PARADIGM SHIFTS IN OPHTHALMIC DIAGNOSTICS*

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ABSTRACT

Purpose: Future advances in ophthalmology will see a paradigm shift in diagnostics from a focus on dysfunction and disease to better measures of psychophysical function and health. Practical methods to define genotypes will be increasingly important and non-invasive nanotechnologies are needed to detect molecular changes that predate histopathology.

Methods: This is not a review nor meant to be comprehensive. Specific topics have been selected to illustrate the principles of important paradigm shifts that will influence the future of ophthalmic diagnostics. It is our impression that future evaluation of vision will go beyond visual acuity to assess ocular health in terms of psychophysical function. The definition of disease will incorporate genotype into what has historically been a phenotype-centric discipline. Non-invasive nanotechnologies will enable a paradigm shift from disease detection on a cellular level to a sub-cellular molecular level.

Results: Vision can be evaluated beyond visual acuity by measuring contrast sensitivity, color vision, and macular function, as these provide better insights into the impact of aging and disease. Distortions can be quantified and the psychophysical basis of vision can be better evaluated than in the past by designing tests that assess particular macular cell function(s). Advances in our understanding of the genetic basis of eye diseases will enable better characterization of ocular health and disease. Non-invasive nanotechnologies can assess molecular changes in the lens, vitreous, and macula that predate visible pathology. Oxygen metabolism and circulatory physiology are measurable indices of ocular health that can detect variations of physiology and early disease.

Conclusions: This overview of paradigm shifts in ophthalmology suggests that the future will see significant improvements in ophthalmic diagnostics. The selected topics illustrate the principles of these paradigm shifts and should serve as a guide to further research and development. Indeed, successful implementation of these paradigm shifts in ophthalmology may provide useful guidance for similar developments in all of healthcare.


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I. INTRODUCTION

Medicine is a discipline that traditionally uses patient evaluations to find disease. In the absence of abnormal findings, health is assumed. This is especially true in ophthalmology where physical examination, diagnostic tests, and imaging are designed to seek abnormalities. Indeed, it is often glibly said that in ophthalmology what you see is what you get. Consequently, the philosophy of our approach is often shaped by direct visualization of disease. Health is defined as the absence of visible manifestations of disease. Advances will shift our focus to the molecular level for assessment of structure and function. Such a paradigm shift enables the establishment of ocular health as a distinct entity (diagnosis). Better measurements will lead to better understanding of the psychophysical basis of vision and better metrics of structure and physiologic function will allow for better management including preventive treatments. With this shift, a new paradigm of health promotion could provide a powerful means to not only prevent disease and the visual consequences associated with aging, but also relate (link) disease prevention and health promotion initiatives to performance-based measures such as reading speed, driving simulators, and tests of actual life function such as CAARV.

The theme of the 2016 Knapp Symposium of the American Ophthalmological Society was "Innovative Paradigm Shifts in Ophthalmology". Paradigm Shift is a term coined in 1962 by T.S. Kuhn to mean "a fundamental change in the basic concepts and experimental practices of a scientific discipline". The term has now become part of the business and political lexicons, but it was initially used as a means of re-considering established scientific models to provide better explanations of available information. The 2016 Knapp symposium addressed both diagnostic and therapeutic paradigm shifts in ophthalmology. Although technologic advances in therapy, such as laser therapy to replace surgery in glaucoma, the transition from surgery to pharmacotherapy with anti-VEGF injections for exudative maculopathies, pharmacologic vitreolysis to replace vitrectomy for vitreo-maculopathies, and new technologies for drug delivery were important parts of this symposium, these topics are beyond the scope of this white paper. Further, it should be emphasized that this is not a comprehensive review article. Rather, the topics presented have been selected to illustrate the principles espoused and demonstrate the initial implementation of the paradigm shifts that will influence the future of ophthalmology.

II. BEYOND VISUAL ACUITY – TOMORROW’S MEASURES OF VISION

A. INTRODUCTION

Visual acuity (VA) has long been the cornerstone of measuring vision. Snellen acuity is common, convenient and reliable, yet this method of measuring vision is also problematic. For example, VA is just a sampling of the central few degrees of visual field and generally it is only measured at 100% contrast. There are many occasions when patients with VA of 20/20 are, in fact, functionally blind. In the future, we will see a paradigm shift to assessments that evaluate other aspects of vision. These will better characterize the patient’s visual abilities, will better detect the early onset of disease, and will better measure response to therapy than VA alone. Going beyond VA will afford better phenotype characterization for both research and clinical care. A better understanding of the physiology of vision as reflected by these new testing paradigms may even enable new methods by which to improve vision beyond what is considered ‘normal’ today.

B. Utility and Limitations of Visual Acuity (VA)

The measurement of VA is typically performed with black letters against a white background. However, a prospective study comparing mean Snellen VA and ETDRS VA found scores were significantly better on ETDRS charts especially in patients with
Contrast sensitivity function (CSF) is known to decrease with age, a phenomenon previously thought to reflect aging changes and degenerations within the retina, optic nerve and visual cortex. Nonetheless, CSF is not commonly measured in clinical practice. Recent studies by Sebag et al. have shown that changes in the vitreous body may alter CSF. Patients without any ocular abnormalities, except bothersome vitreous floaters primarily due to posterior vitreous detachment, had reductions of CSF of about 67% in comparison to that found in age-matched controls. It is believed that in addition to provoking symptomatic floaters, these abnormalities, except bothersome vitreous floaters primarily due to posterior vitreous detachment, had reductions of CSF. It is likely that this phenomenon underlies the measurable impairment. Subsequent studies confirmed that age-related gel liquefaction with intravitreal fiber formation and posterior vitreous detachment are associated with diminished CSF. In young myopic individuals there is similar aggregation of collagen fibrils and gel liquefaction constituting myopic vitreopathy that also reduces CSF. It is likely that this phenomenon underlies the measurable negative impact on the quality of life experienced by patients with vitreous floaters. Thus, CSF testing is a useful measure of the impact of various ocular as well as central nervous system disorders on vision. Future advances will hopefully see the integration of CSF testing into clinical practice alongside visual acuity testing.

### 3. CONTRAST SENSITIVITY FUNCTION IN VITREO-RETINAL DISORDERS

#### a. Vitreous and Posterior Vitreous Detachment

Contrast sensitivity function (CSF) is known to decrease with age, a phenomenon previously thought to reflect aging changes and degenerations within the retina, optic nerve and visual cortex. Nonetheless, CSF is not commonly measured in clinical practice. Recent studies by Sebag et al. have shown that changes in the vitreous body may alter CSF. Patients without any ocular abnormalities, except bothersome vitreous floaters primarily due to posterior vitreous detachment, had reductions of CSF of about 67% in comparison to that found in age-matched controls. It is believed that in addition to provoking symptomatic floaters, these vitreous degenerations produce optical effects that reduce CSF but with normal or nearly normal visual acuities. That this impairment strikingly normalized within one week after limited vitrectomy strongly suggests that vitreous was the cause of the impairment. Subsequent studies confirmed that age-related gel liquefaction with intravitreal fiber formation and posterior vitreous detachment are associated with diminished CSF. In young myopic individuals there is similar aggregation of collagen fibrils and gel liquefaction constituting myopic vitreopathy that also reduces CSF. It is likely that this phenomenon underlies the measurable negative impact on the quality of life experienced by patients with vitreous floaters. Thus, CSF testing is a useful measure of the impact of various ocular as well as central nervous system disorders on vision. Future advances will hopefully see the integration of CSF testing into clinical practice alongside visual acuity testing.

#### b. Retinopathies

In addition to being directly measured, CSF can be integrated into the measurement of visual fields. In this sense, contrast can be the “Z” axis for the construction of a 3-dimensional visual field display done through a computerized presentation and analysis, called 3-dimensional threshold Amsler Grid (3-D TAG) testing. This system has shown characteristic contrast/visual field defects in diabetic retinopathy, plaqueunol toxicity, glaucoma, age-related macular degeneration, and vitreo-macularopathies. In glaucoma, 3D-TAG can detect changes prior to conventional visual field loss (Figure 1, left), as can Pelli-Robson, Mars, Arden Gratings, SPARCS, and other tests of contrast sensitivity function. In maculopathies, disease severity and the response to therapy can be quantified with 3D-TAG, as has been shown for pharmacologic vitreolysis therapy of vitreo-macular traction and surgery for macular pucker.
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(Figure 1, right) Further advances could be realized by relating such visual function to quality of life and ability to function in daily living.3

**FIGURE 1: 3-DIMENSIONAL THRESHOLD AMSLER GRID (3D-TAG)**

Left: 3-dimensional Threshold Amsler Grid testing is found superior and inferior nasal steps in the right eye of glaucoma-suspect patient with normal (B) Humphrey visual field. The 3D-TAG is much more sensitive. Grayscale figure on right is a second patient tested by 3-D TAG on two subsequent days that shows identical stair-case pattern of inferior nasal steps in the right eye only seen at lower contrast levels (the lower the contrast, the larger the field defect). Again this was in a patient with normal Humphrey visual field. The similarity between these two tests on subsequent days confirms the test/retest reproducibility.


Right: Volumetric 3-dimensional plots demonstrate the location and extent of visual distortions before and after surgery for macular pucker. The volume inside the 3-dimensional tracing is calculated and expressed as the percent of the hill-of-vision afflicted with metamorphopsia. Displayed are the plots of the patient’s metamorphopsia before surgery (pre-op) and at 1, 3, 6, and 12 months after surgery (post-op). The distortions index (percent volume loss relative to the entire hill of vision) reduced from 15.80% before surgery to 4.74% at 1 month, 1.33% at 3 months, and 0.34% 12 months after surgery. [Reprinted from Nguyen J, Yee KMP, Sadun AA, Sebag J: Quantifying visual dysfunction and the response to surgery in macular pucker. Ophthalmology 2016; 123:1500-10.]

D. COLOR VISION

Neuro-ophthalmologists refer to a triad of tests that are particularly sensitive to optic nerve dysfunction - color vision, afferent pupillary defect and brightness-sense. Acquired impairments of color (dyschromatopsia) often reflect optic nerve dysfunction, especially in the presence of good visual acuity.33 The Ishihara pseudoisochromatic color plates are commonly used because of the simplicity of this test. However, such testing may not detect subtle disease. More sophisticated testing, such as with the Cambridge Colour testing system, the Farnsworth D-15, the Lanthony 15-Hue Desaturated Test, or the Farnsworth-Munsell 100 Hues test is tedious, as it requires specialized lighting and a commitment of time. But such testing may reveal early glaucoma, autosomal dominant optic atrophy, optic neuritis, and even Alzheimer’s optic neuropathy.34, 35 Furthermore, color vision may also be reduced in macular disease.36,37 Thus, future paradigms of measuring vision will benefit from developing simple yet more sensitive tests of color vision.
E. MACULAR FUNCTION

Measuring macular function can provide insight into the early manifestation(s) of many diseases. Deficient macular function can produce impairments of contrast sensitivity, dyschromatopsia, and especially central depressions on 3-D TAG testing. Furthermore, metamorphopsia are common in macular disease and this too can be characterized, quantitated, and monitored with 3-D TAG testing. Indeed, the three-dimensional shape of a metamorphopsia defect may look very different from that of scotomatous defect. The latter is often cylindric (the same at all contrast levels) while the former is often conical with the tip pointing “down” (denotes increased central visual field abnormality with lower contrast levels between the grid and the background). The 3-dimensional zone of metamorphopsia may be cylindric, conical, or even in an hour-glass shape at times, as the larger zone of metamorphopsia may be brought out by higher or lower contrast levels. Diabetic macular edema was found to be associated with multi-focal, conical 3D-TAG abnormalities, while exudative age-related macular degeneration was associated with unifocal, quasi-cylindric abnormalities. 3D-TAG was also able to distinguish between wet and dry macular degeneration. Insofar as amplification of the zone of metamorphopsia can occur at various contrast levels, it may be reasonable to obtain an index reflecting the total volume of the 3-D cylinder or cone of metamorphopsia. This has been done for macular pucker where the distortions index (%volume lost of the hill-of-vision) decreased progressively following vitreous surgery. (Figure 1, right)

The problem of quantifying distortions is made even more difficult when adding the fourth dimension of spatial frequency. In the future, this should be integrated, since certain lesions may produce more metamorphopsia when looking at finer lines, while others induce more distortions when tested with coarser and broader lines. Thus, future paradigms of measuring vision may be provide even greater insight into the physiology of vision by utilizing 4-dimensional assessments.

1. Retinal Ganglion Cells

Different types of retinal ganglion cells subserve separate visual functions. One major distinction is defined by the layers of the lateral geniculate nucleus (LGN) to which the cells project. M-cells are fast-conducting and project to the Magnocellular layers of the LGN as well as the superior colliculus, while P-cells are slow firing and project to the Parvocellular layers of the LGN. Distinguishing the different functions of M-cells from the P-cells is both complicated and controversial. However, in the simplest sense, P-cells furnish frequency information, and color vision. Since the proportion of P-cells is highest in the macula, high contrast/high spatial frequency rhythms, the pupillary reflex, and other non-image forming functions of the eye. What we would consider as conscious visual elements needed for discrimination, since they provide high contrast, high spatial frequency information, and color vision. Since the proportion of P-cells is highest in the macula, high contrast/high spatial frequency information and color vision are often considered macular functions. M-cells, in contradistinction, help us perceive stimuli that express retinal ganglion cells (mRGCs) represent a new class of photoreceptors that subserve the photoentrainment of circadian rhythms, the pupillary reflex, and other non-image forming functions of the eye. They do not contribute to image resolution. There has been a growing research interest in these cells (Figure 2), including a few studies on the relevance of the mRGC system in humans in aging and in different optic neuropathies. For example, mRGCs resist neurodegeneration in inherited mitochondrial disorders that cause blindness. Measuring their dysfunction requires specialized psychophysics such as pupillometry with light stimuli at different wavelengths, representing another paradigm to measure vision beyond visual acuity.

F. OTHER MEANS OF VISUAL ASSESSMENTS

There are at least eight different retinal projections to different primary nuclei in the human brain, corresponding to different visual functions. To some extent, different visual testing modalities can parse out these pathways and functions. However, the physiology of other projections, such as to the superior colliculus and pulvinar, is still obscure. Visual parallel processing continues in the human cortex. Hence, there can be relatively specific visual impairments in the face of normal VA. Some techniques, like frequency doubling technology perimeter or quantitative layer-by-layer perimeter lay claim to separating out the pathways. But the former is probably not as specific for M-cell function as purported, and the latter rather too tedious to perform. There are old electrophysiological tests, such as critical flicker fusion frequency (the speed at which flickering light is perceived as steady) and new ones, such as the “C” wave or photopic negative response, that may lead to more reliable measures for the detection of specific inner layer impairments of contrast sensitivity, dyschromatopsia, and especially central depressions on 3-D TAG testing.

G. CONCLUSIONS

There will never be a shortage of new investigational tools for the assessment of human visual function, but the tests that matter most to ophthalmologists will be those that possess several critical attributes: they should be quantitative and reliable; they should be low maintenance in terms of cost, hardware and time; they should be sensitive and specific and provide insights into pathophysiology; but most of all, effective tests will allow for the early detection, accurate characterization and accurate monitoring of the progression or resolution of various ocular and visual diseases. The measurement of visual acuity has served us well, but it is now time to expand our basic armamentarium to include new assessments for the evaluation of vision. As an exciting byproduct, the subsequent increased understanding of vision provided by these developments may lead to the enhancement of ‘normal’ vision beyond visual acuity.
III. PHENOTYPE/GENOTYPE - TOMORROW’S DEFINITION OF DISEASE

"Only with the discovery of the double helix and the ensuing genetic revolution have we had grounds for thinking that the powers held traditionally to be the exclusive property of the gods might one day be ours."


A. CLASSIC CONCEPTS OF DISEASE

Medicine, in general, and ophthalmology, in particular, have a long history of describing diseases based on the observable signs in affected patients. For example, at different times during recorded history the disease we now know as tuberculosis (TB) has been called phthisis and consumption, among other names, based on the features care providers felt were most prominent.48,49 Similarly, inherited retinal degenerative disorders have been defined by changes detected in pupil responses and observed on fundus exam, producing names such as Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP), respectively. In short, diseases have been defined by their phenotypes.50 There are problems with this approach, however, as phenotypic descriptions often do not contain information about disease etiology, and thus are limited with regard to informing medical care providers and patients about prognosis and potential therapies for their disorders. For example, only with the discovery of the TB bacillus (Mycobacterium tuberculosis) was it possible to make some headway with treatment and control this disease.47,48 Improved understanding of the etiologies of different diseases provides opportunities for a paradigm shift to define diseases differently, with a focus on etiology rather than disease manifestation(s) alone.

Historically, methods to define diseases have changed as new information about disease etiologies have been discovered. For example, since the identification of infectious etiologic agents provides critical information about therapy and prognosis, the World Health Organization issued updated guidelines for naming infectious disorders in 2015.51 These guidelines (http://www.who.int/topics/infectious_diseases/naming-new-diseases/en/) indicate that terms used for naming infectious diseases should:

1. be descriptive
2. include the etiologic agent, if known
3. avoid eponyms, names of people or places or animals

For example, the disease previously called “swine flu” would now be called H1N1 influenza or Legionnaires’ disease would be called Legionella associated pneumonia. The premise of this treatise is that the same approach used for infectious etiologies should be applied to non-infectious disorders, particularly eye diseases. Specifically, it is proposed that the genetic etiologies of eye disorders should and will be included in their names going forward. The example of inherited retinal degenerations will be used to illustrate the reasons for this recommendation, which the authors hope can also be applied more broadly.
B. GENETICS OF INHERITED RETINAL DEGENERATIONS

Inherited retinal degenerations (IRDs) are important causes of vision loss. This group of Mendelian disorders is characterized by progressive dysfunction and death of rod and cone photoreceptor cells. Several types of IRD, such as those that have been called LCA, cause vision loss in infancy or childhood. Other IRD subtypes, such as RP, account for up to 25% of blindness or visual impairment in working age people (21-60 years). Since the identification of rhodopsin (RHO) as an IRD disease gene in 1990, over 250 genes that harbor mutations which cause retinal degenerative disorders have been identified, making IRDs one of the most genetically diverse groups of inherited disorders.

In the past several years, there has been increasing interest in identifying the genetic cause of disease for patients with IRDs, since several clinical trials have demonstrated the potential benefit of gene augmentation therapy for patients with specific genetic forms of IRD. Given the genetic heterogeneity of IRDs, next generation sequencing (NGS) methods are now being used for genetic diagnostic testing of patients with IRDs. The use of these unbiased testing approaches, which usually include all known IRD disease genes, has confirmed that the traditional phenotype-based definitions of disease do not provide accurate information regarding disease etiology. That is, mutations in disease genes originally identified to be associated with one phenotypic sub-type of IRD can cause disease that manifests clinically as a different sub-type of IRD. As an example of this, we recently evaluated two siblings with teenage onset retinal degeneration whose disease was found to be caused by mutations in the CEP290 gene (Figure 3). The clinical manifestations in these patients with teenage onset of disease were bone spicule pigmentation on fundus exam, mid-peripheral visual field loss, and relative preservation of retinal function as measured by electroretinography (ERG) are consistent with a clinical diagnosis of RP. Yet, mutations in CEP290 have been predominantly associated with early onset, severe retinal degeneration since mutations in CEP290 were originally reported to cause LCA, as well as syndromic ciliopathies. But, until NGS-based approaches for genetic testing of patients with IRDs became available, patients with other phenotypes such as RP were generally not tested for mutations in CEP290. Thus, the range of phenotypes produced by mutations in CEP290 has not been fully appreciated.

Similarly, mutations in genes originally described to be associated with mild or stationary disease can cause severe, early onset retinal degeneration. An example is the recent identification of two patients with early onset, severe retinal degeneration (LCA) due to mutations in the CACNA1F gene, which was initially associated with congenital stationary night blindness (CSNB), a notably milder form of retinal degeneration. In both of these cases, the patients presented with nystagmus in infancy, and were found to have reduced or absent ERG responses (Figure 4). These findings led to the clinical diagnosis of LCA in both patients. NGS-based genetic testing subsequently identified mutations in CACNA1F in both patients. The observation that mutations in the same gene can cause...
clinically different forms of retinal degeneration should not be surprising, since many diseases display a spectrum of severity, and the distinction between the disorders we have called LCA and RP or LCA and CSNB is based on the severity of the disease phenotype. An example from outside of ophthalmology is the clinical presentation of cystic fibrosis, which can range from chronic sinusitis in adulthood to chronic respiratory tract infections and failure to thrive starting during infancy, yet all forms of the disease are due to mutations in the \textit{CFTR} gene.80 What these findings demonstrate is that LCA and RP are not different disorders, but rather the opposite ends of the phenotypic spectrum of the same genetic diseases that cause vision loss via dysfunction and death of the photoreceptor and RPE cells of the retina.

\textbf{FIGURE 4: CACNA1F-ASSOCIATED RETINAL DEGENERATION}

Top: Fundus images from a 4 year old with CACNA1F-associated retinal degeneration  
Bottom: Full-field flash electroretinography according to ISCEV standards at age 3.5 months. ERG responses were recording in response to single flashes of 0.01 cd.s/m² (rod response), 3.0 cd.s/m² (rod-cone responses), or repeated flashes of 3.0 cd.s/m² (30Hz cone responses). All responses were severely attenuated, consistent with the clinical diagnosis of LCA.  

C. PROPOSAL TO UPDATE DEFINITIONS OF INHERITED EYE DISEASES

The genetic data described above are important for several reasons. First, they demonstrate that broad genetic testing is more accurate than focused testing, which can be limited by traditional phenotypic diagnostic categories. At present, options for broad testing include panel-based tests and medical exomes. It is likely that whole genome sequencing will be used more routinely in the future given the ability of this approach to accurately detect structural variants.81 Second, the data indicate that historical definitions of disease are inadequate because the underlying cause of disease cannot be predicted by clinical findings. Information provided to patients about prognosis and possible treatments based on clinical diagnoses alone may be inaccurate, because phenotypic clinical definitions of disease alone are not precise. Finally, the advent of gene therapies for some genetic forms of IRD makes it important to determine the precise genetic basis of disease, and to include this in the description of disease. Moreover, the inclusion of a description of etiology in disease names has therapeutic importance as well. It is proposed that genetic diagnostic testing is part of optimal care for patients with IRDs, and that the descriptions of different forms of IRD be updated to include genetic etiology, when this is available. Examples include:

1. \textit{RHO}-associated retinal degeneration (or \textit{RHO}-associated RP)  
2. \textit{CEP290}-associated early onset retinal degeneration (or \textit{CEP290}-associated LCA); \textit{CEP290}-associated retinal degeneration if the disease has a later onset or less severe phenotype; or \textit{CEP290}-associated ciliopathy if the disease is syndromic  
3. \textit{ABCA4}-associated macular degeneration (or \textit{ABCA4}-associated Stargardt disease)
D. BROADER APPLICATION OF GENETIC DISEASE DEFINITIONS TO OPHTHALMOLOGY

The same paradigm can be applied to other inherited forms of eye disease, such as glaucoma and other optic neuropathies. For example, mutations in 6 genes have been identified to cause early-onset glaucoma, and account for approximately 20% of patients with early-onset disease. Identification of individuals with mutations in one of these genes is valuable, as appropriate monitoring and timely treatment of early disease can reduce vision loss in many cases. Further, early studies suggest that early-onset glaucoma due to mutations in the myocilin (MYOC) gene may be amendable to treatment with agents that reduce endoplasmic reticulum stress. Identification of patients with MYOC-associated glaucoma may thus help identify individuals who could be eligible to participate in clinical trials of novel therapies for this form of disease. Identification of the genetic cause of disease for patients with other forms of optic neuropathy can be similarly, worthwhile. In addition to defining the inheritance pattern so that accurate risk assessment for family members can be provided, clinical trials of gene augmentation therapy for ND4-associated optic neuropathy (Leber Hereditary Optic Neuropathy) due to mutations in the ND4 gene are currently underway. Patients with ND4-associated disease may also benefit from avoidance of certain environmental exposures such as smoking and alcohol consumption.

It may also be desirable to classify common, complex disorders by the genetic contributors to their etiology, although this will be more difficult to achieve because multiple genetic variants with small effect sizes are likely to contribute to these disorders. As an example, over 34 genetic variants have been associated with age-related macular degeneration (AMD). As these and other genetic associations are studied in more detail, it is likely that some will be found to influence the response to therapy. Lorens-Motta and colleagues reported that single nucleotide polymorphisms in the NRPI and VEGFR2 genes are significantly associated with response to anti-VEGF therapy with ranibizumab, and thus can be used to guide treatment decisions for individual patients. It is worth pointing out that numerous other genetic risk scores for AMD have been developed, and many are predictive of the measured outcome when applied to subsets of patients. It has been suggested that additional progress in this area will depend on use of more uniform phenotype and genotype information so that the resulting risk scores are useful for a wide variety of patients. For example, obtaining consistent genotype information for a larger numbers of patients with AMD could allow for development of more robust risk scores related to disease progression or differential response to therapies. Similarly, adoption of consistent methods for assessing phenotype, such as one of the contrast sensitivity or macular function measures described above, could facilitate aggregation of data from multiple centers. Thus, it is possible that tomorrow’s definition of common diseases such as AMD and glaucoma will also include genetic etiology.

IV. OPHTHALMIC NANOTECHNOLOGIES - TOMORROW’S EYE DIAGNOSTICS

*Any sufficiently advanced technology is indistinguishable from magic.*

Arthur C. Clarke, "Profiles of The Future", 1961 (Clarke's third law)

Throughout history, ophthalmology has benefited from technologic advances in imaging. Indeed, it could be argued that ophthalmology was born as a result of imaging. The invention of ophthalmoscopy by Purkinje in 1823 and the subsequent development of the ophthalmoscope by Babbage in 1847 and Helmholtz in 1851 enabled practitioners to actually visualize structures within the eye. Gullstrand’s slit lamp biomicroscope additionally enabled detailed examination of the anterior segment. Hans Goldmann’s classic works employed biomicroscopy and contact lens viewing of the posterior segment to generate great advances in or understanding of eye diseases.

Yet, in the wake of great progress during the past century and a half, the fundamental philosophy of our approach to ophthalmic diagnostics has regrettably plateaued. Even modern imaging technologies such as optical coherence tomography are idled at the level of cell and tissue pathology. This is quite limiting, as disease begins on the molecular level and thus our current approach is only able to diagnose ocular disease at fairly advanced stages in the natural history. Consider, for example, the case of a patient with diabetes. Our current approach to evaluating such a patient’s eyes is basically an effort to identify cataracts, iris neovascularization, vitreous membranes and/or hemorrhage, retinal microhemorrhages, retinal microaneurysms, macular hard exudates/cysts/ thickening, and neovascularization of the retina and/or optic disc. In the absence of these findings we advise the patient that there is no evidence of diabetic eye disease. Yet, it is well-known that this disease begins on a physiologic and biochemical level with molecular changes that predate the aforementioned cellular changes by years, if not decades. Thus, in search of identifying/defining ocular health as a diagnosis (see above) and thereby enabling identification of the earliest departure from this ideal state, new diagnostic technologies are necessary. Given the need for molecular diagnostics, nanotechnology offers great promise. Of particular importance is the ability of laser light to access and leave the eye, providing great opportunities for laser-based nanotechnologies to play an important role in tomorrow’s eye diagnostics. Lastly, with tissues representative of nearly every tissue type in the body, the eye can serve as a window to the body, if not indeed the soul.
A. Anterior Segment

The tear film is a complex structure that plays an important role in ocular health.\textsuperscript{107,108} Non-invasive spectral analysis of the ocular surface could be performed with relatively high levels of incident light energy since off axis laser irradiation poses little risk to the inner eye.

Conjunctival blood vessels can be safely illuminated with relatively high levels of energy and circulatory physiology could be readily evaluated in various conditions of health and disease.\textsuperscript{109,110,111,112,113}

The cornea consists largely of extracellular matrix molecules, primarily collagen. As such, it is representative of extracellular matrix biology throughout the body and can be a useful source of information regarding the molecular effects of systemic diseases such as diabetes on the extracellular matrix. One of the most fundamental molecular effects of diabetes is the formation of advanced glycation endproducts of collagen, which is ubiquitous in the human body.\textsuperscript{114,115,116} Thus, evaluation of the changes in corneal collagen induced by diabetes might provide an index of protein glycation throughout the body, just as the glycated hemoglobin test provides important information regarding recent (2 – 3 months) glycemic control. Corneal collagen, however, does not have the rapid turnover of red blood cells and thus glycation effects here would represent chronic influences. Since glycated collagen demonstrates autofluorescence, a system has been developed to measure corneal autofluorescence\textsuperscript{117} and studies have evaluated diabetic patients\textsuperscript{117,118,119} with the results correlated to diabetic eye disease.\textsuperscript{121} Future studies of corneal autofluorescence and other biophysical properties of key molecules in diabetes should be directed to detecting molecular changes prior to the onset of complications such as diabetic retinopathy, in order to develop new strategies to prevent disease, perhaps with agents that inhibit protein glycation.\textsuperscript{122,123,124}

Aqueous is an ultrafiltrate of blood. Thus, the anterior chamber offers a unique opportunity to non-invasively assay a close representative of blood. For example, aqueous glucose levels have been indexed non-invasively by measuring the polarization properties of aqueous \textit{in vivo}.\textsuperscript{125} Raman spectroscopy offers an even more broad-based non-invasive nanotechnology that could potentially be developed to replace the need for drawing blood.\textsuperscript{126,127,128}

The lens is largely acellular, composed primarily of proteins that undergo pathologic cross-linking and aggregation, resulting in cataracts. Studies have suggested that cataract formation may be irreversible once lens opacities develop.\textsuperscript{129} Early detection is thus needed to enable preventative therapy that could obviate the need for surgery.\textsuperscript{130} Dynamic light scattering is a laser-based nanotechnology\textsuperscript{131} that has been developed to provide non-invasive quantitative assessment of particle size distribution in the human lens (Figure 5, left) and detect precataractous alpha-crystallin chaperone reserve in the lens nucleus, reflecting the risk of cataract formation.\textsuperscript{132} Longitudinal studies in a cohort of 45 subjects identified the progression of lens opacification in those individuals with diminishing alpha-crystallin.\textsuperscript{133} (Figure 5, right)

**FIGURE 5 DYNAMIC LIGHT SCATTERING OF THE HUMAN LENS \textit{IN VIVO}**

Left: Particle size distribution of the human lens at different ages

The histograms display the particle size distribution obtained with non-invasive dynamic light scattering in 3 individuals of different ages. There is a shift to the right denoting larger particle sizes with advancing age. [courtesy of Dr. Rafat Ansari and Dr. Manuel Datiles]

Right: Progression of Nuclear Sclerosis in a 43 year-old patient over 20 months. There is a decrease in the a-crystallin index (ACI) corresponding to an increase in lens opacification (NO=nuclear opacification) as assessed by AREDS nuclear opacification grading.

[AREDS: Age-Related Eye Disease Study; OD=right eye; OS=left eye]


B. Vitreous

Invisible by design, the vitreous body represents perhaps the most challenging of all ocular structures to image, owing to the very low concentration of structural macromolecules in this highly hydrated (98% water) tissue.\textsuperscript{134} Although swept source OCT has recently improved clinical evaluation of the posterior vitreous, it is to date unable to image the entire vitreous body and no studies have...
demonstrated the ability to enable quantitative analysis on a molecular level. Ultrasound is able to image the entire vitreous body and studies have recently shown efficacy in quantifying vitreous echodensity as a metric of vitreous pathology. These are relatively macroscopic changes, however, and more detailed molecular changes need to be measured for health assessment and early diagnosis of disease. Experimentally, dynamic light scattering has been employed to non-invasively evaluate the molecular structure of vitreous, but no clinical studies have been performed to date.

C. Fundus

1. Blood Flow and Oxygenation

Normal physiology in the retina, choroid, and optic nerve is critical to good visual function. There are currently no non-invasive technologies that are routinely employed in patient care, but these are available. Based upon the pioneering work of George Benedek at MIT, the non-invasive nanotechnology of laser Doppler velocimetry has been used to measure blood flow in the human retina, optic nerve, and choroid. Also available are in vivo measures of oxygenation as developed by Delori et al. and Stefánsson. Clinical application of non-invasive evaluation of circulatory physiology and ocular metabolism will enable the paradigm shift in diagnostics from its current focus on histopathology to defining health and disease on the basis of physiopathology, rather than histopathology, hopefully enabling intervention at more reversible stages of disease. Non-invasive evaluation of ocular circulation is currently increasing substantially with the development of OCT angiography. Applications to date include glaucoma (Figure 6) and age-related macular degeneration. (Figure 7)

![FIGURE 6: OCT ANGIOGRAPHY (OCTA) IN GLAUCOMA](image)

Disc photographs (A1, A2), optical coherence tomography (OCT) reflectance (B1, B2), whole-depth OCT angiograms (C1, C2, en face maximum projection), and cross-sectional angiograms (D1, D2, overlaying on OCT reflectance in gray scale) in the right eye of a normal subject (A1) and the left eye of a perimetric glaucoma (PG) subject (A2). Disc margins are marked by the black elliptical outlines (B1, B2, C1, C2). The position of the cross-section is shown by dotted blue lines (C1, C2). A dense microvascular network is visible on the OCTA of the normal disc (C1). This network was greatly attenuated in the glaucomatous disc (C2).


2. Macula

Age-related macular degeneration (AMD) is believed to result from light-induced and metabolism-related oxidative injury to retinal and retinal pigment epithelial cells. Genetic predisposition(s) influences the contributory inflammatory sequelae. It has been proposed that dietary carotenoids, such as lutein and zeaxanthine, could impart protection against AMD owing to the blue light-filtering properties of carotenoids. Non-invasive technologies are being developed to measure the levels of macular pigment as a means of identifying individuals at risk as well as monitoring the response to therapy. Such an approach would truly embody the principles of quantitatively characterizing relative levels of wellness and enabling monitored strategies to promote better health.
**FIGURE 7: OCT ANGIOGRAPHY (OCTA) IN AGE-RELATED MACULAR DEGENERATION**

(Top, left) En face OCTA of the inner retina. (Top, center) En face OCTA of the outer retina showing the CNV. (Top, right) Early-phase fluorescein angiography (FA). (Bottom, left) Composite en face OCTA showing intraretinal fluid (light blue) over the CNV (yellow). (Bottom, center) Retinal thickness deviation map showing thickening over the CNV.

[courtesy of Dr. Liang Liu, Dr. Yali Jia, and Dr. David Huang; Reprinted with permission from Jia Y, Bailey ST, Wilson DJ, Tan O, Klein ML, Flaxel CJ, Potsaid B: Quantitative optical coherence tomography angiography of choroidal neovascularization in age-related macular degeneration. Ophthalmology 2014;121(7):1435-44]

**D. Implementation Platform**

The implementation of advanced non-invasive nanodiagnostics via tele-ophthalmology platforms is an important aspect of this paradigm shift. Such a platform will enable disease (ophthalmic and systemic) detection remotely rather than in the office or clinic. Not only will this provide better outreach into the community, but should enable earlier disease detection and therapy during more reversible stages of disease, which is the ultimate paradigm shift in Medicine.

**CONCLUDING REMARKS**

The foregoing provides an overview of paradigm shifts that promise to improve not only ophthalmic diagnostics, but all aspects of eye and vision care. This white paper is not intended as a review nor to be comprehensive. Rather, topics have been selected to illustrate the principles of the paradigm shifts the authors felt were important for the future of ophthalmic diagnostics. Indeed, successful development and implementation of these paradigm shifts may also provide useful guidance for similar developments in all of healthcare. These transformations must not only be embraced by the ophthalmic community but actively promoted by those most skilled in the art, for the betterment of the science as well as patient care. Eventually, powerful diagnostic capabilities will shift our focus from disease detection to prevention and health promotion, enabling us to help people feel and function better, thereby fulfilling what should be the ultimate mission of medicine:

"...to help people die young, as late in life as possible.

Ernst L. Wynder, President American Health Foundation, 1976"
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