2004 and was an analysis of the study design and baseline factors. It was concluded that HRT measurements are strongly correlated with stereophotographic assessment of horizontal and vertical cup-disc ratios based on the photo in OHTS patients with normal appearing optic discs (Figure 2).

As with the OHTS, racial factors were taken into consideration in the second paper and the relevant adjustments were made for disc size. "Considering disc size is important when evaluating the appearance of the optic disc in glaucoma," emphasized Dr Zangwill.

It was found that, for predictive models, baseline GPS, MRA and stereometric parameters alone or combined with baseline clinical and demographic factors can be used to predict the development of primary open-angle glaucoma (POAG) endpoints in OHTS participants (Figure 3). Also, HRT parameters were found to be as effective as stereophotographs reviewed by experts at the OHTS Optic Disc Reading Center for estimating the risk of developing POAG in ocular hypertensive patients. “Disc area was not found to be predictive in these models,” Dr Zangwill added.

Rates of change were another consideration for the team. She said, “It’s really important for clinicians, not only to know whether your patient is progressing, but also how fast they are progressing.” To identify factors that influence the rates of structural change the team compared the rate of change of HRT parameters of eyes that developed glaucoma or POAG and those that did not.

The rate of structural change measured with the HRT was found to be 4 to 7 times faster in eyes that developed POAG compared with those that did not and was fastest in the inferior temporal region. In the African-American participants, a faster rate of rim area change was found but this was explained in part by larger disc size. Several factors including larger baseline stereophotograph based cup-to-disc ratios, and higher IOP during follow-up were found to be associated with faster loss of rim area.

“Our conclusions about the rate of change as measured with the HRT can provide important information for the clinical management of ocular hypertensive patients and the challenge is how can we best identify fast progressors,” summarized Dr Zangwill.

European glaucoma prevention study (EGPS)
The European Glaucoma Prevention Study was designed in a similar way as the OHTS, including ocular hypertensive patients with annual HRT examinations. However in contrast to the OHTS, a significant difference between medical therapy and placebo in reducing the incidence of glaucoma was not detected in EGPS.

From pooled CSLO data of OHTS and EGPS, a predictive model was developed for the evaluation of the risk of glaucoma development based on several clinical and ocular factors. From this combined data it was concluded that the OHTS predictive model that included CSLO measurements was validated in the EGPS placebo group and can be used to predict the onset of glaucoma in patients with ocular hypertension.

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**OHTS Predictive accuracy during follow-up period**

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<tr>
<td>GPS Global</td>
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**Figure 3**: OHTS predictive accuracy during follow-up period (% of POAG and non-POAG correctly classified at baseline).
It is necessary to measure functional aspects of glaucoma in terms of a patient’s quality of life, according to Dr John Flanagan of the Department of Ophthalmology and Vision Sciences, University of Toronto, Canada and professor at the School of Optometry, University of Waterloo, Canada.

“We may well have structural measures that will take over in clinical trials but we will always need functional evaluations,” Dr Flanagan said. However, is it possible to find functional measures that can pick up some of the early defects as well?

**Design philosophy behind the Heidelberg Edge Perimeter**

One of the two stimuli used in the Heidelberg Edge Perimeter (HEP) is called Flicker-Defined Form (FDF), which targets sub-systems of the visual system to determine functional loss in very early glaucoma.

“We know quite a bit about the stimulus,” Dr Flanagan added, “We can manipulate it in terms of its activity, change the characteristics by adjusting the background, frequency, number of random dots on the screen and the organization of those dots and it is also known that its efficacy is not reduced by optical blur.”

Using FDF, Dr Flanagan wondered whether it is possible to preferentially stimulate some of the different ganglion cell types within the retina. Although some manipulation is possible, it would be naïve to think isolation of any one system is possible. “The idea is to preferentially stimulate sub sets of the ganglion cells in the hope of being able to find defects just that little bit earlier,” he said.

In some collaborative work, Dr Flanagan is comparing different types of parameters, including FDF, in a structurally defined group. The preliminary results have shown that at a certain level of sensitivity, specificity was higher for FDF than Standard Automated Perimetry (SAP). “Obtaining a visual field study of a group defined by structure alone is a relatively novel approach, but some differences have shown up already and of course those differences will be dependent on multiple factors,” explained Dr Flanagan.

“One of the nice things moving forward,” he continued, “is that we have both types of perimetry, SAP and FDF, on the HEP so it has been possible to examine the differences in SAP and FDF for the same patient on the same device.”

**Conclusion**

In Dr Flanagan’s opinion special measures, like FDF, and looking at the nerve fibre layer may be useful in early disease detection but once disease has been established SAP and other ways of looking at function would be better. “That was really the idea of having the two stimuli on the HEP,” he concluded.
ONH: Morphology and biomechanics and clinical importance in glaucoma diagnosis

The current definition of glaucoma from one of the big societies is ‘progressive structural optic nerve damage’. According to Dr Alexander Scheuerle from the Department of Ophthalmology in Heidelberg University, Germany, “It is clear that glaucomatous optic neuropathy leads to changes in the intrapapillary and parapapillary regions of the optic nerve head (ONH).”

Changes in optic disc
In Dr Scheuerle’s opinion, “A major improvement in detecting changes in the optic disc is reducing the number of features you look at in clinical routine.” Dr Scheuerle suggested there be only five parameters to examine optic disc changes. These are disc size, rim size, disc haemorrhage, parapapillary atrophy and retinal nerve fibre layer (RNFL).

Disc size: There is a large variability in ONH regarding disc size and it is dependent upon the patient’s ethnic background. To evaluate an optic disc for glaucoma it is crucial to be aware of the individual disc size. Glaucomatous damage tends to be overestimated in large discs while a pronounced cup–disc-ratio in a small disc is always suspicious for advanced glaucomatous disease. However, disc size is not important for glaucoma susceptibility.

Rim size: Defined as the distance between the scleral ring and the location where the vessels bend. It is dependent on the disc size and can be used for semi-quantitative analysis of glaucomatous damage.

Disc haemorrhage: This isn’t as difficult to detect as some other parameters but can be easily missed. Disc haemorrhages are very often associated with localized nerve fibre defects, rim notches and circumscribed perimetric loss but it is not always associated with glaucoma. However, a disc haemorrhage in a glaucoma patient is always a sign of progression.

Parapapillary atrophy: Alpha zone (irregular hypo- and hyperpigmentation next to the optic disc) and beta zone (parapapillary atrophy) can be seen in healthy patients as well as in glaucoma patients. In unilateral glaucoma patients this type of atrophy is more predominant in the affected eye so it must be connected to the disease process. An increasing beta zone can indicate glaucomatous progression and might precede visual field defects.

RNFL: Qualitative measurements can be obtained with the Heidelberg Retina Tomograph (HRT), using the reflectivity image. It enables the experienced examiner to detect and localize defects of the retinal nerve fibre layer. However, with the HRT it is not possible to obtain a direct quantitative measurement of RNFL thickness. In this area, the future direction will lean towards SD-OCT.

“All eyes with high myopia have localized RNFL defects that might be associated with glaucoma until proven otherwise,” Dr Scheuerle emphasized.

RNFL
“RNFL measurements offer higher sensitivity and specificity than measuring the optic nerve disc with either Moorfield’s...”

Figure 1: A boxplot diagram showing the RNFL thickness and relative loss in groups ranging from normals to patients with premetric glaucoma.

Figure 2: RNFL thickness classification with age adjusted normative database.
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On the road to in vivo histology

Laser-scanning tomography has become the gold standard in three-dimensional optic disc assessment, according to Dr Reinhard Burk, professor of ophthalmology in Bielefeld, Germany. Earlier technologies only allowed the two-dimensional analysis of the optic disc and nerve fibre layer obtained using photogrammetry and were incredibly time consuming. "The possibility of laser scanning, digital, non-mydriatic imaging (HRT and SPECTRALIS) was a big step forward from stereophotography, which required wider dilated pupils," explained Dr Burk. Additionally, three-dimensional information can be detected by confocal laser scanning tomography. Dr Burk said, "With this technology, ophthalmologists are now able to accurately diagnose and track different types of glaucoma and retina diseases with confidence." For example, in topographical analysis with just one measurement cup or GPS, explained Dr Christian Mardin, professor of ophthalmology, University Eye Hospital, Erlangen, Germany.

Dr Mardin and colleagues investigated the three-dimensional morphology of the ONH and looked at the RNFL around the optic disc. A slight significance was found in the tendency to thinner RNFL for pre-perimetric and perimetric glaucoma patients as well as ocular hypertensives (Figure 1).

So why is RNFL better for the diagnosis of early glaucoma? Dr Mardin hypothesized, "If you lose ganglion cells in glaucoma it is followed by an axon nerve fibre layer loss, which all culminates in a loss of retinal tissue and disc." Dr Mardin's team applied their measurements of RNFL damage, taken with Heidelberg's SPECTRALIS OCT, to the San Diego group's work on disc and visual field damage and they found that RNFL loss can be measured in the early stages of glaucoma. Once the disease has progressed, however, visual field is a better method of monitoring and diagnosis.

"The technique in the software has improved greatly over the past years and so in the future using the HRT to monitor changes in the optic disc will prove to be a more sensitive detection method than predicting the nerve fibre layer," said Dr Mardin.

In his research, Dr Claude Burgoyne, from Devers Eye Institute, Portland, Oregon, USA, van Burskirk chair in ophthalmic research and research director, ONH research laboratory, is using HRT progression analysis and SPECTRALIS OCT to test the deformation of the lamina cribrosa and the bowing back of Bruch's membrane in early glaucoma.

Dr Burgoyne reported, "In the animals we've studied so far the prelaminar neural tissues are not thinned, they are in fact thickened." He noted that the bowing back of Bruch's membrane is a result of the posterior deformation of the lamina and the sclera. "The lamina itself is profoundly thickened at this early stage, not thinned," he added.

However, it was also realised that something else was occurring in addition to the deformation. "We're just beginning to understand," he continued, "that, at least regionally, the lamina cribrosa is migrating out of the sclera and posteriorly at least partially to the pia." This is something that Dr Burgoyne believes will be a target for OCT imaging.

OCT imaging

In examining the concepts underlying deep and shallow cupping in a group of animals Dr Burgoyne explained, "The structural stiffness of the connective tissue of the ONH contributes to the depth of cupping at all ages and shallow or senile sclerotic cupping of aged eyes is a manifestation of this stiffness."

To test this hypothesis, Dr Burgoyne's group used SD-OCT in young and old monkey eyes. A group of eight monkeys, four young and four old, received baseline HRT, SPECTRALIS OCT, GDx, stereo photos and ERG four or five times prior to lasering. Chronic unilateral IOP evaluations were delivered by meshwork lasering and the same imaging protocol was applied every two weeks to remove the acute component of deformation. "We used HRT onset by either our parameter, mean position of the disc, or the TCA parameters and confirmation was required on two subsequent occasions," Dr Burgoyne said.

From the results it was implied that optic nerve and parapapillary retinal structural change for clinical cupping has both neural and connective tissue components and occurs very early in experimental glaucoma. "We think that SD-OCT imaging of the ONH and peripapillary retina will provide insight into these two components of structural change," Dr Burgoyne concluded.

Summary

There are many factors that can affect the optic disc, however, reducing the number of parameters examined has helped researchers document changes. Of these parameters RNFL is better for early diagnosis of glaucoma but as the disease progresses this changes. It is believed that the HRT will become a more sensitive tool for specialists working with the optic disc. Additionally, work with OCT imaging will be at the fore for experimental ophthalmology and aiding in the understanding of the internal limiting membrane.
Ocular blood flow

The retina is "some sort of gateway to medical wisdom," according to Dr Georg Michelson, professor of ophthalmology at the University Eye Hospital in Erlangen, Germany.

Measuring ocular blood flow
About eighteen years ago Dr Michelson contacted Heidelberg about the possibility of using the scanning laser system to measure the retina blood flow.

"In scanning laser Doppler flowmetry you measure the Doppler shift in each point of the retina and then calculate the blood flow to reconstruct an image with each pixel proportional to the blood flow at each specific point," Dr Michelson remarked. Using this calculated image it is possible to work out the capillary blood flow in different areas of the retina.

Three options with the Heidelberg Retina Flowmeter were determined:
1. **Functional imaging of the vessels:** Using this option it is possible to view even very small perfused vessels and capillaries. However, these small vessels can be obscured, for example, by an arterial occlusion.
2. **Flow measurements in capillaries:** For option two the overall Doppler shift is calculated. This relative measure gives a mean value of the retinal blood flow.
3. **Assessment of the wall thickness:** The idea behind this option is to offer two images at one time point, so the computer can see the reflectivity and flow image. Thus the vessel wall thickness can be measured using the diameter of the perfused vessel column and total vessel diameter. The wall-to-lumen ratio can be calculated from the vessel wall thickness and the total vessel diameter.

**Wall-to-lumen ratio**
This ratio is a quantitative measure that represents an arteriolar parameter. An increase in this ratio is theoretically a consequence of wall thickening, narrowing of the lumen or a combination of both. "Using this approach, we found a very robust parameter to characterize the small vessels," explained Dr Michelson.

In studies of this ratio, Dr Michelson and colleagues found that arterial hypertension affects the walls of the retinal arteries. They compared their results of arterial hypertension patients with a group who had suffered a cerebrovascular event. "We found that the group with cerebral change or disease have a higher increase in wall-to-lumen ratio in the eye, indicating that the disease we can see in the brain is visible to some degree in the retina vessels," said Dr Michelson.

When examining why the wall-to-lumen ratio changes, Dr Michelson and colleagues found that the so-called urinary sodium excretion of the kidney is one factor. Dr Michelson explained, "With increased salt intake there is an increase in the wall-to-lumen ratio, which means the more salt you eat the higher the risk is of getting arterial hypertension and after a while the probability also increases of changes occurring in the small vessels of the eye and brain."

**Conclusion**
According to Dr Michelson the scanning laser Doppler flowmetry enables surgeons to obtain functional imaging of the retinal vasculature and also of the deep lamina cribrosa vessels. He added, "Software development has enabled automatic assessment of the blood flow of the retinal capillaries. Now, we can automatically assess morphological parameters of the big vessels, we can calculate the wall-to-lumen ratio, which indicates that changes in the brain’s vessels can be observed in the eye."

In the future, Dr Michelson and colleagues will be examining these findings in progressive glaucoma patients.

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depth, steepness, size and shape can be defined. This information enables the clinician to calculate the stage and progression of glaucoma.

Using the imaging information obtained from this technology it is possible to perform a statistical analysis to look for clusters selected by certain topometric parameters. With this procedure seven sub types of discs are found by the computer. The normal, the large normals, the supernormals, with no cupping at all, the pseudonormals, which have a decreased contour line variations, the glaucomatous flat and the glaucomatous steep and the small sample of macrodiscs.

“For an automatic differentiation between normal and glaucomatous the Vancouver group proposed the glaucoma probability score, which is highly sensitive in daily routine but has a limited specificity,” added Dr Burk.

“In this respect,” he continued, “we have to follow up patients and based on our clinical knowledge we can diagnose abnormal discs from certain signs, such as vertical orientated cupping, which is a sign of already established progression.”

Therefore, this technology allows ophthalmologists to identify glaucoma type, stage and also to track progression.
Pre-perimetric glaucoma

In glaucoma a considerable loss of retinal ganglion cells (RGCs) has occurred by the time visual field defects are clinically detected,” emphasized Dr Masanori Hangai of the Department of Ophthalmology and Visual Sciences at Kyoto University Graduate School of Medicine, Kyoto, Japan.

“It has been shown in recent studies that the macular retinal ganglion cell (RGC) complex thickness has comparable glaucoma discriminating ability to circumpapillary retinal nerve fibre layer (cpRNFL) thickness,” Dr Hangai said. However, difficulties have been experienced observing the RGC complex, especially the ganglion cell layer (GCL) on OCT instruments.

OCT imaging and speckle-noise reduction

When viewing an SD-OCT image sometimes the layer boundaries are not clear as a result of black spots, which are called speckle noise. These spots hide each layer boundary.

“To reduce the speckle noise we employed a multiple B-scan averaging method, this was particularly useful in conjunction with eye tracking where multiple OCT scans can be performed at the same location and averaged. This unique technique of the SPECTRALIS OCT allows for improved visualization of retinal layers and small pathological lesions,” Dr Hangai added. They found that there was asymmetry particularly along the vertical scans for GCL thickness, which indicates glaucomatous RGC damage.

Next Dr Hangai examined GCL scans in patients with early glaucoma. He found characteristic changes in macular GCL and RNFL in the OCT scan that corresponded to the location of visual field defects. Additionally, macular GCL thinning was abrupt compared to more diffuse macular RNFL thinning.

Pre-perimetric glaucoma

Dr Hangai’s team examined pre-perimetric glaucoma and found there was a statistically significant difference in the mean GCL thickness between normal and pre-perimetric glaucomatous eyes. This difference was found in both the superior and inferior hemispheres but it was larger in the latter.

“However,” continued Dr Hangai, “we observed more severe thinning in each individual eye, which I believe is a result of the variation in location for local GCL atrophy of each eye.”

Sensitivity and specificity of macular GCL, and RNFL thicknesses were compared to that of cpRNFL thickness on the SPECTRALIS and GDx-VCC. Dr Hangai explained, “Although specificity was comparable for all parameters, sensitivity was much better in macular GCL, and RNFL thicknesses than cpRNFL thickness.”

Conclusion

“Macular RGC structures, including GCL and RNFL, were diminished before visual field defects were detectable in SAP,” Dr Hangai summarized. This observation is consistent with the results of previous histopathological studies.1

Additionally, he emphasized, “Pre-perimetric GCL loss appears to be characterized as abrupt and local. So my conclusion is that macular RGC structure is a potentially useful target for detecting pre-perimetric glaucoma.”

Reference

Clinical perspectives

We need to combine our clinical information with technological information,” said Dr Raymond LeBlanc, professor of ophthalmology and vice president of the Capital Health District Authority, Halifax, Nova Scotia, Canada.

He questioned, “But what are the challenges for clinicians? Is it diagnosis of glaucoma or follow up and has imaging really helped us to address the function/structural relationship?”

HRT

“This system has brought structural changes to the fore and offers a robust and quick method that is user friendly for both operators and patients,” Dr LeBlanc added.

It is now commonplace to view HRT images in the office at the patient’s side. Not only does this help with patient education in general, but it also provides valuable information to both patient and clinician about stability or progression. Dr LeBlanc continued, “Of course when you put structure and function together on the same screen, or flick back and forth, it becomes a very powerful tool.”

OCT

“In the future, I see there will be some long-term data and progression algorithms for SD-OCT,” said Dr LeBlanc.

He predicts that the clarity of the nerve fibre layer and other retinal structures will be improved and that adaptive optics will further improve the level of detail in imaging. “I also predict,” he continued, “that we’ll have additional functional elements that will allow us to look at how the retina is actually functioning.”

Glaucoma diagnosis

Thanks to the OHTS and the ancillary CSLO studies, predictive factors in glaucoma development are well recognized. However, Dr LeBlanc believes that the critical question for clinicians is not whether a patient has glaucoma but more about the detection of progression. “I don’t think it hurts to remind us that primary open angle glaucoma is a chronic disease in which progression is usually slow,” Dr LeBlanc said.

“We need a very objective metric,” he continued, “Testing needs to be patient and user friendly, accessible and affordable, robust over time and easy to interpret, and you know the HRT and the SPECTRALIS OCT are falling into this category very nicely.”

Management of glaucoma

With the worldwide introduction of standardised automated perimetry, the use of prostaglandins, and newer modalities of treatment, the management of glaucoma has changed over the last two decades. “Additionally, our ability to clearly image the structures of the inside of the eye has made a major difference in the clinical management of this chronic disease” Dr LeBlanc explained.

Summary

“There is now good evidence of predictability of developing glaucoma, and there is also evidence of real help in making a diagnosis. But in my opinion, what’s even more important, are rates of progression. I think we now have tools that allow us to determine rates and I really do believe that’s where we need to go,” he concluded.
Innovative in vivo imaging techniques are a prerequisite for successful personalised medicine, according to Dr Frank Holz, professor of ophthalmology and chair of the Department of Ophthalmology at the University of Bonn, Germany. “By having in vivo probing tools to hand,” he continued, “we can better define diseased areas, gain insight into pathogenesis, identify predictors for progression and surrogate markers for intervention trials.”

ICGA significance in AMD

“Indocyanine green angiography (ICGA) enables better diagnosis,” said Dr Giovanni Staurenghi, professor at the University of Milan, Italy. “It is possible to see various types of lesions using ICGA on the SPECTRALIS HRA+OCT that maybe aren’t visible with fluorescein angiography (FA) as a result of the different wavelength used,” he explained.

The importance of lesion detection can be seen in the work of Dr Nagahisa Yoshimura, professor of ophthalmology and chair of the Department of Ophthalmology and Visual Sciences at Kyoto University Graduate School of Medicine in Kyoto, Japan, with newly diagnosed exudative AMD patients who had polypoidal choroidal vasculopathy (PCV). This form of exudative AMD is common in Asia, however, diagnosis has proven to be difficult. In his work Dr Yoshimura followed 88 cases using ICGA and FA and found that the rate at which the lesion size increased was dependent upon type of PCV. However, he believes that ICGA is imperative in the diagnosis and monitoring of PCV. He emphasized, “PCV can be overlooked if you do not use ICGA routinely.”

“Not only does ICGA provide better diagnosis and lesion visualization,” Dr Staurenghi added, “But it also provides better treatment evaluation.” For example, in RAP lesions it is possible to clearly show the size of the retinal vessels, which modify after treatment, and the modification at the level of the angiomatous lesion in the retina.

Dr Staurenghi concluded, “ICGA and FA should both be used to better identify CNV lesions that have not responded to conventional treatment but in our understanding of what is happening ICGA is probably more important.”

Normalization in autofluorescence imaging

Fundus autofluorescence is caused in large part by the fluorescence of lipofuscin compounds in the RPE. Therefore, if RPE is missing, autofluorescence is markedly reduced, which is a good marker for outer retinal atrophy and diseases affecting the RPE.

Retina structural changes in neurological diseases

Injury to the retina is an active and dynamic process in multiple sclerosis (MS), according to Dr Ari Green, assistant professor of neurology and ophthalmology, University of California, San Francisco, USA.

Background

In MS, the immune system targets myelin which is the fatty substance surrounding axons. Disability from the disease correlates much better with the reduction of brain volume than with the number or volume of inflammatory brain lesions detected on MRI. It has been suggested that circumpapillary RNFL OCT scans may be useful for quantifying axon loss in the visual system.

Visual symptoms are very common in MS. Dr Green said, “The majority of patients will suffer from some symptoms from injury to the sensory visual pathway and 100% of MS patients have demyelinated plaques in the optic nerve at death.” So, optic nerve injury has been proposed as a model for understanding the disease given its unique anatomical structure.

OCT and MS

“During the last decade people became interested in OCT and MS but there wasn’t a lot of pathological evidence to base our conclusions on,” Dr Green explained. So, his team collaborated with researchers from Queen’s University, Belfast, Ireland, to analyse the optic nerves and retinas of 100 patients who had MS at the end of their lives.
“Even though it has not yet been proven that lipofuscin plays a role in AMD, it is hypothesized that it could act as a trigger of complement activation and inflammation in the disease,” explained Dr Francois Delori, associate professor of ophthalmology at Schepens Eye Research Institute, Harvard Medical School, Boston, Massachusetts, USA.

Drug companies are currently developing therapies that will decrease lipofuscin accumulation in AMD and retinal dystrophies and as such will have lipofuscin or autofluorescence levels as an endpoint. Therefore, standardization is required.

“To make our autofluorescence measurements quantitative a reference is needed,” Dr Delori said. A fluorescent reference is incorporated in the HRA or SPECTRALIS devices allowing fundus autofluorescence to be corrected for changes in sensitivity, laser power, and further corrections are required to account for differences in magnification and media absorption among patients.

“The reference must emit in the same wavelength range as the lipofuscin,” Dr Delori continued, “if possible, the

Figure 1: Blue laser autofluorescence image with reference standard for quantitative analysis

Figure 2: Autofluorescence imaging demonstrating growth of the area of geographic atrophy.

It was found from these studies that all the pathological hallmarks of MS other than demyelination (not just axon loss) can be found in the retina in MS. Green and his colleagues identified profound loss of axons during the course of the disease and that the injury to the eye is not limited to the retinal nerve fibre layer (RNFL) but can also be seen in the loss of the cell bodies of ganglion cells. “In addition to the loss of ganglion cells, there’s loss of cells in the inner nuclear layer,” added Dr Green. Furthermore, gliotic injury in the optic nerve head was observed in some cases an perivascular inflammation around retinal blood vessels was observed.

Dr Green has also been using the Heidelberg SPECTRALIS system to obtain OCT images in patients with MS. They found that injury was primarily located in the temporal quadrant of the RNFL scan. He noted that the typical circle scan of the system had some vignetting at the edges of the image, which led to degradation of image quality in the most significant region of the papillo-macular bundle (PMB). “Working together with Heidelberg, we designed a new method for putting the temporal portion of the RNFL scan for MS patients in the middle of the image,” Dr Green said. By doing this, the loss of RNFL in the PMB becomes easier to detect.

However, a cautionary tale in interpreting OCT images is that perivascular inflammation is visible in MS patients until the end of life.

Dr Green emphasized, “The OCT is measuring from the internal limiting membrane to the boundary between the ganglion cell layer and RNFL so it will be influenced by any other cellular elements found between the internal limiting membrane and the boundary with the RGC layer.”

Conclusion
Dr Green summarized that injury from MS is not limited to the inner retina but there may also be transynaptic degeneration driving loss in the deeper layers. “The most important lesson is that the visual system is anatomically distinct, easy to study and hence is a very nice model for applied neural science,” he concluded.
fluorescence should be at the same level as the fundus autofluorescence. We have not managed to achieve this but we have found a fluorophore with a similar efficiency to the fundus.” Additionally, this reference must be thin and stable after long light exposures.

According to Dr Delori, “It is possible to perform quantitative analysis of autofluorescence on a modified HRA or SPECTRALIS instrument, however, multiple factors affect the reliability of the method: quality of the image, field uniformity, amplifier linearity and noise, measurement of ‘zero grey level’ and the magnification, and, of course, stability of the reference”.

“The methodology may play an important role in the diagnosis and progress monitoring for patients with AMD and retinal dystrophies,” Dr Delori said. Additionally, the technique may be essential in monitoring treatment by drugs and gene therapy.

Imaging metrics

“As of yet we don’t cure disease, most of the time we are too late and our treatment is too broad for any type of manifestation,” emphasized Dr Holz. To meet patient’s expectations it is vital that outcomes are improved and therapies are more efficacious he explained.

In Dr Holz’s opinion refined phenotyping is a highly relevant development in the field. He said, “Imaging tools have done a lot to bring us closer to the development of specific pathway-targeted interventions and our understanding of these pathways.”

Previous work by Dr Holz’s group has demonstrated that the loss of luteal pigment is an essential hallmark of the disease macular telangiectasia Type II. The proof of this was possible through the use of Heidelberg Engineering’s HRA 2 instrument. This level of early detection is paving the way for effective therapies and outcomes.

In the case of geographic atrophy, which is not only a problem for pure dry AMD but is also an important cause of treatment failure in wet AMD, in vivo probing tools can help define diseased areas. In a multicentred natural history (FAM-) study using the HRA 2 Dr Holz and colleagues found that it is possible to distinguish between different abnormal types of autofluorescence surrounding the atrophic patch and also, that there is a huge variability in progression (Figure 2).

These findings were reproduced in another large natural history study, the Geographic Atrophy Progression study (GAP), where each site used the HRA 2 or SPECTRALIS. “Therefore, autofluorescence imaging is useful in identifying fast progressors by looking at hyperautofluorescent patterns surrounding the area of GA,” according to Dr Holz.

Conclusion

ICGA is a useful technique in the diagnosis and understanding of AMD. However, it is beneficial to use this technique in conjunction with FA to gain the most information and should be used to better identify and classify lesions.

Fundus autofluorescence can be used to address the pathophysiological role of lipofuscin accumulations in the RPE and to identify eyes with fast GA expansion rates. Recruitment of fast progressors will make interventional trials feasible with lesser duration and, thus, less cost. This technique has also proven to be useful in various complex and monogenetic retinal diseases.

“The SPECTRALIS HRA has become the gold standard imaging tool for geographic atrophy. Autofluorescence imaging with semi-automated measurements based on imaging processing methods (RegionFinder) is now used for all of the trials coming up. It is a useful enrichment tool to plan and perform clinical trials, and is accepted as an anatomic endpoint,” concluded Dr Holz.

Therapeutic monitoring and control

The gold standard for primary diagnosis of exudative AMD remains fluorescein angiography (FA). This was agreed upon by Dr Daniel Pauleikhoff, professor of ophthalmology, Münster, Germany, and Dr Sebastian Wolf, professor of ophthalmology and head of the University Eye Hospital in Bern, Switzerland.

However, there are many different imaging techniques available and certainly in patient follow-up other techniques are more sensitive at detecting structural parameters.

According to Dr Wolf, “One of the most important features of the Heidelberg SPECTRALIS system is that it allows us to perform multimodal digital imaging.” This level of integration not only enables everything to be performed on one instrument, but the images can also be viewed together and related to each other, which means easier detection of changes and, therefore, monitoring progression.

In age-related macular degeneration (AMD) these various imaging modalities are vital for correct diagnosis and follow-up but atrophic and exudative AMD require different approaches.
Atrophic AMD

Fundus colour photographs are still vital in this area, and these are taken by a digital fundus camera and fed into the Heidelberg system. "But, probably today the most important tool is retinal autofluorescence," said Dr Wolf.

"Autofluorescence gives us a clue about the metabolism of the retinal pigment epithelium (RPE), so a normal autofluorescence will suggest some kind of regular RPE function," added Dr Pauleikhoff.

Comparing information from the fundus appearance and the autofluorescence pattern can demonstrate a lack in RPE, which leads to delineation of the area of atrophy. Autofluorescence imaging can be used to monitor progression in patients over time. Autofluorescence images portray RPE damage and correlate with visual acuity.

Exudative AMD

Diagnosis of exudative AMD can be performed using FA with the scanning laser system and what is obtained is a high contrast image where the borders of neovascularisation are clearly visible (Figure 1).

Anti-VEGF treatment

In Lucentis studies OCT was used and demonstrated that the retina is primarily thinned from anti-VEGF treatment but once the vessels became more permeable again the thickness increased. "This variability in the retina thickness negatively affects visual acuity," explained Dr Pauleikhoff.

His group examined this variance in retinal thickness and its effect on visual acuity to try to determine an effective retreatment strategy. The team looked into several different imaging possibilities. Using autofluorescence and microperimetry they found that patients with abnormalities on autofluorescence or large decrease in central retinal sensitivity did not benefit as much from anti-VEGF therapy as patient with normal fundus autofluorescence.

Even though retinal thickness was an important factor in some patients it was noted that intraretinal changes were also vital. Using Heidelberg’s SPECTRALIS system the team found that there was a trend in cyst position and visual acuity. Cysts in the inner retina were associated with a better prognosis of visual acuity in comparison with cysts in the outer retina. This was also true when the team looked at the size and numbers of cysts.

Additionally, the team noted that subretinal fluid and the inner/outer photoreceptor segment junctions were important in patient follow up and changes in visual acuity. "This type of diagnostic techniques would give us a clue as to how the retina functions or about the photoreceptor structure, whether they have been destroyed or not," said Dr Pauleikhoff.

Conclusion

In conclusion, the gold standard for initial diagnosis of exudative AMD is still FA but an integrated device offers more comprehensive diagnosis and follow-up. OCT has been agreed upon to be a good follow-up strategy and has the potential to reduce the amount of visual acuity lost by the patient when used to guide anti-VEGF therapy.

“I think for exudative AMD FA is still the gold standard for imaging the choroidal neovascularization (CNV) for diagnosis. We can detect the CNV, we can look at the lesion type, the lesion size, components and the location,” Dr Wolf said.
Imaging techniques are equally important in experimental as they are in clinical ophthalmology, according to Dr Mathias Seeliger, professor of ophthalmology at the Institute for Ophthalmic Research, Centre for Ophthalmology, in Tübingen, Germany.

“Our work focuses on retinal degenerations, especially of the hereditary kind,” Dr Seeliger said. Only 10 years ago, the treatment of such severe, and in part blinding, disorders seemed impossible. “It sounds paradoxical: It now looks like we are nearly at the point of truly curing patients in contrast to many other ocular diseases,” he added.

According to Dr Seeliger, only a combined functional and imaging approach will meet the requirements of the complex findings in treatment situations in both patients and disease models. “The depth of scientific insights always depends on the quality of the diagnostic tools available,” he said. “As we started out as a purely electrophysiologically oriented group, Heidelberg Engineering’s HRA 1 SLO and SPECTRALIS OCT were instrumental for us to establish in vivo imaging on the same level.”

How it all started
Dr Seeliger’s early work on retinal diagnostics used multifocal electroretinography (mfERG) to give information about central vision. “You can obtain a topographic map of the central area, which is particularly helpful in macular disorders where you don’t see much of the pathology, and in concentric peripheral diseases like retinitis pigmentosa where only a small central area remains functional,” he explained.

It was at that time that Dr Seeliger’s team initially collaborated with Heidelberg Engineering to obtain comparable information in animal models. “We began by combining retinal SLO imaging with mfERG electrophysiology for the use in mouse disease models,” Dr Seeliger said. However, cone response timing turned out to be too slow for that in mice, but the innovative approach helped eventually to evaluate treatment success in the Briard dog model genetically deficient for RPE65. “This proof of a local functional improvement was an important step towards human gene therapy,” he emphasized.

SLO
Over the following years, SLO in vivo imaging in animal models was established as a complementary technique to full field ERG. Dr Seeliger and his team were involved in a number of worldwide collaborations using their “travelling” HRA 1 to assess the scale and progression of retinal disease in a variety of species, including monkey, dog, cat, rabbit, rat, gerbil and bird, in addition to their own work on mice.1

In extension of the conventional fundus imaging, the angiography modes turned out to be of particular importance for the assessment of vascular structures. The differences in binding of dyes to plasma proteins (fluorescein only around 60%, indocyanine green (ICG) about 98%) allow detection of sites of ultrafiltration, a common feature of new vessel formation. Such sites will show leakage in fluorescein angiography (FA), but not in ICG angiography (ICGA). Dr Seeliger added, “It is thus useful to compare ICGA and FA to determine both structure and function of vessels during the course of a disease.”

The autofluorescence mode opens further possibilities in animal models. One that Dr Seeliger believes to be important is the specific labelling of tissues by the transgenic expression of green fluorescent protein (GFP). “The protein-producing cells light up when using the autofluorescence imaging mode of the HRA, so no dye is required,” he explained. Further, a loss of GFP-positive cells in sequential examinations does indicate disease progression, like in neuronal cells. Dr Seeliger continued, “If you stain degenerating cones with GFP, it is possible to see them disappear over time.”2

OCT
“OCT is an important innovation for experimental ophthalmology, particularly for longitudinal studies,” commented Dr Seeliger. “In this regard, the capabilities of the SPECTRALIS to repeat measurements at the same position are very important. By using...”
OCT, researchers can track inner and outer retinal thickness changes with treatment, obtaining sufficient information to conduct a powerful statistical analysis,” Dr Seeliger emphasized.3

“Our published studies4-6 show that OCT allows detection and non-invasive analysis of a wide range of mouse retinal pathologies, comparable with histological studies,” Dr Seeliger said. The comparative results and the level of detail currently achievable indicate that OCT could replace a large portion of standard histology in the future (Figure 1). Supported by the Federal Ministry of Education and Research, the efforts of Dr Seeliger’s team to reduce animal numbers have been recognized with a major animal welfare research award.

Monitoring therapeutic approaches
Dr Seeliger believes that monitoring therapy will be a major field of application for clinical and experimental in vivo techniques, like in gene therapy. In this approach, a viral vector is injected subretinally that transduces specific retinal cells, leading to the expression of the gene missing due to a hereditary defect. “So it’s a very promising therapy but it is vital to use the OCT for immediate quality control (i.e., to track accurate administration) particularly with regards to the long-term success,” he added.

“Microbeads are another form of treatment,” he continued. “These are alginate spheres that contain living cells (programmed to produce and secrete active substances) that can be used either subretinally or epiretinally.” In contrast to retinal cells, these cells are not designed for optical translucency, which makes them particularly visible with the OCT, and facilitates observation of their integrity over time.

Conclusion
In Dr Seeliger’s opinion, “The different in vivo imaging techniques are very important to understand and monitor vascular as well as neurodegenerative retinal diseases, offering even more possibilities with transgenic expression of markers like GFP. I think that we have been helped immensely by the imaging technologies available, both for OCT and autofluorescence,” he concluded.

References

Retinal cell apoptosis

“Apoptosis has been shown to signal the first degree of disease in animal models and patients,” said Dr Francesca Cordeiro, professor of glaucoma and retinal neurodegeneration at UCL, London, UK.

Currently, Dr Cordeiro and colleagues are using Heidelberg’s wide-angled SPECTRALIS HRA and have seen apoptosis in retinal ganglion cells in different experimental models, including rat glaucoma, where peaking apoptosis occurs at three weeks after intraocular pressure (IOP) elevation (Figure 1). This technology called DARC (detection of apoptosing retinal cells) is currently going into early phase clinical trials in glaucoma patients. In another model of neurodegeneration, Dr Cordeiro’s group found that the retinal ganglion cells die through apoptosis in association with Alzheimer’s disease, and in early disease. “Ultimately what we’re aiming for is to use DARC as a screening mechanism to stop people ever losing their retinal ganglion cells and visual field in the first place,” Dr Cordeiro explained.

However, the mode by which these cells die is very important because there are, in fact two phases in apoptosis, an early and a late phase. Early apoptosis is reversible, and may be more amenable to targeting with treatment strategies.

Dr Cordeiro is also examining whether these results can be related to the varying stages of disease. For example, in Alzheimer’s there are multiple processes occurring at the same time so if you were to add stress, such as oxidative stress, more apoptosis occurs. Not only does this happen but it also shifts towards more early apoptosis, which may be more treatable.

Conclusion
“So in glaucoma our expectations for DARC at the moment are still expectations because we need to prove the role of DARC,” Dr Cordeiro added. “However, we think that ultimately we can increase the diagnosis level to the point where clinical trials would be shortened by being able to see the efficacy of treatment more quickly and monitor patients more frequently.”

Figure 1: A SPECTRALIS-acquired rat retinal image of fluorescent apoptosing cells with the DARC technique.
Anterior segment imaging

The slit lamp is still the gold standard for anterior segment imaging but it is not without its limitations primarily, low level magnification. Hence, in vivo confocal microscopy has offered a number of benefits, providing anterior segment surgeons the ability to differentiate cellular structures within the cornea to identify new structures and pathways to certain prevalent diseases. It can even help define how a LASIK flap heals and it was this technology that found that the epithelial nerve plexus was not as described in textbooks but actually a whirl-like structure.

Dr Rudolf Guthoff, professor of ophthalmology at Rostock University, Germany, is currently using this technology developed in his department together with Heidelberg Engineering to further investigate the subepithelium nerve plexus. The attachment is called Rostock Cornea Module.

“Each fluorophore can be characterized by the absorption spectrum, the emission spectrum and the lifetime of fluorescence in the excited state,” explained Dr Guthoff. “You can see the regeneration of the nerves and even though it isn’t much it is still more than what was available before,” said Dr Guthoff.

Crosslinking

This is currently a big topic and now with confocal microscopy it is possible to view what is happening to the cells after crosslinking has been performed. Importantly, it is

Fluorescence lifetime imaging

According to Dr Dietrich Schweitzer, docent of experimental ophthalmology at the University of Jena, Germany, pathological alterations occur first in the metabolism at which stage alterations are reversible.

Based on previous studies and their own work, Dr Schweitzer and his colleagues were able to determine that the co-enzymes FAD, NADH and other fluorophores like lipofuscin, advanced glycation end products, collagen and elastin were of particular interest in the metabolic processes of disease. Dr Schweitzer questioned, “How can we distinguish different fluorophores?”

Fluorescence

“Each fluorophore can be characterized by the absorption spectrum, the emission spectrum and the lifetime of fluorescence in the excited state,” explained Dr Schweitzer. “The fluorescence lifetime has a very important property,” he continued, “it is independent of the concentration and intensity. Moreover, two fluorophores with the same emission spectrum can have sufficiently different lifetimes.”

“In previous studies, we have measured the excitation and emission spectrum as well as the fluorescence lifetime of all fluorophores that we expect in the eye,” Dr Schweitzer said.

It was found from these studies of separated ocular structures of porcin eyes that the mean fluorescence lifetimes of all anatomical structures of the eye are as follows: the shortest lifetime is observed in the RPE, then the neural retina and then the vitreous and finally, as would be expected, the longest lifetimes occur in the lens and the cornea, which are reasonably similar.

Figure 1: Composite image of the human cornea after fs-LASIK.
“According to this primary information,” Dr Schweitzer added, “we have developed a fluorescence lifetime laser scanning ophthalmoscope, based on the HRA.” The decay of fluorescence intensity is simultaneously detected in two spectral channels by time correlated single photon counting.

Clinical results
Dr Schweitzer explained some clinical results obtained with this instrument, “In case of branch arterial occlusion, a difference was found between the supplied and non-supplied regions in the lifetime of short wavelength autofluorescence.” This corresponded with his assumptions that the lifetime in the neuronal retina is elongated and that an increased contribution of protein-bound NADH, which has a longer lifetime of at least one nanosecond, is expected. He said, “In the long wavelength channel we found no difference in lifetime between these regions.”

Presbyopia
In presbyopia, softening the lens is beneficial but the depth of the femtosecond laser cuts is definitely a consideration and is something that Dr Guthoff and colleagues are looking into.

Conclusion
Dr Guthoff emphasized, “Confocal microscopic differentiation of cellular structures is good. We are now able to perform microbiometry of corneal substructures and can quantify cells three dimensionally. Additionally, we can evaluate cells that we didn’t even know existed in the cornea like the dendritic cells.” He explained that this technology has enabled the mapping of the subepithelial nerve plexus, which he hopes will soon be quantified to help aged patients and the understanding of when they will start to experience nerve degeneration.

“Confocal in vivo microscopy has brought us closer to in vivo histology and histopathology. Even though it is still difficult to interpret unstained surface parallel images our efforts are justified because in vivo confocal images represent real life and not an interaction between staining techniques,” he concluded.
When looking at diabetic retinopathy, no alterations were found in the fluorescence intensity but a difference was observed in the lifetime, again in the short wavelength channel. There was an increased value of lifetime in the superior temporal quadrant.

Dr Schweitzer and his colleagues compared their measurements from branch arterial occlusion and diabetic retinopathy. They selected a lifetime range that they assumed to be a sign of reduced metabolism and found several characteristic locations in early diabetic retinopathy (Figure 1).

Dr Schweitzer continued, “Our assumption is that these regions can be used for therapy control.”

In their examinations of AMD, the team only found statistical differences in the short wavelength range between healthy subjects and early AMD. Dr Schweitzer said, “According to our interpretation it means that in the early stages of AMD alterations begin in the neuronal retina, not in the RPE.”

**Conclusion**

“Fluorescence lifetime characterises each fluorophore and is independent of concentration and absorption of its surroundings. That means we can also measure the fluorescence behaviour below the macular pigment,” Dr Schweitzer summarized.

“In the future we hope to combine lifetime measurements with tomography creating functional tomography. Thus, we can determine functionality in single fundus layers,” he concluded.

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**Two-photon imaging**

Heidelberg Engineering and Dr Bille, professor at the University of Heidelberg have developed a unique imaging technology based on the principle of two-photon imaging; technology that has yielded enhanced optical resolution. A two-photon microscope is based on the architecture of a conventional scanning laser ophthalmoscope (SLO) such as the HRA with the addition of femtosecond laser technology.

**Second harmonic generation imaging**

In second harmonic generation (SHG) imaging two photons are effectively ‘combined’ to form a new photon with twice the energy and half the wavelength.

Dr Bille explained, “SHG is non invasive and the longer primary wavelength is more gentle.” Using SHG imaging in the human cornea, for example, allows the clear visualization of differences in collagen structures in Bowman’s membrane and corneal stroma (Figure 1).

**Two photon excited fluorescence**

Two-photon fluorescence excitation, based on the two-photon process, has been shown to be a superior alternative to confocal microscopy for deeper tissue penetration, efficient light detection and autofluorescence. The localized two-photon effect offers intrinsic three-dimensional resolution and eliminates out-of-focus and scattered fluorescence (Figure 2).

The principle of two-photon retina tomography was developed based on previous imaging studies performed on a conventional two-photon microscope. The newly developed device exhibits a femtosecond fibre-laser with a wavelength of 780 nm attached to a Heidelberg HRA 2 instrument.

“By increasing the femtosecond laser power from 40 mW to 100 mW, the image contrast can be greatly enhanced,” said Dr Bille. “Employing an intensity window technique and adaptive optical beam shaping, a similar augmentation of image contrast for imaging retinas in human eyes is anticipated.”

**Adaptive optics two-photon ophthalmoscope**

The newly developed two-photon ophthalmoscope, which is based on the SPECTRALIS HRA is equipped with a femtosecond fibre-laser and a two-photon detector, and additionally exhibits a Hartmann-Shack wavefront sensor, a phase plate precompensator and a MEMS mirror for dynamic compensation of higher order optical aberrations. “Initially, a static compensation with customized phase plates is foreseen, omitting the MEMS mirror,” Dr Bille added.

In experimental work Dr Bille and colleagues looked at in vivo IOL adaptation. According to the 2010 Market Scope Report, approximately 10% of all cataract surgeries result in residual refractive errors of more than 1 D. Thus, a method
A history of Heidelberg Engineering

After deciding there must be an easier way to perform confocal microscopy at the back of the eye and that there would be a place in the market for this, Dr Gerhard Zinser and Christoph Schoess co-founded Heidelberg Engineering in 1990.

Their first device, the Heidelberg Retina Tomograph (HRT), was developed in 1991 and that same year they showcased it at the American Academy of Ophthalmology.

In 1992, the first devices were delivered and numerous collaborations with leading researchers were established. Dr Zinser emphasized, "These collaborations provided invaluable input in developing methods to analyse the acquired confocal 3D images and identifying the potential clinical applications."

Further developments were made, including the Heidelberg Retina Angiograph (HRA) and the Heidelberg Retina Flowmeter (HRF), however, the company’s focus was always on the application of the HRT in glaucoma, especially the topography of the optic nerve head (ONH).

By 1999 over 200 papers had been published, which demonstrated that the HRT measurements of the ONH topography were reproducible, correlated with histology and with visual field, and were useful to quantify glaucomatous damage and progression.

Then, that same year, a turning point happened for the company. Not only was ONH morphological analysis considered important for glaucoma diagnosis and follow-up but also the next generation of HRT was introduced, the HRT 2.

"In the HRT 2 we really tried to condense all that we learnt and decided to dedicate the device to ONH analysis and that allowed us to make it more compact, easy-to-use and less expensive. This led to an explosion," added Dr Zinser.

Today, the HRT is in its third generation and around 10000 ophthalmologists worldwide have access to it. Dr Zinser continued, “We have developed additional applications for the HRT 3 to assess retinal oedema and perform corneal microscopy and we have also introduced a second generation of the angiography system, the HRA 2.”

In 2005, Heidelberg Engineering acquired 4Optics AG, a company in Lubeck, Germany, specialized in OCT technology. The vision was to combine confocal laser scanning and SD-OCT technologies to create something new.

The outcome was the SPECTRALIS HRA+OCT in the autumn of 2006. The combination of confocal laser scanning and spectral-domain OCT in the SPECTRALIS accounts for a variety of unique features, such as real-time eye tracking, unparalleled OCT image quality, precise follow-up examinations, simultaneous angiography and OCT imaging.

“We are of course still in the middle of developing appropriate applications of all the capabilities of the SPECTRALIS but it can already be used in glaucoma, retinal imaging and anterior segment imaging,” said Dr Zinser.

“Our latest product is the Heidelberg Edge Perimeter (HEP) for visual field examination. We have a new stimulus — the Flicker Defined Form, which appears to be able to detect visual field defects quite early. Of course the idea is not just to stop here but develop an intelligent combination of structure and function,” he emphasized.